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Research Article

Novel Anlytical Rp-Hplc Method for Simultaneous Determination of Antiretroviral Drugs

USHA KONDLA[1], VANKUDOTH KAVITHA[2],GOPI SWAPNA[3],PITTA LAVANYA[4]
Avanthi Institute of Pharmaceutical Sciences, Approved by AICTE, PCI, NAAC, JNTUH, Gunthapally, Hayathnagar, Hyderabad-501512
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ABSTRACT
A simple, precise, rapid and accurate reverse phase HPLC method was developed for the simultaneous estimation of cobicistat and elvitegravir in the pharmaceutical dosage form. A column of ODS (250mm 4.6mm; i.d and 5µ particle size) was used along with the mobile phase comprising of 0.02M dipotassium hydrogen orthophosphate buffer (pH adjusted to 3.3) and methanol in the ratio of 80:20 (v/v). The flow rate was maintained at 1.0 ml/min and the effluents monitored at 254 nm. The retention time for cobicistat was found to be 2.58 ± 0.3 min and elvitegravir was 3.71 ± 0.3 min. The detection concentration was linear over 125-750 µg/ml for cobicistat and 12.5-75 µg/ml for elvitegravir. Regression equations of cobicistat and elvitegravir were found to be y = 25883x + 19711 and y = 27696x + 6046 respectively with regression co-efficient 0.999. The % RSD for Intra and Inter day precision was < 2%. The accuracy of method was validated by recovery studies and found to be significant within acceptable range 98-102%. The developed method was successfully validated in accordance with ICH guidelines.

Keywords: Cobicistat, Elvitegravir, Anti-HIV agent, CYP3A Inhibitors, Validation, ICH guidelines

INTRODUCTION

Cobicistat and Elvitegravir combined dosage form is used for the treatment of HIV infection in adult patients. Cobicistat is chemically as 1,3-thiazol-5-ylmethyl N-[(2R,5R)-5-[[(2S)-2-[[methyl [(2propa2-yl-1, 3-thiazol-4-yl) methyl] carbamoyl] amino]-4 morpholin-4-yl buta-noyl] amino]-1, 6- diphenylhexan-2-yl] carbamate which acts as an HIV integrase inhibitor [1, 2].

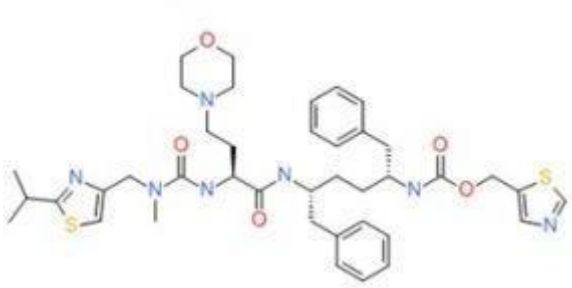

Fig. 1: Chemical Structure Of Cobicistat

It has a molecular formula of C40H53N7O5S2 and a molecular weight of 776.0 g/mol **Fig. 1**. It is a mechanism- based inhibitor of cytochrome P450 3A (CYP3A) isoforms. Cobicistat does not have any anti-HIV activity on its own. It is a new pharmacokinetic enhancer, metabolized by CYP3A and especially used to increase elvitegravir levels when administered.

Elvitegravir is chemically 6-(3-chloro-2-fluorobenzyl) -1- [(2S) -1-hydroxy-3-methyl butan -2- yl] -7-methoxy -4- oxo-1, 4 dihydro quinoline -3- carboxylic acid. It has a molecular formula of C23H23ClFNO5 and a molecular weight of 447.883 g/mol **Fig. 2**. Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme that is required for viral replication.

Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection [3-5].

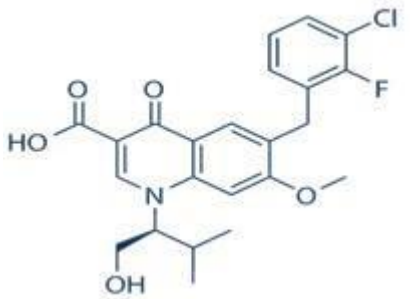

Fig. 2: Chemical Structure Of Elvitegravir

Various HPLC and HPTLC methods were reported in the literature for the estimation of cobicistat and elvitegravir individually [6-8], simultaneously [9-10] and other antiretroviral drugs [10-20]. The present method is novel and was successfully validated in accordance with ICH guidelines [21]. The results of the study showed that the proposed RP-HPLC method is useful for the routine simultaneous determination of cobicistat and elvitegravir in the pharmaceutical dosage form.

MATERIALS AND METHODS

Materials: Cobicistat and elvitegravir were obtained as a gift sample from Hetero Drugs Ltd. Hyderabad. Methanol (Merck Specialities Private Limited, India), potassium dihydrogen phosphate and ortho- phosphoric acid (Rankem Ltd., India) used were of analytical grade. Commercially available cobicistat tablets (TYBOST® 150mg) and elvitegravir tablets (VITEKTA ®-150mg) were procured from the local market.

Instruments

Quantitative HPLC was performed on Waters Alliance 2695 separations module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with Empower-2 Software. An ODC column of 250mm 4.6mm: i.d and 5μ particle size was used. PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz was be used for UV measurements.

Selection of UV Wavelength

The sensitivity of the method that uses UV-Visible detector depends upon the proper selection of wavelengths. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected.

Standard solutions of cobicistat and elvitegravir were scanned in the UV range (200-400nm), and the spectrums obtained were overlaid, and the overlain spectrum was recorded. From the overlain spectrum, 254 nm was selected as the detection wavelength for the present study **Fig. 3**.

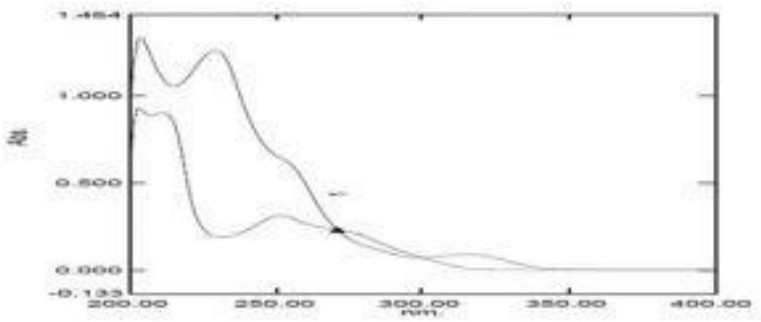

Fig. 3: Over Line Spectrum Of Cobicistat And Elvitegravir

Preparation of Standard Solution

Accurately weighed and transferred 50 mg of cobicistat and 5 mg of elvitegravir working Standards into 10 ml clean dry volumetric flasks, added 3/4 ml of diluent, sonicated for 5 min and makeup to the final volume with diluents. 1 ml each from the above two stock solutions was taken into a 10 ml volumetric flask, made up to the mark to obtain the final concentration of 500 µg/ml of cobicistat and 50 µg/ml of elvitegravir respectively.

Preparation of Working Standard

Twenty tablets of cobicistat and two tablets of elvitegravir were accurately weighed, the average weight of tablets were found and crushed to a fine powder. From the triturate of tablets, an amount equivalent to 2500 mg of cobicistat and 250 mg of elvitegravir were weighed and transferred into 100ml volumetric flask and make up to the mark with diluent. The solution was sonicated for 25 min and filtered through Whatman filter paper no. 41. From both the solutions pipette out
0.2 ml each, transfer into a 10 ml volumetric flask, made up to the mark with diluent to obtain final concentration of 500 µg/ml of cobicistat and 50 µg/ml of elvitegravir working standards.

Preparation of Mobile Phase

Prepare a mixture of 80 ml buffer and 20 ml methanol, degas in an ultrasonic water bath for 5 min. Then this solution is filtered through 0.45 µ filter under vacuum filtration. The mobile phase is used as diluent.

Preparation of Buffer (0.01 KH_2PO_4)

Accurately weigh 1.36 gm of Potassium di-hydrogen orthophosphate in a 1000ml of volumetric flask, add about 900 ml of Milli-Q water and degas to sonicate. Finally, make up the volume with water and pH adjusted to 3.3 with orthophosphoric acid solution.

Analytical Method Validation

The HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines (2005) [21]. The following characteristics were considered for validation: specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness.

The specificity was evaluated by comparing the representative chromatograms of samples containing possible interfering substances and samples containing cobicistat and elvitegravir. Linearity was determined from the plot peak area *vs*. concentration for the six concentrations of cobicistat and elvitegravir. The regression equation and regression coefficient were calculated using least square methodology.

Precision is of two types: repeatability or intra-day variability and intermediate precision or inter-day variability. The intraday precession was testing for six different solutions of cobicistat and elvitegravir on the same day. Inter day precision tested by analyzing solutions of both drugs six times on different days. The results were reported as % RSD.

The LOD and LOQ were determined from the specific calibration curve obtained using six standard solutions that were the closest to the LOQ.

Robustness was evaluated by deliberately varying the temperature of the analytical column, the flow rate, and by using similar columns.

RESULTS AND DISCUSSION

System Suitability

The system suitability studies were evaluated by comparing with standard chromatogram and by obtaining the parameters retention time, column efficiency, and tailing factor **Table**

All the system suitability parameters are within range and satisfactory as per ICH guidelines **Fig. 4,5** [21].

Table 1: System Suitability Data

Property	Cobicistat	Elvitegravir
Retention time (tR)	2.58 ± 0.3 min	3.71 ± 0.3min
Theoretical plates (N)	6477 ± 163.48	7979 ± 163.48
Tailing factor (T)	0.86 ± 0.117	1.34 ± 0.117

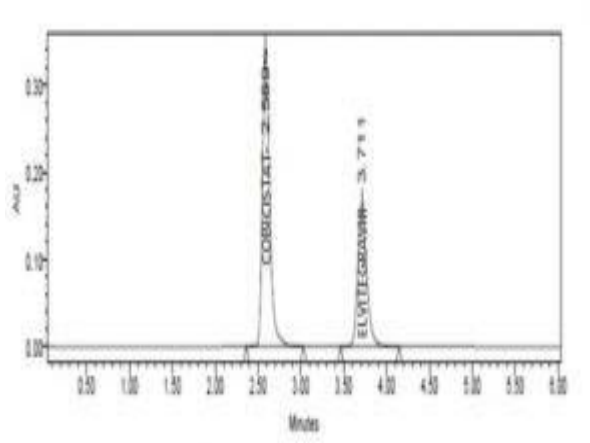

Fig. 4: Optimized Chromatogram Of Cobicistat And Elvitegravir Standards

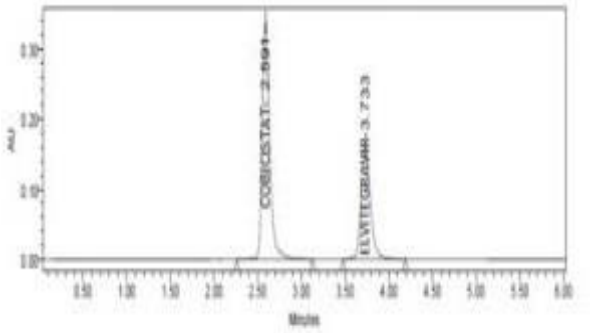

Fig. 5: Optimized Chromatogram Of Cobicistat And Elvitegravir Sample

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of the analyte in the sample. A value of correlation coefficient (r^2) > 0.998 is considered as the evidence of an acceptance of the data to the regression line. Serial dilutions of cobicistat (125-750 µg/ml) and elvitegravir (12.5-75 µg/ml) were injected into the column and detected at a wavelength set at 254 nm Table 2. The calibration curve was obtained by plotting the concentration *vs.* peak area Fig. 6, 7.

Regression equation of cobicistat and elvitegravir are found to be y = 25883x + 19711 and y = 27696x + 6046 respectively. The regression co-efficient was 0.999.

Table 2: Calibration Data Of Cobicistat And Elvitegravir

S. no.	Concentration of Cobicistat (µg/ml)	Response	Concentration of Elvitegravir (µg/ml)	Response
1	0	0	0	0
2	125	3481464	12.5	352868
3	250	6645306	25	689638
4	375	10066263	37.5	1052360
5	500	13408035	50	1405358
6	625	16372442	62.5	1753133
7	750	19348128	75	2059078

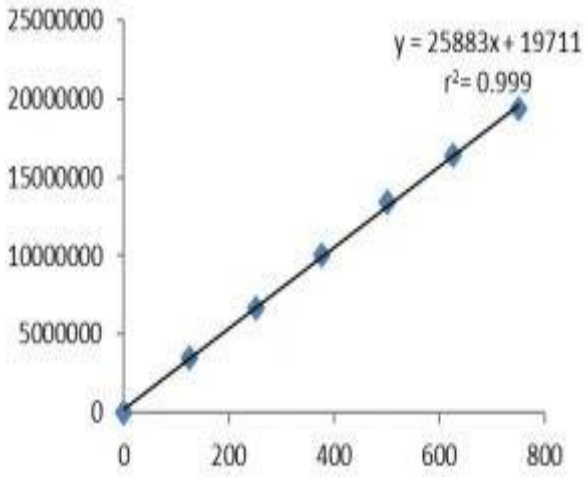

Fig. 6: Calibration Curve Of Cobicistat (X-Axis = Concentration, Y-Axis = Peak Area)

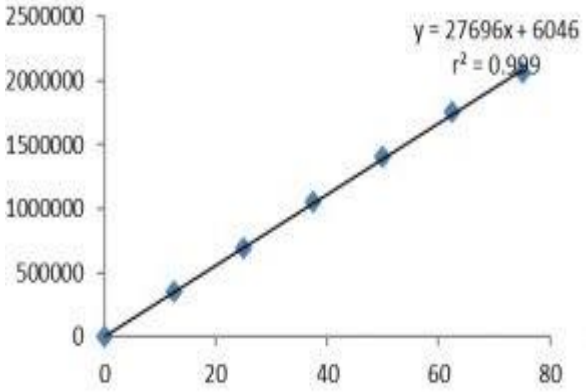

Fig. 7: Calibration Curve Of Elvitegravir (X-Axis = Concentration, Y-Axis = Peak Area)

Assay Studies

Six homogeneous samples of both samples and standards were injected. The percentage assay of the drugs in the formulation was estimated. The average % assay was calculated and found to be 99.87% and 100.16% for cobicistat and elvitegravir respectively Fig. 8. The assay data were tabulated in Table 3.

Assay % = AT / AS × WS / DS × DT / WT × P / 100 × Avg. Wt / Labelled Claim (LC) × 100 Where,

AT = average area counts of sample preparation. AS = average area counts of standard preparation. WS = Weight of working standard taken in mg.

DS = Weight of sample taken in mg.

P = Percentage purity of working standard LC = Label claim of a drug in mg/ml.

Table 3: Assay Data Cobicistat And Elvitegravir

S. no.	Cobicistat % Assay	Elvitegravir % Assay
1	99.24	100.13
2	100.61	99.55
3	99.61	100.92
4	100.53	99.50
5	100.16	100.54
6	99.07	100.31
Avg.	99.87	100.16
Std. Dev	0.66	0.56
% RSD	0.66	0.56

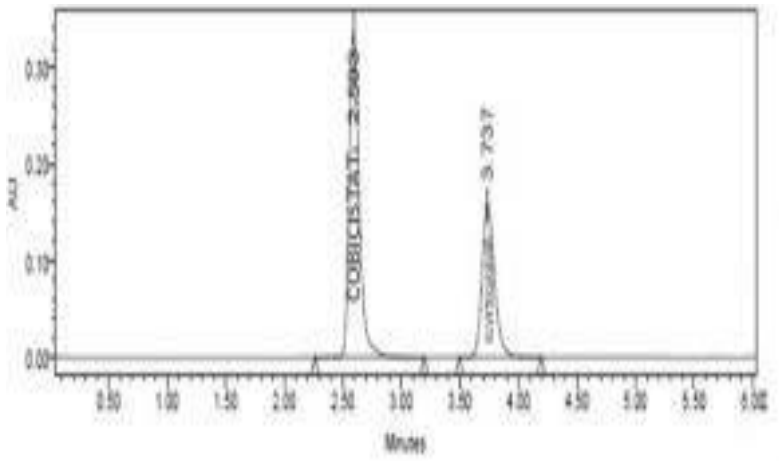

Fig. 8: Standard Assay Chromatogram Of Cobicistat And Elvitegravir

Table 4: Inter Day Precision Studies Of Cobicistat And Elvitegravir

S. no.	Cobicistat (500 µg/ml)	Elvitegravir (50 µg/ml)
1	13541278	1387585
2	13515981	1412087
3	13030796	1353382
4	12991186	1350963
5	13056331	1357619
6	13087151	1362676
Mean	13203787	1370719
Std. Dev.	253708	24178.3
%RSD	1.92	1.76

Precision

The precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of both standard and sample solutions. Precision was determined in six replicates of the analyte on the same day (intra-day precision) and daily for 6 times over a period of one week (inter-day precision). The results were expressed as % RSD of the measurements. The interday and intraday precession studies of cobicistat and elvitegravir were performed at concentrations of and found within the acceptable limit.

Interday precision was performed with 24 h time lag and the % RSD obtained for cobicistat and elvitegravir were 1.92% and 1.76% Table 4.

Intraday Precision Studies (Repeatability)

Interday precision was performed and % RSD for cobicistat and elvitegravir were found to be 0.66% and 0.56% respectively Table 5.

Table 5: Repeatability Results For Cobicistat And Elvitegravir

S. no.	Cobicistat (500 µg/ml)	Elvitegravir (50 µg/ml)
1	13347448	1386574
2	13531146	1378592
3	13397408	1397495
4	13520404	1377842
5	13470132	1392342
6	13324749	1389042
Mean	13431881	1386981
Std. Dev.	88258	7714.8
%RSD	0.66	0.56

Recovery

Three different concentrations (50%, 100%, 150%) of cobicistat and elvitegravir were injected in a triplicate manner and amount recovered, and percentage recovery were tabulated in Table 6.

Table 6: Accuracy Results For Cobicistat And Elvitegravir

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
	250	250.70	100.28	0.11
Cobicistat	500	499.78	99.96	0.24
	750	749.4	99.92	0.43
Elvitegravir	25	25.08	100.33	0.55
	50	49.89	99.79	0.42
	75	74.97	99.97	0.86

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of the y-intercepts of regression lines.

* LOD = 3.3 × /S

**LOQ = 10 × ϰS

Where;

ϱ= the standard deviation of the response * S = Slope of the calibration curve

LOD for Cobicistat and Elvitegravir were found to be 2.51 µg/ml and 0.72 µg/ml respectively **Table 7**.

Table 7: Lod Results For Cobicistat And Elvitegravir

S.no.	Drug name	Conc. (µg/ml)	RT (min)	Area (µV* sec)
1	Cobicistat	2.51	2.589	56211
2	Elvitegravir	0.72	3.732	5016

LOQ

Limit of quantification of cobicistat and elvitegravir was calculated by method and LOQ for cobicistat and elvitegravir were found to be 7.62 µg/ml and 2.18 µg/ml respectively Table 8.

Table 8: Loq Results For Cobicistat And Elvitegravir

S. no.	Drug name	Conc. (µg/ml)	RT (min)	Area (µV* sec)
1	Cobicistat	7.62	2.590	56211
2	Elvitegravir	2.18	3.733	5016

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

For the determination of a method's robustness, deliberate change in the Flow rate was made to evaluate the impact on the method. Small deliberate changes in a method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized changes in the result and are within range as per ICH Guidelines Table 9, 10.

Table 9: Robustness Results For Cobicistat And Elvitegravir

S. no.	Robustness condition	Cobicistat %RSD	Elvitegravir %RSD
1	Flow minus	0.3	0.0
2	Flow Plus	0.5	0.6
3	Mobile phase minus	0.0	0.1
4	Mobile phase Plus	0.3	0.2
5	Temperature minus	0.2	0.1
6	Temperature Plus	0.5	0.1

Table 10: Detection Characteristics Of Cobicistat And Elvitegravir

Parameters	Cobicistat	Elvitegravir
Calibration range (µg/ml)	125-750 µg/ml	12.5-75 µg/ml
Optimized wavelength	254nm	254nm
Retention time	2.58±0.3min	3.71±0.3 min
Regression equation (Y)	y = 25883x + 19711	y = 27696x + 6046
Correlation coefficient (r^2)	0.999	0.999
Precision (% RSD)	1.92%	1.76%
% Assay	99.87%	100.16%
Limit of Detection (µg/ml)	2.51 µg/ml	0.72 µg/ml
Limit of Quantitation (µg/ml)	7.62 µg/ml	2.18 µg/ml

CONCLUSION

From the typical chromatogram of drugs, it is shown that the retention time for cobicistat is 2.58 min and elvitegravir is 3.71min. The mobile phase comprises of 0.02M di-potassium hydrogen orthophosphate buffer (pH adjusted to 3.3) and methanol in the ratio of 80:20 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 254 nm. Over 1.0 ml/min gradient mode of separation, which was found to be most suitable to obtain a peak well defined and free from tailing.

In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction was involved. A good linear relationship (r^2=0.999) was observed between the concentration range of 125-750 µg/ml for cobicistat and 12.5-75 µg/ml for elvitegravir. Low values of standard deviation are indicative of the high precision of the method. The percentage assay of Cobicistat is 99.87%, and Elvitegravir is 100.16%. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The limit of detection (LOD) and limit of quantification (LOQ) for cobicistat were found to be 2.51 µg/ml and 7.62 µg/ml; for elvitegravir were 0.72 µg/ml and 2.18 µg/ml respectively. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of the pharmaceutical dosage form of the drugs within a short analysis time.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

Nil

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Research Article

Cytotoxicity of *Taxillus tomentosus* and *Spilanthes calva* in Different Cancer Cell Cultures *In-vitro*

Rambabu Boda[1]*, Rakeshkumar Jat[1], Harikiran Lingabathula[2]

[1]*Department of Pharmacology, Shri JJT University, Jhunjhunu, Rajasthan, India*
[2]*Department of Pharmacology, Princeton College of Pharmacy, Ghatkesar, Telangana, India*

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ABSTRACT

Cancer is one of the leading diseases to cause death globally. Most of the synthetic drugs used to treat cancer are having serious side effects. So, there is a need to develop the drugs which are used to treat cancer with little side effects. This can lead to investigating the plant products having fewer side effects, for their anti-cancer activity. The present investigation evaluated the in vitro toxicity of leaf extracts and fractions of *Spilanthes calva* and *Taxillus tomentosus* in 5 totally different neoplastic cell cultures, like HeLa (cervical cancer cells), HEK 293 (kidney cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) and Hep G2 (hepatic cancer cells) by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays. Among all the test extracts and fractions, petroleum ether and n-hexane fractions of *S. calva*, and ethyl acetate and n-hexane fractions of *T. tomentosus* have been shown maximal cytotoxic activities against all tested cell cultures. The IC50 values of SCPEF, SCNHF, and TTEAF were found to be 41.79, 42.26 and 41.01 µg/mL on HEK 293 cells, which were very close to standard drug PCTXL has been found to be 35.22 µg/mL on HEK 293 kidney cancer cells. The TTNHF IC50 value was found to be 39.29 µg/mL which is comparable with the standard paclitaxel (PCTXL) 39.18 µg/mL on A549 lung cancer cells.

Introduction

Cancer is one of the most dangerous diseases found and is the second reason for death worldwide characterized by uncontrollable cellular growth.[1] Therapies for cancer malady embrace surgery, chemotherapy, radiation therapy, and hormonal therapy have shown restricted success and have some serious aspect effects. Thus, it's necessary to use different methodologies for the treatment of cancers.[2,3] The assumption that natural materials are safer than artificial medication has a basis for the extraordinary development in human exposure to natural medicines like plants, phytotherapeutic agents, and phytopharmaceutical products.[4] Research has developed into assessing the potential properties and uses of plant extracts for the treatment of cancers In parallel, there is huge evidence for the potential of plant-based products as inhibitors of various stages of cancers.[5,6]

To date, many plant-based compounds have been identified that have anti-cancer properties, such as, inhibition of cell proliferation and induction of apoptosis which finally reduce the cancer risk. Furthermore, many phytochemicals have been known that can inhibit tumor progression via various mechanisms.[7-10] Paclitaxel, Vincristine and vinblastine are well-known examples of derived from plants clinically useful anti-cancer drugs. Free radical scavenging properties of different plant extracts are also of great importance because herbal compounds with free radical scavenging activity can protect against different cancers.[5] Plant-derived natural drug merchandise have received important attention in recent years, thanks to their numerous pharmacologic assets as well as cytotoxic and cancer chemo-preventive effects. Therefore, knowing and assessing the potentials of plant-derived bioactive compounds is

***Corresponding Author:** Rambabu Boda

Address: Department of Pharmacology, Shri JJT University, Jhunjhunu, Rajasthan, India

Email ✉**:** rambabuboda34@gmail.com

Tel. : +91 7893151551

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very important for additional drug development.[11,12] *Spilanthes calva* belongs to the family *Asteraceae*, is an herb having 60 cm in height. The plant is having high medicinal values and is used for the treatment of various diseases. This plant is having anti-ageing properties, antimicrobial and antifungal activities, alleviates toothache and dysentery, as well as enhances immunity. The plant leaves stimulate salivation, which is due to containing an active chemical spilanthol.[13,14] *T. tomentosus* is one of the plants that belong to the family *Loranthaceae*. Recent studies revealed that this plant have diverse pharmacological activities, such as antidiabetic, hepatoprotective, neuroprotective, cardioprotective, antistress, nootropic, and anti-urolithiasis activities.[15-17]

In this research, we tend to assess the *in vitro* toxicity of leaf extracts and fractions of *S. calva* and *T. tomentosus* using five different cancer cell cultures, such as HeLa (cervical cancer cells), (HEK 293) (Human embryonic kidney cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) and Hep G2 (hepatic cancer cells) by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) leakage assays.

Materials and Methods

Chemicals

The fetal bovine serum, DMEM/RPMI 1640 culture media, L-glutamine, penicillin-streptomycin antibiotic solution, phosphate buffer saline (PBS), trypsin-EDTA, and ascorbic acid were purchased from Himedia, India. MTT was purchased from Sigma-Aldrich, Banglore, Karnataka, India. LDH assay kit was acquired from Cayman Chemicals, USA. All the neoplastic cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India.

Plant Collection, Extract Preparation and Fractionation

The recent healthy, sickness-free leaves of *S. calva* and *T. tomentosus* were collected from the hills of Tirumala, Tirupati, India, and were authenticated by Dr. M. Madhava Chetty, Department of Biological Sciences, Sri Venkateswara University, Andhra Pradesh, India. The leaves of each plant were shade dried for 2 weeks and ground into a rough powder employing a grinder. The 100 g of powder of all plant leaves were macerated for 24 hours with continuous stirred in 500 mL of ethyl alcohol employing a shaker at 28°C. Then, the supernatant was recovered by filtration through muslin cloth and Whatman paper.

Further, the filtrates were utterly dried by a rotary vacuum evaporator. The solvents were evaporated to dryness at room temperature to produce the ethanol extracts of both plants. The ethanol extracts of *S. calva* (SCEE) and *T. tomentosus* (TTEE) were subjected to fractionation by partitioning the aqueous suspension of the drug with petroleum ether, ethyl acetate, and n-hexane to get respective fractions such as petroleum ether fraction (SCPEF and TTPEF), ethyl acetate fraction (SCEAF and TTEAF), n-hexane (SCNHF and TTNHF) and aqueous fraction (SCAF and TTAF). Further, the extract and fractions were stored at -4°C until further use. Both plants' different concentrations of methanolic extract and their fractions (10, 20, 40, 80, and 160 µg/mL) were prepared in 1% DMSO for determining in vitro cytotoxicity.

Cell Culture and Treatment

HeLa, HEK 293, MCF-7, A549, and Hep G2 cells were obtained from NCCS, Pune. These cells were used between passages ten and fifteen. All these cells were fully grown in culture media (RPMI 1640/DMEM) along with 10% fetal bovine serum (FBS), 1% of L-glutamine, and 1% of the penicillin-streptomycin antibiotic solution. The neoplastic cells were planted at 2,50,000 cells/flask within the total volume of 10 mL. After sufficient growth, all the cells were trypsinized using trypsin-EDTA.

The cells were seeded in 96 well plates (Parsons, India) at the rate of 1.0 x 104 cells/100 µL. All the neoplastic cell lines were maintained in a 5% CO_2 + 95% O_2 incubator (WTC Binder, Germany) at a temperature of 37°C. The test extracts and their fractions were prepared in 1% DMSO. Various concentrations of test extracts, fractions, and standard drug, paclitaxel (PCTXL) in the corresponding culture medium were freshly ready and used for *in vitro* cytotoxic activity.

In-vitro Cytotoxicity by MTT Assay

The impact of *S. calva* and *T. tomentosus* test extracts and fractions on the cellular propagation and viability was decided by using the MTT assay technique. For performing this assay, the neoplastic cells were seeded in 96 well plates and each well solvent/extract/fraction solution (0.1 mL, in triplicate) in respected media was added.

This 96 well plate was subsequently incubated at 37 ± 2°C for 48 h in a 5% CO_2 incubator and the MTT (5 mg/mL) was added to all wells. Further, the 96 well plate was another time incubated for 2 hours, and DMSO (80 µL) was added to every well, the microtiter plate was enveloped with aluminum foil to avoid the oxidation of the MTT dye and the microtiter plate was kept on a rotary shaker (Remi equipment's, India) for 2 hours. The resulting absorbances were documented using the enzyme-linked immunoassay (ELISA) reader (Anthos, Germany) at 562 nm.[18,19] The absorbance produced by the test extracts and fractions compared with the solvent control to calculate the percent cytotoxicity.

LDH Leakage Assay

Lactate dehydrogenase is a soluble enzyme that contains zinc and is situated in the cytosol of cells. This enzyme is released/leaked into the surrounding culture medium only upon cell death, may be used as a symbol of cell

wall integrity, and therefore a measurement of cell toxicity. For this assay, the cells were seeded in 96 well microtiter plates at a density of 1.0×10^4 cells/well in100 µL of corresponding culture medium. 100 µL of growing concentrations of test extracts/fractions/standard drug were added to each well in triplicate. Subsequently, the microtiter plate was incubated in a 5% CO_2 incubator at 37 ± 2°C for 48 hours.[20] The supernatants of microtiter plate continued shifted into new 96 well microtiter plates and assayed giving to the producer's protocol.

Statistical Analysis

The IC50 was calculated as soon as at least 2 viability values were beneath 50% of the control condition, utilizing the GraphPadPrism software. The statistical analysis remained performed employing one-way ANOVA subsequently Bonferroni posttests. The data were represented as mean ± standard deviation (S.D.) of 3 independent experiments. Test significance was designated by *p < 0.01 and ** p < 0.001 compared to control.

Results

MTT Assay

The *in vitro* toxicity of leaf extracts and fractions of *S. calva* and *T. tomentosus* using different cancer cell cultures by MTT assay were performed and calculated the IC50 values were shown in Table 1. The percent cytotoxicity induced by *Spilanthes calva* and *T. tomentosus* plant extracts and fractions were shown in Figs. 1 and 2. The percent cytotoxicity was increased in a dose-dependent manner related to all tested cancer cell cultures for both plant extracts and fractions tested.

LDH Leakage Assay

The cytotoxicity of *S. calva* and *T. tomentosus* using different cancer cell cultures by LDH leakage assay were performed and shown in Fig. 3 and 4, respectively. The leakage of LDH is increased in a dose-dependent manner.

Discussion

The plant-based most natural products efficiently induce cytotoxicity in neoplastic cells are promising to play a substantial role in managing and curing cancer. Various reports showed several extracts, fractions, and isolates derived from medicinal plants demonstrated their anti-cancer activities.[21] So, the present experiment investigated the potential in vitro cytotoxicity induced by the leaf extracts and fractions of *S. calva* and *T. tomentosus* using different cancer cell cultures, such as HeLa (cervical carcinoma cells), HEK 293 (Human embryonic kidney carcinomas cells), MCF-7 (breast carcinoma cells), A549 (lung carcinoma cells) and Hep G2 (hepatic carcinoma cells) by MTT and LDH leakage assays.

Our MTT assay study reveals that both plant extracts and fractions were shown increased cytotoxic activity in a dose-dependent manner. The SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown to promise cytotoxic activities in all the cell cultures tested. For *S. calva*, petroleum ether and n-hexane fractions showed the highest cytotoxic activities compared to other fractions and ethanolic extract. Also, both SCPEF and SCNHF have shown maximum cytotoxic activity towards HEK 293 cells so that these fractions have more cytotoxicity against kidney cancer cells. For *T. tomentosus*, fractions of n-hexane and ethyl acetate showed superior cytotoxic activities compared to other factions.

TTEAF has been shown maximum cytotoxicity towards HEK 293 cells, whereas TTNHF has been shown maximum cytotoxic activity towards A549 cells. So that, TTEAF has more cytotoxic activity against kidney cancer cells, whereas TTNHF has more cytotoxicity against lung cancer cells. The IC50 values of SCPEF, SCNHF, and TTEAF were found to be 41.79, 42.26 and 41.01 µg/mL on HEK 293 cells, which were very close to standard drug PCTXL is 35.22 µg/mL on HEK 293 kidney cancer cells. The TTNHF IC50 value was 39.29 µg/mL, which is comparable with the

Table 1: IC50 values (µg/mL) of various extracts on different human cell cultures

	IC50 values in different cell lines (µg/mL)				
Plant extracts	*HeLa*	*HEK 293*	*A549*	*MCF 7*	*Hep G2*
SCEE	46.52	47.83	47.70	48.11	48.77
SCPEF	46.81	41.79	43.86	46.37	47.26
SCEAF	53.07	49.56	47.93	51.97	51.67
SCNHF	45.16	42.26	43.34	46.38	51.60
SCAF	50.18	52.27	56.26	48.85	48.52
TTEE	52.65	50.17	50.23	52.29	48.42
TTPEF	55.88	48.71	46.32	55.13	50.48
TTEAF	47.94	41.01	43.86	47.78	44.36
TTNHF	43.06	42.26	39.29	44.49	46.83
TTAF	54.56	54.30	59.73	51.56	49.66
PCTXL	40.84	35.22	39.18	41.04	39.94

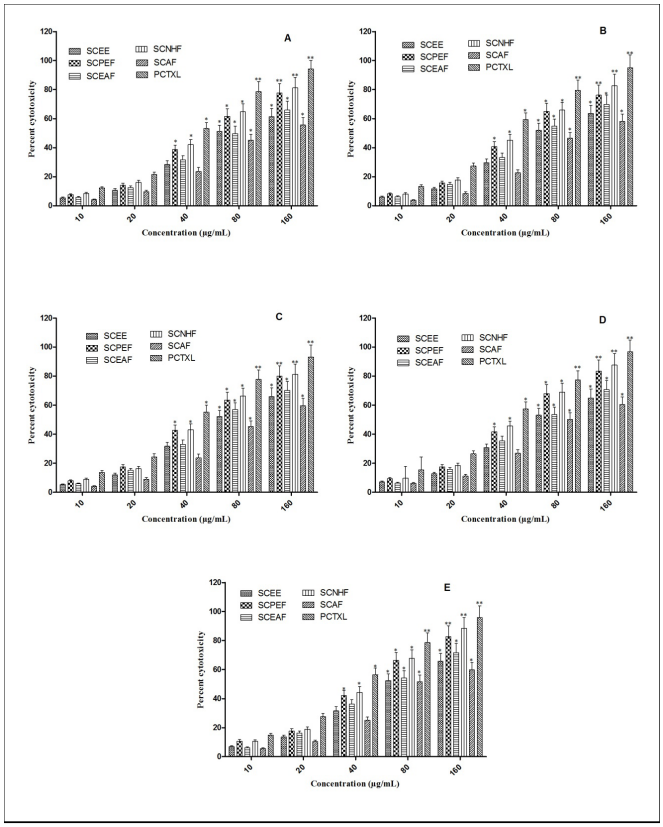

Fig. 1: Percent cytotoxicity measured by MTT assay on (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7, and (E) Hep G2 carcinoma cells, after treatment with different extracts of *Spilanthes calva* (10-160 µg/mL) for 48 h

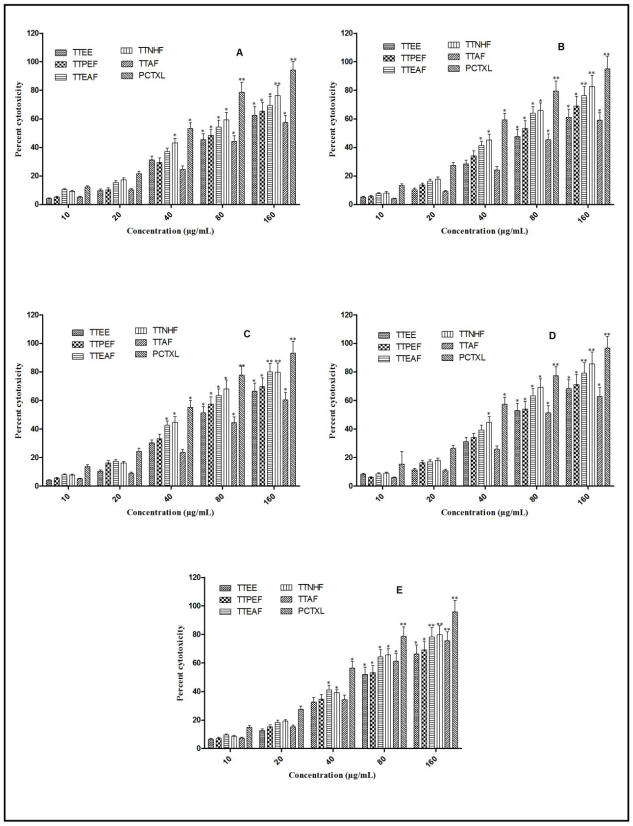

Fig. 2: Percent cytotoxicity measured by MTT assay on (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7, and (E) Hep G2 carcinoma cells, after treatment with different extracts of *Taxillus tomentosus* (10-160 µg/mL) for 48 h.

standard PCTXL 39.18 µg/mL on A549 lung cancer cells.

Effect of anti-cancer activity raised considered by using MTT assay on HT- 29 cell lines of ethanolic extract of *Reissantia indica*. Different quantities of plant extracts and standard remained taken, and existing into cancer cells remained recorded at 24 hours respectively. It visibly showed U's the dose needed for the inhibition of cells. The utmost potent anti-cancer activity is shown colon cancer cell line at the concentration of 1000 µg/mL of *R. indica* extract on HT-29.[5] Dikamaliartane, a plant product assessed for the cytotoxic activity by MTT assay on HeLa and MCF 7 cell lines, and the study revealed the potential for its cytotoxic activity against tested cancer cell lines. These studies support our cytotoxic activity results using MTT assay with various cell cultures.

S. calva and *T. tomentosus* extracts and fractions upon treatment with different cancer cell cultures revealed the dose-dependent leakage of LDH from the cells leading to cytotoxicity. As LDH is an enzyme present in the cytoplasm in cells, can release into extracellular sites only after cell membrane damage leading to cell mortality, an indication of cytotoxicity.[18] Similar to cytotoxicity induced through MTT assay, SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown increased leakage of LDH in all the cell cultures tested. For *S. calva*, petroleum ether and n-hexane fractions showed the highest LDH leakage, whereas, for *T. tomentosus*, ethyl acetate and n-hexane fractions showed superior LDH leakage, which was comparable to the standard drug paclitaxel.

One of the studies investigated the in vitro cytotoxic activity of methanol extracts of *Morus alba*, *Eucalyptus camaldulensis*, *Zataria multiflora* and *Cichorium intybus* against P19 embryonal cancer cells using various cytotoxic assays like MTT, LDH leakage, etc. In this investigation, *Morus alba* showed the highest cytotoxicity, whereas *C. intybus* showed the least cytotoxicity.[2] Other studies also revealed that the plant extracts and their fractions have shown the in vitro toxicity against different types of carcinoma cell lines using MTT and LDH leakage assays[22,23] supports our results. Upon treatment with five different carcinoma cells, *S. calva* and *T. tomentosus* extracts and fractions showed the potent in vitro cytotoxic activities in a concentration-dependent manner. Among all the test extracts and fractions, SCPEF, SCNHF, TTEAF, and TTNHF fractions have been shown maximal cytotoxic activities against tested cell cultures by using both MTT and LDH assays. These fractions upon further research may be useful for future drug candidates to treat cancer.

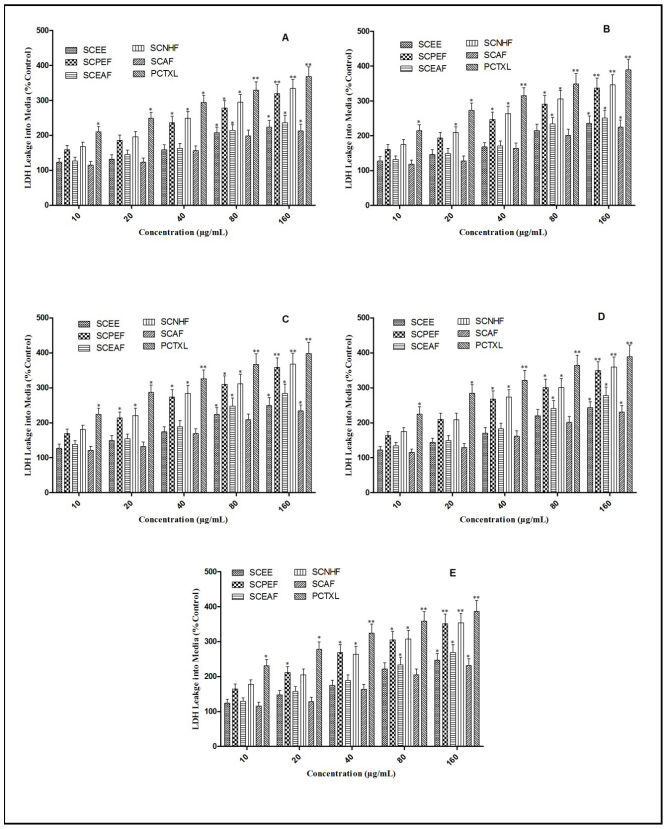

Fig. 3: LDH leakage from (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7 and (E) Hep G2 carcinoma cells, after treatment with different extracts of Spilanthes calva (10-160 µg/mL) for 48 hours.

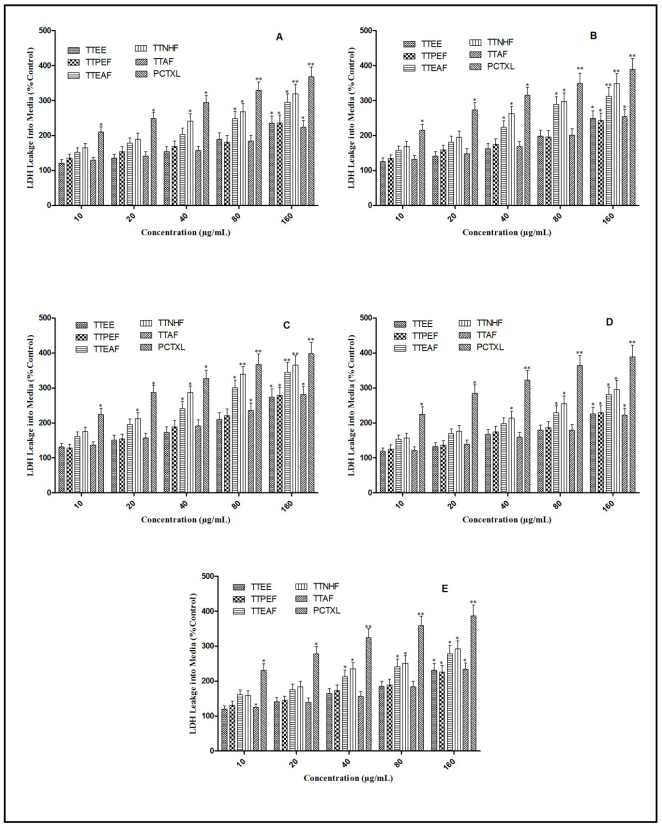

Fig. 4: LDH leakage from (A) HeLa, (B) HEK 293, (C) A549, (D) MCF-7 and (E) Hep G2 carcinoma cells, after treatment with different extracts of Taxillus tomentosus (10-160 µg/mL) for 48 hours.

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Design and Fabrication of Topical Niosomal Gel Containing Aceclofenac and Serratiopeptiase

P.V. Pavan Kumar[1] , Pitta Lavanya[2] , Vankudoth Kavitha[3], Usha Kondla[4]

[1 2 3 4] Avanthi Institute of Pharmaceutical Sciences.,

Approved by AICTE, PCI, NAAC, JNTUH,Gunthapally, Hayathnagar, Hyderabad-501512.

ABSTRACT: Aceclofenac is classified as a BCS Class II drug because of its low water solubility. Systemic adverse effects, such as anticoagulant effects, have been described when SRP is taken orally. The goal of this study was to see how well a topical Niosomal gel containing Aceclofenac (ACE) and Serratopeptidase (SRP) could deliver the drugs. Particle size, shape, entrapment efficiency, and in vitro properties of Niosomal formulations produced using the thin film hydration process at varied cholesterol and Span 40 ratios were studied.The average particle size of the Niosomal formulation was determined to be between 1 µm - 2µm. The entrapment efficiency of the Niosomal formulations F2 (1:1:1) and F6 (1: 2: 1) of cholesterol and surfactant was 65 percent and 66.4 percent, respectively. Niosomal formulation (F2 and F6) displayed high percentage of drug release after 12 hr. For the convenience of application, greater stability, reduced aspect effects, greater affected person compliance, and the convenience of discontinuation on desire, there were numerous advantages to deliver ACE through topical route. Therefore, topical therapy isn't most effective promising at the protection and efficacy fronts however additionally at the financial fronts too. Niosomal gel had greater pores and skin penetration, according to an in vitro study results.

Keywords: ACE; SRP; Topical Niosomal gel; Niosomal gel.

Introduction

Topical drug delivery systems include a large variety of pharmaceutical dosage form like semisolids, liquid preparation, sprays and solid powders. Most widely used semisolid preparation for topical drug delivery includes gels, creams and ointments [1]. Topical preparations are applied to the skin for surface, local or systemic effects. In some cases, the base may be used alone for its therapeutic properties, such as emollient, soothing or protective action. Many topical preparations, however, contain therapeutically active ingredients which is dispersed or dissolved in the base. The combination of active ingredients and base provides the opportunity for a wide range of topical preparations, appropriate for many types of drug delivery and therapy terms used to classify the bases of topical preparations in which therapeutically active ingredients are incorporated, may be based on their physical properties (suspension) or on their intended use (liniments) or on their composition (hydrophilic creams)[2].Topical preparation prevents the GI-irritation; prevent the metabolism of drug in the liver so as increase the bioavailability of the drug. Topical preparations give its action directly at the site of action. It can penetrate deeper into skin and hence give better absorption. Topical application has no of advantages over the conventional dosage forms [3].

The term ‘Gel’ was introduced in the late 1800 to name some semisolid material according to their physiological characteristics rather than molecular composition [5-6]. A gel is a two-component, cross linked three-dimensional network consisting of structural materials. The structural materials that form the gel network can be composed of inorganic particles or organic macromolecules, primarily polymers. U.S.P. defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule

enclosing and interpenetrated by liquid. Gels consist of two phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved in the continuous phase, randomly coiled in the flexible chains [7]..

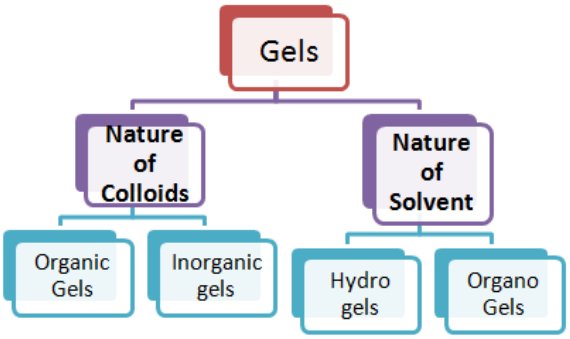

Fig. 1 General Classification of Gels

Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are widely used throughout the world. Generally, they are well tolerated, but the occurrence of sometimes life threatening adverse events limits their use and results in substantial morbidity, mortality and increasing health care cost [8]. To increase the effect–risk ratio of NSAIDs, topical formulations have been developed. NSAIDs are medicines that are widely used to relieve pain, reduce inflammation, and bring down a high temperature [9]. They are often used to relive symptoms of headaches, painful periods, sprains and strains, colds and flu, arthritis, and other causes of long – term pain [10].

Aceclofenac Sodium

Aceclofenac is an oral non-steroidal anti-inflammatory drug (NSAID) with marked anti-inflammatory and analgesic properties used to treat osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Aceclofenac belongs to BCS Class II as it possesses poor aqueous solubility [11-13].

Serratopeptidase

Enzymes play an integral role in a biological world by offering their potential as a biocatalyst [14]. With their broad substrate affinity and robust catalyst, activity enzyme has been used for many decades in different areas including industries, agriculture, research, and development[15-16]. Serratiopeptidase has a long history in medicine and is widely used to combat various kinds of inflammation and inflammatory disorders [17]. Serratiopeptidase or serrapeptase is a protein (proteolytic) enzyme isolated from the non-pathogenic enterobacteria *Serratia E15* found in silkworms. Serratiopeptidase often prescribed in various specialties like surgery, orthopedics, otorhinolaryngology, gynecology and dentistry for its anti-inflammatory, anti-endemic and analgesic effects [18]. A recent finding has suggested that serratiopeptidase reduces capillary permeability induced by histamine, bradykinin, and serotonin; breaks down abnormal exudates and proteins; facilitates the absorption of decomposed products through blood and lymphatics [19].Further, enzyme promotes wound healing and repair and restores the skin temperature of the inflamed area, burn or trauma to normal. The activity of serratiopeptidase remains stable and offer more efficiency in combination with the addition of metal ions like zinc and manganese [20].

Rheumatoid arthritis (RA)

Rheumatoid Arthritis is an autoimmune, inflammatory disease that causes pain, swelling, stiffness, and loss of function in various joints (most commonly in the hands, wrists, and knees). Patients can experience an acute worsening of their symptoms—*disease flare*—but with early intervention and appropriate treatment, symptoms can be ameliorated for a certain duration [21-24].

Material and Method

Material

Aceclofenac was obtained as a gift sample from Welcure Pharmaceuticals, Indore. Serratiopeptidase (SRP) was obtained as a gift sample from Advanced Enzyme Technology, Thane, India. Sorbitan monopalmitate (Span 40) was a gift sample from Nikko Chemicals, India. Cholesterol was purchased from Loba Chemicals Ltd, India. Other materials and solvents used were of analytical grade.

Method

Niosomes were prepared by a thin film hydration method. The accurately weighed amount of span 40 and CHO were dissolved in 10 ml of the chloroform: methanol mixture (2:1) along with Serratopeptidase (5mg) and Aceclofenac (5 mg) in a round bottom flask (100ml) and the solvent was evaporated under

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reduced pressure at room temperature, rotated continuously until the formation of thin lipid film. The formed film was hydrated with 10ml of PBS (pH 7.4). The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain niosomal dispersion containing both free and untrapped drugs of varying size. The unentrapped drug was extracted using centrifugation, and the drug-loaded niosomes were kept at 2–8°C for future analysis.

Compatibility Test

At a neutral pH, this study was carried out with specified excipients. Carbopol 934 was chosen as the polymer. The compatibility tests were all carried out at room temperature. The polymer dispersion (0.5 percent w/v) was treated with an SRP solution (1 percent w/v). The mixture was continually stirred (with a Remi Overhead Stirrer) and kept at room temperature for 12 hours. Using the proteolytic test method, the proteolytic activity of the mixture was evaluated at zero hour and after 12 hours.

Characterization of Niosomes

Microscopy & vesicle size analysis of Niosomes: Optical microscopy with a resolution of 45X confirmed niosome vesicle production. The dried thin layer of Niosomal suspension was noticed after the prepared niosomal suspension was placed over a slide and fastened over it. The niosomes were microphotographed using digital cameras attached to the microscope [25]. Ocular and stage micrometres were used to measure the size of the vesicles [26].

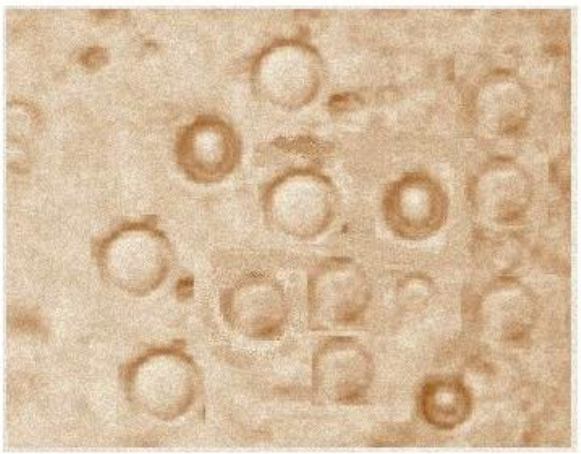

Fig.2 Showing microscopy image of drug loaded Niosomes.

Entrapment Efficiency: Entrapment efficiency was determined using micro-centrifuge RM-12CDX. Niosomal suspension was centrifuged at 12000 rpm for 20 min. The wavelength of maximal absorption was determined in the range of 200-600 nm. At 375 nm, Aceclofenac's absorbance peaks. At 316 nm, the Isoabsorptive point was found. Absorbance of the drug was compared with the standard calibration curve and dilution factor were also calculated [27] :

% of drug encapsulated was calculated by following equation –

$$EE\% = [(Ct-Cf)/Ct)] \times 100$$

Where,

C_t is the concentration of total drugs

C_f is the unentrapped drug.

Table 1. Effect of Span 40 : CHO ratio on percent entrapment efficiency and vesicle size.

Formulation Code	ACE+SRP (10mg):Span 40 : CHO: Composition Ratio	Vesicle Size (µm)	Entrapment Efficiency (%)
F_1	1:0.5:1	1.5± 0.5	58.23±4.61
F_2	**1:1:1**	2.0±0.3	**65.10±4.06**
F_3	1:1.5:1	1.6 ±0.1	37.52±2.16
F_4	1:1:0.5	1.6 ±0.3	45.65±2.15
F_5	1:1:1.5	1.8 ±0.5	38.64±3.21
F_6	**1:2:1**	**1.9±0.3**	**66.44±0.15**
F7	1:0.5:1	2.1±0.7	51.22±4.21
F8	1:1:1	1.5±0.8	49.75±1.23
F9	1:1.5:1	1.8±0.6	48.12±2.05
F10	1:1:0.5	2.1±0.4	39.54±4.15

Assessment of the physical stability of topical niosomal gel: Physical stability study of the prepared topical niosomes gel was carried out to observe the leaching out of drug from niosomes at different temperature refrigerator condition (4±5˚C) and at room temperature (25±31˚C). Niosomes were observed for drug content respectively.

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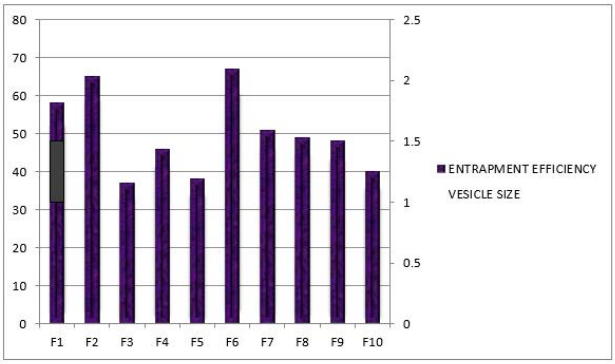

Fig.3 Niosome Optimization in Relation to Vesicle Size (µm) and Entrapment Efficiency (%)

Table 2. Observation table of physical stability of topical niosomes based gel with respect to vesicle size & EE%.

F2	T_1		T_2	
	1 month	2 month	1 month	2 month
Chart Area ize	2.0±0.3	2.0±0.4	3.2 ±0.4	4.0±0.5
% Entrapment efficiency	65.10±4.06	65.10±3.7	65.10±4.06	65.09±4.02
F6	T_1		T_2	
	1 month	2 month	1 month	2 month
Vesicle size	1.9±0.3	1.5.0±0.2	1.9 ±0.3	1.6±0.2
% Entrapment efficiency	66.44±0.15	66.40±0.11	66.44±0.15	64.09±0.10

Where, T_1=Refrigerator condition, T_2= Room temperature

Preparation of Gel Base

1gm of carbopol 934 were taken and dispersed into double distilled water (80ml). This solution was stirred, at 800 rpm for 1 hour. 10ml of propylene glycol was added to this solution. This mixture was neutralized by drop wise addition of 10% NaOH solution, while mixing was continued until a transparent gel appeared and finally volume was adjusted to 100ml. pH of the gel base was adjusted to 6.5 [28].

Physical Examination of Gel

The colour, homogeneity, consistency, spreadability, and phase separation of the prepared Niosomal gel containing Acecelofenac and Serratopeptidase were visually evaluated. Each gel's pH was determined using a pH meter that had been calibrated with standard buffer solutions at pH 4, 7, and 9 before each usage. At room temperature, the electrode was put into the sample 10 minutes before the reading was taken.

Preparation of Aceclofenac and Serratopeptidase Gel

The prepared base gel was precisely weighed and added to the Niosomal dispersion while stirring. By adding distilled water drop by drop, the weight of the combination was adjusted up to 10 gm. The mixture was kept at room temperature for 2 hours after completion of addition to allow it to hydrate to its maximum capacity.

Measurement of viscosity: Viscosity measurements of prepared topical Niosomal based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity was found to be 2890 cps.

Drug content: Accurately weighed equivalent to 10 mg of topical Niosomal gel of ACE and SRP was taken in beaker and added 20 mL of 0.01N HCl. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with 0.01 N HCL. This solution was analyzed using UV-Spectroscopy at λ_{max} 251 nm. Drug content of topical Niosomal based gel was found to be 7.82%.

Drug release study: Release study of prepared Niosomal gel was determined by Franz diffusion cell which consisted PBS (pH 7.4).1 mL of Niosomal gel were applied in a cellophane membrane (1200 Daltons) of diameter 2.5 cm & observed at different intervals of time [15]. Zero order, First order, Hixson's crowell, Higuchi, Krosmeyer peppas equations were applied to in-vitro release results and correlation coefficient were found [29].

Table 3. The Cumulative percentage release of Optimized formulations

Time(hr)	% Release of topical niosomal gel (F_2)	% Release of topical niosomal gel (F_6)
0.30	4.25±1.12	5.75±1.12
1	12.25±0.45	12.10±1.2
2	16.75±4.40	15.5± 1.4
4	24.73±1.56	26.73±1.5
6	29.9±1.77	31.20±1.2
8	32.56±2.60	32.40.±1.25
10	35.45±4.20	34.45±1.5
12	40.2±2.44	39.5±1.51

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Table 4. Observation table for release order of optimized formulation of F_2

S.no	Release order	Equation	r^2 value
1.	Zero order	y=2.307x+ 19.988	0.7433
2.	First order	y=-0.0104x+1.9063	0.8663
3.	Hixson crowell	y=0.2286x+4.5943	0.9609
4.	Higuchi	y= 10.395x+6.7999	0.8733
5.	Krosmeyer peppas	y=0.4722x+1.1535	0.8750

Table 5 Observation table for release order of optimized formulation of F_6

S.No	Release order	Equation	r^2 value
1.	Zero order	y=1.2763x+ 24.881	0.6582
2.	First order	y=-0.0108x+1.8773	0.8443
3.	Hixson crowell	y=0.2385x+4.5181	0.9716
4.	Higuchi	y= 10.477x+11.227	0.8556
5.	Krosmeyer peppas	y=0.4059x+1.2764	0.8876

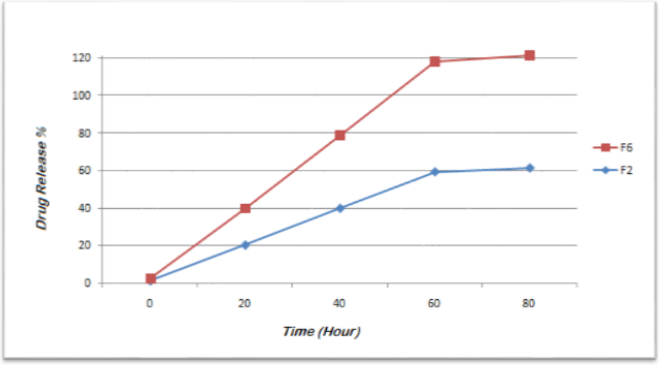

Fig. 4 *In-vitro* Drug Release Profile of Topical Niosomal Gel containing Aceclofenac and Serratiopeptiase.

Ex-vivo diffusion studies

Franz Diffusion cell was used to performed ex-vivo skin permeation studies for entrapped niosomal gel formulations (). The receptor compartment contained phosphate buffer (pH 5.8) which was constantly stirred at 100 rpm with a small magnetic stirrer and controlled temperature at 37 ± 2 °C throughout the experiment. The abdominal skin of male rats (weighing 200 g) was taken for this study. The shaved rat skin was mounted with the stratum corneum side facing upwards to the donor compartment, and the subcutaneous side was in contact with the receiver medium. The gel was placed into the donor compartment and covered with paraffin film. The sample aliquots from the receiver chamber were collected at 30 min 1, 2, 3, 4, 5, 6, 7, and 8 h respectively and analyzed by UV-VIS Spectroscopy at 221nm. The amount of drug permeated, drug flux, and the permeation coefficients were calculated for niosomes loaded gel formulations ().

In-vivo anti-inflammatory study

The animal study has been conducted as per the Animals (Scientific Procedures) Act. All the animals used in research work were cared for by trained, accountable staff, and housed in proper facilities. Before the animal study, the animal approval was obtained by IAEC (CPCSEA No. CPCSEA) Anti-inflammatory study was performed by "Carrageenan induced rat paw edema" model using 24 albino rats of either sex weighing (100-150 g) and divided into 4 groups [shown in Table In all groups, acute inflammation was induced by a sub-planter injection of 0.1 mL of freshly prepared 1% suspension of carrageenan in normal saline in the left hind paw of the rats. The paw edema volume was measured using "Plethysmometer" at every 15 min interval for 2 h after the injection of carrageenan. The average paw edema volume of all the groups were calculated and compared with that of control .

Table 3

Various animal groups used for *in-vivo* anti-inflammatory study

Groups	No of animals	Description
1	6	Served as normal or Untreated control group
2	6	Received standard topical Gel (A+S) equivalent to 720 µg/100 g (25 mg of gel) of the Ibuprofen topically
3	6	Received F2 loaded niosomal gel formulation
4	6	Received F6 loaded niosomal gel formulation

The percent inhibition of edema was calculated by using the following Equation 8:

Edema inhibition% = 1 – VtVc × 100

(Equation 8)

Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control.

Skin irritancy study

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The skin irritation test was performed on the healthy albino rat (200 g) for the best formulation by applying niosomes loaded gel formulation on the shaved portion of rat skin. The test was performed primarily by examining the rat to notice any changes after the application of the formulation. Then photographic imaging of an exposed portion of rat skin was taken out before and after subsequent application for 72 h that is after the study period and these images were compared determining the difference with the images taken before applying the formulation

Ex-vivo diffusion studies

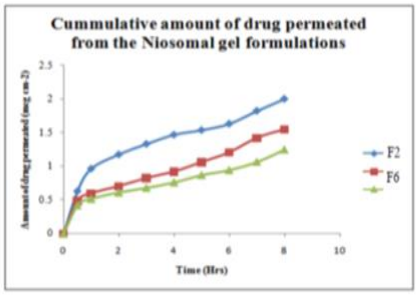
The selected gels were evaluated for *in-vitro* skin permeation studies. *In-vitro* drug release of niosomal gel formulations were observed up to 8 h and showed a slow release pattern in all of the three niosomal gel formulations as shown in table . An inverse relationship was observed between the permeation rate and the viscosity of the gel formulation. The data is graphically represented in fig

Table

Results for Amount permeated, Drug flux, and Log permeability coefficient of the loaded Niosomal gel formulations

Gel Formulations	Amount of drug permeated[Q] (mg cm $^{-2}$) ± SD	Drug flux [Jss] (mg cm $^{-2}$ h $^{-1}$) ± SD	Log Permeability Coefficient (Log Kp) ± SD
F2			
	1.999 ± 1.34	10.66 ± 1.82	0.546 ± 0.58
F6			
()	1.548 ± 1.09	8.256 ± 1.15	0.433 ± 0.76

Figure



Cumulative amount of Drug Permeation from all Ibuprofen loaded niosomal gel system (F2,F6).

In-vivo anti-inflammatory activity

In-vivo anti-inflammatory activity was performed using the "Carrageenan induced rat paw edema" model. Percentage inhibition of inflammation in control and treated groups was calculated for different formulations (Standard, F2, and F6) and results were shown in FIG AND TABLE . Percentedema inhibition was found maximum in both the niosomal gel formulations (F2 and F6) as compared to the standard (marketed plain gel) up to 2 h. Data significance was calculated for all formulations (standard, and test (F2 and f_6)) to control groups and the test formulations (f2 and f6) were also compared.

Table 5

Percentage inhibition of the edema in albino rats

Time (min)	Control (%)	Standard (%)	f_2 (%)	f_6(%)
Basal	-	0	0	0
30	-	6.2	2.8	3.17
45	-	8.0	3.5	8.46
60	-	14.4	5.2	12.5
75	-	15.7	7.16	13.32
90	-	16.89	9.3	15.43
105	-	18.67	10.5	17.97
120	-	21.0	12.7	18.66

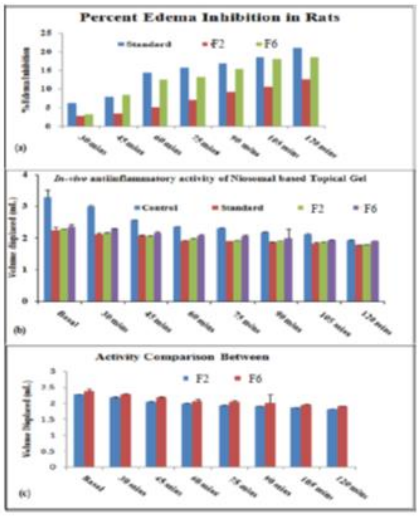


FIg.Comparison plots representing in-vivo studies: Percent edema Inhibition and anti-inflammatory activity in animal groups for Ibuprofen loaded niosomal gel system (F2 and F6).

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Skin-irritancy study

Based on the results obtained from the above studies, it has been found that formulation F2 showed optimum results within the desired range. Hence, formulation F2 was subjected to skin irritancy study on animals (albino rat). Following 3 days application of the niosome loaded gel; the results of the skin irritation test indicated that the f6 gel did not cause any skin reaction. It can be assured that Ibuprofen loaded noisome loaded gel did not cause any skin irritation and can be used as topical gel formulation.

Result and Discussion

Because of the higher drug entrapment efficiency and smaller vesicle size, hand shaking was used to make Niosomes in the current investigation. For the production of Niosomes, we chose Spans (surfactants). The entrapment efficiency of Span 40 was assessed using different molar ratios. F2 and F6 have improved drug entrapment by 65.10 % and 66.44 % respectively. The current study was conducted with the goal of overcoming issues associated with the oral mode of administration, as well as enhancing patient compliance by delivering sustained drug release [30-31].In the transdermal drug delivery system, the solubility and chemical structure of the molecule are crucial. The physical stability of Niosomes shows that at room temperature, the medication leaches more readily, but that it is stable when chilled. Because it prevents fusion, adding Niosomes to a gel basis improves their stability.

After optimizing niosomes formulations; the best formulation was transferred into the gel system. All the formulations were shear thinning and exhibited pseudoplastic behavior. The flow behavior of the formulations was shown to be non-Newtonian pseudoplastic flow and thus said to have good rheological properties. The spreadability of formulated gels was decreased as the concentration of polymer increased. The values of spreadability indicate that the gel is easily spreadable by the small amount of shear. *Ex-vivo* diffusion study showed a linear relationship between the cumulative amount permeated and time, indicating zero-order permeation kinetics and the permeation of Ibuprofen was based on diffusion-controlled mechanism. The significantly low flux values of Ibuprofen were found in gels f_2 and f6 as compared to that of niosomes loaded gels. The results showed decrease in drug permeation and drug flux values with increased concentration of *xxxxxxxxx* that suggestsmucilage have a good binding to the system and therefore controlled the release of drug from the niosomal gel system . The *in-vivo* results of test formulations (f2 and f6) indicated high skin retention and enhanced penetration rates within the skin. According to both *ex-vivo* and *in-vivo* studies f6 niosomal gel formulation showed better permeation and effectiveness as compared to F2 niosomal gel formulation. This probable reason may be due to higher skin retention and deposition of the f2 niosomal gel formulation resulting in higher partitioning of the drug into the rat paw which may be responsible for its prolonged and enhanced anti-inflammatory activity. Thus, formulation (f_2) may help in improving the therapeutic index and is also expected to minimize the side effects due to selective build-up of drug concentration at the site of action. Skin irritancy results indicated that the f2 gel did not cause any skin reaction and can be used as gel formulation.

Conclusion

ACE displays high permeability to penetrate into synovial joints where in patients with osteoarthritis and related conditions, the loss of articular cartilage in the area causes joint pain, tenderness, stiffness, crepitus, and local inflammation. Aceclofenac is also reported to be effective in other painful conditions such as dental and gynaecological conditions [42-43]. Orally administered SRP has been reported to show systemic side effects like anticoagulant effects and is conventionally given in the form of enteric coated tablet formulations Topical formulations of Aceclofenac and SRP would be useful to treat local inflammations and may prove to be more effective compared to non steroidal anti -inflammatory agents. Topical gel can be clinically effective, safe, and cost-efficient treatment compared to an oral formulation. NSAID's having excellent anti-inflammatory and analgesic activity but NSAID's produces undesirable serious adverse effects like GIT ulceration, liver and kidney trouble especially in case of oral administration. In view of adverse drug reaction associated with oral formulations, many NSAID's are increasingly administered by topical route.

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The developed formulation G_2 is a viable alternative to conventional topical gels as it provides sustained delivery. Moreover, the natural mucilage, used in the gel, possess anti-inflammatory properties suggesting to exert a synergistic action of niosomal gel system at the target site to improve overall effect. Thus, it can be concluded that the niosomes loaded gel can be a promising potential drug delivery system for topical application to efficiently target local pains for prolonged periods and to reduce the frequency of application of gels as compared to conventional gels.

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Evaluation of the bacterial, antioxidant and anticancer activity of pyrene derivatives and their synthesis

Nooreen Unissa,Gopi Swapna,Usha Kondla,P.V.Pavan Kumar.
Avanthi Institute of Pharmaceutical Sciences
Approved by AICTE, PCI, NAAC, JNTUH,Gunthapally, Hayathnagar, Hyderabad-501512

Abstract

Using ethanol as a solvent, heterocyclic hexagonal rings for 2-amino-pyran derivatives (I33-I40) were produced by reacting a mole of chalcones derivatives with a mole of malononitrile. Physical properties such as melting point, colour, and molecular weight and spectroscopic measurements such as the infrared spectrum, [^{1}H-NMR], [^{13}C-NMR] spectrum were used to confirm the accuracy of the prepared compounds' compositions. The biological activity of various produced compounds was investigated in two species of pathogenic bacteria, one of which is Gram-positive, Staphylococcus aureus, and the other, Gram-negative, Escherichia coli. Chemical solutions for the two substances (I25, I32) with concentrations (0,01, 0.001, 0.0001) mg /ml were created using a solvent DMSO and Multer Hinton Agar growth medium. The antibiotic Ciprofloxacin was utilized as a control sample for the diffusion sensitivity test of the bacterial isolates used in the investigation. Using DPPH root and varied concentrations, the impact of chemical (I32) on the elimination of free radicals was also investigated. Breast cancer cells were taken from Baghdad's Medical City and used to test the efficiency of several produced chemicals against them. 200 µl of the prepared concentrations of the compounds produced for the research (25, 50, 100, 200) g/ml were added to the pits at three concentrations, cytotoxicity tests were conducted in three duplicates, and (IC50) values were computed. The cytotoxicity impact of the compounds was evaluated and produced using MTT and solute solutions on breast cancer cells and the ordinary cell line WRL68 for comparison.

Keywords 2-amino-pyran, staphylococcus aureus, escherichia coli, antioxidant activity, breast cancer.

Introduction

Pyrene or pyran is a heterocyclic, unsaturated organic compound consisting of six central atoms, five of which are carbon atoms and one oxygen atom, and contains two double bonds. It has the chemical formula C_5H_6O, and the molar mass is
82.10 g/mol [1]. Its common name is (IUPAC) 2H-Pyran, 4H-Pyran, and its other names are 2H-Oxine and 4H-Oxine [2]. There are two isomers of pyran that differ in the location of the double bonds. In 2H- pyran, the saturated carbon is in position 2 [3]. In contrast, in 4H-pyran, the saturated carbon is in position 4. 4H- pyran was first isolated, prepared and distinguished in 1962 by the pyrolysis of 2- acetoxy-3,4-dihydro-2H-pyran [4], and found to be very unstable, particularly in the presence of air, 4H-pyran does not readily match the corresponding dihydropyran and beryllium ion, which readily decomposes in an aqueous medium [5]. Although pyrenes themselves are of little importance in chemistry, many of their derivatives are essential biological molecules [6], such as pyranoflavonoids, and pyranones are also important derivatives, which are natural products, an

example of which is coumarin [7]. As the term pyran is applied to its saturated ring analog, which is more appropriately referred to as tetrahydropyran (oxane), in this context, monosaccharides containing the six- membered ring system are known as pyranose [8]. Piran compounds are prepared in many ways, including through a series of reactions as the response begins with propylene oxide, which was converted directly through three steps to acetylene [9]. Then, heating gave chitin dimethyl acetal diene, which regularly reacts with 2- chloro-1,4- naphthoquinone to form anthraquinone, an aromatic compound, which reacts with sulfuric acid to produce lactone [10]. Bacteria are micro- organisms that can only be seen with a microscope. Bacteria are found everywhere, in the air, in the water, in the human body, and inside the digestive canal and respiratory system [11]. Bacteria may persist for many years, enduring all kinds of improper circumstances including extreme heat or cold, as well as other severe environmental factors. When the bacteria's environment improves, they shed the thick membrane and resume their prior activity and vigor [12]. Staphylococcus aureus is a Gram-positive bacterium that has spherical germ cells with a diameter of approximately 3 m, is extremely thick, immobile, non- sporulating, and ferments sugars [13, 14]. Escherichia coli is a Gram-negative bacillus that thrives on ordinary culture medium without the need of growth factors. These bacteria are killed by heating them to 60°C for 30 minutes. On MacConkey agar culture, this type of bacterium may thrive, and some strains of this bacteria can produce capsules [15]. This bacterium is one of the most well- known forms of intestinal bacteria in people and animals, and it causes bladder, urinary tract, meninges, and bile sac inflammation [16]. Cancer is a group of diseases characterized by the unrestricted growth and division of cells in the body's tissues, as well as the ability of these cells to invade and destroy neighboring tissues or spread to distant tissues via the blood or lymphatic system, which are the characteristics of a malignant tumor. They differ from Benign's adenoma in that they have a distinct development pattern, are unable to penetrate, and cannot metastasis. A benign tumor may become cancerous in certain cases [17]. Cancer may strike anybody at any age, but the risk rises with age, and it is one of the top causes of mortality in both industrialized and developing nations [18]. Breast cancer is a kind of malignant tumor that affects the breast tissue and manifests itself in the ducts of the tubes that transport milk to the nipple and milk glands [19]. It affects both men and women, although the frequency in men is uncommon, with just one male injury for every 200 female injuries [20]. Breast cancer is classed as invasive or non-invasive, depending on whether it is found in the duct or the lobes [21]. Modifiable risk factors (such as alcoholic beverage intake) and fixed risk factors (such as age and biological sex) are the two types of risk factors [22]. The greatest risk factor for breast cancer is gender, since women are more likely than men to acquire it and the risk increases with age [23].

Experimental

Material: All chemicals used in this work were purchased from Fluka, Aldrich, and BDH and used without further purification.
Devices used: The melting points were measured using Electrothermal Melting Apparatus 9300. The FT-IR spectra were captured using a Shimadzu FT-IR 8400S spectrophotometer with a scale of (400-4000) cm^{-1} by KBr disc. DMSO-d_6 as solvents were used to capture ^{1}H-NMR and ^{13}C-NMR spectra on Bruker instruments running at 400 MHZ.

Preparation of 2-amino-pyran derivatives (I33-I40) [24]

In a round flask of 100 ml volume, 0.01 mole of the prepared chalcone derivatives (I1-I8) is dissolved in 10 ml of ethanol. A solution of 0.01 mole of malononitrile dissolved in 10 ml of ethanol is added, then 10 ml of ethanol is added. 10% sodium hydroxide solution, and the mixture rises for 6 hours with continuous stirring, then concentrates the solvent, cools the answer, and is added to crushed ice. It is equalized by adding drops of concentrated hydrochloric acid HCl and noting the product's precipitation. The precipitate is separated by filtering, washed with cold water, and recrystallized methanol, and table (1) shows some physical properties of 2-amino-pyrene derivatives

(I25-I32).

Table (1): Physical properties of 2-amino-pyran derivatives (I33-I40)

Comp. No.	X	R	Molecular Formula/ M.Wt g/mol	Color	M.P. (^{0}C)	Yield (%)
I25	F	Cl	$C_{18}H_{12}FN_2OCl$ 326.76	Drack yellow	139-141	78
I26	F	Br	$C_{18}H_{12}N_2OFBr$ 371.21	Light brown	134-135	59
I27	F	CH_3	$C_{19}H_{15}N_2OF$ 306.34	Light brown	171-173	71
I28	F	OCH_3	$C_{19}H_{15}N_2O_2F$ 322.34	Yellow	185-187	63
I29	Cl	Cl	$C_{18}H_{12}N_2OCl_2$ 343.21	Brown	99-101	55
I30	Cl	Br	$C_{18}H_{12}N_2OClBr$ 387.66	Light brown	159-160	60
I31	Cl	CH_3	$C_{19}H_{15}N_2OCl$ 322.79	Light brown	157-158	66
I32	Cl	OCH_3	$C_{19}H_{15}N_2O_2Cl$ 338.79	White	191-193	69

Biological activity study

This research employed two kinds of harmful bacteria, one of which is Gram- positive, Staphylococcus aureus, and the other of which is Gram-negative, Escherichia coli; and these microorganisms. It is essential in medicine because it is resistant to antibiotics. These bacteria were collected from the Department of Life Sciences at the College of Education for Pure Sciences. The culture medium was utilized as a form of Multer Hinton Agar, which is used to test the biological activity of antibiotics and other chemicals. Chemicals have therapeutic potential. Chemical solutions of (I25, I32) were prepared in concentrations of (0,01, 0.001, 0.0001) mg/ml and using a solvent Dimethyl sulfoxide to measure and determine the minimum inhibitory concentration (MIC) (DMSO). The bacteria isolates used in the study were sensitivity tested using the diffusion method in the nutrient medium Mueller- Hinton agar, which is a transparent food medium with a dark yellow color that is useful in testing the sensitivity of microorganisms to antibiotics because it contains casein and starch extracted from an animal infusion. It allows most bacteria and germs to thrive. The medium was prepared and sterilized in an autoclave, then distributed in dishes, and allowed to harden before making four small pits in each plate. It was then incubated for 48 hours at 37 degrees Celsius (24 hrs). The results were read the next day to show the sensitivity derivatives used, which are dependent on the diameter of the inhibition visible in the dishes around the holes used as the diameter of the inhibition increases. Inhibition refers to the rise in the biological activity of the prepared compounds, as opposed to antibiotics' diameter of inhibition [25, 26].

Measuring the antioxidant activity of some compounds prepared ex vivo (DPPH inhibition activity test)

DPPH root (2,2-Diphenyl-2-Picryl Hydrazyl) was used to evaluate natural oxidation's free radical scavenging activity. At a concentration of 0.1 mM by dissolving 4 mg in 100 mL of methanol, then 3 mL of DPPH prepared solution was added to 1 ml of the prepared compounds of several concentrations ranging from (25, 50, 100, 200) µg/mL, as well as Ascorbic acid prepared from dilute
concentrations of (25, 50, 100, 200) µg/ml, left the mixture in the dark for 30 minutes, then read the absorbance at a wavelength of 516 nm, and calculated the percentage of scavenging ability of the prepared compounds DPPH for free radicals for each compound as well as for Ascorbic acid AA [27], which represents the positive witness for comparison through the following equation:

$I\% = (Abc_0 – Abc_1) / Abc_0 \times 100$

The standard solution was made by adding DPPH solution without ascorbic acid or extracts.

Testing the cytotoxicity of I32 on breast cancer cells (MCF-7)

Cancer cells were obtained from the Medical City - Baghdad, and the cancer cells were preserved in liquid nitrogen; they were perpetuated, grown, and tested at the Biotechnology Research Center at Al-Nahrain University; and work began with steps:

First: The natural cell line WRL 68 Cell Line: The human liver cell line represents a thank similar to hepatocytes and primary liver transplants. It has been proven that the cells secrete albumin and alpha-fetoprotein and express enzymes specific to the liver. Like alanine aminotransferase

Second: Solutions Used in Tissue Culture Technique: Several solutions used for cell culture were prepared, namely: Antibiotic Solution (Streptomycin (1g/vial),

Benzyl Penicillin, Sodium Bicarbonate Solution, Phosphate Buffer Saline (PBS), Trypsin Solution, EDTA, Trypsin - EDTA
Third: Media: several media attended, namely: Roswell Park Memorial Institute - 1640 Medium (RPMI), Serum-Free Medium, and Freezing Medium [28].

Breast Cancer Cell Growth (MCF-7): The freshener method was used to grow cancer cells as follows

The tumor cells were thawed using a water bath at 37 ^{0}C. Then, the tumor cells were placed in a 25 cm^2 animal cell culture vessel (Falcon) containing culture medium (RPMI-1640) and 10% calf serum. Then the culture vessels containing the cell suspension and medium were incubated in a 5% CO_2 incubator at 37 ^{0}C for 24 hours. After incubation, and when it was confirmed that there was growth on the farm and that it was free from pollution, secondary farms were conducted for it. The cells were examined using an inverted microscope to ensure their viability, freedom from contamination, and growth to approximately 500000- 800000 cells/ml. Then the cells were transferred to the growth booth, and the used culture medium was discarded. Cells were washed using PBS solution and discarded, and the process was repeated twice for 10 minutes each time. A sufficient amount of trypsin/EDTA enzyme solution was added to the cells and incubated for (30-60) seconds at a temperature of 37 ^{0}C and monitored until they changed from a monolayer of cells to single cells. Then, the enzyme was stopped by adding a new development medium containing serum. Then the cells were collected in centrifugal tubes and placed in the apparatus at a speed of 2000 rpm for 10 minutes at room temperature to precipitate the cells and get rid of the trypsin and the used medium. The filtrate was discarded, and the cells were suspended in a fresh medium containing 10% serum [29]. The cell number was examined by taking a specific volume of the cell suspension and adding to it the same volume of Trypan Blue dye to find out the number of cells and their vitality percentage using a Haemocytometer chip, according to the equation:
$C = N \times 10^4 \times F/ml$
The cell suspension was distributed in new containers and then incubated in a 5% CO_2 incubator at 37 ^{0}C for 24 hours: Total Cell Count/ ml = Cell Count x dilution factor (Sample Volume) x 10^4.

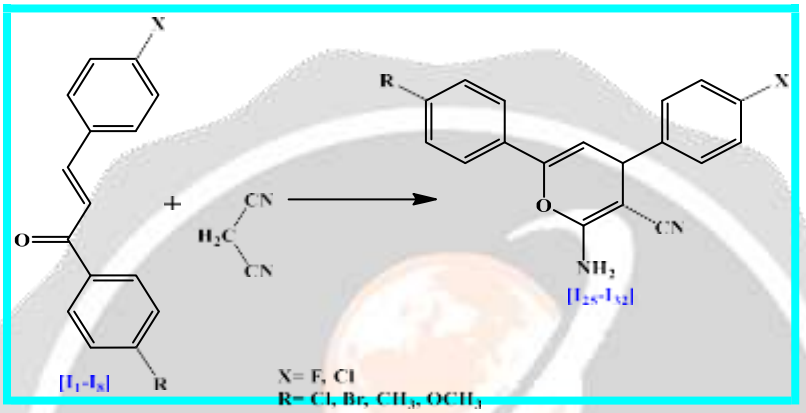
MTT Cytotoxicity Assay

Cytotoxicity tests were performed in three replicates, and the (IC_{50}) values were calculated. 3-(4,5 dimethylthiazol - 2-yl) 2,5-Diphenyl tetrazolium bromide (MW = 414), and solute solution [30]. The manufacturer's instructions were followed as they prepared breast cancer cells as previously mentioned, then placed the cell suspension at a concentration of (1×10^4 to 1×10^6) cells/ml in a 96-hole newspaper to a final volume of 200 µl of complete culture medium for each hole and the plates were covered with sterile parafilm, gently stirred and incubated. After incubation, the medium was removed, and 200 µl of the prepared concentrations of the studied compounds (25, 50, 100, 200) □g/ml were added to the pits at three pits for each engagement with the control sample and incubated in a 5% incubator. CO_2 at 37 ^{0}C for 24 hours. After exposure to the compounds under study, ten µl of MTT solution was added to each hole; then, the plate was incubated in a 5% CO_2 incubator at 37 ^{0}C for 4 hours. 100 µl of DMSO was added

to each pit and set for 5 minutes. Then, the absorbance was read using an ELISA device at a wavelength of 570 nm [31]. Statistical analysis was performed on the optical density readings to calculate the IC50.

Results and Discussion

The 2-amino-pyran derivatives (I33-I40) were prepared by reacting a mole of chalcone derivatives with a mole of malononitrile and using ethanol as a solvent, as in the following equation:



Scheme (1): Route of prepared compounds (I25-I32)

Diagnosis of 2-amino-pyran derivatives (I33-I40)

The reaction to the 2-amino-pyran derivatives (I33-I40) was confirmed by observing the changes in the physical properties of the melting point and the significant color difference. During measurements of infrared (IR), (^{1}H-NMR), and (^{13}C-NMR) spectra.

When studying the infrared (FT-IR) spectrum of 2-amino-pyrane derivatives (I25- I32), two absorption bands were observed at the frequency (3239-3270) cm^{-1}, and (3176-3195) cm^{-1} due to the stretching of the (NH2) bond, and the appearance of an absorption band at the frequency (3007-3098) cm^{-1} due to the extension of the aromatic (CH) bond, as well as the appearance of two absorption bands at the frequency (2913-2997) cm^{-1} and (2818-2891) cm^{-1}, are due to the stretching of the aliphatic (CH) bond, in addition to the appearance of an absorption band at the frequency (2254-2264) cm^{-1} due to the stretching of the (CN) bond, as well as the appearance of two absorption bands at the frequency (1581-1599) cm^{-1} and (1477-1499) cm^{-1} were due to the stretching of the aromatic (C=C) bond, and there was an absorption band at the frequency (1302-1387) cm^{-1} that was due to stretching (C-O) group bond, as shown in table (2), and these bundles were close to what is found in the literature [32, 33].

Table (2): Infrared absorption results (cm-1) 2-amino-pyran derivatives (I33-I40)

Comp. No.	X	R	NH2	(CH) Arom.	(CH) Aliph.	(CN)	(C=C) Arom.	C-O	Others
I25	F	Cl	3261 3180	3043	2966 2825	2262	1583 1491	1332	(C-F) 1093 (C-Cl) 700
I26	F	Br	3255 3198	3012	2945 2879	2256	1599 1481	1387	(C-F) 1061 (C-Br) 633
I27	F	CH3	3270 3183	3032	2913 2865	2260	1583 1497	1302	(C-F) 1070
I28	F	OCH3	3239 3177	3071	2997 2818	2264	1594 1486	1365	(C-F) 1087
I29	Cl	Cl	3254 3191	3007	2920 2891	2257	1581 1477	1366	(C-Cl) 734
I30	Cl	Br	3259 3182	3066	2943 2820	2254	1587 1485	1340	(C-Cl) 773 (C-Br) 678
I31	Cl	CH3	3241 3195	3098	2942 2857	2261	1590 1499	1349	(C-Cl) 769
I32	Cl	OCH3	3260 3176	3011	2990 2831	2259	1592 1488	1358	(C-Cl) 741

When studying the nuclear magnetic resonance spectrum of the proton for the compound (I30), it was observed that multiple signals appeared in the range (7.037-7.903) ppm attributed to the aromatic ring protons, and the appearance of a binary signal in the position (6.864 and 6.872) part of The ppm is attributed to the proton of the (CH) group of the double bond, the appearance of a binary signal at the position (6.829 and 6.853) ppm is attributed to the proton of the (CH) group adjacent to the benzene ring, and the appearance of a single signal at the site (3.406) ppm attributed to the protonation of the (NH_2) group, and the appearance of a signal at the site (2.502-2.512) ppm attributed to the protons of the solvent (DMSO-d^6) [34], and as in figure (3).

When studying the nuclear magnetic resonance spectrum of carbon for the compound (I30), it was observed that a signal appeared at the site (187.60) ppm attributed to the carbon of the carbonyl group (C-O) far from the group (NH_2). The appearance of a signal at the site (142.45) ppm was attributed to the group carbon (CN), and the formation of a signal at the site (135.79) ppm was attributed to the group carbon (=C-NH_2), as well as the appearance of signals At the location (127.37-134.66) ppm attributed to the carbons of the aromatic benzene ring, and the formation of a signal at the site (126.85) ppm attributed to the carbon of the group (=CH) of the double bond in the pyran ring, and a signal appeared at the site (121.81) ppm attributed to the carbon of the pyran ring which is related to the benzene ring, and the appearance of signals at the range (39.19-41.23) ppm attributed to the solvent carbonate (DMSO-d^6) [35], and as in figure (4).

Biological activity of some prepared compounds

The study of the biological activity of the compounds prepared at certain concentrations showed that most of these compounds contain antagonistic

activity against the types of bacteria studied compared with the antibiotic (Ciprofloxacin), which is a broad-based antibiotic, especially these two types of bacteria studied in addition to many types. It also has an inhibitory diameter. It is great as it gives a high selectivity when studying the sensitivity of bacteria to the prepared compounds since this antibiotic is used to treat many infections and diseases such as infections of the urinary tract, especially those that occur as a result of infection with colon bacteria and Staphylococcus aureus bacteria. It also treats simple cystitis in females caused by bacteria Colon. It treats chronic bacterial prostatitis caused by colon bacteria and Staphylococcus aureus and infections of the lower respiratory tract, sinusitis, arthritis, and bones. It is also used to treat diarrhea caused by colon bacteria and effectively treat typhoid. Therefore, two compounds of the compounds prepared in this research (I25, I32) were studied on different types of chromium-positive and negative bacteria, which recorded a global antagonistic activity against the bacteria studied and compared with the mentioned antibiotic, it is possible to use this Compounds as a treatment for the same infections and pathological conditions above after investigating the biological pathway of these compounds, their side effects, and the amount of their accumulation in animal tissues (0.01, 0.001, 0.0001) mg/ml where the inhibition diameter ranges between (10 mm the lowest inhibition diameter, to 35 mm, the highest measured inhibition diameter) and the table below shows the inhibitory activity of some of the prepared compounds [36], and as in table (3).

Table (3): The inhibitory activity of the two compounds (I9, I16) in the growth of several positive and negative bacteria (the diameter of inhibition is measured in mm)

Comp. No	*E. coil*			*Staph. aur*		
	0.0001	0.001	0.01	0.0001	0.001	0.01
I25	18	23	29	10	17	21
I32	16	24	36	15	19	26
Ciprofloxacin	18	25	27	10	15	20

Antioxidant Activity

The qualitative evaluation of the anti-oxidant activity of free radical scavenging is done by utilizing the TLC method to determine the prepared compound (I32) ability to reduce the oxidative stress, besides studying the quantitative free radical scavenging activity. Any compound's action is shown when it changes the purple color of DPPH to yellow color, and the intensity of the spot color stated the positive marker. The results indicate that the prepared compound (I32) has an excellent scavenging ability. There is a change in color for yellow, and that is detected that (I32) was a good source of anti-oxidant, but with the least activity than ascorbic acid (as standard). It is clear from the collected data that the free radical scavenging activity of the compound under search increased with increasing concentration exhibiting; for that, the highest free radical scavenging percentage is given with 200 µg\mL of with 80 %, and had the lowest percentage of 40% when 25 µg\mL. Also, this search displays that 2-amino-pyran derivatives were a leading source of anti-oxidant, besides it has an excellent free radical scavenging activity and functions as an anti-bacterial [37,38]. As shown in figure (6).

Results of the breast cancer cell cytotoxicity (MCF-7) test

The test results for compound (I32) showed good inhibitory activity against (MCF-7) breast cancer cells and normal cell line (HdFn). When calculating the IC50 of the compound (I32) against breast cancer cells was 161.4. Its value was 231.4 against normal cells. When calculating IC50, the results showed significant differences, P≤0.0001, when treated with the compound (I32) for breast cancer cells and normal cells [39], as shown in figures (6, 7, and 8).

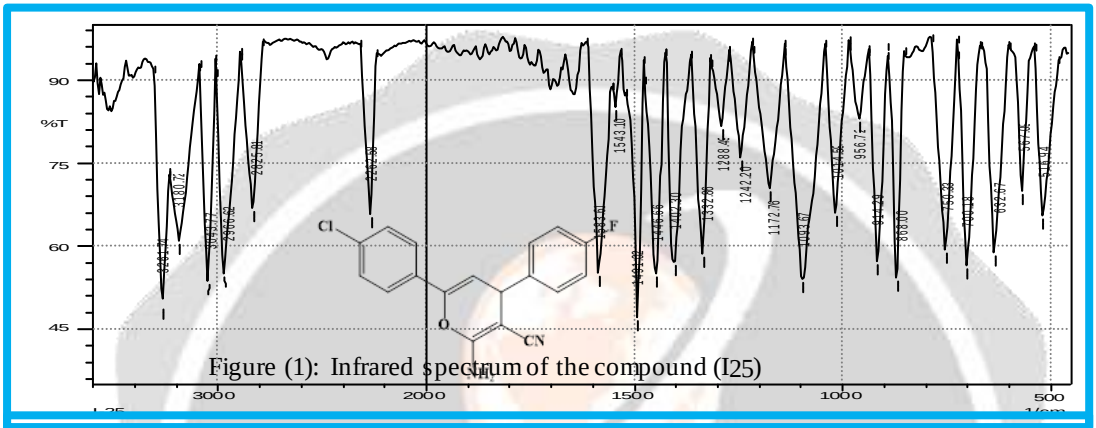


Figure (1): Infrared spectrum of the compound (I25)

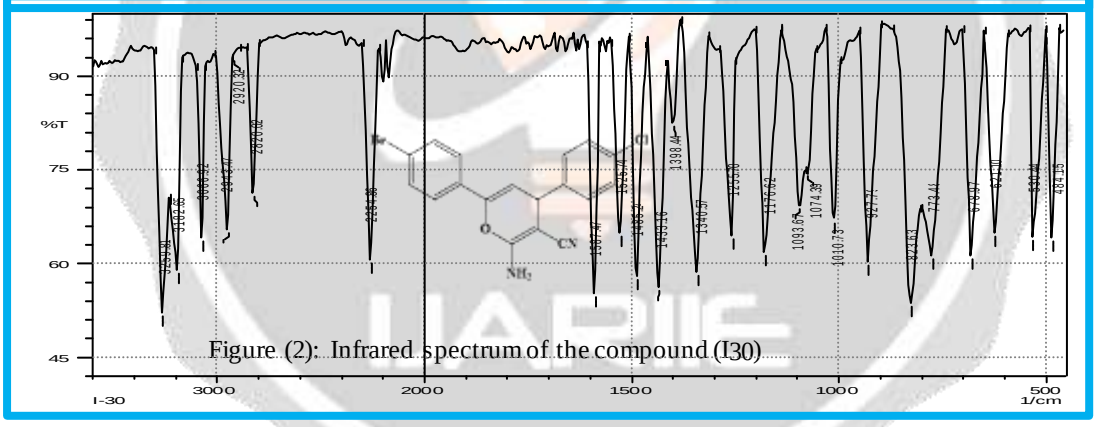


Figure (2): Infrared spectrum of the compound (I30)

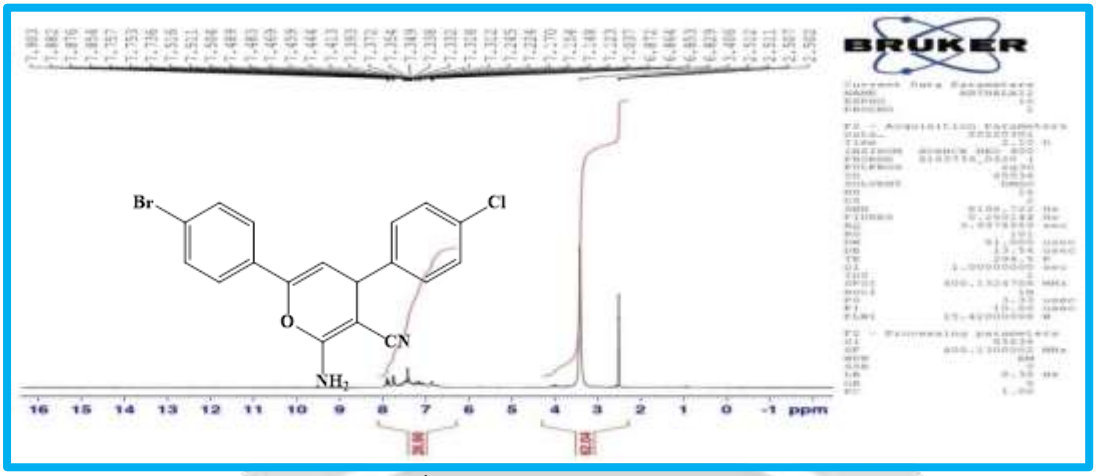


Figure (3): The ^{1}H- NMR spectrum of compound (I30)

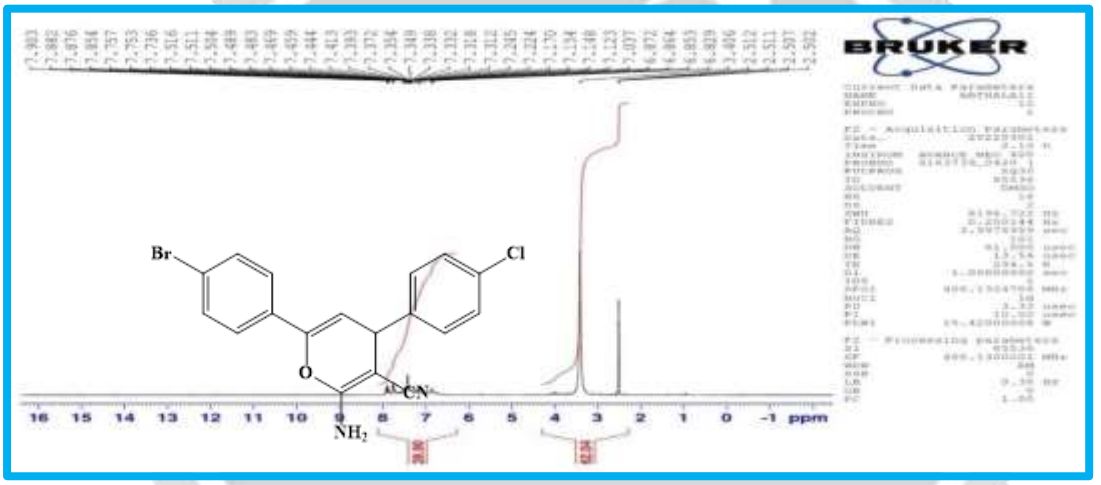


Figure (4): The ^{13}C- NMR spectrum of compound (I30)

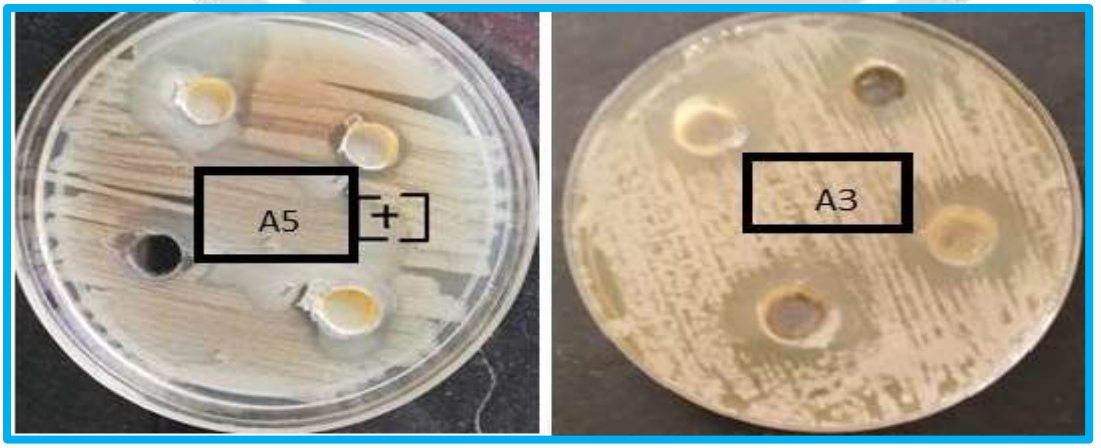


Figure (5): Inhibition of compound (I25) against *Escherichia coli* and inhibition of compound (I32) against *Staphylococcus aureus*

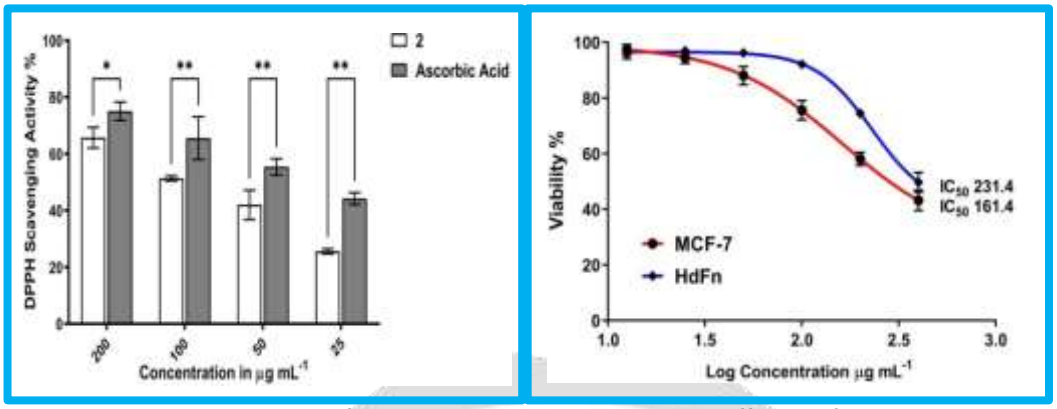


Figure (6): Antioxidant activity for compound (I32) and anticancer efficacy of MCF- 7 and HdFn for compound (I32)

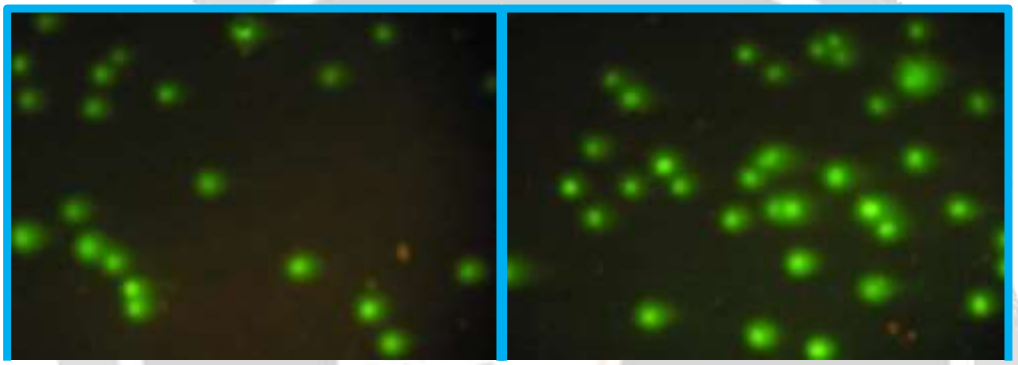


Figure (7): Anticancer efficacy of control sample

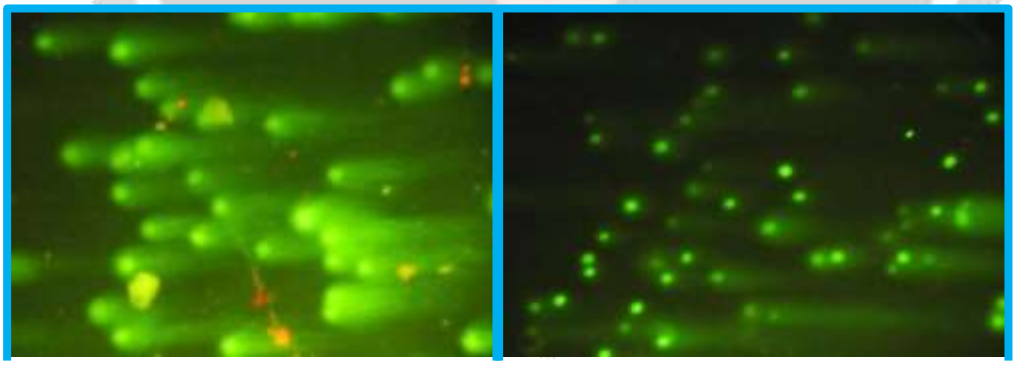


Figure (8): Anticancer efficacy of compound (I32)

Conclusions

Through spectroscopic and physical measurements, it was found that the accuracy and validity of the prepared compounds. The biological activity study showed that the compounds had the effect of inhibiting Gedo compared to the control sample. (I32) compound gave the highest percentage of 35 mm against E. coli bacteria, and the two compounds gave the same percentage of inhibition of 26 mm against Staphylococcus aureus bacteria. The results indicate that the

prepared compound (I32) has an excellent scavenging ability. That the free radical scavenging activity of the compound under search increased with increasing concentration exhibiting; for that, the highest free radical scavenging percentage is given with 200 µg\mL of with 80 %, and had the lowest percentage of 40% when 25 µg\mL. The test results for compound (I32) showed good inhibitory activity against (MCF-7) breast cancer cells and normal cell line (HdFn). When calculating the IC50 of the compound (I32) against breast cancer cells was 183.4. Its value was 561.5 against normal cells. When calculating IC50, the results showed significant differences, $P \leq 0.0001$, when treated with the compound (I32) for breast cancer cells and normal cells.

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RP-HPLC ANLAYTICAL METHOD DEVELOPMENT, VALIDATION AND ESTIMATION OF ANAGRELIDE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

Usha.kondla*, Neeradi Ajay, Nenavathi sujatha, P.Divya, Pabboju Snehitha

Avanti institute of pharmaceutical sciences, gunthapally , rangareddy district.

ABSTRACT

A simple, fast and reproducible reverse phase liquid chromatography (RP-HPLC) method was developed for the determination of Anagrelide Hydrochloride in pharmaceutical dosage form. The method was developed Kromasil C18 column (150 x 4.6 mm; 5µm) and a diode array detector (Model 2996) was employed for the study **Mobile phase**: phosphate buffer (pH 2.5) - acetonitrile (75:25 v/v) with isocratic elution at a flow rate of 1 mL/min. System Suitability test were performed for the assurance of quality performance of method. The drug was subjected to accelerated degradation for photolytic, hydrolytic, thermal, oxidative conditions. The retention time of Anagrelide Hydrochloride was found to be 4.8 min. The method was validated for accuracy, precision, specificity, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines. All the parameters were within limits. The proposed method gave good resolution of Anagrelide Hydrochloride and its degradation products. The developed method can be used for the routine quality control analysis.

Keywords*: RP-HPLC, Anagrelide, Validation, ICH.*

1. INTRODUCTION

Anagrelide Hydrochloride [1] Monohydrate 6,7-dichloro-1H,2H,3H,5H-imidazolidino[2,1-b]quinazolin-2-one C10H7Cl2N3O. HCl.H2O Anagrelide HCl [2,3] inhibits phosphodiesterase III which is found in thrombocytes and cause that few analytical methods available for determination of anagrelide [4-8] by RP-HPLC, LC-MS, UV spectrophotometry. The aim of the present work was the development of a RP-HPLC method for the estimation of Anagrelide Hydrochloride in a pharmaceutical dosage form.

2. MATERIALS AND METHODS

A Waters Alliance liquid chromatography (Model 2695) equipped with a Kromasil C18 column (150 x 4.6 mm; 5µm) and a diode array detector (Model 2996) was employed for the study. Sample injections were done with an automatic injector. Empower2 software was used for data handling. Solubility of the substances was enhanced by sonication on an ultrasonicator (Ultrasonics 3.51). Weighing of the substances in the experiments was done on a Sartorius balance (Model CPA225D).

Drugs, chemicals and solvents:

A pure sample of anagrelide (99.4% purity) obtained from Aarey Drugs & Pharmaceuticals Ltd, Boisar, Maharashtra was used as the reference standard. The commercial capsule formulation of anagrelide „Agrylin (1.0 mg)" manufactured by Shire Manufacturing Inc. was used in this study. Potasium dihydrogen orthophosphate, orthophosphoric acid, sodium hydroxide, HPLC grade acetonitrile and methanol were purchased from Rankem Fine Chemicals Ltd., Mumbai. HPLC grade water was prepared by using Millipore Milli-Q system.

Preparation of the buffer solution (pH 2.5):

6.8 g of potassium dihydrogen orthophosphate was transferred into a beaker containing 800 mL of water. The contents were mixed well and the volume was made up to 1000 mL with water. The pH of the solution was adjusted to 2.5 with orthophosphoric acid. The solution was then filtered through a 0.45µ membrane filter.

Preparation of the mobile phase:

A mixture of the above phosphate buffer (pH2.5) and acetonitrile in the ratio of 75:25 v/v was prepared by mixing 750 mL of the buffer with 250 mL of acetonitrile in a one liter flask. The contents were degassed in an ultrasonic bath for 5 min, and then filtered through a 0.45 µ membrane filter. This mixture was used as the mobile phase in the chromatography.

Stock and working standard solution of anagrelide:

About 10 mg of the reference sample of anagrelide was accurately weighed and transferred into a 10 mL volumetric flask. To this, 2.0 mL of 0.1N

Sodium hydroxide solution was added and sonicated for 5 minutes to dissolve the drug. The volume was made up with methanol and mixed well. This solution was used as the stock solution.

The working standard solution was prepared by transferring 1.0 mL of the stock solution into a 10 mL volumetric flask and diluting to volume with the diluent. This solution (100 µg/mL) was used as the working standard solution of anagrelide.

Preparation of the diluents:

A mixture of the phosphate buffer (pH 2.5) and acetonitrile in the ratio of 50:50 v/v was used as the diluent.

3. OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS AND METHOD DEVELOPMENT

After a number of trials a mixture of phosphate buffer (pH 2.5) and acetonitrile in 75:25 v/v ratio was selected as the mobile phase for separation of anagrelide. The solvent was pumped at a flow rate of 1.0 mL/min. The injection volume was 10 µL and the column temperature was maintained at 25°C. The detector wavelength was set at 250 nm for monitoring the analytes. Prior to injection of the drug solution, the column was equilibrated for atleast 20 min by pumping the mobile phase through it. Typical chromatogram of the working standard solution of anagrelide is shown in Fig. 2.2.

Table 1. Optimized chromatographic conditions of the proposed method

Stationary phase	: Kromasil C18 (150 x 4.6 mm, 5 µm)
Mobile phase	: phosphate buffer (pH 2.5) - acetonitrile (75:25 v/v)
Flow rate	: 1.0 mL/min
Column temperature	: 25°C Injection
volume	: 10 µL
Detection wavelength	: 250 nm
Run time	: 8 min

The retention time (R_t) obtained for anagrelide under the above optimized conditions was **4.818** min.

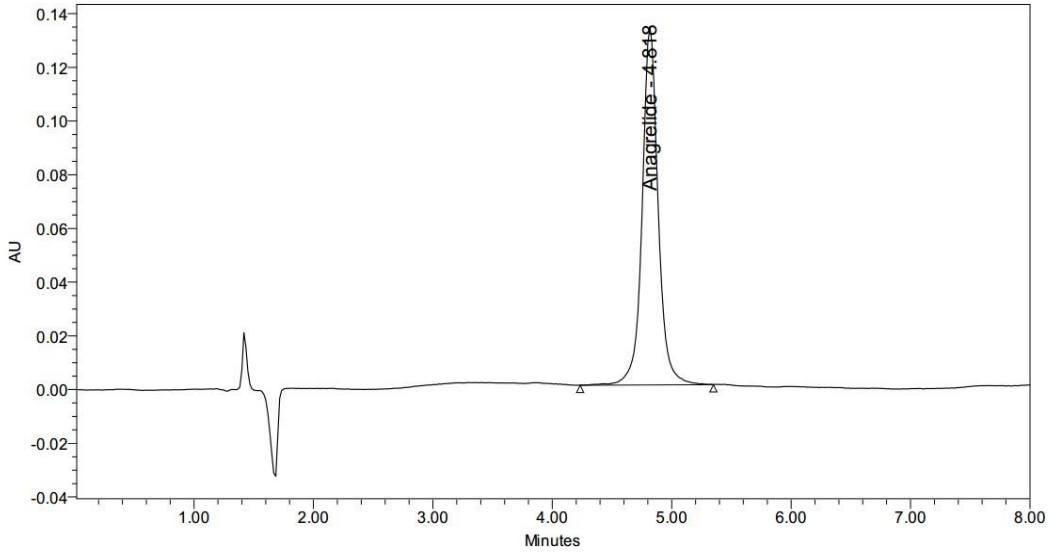

Figure 1 A representative chromatogram of the working standard solution of anagrelide

3.1 VALIDATION OF THE PROPOSED METHOD

Linearity:

Solutions of anagrelide at different concentration levels including the working standard concentration were prepared in the diluent. Ten microlitres of each concentration was injected three times into the HPLC system (n=3). The response was read at 250 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas at the different concentration levels were calculated and the linearity plot of the mean peak areas over concentration was constructed.

Linearity data for anagrelide is presented in the Table 2. The corresponding plot for anagrelide is depicted in the Fig. 2.

Table 2 Linearity data for anagrelide

Concentration of anagrelide (µg/mL)	Peak area	Mean peak area	SD	%RSD
50	612498	610962.3	1438.595	0.23
	610743			
	609646			
70	859785	860698.3	1149.43	0.13
	860321			
	861989			
	1239846			
	1223570			

100	1219789	1227735	10657.45	0.86
120	1492725	1492404	2314.257	0.15
	1494541			
	1489946			
150	1839412	1848889	11683.09	0.63
	1845312			
	1861942			

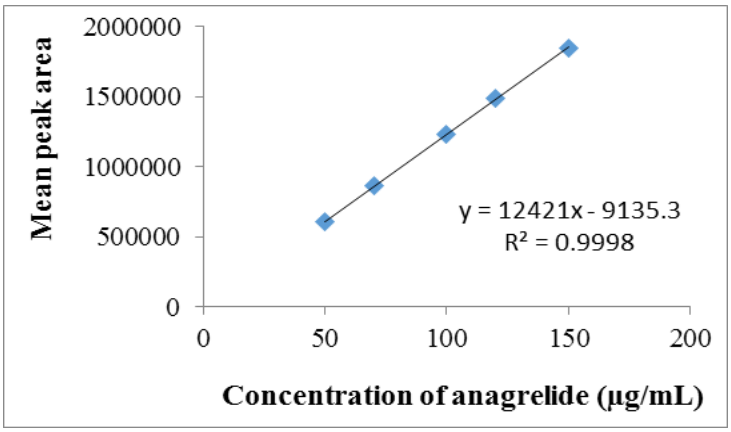

Fig. 2. Linearity plot for anagrelide

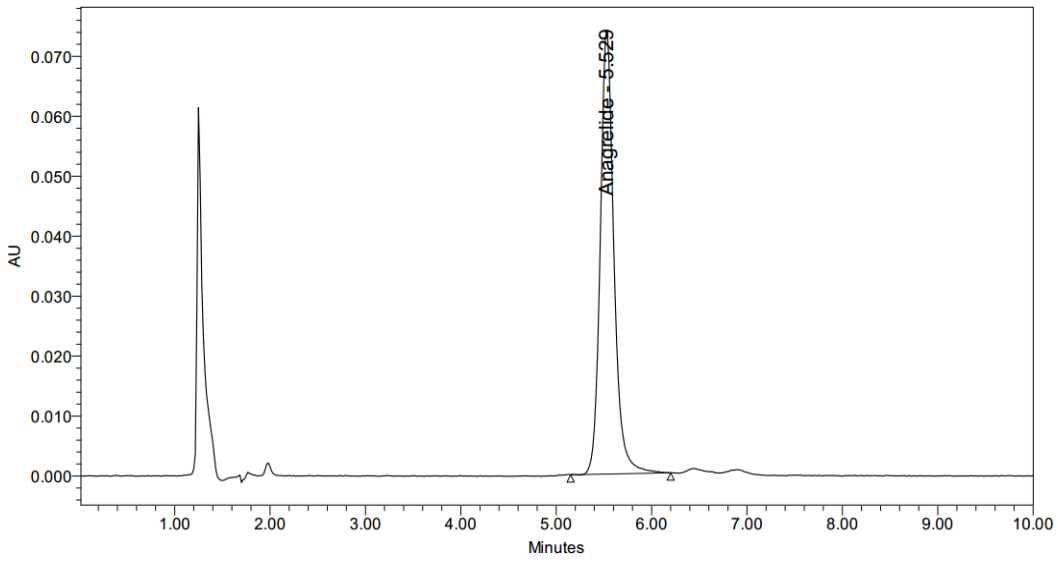

Figure 3.1. A representative chromatogram of 50 µg/mL concentration of anagrelide

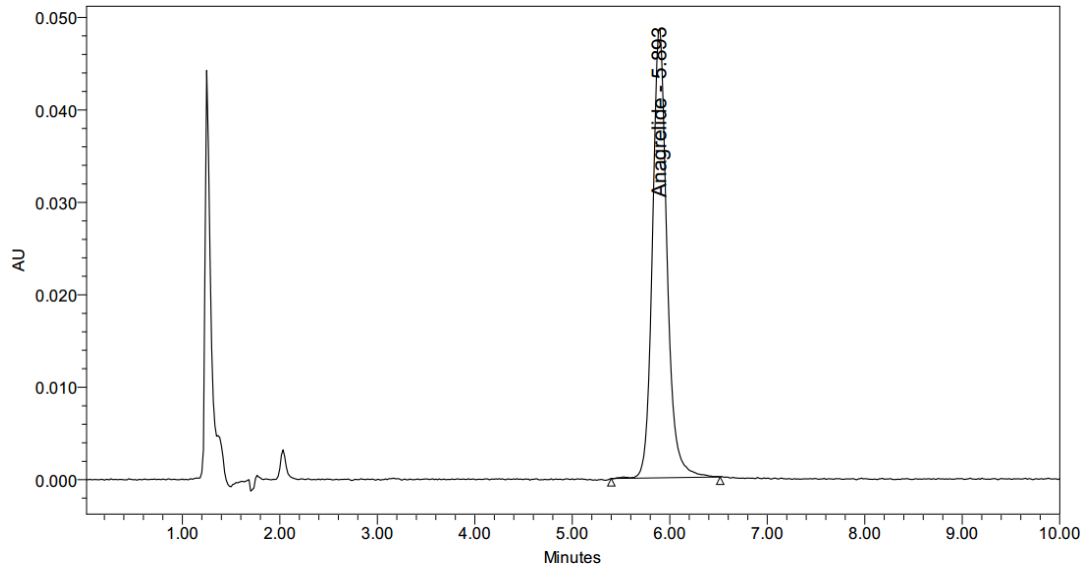

Figure 3. A representative chromatogram of 70 µg/mL concentration of anagrelide

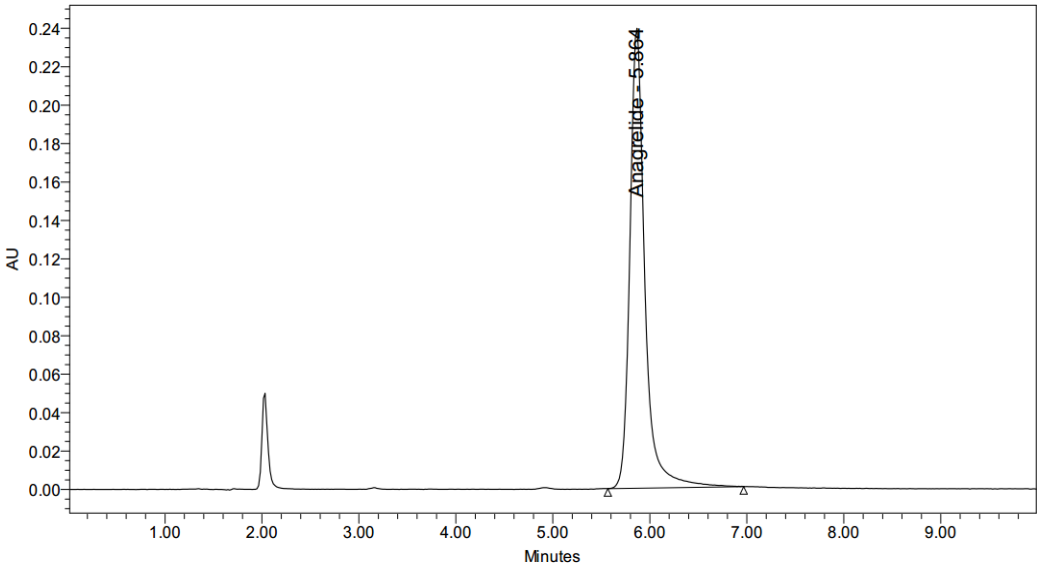

Figure 3. A representative chromatogram of 100 µg/mL concentration of anagrelide

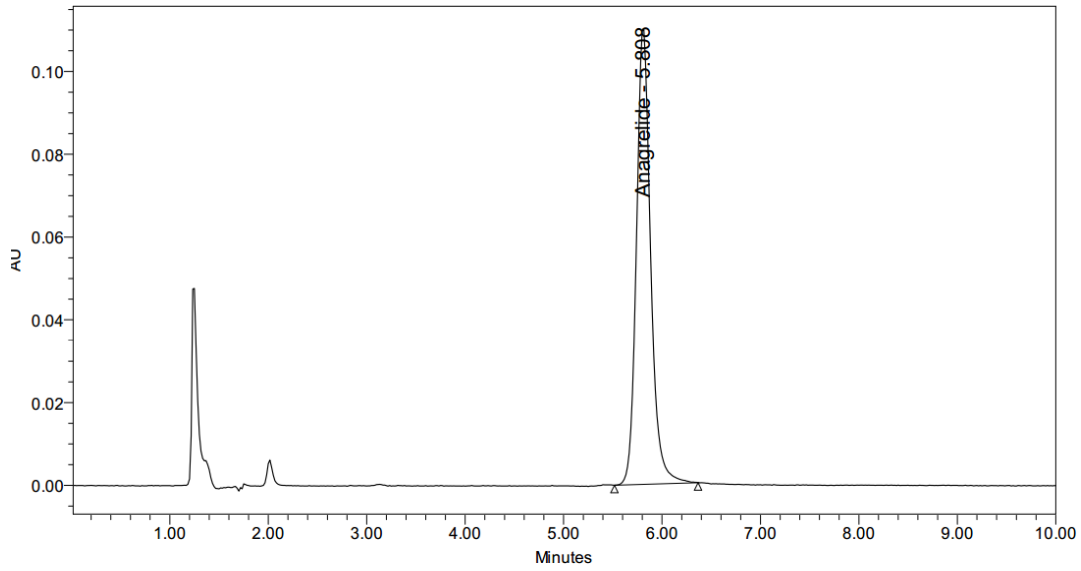

Figure 4. A representative chromatogram of 120 µg/mL concentration of anagrelide

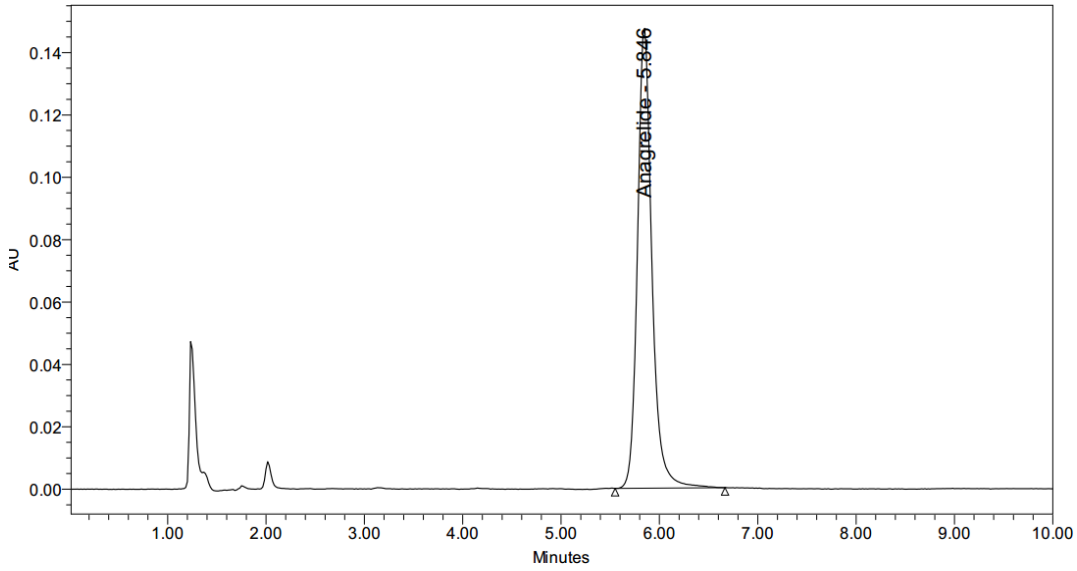

Figure 5. A representative chromatogram of 150 µg/mL concentration of anagrelide

Precision:

Repeatability and intermediate precision were assessed by analyzing standard solutions of anagrelide on the same day (n=6) and on two consecutive days respectively. The results of repeatability and intermediate precision studies are depicted in the Tables 2.4 and 2.5.

Table 3. Repeatability data

S. No.	Peak areas of anagrelide
1	1239846
2	1223570

3	1219789
4	1219985
5	1224567
6	1231256
Average	1226502
SD	7752.927
%RSD	0.63

Table 4. Intermediate precision data

Day	**Average peak areas of anagrelide (n=6)**
1	1220080
2	1235106
Overall average	1227593
SD	10865.18
%RSD	0.88

Accuracy:

The accuracy of the method was determined by analyzing in triplicate known concentrations of the drug corresponding to 50, 100 and 150 % levels of anagrelide (50, 100 and 150 µg/mL). The percent recovery was calculated. The results are incorporated in the Table 2.6.

Table 5. Recovery data of anagrelide

Concentration (µg/mL)	**Peak area**	**Recovery**	**Mean recovery**	**SD of recoveries**	**% recovery**
50	615394	50.00	99.79	0.23	100.00
	614378	49.92			99.83
	612591	49.77			99.54
100	1221473	99.24	100.20	0.94	99.24
	1233651	100.23			100.23
	1244693	101.13			101.13
150	1815748	147.53	98.47	0.47	98.35
	1827314	148.47			98.98
	1810526	147.10			98.07

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated by using residual standard deviation of the response and the slope of the regression line. The LOD and LOQ of anagrelide were found to be 2.035 and 6.168 µg/mL respectively.

Robustness study:

The robustness of the method was determined as per ICH guidelines under three conditions i.e. flow rate, temperature and mobile phase composition. The results obtained by deliberate variation in method conditions are summarized below.

Table 6. Robustness data

Chromatographic condition	Value	Retention time (min)	Tailing factor	Number of theoretical plates
Flow rate (mL/min)	0.9	5.648	1.15	4019
	1.0(O)	4.818	1.12	4203
	1.1	3.614	1.12	4377
Temperature (°C)	23	4.420	1.14	4294
	25(O)	4.818	1.12	4325
	27	4.265	1.11	4385
Mobile phase composition (Phosphate buffer : Acetonitrile (% v/v))	76:24	4.991	1.14	4184
	75:25(O)	4.818	1.12	4357
	74:26	4.729	1.13	4682

(O) – Optimised value.

Specificity of the proposed method:

The specificity of the method was evaluated with regard to interference due to presence of excipients in tablet formulation. The HPLC chromatograms recorded for the drug matrix did not show any interfering peak within retention time ranges. Fig. 2.2 and 2.9 show the representative chromatograms obtained from the analysis of anagrelide from working standard solution and the formulation sample solution. The figures show that the selected drug was clearly separated.

System suitability:

For finding out system suitability, six replicates of the working standard sample were injected and the parameters like peak retention time, tailing factor, number of theoretical plates (N) and HETP of the peak were generated. These results are shown in Table 2.8.

Table 7. System suitability parameters of the proposed method

S. No.	Parameter	Result
1	Retention time (min)	4.818

2	Tailing factor	1.12
3	Theoretical plates	4357
4	HETP	0.03271

Estimation of the drug from capsule dosage forms:

The contents of five capsules of ``Agrylin`` were transferred into a 50 mL volumetric flask. To this, 30 mL of the diluent was added and sonicated for 25 min. Then the volume was made up with the diluent and the contents were mixed well to get a final concentration of 100 µg/mL. This mixture was filtered through a 0.45µ membrane filter (discarding the first few mL of the filtrate).This solution was then chromatographed six times and from the chromatograms obtained, the average drug content in the formulation was calculated. A typical chromatogram obtained from the analysis of Agrylin capsule is shown in the Fig. 6.

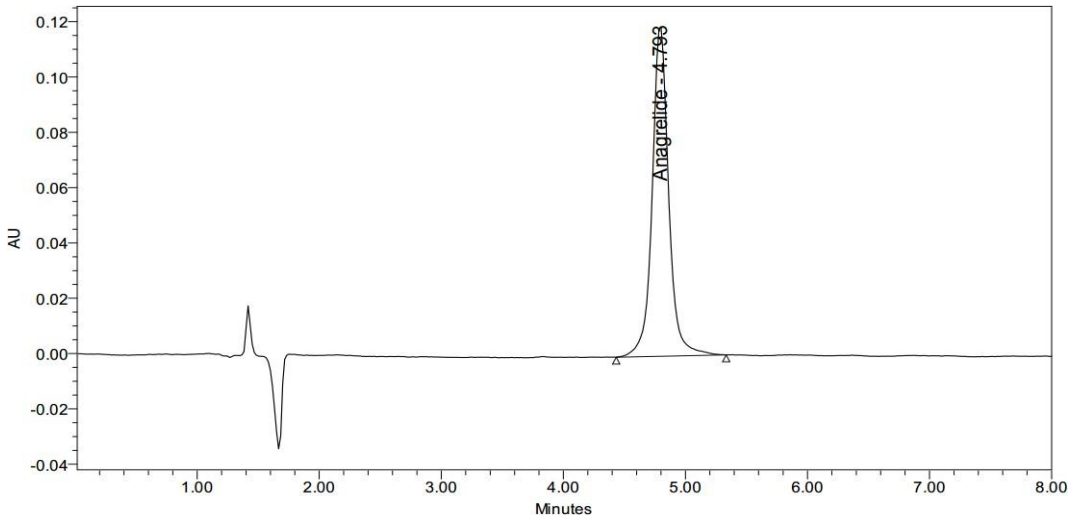

Figure 6. A representative chromatogram of the capsule formulation of anagrelide

Method suitability:

Studies for recovery of anagrelide from its commercial capsule formulations (Agrylin) were carried out by the proposed method and the results are shown in Table 2.9. The values obtained were found to be in good agreement with the labelled amounts. This confirms the suitability of the method for the analysis of anagrelide in capsule dosage form.

Table 2.9 Recovery of the drug from the capsule dosage form Agrylin

Drug	Labelled amount (mg)	Average Amount recovered (mg) (n=6)	% Recovery
Anagrelide	1.0	0.99	99

4. APPLICABILITY OF THE METHOD FOR MONITORING FORCED DEGRADATION STUDIES ON THE DRUG

In order to evaluate the stability of anagrelide and ability of the proposed method to separate anagrelide from its degradation products, anagrelide was subjected to various stress conditions such as acidic condition (2N hydrochloric acid), alkaline condition (2N sodium hydroxide), dry heat

condition (105 ° C for 6 hr), oxidation (20 % w/v of hydrogen peroxide), photolysis (exposure to ultraviolet radiation) and neutral degradation. The chromatograms are presented in Fig.2.10 to 2.16**.** The results are summarized in Table 2.10 and Table 2.11.

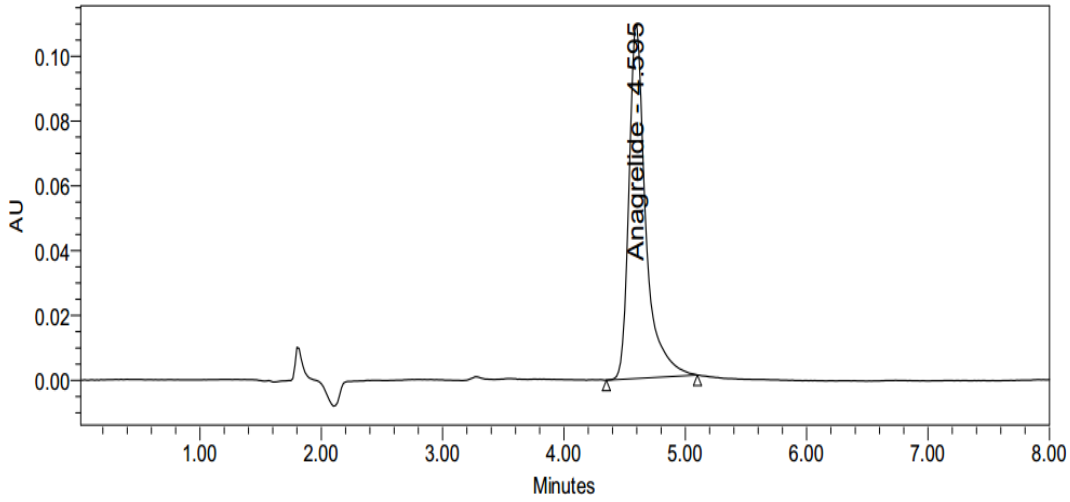

Fig.7. Chromatogram: Standard drug solution of anagrelide.

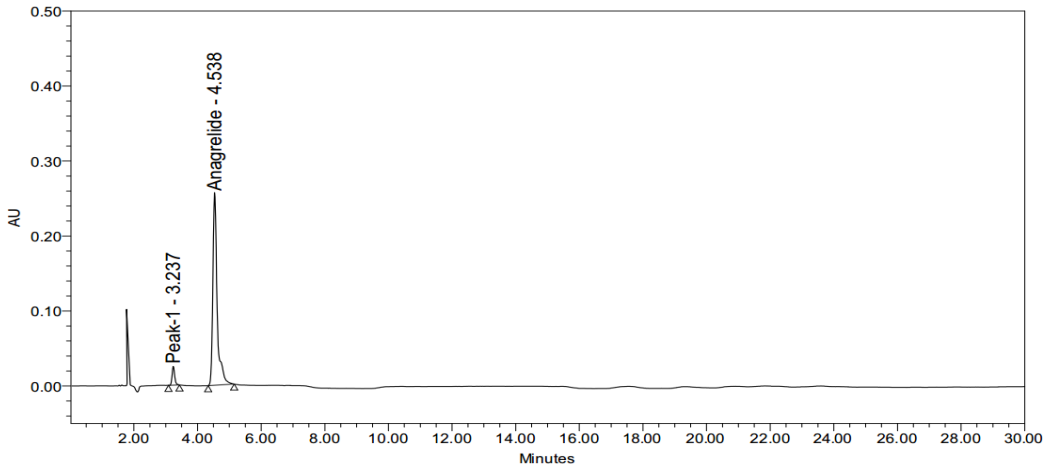

Fig.8. Chromatogram : Acid degradation of anagrelide

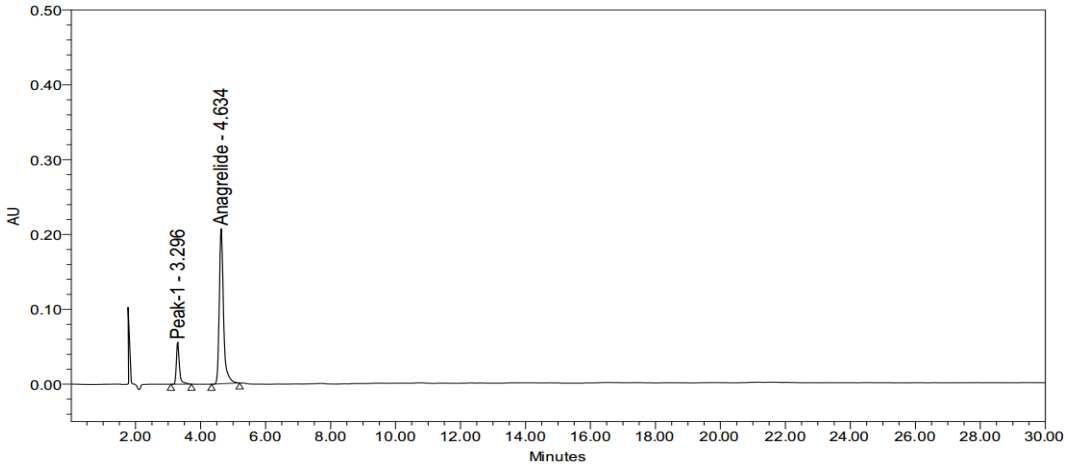

Fig.9. Chromatogram : Base degradation of anagrelide

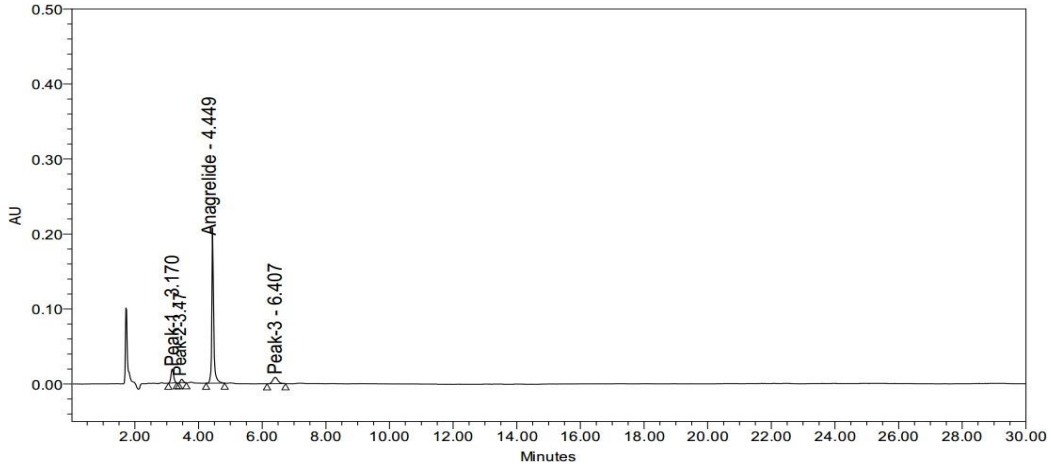

Fig.10. Chromatogram : Oxidative degradation of anagrelide

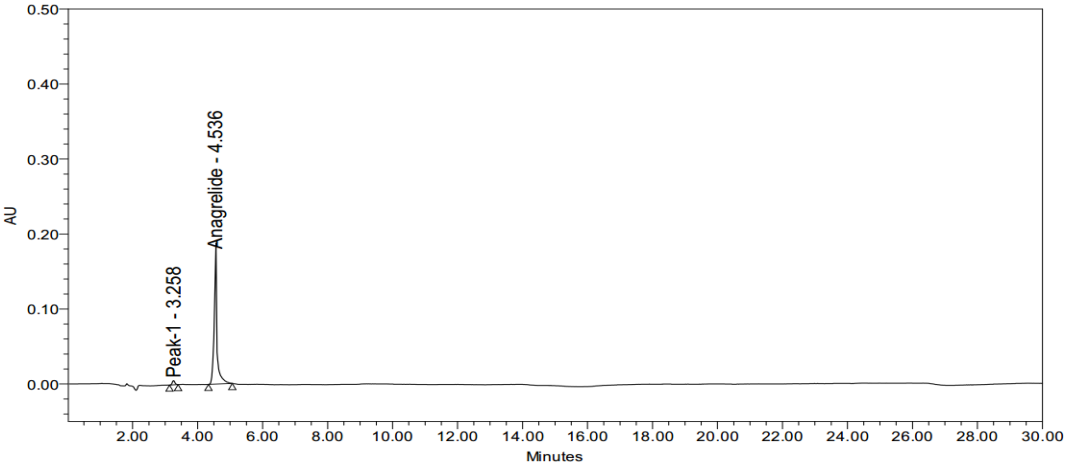

Fig.11. Chromatogram : Thermal degradation of anagrelide

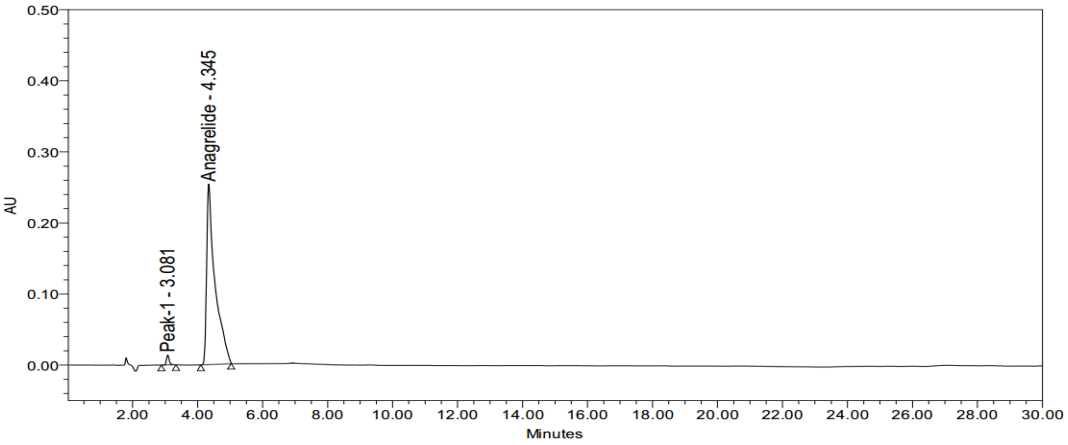

Fig.12. Chromatogram: Photolytic degradation of anagrelide

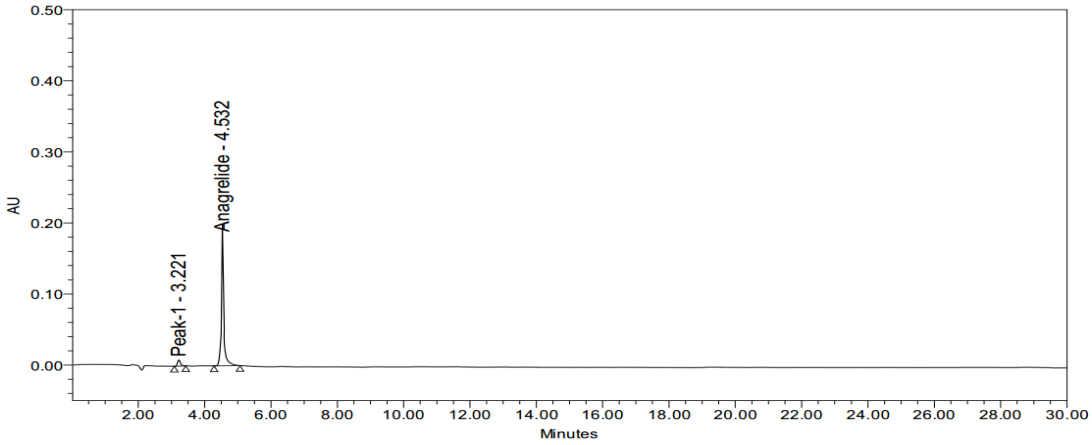

Fig.13. Chromatogram: Neutral degradation of anagrelide

Table 8. Forced degradation data for anagrelide

S.No.	Degradation	Retention time	Area	USP plate count	USP tailing	Purity angle	Purity threshold
1.	Acidic	4.538	1121328	6947	1.5	0.204	1.282
2.	Basic	4.634	1135058	6895	1.4	0.088	0.289
3.	Thermal	4.536	1174331	6249	1.5	0.181	0.369
4.	Oxidative	4.449	1163107	6455	1.4	1.133	2.582
5.	Photolytic	4.345	1216169	3426	1.5	0.128	0.585
6.	Neutral	4.532	1216729	6243	1.6	0.228	0.467

7.	Standard drug	4.595	1245618	6370	1.52	------	------

Table 9 Percentage of degradation of anagrelide

S.No.	Degradation Studies	% Degradation
1.	Acidic	9.4
2.	Basic	8.3
3.	Thermal	5.1
4.	Oxidative	6.0
5.	Photolytic	1.7
6.	Neutral	1.6

Table 10 A comparison chart of the proposed (current) method with the reported methods for determination of anagrelide

Method	Column	Mobile phase	Flow rate	Retention times	Linearity range	Run time
Proposed method	Kromasil C18 (150 x 4.6 mm; 5□m)	Phosphate buffer (pH 2.5) and acetonitrile (75:25 v/v)	**1.0 mL/min**	**4.818 min.**	50–150 µg/mL	**8 min**
Kalaichelvi *et al* [4]	Agilent Eclipse XDB C18 column (150 x 4.6 mm ; 5µ)	phosphate buffer (ph 4.0) and acetonitrile (70:30 v/v)	1.0 mL/min	5.8 min.	50–150 µg/mL	above 9 min
Venugopal *et al* [5]	Chromosil C18 column(250 mm x 4.6 mm; 5µ).	methanol, acetonitrile and water in the ratio 80:15:05(v/v)	1.0 mL/min	4.46 min.	20-120 µg/ml.	10 min
Sudhakar *et al* [6]	C18 Inertsil (250 x 4.6 mm ; 5µ)	phosphate buffer methanol: acetonitrile (90:5:5, v/v/v)	1.0 mL/min	8.376 min	0.05–152 µg /ml.	above 10 min

Ramanjaneyulu *et al* [7]	XTerra symmetry C18 (150×4.6 mm; 5µm)	acetonitrile and water (ph 3.0) in a ratio of 40:60 v/v	1.2 mL/min	2.349 min.	5-30 µg/mL	above 10 min
Kakadiya *et al* 8	Kromasil C18 (250mm x 4.6 mm; 5µm) column	methanol: phosphate buffer (ph-3) in a ratio of 70:30 v/v	1.0 mL/min	9.46 min	10-50 µg/mL	above 10 min

5. DISCUSSION ON THE RESULTS AND CONCLUSION

The aim of the present study was to develop a precise, accurate and sensitive HPLC method for the analysis of anagrelide in bulk drug and in pharmaceutical dosage forms. To analyse the component peaks, different combinations of mixtures of acetonitrile with phosphate buffer were tested as mobile phase on a Kromasil C18 (150 x 4.6 mm; 5µm) stationary phase. A binary mixture of acetonitrile and phosphate buffer (P^H 2.5) in a proportion of 25:75 v/v was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time obtained for anagrelide was 4.818 min. Each of the samples was injected six times and the same retention times were observed in all cases. The peak areas of anagrelide were reproducible as indicated by low coefficient of variation. A good linear relationship (r=0.9991) was observed between the concentration of anagrelide and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was Y = 12421X - 9135 (where y gives peak area and x is the concentration of the drug). The regression characteristics are given in Table 2.3**.** When anagrelide solution containing 100 µ g /mL was analysed by the proposed method for finding out intra and inter- day variations, low coefficient of variation was observed. High recovery values obtained from the different dosage forms by the proposed method indicates the method is accurate. The absence of additional peaks indicates non- interference of common excipients used in the capsules.

The drug content in capsules was quantified using the proposed analytical method. The capsules were found to contain an average of 99.6 % of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of anagrelide in dosage forms.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The lowest values of LOD and LOQ as obtained by the proposed method indicate the method is sensitive. The standard solution of the drug was stable up to 24 hours as the difference in percent assay is within limit.

6. CONCLUSION

System suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor, the number theoretical plates and HETP are in the acceptable limits.

Table 2.12 compares the present proposed method with the methods published earlier for anagrelide. An observation of the chart reveals some advantages of the proposed method. A Kromasil C18 column was used for the study. The retention time obtained is less than many other methods. The upper limit of quantitation is 150 µg/mL which is higher than three of the methods mentioned. The run time is also very less.

Hence the author concludes that the proposed HPLC method is sensitive and reproducible for the analysis of anagrelide in pharmaceutical dosage forms with short analysis time.

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Synthesis, Characterization of New 3-Chloro- Azetidine-2-One and 1, 3-Thiazinan-4-One Derivatives from Di Imines

[1]Gopi Swapna, [2]Thejovathi. B, [3]Usha Kondla, [4]Vankudoth Kavitha

Avanthi Institute of Pharmaceutical Sciences

ABSTRACT

The study Included synthesis of some new Derivatives of (benzylideneamino)-3-chloro-4-phenylazetidin-2-one and 3-(3-chloro- 2-oxo-4-phenylazetidin-1-yl)-2-phenyl-1,3-thiazinan-4-one by tow steps; The first include amino group of the di amino was condensed with different aromatic aldehydes in the presence of absolute ethanol to give new Schiff bases derivatives [1– 3] respectively. The second step , the resulting imines derivatives [1 – 3] were reacted with chloro acetyl chloride in presence of triethylamine in dry benzene by per cyclic reaction to give novel 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl) derivatives (A_1-A_3) and reacted with 3-mercapto propanoic acid with (Schiff-base) in dry benzene to give1,3-thiazinan-4-one derivative's(Z_1- Z_9) The composites prepared were described by melting point .Most of these derivatives were confirmed by "FT-IR, 1HNMR spectra.

Keywords---Schiff's bases, (benzylideneamino)-3-chloro-4- phenylazetidin-2-one, 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2- phenyl-1,3-thiazinan-4-one

Introduction

Azetidine, a four-member heterocyclic ring system with (N) as a heteroatom, is the parent heterocyclic ring of azetidinone. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics [1].Although the ring of azetidinone was known since (1907) but the realization of their chemistry began from (1947) only. These are presently used for chemotherapy of bacterial infections [2-4]. Realization of their chemistry began from (1947) only. These are presently used for chemotherapy of bacterial infections [2-4].] cycloaddition, also known as the Staudinger reaction, is a reaction between imine and ketene that is one of the most important and versatile techniques for the synthesis of structurally diverse 2-azetidinone derivatives[5]. The Staudinger reaction is thermally or photochemically enhanced by utilizing acid chlorides in the presence of (Et3N) triethylamine or a-diazoketones as ketene precursors[6]. Azetidinone is a four-membered cyclic that has been used as a useful building block for the preparation of a variety of chemical compounds by utilizing the strain energy associated with it[7] . Sulfadiazine is a sulfonamide antibiotic that is listed on the WHO's "List of Essential Medicines." It kills bacteria that cause infections by preventing the bacterial cell from producing folic acid, and it's commonly used to treat "urinary tract infections" (UTIs) and burns[8,9]. The four-membered cyclic amide azetidinone, often known as "-lactam," is produced from 3-amino-propanoic acid [10,11]. Azetidine is the parent heterocyclic ring of azetidinone, which is a four-membered heterocyclic ring system with (N) as the heteroatom. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics[10]. Although the ring of azetidinone has been known since 1907, its chemistry has only recently been discovered (1947) Azetidine, a four- member heterocyclic ring system with (N) as a heteroatom, is the parent heterocyclic ring of azetidinone. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics[12]. Although the ring of azetidinone has been known since 1907, the chemistry of the compound was finally discovered in 1947. These are currently being utilized to treat bacterial infections [13-15]. Thiazinanones (six- membered heterocyclic) have not been extensively studied in the past, but they have important biological properties such as immunopotentiating [16], anti- inflammatory [17], antimalarial [18], and antibacterial [19]. The current study additionally looked at how thiazolidinones have been synthesized in recent years [19, 20]. The methods utilized in nonconventional sonochemistry were of great interest to the researchers [21, 22]. The research is the first to look at the thiazinanone ring's chemistry. Thus, using 2-picolylamine, aldehydes, and MercaptoPropanoic acid, the current work produced 15 novel thiazinanones. The goal of this research is to look at the antioxidant properties of thiazolidinones [23] and novel thiazinanones that have been synthesized in the past. N-bromo compounds are antibacterial, antifungal, and anti-HIV chemicals that have a bromine atom linked to nitrogen [22-27]. The antibacterial activity of 2-(4-((1-aryl- 1H-1, 2, 3-triazol-4-yl)methoxy)phenyl)2-(2-oxoazetidin-1-yl) acetamide against different G-positive (Staphylococcus aureus and Bacillus subtilis) acetates was investigated.The 2-(4-((1-aryl-1H-1, 2, 3-triazol-4-yl)methoxy)phenyl)2-(2-oxoazetidin-1-yl) acetamide product was characterized and their antibacterial activities were evaluated against various G-positive (Staphylococcus aureus and Bacillus subtilis) and G-negative (Pseudomonas aeruginosa and Escherichia coli) bacteria, using minimal inhibition concentration.[28].

Steps of the theoretical study to estimate the biological effectiveness

In this study, the structural formulas of the prepared compounds were drawn using ChemDraw Professional 16.0 program. After that, the structural formula was modified and the files were converted to mol format using Chem3D 16.0 program. In the next step, Discovery Studio 4.0 Client converted the files to pdb. The last step, the files of the structural formulas were uploaded to the website www.dockthor.com to complete the process of molecular docking of the compounds after specifying the email to which the results will be sent after the bond strength assessment is completed. After receiving the results, they were included in Table No. (7).

Materials and Methods

Using the electro thermal 9300 melting point LTD, UK, the melting points were recorded and reported in degrees (0c). TLC was carried out on aluminum and glass plates that were coated with a 0.25mm layer of silica gel (Fluka). Iodine vapor was used to detect some of the derivatives. Fourier transform infrared (SHIMADZU, 8400) spectrophotometer, Japan the prang 4000-600cm-1 FT-IR spectra The samples were put through their paces on a KBr disc. The 13c and 1H-NMR spectra were measured in (ppm) units in DMSO-d6 as the solvent (Bruker- Ultra Shield 300 MHz Switzerland).

Synthesis of Schiff-bases [1-9]:

Mixture of diamines (0.01 mol) and the corresponding aldehydes (0.02mol) in ethanol (30ml) was treated with (3-5) drops glacial acetic acid and refluxed for (5h). The reaction of mixture was cooled and filtered. The final compounds were purified by recrystallization from ethanol [**29**].The physical properties for these compounds are listed in table (): In the same way, the rest of Schiff's rules were prepared. (1-3)

Synthesis of Heterocyclic Compounds:-

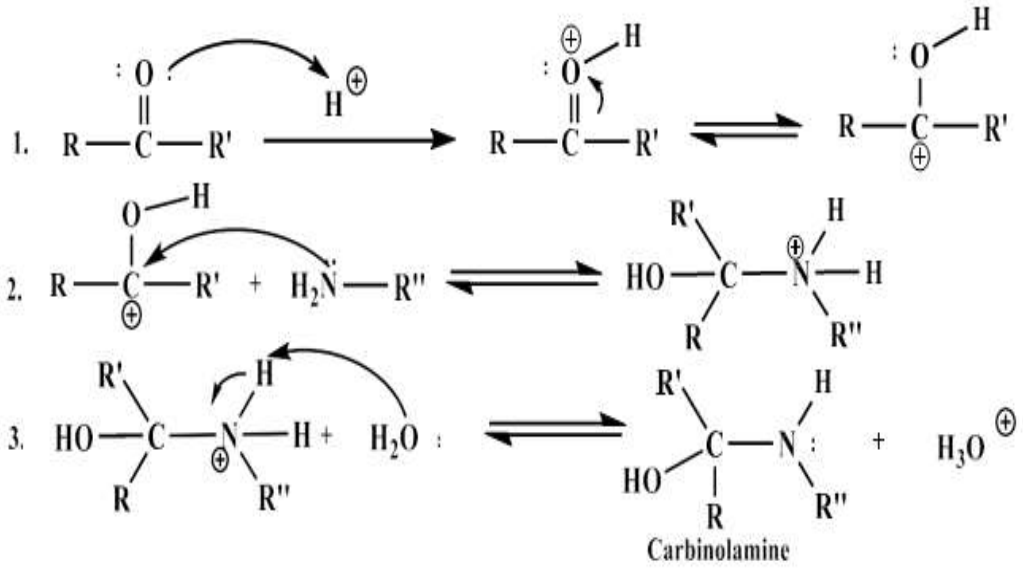
Synthesis of 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl) derivatives (A_1-A_9). A mixture of Schiff bases (0.001mol) with (0.002 mol) of chloroacetyl chloride in dry benzene (30ml) was added drop wise at room temperature. and (3-5) drops of triethyl amine .Content was stirred vigorously for 15 minutes and refluxed for 8hrs. Mixture was cooled at room temperature, filtered, washed with ice-cooled water, dried and recrystallized from ethanol [**30**]. In the same way, the rest of Schiff's rules were prepared. (1-8)Some of the physical properties and yield of compounds are listed in table ():

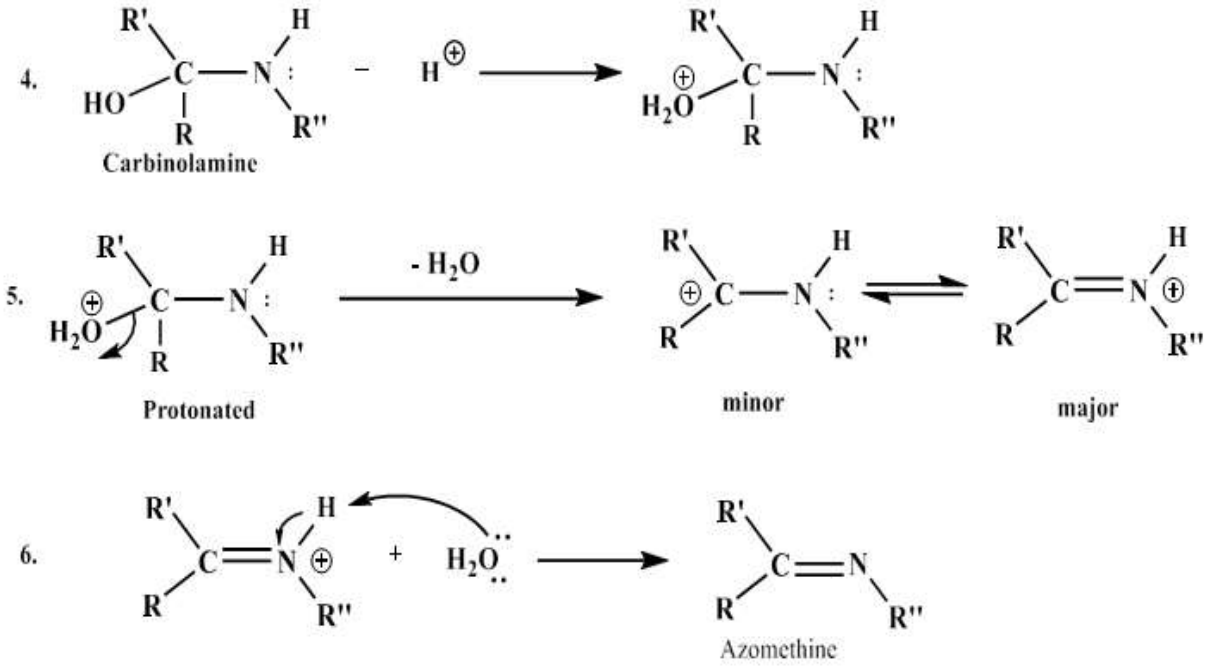
Synthesis of 1, 3-Thiazinane -6-one Derivatives (Z1-Z9)

"(0.01mole) of Schiff bases (A1-A9) with (0.01 mole, 1.085 g) of (3- Mercaptopropanoic acid) in (20 mL)" dry benzene and two drops of ZnCl2 , refluxed for 6 hours, then the solvent was dissolved. After that, 100% ethanol was used to recrystallize the molded precipitate. The rest of Schiff's regulations were prepared in the same way. (A1-A9) Table 1 shows the physical characteristics (3).

Results and Discussion

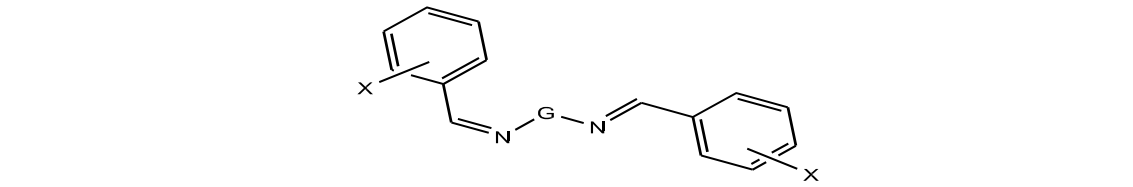
In this work, A number of diamines compounds which on condensation with various selected aromatic aldehydes in the presence of absolute ethanol and few drops of glacial acetic acid formed Schiff bases derivatives [1 -9]. The spectral data respectively. and is illustrated in scheme 1. Of FT.IR of [1

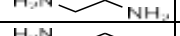
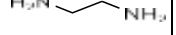
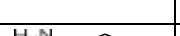
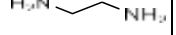
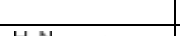
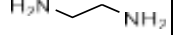
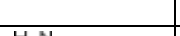
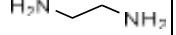




Scheme-(1): Mechanism of Imine formation

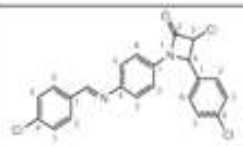
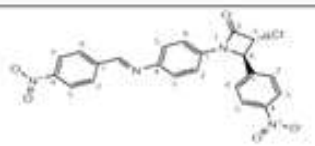
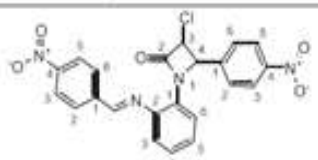
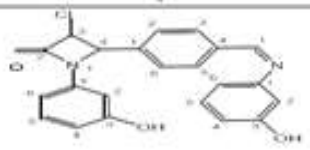
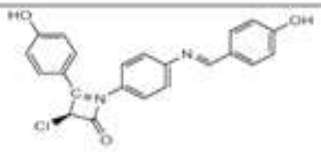
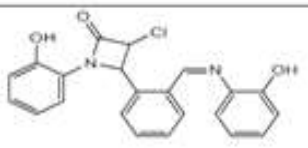
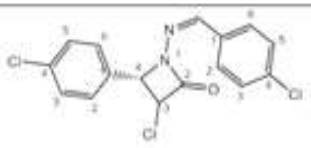
Table (1): values of FT-IR absorption bands for Schiff bases (1-9) measured in cm-

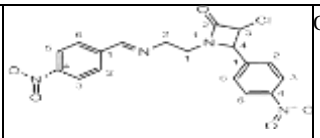


Code	X	G	Molc. Formula	M.Wt	m.p	Yelid %	Colour
6	o-OH		$C_{16}H_{16}N_2O_2$	268.31	621-621	60	Yellow
2	P-NO2		$C_{16}H_{14}N_4O_4$	326.31	184-186	80	Golden
3	P-B r		$C_{16}H_{14}Br_2N_2$	394.11	150-152	78	yellow
4	P-OH		$C_{16}H_{16}N_2O_2$	268,31	218-220	73	white
5	P-Cl		$C_{16}H_{14}Cl_2N_2$	305.20	172-174	70	brown
6	o-OH		$C_{14}H_{12}N_2O_2$	240	204-206	69	white
7	*P*-Cl		$C_{14}H_{10}Cl_2N_2$	277	212-214	70	Yellow
8	*P*-NO_2		$C_{14}H_{10}N_4O_4$	298	273-275	74	Golden

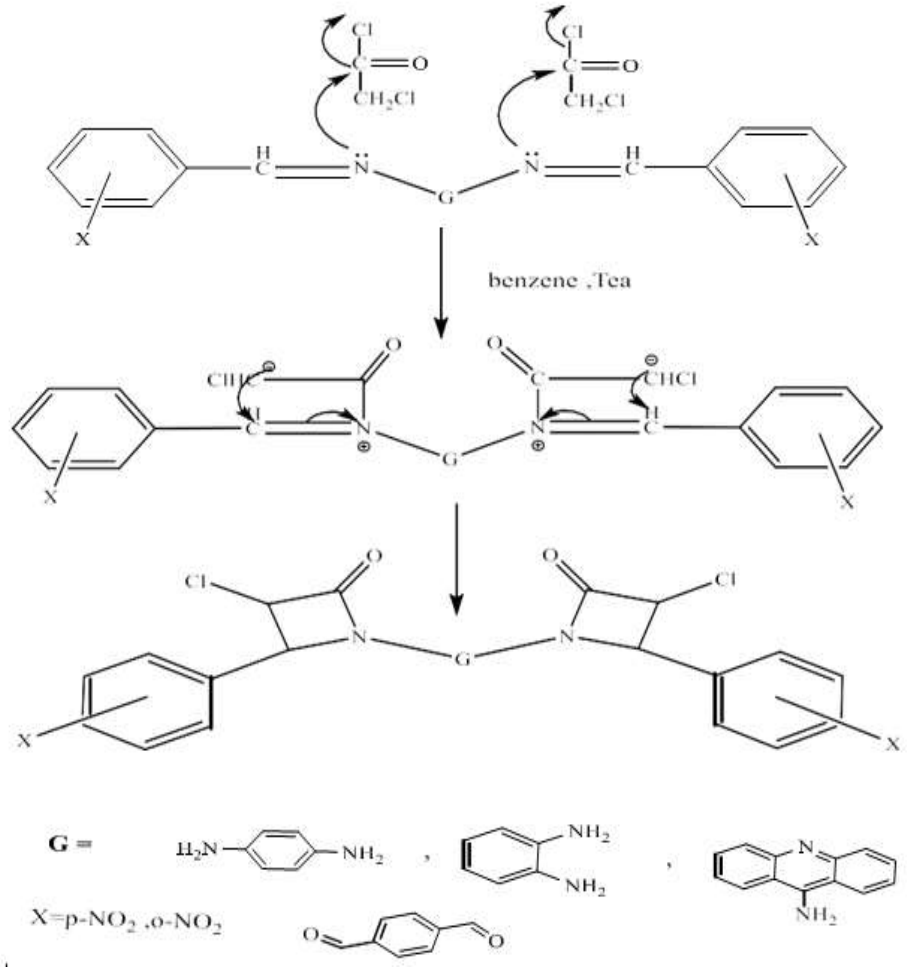
9	*P*-OH	H_2N—NH_2	$C_{14}H_{12}N_2O_2$	240.26	272-274	70	orange
10	*P*-NO_2		$C_{20}H_{14}N_4O_4$	374	206-208	75	Yellow
11	*P*-Cl		$C_{20}H_{14}Cl_2N_2$	352	138-140	74	Orange

Table (2): The chemical formula, molecular weights % yield, melting points, colours, of the azetidine compounds (A_1-A_9)

Comp.No	Nomenclature	Structural formula	Molec. formula	M.p. Co	Color
A_1	(E)-3-chloro-1-(4-((4-chlorobenzylidene)amino)phenyl)-4-(4-chlorophenyl)azetidin-2-one		$C_{22}H_{15}N_2OCl_3$	208-210	Green
A_2	**3**R,4R)-3-chloro-1-(4-(((E)-4-nitrobenzylidene)amino)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{22}H_{15}N_4O_5Cl$	248-246	yellow
A_3	(Z)-1-(acridin-9-yl)-4-(4-((acridin-9-ylimino)methyl)phenyl)-3-chloroazetidin-2-one		$C_{36}H_{23}N_4OCl$	Dec.	yellow
A_4	(E)-3-chloro-1-(2-((4-nitrobenzylidene)amino)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{22}H_{15}N_4O_5Cl$	206-204	orange
A_5	(Z)-3-chloro-1-(3-hydroxyphenyl)-4-(4-(((3-hydroxyphenyl)imino)methyl)phenyl)azetidin-2-one		$C_{22}H_{17}N_2O_3Cl$	211-213	red
A_6	(S,E)-3-chloro-1-(4-((4-hydroxybenzylidene)amino)phenyl)-4-(4-hydroxyphenyl)-114-azet-2(3H)-one		$C_{22}H_{17}N_2O_3Cl$	220-219	yellow
A_7	(Z)-3-chloro-1-(2-hydroxyphenyl)-4-(2-(((2-hydroxyphenyl)imino)methyl)phenyl)azetidin-2-one		$C_{22}H_{17}ClN_2O_3$	162-160	orange
A_8	(**4**S)-3-chloro-1-(((Z)-4-chlorobenzylidene)amino)-4-(4-chlorophenyl)azetidin-2-one		$C_{16}H_{11}N_4O_5Cl$	222-218	yellow

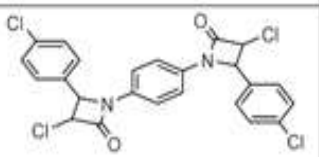
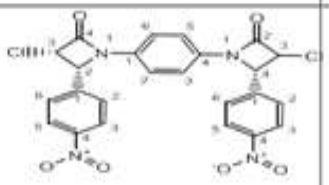
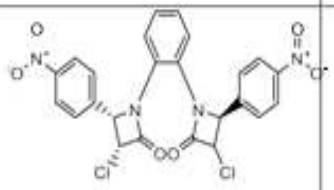
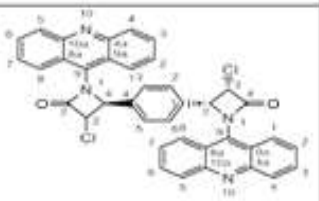
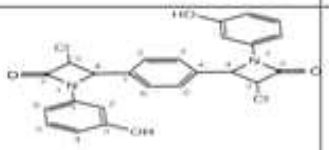
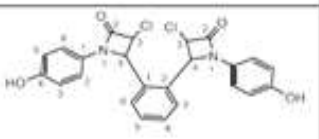
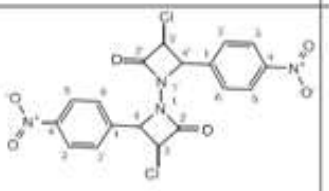
A_9	(E)-3-chloro-1-(2-((4-nitrobenzylidene)amino)ethyl)-4-(4-nitrophenyl)azetidin-2-one		C16H11N2OCl3	292-290	orange

The four-membered β-lactam ring was introduced by the cycloaddition of [1 – 9] and chloroacetyl chloride in the presence of triethylamine catalyst to give (benzylideneamino)-3-chloro-4-phenylazetidin-2-one [A_1 – A_{18}] . Title compounds [A_1 – A_9] shown IR bands at (1695 – 1799) cm-1 confirming the formation of (C=O) β-lactam and appearance of the vibration between (702 – 784) cm-1 was due to the (C-Cl) β-lactam which was further substantiated with the help of 1H-NMR data with the signals at δ (5.09 – 5.16) shown the presence of (N-CH) β-lactam(4.8- 6.50),(5.93-5.95) , and signal was observed for δ ppm for (HC-Cl) β-lactam (6.48- 6.50),(6.08-6.10)



Scheme-2: Mechanism of 3-Chloro-Azetidine-2-One formation

Table (4): Show the nomenclature, melting points, structural and molecular formula for compounds (A_{10}-A_{18}).

Comp.No	Nomenclature	Structural formula	Molec. formula	M.p. Co	Color
A_{10}	-'**1,1(1,4-**phenylene)bis(3-chloro-4-(4-chlorophenyl)azetidin-2-one)		$C_{24}H_{16}N_2O_2Cl_4$	212-210	green
A_{11}	(**4**S)-3-chloro-1-(4-((2R,3S)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{24}H_{16}N_4O_6Cl_2$	240-238	yellow
A_{12}	(**3**R,4S)-3-chloro-1-(2-((2S)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{24}H_{16}N_4O_6Cl_2$	222-220	orange
A_{13}	(**4**S)-1-(acridin-9-yl)-4-(4-((2R,3S)-1-(acridin-9-yl)-3-chloro-4-oxoazetidin-2-yl)phenyl)-3-chloroazetidin-2-one		$C_{38}H_{24}N_4O_2Cl_2$	Dec.	yellow
A_{14}	-'**4,4(1,4-**phenylene)bis(3-chloro-1-(3-hydroxyphenyl)azetidin-2-one)		$C_{24}H_{18}N_2O_4Cl2$	250-252	red
A_{15}	-'**4,4(1,1-**phenylene)bis(3-chloro-1-(4-hydroxyphenyl)azetidin-2-one)		$C_{24}H_{18}N_2O_2Cl_2$	226-224	brown
A_{16}	-'**3,3**dichloro-4,4'-bis(4-nitrophenyl)-[1,1'-biazetidine]-2,2'-dione		$C_{18}H_{14}N_2O_6Cl_2$	290-288	yellow

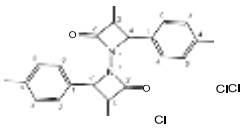
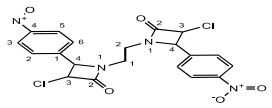
A17	**-'3,3**dichloro-4,4'-bis(4-chlorophenyl)-[1,1'- biazetidine]-2,2'-dione		C18H12N2O2Cl4	210-208	Light green
A18	**-'1,1**(ethane-1,2-diyl)bis(3-chloro-4-(4-nitrophenyl)azetidin-2-one)		C24H16N4O6Cl2	222-220	orange

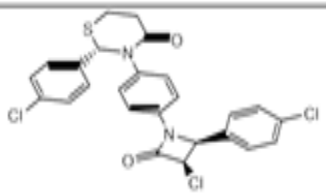
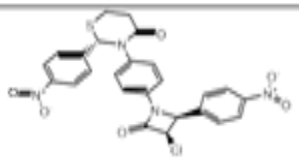
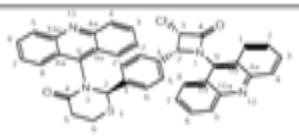
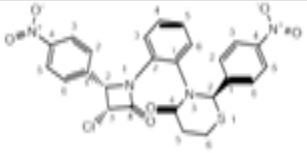
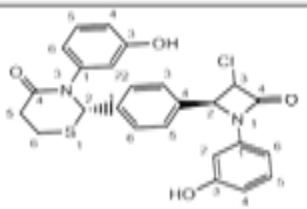
Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (A_1-A_9)

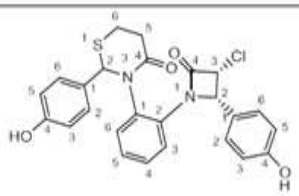
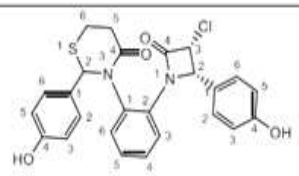
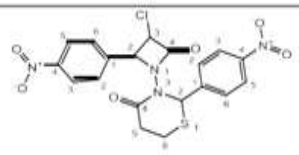
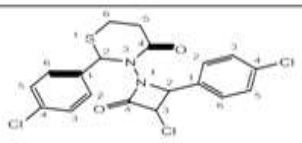
Comp. NO.	ν C-H arom.	ν C-H (-CH_2-) aliphatic		ν C=O lactam	ν C=N	ν C=C ring		ν C-N	ν C-Cl	Others
		Asym.	Sym.							
A_1	3081	2991	—	1668	1612	1582	1485	1270	680	766 C-Cl
A_2	3081	2853	—	1670	1621	1593	1492	1265	648	1415,1339 C- NO_2
A_3	3100	2913	—	1670	1647	1586	1480	1263	679	
A_4	3040	2884	—	1680	1635	1594	1511	1275	684	1446,1340 C- NO_2.
A_5	3000	2950	—	1686	1686	1597	1505	1280	688	3594 O-H
A_6	3030	2922	—	1654	1654	1594	1463	1241	740	3237 O-H
A_7	3068	2985	—	1675	1620	1609	1474	1234	734	3465 O-H
A_8	3052	2986	—	1670	1635	1605	1491	1278	748	1457,1320 C-NO_2
A_9	3046	2997	—	1660	1618	1590	1483	1285	708	780 C-Cl

Comp. No.	ν C-H arom.	ν C-H (-CH_2-) aliphatic		ν C=O lactam	ν C=C ring		ν C-N	ν C-Cl	Others
		Asym.	Sym.						
A10	3102	2939	2848	1635	1597	1514	1205	742	,-NO2 Asy **1114**,**1114**
A11	3053	2987	—	1670	1585	1488	1271	681	740C-Cl
A12	3082	2853	—	1688	1592	1493	1190	683	1414,1338 C-NO_2 Asy.
A13	3063	2933	—	1680	1590	1488	1270	688	1435, 1347C-NO_2 Asy.
A14	3136	2945	—	1648	1588	1480	1265	755	
A15	3165	2988	—	1684	1604	1503	1236	717	3387 O-H
A16	3060	2945	—	1665	1590	1470	1256	735	3457 O-H
A17	3092	2990	—	1680	1601	1478	1245	727	1430,1335 C-NO_2 Asy.
A18	3086	2956	—	1684	1590	1518	1238	718	734C-Cl

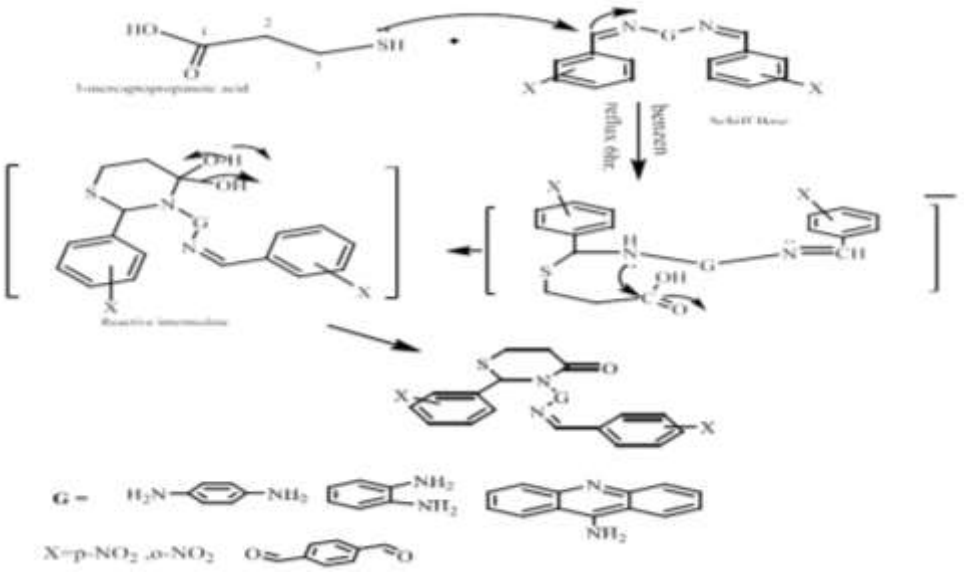
Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (A_{10}- A_{18})

Table (3): Show the nomenclature, melting points, structural and molecular formula for Compounds (Z_1-Z_9).

Comp.No.	Nomenclature	Structural formula	Mole. formula	M.p.	Color
Z_1	(S)-3-(4-((2S,3R)-3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_2O_2Cl_3S$	208-210	yellow
Z_2	(S)-3-(4-((2S,3R)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_4O_6ClS$	248-246	yellow
Z_3	(R)-3-(acridin-9-yl)-2-(4-((2R,3S)-1-(acridin-9-yl)-3-chloro-4-oxoazetidin-2-yl)phenyl)-1,3-thiazinan-4-one		$C_{39}H_{27}N_4O_2ClS$	Dec.	yellow
Z_4	(R)-3-(2-((2S,3R)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_4O_6ClS$	206-204	orange
Z_5	(1S)-2-(4-((2S)-3-chloro-1-(3-hydroxyphenyl)-4-oxoazetidin-2-yl)phenyl)-3-(3-hydroxyphenyl)-1,3-		$C_{25}H_{21}N_2O_4Cl\ S$	211-213	red

	thiazinan-4-one				
Z_6	(((**3**R,4S)-3-chloro-1-(2-(((E)-4-hydroxybenzylidene)amino)phenyl)-4-(4-hydroxyphenyl)azetidin-2-one		$C_{25}H_{21}N_2O_4ClS$	220-219	yellow
Z_7	**-3**(**1**-((**1**S,3R)-3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-hydroxyphenyl)-1,3-thiazinan-4-one		$C_{19}H_{15}N_4O_6ClS$	162-160	orange
Z_8	**-3**(**3**-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{19}H_{15}N_2O_2Cl_2S$	222-218	yellow
Z_9	**-3**(**3**-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one		$C_{21}H_{19}N_4O_6ClS$	292-290	orange

1,3-Thiazinan-6-one compounds [Z_1-Z_9] prepared by reaction of 3- mercaptopropanoic acid compound with [A_1-A_9] by using dry benzene as a solvent and ammonia. FT-IR spectrum showed bands at (3020 –3055) cm-1 for benzene ring, at (1640-1674) cm-1 for(C=O) lactone and lactam compounds, at (1360- 1385) cm-1 for (C-N) and (1587–1592) cm-1 for (C=C) aromatic ring. and appearance of band at (684-692) cm-1to C-S.

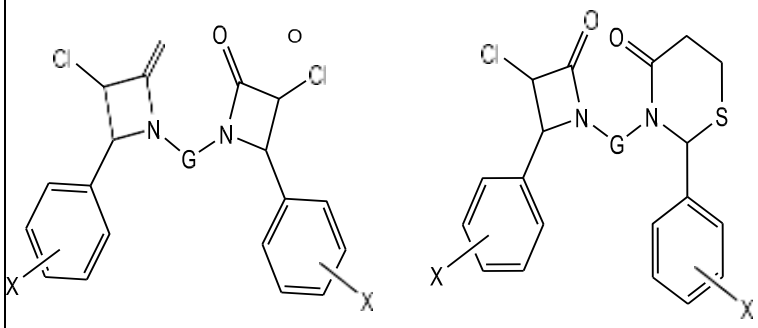


Scheme-2: Mechanism of 1,3-Thiazinan-6-one formation

Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (Z_1-Z_9)

Comp. N.O	ν C-H arom.	ν C-H (-CH2-) aliphatic		ν C=O lactam	ν C=O lacta m	ν C=C ring		ν C-N	ν C-Cl	ν C-S	Others
		Asym.	Sym.								
Z1	3050	2978	2845	1650	6165	1598	1478	1265	740	682	755 =C-Cl
Z2	3065	2985	2875	1678	6120	1589	1485	1275	729	681	1430 =C-NO2 Asy. 1345 =C-NO2 sy.
Z3	3048	2965	2890	1666	6160	1592	1465	1255	735	692	
Z 4	3044	2968	2838	1648	6122	1587	1495	1248	727	684	1432 =C-NO2 Asy. 1350 =C-NO2 sy
Z 5	3070	2976	2865	1645	6166	1592	1475	1269	718	695	3394 O-H
Z 6	3025	2985	2895	1635	6101	1586	1468	1238	708	682	3347 O-H
Z 7	3035	2980	2845	1670	6165	1601	1488	1285	735	697	3365 O-H
Z 8	3064	2990	2866	1665	6166	1592	1487	1278	740	685	1436 =C-NO2 Asy. 1340 =C-NO2 sy.
Z 9	3075	2946	2878	1652	6162	1589	1478	1257	738	678	743=C-Cl

Table (6): 1H-NMR Data of 3-Chloro-1-(Pyrimidin-2-Yl) Azetidin-2- One and thiazinan-4-One Compounds (A_1, A_3, Z_1, Z_3).



Comp. No.	δ(CH2 Thiazine) ppm 4H,t	δ(HC-Cl) β-lactam ppm 1H,s	δ(CH-Aromatic) ppm nH,m	δ(HC-N) lactam ppm 3H,m	δ (CH-S) Ppm 1H,s
A_1	-	d6.48-6.50	dd8.00-8.76	d.4.8-6.50	-
A_3	-	d6.08-6.10	m6.44-8.02	d5.93-5.95	-
Z_1	1.72-1.74	5.60-5.56	7.26-7.98	4.06-4.07	6.79
Z_3	.48-2.61	5.45-5.47	6.38-7.58	5.30-5.32	6.79

Applied efficacy

It has been found from the results that we obtained from the compounds prepared in the laboratory as in the table ( ), which are expressed in the unit (Kcal/mole), some of them have varying effectiveness,. Lung and chest cancer and with the anti-fungal, as well as with the bacteria E coli, Staphyll l, so it can be considered in the future as good pharmaceutical medical compounds, especially that some of them have carcinogenic activity. it was effective with all except with breast cancer Compound A_2(it gave good efficacy with all except for E coli bacteria and compound A_4 ,A_8,Z_8) gave good efficacy with all, especially with breast cancer, but it did not give with anti-fungal type.

Table (7):- Represents the applied effectiveness of some prepared compounds with the values of some drugs

Code	Breast cancer	Lung cancer	Anti-Fungal	E. coli	Staphyll
A2	-	-72116	-32113	-	-
A4	-112746	-12643	-42064	-	-62637
A8	-11.908	-7.022	-	-8.505	-8.79
A11	-	-	-	-42117	-62144
A12	-	-42311	-	-	-102411
A14		-4271	-	-12414	-12133
A15			-32616	-	-
Z1				-42111	
Z4		-72633		-72163	
Z8	-11211				
Tamoxifen	-112371				
Fulvestrant	-112441				
Raloxifene	-62443				
Toremifene	-112743				
Gefitinib		-42341			
Erlotinib		-4243			
Terbinafine			-42746		
Fluconazole			-12613		
Miconazole			-42166		
Econazole			-62316		
Clotrimazole			-42441		
Cephalexin				-72461	
Trimethoprim				-12661	
Trimethoprim					-62447
Cephalexin					-12371

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Synthesis, Characterization of New 3-Chloro- Azetidine-2-One and 1, 3-Thiazinan-4-One Derivatives from Di Imines

[1]Gopi Swapna, [2]Thejovathi. B, [3]Usha Kondla, [4]Vankudoth Kavitha

Avanthi Institute of Pharmaceutical Sciences

ABSTRACT

The study Included synthesis of some new Derivatives of (benzylideneamino)-3-chloro-4-phenylazetidin-2-one and 3-(3-chloro- 2-oxo-4-phenylazetidin-1-yl)-2-phenyl-1,3-thiazinan-4-one by tow steps; The first include amino group of the di amino was condensed with different aromatic aldehydes in the presence of absolute ethanol to give new Schiff bases derivatives [1– 3] respectively. The second step , the resulting imines derivatives [1 – 3] were reacted with chloro acetyl chloride in presence of triethylamine in dry benzene by per cyclic reaction to give novel 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl) derivatives (A_1-A_3) and reacted with 3-mercapto propanoic acid with (Schiff-base) in dry benzene to give1,3-thiazinan-4-one derivative's(Z_1- Z_9) The composites prepared were described by melting point .Most of these derivatives were confirmed by "FT-IR, 1HNMR spectra.

Keywords---Schiff's bases, (benzylideneamino)-3-chloro-4- phenylazetidin-2-one, 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2- phenyl-1,3-thiazinan-4-one

Introduction

Azetidine, a four-member heterocyclic ring system with (N) as a heteroatom, is the parent heterocyclic ring of azetidinone. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics [1].Although the ring of azetidinone was known since (1907) but the realization of their chemistry began from (1947) only. These are presently used for chemotherapy of bacterial infections [2-4]. Realization of their chemistry began from (1947) only. These are presently used for chemotherapy of bacterial infections [2-4].] cycloaddition, also known as the Staudinger reaction, is a reaction between imine and ketene that is one of the most important and versatile techniques for the synthesis of structurally diverse 2-azetidinone derivatives[5]. The Staudinger reaction is thermally or photochemically enhanced by utilizing acid chlorides in the presence of (Et3N) triethylamine or a-diazoketones as ketene precursors[6]. Azetidinone is a four-membered cyclic that has been used as a useful building block for the preparation of a variety of chemical compounds by utilizing the strain energy associated with it[7] . Sulfadiazine is a sulfonamide antibiotic that is listed on the WHO's "List of Essential Medicines." It kills bacteria that cause infections by preventing the bacterial cell from producing folic acid, and it's commonly used to treat "urinary tract infections" (UTIs) and burns[8,9]. The four-membered cyclic amide azetidinone, often known as "-lactam," is produced from 3-amino-propanoic acid [10,11]. Azetidine is the parent heterocyclic ring of azetidinone, which is a four-membered heterocyclic ring system with (N) as the heteroatom. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics[10]. Although the ring of azetidinone has been known since 1907, its chemistry has only recently been discovered (1947) Azetidine, a four- member heterocyclic ring system with (N) as a heteroatom, is the parent heterocyclic ring of azetidinone. The second position of 2-azetidinone has a carbonyl group, which is one of the most prevalent heterocyclic rings found in many antibiotics[12]. Although the ring of azetidinone has been known since 1907, the chemistry of the compound was finally discovered in 1947. These are currently being utilized to treat bacterial infections [13-15]. Thiazinanones (six- membered heterocyclic) have not been extensively studied in the past, but they have important biological properties such as immunopotentiating [16], anti- inflammatory [17], antimalarial [18], and antibacterial [19]. The current study additionally looked at how thiazolidinones have been synthesized in recent years [19, 20]. The methods utilized in nonconventional sonochemistry were of great interest to the researchers [21, 22]. The research is the first to look at the thiazinanone ring's chemistry. Thus, using 2-picolylamine, aldehydes, and MercaptoPropanoic acid, the current work produced 15 novel thiazinanones. The goal of this research is to look at the antioxidant properties of thiazolidinones [23] and novel thiazinanones that have been synthesized in the past. N-bromo compounds are antibacterial, antifungal, and anti-HIV chemicals that have a bromine atom linked to nitrogen [22-27]. The antibacterial activity of 2-(4-((1-aryl- 1H-1, 2, 3-triazol-4-yl)methoxy)phenyl)2-(2-oxoazetidin-1-yl) acetamide against different G-positive (Staphylococcus aureus and Bacillus subtilis) acetates was investigated.The 2-(4-((1-aryl-1H-1, 2, 3-triazol-4-yl)methoxy)phenyl)2-(2-oxoazetidin-1-yl) acetamide product was characterized and their antibacterial activities were evaluated against various G-positive (Staphylococcus aureus and Bacillus subtilis) and G-negative (Pseudomonas aeruginosa and Escherichia coli) bacteria, using minimal inhibition concentration.[28].

Steps of the theoretical study to estimate the biological effectiveness

In this study, the structural formulas of the prepared compounds were drawn using ChemDraw Professional 16.0 program. After that, the structural formula was modified and the files were converted to mol format using Chem3D 16.0 program. In the next step, Discovery Studio 4.0 Client converted the files to pdb. The last step, the files of the structural formulas were uploaded to the website www.dockthor.com to complete the process of molecular docking of the compounds after specifying the email to which the results will be sent after the bond strength assessment is completed. After receiving the results, they were included in Table No. (7).

Materials and Methods

Using the electro thermal 9300 melting point LTD, UK, the melting points were recorded and reported in degrees (0c). TLC was carried out on aluminum and glass plates that were coated with a 0.25mm layer of silica gel (Fluka). Iodine vapor was used to detect some of the derivatives. Fourier transform infrared (SHIMADZU, 8400) spectrophotometer, Japan the prang 4000-600cm-1 FT-IR spectra The samples were put through their paces on a KBr disc. The 13c and 1H-NMR spectra were measured in (ppm) units in DMSO-d6 as the solvent (Bruker- Ultra Shield 300 MHz Switzerland).

Synthesis of Schiff-bases [1-9]:

Mixture of diamines (0.01 mol) and the corresponding aldehydes (0.02mol) in ethanol (30ml) was treated with (3-5) drops glacial acetic acid and refluxed for (5h). The reaction of mixture was cooled and filtered. The final compounds were purified by recrystallization from ethanol [**29**].The physical properties for these compounds are listed in table (): In the same way, the rest of Schiff's rules were prepared. (1-3)

Synthesis of Heterocyclic Compounds:-

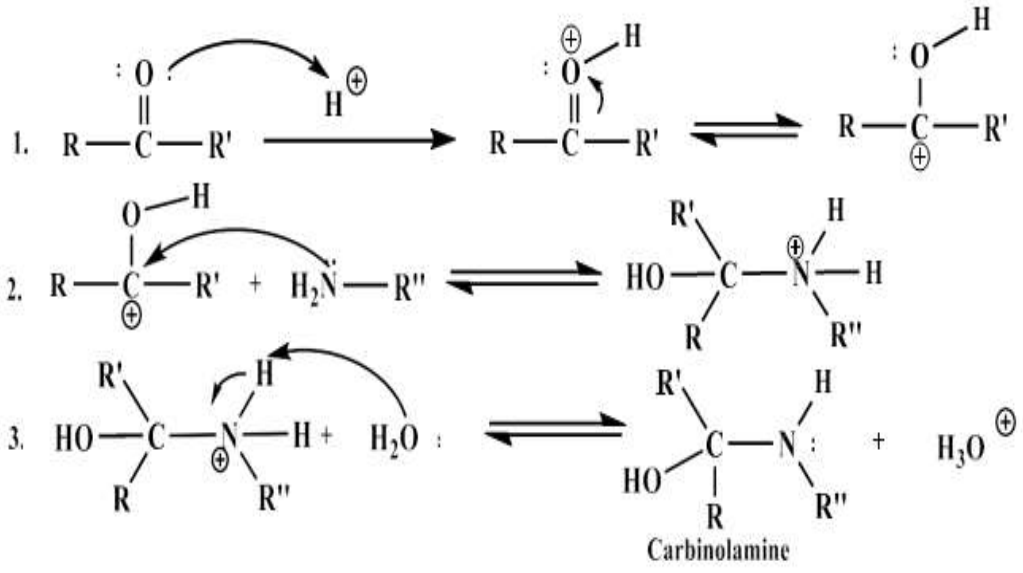
Synthesis of 3-(3-chloro-2-oxo-4-phenylazetidin-1-yl) derivatives (A_1-A_9). A mixture of Schiff bases (0.001mol) with (0.002 mol) of chloroacetyl chloride in dry benzene (30ml) was added drop wise at room temperature. and (3-5) drops of triethyl amine .Content was stirred vigorously for 15 minutes and refluxed for 8hrs. Mixture was cooled at room temperature, filtered, washed with ice-cooled water, dried and recrystallized from ethanol [**30**]. In the same way, the rest of Schiff's rules were prepared. (1-8)Some of the physical properties and yield of compounds are listed in table ():

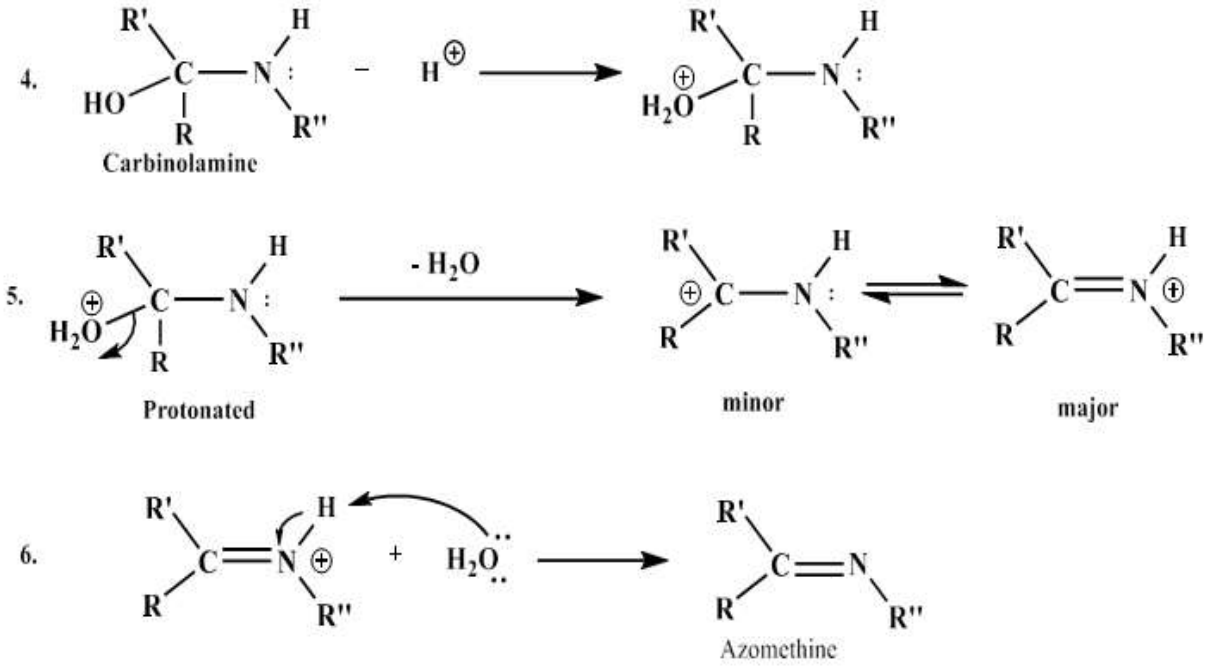
Synthesis of 1, 3-Thiazinane -6-one Derivatives (Z1-Z9)

"(0.01mole) of Schiff bases (A1-A9) with (0.01 mole, 1.085 g) of (3- Mercaptopropanoic acid) in (20 mL)" dry benzene and two drops of ZnCl2 , refluxed for 6 hours, then the solvent was dissolved. After that, 100% ethanol was used to recrystallize the molded precipitate. The rest of Schiff's regulations were prepared in the same way. (A1-A9) Table 1 shows the physical characteristics (3).

Results and Discussion

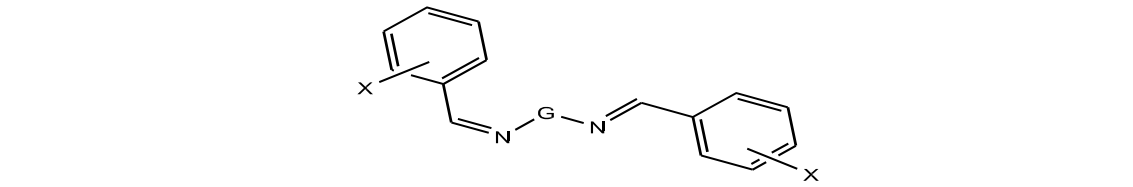
In this work, A number of diamines compounds which on condensation with various selected aromatic aldehydes in the presence of absolute ethanol and few drops of glacial acetic acid formed Schiff bases derivatives [1 -9]. The spectral data respectively. and is illustrated in scheme 1. Of FT.IR of [1

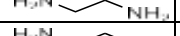
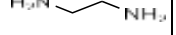
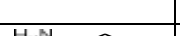
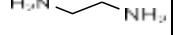
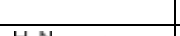
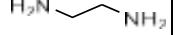
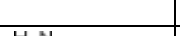
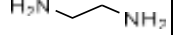




Scheme-(1): Mechanism of Imine formation

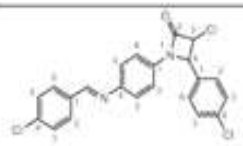
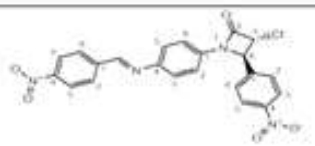
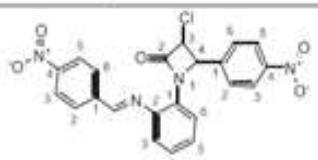
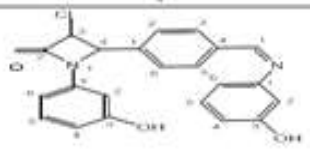
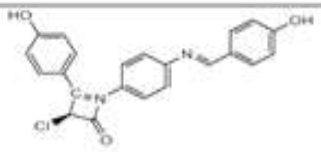
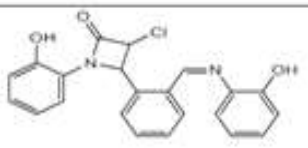
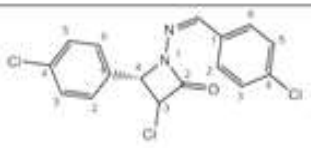
Table (1): values of FT-IR absorption bands for Schiff bases (1-9) measured in cm-

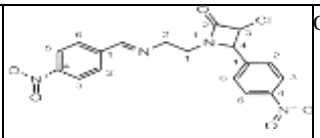


Code	X	G	Molc. Formula	M.Wt	m.p	Yelid %	Colour
6	o-OH		$C_{16}H_{16}N_2O_2$	268.31	621-621	60	Yellow
2	P-NO2		$C_{16}H_{14}N_4O_4$	326.31	184-186	80	Golden
3	P-B r		$C_{16}H_{14}Br_2N_2$	394.11	150-152	78	yellow
4	P-OH		$C_{16}H_{16}N_2O_2$	268,31	218-220	73	white
5	P-Cl		$C_{16}H_{14}Cl_2N_2$	305.20	172-174	70	brown
6	o-OH		$C_{14}H_{12}N_2O_2$	240	204-206	69	white
7	*P*-Cl		$C_{14}H_{10}Cl_2N_2$	277	212-214	70	Yellow
8	*P*-NO_2		$C_{14}H_{10}N_4O_4$	298	273-275	74	Golden

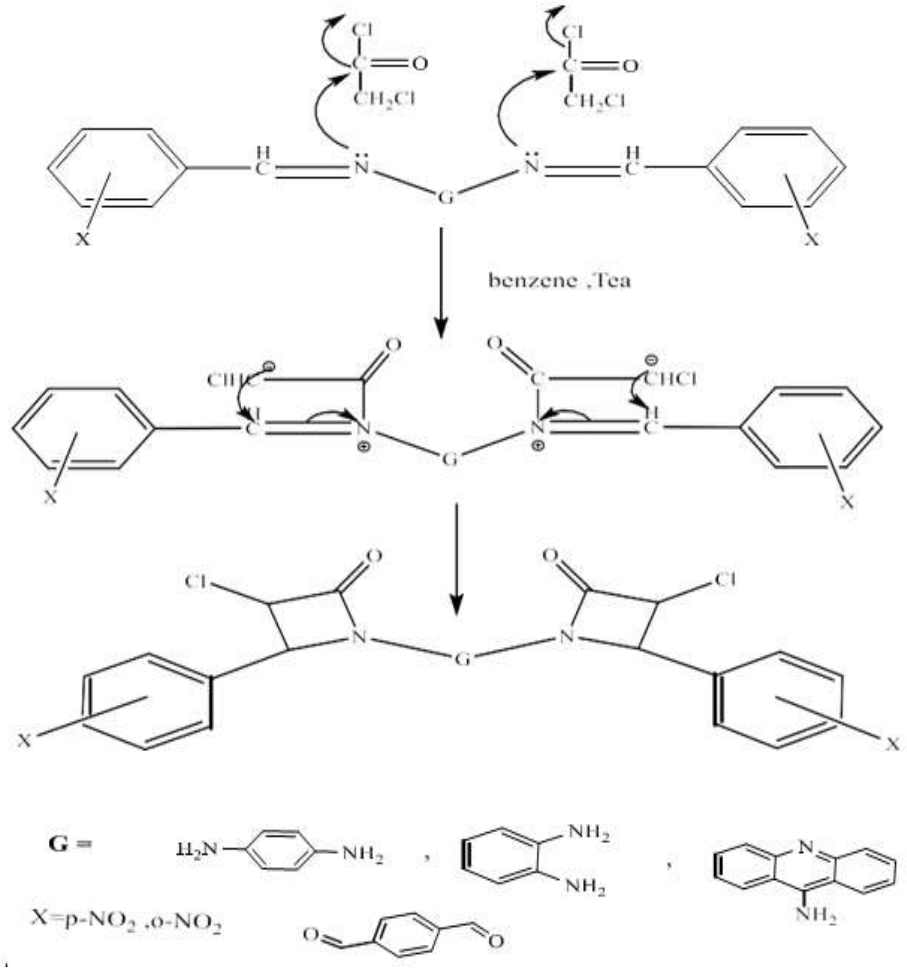
9	*P*-OH	H_2N—NH_2	$C_{14}H_{12}N_2O_2$	240.26	272-274	70	orange
10	*P*-NO_2		$C_{20}H_{14}N_4O_4$	374	206-208	75	Yellow
11	*P*-Cl		$C_{20}H_{14}Cl_2N_2$	352	138-140	74	Orange

Table (2): The chemical formula, molecular weights % yield, melting points, colours, of the azetidine compounds (A_1-A_9)

Comp.No	Nomenclature	Structural formula	Molec. formula	M.p. Co	Color
A_1	(E)-3-chloro-1-(4-((4-chlorobenzylidene)amino)phenyl)-4-(4-chlorophenyl)azetidin-2-one		$C_{22}H_{15}N_2OCl_3$	208-210	Green
A_2	**3**R,4R)-3-chloro-1-(4-(((E)-4-nitrobenzylidene)amino)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{22}H_{15}N_4O_5Cl$	248-246	yellow
A_3	(Z)-1-(acridin-9-yl)-4-(4-((acridin-9-ylimino)methyl)phenyl)-3-chloroazetidin-2-one		$C_{36}H_{23}N_4OCl$	Dec.	yellow
A_4	(E)-3-chloro-1-(2-((4-nitrobenzylidene)amino)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{22}H_{15}N_4O_5Cl$	206-204	orange
A_5	(Z)-3-chloro-1-(3-hydroxyphenyl)-4-(4-(((3-hydroxyphenyl)imino)methyl)phenyl)azetidin-2-one		$C_{22}H_{17}N_2O_3Cl$	211-213	red
A_6	(S,E)-3-chloro-1-(4-((4-hydroxybenzylidene)amino)phenyl)-4-(4-hydroxyphenyl)-114-azet-2(3H)-one		$C_{22}H_{17}N_2O_3Cl$	220-219	yellow
A_7	(Z)-3-chloro-1-(2-hydroxyphenyl)-4-(2-(((2-hydroxyphenyl)imino)methyl)phenyl)azetidin-2-one		$C_{22}H_{17}ClN_2O_3$	162-160	orange
A_8	(**4**S)-3-chloro-1-(((Z)-4-chlorobenzylidene)amino)-4-(4-chlorophenyl)azetidin-2-one		$C_{16}H_{11}N_4O_5Cl$	222-218	yellow

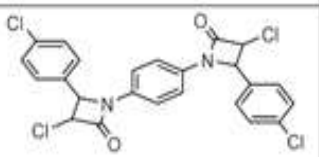
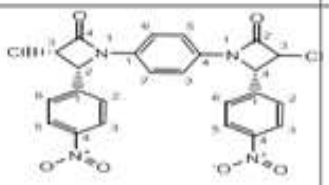
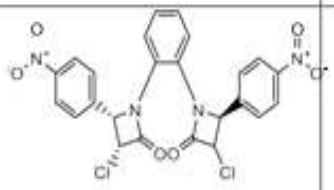
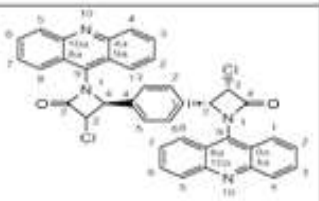
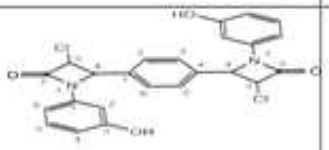
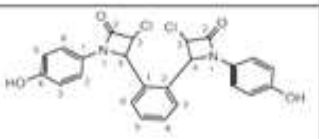
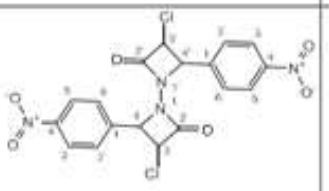
A_9	(E)-3-chloro-1-(2-((4-nitrobenzylidene)amino)ethyl)-4-(4-nitrophenyl)azetidin-2-one		C16H11N2OCl3	292-290	orange

The four-membered β-lactam ring was introduced by the cycloaddition of [1 – 9] and chloroacetyl chloride in the presence of triethylamine catalyst to give (benzylideneamino)-3-chloro-4-phenylazetidin-2-one [A_1 – A_{18}] . Title compounds [A_1 – A_9] shown IR bands at (1695 – 1799) cm-1 confirming the formation of (C=O) β-lactam and appearance of the vibration between (702 – 784) cm-1 was due to the (C-Cl) β-lactam which was further substantiated with the help of 1H-NMR data with the signals at δ (5.09 – 5.16) shown the presence of (N-CH) β-lactam(4.8- 6.50),(5.93-5.95) , and signal was observed for δ ppm for (HC-Cl) β-lactam (6.48- 6.50),(6.08-6.10)



Scheme-2: Mechanism of 3-Chloro-Azetidine-2-One formation

Table (4): Show the nomenclature, melting points, structural and molecular formula for compounds (A_{10}-A_{18}).

Comp.No	Nomenclature	Structural formula	Molec. formula	M.p. Co	Color
A_{10}	-'**1,1(1,4-**phenylene)bis(3-chloro-4-(4-chlorophenyl)azetidin-2-one)		$C_{24}H_{16}N_2O_2Cl_4$	212-210	green
A_{11}	(**4**S)-3-chloro-1-(4-((2R,3S)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{24}H_{16}N_4O_6Cl_2$	240-238	yellow
A_{12}	(**3**R,4S)-3-chloro-1-(2-((2S)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-4-(4-nitrophenyl)azetidin-2-one		$C_{24}H_{16}N_4O_6Cl_2$	222-220	orange
A_{13}	(**4**S)-1-(acridin-9-yl)-4-(4-((2R,3S)-1-(acridin-9-yl)-3-chloro-4-oxoazetidin-2-yl)phenyl)-3-chloroazetidin-2-one		$C_{38}H_{24}N_4O_2Cl_2$	Dec.	yellow
A_{14}	-'**4,4(1,4-**phenylene)bis(3-chloro-1-(3-hydroxyphenyl)azetidin-2-one)		$C_{24}H_{18}N_2O_4Cl2$	250-252	red
A_{15}	-'**4,4(1,1-**phenylene)bis(3-chloro-1-(4-hydroxyphenyl)azetidin-2-one)		$C_{24}H_{18}N_2O_2Cl_2$	226-224	brown
A_{16}	-'**3,3**dichloro-4,4'-bis(4-nitrophenyl)-[1,1'-biazetidine]-2,2'-dione		$C_{18}H_{14}N_2O_6Cl_2$	290-288	yellow

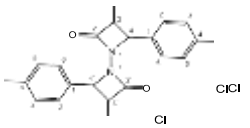
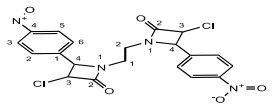
A17	**-'3,3**dichloro-4,4'-bis(4-chlorophenyl)-[1,1'- biazetidine]-2,2'-dione		C18H12N2O2Cl4	210-208	Light green
A18	**-'1,1**(ethane-1,2-diyl)bis(3-chloro-4-(4-nitrophenyl)azetidin-2-one)		C24H16N4O6Cl2	222-220	orange

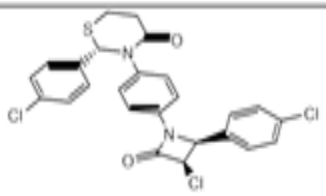
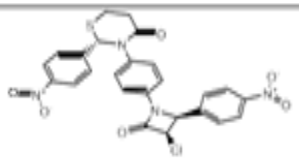
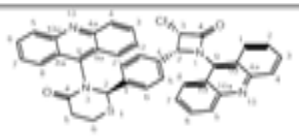
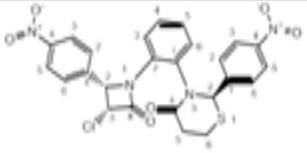
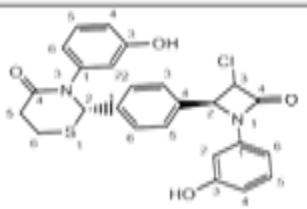
Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (A_1-A_9)

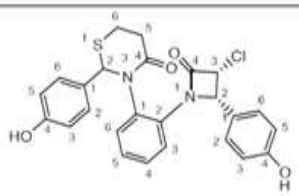
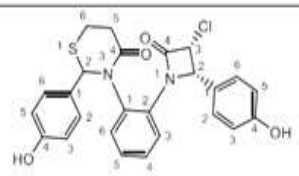
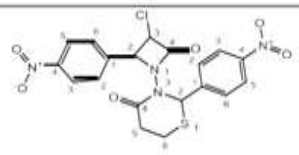
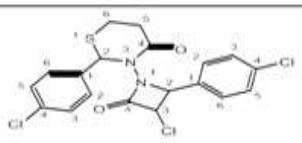
Comp. NO.	ν C-H arom.	ν C-H (-CH_2-) aliphatic		ν C=O lactam	ν C=N	ν C=C ring		ν C-N	ν C-Cl	Others
		Asym.	Sym.							
A_1	3081	2991	—	1668	1612	1582	1485	1270	680	766 C-Cl
A_2	3081	2853	—	1670	1621	1593	1492	1265	648	1415,1339 C- NO_2
A_3	3100	2913	—	1670	1647	1586	1480	1263	679	
A_4	3040	2884	—	1680	1635	1594	1511	1275	684	1446,1340 C- NO_2.
A_5	3000	2950	—	1686	1686	1597	1505	1280	688	3594 O-H
A_6	3030	2922	—	1654	1654	1594	1463	1241	740	3237 O-H
A_7	3068	2985	—	1675	1620	1609	1474	1234	734	3465 O-H
A_8	3052	2986	—	1670	1635	1605	1491	1278	748	1457,1320 C-NO_2
A_9	3046	2997	—	1660	1618	1590	1483	1285	708	780 C-Cl

Comp. No.	ν C-H arom.	ν C-H (-CH_2-) aliphatic		ν C=O lactam	ν C=C ring		ν C-N	ν C-Cl	Others
		Asym.	Sym.						
A10	3102	2939	2848	1635	1597	1514	1205	742	,-NO2 Asy **1114**,**1114**
A11	3053	2987	—	1670	1585	1488	1271	681	740C-Cl
A12	3082	2853	—	1688	1592	1493	1190	683	1414,1338 C-NO_2 Asy.
A13	3063	2933	—	1680	1590	1488	1270	688	1435, 1347C-NO_2 Asy.
A14	3136	2945	—	1648	1588	1480	1265	755	
A15	3165	2988	—	1684	1604	1503	1236	717	3387 O-H
A16	3060	2945	—	1665	1590	1470	1256	735	3457 O-H
A17	3092	2990	—	1680	1601	1478	1245	727	1430,1335 C-NO_2 Asy.
A18	3086	2956	—	1684	1590	1518	1238	718	734C-Cl

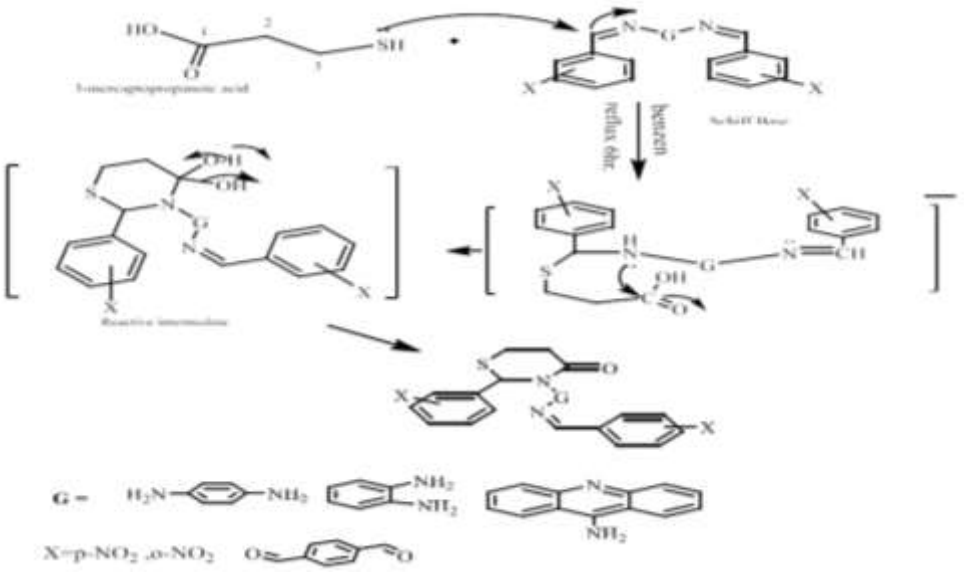
Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (A_{10}- A_{18})

Table (3): Show the nomenclature, melting points, structural and molecular formula for Compounds (Z_1-Z_9).

Comp.No.	Nomenclature	Structural formula	Mole. formula	M.p.	Color
Z_1	(S)-3-(4-((2S,3R)-3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_2O_2Cl_3S$	208-210	yellow
Z_2	(S)-3-(4-((2S,3R)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_4O_6ClS$	248-246	yellow
Z_3	(R)-3-(acridin-9-yl)-2-(4-((2R,3S)-1-(acridin-9-yl)-3-chloro-4-oxoazetidin-2-yl)phenyl)-1,3-thiazinan-4-one		$C_{39}H_{27}N_4O_2ClS$	Dec.	yellow
Z_4	(R)-3-(2-((2S,3R)-3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{25}H_{19}N_4O_6ClS$	206-204	orange
Z_5	(1S)-2-(4-((2S)-3-chloro-1-(3-hydroxyphenyl)-4-oxoazetidin-2-yl)phenyl)-3-(3-hydroxyphenyl)-1,3-		$C_{25}H_{21}N_2O_4Cl\ S$	211-213	red

	thiazinan-4-one				
Z_6	(((**3**R,4S)-3-chloro-1-(2-(((E)-4-hydroxybenzylidene)amino)phenyl)-4-(4-hydroxyphenyl)azetidin-2-one		$C_{25}H_{21}N_2O_4ClS$	220-219	yellow
Z_7	**-3**(**1**-((**1**S,3R)-3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)phenyl)-2-(4-hydroxyphenyl)-1,3-thiazinan-4-one		$C_{19}H_{15}N_4O_6ClS$	162-160	orange
Z_8	**-3**(**3**-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one		$C_{19}H_{15}N_2O_2Cl_2S$	222-218	yellow
Z_9	**-3**(**3**-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one		$C_{21}H_{19}N_4O_6ClS$	292-290	orange

1,3-Thiazinan-6-one compounds [Z_1-Z_9] prepared by reaction of 3- mercaptopropanoic acid compound with [A_1-A_9] by using dry benzene as a solvent and ammonia. FT-IR spectrum showed bands at (3020 –3055) cm-1 for benzene ring, at (1640-1674) cm-1 for(C=O) lactone and lactam compounds, at (1360- 1385) cm-1 for (C-N) and (1587–1592) cm-1 for (C=C) aromatic ring. and appearance of band at (684-692) cm-1to C-S.

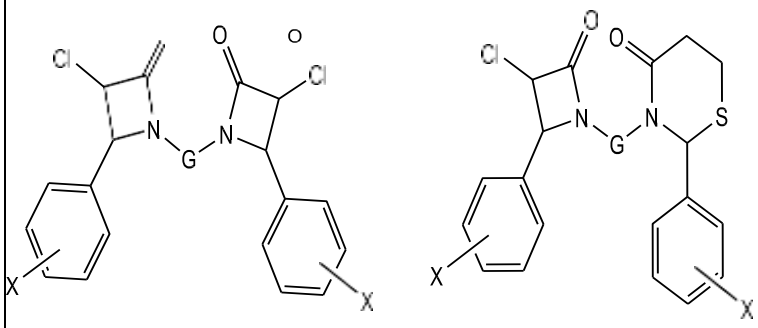


Scheme-2: Mechanism of 1,3-Thiazinan-6-one formation

Table (5): Wave numbers in cm-1 of I.R spectrum for prepared compounds: (Z_1-Z_9)

Comp. N.O	ν C-H arom.	ν C-H (-CH2-) aliphatic		ν C=O lactam	ν C=O lacta m	ν C=C ring		ν C-N	ν C-Cl	ν C-S	Others
		Asym.	Sym.								
Z1	3050	2978	2845	1650	6165	1598	1478	1265	740	682	755 =C-Cl
Z2	3065	2985	2875	1678	6120	1589	1485	1275	729	681	1430 =C-NO2 Asy. 1345 =C-NO2 sy.
Z3	3048	2965	2890	1666	6160	1592	1465	1255	735	692	
Z 4	3044	2968	2838	1648	6122	1587	1495	1248	727	684	1432 =C-NO2 Asy. 1350 =C-NO2 sy
Z 5	3070	2976	2865	1645	6166	1592	1475	1269	718	695	3394 O-H
Z 6	3025	2985	2895	1635	6101	1586	1468	1238	708	682	3347 O-H
Z 7	3035	2980	2845	1670	6165	1601	1488	1285	735	697	3365 O-H
Z 8	3064	2990	2866	1665	6166	1592	1487	1278	740	685	1436 =C-NO2 Asy. 1340 =C-NO2 sy.
Z 9	3075	2946	2878	1652	6162	1589	1478	1257	738	678	743=C-Cl

Table (6): 1H-NMR Data of 3-Chloro-1-(Pyrimidin-2-Yl) Azetidin-2- One and thiazinan-4-One Compounds (A_1, A_3, Z_1, Z_3).



Comp. No.	δ(CH2 Thiazine) ppm 4H,t	δ(HC-Cl) β-lactam ppm 1H,s	δ(CH-Aromatic) ppm nH,m	δ(HC-N) lactam ppm 3H,m	δ (CH-S) Ppm 1H,s
A_1	-	d6.48-6.50	dd8.00-8.76	d.4.8-6.50	-
A_3	-	d6.08-6.10	m6.44-8.02	d5.93-5.95	-
Z_1	1.72-1.74	5.60-5.56	7.26-7.98	4.06-4.07	6.79
Z_3	.48-2.61	5.45-5.47	6.38-7.58	5.30-5.32	6.79

Applied efficacy

It has been found from the results that we obtained from the compounds prepared in the laboratory as in the table ( ), which are expressed in the unit (Kcal/mole), some of them have varying effectiveness,. Lung and chest cancer and with the anti-fungal, as well as with the bacteria E coli, Staphyll l, so it can be considered in the future as good pharmaceutical medical compounds, especially that some of them have carcinogenic activity. it was effective with all except with breast cancer Compound A_2(it gave good efficacy with all except for E coli bacteria and compound A_4 ,A_8,Z_8) gave good efficacy with all, especially with breast cancer, but it did not give with anti-fungal type.

Table (7):- Represents the applied effectiveness of some prepared compounds with the values of some drugs

Code	Breast cancer	Lung cancer	Anti-Fungal	E. coli	Staphyll
A2	-	-72116	-32113	-	-
A4	-112746	-12643	-42064	-	-62637
A8	-11.908	-7.022	-	-8.505	-8.79
A11	-	-	-	-42117	-62144
A12	-	-42311	-	-	-102411
A14		-4271	-	-12414	-12133
A15			-32616	-	-
Z1				-42111	
Z4		-72633		-72163	
Z8	-11211				
Tamoxifen	-112371				
Fulvestrant	-112441				
Raloxifene	-62443				
Toremifene	-112743				
Gefitinib		-42341			
Erlotinib		-4243			
Terbinafine			-42746		
Fluconazole			-12613		
Miconazole			-42166		
Econazole			-62316		
Clotrimazole			-42441		
Cephalexin				-72461	
Trimethoprim				-12661	
Trimethoprim					-62447
Cephalexin					-12371

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Formulation and Evaluation of Colon Specific Drug Delivery System of Sulfasalazine Loaded Microspheres

[1]Pitta Lavanya, [2]P.V. Thejovathi.B, [3]Pavan Kumar, [4]Usha Kondla
[1,2,3,4]Avanthi Institute of Pharmaceutical Sciences
[1,2,3,4]Approved by AICTE, PCI, NAAC, JNTUH,Gunthapally, Hayathnagar, Hyderabad-501512

***Abstract*--**Oral colon-targeted microsphere based drug delivery system containing sulfasalazine was prepared, optimized and characterized. The microspheres were successfully prepared by simple emulsification phase-separation technique followed by crosslinking. The formulations were optimized on the basis of drug: polymer ratio, stirring speed, concentration of glutraldehyde. The prepared microspheres were characterized on the basis of morphology, entrapment efficiency, particle size and in-vitro release.

***Keywords*--** *Microspheres, colon-targeted drug delivery systems, double-emulsion solvent diffusion method, chitosan, salfasalzine*

I. INTRODUCTION

Oral colon-targeted drug delivery systems have recently gained recognition for efficient delivery of therapeutic agent for both local as well as systemic action for the treatment of various colonic inflammatory diseases. The successful targeted delivery of drugs to the colon via the gastrointestinal tract requires the protection of a drug degradation and release in the stomach and small intestine and ensures immediate controlled release in the proximal colon [1, 2]. Microsphere based drug delivery systems are known to increase the life span of active pharmaceutical ingredient (API) and also involved in controlled release of API. Small particle size of microspheres with large surface area attributed for controlled release of insoluble drugs [3, 4]. Thus microspheres targeted to colon would be a promising for both local and systemic drug delivery. The prepared microspheres may be advantageous in term of reduced dose frequency, improved patient compliances, reduced side effects, high drug loading, and improves bioavailability [5]. Sulfasalazine (SLZ) is the anti-inflammatory drugs used to treat various inflammatory bowel diseases such as ulcerative colitis, and Crohn's disease due to induction of T-lymphocyte apoptosis modulates inflammatory mediators. It is poorly absorbed drug with approximately 5-19 hr elimination half-life [6]. Sulfasalazine is a derivative of mesalazine and also a prodrug of 5 aminosalicylic acid that is covalently linked to the antibiotic sulfapyridine by an azo bond. The objective of 54 present research works is to prepare, optimize and characterize the mucoadhesive microspheres for enhanced delivery of active ingredients [7-9].

II. MATERIALS AND METHODS

Materials

Sulfasalazine (SLZ) was received as a gift sample from Syntho Pharmaceuticals, Lucknow, India. Chitosan, light liquid paraffin, heavy liquid paraffin, Span 85, lsoproyl alcohol and glutraldehyde were procured from Himedia, Mumbai, India. All other chemicals, reagents and solvents used were of analytical grade.

Preparation of Sulfasalazine Microspheres

The sulfasalazine loaded microspheres were prepared by simple emulsification method followed by cross-linking method. Chitosan solution was prepared by dissolving the 100 mg of chitosan 1% v/v acetic acid (50 ml). The sulfasalazine (100 mg) was added to the disperse phase (chitosan solution). The drug-chitosan solution was extruded through a syringe (No. 20) in liquid paraffin (100 ml, heavy and light, 1 : 1 ratio) containing Span 85 (0.5%), and it was stirred at 1500 rpm using mechanical shaker. After 15 minutes, crosslinking agent (v/v aqueous solution) was added and stirring was continued for next 3 hours. The obtained microsphere were filtered and washed with isopropyl alcohol to remove traces of oil. They were finally washed with water to remove excess of crosslinking agent. The microspheres were then dried at 25°C and 60% relative humidity for 24 hrs [10].

Optimization of SLZ Microspheres

The SLZ microspheres were optimized by preparing six formulations (Table 1) using different variables such as drug: polymer ration, stirring speed, volume of gluteraldehyde. The resultant particle size, entrapment efficiency and drug release studies were considered for optimization process.

Table 1: Optimization of SLZ Microspheres

Formulation Code	**Variables**		
	(Drug: polymer)	**(stirring speed) rpm**	**(Vol. of gluteraldehyde) (v/v)**
SLZ-1	(1:1)	(500)	(0.5)
SLZ-2	(1:1)	(1000)	(1.0)
SLZ-3	(1:1)	(1500)	(1.5)
SLZ-4	(1:2)	(500)	(1.0)

SLZ-5	(1:2)	(1000)	(1.5)
SLZ-6	(1:2)	(1500)	(0.5)

Characterization of Microspheres

Morphological Characterization of Microspheres

Scanning electron microscopy is the very adequate method for the investigation of surface morphology of the prepared microspheres. The microsphere samples were prepared by smattering the powder on a double-sided adhesive tape stuck to an aluminum stub. The coating of gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in high-vacuum evaporator. The coated 55 samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (LEO-430, Cambridge, UK).

Particle Size Analysis

The particle size was determined by microscopic method. For each batch of the microsphere, 100 particles were randomly selected using an optical microscope fitted with a camera (Yoko CDD camera, Taiwan) and Medical Pro software (Version 3.0).

Determination of Encapsulation Efficiency

Weighed amount of microspheres was triturated with 100 ml of phosphate buffer (pH 6.8). The resulting mixture was stirred by magnetic stirrer for 2h. The solution was filtered through a membrane filter (0.45 mm pore size). 1 ml of the filtrate was suitably diluted using phosphate buffer (pH 6.8) and analyzed spectrophotometrically at 359 nm using UV-1700 Pharmaspec, Shimadzu UV- Visible spectrophotometer.

The EE was calculated using the formula.

$$\%EE = \frac{\text{Initial amount of drug in NPs} - \text{free drug}}{\text{Initial amount of drug in NPs}} \times 100$$

In-vitro drug release study

A weighed quantity of the microspheres was suspended in 200 ml of phosphate buffer pH 6.8 for 24 hrs using United States Pharmacopoeia basket-type dissolution rate test apparatus. Sample solution (5 ml) was withdrawn at predetermined time intervals and filtered through whatman filter paper. The samples were diluted suitably and analyzed spectrophotometrically with UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu) at 359 nm. % drug release for the batch 5 (SLZ-5).

Stability studies

Six batches of optimized formulation SLZ-5 were stored in amber colored screw capped glass vials in stability chamber at 40±1°C and 75%±5 relative humidity, room temperature and 4±0.5°C (refrigerator) for 3 months. Samples were analyzed for physical appearance, residual drug content after a period of 0, 7, 15, 30, 60 and 90 days. Initial drug content was taken as 100 % for each formulation.

Results and Discussion

Microspheres of SLZ have been successfully prepared using by simple emulsification method followed by cross-linking method due to high entrapment efficiency. The various variables (drug: polymer ratio, stirring speed, concentration of crosslinking agent play an important role in the formulation of microspheres and their characteristics.

Morphological characterization of microspheres

The surface topography of the microspheres was investigated by SEM (LEO-430, Cambridge, U. K). Microparticles containing sulfasalazine were small and uniform in size with surface cross- linked and almost spherical and free flowing (Figure 1).

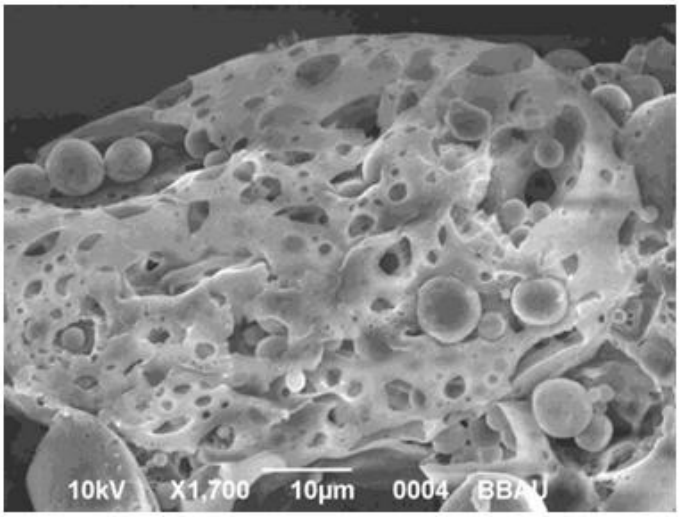

Fig 1: SEM photomicrograph of Chitosan microspheres of SLZ-6

Particle size analysis

The particle size and size distribution of the 100 randomly selected microspheres were determined using an optical microscope fitted with a camera (Yoko CDD camera, Taiwan) and Medical Pro software (Version 3.0). The particle size of optimized batch (SLZ 5) was found to be 135 µm as shown in Table 2.

Table 2: Experimental data (Optimization of particle size, µm)

Formulation	**Observed response value of particle size (µm**			**Mean (µm)**
SLZ-1	360	372	385	372.33
SLZ-2	257	255	270	294
SLZ-3	275	267	245	262.33
SLZ-4	167	162	163	164
SLZ-5	135	131	139	135

SLZ-6	171	167	152	163.33

Drug Entrapment Efficiency

The entrapment efficiency of optimized batch (SLZ-5) was found to be 78.26±0.87%). Entrapment efficiency of microparticles depends on drug: polymer ration, stirring speed and concentration of crosslinking agent (gluteraldehyde).

Table 3: Experimental data and (Optimization of entrapment efficiency, %)

Formulation	Observed response value of encapsulation efficiency (%)			Mean (%)
SLZ-1	45.16	42.17	43.11	43.48
SLZ-2	50.00	51.22	55.00	52.07
SLZ-3	61.09	62.11	67.55	63.58
SLZ-4	70.00	69.15	73.23	70.79
SLZ-5	79.11	78.26	78.27	78.54
SLZ-6	62.00	65.23	67.12	64.78

In-vitro Drug Release Study

The *in vitro* drug release studies were performed in simulated colonic fluid (pH 6.8). The amount of the drug released from the formulation in dissolution medium without rat caecal contents was found to be (Fig. 2) only 57.55± 0.19 of SLZ-5 respectively.

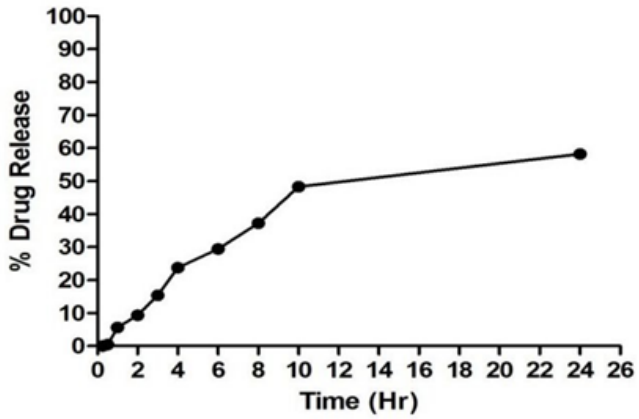

Fig 2: *In-vitro* release profile of optimized batch (SLZ-5) microspheres in simulated colonic fluid (pH 6.8)

Stability studies

The dried SLZ loaded microspheres (SLZ-5) exhibit no considerable difference in residual drug content presented in Table 4. The stability result suggested that microspheres were stable upto 90 days at 4°C and 25°C.

Table 4: Stability data for optimized formulation (SLZ-5)

S. No.	Sampling interval (days) %	Residual Drug Content Mean ± S.D. (n=3)		
		4±0.5 °C	Room temp. 40±0.5 °C	75%±5 RH
1	0	100	100	100
2	7	99.68±0.12	99.76±0.20	99.16± 0.15
3	15	99.45±0.06	98.92±0.08	98.45± 0.16
4	30	99.10±0.11	98.65±0.06	97.28± 0.17
5	60	98.45±0.09	98.78±0.05	96.56± 0.19
6	90	98.05±0.07	96.32±0.10	95.10± 0.27

CONCLUSION

Microspheres loaded SLZ have been prepared by simple emulsification method followed by cross-linking method. The variables such as drug: polymer ratio, stirring speed and concentration of glutraldehyde were optimized on the basis of particle size, entrapment efficiency. The prepared microspheres were stable, spherical particles and exhibited favorable release profiles in simulated colonic fluid. 58 However, further evaluation of these carriers can be performed for their potential to treat colonic diseases, as a future scope.

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Formulation and Evaluation of Colon Specific Drug Delivery System of Sulfasalazine Loaded Microspheres

[1]Pitta Lavanya, [2] Thejovathi.B, [3]Pavan Kumar.PV, [4]Usha Kondla
[1,2,3,4]Avanthi Institute of Pharmaceutical Sciences
[1,2,3,4]Approved by AICTE, PCI, NAAC, JNTUH,Gunthapally, Hayathnagar, Hyderabad-501512

***Abstract*--**Oral colon-targeted microsphere based drug delivery system containing sulfasalazine was prepared, optimized and characterized. The microspheres were successfully prepared by simple emulsification phase-separation technique followed by crosslinking. The formulations were optimized on the basis of drug: polymer ratio, stirring speed, concentration of glutraldehyde. The prepared microspheres were characterized on the basis of morphology, entrapment efficiency, particle size and in-vitro release.

***Keywords*--** *Microspheres, colon-targeted drug delivery systems, double-emulsion solvent diffusion method, chitosan, salfasalzine*

I. INTRODUCTION

Oral colon-targeted drug delivery systems have recently gained recognition for efficient delivery of therapeutic agent for both local as well as systemic action for the treatment of various colonic inflammatory diseases. The successful targeted delivery of drugs to the colon via the gastrointestinal tract requires the protection of a drug degradation and release in the stomach and small intestine and ensures immediate controlled release in the proximal colon [1, 2]. Microsphere based drug delivery systems are known to increase the life span of active pharmaceutical ingredient (API) and also involved in controlled release of API. Small particle size of microspheres with large surface area attributed for controlled release of insoluble drugs [3, 4]. Thus microspheres targeted to colon would be a promising for both local and systemic drug delivery. The prepared microspheres may be advantageous in term of reduced dose frequency, improved patient compliances, reduced side effects, high drug loading, and improves bioavailability [5]. Sulfasalazine (SLZ) is the anti-inflammatory drugs used to treat various inflammatory bowel diseases such as ulcerative colitis, and Crohn's disease due to induction of T-lymphocyte apoptosis modulates inflammatory mediators. It is poorly absorbed drug with approximately 5-19 hr elimination half-life [6]. Sulfasalazine is a derivative of mesalazine and also a prodrug of 5 aminosalicylic acid that is covalently linked to the antibiotic sulfapyridine by an azo bond. The objective of 54 present research works is to prepare, optimize and characterize the mucoadhesive microspheres for enhanced delivery of active ingredients [7-9].

II. MATERIALS AND METHODS

Materials

Sulfasalazine (SLZ) was received as a gift sample from Syntho Pharmaceuticals, Lucknow, India. Chitosan, light liquid paraffin, heavy liquid paraffin, Span 85, lsoproyl alcohol and glutraldehyde were procured from Himedia, Mumbai, India. All other chemicals, reagents and solvents used were of analytical grade.

Preparation of Sulfasalazine Microspheres

The sulfasalazine loaded microspheres were prepared by simple emulsification method followed by cross-linking method. Chitosan solution was prepared by dissolving the 100 mg of chitosan 1% v/v acetic acid (50 ml). The sulfasalazine (100 mg) was added to the disperse phase (chitosan solution). The drug-chitosan solution was extruded through a syringe (No. 20) in liquid paraffin (100 ml, heavy and light, 1 : 1 ratio) containing Span 85 (0.5%), and it was stirred at 1500 rpm using mechanical shaker. After 15 minutes, crosslinking agent (v/v aqueous solution) was added and stirring was continued for next 3 hours. The obtained microsphere were filtered and washed with isopropyl alcohol to remove traces of oil. They were finally washed with water to remove excess of crosslinking agent. The microspheres were then dried at 25°C and 60% relative humidity for 24 hrs [10].

Optimization of SLZ Microspheres

The SLZ microspheres were optimized by preparing six formulations (Table 1) using different variables such as drug: polymer ration, stirring speed, volume of gluteraldehyde. The resultant particle size, entrapment efficiency and drug release studies were considered for optimization process.

Table 1: Optimization of SLZ Microspheres

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Six batches of optimized formulation SLZ-5 were stored in amber colored screw capped glass vials in stability chamber at 40±1°C and 75%±5 relative humidity, room temperature and 4±0.5°C (refrigerator) for 3 months. Samples were analyzed for physical appearance, residual drug content after a period of 0, 7, 15, 30, 60 and 90 days. Initial drug content was taken as 100 % for each formulation.

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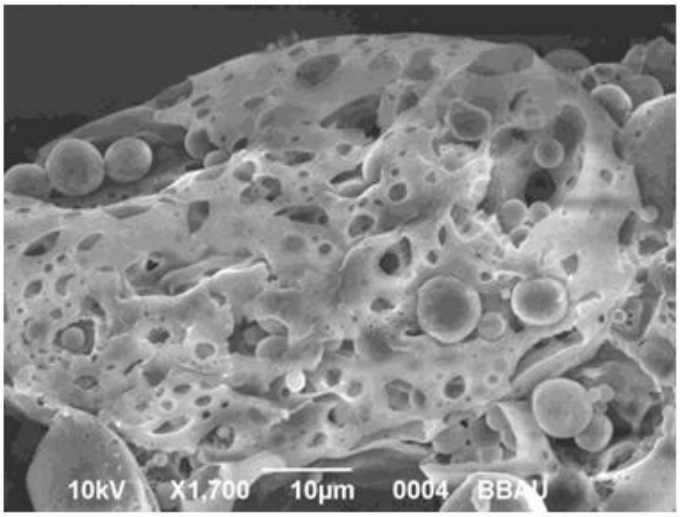

Fig 1: SEM photomicrograph of Chitosan microspheres of SLZ-6

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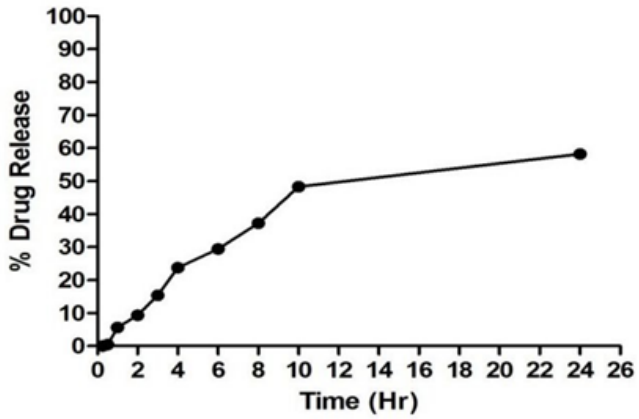

Fig 2: *In-vitro* release profile of optimized batch (SLZ-5) microspheres in simulated colonic fluid (pH 6.8)

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S. No.	Sampling interval (days) %	Residual Drug Content Mean ± S.D. (n=3)		
		4±0.5 °C	Room temp. 40±0.5 °C	75%±5 RH
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2	7	99.68±0.12	99.76±0.20	99.16± 0.15
3	15	99.45±0.06	98.92±0.08	98.45± 0.16
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Microspheres loaded SLZ have been prepared by simple emulsification method followed by cross-linking method. The variables such as drug: polymer ratio, stirring speed and concentration of glutraldehyde were optimized on the basis of particle size, entrapment efficiency. The prepared microspheres were stable, spherical particles and exhibited favorable release profiles in simulated colonic fluid. 58 However, further evaluation of these carriers can be performed for their potential to treat colonic diseases, as a future scope.

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Research Article

Formulation And Evaluation of Mucoadhesive Buccal Tablets (Repaglinide) For Management of Diabetes

THEJOVATHI B[1]
[1]LNCT University, Bhopal, MadhyaPradesh State, India.
[2]Department of Pharmaceutics, Princeton college of Pharmacy, Narapally(V), Ghatkesar, Hyderabad-500088, Telangana State, India.
Email : thejovathi.pharmaceutics@gmail.com
Received: 10.08.21, Revised: 15.11.21, Accepted: 14.01.22

ABSTRACT
Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus. The main objective of the study was to formulate and evaluate bioadhesive buccal tablets to avoid the first pass metabolism in liver. Bioadhesive buccal tablets were prepared by direct compression method using bioadhesive polymers like HPMC K4 M, HPMC K15 M, Chitosan, HPMC K100 M, Sodium CMC, Carbopol 974 P, Sodium Alginate, Gum karaya and Carbopol 941NF in different ratios. The physicochemical compatibility of drug and polymers was studied by FT-IR spectroscopy. Prepared tablets were evaluated for permeation study through porcine buccal mucosa, in vitro drug release, bioadhesion strength, swelling index, moisture absorbance, surface pH.

Keywords: Formulation, Management, Tablets.

1. INTRODUCTION

Buccal Delivery involves the administration of drug through buccal mucosal membrane (the lining in the oral cavity).[1]The drug directly reaches to the systemic circulation through the internal jugular vein and bypasses the drugs from the hepatic first pass metabolism, which leads to high bioavailability.[2] A suitable buccal drug delivery system should be flexible and should posses good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. Bioadhesive formulations have been developed to enhance the bioavailability[8,9] of drugs that undergo substantial first pass hepatic effect and to control the drug release to a constant rate.[10] In addition ,it should release the drug in a controlled and predictable manner to elicit the required therapeutic response.[11-13] Various buccal mucosal dosage forms are suggested for oral delivery which includes: buccal tablets, buccal Patches and buccal gels.[14,15]

Advantages [6,9]

- Significant reduction in dose related side effects.
- It provides direct entry of drug into systemic circulation.
- Drug degradation in harsh gastrointestinal environment can be circumvented by administering the drug via buccal route.
- Drug absorption can be terminated in case of emergency.
- It offers passive system, which does not require activation.
- Rapid cellular recovery following local stress or damage.
- Ability to withstand environmental extremes like change in pH, temperature etc. Sustained drug delivery.
- The potential for delivery of peptide molecules unsuitable for the oral route.

General criteria for candidate's drug

One of the drug properties required for the practical buccal formulation will be high pharmacological activity or a low dose requirement. The Limit size of the dosage form should not exceed 12 cm^2 for buccal application or 3cm^2 for sublingual or gingival application. The following properties will make the drug suitable candidate for buccal delivery:

- In general, any drug with a daily requirement of 25mg or less would make a good candidate
- Relatively short biological half-life:- Drugs with biological half-life 2-8 hr will in general be good candidates for sustained release dosage forms
- The maximal duration of buccal delivery is approximately 4–8 hr
- Drug must undergo first pass effect or it should have local effect in oral cavity.
- Drugs susceptible to degradation:-Drug degradation either by stomach/intestinal enzymes or by first pass hepatic metabolism

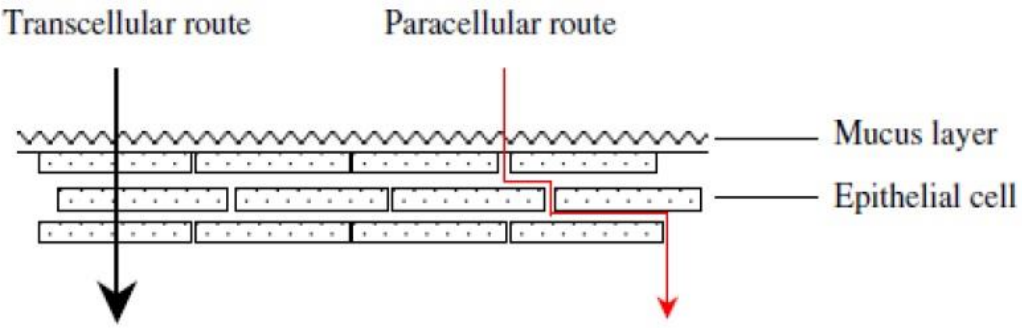
will be assured protection in buccal dosage form.

- Drug must undergo first pass effect or it should have local effect in oral cavity.

Absorption pathways

Studies with microscopically visible tracers such as small proteins and dextrans suggest that the major pathway across stratified epithelium of large molecules is via the intercellular spaces and that there is a barrier to penetration as a result of modifications to the intercellular substance in the superficial layers. However, rate of penetration varies depending on the physicochemical properties of the molecule and the type of tissue being traversed. This has led to the suggestion that materials uses one or more of the following routes simultaneously to cross the barrier region in the process of absorption, but one route is predominant over the other depending on the physicochemical properties of the diffusant.[11]

- Passive diffusion
  - Transcellular or intracellular route (crossing the cell membrane and entering the cell).
  - Paracellular or intercellular route (passing between the cells).
- Carrier mediated transport.
- Endocytosis.



Penetration routes in buccal delivery[26]

Factors Important For Bioadhesion

I . Polymer related factors

1.Molecular weight:[9] The inter penetration of polymer molecules is favorable for low molecular weight polymers where as entanglements are favored for high molecular weight polymers. The optimum molecular weight for the maximum bioadhesion depends on the type of polymers. Their nature dictates the degree of swelling in water in turn determines interpenetration of polymer molecules within the mucus. The bioadhesive force increases with the molecular weight of the bioadhesive polymer up to 100,000 and beyond this level there is not much effect.

2.Concentration of active polymer:[9,10] There is an optimum concentration of polymer corresponding to the best bioadhesion. In highly concentrated systems the adhesive strength drops significantly. In fact, in concentrated solutions the coiled molecules become solvent poor and the chains available for interpenetration are not numerous.

3.Flexibility of polymer chain:[10] As water-soluble polymers become crossed linked, the mobility of the individual polymer chain decreases. As the cross linking density increases, the effective length of the chain which can penetrate into the mucus layer decreases even further and mucoadhesive strength is reduced

4.Spatial conformation:[11] Despite a high molecular weight of 19,500,000 for dextrans, they have similar adhesive strength to that of PEG with a molecular weight of 200,000.

5.Swelling:[10,11] This characteristic is related to the polymer itself, and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Swelling depends both on polymer concentration and on presence of water. When swelling is too great, a decrease in bioadhesion occurs. Such a phenomenon must not occur too early in order to lead to a sufficient action of the bioadhesive system. Its allows easy detachment of the bioadhesive system after the discharge of the active ingredient.

II. Environment related factors

1. pH: It was found to have a significant effect on the mucoadhesion as observed in studies of polyacrylic polymers cross linked with –COOH groups. pH influences the charge on the surface of both mucus and polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups

on the carbohydrate moiety and amino acids of the polypeptide back bone.

2. **Applied strength**: To place a solid bioadhesive system, poly (Acrylic acid /Divinyl benzene) or Carbopol 934, the adhesion strength increases with the applied strength. The pressure initially applied to the mucoadhesive tissue contact site can affect the depth of interpenetration.

3. **Initial contact time**: The initial contact time between mucoadhesives and the mucus layers determines the extent of swelling and interpenetration of polymer chains. Along with internal pressure, the initial contact time can dramatically affect the performance of the system.

4. **Selection of the model substrate surface**: The handling and treatment of biological substrates during the testing of mucoadhesives in an important factor, since physical and biological changes may occur in the mucus gels or tissues under the experimental conditions.

2. MATERIALS & METHODS

Materials

HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, sodium alginate, Gum karaya, Carbopol 941NF. All other chemicals used in the study were of analytical grade which are obtained from SURA Labs Pvt Ltd.

Method

Buccal Tablet Preparation

Buccal tablets were prepared by a direct compression method, before going to direct compression all the ingredients were screened through sieve no.100. HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, Sodium alginate, Gum karaya, Carbopol 941NF are the mucoadhesive and biodegradable polymers used in this preparation of buccal mucoadhesive drug delivery systems. Repaglinide was mixed manually with different ratios of HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, sodium alginate, Gum karaya, and Microcrystalline Cellulose as diluent for 10 min. In every formulation constant amount of PVPK30 was added as binding agent. The blend was mixed with aerosil and magnesium stearate for 3-5 min. Buccal tablets were compressed by a Single punch tablet machine. Tablet weight was kept constant 100mg, and the thickness of tablet was adjusted to 2.9 mm. Tablets were stored in air tight container away from the light for further studies. The Composition of 24 formulation of Buccal Tablets are mentioned in the following Table 1 & Table 2.

Table: 1

Ingredients (mg)	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	12	16	20	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	12	16	20	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	12	16	20	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	12	16	20
Sodium CMC	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	-	-	-	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	-	-	-	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

Table: 2

Ingredients (mg)	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	-	-	-	-	-	-
Sodium CMC	12	16	20	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	12	16	20	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	12	16	20	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	12	16	20
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

3. Pre formulation Studies:

3.1. Physical Characterisation of Compressed blend: After the Blend preparation there are many formulations and process variables involved in mixing and all these can affect the characterization of blends produced. Prior to compression, granules were evaluated for their characteristic parameter such as Tapped density, Bulk density, Carr's index, Angle of repose, Hausner's ratio. For Bulk density determination 30 gm of powder blend introduced into a dry 100 mL cylinder, without compacting and powder volume was recorded. Tapped density was obtained by using tapped density apparatus----, with a fixed drop of 14±2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped 500 times initially followed by an additional tap of 750 times until difference between succeeding measurement is less than 2%. Compressibility index(carr's index), Hausner's ratio was calculated from the bulk and tapped density using the equations 1& 2

$$\text{Carr's index} = [(\rho_{tap}-\rho b)]/\rho_{tap}]\times 100 \quad (1)$$

$$\text{Hausner's Ratio} = \rho_{tap}/\ \rho b \quad (2)$$

Where, ρ_{tap} = Tapped density.

ρb = Bulk density.

3.2. Drug-excipient compatibility studies

A Fourier Transform-Infra Red spectrophotometer was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The spectrum of each sample was recorded over the 450-4000 cm^{-1}. Pure drug of Repaglinide, Repaglinide with physical mixture (excipients) compatibility studies were performed.

3.3. Analytical Quantification of Repaglinide in UV Spectroscopy

100 mg of Repaglinide was dissolved in small amount of phosphate buffers 6.8 and 7.4 seperately and make the volume up to 100 mL with phosphate buffer pH 6.8 and 7.4 in two Volumetric flasks. From this two primary stock (1mg/mL), 10 mL solution was transferred to another volumetric flask 1 made up to 100 mL with Phosphate buffer pH 6.8 and in another flask 2 with Phosphate buffer pH 7.4. From this secondary stock of two flasks 0.4 ,0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 mL was taken separately and made up to 10 mL with phosphate buffer pH 6.8 and Phosphate buffer pH 7.4 to produce 4, 8, 12,16, 18, 20, 24, 28 μg/mL respectively. The absorbance was measured at 282 nm using a UV spectrophotometer.

3.4. Solubility Studies

The solubility of Repaglinide in phosphate buffer solution pH 6.8 was determined by phase equilibrium method. An excess amount of drug was taken into 20 mL vials containing 10 mL of phosphate buffers (pH 6.8). Vials were closed with rubber caps and constantly agitated at room temperature for 24 hr using rotary shaker. After 24 hr, the solution was filtered through 0.2 μm Whitman's filter paper. The amount of drug

solubilized was then estimated by measuring the absorbance at 282 nm using a UV spectrophotometer. The standard curves for Repaglinide were established in phosphate buffer (pH 6.8) and from the slope of the straight line the solubility of Repaglinide was calculated. The studies were repeated in triplicate (n = 3), and mean was calculated.

4. Evaluation of Buccal Tablets

4.1. Physicochemical characterization of tablets: The prepared Repaglinide buccal tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability and drug content(assay).

4.2. Weight variation: The weight variation test is done by taking 20 tablets randomly and weighed accurately. The composite weight divided by 20 provides an average weight of tablet. Not more than two of the individual weight deviates from the average weight by 10 %. The percent deviation was calculated using the following formula:

% Deviation = (Individual weight – Average weight / Average weight) X 100

4.3. Tablet Thickness: The thickness and diameter of the tablets was determined using a Digital Vernier caliper. Ten tablets from each formulation were used and average values were calculated.

4.4. Tablet Hardness: Six tablets were taken from each formulation and hardness was determined using Monsanto hardness tester and the average was calculated. It is expressed in Kg/cm2.

4.4. Friability: A sample of preweighed tablets was placed in Roche friabilator which was then operated for 100 revolutions at a speed of 25 rpm for 4 minutes, dropping the tablets to a distance of 6 inches in each revolution. Percent friability (% F) was calculated as

$$\text{Friability (\%)} = \frac{\text{Initial weight of 10 tablets} - \text{final weight of 10 tablets}}{\text{Initial weight of 10 tablets}} \times 100$$

$$F\ (\%) = [W_o - W/W_O] \times 100$$

Where, W_0 is the initial weight of the tablets before the test and

W is the final weight of the tablets after test.

4.4. Assay: Six tablets of each formulation were taken and amount of drug present in each tablet was determined. Powder equivalent to one tablet was taken and added in 100 mL of pH 6.8 phosphate buffer followed by stirring for 10 minutes. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured by using UV-Visible spectrophotometer at 282 nm using pH 6.8 phosphate buffer.

5. In vitro release studies

The drug release rate from buccal tablets was studied using the USP type II dissolution test apparatus. Tablets were supposed to release the drug from one side only; therefore an impermeable backing membrane was placed on the other side of the tablet. The tablet was further fixed to a 2x2 cm glass slide with a solution of cyanoacrylate adhesive. Then it was placed in the dissolution apparatus. The dissolution medium was 500 mL of pH 6.8 phosphate buffer at 50 rpm at a temperature of 37 ± 0.5 °C. Samples of 5 mL were collected at different time intervals up to 8 hrs and analyzed after appropriate dilution by using UV Spectrophotometer at 282 nm.

6. Kinetic Analysis of Dissolution Data: [14,15]

To analyze the in vitro release data various kinetic models were used to describe the release kinetics.

1. Zero – order kinetic model – Cumulative % drug released versus time.

$$A_t = A_0 - K_0 t$$

Where, A_t = Drug release at time't'.

A_0 = Initial drug concentration

K_0 = Zero – order rate constant (hr^{-1}).

2. First – order kinetic model – Log cumulative percent drug remaining versus time.

$$\text{Log } C = \log C0 - Kt / 2.303$$

Where, C = Amount of drug remained at time't'.

C0 = Initial amount of drug.

K = First – order rate constant (hr-1).

3. Higuchi's model – Cumulative percent drug released versus square root of time.

$$Q = [D\varepsilon / \tau\ (2A - \varepsilon Cs)\ Cst]^{1/2}$$

Where, Q = Amount of drug released at time't'.

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = the solubility of the drug in the matrix.

ε = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs) at which 'q' amount of drug is released.

4. Korsmeyer equation / Peppa's model – Log cumulative % drug released versus log time.

$$M_t / M_a = Kt^n$$

Where, M_t / M_a = the fraction of drug released at time't'.

K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

7. Swelling Studies

Buccal tablets were weighed individually (designated as W_1) and placed separately in Petri

dishes containing 15 mL of phosphate buffer (pH 6.8) solution. At regular intervals (0.5, 1, 2, 3, 4, 5 and 6 hr), the buccal tablets were removed from the Petri dishes and excess surface water was removed carefully using the filter paper. The swollen tablets were then reweighed (W_2). This experiment was performed in triplicate. The swelling index (water uptake) calculated according to the following Eq.

Swelling index = $\frac{(W_2-W_1)}{} \times 100$

8. In vitro bioadhesion strength

Bioadhesion strength of tablets were evaluated using a microprocessor based on advanced force gauge equipped with a motorized test stand (Ultra Test Tensile strength tester, Mecmesin, West Sussex, UK) according to method describe as it is fitted with 25 kg load cell, in this test porcine membrane was secured tightly to a circular stainless steel adaptor and the buccal tablet to be tested was adhered to another cylindrical stainless steel adaptor similar in diameter using a cyanoacrylate bioadhesive. Mucin 100 μl of 1 % w/v solution was spread over the surface of the buccal mucosa and the tablet immediately brought in contact with the mucosa. At the end of the contact time, upper support was withdrawn at 0.5mm/sec until the tablet was completely detached from the mucosa. The work of adhesion was determined from the area under the force distance curve.

The peak detachment force was maximum force to detach the tablet from the mucosa.

$$\text{Force of adhesion} = \frac{\text{Bio adhesion strength}}{1000} \times 9.8$$

$$\text{Bond strength} = \frac{\text{Force of adhesion}}{\text{surface area}}$$

9. Surface pH

Weighed tablets were placed in boiling tubes and allowed to swell in contact with pH 6.8 phosphate buffer (12 mL). Thereafter, surface pH measurements at predetermined intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h were recorded with the aid of a digital pH meter. These measurements were conducted by bringing a pH electrode near the surface of the tablets and allowing it to equilibrate for 1 min prior to recording the readings. Experiments were performed in triplicate (n=3).

10. Moisture absorption

Agar (5% m/V) was dissolved in hot water. It was transferred into Petri dishes and allowed to solidify. Six buccal tablets from each formulation were placed in a vacuum oven overnight prior to the study to remove moisture, if any, and laminated on one side with a water impermeable backing membrane. They were then placed on the surface of the agar and incubated at 37 °C for one hour. Then the tablets were removed and weighed and the percentage of moisture absorption was calculated by using following formula:

$$\% \text{ Moisture Absorption} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

RESULTS & DISCUSSION

Preformulation study

FTIR Compatibility Studies: FTIR spectra of pure drug and formulation with other ingredients were recorded. The spectrum of each sample was recorded over the 450-4000 cm^{-1}. There was no appearance or disappearance of any characteristics peak in the FTIR spectrum of drug and the polymers used. This shows that there is no chemical interaction between the drug and the polymers used. The FTIR Spectra of pure Repaglinide drug and polymer was compared with the FTIR spectrum of drug and optimised in the Figures 1,2.

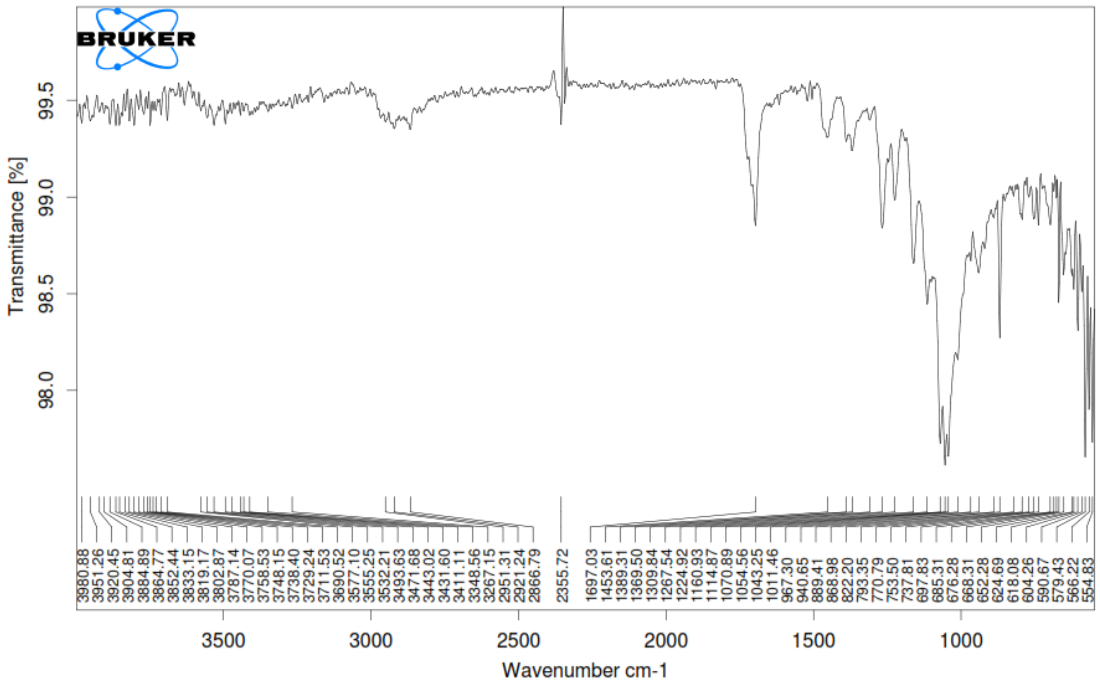


Fig 1: FTIR studies of pure drug Repaglinide

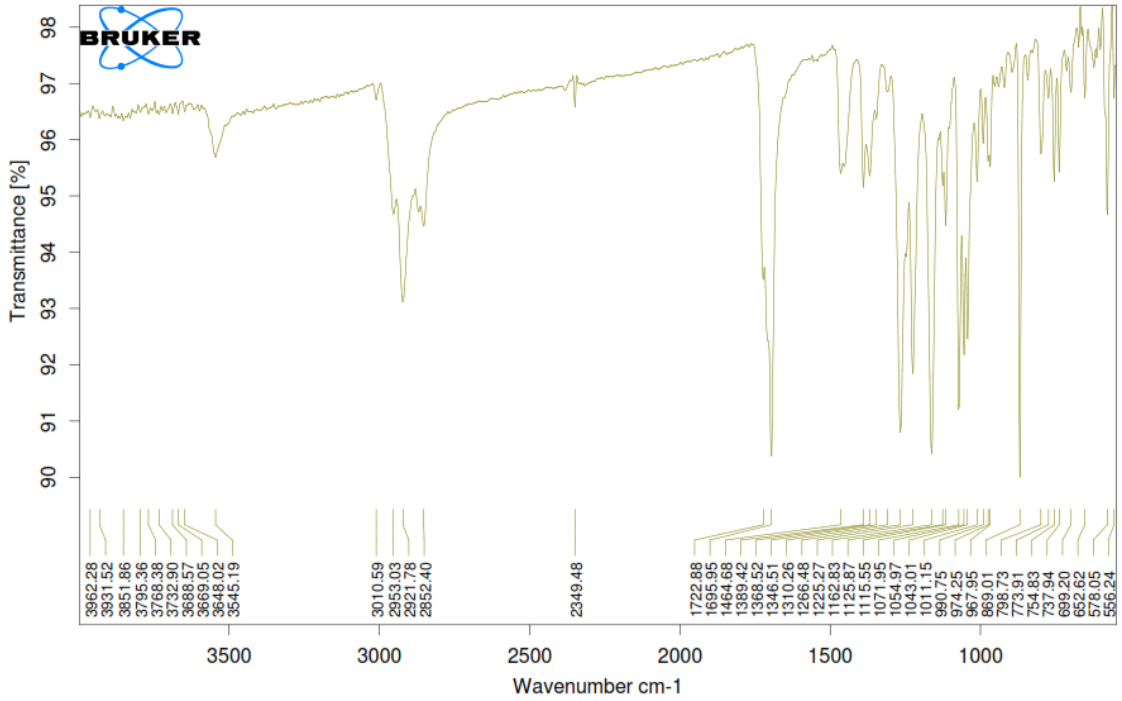


Fig 2: FTIR compatibility studies of optimized formulation

Solubility studies

The solubility of the Repaglinide in phosphate buffer pH 6.8 is 88 μg/mL and it was selected as the suitable media for the release studies because the pH of the phosphate buffer pH 6.8 is nearer to that of buccal mucosa pH. The results revealed that the solubility of the Repaglinide was increased from pH 6.8 to 7.4. The studies were shown in the Table3.

Table 3: Solubility studies of Repaglinide

Medium	Amount present (µg/mL)
Distilled water	22.59
Phosphate buffer pH 6.8	88
Phosphate buffer pH 7.4	94

Standard graph in phosphate buffer pH 6.8 (λ_{max} 282 nm): The standard graph of Repaglinide showed good linearity with R^2 of 0.999, which indicates that it obeys "Beer- Lamberts" law in the range 0-28 µg/mL. Its linearity is shown in Table 4 and Figure 3.

Table 4: Standard graph of Repaglinide in phosphate buffer pH 6.8

Concentration (µg/mL)	Absorbance
0	0
4	0.102
8	0.198
12	0.275
16	0.365
20	0.444
24	0.532
28	0.622

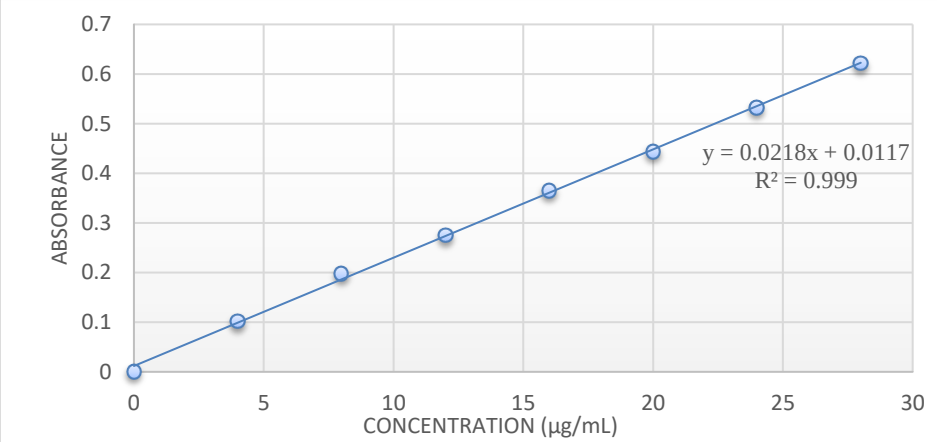


Figure 3

Characterization of Precompression Blend

The precompression blend for Buccal tablets were characterized with respect to Angle of repose was less than 33.65°, Carr's index values were less than 19.39 and Hausner's ratio was less than 1.24 for all formulations indicating good to fair flowability, compressibility and flow property shown in Table 5.

Table 5: Physical Properties of Precompression Blend

Formulation Code	Angle of repose (θ)	Bulk density (g/mL)	Tapped density(g/mL)	Carr's index (%)	Hausner's ratio
RF1	25.49	0.214	0.251	14.74	1.17
RF2	26.24	0.308	0.364	15.38	1.18
RF3	29.05	0.276	0.322	14.28	1.16
RF4	26.97	0.341	0.388	12.11	1.13
RF5	29.25	0.324	0.376	13.82	1.16
RF6	32.27	0.320	0.397	19.39	1.24
RF7	33.65	0.521	0.629	17.17	1.20
RF8	33.21	0.518	0.627	17.38	1.21
RF9	26.56	0.422	0.506	16.60	1.19

RF10	28.75	0.481	0.572	15.90	1.18
RF11	27.33	0.475	0.566	16.07	1.19
RF12	25.38	0.524	0.599	12.52	1.14
RF13	26.43	0.412	0.483	14.69	1.17
RF14	24.77	0.488	0.537	9.12	1.10
RF15	26.42	0.439	0.521	15.73	1.18
RF16	28.19	0.559	0.649	13.94	1.16
RF17	29.58	0.331	0.393	15.77	1.18
RF18	28.73	0.362	0.428	15.42	1.18
RF19	30.45	0.386	0.473	18.39	1.22
RF20	26.43	0.375	0.442	15.15	1.17
RF21	19.29	0.434	0.497	12.67	1.14
RF22	21.25	0.520	0.582	10.65	1.11
RF23	26.27	0.487	0.561	13.19	1.15
RF24	25.49	0.494	0.566	12.72	1.14

Each value represents the mean value (n =3).

Physicochemical Characterization of Buccal Tablets

Acceptable physicochemical properties were observed for the prepared buccal tablets all the formulated tablets passed the weight variation test within the limits as per USP, thickness varied from 2.00 to 2.98, hardness compression force applied (5.0 – 6.2 kg/cm^2), friability was below 1%, assay values in the range of 97.42 to 100.14 of all compressed tablets were within the limits as per USP shown in Table 6.

Table 6: Physico-chemical parameters of Repaglinide buccal tablets

Formulation Code	Weight variation (mg)	Thickness (mm)	Hardness (kg/cm$^{2)}$	Friability (%)	Assay (%)
RF1	100.12	2.61	5.1	0.62	98.61
RF2	99.86	2.14	5.9	0.54	99.74
RF3	100.25	2.81	6.0	0.38	98.32
RF4	98.68	2.36	5.6	0.49	99.64
RF5	100.21	2.80	6.2	0.51	99.56
RF6	99.86	2.91	5.4	0.37	97.42
RF7	99.57	2.05	5.3	0.61	98.61
RF8	100.08	2.34	6.1	0.59	99.82
RF9	100.31	2.62	5.9	0.34	99.24
RF10	99.87	2.19	5.7	0.52	98.46
RF11	97.59	2.00	6.1	0.49	99.29
RF12	99.38	2.78	5.4	0.63	99.52
RF13	100.25	2.81	5.3	0.71	98.68
RF14	100.47	2.54	5.5	0.65	99.87
RF15	99.34	2.98	5.6	0.48	98.46
RF16	100.09	2.33	5.8	0.59	99.81
RF17	99.37	2.50	6.1	0.39	99.37
RF18	98.65	2.18	5.7	0.51	100.14
RF19	97.34	2.51	5.3	0.48	99.52
RF20	100.19	2.64	5.0	0.28	98.64
RF21	100.02	2.08	6.2	0.34	100.01
RF22	99.89	2.11	5.4	0.29	99.64
RF23	99.72	2.34	5.8	0.41	98.37
RF24	100.05	2.52	5.9	0.62	99.81

Each value represents the mean value (n =3).

In vitro drug release studies : In vitro drug release studies revealed that the release of Repaglinide from different formulations varied according to the type and ratios of the matrix forming polymers.From the dissolution studies, it was observed that as the concentration of repaglinide increases drug release was prolonged indicating the drug release retarding ability of the HPMC K100M. Formulation RF11 containing drug to HPMC K100M in the ratio of 1:8 and extended the drug release up to 6 h and was considered as preliminary optimized formulation (Table7 to 14) & (Figure 4 to 9).

Table 7. Repaglinide formulations with HPMC K4M

Time (hrs)	Cumulative percentage drug release		
	RF1	RF2	RF3
0	0	0	0
1	51.35	43.64	31.85
2	68.18	53.25	42.15
3	76.92	62.98	57.62
4	85.06	68.16	61.22
5	90.15	78.50	67.85
6	93.73	80.14	72.98

Table 8. Repaglinide formulations with HPMCK15M

Time (hrs)	Cumulative percentage drug release		
	RF4	RF5	RF6
0	0	0	0
1	41.12	47.35	44.36
2	52.81	59.19	56.91
3	61.63	65.02	62.89
4	69.19	79.56	70.05
5	75.06	82.18	76.20
6	85.14	96.36	94.64

Table 9. Formulations with HPMC K100 M.

Time (hrs)	Cumulative percentage drug release		
	RF7	RF8	RF9
0	0	0	0
1	38.68	46.35	55.05
2	46.55	52.19	64.56
3	58.62	65.56	73.25
4	62.18	73.29	83.53
5	65.72	79.86	89.50
6	72.35	83.21	98.42

Table 10. Formulations with Chitosan.

Time (hrs)	Cumulative percentage drug release		
	RF10	RF11	RF12
0	0	0	0
1	33.83	52.15	31.97
2	48.16	61.83	45.56
3	56.93	74.19	53.82
4	60.18	84.43	65.19
5	66.08	96.19	77.34
6	71.11	99.43	82.97

Table 11. Formulations with Sodium CMC

Time (hrs)	Cumulative percentage drug release		
	RF13	RF14	RF15
0	0	0	0
1	53.18	41.81	31.92
2	63.34	52.96	44.75
3	74.19	60.47	50.29
4	80.62	67.36	56.09
5	86.36	72.97	67.79
6	96.17	81.83	73.34

Table 12. Formulations with Carbopol974P

Time (hrs)	Cumulative percentage drug release		
	RF16	RF17	RF18
0	0	0	0
1	30.96	42.62	53.39
2	46.14	48.92	64.14
3	51.83	53.06	73.81
4	57.23	62.53	81.08
5	64.09	73.85	87.43
6	71.18	81.32	95.27

Table 13. Formulations with Sodium alginate

Time (hrs)	Cumulative percentage drug release		
	RF19	RF20	RF21
0	0	0	0
1	38.89	55.31	45.91
2	45.17	60.85	62.03
3	50.36	75.96	68.75
4	56.89	82.36	75.19
5	61.26	87.19	92.43
6	64.19	97.54	95.79

Table 14. Formulations with Gum Karaya

Time (hrs)	Cumulative percentage drug release		
	RF22	RF23	RF24
0	0	0	0
1	50.38	39.62	32.46
2	68.17	52.92	44.11
3	74.63	58.06	53.38
4	80.27	63.53	56.19
5	86.88	70.85	62.76
6	92.09	82.32	70.39

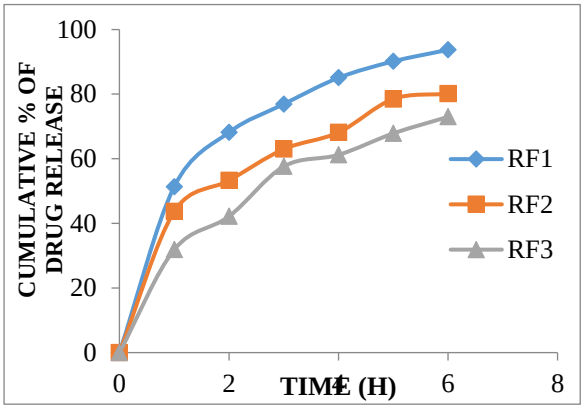


Figure 4

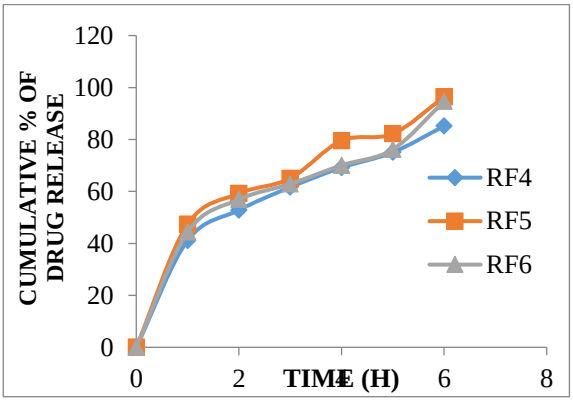


Figure 5

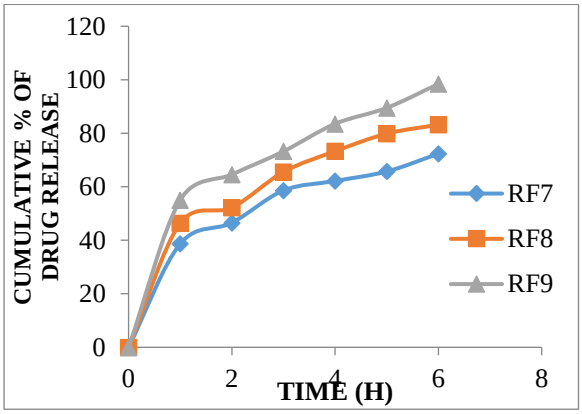


Figure 6

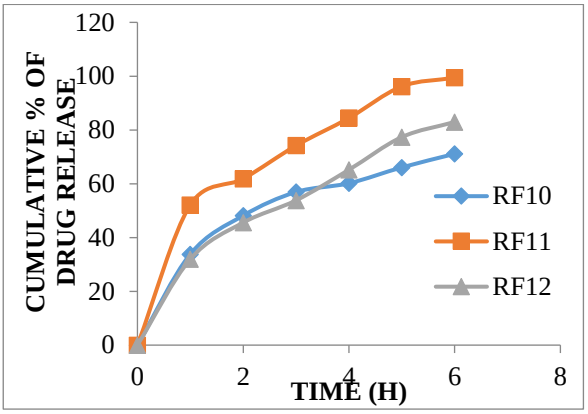


Figure 7

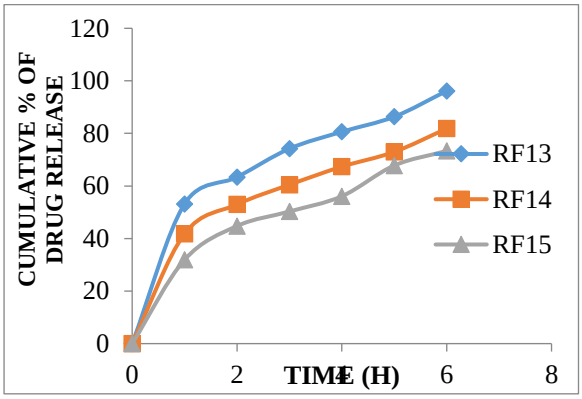


Figure 8

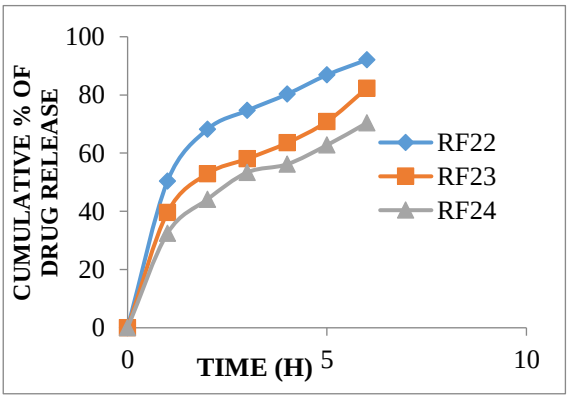


Figure 9

Surface pH Study: The surface pH of the formulations was found to be 6.0 to 6.9 and the pH was near to the neutral. These results suggested that the polymeric blend identified was suitable for oral application and formulations were not irritant to the buccal mucosa. Surface pH values for all the formulations shown in Table-----

Moisture absorption test: Moisture absorption of the mucoadhesive buccal tablets is in the range of 42.1% to 86.9%, which shows that the tablets have suitable moisture absorption capacity. The highest moisture absorption of formulation was RF11.

Ex vivo bioadhesive strength measurement: The results revealed that the carbopol 974 P containing formulations showed better residence time than the other polymer formulations shown in Table.

Ex vivo bioadhesive strength measurement: From the results, finally It was concluded that, the

more bioadhesive strength of formulation was RF 11. Hence the formulation with optimized bioadhesive strength should be chosen i.e. formulation containing HPMC K100M shown in Table 14.

Table 15: Ex vivo residence time, Moisture absorption, Surface pH, Bioadhesive strength values of Repaglinide buccal tablets.

Formulation code	Ex vivo residence time	Moisture absorbance	Surface pH	Bioadhesive strength	
				Peak detachment force (N)	Work of adhesion (mJ)
RF1	4Hrs 32 min	56.3	6.2	1.28	0.52
RF2	4Hrs 45 min	62.9	6.8	2.62	0.42
RF3	5Hrs 10 min	71.2	6.1	2.38	0.36
RF4	4Hrs 45 min	43.1	6.0	2.10	0.71
RF5	4Hrs 58 min	50.6	6.5	2.54	0.39
RF6	5Hrs 25 min	68.2	6.9	2.67	0.54
RF7	4Hrs 18 min	65.7	8.5	1.36	0.38
RF8	4Hrs 36 min	70.5	8.6	2.01	0.49
RF9	4Hrs 42 min	72.6	8.5	2.36	0.54
RF10	5Hrs 30 min	76.1	6.3	2.16	0.62
RF11	6Hrs 49 min	80.0	6.5	2.99	0.89
RF12	7Hrs 10 min	86.9	6.4	2.51	0.19
RF13	5Hrs 35 min	46.6	6.9	1.97	0.27
RF14	5Hrs 48 min	52.1	6.2	2.64	0.39
RF15	5Hrs 53 min	61.8	6.4	2.82	0.30
RF16	8Hrs 48 min	44.6	6.3	2.71	0.42
RF17	9 Hrs 23 min	42.1	6.2	2.61	0.40
RF18	9 Hrs 53 min	53.6	6.0	3.30	1.24
RF19	4Hrs 43 min	56.1	6.1	2.41	0.31
RF20	4Hrs 59 min	62.8	6.8	1.66	0.38
RF21	5Hrs 50 min	68.5	6.3	1.89	0.20
RF22	5Hrs 12 min	46.4	6.4	1.91	0.35
RF23	5Hrs 26 min	50.1	6.9	2.15	0.42
RF24	5Hrs 41 min	53.9	6.2	2.45	0.58

Each value represents the mean value (n =3)

Swelling Studies of buccal tablets: Therefore, formulations containing HPMC K 100M showed higher swelling index values (higher water uptake) of all the formulations were given in (Table 15 & 16). Swelling behavior of buccal tablets of all formulations as a function of time is shown in Figures 10 to 17.

Table 16: Swelling studies of buccal tablets

Time (hr)	% Swelling index											
	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	38.63	31.91	28.36	21.23	32.46	28.82	30.90	42.92	58.83	29.19	42.46	35.82
2	48.97	42.65	35.73	38.60	42.20	40.49	45.93	59.31	66.39	38.53	57.20	51.49
3	56.16	54.53	44.15	46.01	55.85	46.96	55.56	62.05	72.2	42.16	65.34	58.96
4	68.41	67.92	58.86	56.83	65.41	56.46	63.12	72.96	81.16	56.73	74.12	63.49
5	83.69	75.90	62.21	64.07	77.86	62.32	67.94	87.56	89.42	63.98	85.59	78.35
6	92.79	86.19	75.36	76.29	91.59	86.79	72.26	93.72	97.73	76.46	98.53	86.72

Each value represents the mean value (n =3)

Table 17: Swelling studies of buccal tablets

Time (hr)	% Swelling index											
	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
0	0	0	0	0	0	0	0	0	0	0	0	0
1	45.19	31.82	28.19	28.59	33.92	48.83	51.09	55.53	58.96	56.74	45.02	36.20
2	57.56	45.49	35.82	39.96	40.31	56.03	62.56	66.90	61.17	65.90	52.26	49.12
3	68.15	50.96	42.54	45.34	56.99	68.99	67.85	73.14	70.45	79.35	65.62	51.52
4	78.30	66.46	56.19	65.71	72.25	76.34	73.42	81.64	78.24	84.39	71.75	62.64
5	87.84	72.39	68.06	76.43	83.06	87.92	81.63	89.76	83.41	87.01	77.26	65.91
6	96.93	84.72	75.96	82.35	93.13	95.67	85.74	94.53	90.63	96.34	81.79	72.20

Each value represents the mean value (n =3)

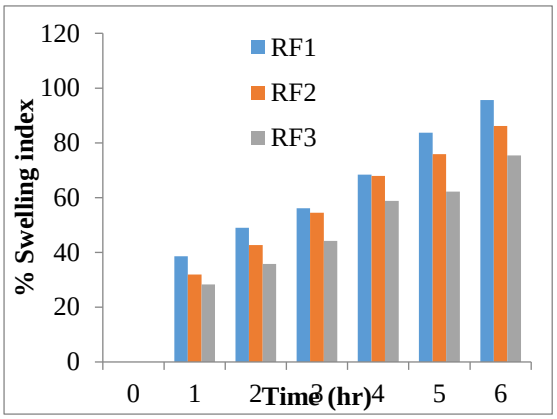


Figure 10

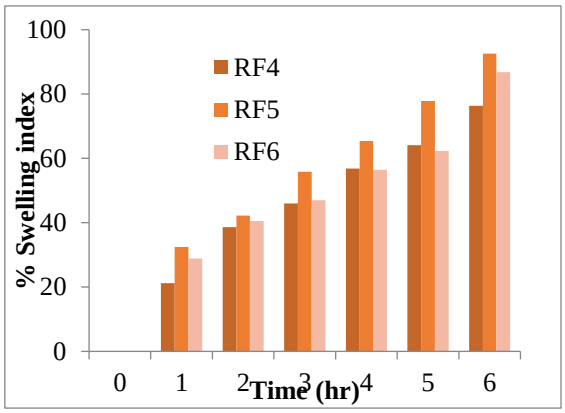


Figure 11

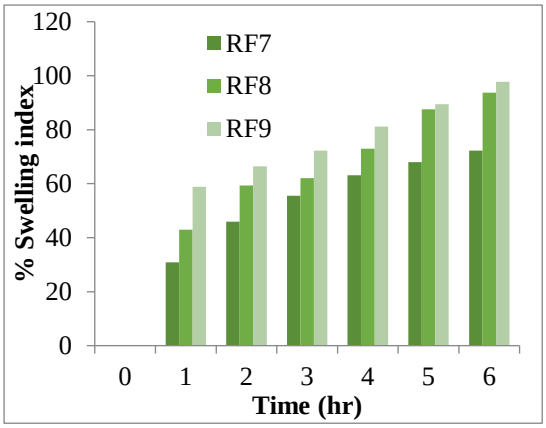


Figure 12

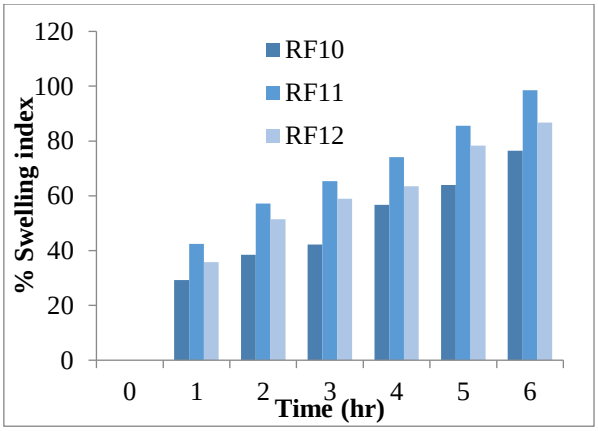


Figure 13

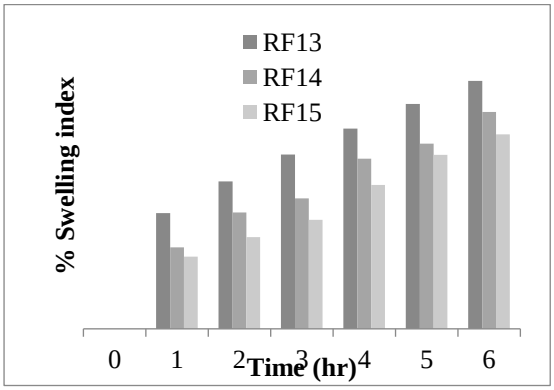


Figure14

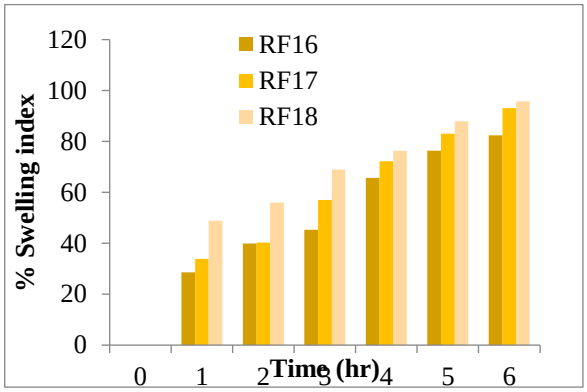


Figure 15

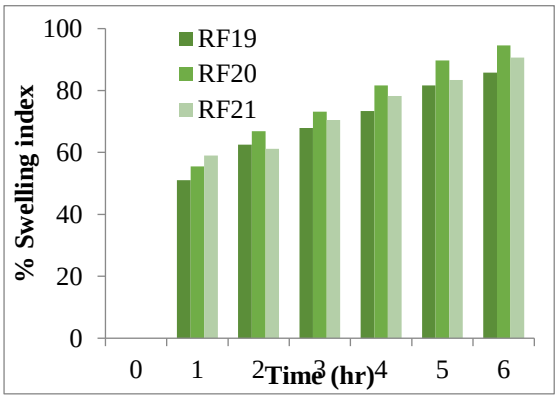


Figure 16

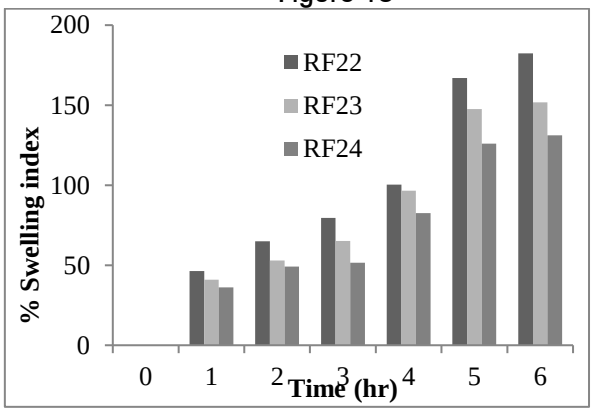


Figure 17

Ex vivo permeation studies of Repaglinide buccal tablet: The formulations containing HPMC K4M (RF1), HPMC K 100M (RF11) and Carbopol 974 P (RF18) showed highest flux at 6th hr i.e., 0.488 mg $hr^{-1}cm^{-2}$, 0.522 mg $hr^{-1}cm^{-2}$ and 0.487 mg $hr^{-1}cm^{-2}$ respectively shown in Table 17, 18.

Table 18: % Drug permeation of Repaglinide Ex vivo permeated buccal tablets

Time (hr)	Cumulative amount of Repaglinide through buccal mucosa (mg)											
	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.55	0.41	0.36	0.42	0.54	0.43	0.21	0.35	0.53	0.25	0.59	0.43
2	0.68	0.56	0.43	0.56	0.67	0.57	0.38	0.46	0.64	0.43	0.68	0.55
3	1.13	0.98	0.81	0.97	0.84	0.64	0.46	0.53	0.75	0.61	0.76	0.68
4	1.61	1.25	1.12	1.14	1.10	0.83	0.81	0.93	1.24	0.86	1.21	0.93
5	1.72	1.32	1.24	1.36	1.19	0.91	0.99	1.24	1.36	0.93	1.55	1.19
6	1.86	1.65	1.36	1.42	1.26	1.12	1.03	1.39	1.53	1.10	1.99	1.53
*Flux	0.488	0.433	0.356	0.372	0.330	0.293	0.270	0.364	0.401	0.288	0.522	0.401

Each value represents the mean value (n =3) **indicates units for flux: mg $hr^{-1}cm^{-2}$**

Table 19: % Drug permeation of Repaglinide Ex vivo permeated buccal tablets

Time (hr)	Cumulative amount of Repaglinide through buccal mucosa (mg)											
	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.39	0.32	0.23	0.19	0.29	0.39	0.18	0.39	0.35	0.46	0.35	0.26
2	0.56	0.49	0.34	0.26	0.38	0.54	0.22	0.42	0.40	0.58	0.46	0.31
3	0.89	0.73	0.52	0.37	0.52	0.86	0.33	0.58	0.51	0.69	0.57	0.43
4	0.99	0.86	0.61	0.89	0.93	1.57	0.46	0.72	0.64	0.86	0.65	0.51
5	1.65	1.24	1.12	0.92	1.21	1.73	0.62	1.26	1.12	1.35	1.12	0.89
6	1.78	1.30	1.27	0.99	1.82	1.86	0.76	1.54	1.39	1.65	1.36	0.98
*Flux	**0.466**	**0.340**	**0.332**	**0.259**	**0.477**	**0.487**	**0.199**	**0.403**	**0.364**	**0.432**	**0.356**	**0.256**

Each value represents the mean value (n =3)

Release Kinetics: The optimized formulation such as HPMC K100 M (RF11) follows Zero order and Higuchi order of release kinetics governed by Fickian diffusion mechanism shown in Table 19,20 and in Figure 18-22.

Table 20: Release kinetics and correlation coefficients

Formulation code	Mathematical models (Release kinetics)					
	Zero order	First order	Higuchi	Hixson-Crowell Kinetics	Korsemeyer – peppas	
	R^2	R^2	R^2	R^2	R^2	n
RF11	0.904	0.799	0.992	0.926	0.983	0.449

Table 21: RF11 Release kinetics

Cumulative (%) Release Q	Time (T)	Root (T)	Log(%) Release	Log (T)	Log (%) Remain	Release Rate (Cumulative % Release / T)	1/Cum% Release	Peppas Log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
42.46	1	1.000	1.628	0.000	1.760	42.460	0.0236	-0.372	57.54	4.642	3.861	0.781
57.20	2	1.414	1.757	0.301	1.631	28.600	0.0175	-0.243	42.8	4.642	3.498	1.144
65.34	3	1.732	1.815	0.477	1.540	21.780	0.0153	-0.185	34.66	4.642	3.260	1.381
74.12	4	2.000	1.870	0.602	1.413	18.530	0.0135	-0.130	25.88	4.642	2.958	1.684
85.59	5	2.236	1.932	0.699	1.159	17.118	0.0117	-0.068	14.41	4.642	2.433	2.208

98.53	6	2.449	1.994	0.778	0.167	16.422	0.0101	-0.006	1.47	4.642	1.137	3.505

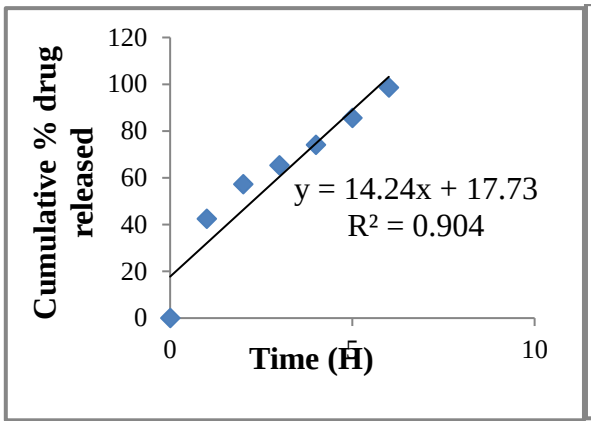


Figure 18: Zero order release kinetics

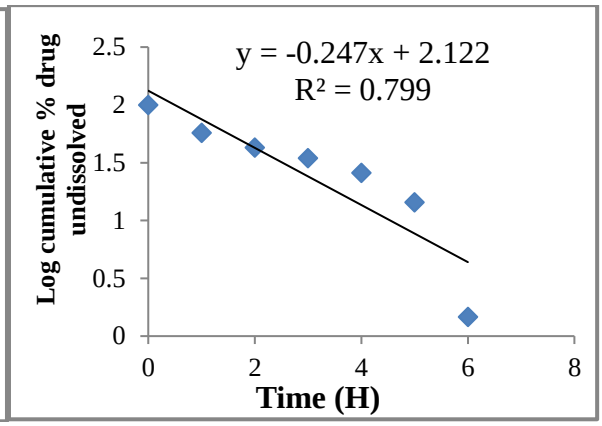


Figure 19: First order release kinetics

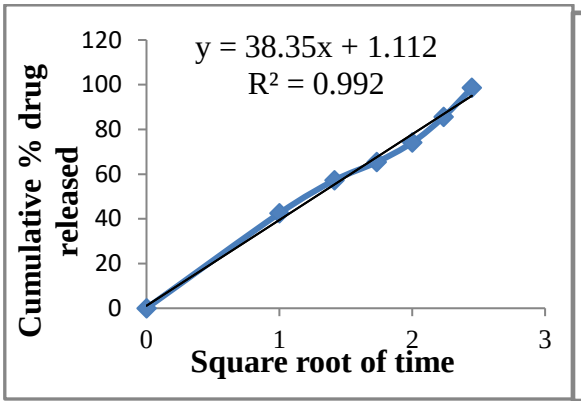


Figure 20: Higuchi order release kinetics

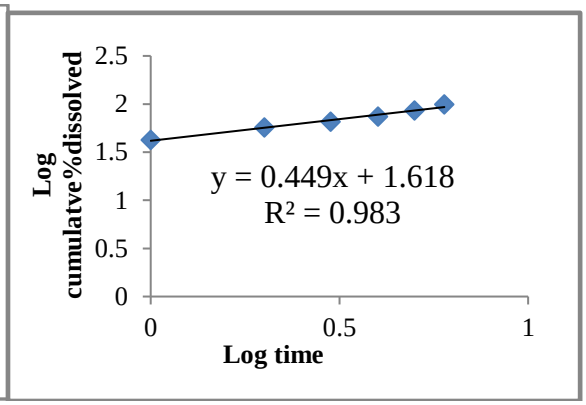


Figure 21: Peppas release kinetics

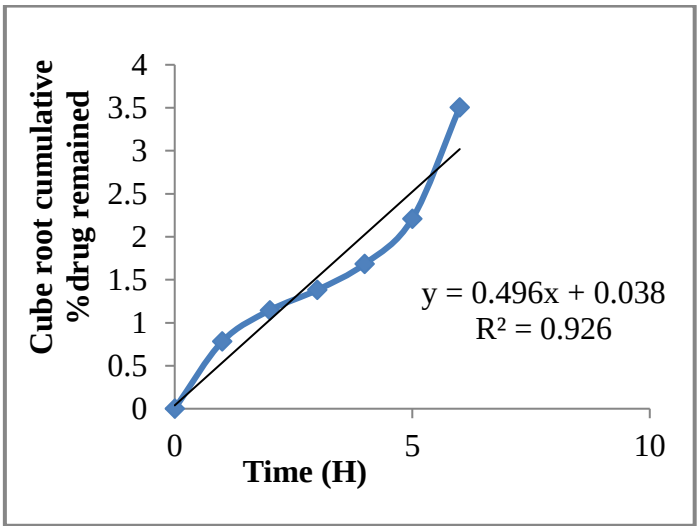


Figure 22: Hixson-Crowell Kinetics

CONCLUSION

Among the prepared formulations, the formulation containing HPMC K 100M (RF11) was found to be best formulation which showed the higher flux 0.522 mg $hr^{-1}cm^{-2}$ than the pure drug solution (0.288 mg $hr^{-1}cm^{-2}$), and bioadhesive strength of 2.99 N (peak detachment force) and 0.89 mJ (work of adhesion). From the results of this study, it may be concluded that the combination of HPMC K 100M and Carbopol 974P polymers are suitable for developing bio adhesive buccal tablets of Repaglinide.

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Research Article

Stability And Invivo Studies Of Mucoadhesive Buccal Tablets (Repaglinide) For Management Of Diabetes

THEJOVATHI B[1*], AKHILESH K SINGHAI[2]
[1,2]Department of Pharmaceutis, School of pharmacy, LNCT University, J.K Town, Kolar Road, Bhopal (M.P)-462 042
*Corresponding Author
Email: thejovathi.pharmaceutics@gmail.com
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ABSTRACT
Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus. The main objective of the study was to formulate and evaluate bioadhesive buccal tablets to avoid the first pass metabolism in liver. Bioadhesive buccal tablets were prepared by direct compression method using bioadhesive polymers like HPMC K4 M, HPMC K15 M, Chitosan, HPMC K100 M, Sodium CMC, Carbopol 974 P, Sodium Alginate, Gum karaya and Carbopol 941NF in different ratios. *In vivo* mucoadhesive behavior of optimized formulation was performed in New Zealand rabbits and pharmacokinetic parameters were evaluated. The Stability studies were carried out for best formulation

Keywords: Repaglinide, buccal tablet, Stability studies, *In vivo* permeation.

INTRODUCTION

Among the various routes of drug delivery, oral route is the most suitable and most widely accepted one by the patients for the delivery of the therapeutically active drugs. But after oral drug administration many drugs are subjected to presystemic clearance in liver, which often leads to a lack of correlation between membrane permeability, absorption and bioavailability.[1-5] Within the oral route, the Buccal cavity is an attractive site for drug delivery due to ease of administration and avoids possible drug degradation in the gastrointesinal tract as well as first pass hepatic metabolism.[6]

The primary objectives of mucoadhesive dosage forms[7]are to provide intimate contact of the dosage form with the absorbing surface and to increase the residence time of the dosage form at the absorbing surface to prolong drug action. Due to mucoadhesion, certain water-soluble polymers become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body including the buccal mucosa, gastrointestinal tract, the urogential tract, the airways, the ear, nose and eye. It represent potential sites for attachment of any mucoadhesive system and hence, the mucoadhesive drug delivery system includes

- Buccal delivery system
- Gastrointestinal delivery system
- Nasal delivery system
- Ocular delivery system
- Vaginal delivery system
- Rectal delivery system

Buccal Delivery System[7]

The unique environment of the oral cavity offers its potential as a site for drug delivery. Because of the rich blood supply and direct access to systemic circulation, the oral mucosal route is suitable for drugs, which are susceptible to acid hydrolysis in the stomach or which are extentensively metabolized in the liver (first pass effect).

Delivery through Sublingual Mucosa

Sublingual delivery traditionally involves systemic administration of drug via membranes of the floor of the mouth or the ventral surfaces of the tongue. The sublingual mucosa is relatively permeable due to thin membrane and large veins, hence allows rapid absorption and acceptable bioavailability of many drugs. Moreover, the sublingual mucosa is a smooth surface and, free of mucous and undigested food, therefore, it is conveniently accessible for application of dosage forms.

Local Delivery to Mouth[8]

Local delivery to mouth includes any system that is applied to the oral mucous membrane in order to treat conditioning of the mouth such as periodontal diseases, gingivitis, oral candidisis and other chronic lesions or topical fungal infections. Traditional methods of delivery to the diseased site include chewing gums, mouthwashes, ointments and gels.

Gastrointestinal drug delivery system[7-8]

The ideal of mucoadhesive began with the clear need to localize a drug at certain sites in the GIT. Therefore, a primary objective of using mucoadhesive system orally would be achieved by obtaining a substantial increase in residence time of the drug for local drug effect and to permit one daily dosing.

Nasal drug delivery system[9]

The nasal mucosa provides a potentially good route for systemic drug delivery. With a surface area of 150 cm^2, a highly dense vascular network, and relatively permeable membrane structure, the nasal route has good absorption potential. One of the most important features of the nasal route is that it avoids first-pass hepatic metabolism, thereby reducing metabolism.

Ocular drug delivery system[6]

Mucin is secreted by conjuctival goblet cells, but there are no goblet cells on the cornea. On this basis, a mucoadhesive polymer will firmly attach to conjuctival mucus but only loosely, if at all, to corneal mucus. Ophthalmic dosage forms can be improved by increasing the time the active ingredient remains in contact with eye tissues.

Vaginal drug delivery system[7]

Vaginal mucoadhesive preparations have been developed as new type of controlled release form for the treatment of both topical and systemic diseases. For drugs that are susceptible to gut or hepatic metabolism or which cause GI side effects, vaginal delivery may offer a number of advantages over the other routes of administration.

Rectal drug delivery system[10]

Another way to deliver the drug by using mucoadhesive polymers is through the mucous membrane of the rectum. Hydrogels administered rectally have proven to be useful for drug deliver.

MATERIALS & METHODS

Materials

HPMC K4M, HPMC K15M, HPMC K100M, Chitosan, Sodium CMC, Carbopol 974P, sodium alginate, Gum karaya, Carbopol 941NF. All other chemicals used in the study were of analytical grade which are obtained from SURA Labs Pvt Ltd.

Method

Preparation of Buccal Tablet

Table 1: Composition of buccal tablets

Ingredients (mg)	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8	RF9	RF10	RF11	RF12
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	12	16	20	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	12	16	20	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	12	16	20	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	12	16	20
Sodium CMC	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	-	-	-	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	-	-	-	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

Table 2: Composition of buccal tablets

Ingredients (mg)	RF13	RF14	RF15	RF16	RF17	RF18	RF19	RF20	RF21	RF22	RF23	RF24
Repaglinide	2	2	2	2	2	2	2	2	2	2	2	2
HPMC K4 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K15 M	-	-	-	-	-	-	-	-	-	-	-	-
HPMC K100 M	-	-	-	-	-	-	-	-	-	-	-	-
Chitosan	-	-	-	-	-	-	-	-	-	-	-	-
Sodium CMC	12	16	20	-	-	-	-	-	-	-	-	-
Carbopol 974 P	-	-	-	12	16	20	-	-	-	-	-	-
Sodium Alginate	-	-	-	-	-	-	12	16	20	-	-	-
Gum karaya	-	-	-	-	-	-	-	-	-	12	16	20
Carbopol 941NF	6	6	6	6	6	6	6	6	6	6	6	6
PVP K30	5	5	5	5	5	5	5	5	5	5	5	5
MCC pH 102	71	67	63	71	67	63	71	67	63	71	67	63
Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	3	3	3	3	3	3	3	3	3	3	3	3
Total Weight (mg)	100	100	100	100	100	100	100	100	100	100	100	100

Stability studies

Stability studies for 3 months were carried out for the best formulation; the best formulation is kept under two different conditions like at 30 ± 2°C & 65 ± 5 % RH and other at 40 ± 2°C & 75 ± 5 % RH. After 30 days first month stability studies were carried out for important parameters like dissolution, *Ex vivo* residence time, drug content, Surface pH. The same study is repeated after completion of 60, and 90 days.

In vivo studies

In vivo studies were carried out in white New Zealand rabbits were taken with a mean weight of 1.85-2.25 kg. The animals were fasted overnight and kept in individual cages before the study and the study animals were anesthetized by giving xylazine 4 mg/kg and ketamine 100 mg/kg intradermal injection upon the introduction of anesthesia, a drop of water was placed on the surface of the tablet, the tablet was applied to the oral cavity by pressing for 30 sec, ensure that the tablet was placed carefully in between the check and gingiva to prevent the animal from spitting out. Blood samples of 0.5 mL were withdrawn from the ear vein of a rabbit using a gauze needle at a regular time interval of 0.5 h, 1 h, 2 h, 4 h, 8 h, 10 h, 12 h, and 24 h. Collected blood samples were taken in heparinized tubes and shaken well their samples were centrifuged at 3000 rpm for 15 min to separate the plasma. The clear supernatant plasma layer was collected in an Eppendorf tube and stored immediately at -20°C until analysis.

RESULTS & DISCUSSION

1. **Stability Studies**: Stability studies are done for best formulation (RF11) as per ICH guideline as follows Acceleration stability studies intermediate storage condition has been changed from 30°C ± 2°C and 60% RH ± 5% RH and 40°C ± 2°C and 75% RH ± 5% RH. It focuses that there's no change in Drug content shown in Table 3 & Figure 1& 2.

Table 3: Acceleration stability studies of formulation RF11

Time in (Min)	30°C ± 2°C and 60% RH ± 5% RH (INTERMEDIATE)				40°C ± 2°C and 75% RH ± 5% RH (ACCELERATED)		
	Initial	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
0	0	0	0	0	0	0	0
1	52.15	52.10	52.01	49.89	52.10	52.02	49.91
2	61.83	61.75	61.70	61.27	61.71	61.60	61.30
3	74.19	74.12	74.05	74.10	74.16	74.10	74.08
4	84.43	84.40	84.38	84.10	84.31	84.26	84.05
5	96.19	96.15	96.11	96.05	96.11	96.03	98.95
6	99.43	99.41	99.35	99.24	99.38	99.28	99.14

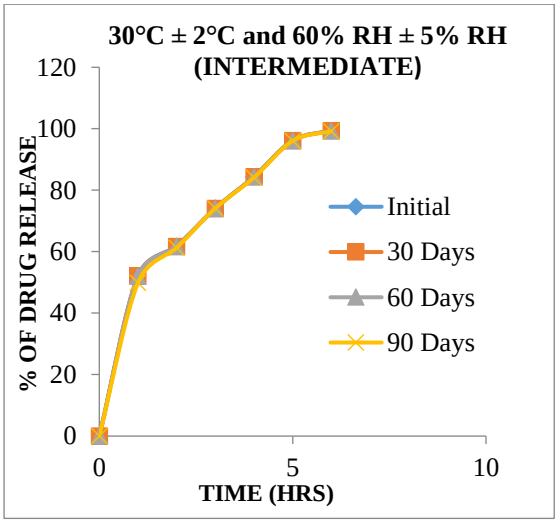

Fig1: RF11 Intermediate stability studies

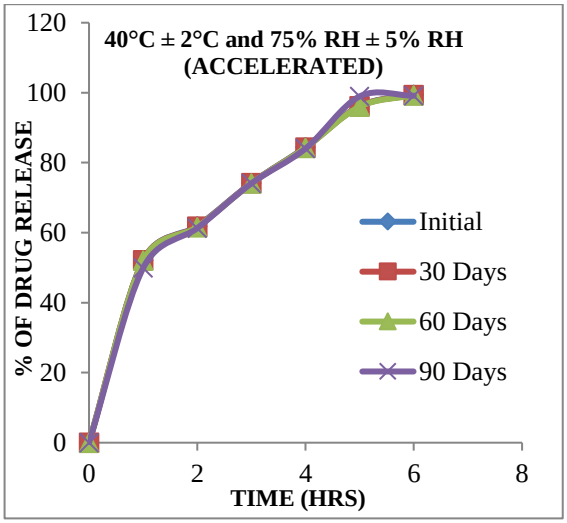

Fig2: RF11 Acceleration stability studies

2. In vivo results

The mean plasma concentration–time curves of Repaglinide pure drug Suspension and optimized buccal tablets (RF11) following the application of the bioadhesive buccal tablet and oral suspension to pigs are shown in Table 4 & Figure 3

The mean peak plasma concentrations (C_{max}) and time to reach peak plasma concentration (T_{max}) for pure drug Suspension, RF11 were calculated to be 46.11 and 131.54 ng/mL respectively, 1 hr, 4hr respectively after administration of oral suspension and bioadhesive buccal tablet. The AUC0–α were found to be, respectively, 340.00 and 1672.64 ng/h/mL. The results of bioavailability study (Table 5) reveal that Repaglinide were released and permeated well from the bioadhesive buccal tablet, as compared with oral suspension. The C_{max}, T_{max}, and AUC profiles were compared, C_{max} was found to be higher by the buccal route than oral route, greater C_{max} values could be attributed due to avoidance of first pass hepatic metabolism after buccal administration.

Table 4: Plasma concentration data for Repaglinide pure drug suspension, Repaglinide optimized buccal tablets

Time (h)	Plasma drug concentration (ng/mL)	
	Repaglinide pure drug Suspension	Repaglinide optimized buccal tablets (RF11)
0	0.00	0.00
0.5	23.29	35.98
1	46.11	62.67
2	33.55	79.22
4	24.75	131.54
8	23.66	57.34
10	12.21	42.06

12	6.05	31.15
24	2.11	28.77

Each value represents the mean value (*n* =6)

Table 5: Pharmacokinetic parameters of Repaglinide pure drug suspension, Repaglinide optimized buccal tablets

Pharmacokinetic parameter	Repaglinide pure drug Suspension	RF11 optimized buccal tablets
C_{max} (ng/mL)	46.11	131.54
t_{max} (h)	1	4
AUC_t(ng-h/mL)	321.21	1225.25
$AUMC_t$(ng-h/mL)	2062.13	10848.97
AUC_i(ng-h/mL)	340.00	1672.64
$AUMC_i$(ng-h/mL)	2680.55	28543.40
Clearance (CL) (mL/min)	0.0058	0.0011
V_d (mL)	0.052	0.018

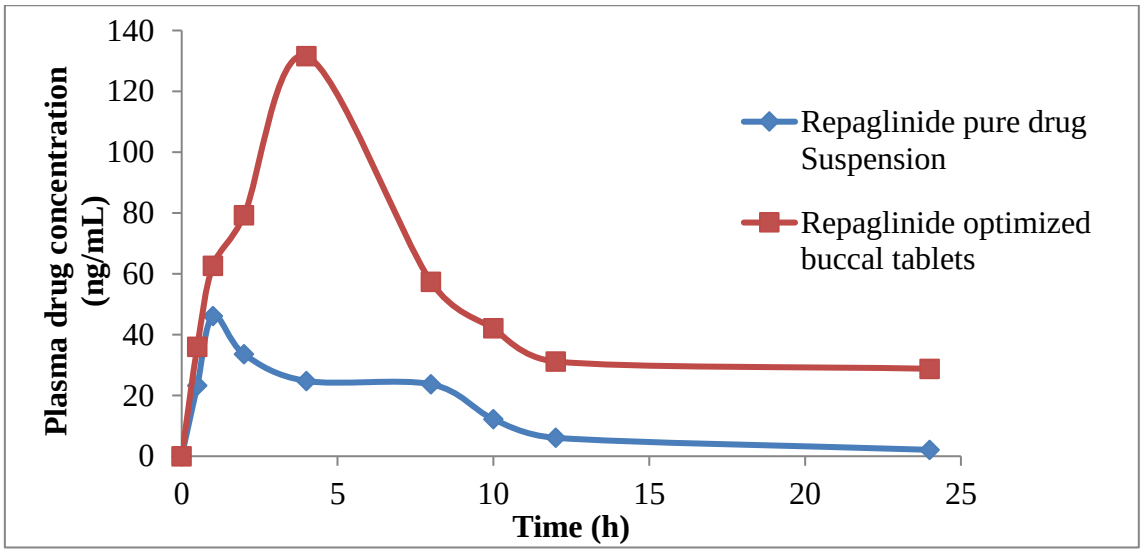

Figure 3: Plasma concentration data for repaglinide pure drug suspension, repaglinide optimized buccal tablets

CONCLUSION

From the results it was conclude that an improvement of bioavailability by the buccal tablet higher than that of the oral route for Repaglinide, respectively, was obtained. Hence, the development of a bioadhesive buccal tablet in tablet dosage form for Repaglinide may be a promising one, as the necessary dose of Repaglinide drug may be decreased, and thus side-effects may be reduced. The Stability of the Repaglinide Buccal tablet(RF11) under accelerated stability condition remains unchanged in Drug content.

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A RAPID NOVEL ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF GRAZOPREVIR AND ELBASVIR BY USING RP-HPLC

Vankudoth Kavitha[1], Usha Kondla[2], P.V Pavan Kumar[3], Gopi Swapna[4]
[1,2,3,4]*Avanthi Institute of Pharmaceutical Sciences, Approved by AICTE, PCI, NAAC, JNTUH, Gunthapally, Hayathnagar, Hyderabad-501512*

ABSTRACT
The current investigation described a sensitive, selective, precise and accurate RP-HPLC method with photodiode array detector for the simultaneous estimation of antiviral drugs, grazoprevir and elbasvir. The separation and analysis were done on Sunsil C18 analytical column (250 mm x 4.6 mm, 5 µ particle size). 0.1M NaH_2PO_4: methanol [60:40 v/v] in isocratic elution mode was used as mobile phase. The pH of the mobile was adjusted to 4.0 with orthophosphoric acid. The elution of grazoprevir and elbasvir was accomplished with a flow rate of 1.2 ml/min. Detection was performed with photodiode array detector set at a wavelength of 260 nm. The detector response was linear in the concentration of 25-75 µg/ml for elbasvir and 50-150 µg/ml for grazoprevir. The limit of detection and limit of quantitation values were found to be 0.137 µg/ml and 0. 574 µg/ml for elbasvir and 0.290 µg/ml and 0.968 µg/ml for grazoprevir, respectively. The method was validated following international conference on harmonization guidelines. The percentage recovery for grazoprevir and elbasvir were found to be in the range of 100.08%-100.45% and 99.60%-100.06%, respectively. The %RSD values are 0.130% and 0.161% for grazoprevir and elbasvir, respectively. The results of validation parameters were found in the acceptance range. The present investigation concluded that the RP-HPLC method with photodiode array detector method was selective for simultaneous estimation of elbasvir and grazoprevir in combined dosage form.
KEYWORD: *Elbasvir , Grazoprevir , Method Development,RP-HPLC.*

INTRODUCTION

Grazoprevir is a NS3/4A protease inhibitor used against different hepatitis C virus genotype variants [1]. Grazoprevir belongs to second generation hepatitis C virus protease inhibitor [2]. By inhibiting NS3/4A protease enzyme, grazoprevir stops the conversion of viral polyprotein into its functional proteins. Elbasvir is a NS5A protein inhibitor used in the treatment of hepatitis C viral infection [3]. NS5A is a protein important for replication of virus and assembly of virion. The combination of elbasvir with grazoprevir was approved by FDA in 2016 in the treatment of chronic Hepatitis C virus genotypes 1 and 4 [4].

The combination of elbasvir with grazoprevir is not listed official in any pharmacopoeia. Only few methods are found in the literature for the quantification of elbasvir and grazoprevir either individually or in combination. Haiyan et al., [5] established an ultra performance liquid chromatography with tandem mass spectrometry method for the quantification of elbasvir in rat plasma using deuterated elbasvir as internal standard. The separation and analysis was achieved with an UPLC BEH C18 column. The mobile phase consisted of acetonitrile–water (containing 5 mM ammonium acetate with 0.01% acetic acid, pH 4.5) at a flow rate of 0.3 ml/min for 3 min in gradient elution mode. This method was applied to the pharmacokinetics study of elbasvir in rats. Haritha et al., [6] described a liquid chromatography with tandem mass spectrometry method for estimation of grazoprevir and elbasvir simultaneously in human plasma. Agilent TC-C18 (4.6 x 75 mm, 3.5 µm, 80 Å) column as stationary phase and acetonitrile: 5 mM ammonium acetate (80:20 *v/v*) as mobile phase was used for the analysis. Akram [7] determinate elbasvir and grazoprevir in bulk and in its pharmaceutical dosage forms using an RP-HPLC method. The separation and analysis are performed using Ineertsil ODS column (4.6 ×2 50 mm, 5 µm). Acetonitrile and phosphate buffer (pH 3) in the ratio of 40:60 (*v/v*) with a flow rate of 1 ml\min was used.

The methods of Haiyan et al., [5] and Haritha et al., [6] were not applied to the quantification of elbasvir and grazoprevir in bulk and pharmaceutical dosage forms. Though the RP-HPLC method of Akram [7] was applied to pharmaceutical dosage forms, this method has disadvantages such as less sensitive, less precise increased retention time of drugs. The present study was aimed to develop a cost effective, sensitive and fully validated RP-HPLC method with photodiode array detection method for the simultaneous determination of elbasvir and grazoprevir in bulk and pharmaceutical dosage forms.

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EXPERIMENTAL

Mobile phase

All the solvents and chemicals are used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. 0.1 M NaH_2PO_4 and methanol (Merck India Ltd., Mumbai) in the ratio of 60:40 (*v/v*) was used as mobile phase. NaH_2PO_4 solution (0.1M) was prepared by dissolving 12 g of NaH_2PO_4 (Sd. Fine Chemicals Ltd., Mumbai) in 300 ml of double distilled water in a 1000 ml volumetric flask and made up to the volume with the same solvent. pH of the mobile phase was corrected to 4.0 with dilute orthophoshoric acid (Sd. Fine Chemicals Ltd., Mumbai). Before use, the mobile phase was filtered through millipore membrane filter and degassed for 15 min.

Instrumentation and chromatographic conditions

Waters 2695 alliance with binary HPLC pump coupled with Waters 2998 PDA detector and Waters Empower2 software was used. Sunsil C18 analytical column (250 x 4.6 mm; 5 µm particle size) was used for separation and analysis of elbasvir and grazoprevir. The temperature of the column was maintained at 25±2^{o}C. Isocratic elution with 1.2 ml/min as flow rate was used. The injection volume was 10 µl. The eluents were detected at 260 nm.

Standard solutions

Elbasvir and grazoprevir reference standard samples were obtained from Lara drugs pvt Ltd. (Hyderabad, Telangana, India). 50 mg of elbasvir and 100 mg of grazoprevir was dissolved in 100 ml of mobile phase in a 100 ml volumetric flask to prepare the stock standard solution. Working standard solutions in the concentrations 25, 37.5, 50, 62.5 and 75 µg/ml of elbasvir and 50, 75, 100, 125 and 150 µg/ml of grazoprevir was prepared from stock solution by aptly diluting the stock solution with the mobile phase.

Calibration curve

10 µl of each of the working standard solutions was injected automatically into the column (n=3) under the chromatographic conditions described. The chromatograms and the peak area response of selected drugs were recorded. The calibration curve was constructed by plotting the mean peak area *vs* concentration of analyte (µg/ml). The results of each drug were subjected to regression analysis to compute the regression equation and regression coefficients.

Assay of elbasvir and grazoprevir content in tablet dosage form:

Zepatier tablets (labeled to contain 50 mg of elbasvir and 100 mg of grazoprevir) are used. Ten tablets were weighed, powdered and an accurate weight of the powder corresponding to 50 mg of elbasvir and 100 mg of grazoprevir was transferred to a 100 ml volumetric flask. The analytes were extracted with 30 ml of mobile phase in an ultrasonic bath for 30 min. The resulting solution was diluted to volume with the mobile phase then filtered through a membrane filter (0.45 µm pore size). One ml of tablet sample solution prepared was diluted to 10 ml with mobile phase in a 10 ml volumetric flask. The resulting tablet sample solution contains 50 µg and 100 µg of elbasvir and grazoprevir, respectively. The solution thus prepared was filtered using membrane filter and then analyzed as described in the section "calibration curve". The content of grazoprevir and elbasvir in the tablets were obtained either the calibration curve or regression equation.

RESULTS AND DISCUSSION

High performance liquid chromatography method parameters optimization

In order to achieve good resolution, better sensitivity, good symmetric peak shape for selected drugs several trails were conducted to optimize the chromatographic method parameters (analytical column, composition of the mobile phase, pH, flow rate and analytical wavelength). 0.1 M NaH_2PO_4 and methanol in different ratios and with different pH were tested. The best separation was obtained on Sunsil C18 (250 x 4.6 mm; 5 µm particle size) using a 0.1 M NaH_2PO_4 and methanol in the ratio of 60:40 (*v/v*) with pH 4.0 as mobile phase pumped with a flow rate of 1.2 ml/min. The column temperature was set at 25±2°C. The maximum response of grazoprevir and elbasvir together was detected at 260 nm and the same wavelength was chosen for the analysis. Using the above described conditions, the retention times for elbasvir and grazoprevir was observed to be 2.853 min and 3.882 min respectively (Figure 1). Total run time of analysis was 6 min.

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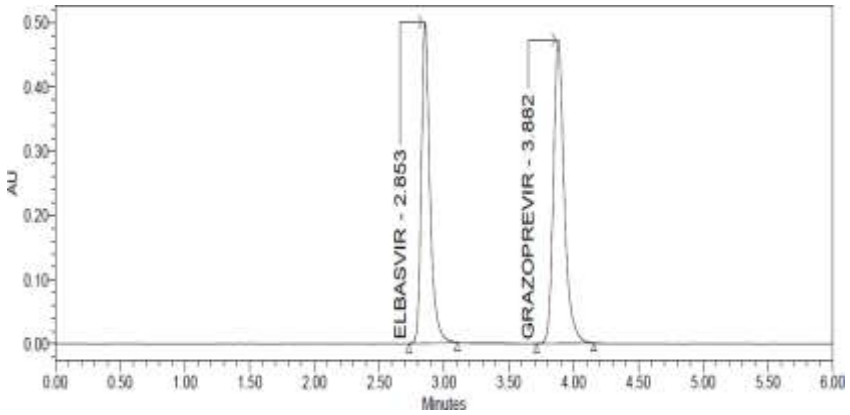

Figure 1: Chromatogram of Elbasvir and Grazoprevir by the Developed Method.

HPLC method validation

Method validation was done in accordance with ICH recommendation [8].

System suitability

Chromatographic parameters associated to the developed method must pass the system suitability limits before the analysis of sample. The relative standard deviation for peak area of drugs, relative standard deviation for retention time of drugs peak response, theoretical plates, resolution and tailing factor for elbasvir and grazoprevir peaks was evaluated using a working standard solution (50 and 100 µg/ml of elbasvir and grazoprevir, respectively). The results (Table 1) pledge the adequacy of the proposed method for routine analysis of grazoprevir and elbasvir simultaneously.

Table 1: System Suitability Results.

Parameters	Elbasvir	Grazoprevir	Recommended limits
Retention time	2.843 (%RSD – 0.435)	3.875 (%RSD – 0.344)	RSD ≤2
Peak area	2299022 (%RSD – 0.082)	2778077 (%RSD – 1.008)	RSD ≤2
USP resolution	-	7.524	> 1.5
USP plate count	9723	10709	> 2000
USP tailing factor	1.370	1.294	≤ 2

Selectivity

The selectivity study was assessed to verify the absence of interference by the components of mobile phase and tablet excipients. For this study, solutions of working standard (50 µg/ml-elbasvir; 100-µg/ml of grazoprevir), tablet sample (50 µg/ml-elbasvir; 100-µg/ml of grazoprevir), placebo blank (contains the tablet excipients and devoid of drugs) and mobile phase blank were injected into the chromatographic system. The chromatograms obtained are shown in Figure 2. The chromatogram confirmed the specificity of the method, because there were no peaks at the retention time of selected drugs in the chromatogram of mobile phase blank and placebo blank. The retention time of selected drug combination in the chromatograms of standard solution and tablet sample solution were almost same.

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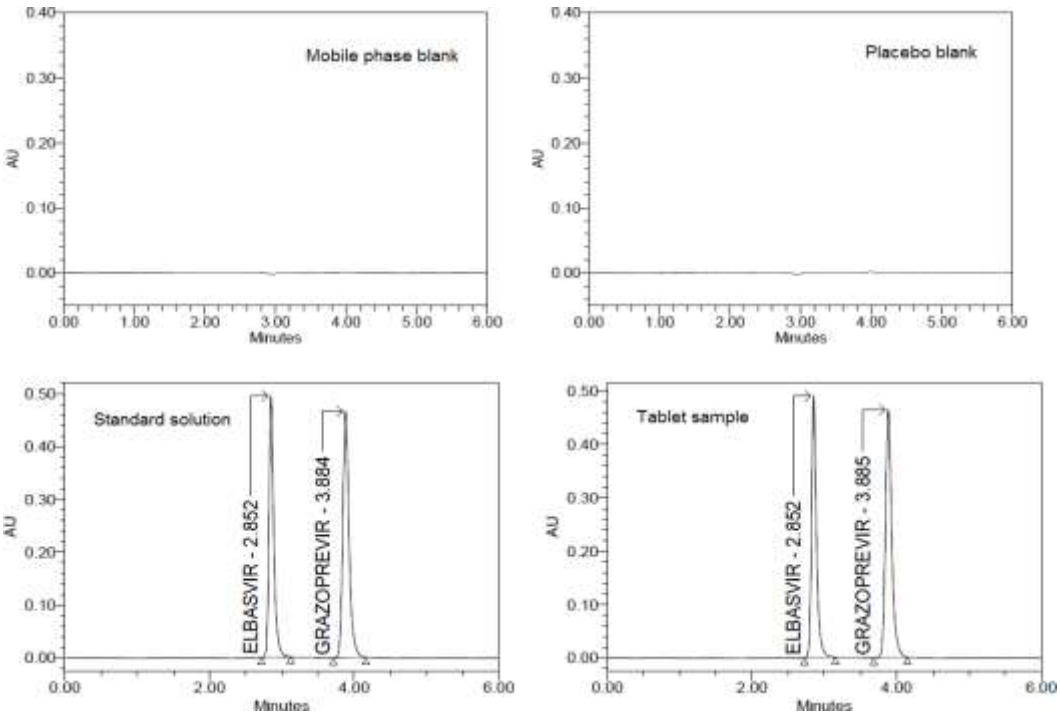

Figure 2: Chromatograms of selectivity studies.

Linearity and sensitivity (Limits of detection and quantification)

Table 2 presents the equation of the regression line, regression coefficient (R2), slope and intercept for each drug. Excellent linearity with good regression coefficient was found between the peak area and concentration. The linearity was found in the range of 25-75 µg/ml and 50-150 µg/ml for elbasvir and grazoprevir, respectively. The high R^2 value was indicative of good linearity.

The limit of detection (LOD) and limit of quantitation (LOQ) represents the sensitivity of the method. they were calculated based on the signal-to-noise ratio. LOD and LOQ were demonstrated by five injections of elbasvir and grazoprevir at concentrations of LOD and LOQ. The results presented in the Table 2 indicated the satisfactory sensitivity of the method for the assay of elbasvir and grazoprevir. The chromatograms of selected drug combination at LOD and LOQ levels are shown in Figure 3.

Table 2: Linearity and sensitivity results.

Drug	Regression equation (Y = m X + c)	Regression coefficient (R^2)	LOD (µg/ml)	LOQ (µg/ml)
Elbasvir	y = 45916x + 2322	0.9998	0.137	0.457
Grazoprevir	y = 27804x - 1757	0.9996	0.290	0.968

X = Concentration (µg/ml); Y=Area; m=slope; c=intercept.

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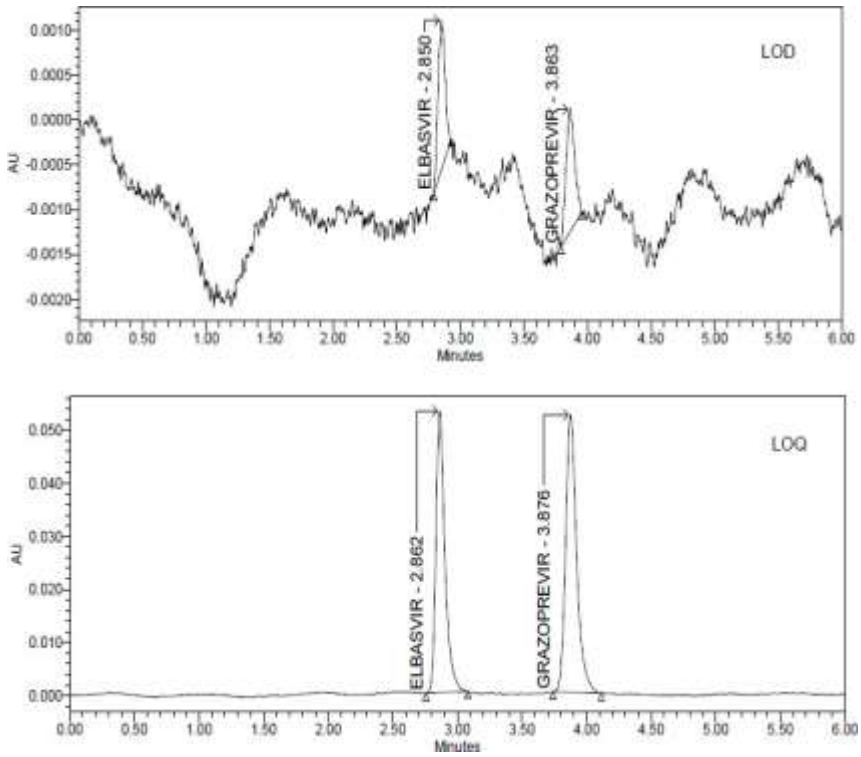

Figure 3: Chromatograms of elbasvir and grazoprevir at LOD and LOQ levels.

Precision

The precision of the method was checked by injecting elbasvir and grazoprevir standard solution 6 times at the 50 µg/ml and 100 µg/ml concentration level. The method precision was expressed as % RSD and found to be 0.161% and 0.130% for elbasvir and grazoprevir, respectively (Table 3). The low percent RSD values indicated the precision of the method.

Table 3: Method Precision Results.

Elbasvir		Grazoprevir	
Peak area		Peak area	
2299045	Mean peak	2778843	Mean peak
2292669	area:	2774658	area:
2297777	2296191	2778971	2777029
2299705		2779893	
2291795	%RSD:	2779769	%RSD:
2299011	0.161	2771858	0.130

Accuracy

A standard working solution containing elbasvir and grazoprevir, at concentration level 50 µg/ml and 100 µg/ml, respectively was prepared. The prepared standards were injected 6 times in the HPLC system as a test sample. From the respective peak area counts, the concentrations of elbasvir and grazoprevir were calculated using the detector responses. The accuracy represented in terms of percentage recovery is listed in Table 4. The good percent recovery values indicated the accuracy of the method.

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Table 3: Method Accuracy Results.

Elbasvir			Grazoprevir		
Concentration taken (µg/ml)	**Recovery (**%)		**Concentration taken(**µg/ml)	**Recovery (**%)	
50	99.20	**Mean**	100	99.53	**Mean recovery(**%**):**
50	98.93	**recovery(**%**):**	100	99.38	99.47
50	99.15	99.10	100	99.53	
50	99.23		100	99.57	**%RSD:**
50	98.89	**%RSD:**	100	99.56	0.120
50	99.20	0.150	100	99.28	

The accuracy of the proposed method was again established by recovery studies through standard addition method. For this, the preanalyzed sample solution was spiked with known concentration of elbasvir and grazoprevir at 3 diverse concentration levels (50%, 100% and 150%). The percentage recovery data presented in Table 5 show that the proposed method was accurate and the excipients present in tablets did not obstruct the assay of elbasvir and grazoprevir.

Table 5: Recovery Study Results.

Spiked level (%)	Elbasvir				Grazoprevir			
	Added (µg/ml)	**Found (µg/ml)**	**Recovery (%)**	**Mean (%)**	**Added (µg/ml)**	**Found (µg/ml)**	**Recovery (%)**	**Mean (%)**
	24.75	24.70	99.79		49.50	49.51	100.02	
50	24.75	24.62	99.49	99.66	49.50	49.60	100.19	100.08
	24.75	24.67	99.69		49.50	49.51	100.02	
	49.50	49.57	100.13		99.00	99.26	100.26	
100	49.50	49.41	99.81	100.01	99.00	99.56	100.57	100.42
	49.50	49.54	100.08		99.00	99.43	100.43	
	74.25	74.23	99.98		148.50	149.14	100.43	
150	74.25	74.28	100.04	100.06	148.50	149.24	100.50	100.45
	74.25	74.36	100.15		148.50	149.14	100.43	

Robustness

The method robustness was established at a concentration of 50 µg/ml (elbasavir) and 100 µg/ml (grazoprevir). To measure the method robustness, the chromatographic conditions were deliberately varied. The studied parameters were: column temperature (±2°C) and flow rate (±0.1). The system suitability parameters were determined to reveal the method robustness. The results shown in Table 6 indicated that the minute change in the chromatographic conditions did not notably affect the system suitability. Thus, the method is robust.

Table 5: Results of method robustness.

Parameter	Retention time	Peak area	USP plate count	USP Tailing	USP resolution
Elbasvir					
Flow rate – 1.1 ml/min	3.572	2841800	10645	1.44	-
Flow rate – 1.3 ml/min	2.384	1898190	9087	1.35	-
Column temperature-23^{o}C	3.576	2855300	10744	1.39	-
Column temperature-27^{o}C	2.387	1897586	9256	1.36	-
Grazoprevir					
Flow rate – 1.1 ml/min	4.815	3479251	11932	1.35	7.64
Flow rate – 1.3 ml/min	3.233	2272806	9903	1.24	7.14
Column temperature-23^{o}C	4.822	3486149	11888	1.31	7.64
Column temperature-27^{o}C	3.236	2303683	9810	1.25	7.14

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CONCLUSION

The RP-HPLC photodiode array detector system with C18 reversed phase column (250 mm x 4.6 mm, 5 μm) was used in this investigation. NaH_2PO_4 (0.1M) and methanol in the ratio of 60:40 (*v*/*v*) with a flow rate of 1.2 ml/min was selected as the mobile phase. Analytical wavelength of 260 nm was used. The method validation was performed following the guidelines of the International Conference on Harmonization and the results of validation parameters were found to be within the acceptance criteria. The components of mobile phase and common tablet excipients did not interfere with the assay. Therefore, the present RP-HPLC method can be helpful for estimating the concentration of elbasvir and grazoprevir simultaneously in tablet dosage forms in quality control laboratories.

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SYNERGISTIC ANTI-TUMOR EFFECTS OF NANO EMULSION FORMULATION CONTAINING CURCUMIN AND BRUCEA JAVANICA OIL: FORMULATION AND CHARACTERIZATION

Chinnabathini Anilkumar
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Nandru Ramya
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract -
Objective: The study's objective was to prepare and further enhance the antitumor effect of a nano emulsion (NE) infused with brucea javanica oil (BJO) and curcumin (CUR).
Methods and Materials: In a shaking incubator, the soluble levels of CUR and BJO in a variety of vehicles were measured. Pseudo-ternary phase diagrams were used to create NE in order to determine the component concentration range. Size, zeta potential, morphologies, physical stability, in vivo antitumor activity, and in vivo pharmacokinetics were used to investigate the NE's physicochemical and biological characteristics.
Conclusions: In optimized NE, the formulation of the BJO-CUR combination was successful. An anti-tumor activity experiment was used to determine whether BJO, which was used as the oil phase, had a synergistic antitumor effect with CUR. When compared to pure CUR, both the dissolution rate and oral bioavailability were enhanced.
Keywords: Curcumin, nano emulsion, pharmacokinetics, antitumor effect, and oil of Brucea javanica.

1 INTRODUCTION

The ripe fruits of Brucea javanica [L.] Merr are the source of the complex mixture of fatty acids and fatty acid derivatives known as Brucea javanica oil (BJO). [Simaroubaceae It is accounted for that BJO has different diseases strong pharmacological exercises, for example, cancer suppressive, mitigating and antimalarial exercises. Additionally, it has effects on the clinical treatment of malignant tumors of intermediate and advanced stages. Bone marrow hematopoietic stem cell safety and improved quality of life for patients undergoing tumor treatment have both been demonstrated by BJO studies.

Curcumin, also known as CUR, is a naturally occurring, hydrophobic polyphenol that comes from the rhizome of the herb Curcuma longa. It includes anti-oxidant and anti-microbial properties in its extensive pharmacological profile [8, 9]. It also suppresses tumors in breast, head, colon, and other cell lines. CUR is a promising drug for clinical treatment because of its low toxicity, as demonstrated by pharmacological studies. However, the dissolution rate, metabolism, and permeability had been impacted by CUR's poor bioavailability and low solubility. The therapeutic effects and clinical application will then be limited by the low solubility. The creation of a formulation loaded with two therapeutic agents has become increasingly common, particularly in the treatment of cancer. In the field of chemotherapy for cancer, there are very few cases in which the disease is treated by a single agent. Both CUR and BJO have extensive anti-tumor spectra and favorable therapeutic effects, making them important players in cancer treatment. The therapeutic superiority of combination treatment over single drug treatment has been demonstrated by a

number of studies. To the best our insight, there is no writing written about the planning both of these two medications as NE.

The CUR and BJO's therapeutic effects have been limited by their low solubility and poor bioavailability. The significance of this article lies in selecting the appropriate drug delivery method. In order to sustain pharmacological action and achieve therapeutic local concentrations in a reasonable amount of time, the rate and extent of drug release from the carrier are crucial. The technology for nanoemulsification of drugs has advanced over the past ten years. The nanoscale droplet diameter, relative high rate of drug release from oil droplets into aqueous media, and high solubilization properties of nanoemulsion were identified as promising delivery systems.

It has received increasing attention for its beneficial properties because it is an efficient drug carrier that increases the absorption and solubility of poorly water-soluble drugs. Water, oil, surfactants, and co-surfactants made up nano emulsions. NE is a formulation that is thermodynamically stable. Depending on the oil phase, it is possible to avoid hepatic first pass metabolism and increase lymph directivity; Consequently, NE can also improve the skin's delivery of lipophilic and hydrophilic drugs. Because of its small droplet sizes, it prevented phase separation and improved membrane adhesion.

The formulation of a nanoemulsion requires a certain amount of oil. However, there were no reports regarding the BJO formulation as an oil for NE. The preparation of a nanoemulsion with BJO acting as the oil phase was the primary focus of this study. After that, a CUR-BJO-NE NE formulation with BJO and CUR was created. We wished mix utilizing of them could accomplish synergistic antitumor impact and improve oral bioavailability. Characterization, a stability test, and an evaluation of the formulation followed. Bioavailability. The portrayal of nanoemulsion was directed by it appearance of utilizing transmission electron microscopy (TEM) and drop size. CUR-BJO-NE's oral bioavailability was studied in rats to compare its absorption to that of CUR suspension (CUR powder dispersed in 0.4% CMC-Na solution).

2 MATERIALS AND METHODS

2.1 Materials

The Sinopharm Chemical Reagent Co. Ltd. in Shanghai, China, supplied the CUR. Yaoda Pharmaceutical Co. Ltd., based in Shenyang, China, supplied BJO. Gattefosse (Shanghai, China) obtained Capryol 90. Aotai Chemical Ltd., based in Jinan, China, supplied the ethyl oleate. Yihai Jiali Food Marketing Ltd. in Beijing, China provided the peanut oil. Polyoxyl 40 hydrogenated castor oil 40 (Cremophor RH 40) and Polyethylene glycol 400 (PEG400) was bought from BASF Co., Ltd. (Germany) and Huadong substance reagent plant (Tianjin, China), individually. Meilin Industry and Trade Co., Ltd., based in Tianjin, China, supplied Tween 80. Fuchen chemical reagent factory in Tianjin, China, supplied the sodium carboxymethycellulose (CMC-Na). Analytical reagent grade was used for all other chemicals and solvents.

2.2 Animals

Vital River Laboratory Animal Center, located in Beijing, China, provided the male Wistar rats (250 20 g). The Laboratory Animal Centre of Hebei Medical University in Shijiazhuang, China, supplied the Kunming mice (weighing between 18 and 22 grams).

2.3 Cell lines

The Chinese Academy of Sciences' cell bank in Beijing, China, provided the mouse ascetic turnout cell line S180.

3 PREPARATION OF NES

3.1 Solubility study

The following measurements were made and carried out on the solubility of CUR and BJO in various vehicles: First, a quantity of BJO was added to each centrifugal tube with 1 mL of selected vehicles. The mixture was then kept in centrifugal tubes for two days at 25°C in a shaking incubator to reach equilibrium. The degree of uniformity in mixing quality was chosen as the vehicle indicator. In addition, excess CUR was separately added to 1 mL of various oils and surfactants in the centrifugal tube, mixed for 48 hours in a shaking incubator at 25°C, and the solution was centrifuged at 10000 rpm for 10 minutes to remove the excess CUR. The UV spectrophotometer measured the concentration of CUR in the supernatant after the appropriate dilution with ethanol.

3.2 Construction of Pseudo-ternary Phase Diagrams

To determine the component concentration range for the current NE region, pseudo-ternary phase diagrams were constructed. Distilled water was added drop by drop to the mixture under proper magnetic stirring at room temperature until the mixture became clear at a certain point. Different weight ratios of oil phase, surfactants, and co-surfactants were mixed together.

3.3 Pharmacokinetics study of CUR-BJO-NE in rats

Two groups of five Wistar rats were randomly divided into. The Institutional Animal Care and Use Committee gave its approval to this animal experiment, which followed all of the regulations. The rodents were abstained for the time being before explore different avenues regarding free admittance to water. CUR-BJO-NE (400 mg/kg of body weight) and CUR suspension (1000 mg/kg of body weight, pure CUR powder dispersed in 0.4% CMC-Na solution) were administered intragastically to rats. At predetermined intervals, 0.5 milliliters of blood were taken from the ophthalmic vein of rats and centrifuged for ten minutes at 4000 rpm. The blood samples were then kept at -20 degrees Celsius until the analysis. 0.2 ml plasma was added with 0.6 ml ethanol and it was for 5 min vortex. The mixture was then centrifuged for ten minutes at 4000 rpm. HPLC analysis was used to determine the supernatant's CUR concentration.

3.4 In Vivo Antitumor Activity

The CUR-NE in vivo antitumor activities were contrasted with the established CUR-BJO-NE against mouse model bearing the S180 cell line. The following was the structure of the experiments: Ascites formed in about seven days after tumor-bearing mice were inoculated intraperitoneally with mouse sarcoma cells from the S180 cell line. Then, at that point, the ascites was removed and washed with PBS and acclimated to cell suspensions in the serum free medium at cell grouping of 2 × 107 cells/ml and 200 μL of the blend was embedded subcutaneously into the flank of bare mice to lay out cancer xenograft. At that point, the tumor mouse model was established. The mice were then evenly divided into four groups based on their respective weights. With ten mice per group, the treatment began.

3.5 Statistical Analysis

The data were presented as the mean minus the standard deviation for each of the study's three experiments. At p 0.05, a two-tailed unpaired Student's t-test was used.

3.6 Characterization of CUR-BJO-NE

CUR-BJO-NE's optimized TEM morphology. NE had a uniform size and appeared to be spherical. The average size of the particles was about 51.5 nm. Additionally, CUR-BJO-NE's physical stability was evaluated. The samples did

not change in appearance, homogeneity, or phase separation, breaking, or drug precipitation after centrifugation. After centrifugation, this demonstrated that the formulation remained physically stable.

4 DISCUSSION

The purpose of the solubility studies was to identify appropriate oils and surfactants in NE with a high drug-loading capacity for both CUR and BJO. When choosing vehicles, other ingredients' emulsification properties were also taken into account. After that, PEG400 was chosen as the co-surfactant rather than Transcutol HP, despite the fact that Transcutol HP was more soluble than PEG400. As surfactants, Cremophor RH40 and Labrasol were able to achieve a greater emulsifying effect when combined than when used separately. This may be connected to a greater reduction in the oil–water interfacial tension. The combination of the following effects probably contributed to the increased bioavailability: First, CUR's dissolution rate was significantly increased in CUR-BJO-NE, and when the drug was kept in a dissolved state in NE, oral absorption could be increased; Second, the effect of P-glycoprotein on the drug efflux effect may also be diminished or inhibited by surfactant use; NE droplets were able to escape the reticuloendothelial system's uptake and phagocytosis due to the smaller droplet size, which also increased the drug's circulation time. The large surface area of NE and the use of surfactants in formulation may be the cause of the increased dissolution rate.

In comparison to the group of blank NE, the in vivo antitumor activity results demonstrated that CUR-BJO-NE and CUR-NE effectively inhibited tumor cell growth. It suggested that the S180 cell was protected from tumor growth by both CUR and BJO. Additionally, this investigated whether utilizing BJO as an oil phase to dissolve CUR had a synergistic anticancer effect. In conclusion, the development of a potential CUR-BJO-NE formulation was strongly supported by all of the obtained results.

5 CONCLUSION

A NE system that successfully incorporated both BJO and CUR for oral administration was developed in this study. An anti-tumor activity experiment was used to determine whether BJO, which was used as the oil phase, had a synergistic antitumor effect with CUR. During the centrifugation and water dilution experiment, the CUR-BJONE exhibited stability. When compared to pure CUR, the dissolution rate and oral bioavailability were significantly improved. The current study proved that the combination of CUR and BJO could be used to treat cancer.

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ASSESSING ANTIBIOTIC PRESCRIBING PATTERNS FOR INPATIENT PNEUMONIA MANAGEMENT: A SAFETY-FOCUSED STUDY

G Satheesh
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
P. Raja Sridhar Rao
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - As it is for many health services worldwide, improving patient safety is a significant challenge for national health systems. Common and potentially fatal, community-acquired pneumonia (CAP) is linked to significant morbidity, mortality, and expenditure of healthcare resources. The study's objective was to clarify and evaluate the frequency with which antibiotics are prescribed to inpatients with CAP, the main reasons why prescriptions change during treatment, and doctors' attitudes toward the patient safety issue. We looked back at the medical records of 107 inpatients who had been hospitalized because of CAP. Pseudo-randomization was the method used; Expert evaluation of the variations' frequencies and non-interventional pharmacoepidemiological evaluation In addition, we divide the reasons why prescriptions for medicines change into five broad categories. More than 33% of patients with CAP have had at least one variation in their drug therapy during their hospital stay, according to the data. Most normal change in the treatment is adding another anti-infection (67%) because of absence of helpful impact (63%) or determining finding (26%). Beginning treatment without a microbiological diagnosis is the most common reason for prescribing additional antibiotics. Concerning patient safety, the interviewed physicians (MDs) mentioned issues with the healthcare system's financing, institutional limitations, diagnostics, and the absence of clear practice rules and standards. The variety of obstacles that healthcare providers encounter on a daily basis should be taken into consideration when making efforts to increase antibiotic use inpatients with CAP.

Keywords: Patient security, local area gained pneumonia, anti-toxins, clinical specialists' disposition.

1 INTRODUCTION

Patient Wellbeing is an umbrella term which dwells numerous classes of possible damages to patients: medication errors, wound infections after surgery, falls, bedsores, technical failures, communication issues, restrictive barriers, and other issues Despite being a significant, largely unnoticed, and understudied issue in Bulgaria up until just recently, the issue has recently gained public attention.

The safety of healthcare is inadequate. As it is for many health services worldwide, improving patient safety is a significant challenge for the national health system. In numerous nations, it has turned into the primary technique for making upgrades in medical care frameworks. Governments must adopt a proactive, preventative, and systematic approach before developing strategies for Patient Safety. A framework based approach surmises the deliberate plan of safe designs, methods and cycles, as well as right responses because of security episodes.

In order to increase patient safety, healthcare organizations are increasingly recognizing the significance of transforming organizational culture. The need for assessment tools that focus on the medical, economic, and cultural

aspects of patient safety improvement efforts has accompanied the growing interest in safety culture.

Hospital patients' safety is only one aspect of a larger trend toward increased risk perception and decreased risk acceptance. The complexity of hospital treatment has increased. Reduced staff, shorter hospital stays, and more intensive treatment have all been a result of increased competition in medical care between providers, hospitals, and third-party payers, as well as pressure to control costs and maximize effectiveness. Dispensing, prescribing, and administering medications involve a variety of healthcare professionals in patient care. An open discourse and correspondence between wellbeing experts is a positive method towards working on tolerant security in prescription use. If a patient safety issue arises, this enables both the patient and medical professionals to monitor the condition and take the necessary actions.

Common and potentially fatal, community-acquired pneumonia (CAP) is linked to significant morbidity, mortality, and expenditure of healthcare resources. To effectively plan for change in order to improve prescribing practices and patient safety, it is necessary to have a better understanding of the factors that influence the best treatment. The administration of the appropriate antibiotics is still the cornerstone of management and has a direct impact on patient safety.

The study's objective was to clarify and assess the factors leading to prescription variation during treatment and the frequency with which antibiotics are prescribed to inpatients with pneumonia. We also looked at how doctors felt about the issue of "patient safety" to emphasize how important it is. During the study were assessed the primary hindrances for working on the wellbeing and the required insurances for establishing a more security medical services climate.

2 METHODS

In a specialized pneumology and phthisiology clinic at a Bulgarian university hospital, we conducted a retrospective review of the medical records of 107 inpatients with community-acquired pneumonia. Pseudo-randomization was the method that was used for the sample selection. Strict criteria for the type of treatment, variations, outcomes, etc. were used to analyze the collected data. Non-interventional pharmacoepidemiological evaluation of the frequency and classification of the factors that lead to changes in antibiotic prescriptions is also included in the survey. For this target group, we used a preliminary created schedule model and the medical records of patients who had been hospitalized with CAP as the sources of the data. Columns for demographic indexes, such as sex and age, the primary diagnosis, which was categorized according to the ICD code, the length of stay in the hospital (in days), the initial drug treatment, variations in therapy (day, type of change, reason), and additional comments on the patient's medical history (side effects, lack of effect, complications and interactions, etc.) were included in the data collection form. The examination has been produced using the existed electronic information base at the college multiprofile emergency clinic between clinical records gathered for patients hospitalized with CAP in the time of October 2008 - Walk 2009. 107 patients' data have finally been processed. For that time period, there were 1006 patients who had been admitted to the hospital (or 10.6% of the total), and there were 572 inpatients who had been diagnosed with CAP.

In this multi-profile university hospital, we also conducted indirect interviews with medical professionals

using a standardized questionnaire. The 15 questions were divided into three sections: personal data; attitudes toward the patient safety issue; and barriers to appropriate antibiotic use. Common decency; attitude and knowledge of the problem being studied. 93 of the 120 inquiry cards were returned and completed (response rate: 77%). We looked at 92 after the validation process. Our sample size was 22%, and the hospital under investigation has a total of 427 medical professionals.

3 PATIENTS' AND DRUG THERAPY DATA ANALYSIS

Worked and analyzed the medical record data that was gathered. For patients with CAP, the predominant gender was female (60 percent), and the predominant age group was 51-65 years old, with an average hospital stay of 8.5 days.

Eliminating the pathogens that are causing the condition, resolving the clinical signs and symptoms, minimizing hospitalization, and preventing re-infection are the primary objectives of pharmacotherapy for these patients. The pharmacokinetic profile, adverse reactions, drug interactions, and cost-effectiveness should all be taken into consideration by physicians when selecting a medication. In addition, the patient's age, co-morbidities, clinical presentation, epidemiologic setting, and previous exposure should all be taken into consideration when evaluating the patient. The most common pathogen(s) associated with CAP are used empirically to treat the majority of CAP patients.

Initially, 74 patients (69 percent) received monotherapy, while 33 patients received multiple medications. According to the analysis of medical records, Ceftriaxone i.v. was the antibiotic that was most frequently prescribed for initial therapy for 48 patients— (44 percent of cases). Combination of intravenous Ceftriaxone + IV azithromycin was given to 26 patients (24 percent) and Azithromycin intravenously for 18 people. The combination of intravenous Ceftriaxon and + oral azithromycin was chosen only in 5% of cases. There are other remedial methodologies (6%), however they are completely individual and steady with a specific case and patient's qualities.

The extra medication generally remembered for the treatment of CAP after assurance of the causative pathogen(s). Ciprofloxacin i.v. was the drug that was added most frequently. for fifteen patients p.o. metronidazol was chosen for 9 patients (26%), Clarithromycin for 5 patients (15%) and Azithromycin i.v. for 6 percent of cases. The ratio of monotherapy to combination therapy was flipped around by the change in treatment (from 48% for monotherapy to 52% for combination therapy).

The "switch" strategy is an important part of making sure that patients with CAP get the best antibiotics. This strategy, according to numerous authors can cut costs associated with drug administration and hospitalization length. In the management of community-acquired pneumonia, one important strategy is to target patients for early hospital discharge and switch from intravenous to oral antibiotic therapy. Sadly doctors at the concentrated on facility stick to intravenous anti-toxin treatment. Ten cases (22 percent) when administered intravenously In 46 cases, macrolide was initially prescribed, but now it is taken orally. In none of the cases where intravenous cephalosporins were prescribed, a switch was made. The sixth day of the therapy has seen the most implementation of this strategy. Despite the fact that numerous studies demonstrate that the patient's therapeutic outcome improves with the early switch.

In addition, our survey revealed that sixty percent (56%) of patients received antibiotic treatment prior to admission and that seventy-two percent (67%) received ambulatory treatment

upon discharge. Ciprofloxacin oral was the drug that was most frequently prescribed for use at home. for 38 individuals p.o. clarithromycin Moxifloxacin orally was prescribed to 24 patients (32%) for 4 patients and Amoxicillin+ Clavulanic corrosive p.o. for 4 percent of patients.

Our hypothesis that inpatients with CAP have a higher proportion of therapy variations is supported by data analysis. 35 percent of 38 patients who were admitted underwent drug therapy adjustments while they were there. Adding another antibiotic to the treatment is the most common change (67%). In 17% of cases, the method of administration was changed, in 7%, a drug from the same chemical or therapeutic subgroup was exchanged, and in 7%, drug administration was stopped. In 2% of the cases doctors had performed tweaking of the measurements routine.

Most of the time, these were cases in which the treatment that was given was different because it didn't work. Specifying the diagnosis was the reason for a change in 26% of cases, administrative issues were the reason for 7%, and adverse drug reactions were the reason for only 4%.

4 CONCLUSIONS

The study demonstrates that physicians are unaware of the significance of patient safety issues and their impact on the hospital level. Yet again we demonstrate the significance of the patient security occurrence revealing framework on medical clinic level. It can be set up as a "stand alone" system or integrated with other systems to report on patient safety. The improvement of patient safety through the application of lessons learned from incidents and errors must be the primary objective of an incident reporting system. Analyzing and evaluating the data is the only way to make incident reporting and data collection meaningful. All professionals and anyone else who is interested in learning more about the events' analysis could receive the feedback.

Parenteral versus oral therapy, reliable switch, minimizing the emergence of penicillin-resistant pneumococci, and reducing the length of hospital stay are the most recent therapeutic considerations in CAP. The variety of obstacles that healthcare providers encounter on a daily basis should be taken into consideration when making efforts to increase antibiotic use inpatients with CAP.

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EXPLORING THE THERAPEUTIC POTENTIAL OF INDIAN MEDICINAL PLANTS IN THE TREATMENT OF ARTHRITIS

Banuvari Sandeep
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Shireesha Bandirala
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Conventional prescriptions are utilized around the world for the board of rheumatoid joint pain since ancient times. This review focuses on the medicinal flora of India and how they have traditionally been used to treat rheumatoid arthritis. Articles from the past three decades that were peer reviewed and classical textbooks were looked at for bibliographic research. The current review examines plant extracts that have traditionally been used to treat arthritis. There are 124 plants that have traditionally been used to treat arthritis. The need to investigate the potential chemical moieties of unexploited plants for arthritic management, as well as their mechanism of action, is reflected in this study.
Keywords: Against ligament action, substance moieties restorative plants.

INTRODUCTION

Chronic, progressive, and systemic inflammatory disorder known as rheumatoid arthritis (RA) It specially influences the synovial layers of joints and in the end prompts bone and ligament annihilation. Worldwide, 0.5–1 percent of adult populations are affected by RA; Although RA can occur at any age, it is more common among people in their fourth to sixth decades of life. The disease affects patients of all ethnic backgrounds and primarily affects females, with a 2- or 3-fold prevalence rate for males. RA is a costly condition that affects both patients and society as a whole because it is a chronic disease that causes irreversible damage to the joints. RA's clinical manifestations can be divided into articular and extra-articular categories. RA is shown by fever, asthenia, exhaustion, myalgia, and weight reduction which show up previously.

1.1 Pathophysiology

Several genetic and environmental factors contribute to the phenotype in various combinations in RA. Metalloproteinases have an impact on RA, which is triggered by immune complexes and the complement system. Cytokines keep the disease going. Through cell-surface signaling, antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokines interleukin-1, interleukin-6, and TNFa and secrete matrix metalloproteinases. Synovial fluid contains a lot of neutrophils in early RA. Hypertrophy and hyperplasia develop projections into the joint capsule over time. IgG/anti-IgG antigen-antibody complexes are frequently present in immune complexes in synovial fluid. Cartilage dissolution is the result of bone erosion caused by osteoclasts and proteolytic enzymes in RA. Key pathogenic markers include rheumatoid factors (IgM and IgA).

2 MATERIAL AND METHODS

For the purpose of this review, a bibliographic investigation was conducted to locate articles in the global scientific databases viz. SCIELO, NISCAIR, PUBMED, SCOPUS, and Google Scholar. The 2014 plant list and the International Plant Names Index were used to verify the botanical names of plants. The following

are the inclusion criteria for the selection of plants: (i) medicinal herbs with reported animal studies on their use in arthritis management; (ii) compounds isolated from medicinal plants with potential for arthritis relief; and (iii) we restricted the number of studies to those that were published in English.

2.1 Alangium Salviifolium Wang. (Family: Alangiaceae)

The small deciduous tree or shrub known as Alangium salviifolium (AS) can be found growing wild in India's hotter regions (The Wealth of India, 1952). Alangine A and B, alangicine, markindine, lamarckinine, and emetine are the plant's main chemical components. In the Indian system of medicine, the root of AS has been used as an astringent, diuretic, and antidote to a number of poisons. The plant's fruit can help alleviate burning and hemorrhages. Various concentrates of stem bark of AS were found to have hostile to joint movement utilizing Freunds adjuant model in rodents.

2.2 Caesalpinia Sappan L. (Family: Leguminosae)

Caesalpinia sappan (CS) is a traditional Asian medicinal plant that is mostly used for its anti-inflammatory and blood-flow-promoting properties. In a rat model of collagen-induced arthritis, the plant was found to have anti-arthritic properties. The arthritis index, radiographic and histopathological changes, and paw swelling were significantly reduced at various doses of ethanolic extract.

2.3 Delonix Elata (Family: Leguminosae)

Delonix elata (DE) (white gold mohur) is utilized generally for joint agonies and in fart. Locals use a paste made of bark and leaves to ease pain and inflammation [9]. Using an incomplete Freund's adjuvant-induced arthritis model in rats, the anti-arthritic activity of Bark of DE was evaluated. Extract that is 40% hydroalcoholic (250 mg/kg p.o.) was found have critical repressing enemy of ligament movement rodent paw edema following 14 and 21 days. When compared to Diclofenac, the outcomes were also significant.

2.4 Semecarpus Anacardium Linn. (Family: Anacardiaceae)

In Indian medicine, the "marking nut," or Semecarpus anacardium (SA), has been used to treat gout, rheumatic pain, and cancer. Using an adjuvant arthritis model, SA nut extract was evaluated. Paw edema in both newly developed and established adjuvant arthritis was reduced by treatment with SA extract (150 mg/kg). The findings suggested that the SA nut extract may have anti-arthritic properties.

2.5 Azima Tetracantha Lam (Family: Salvodoraceae)

In Ayurvedic medicine, Azima tetracantha (AT) is known as Kundali and in kannada as uppimullu. There are reports that the juice from the leaves is effective against ear and toothaches. To treat snakebite, Indian tribes use AT leaves paste. Friedelin, a compound segregated from AT. Wistar rats and 54.5% of the paw thickness were tested for adjuvant-induced arthritis activity in the leaves.

2.6 Chaenomeles Speciosa (Sweet) Nakai (Family: Rosaceae)

Chaenomeles speciosa (CS), also referred to as mugua, tiegenghaitang, tiejiaoli, or zhoupimugua, is a species that can be found in Central, East, and Southwest China. It is now grown in many countries. The warm and sour CS fruit, which is used in traditional Chinese medicine, has the ability to calm the liver, relax the muscles and tendons, balance the stomach, and get rid of dampness. Using a collagen-induced arthritis model in rats, the anti-arthritic activity of the roots of CS was investigated. In arthritic rats, extract doses of 30, 60, and 120 mg/kg

reduced inflammation and restored body weight.

2.7 Ficus Bengalensis Linn (Family: Moraceae)

In Ayurveda, Ficus bengalensis (FB), also known as the Banyan tree, is used to treat diarrhea, dysentery, hypoglycemia, rheumatism, and was applied to the gums to reduce inflammation. Using arthritis models that were induced by Complete Freund's Adjuvant, formalin, and agar, the bark of FB was examined for its antirheumatic activity. The supplement (100, 200, and 300 mg/kg intravenously) inhibited Formalin-induced pain and had a significant inhibitory effect on edema, particularly in secondary immunological arthritis.

2.8 Hemidesmus Indicus R.Br (Family: Asclepiadaceae)

In Ayurvedic and Unani medicine, Hemidesmus indicus (HI) has been used to treat inflammation and blood disorders. Extract of hydrocarbons (450 mg/kg, orally), ethanolic acid (75 mg/kg, orally), residual fractions (270 mg/kg, p.o.), chloroform (60 mg/kg, p.o.), was tested in rat models of Complete Freund's adjuvant arthritis. Body weight, arthritic index, erythrocyte sedimentation rate, serum rheumatoid factor, serum C-reactive protein, and serum nitrite level all decreased significantly in the study.

2.9 Holarrhena Pubescens (Buch.-Ham.) Wall. (Family: Apocynaceae)

Holarrhena pubescens (HP), also known as "kurchi" in India, is a traditional Indian medicinal plant (Kirtikar & Basu, 2006; 2009, Nadkarni) The anti-inflammatory and anti-arthritic properties of ethanolic extract from dried HP seeds were investigated. HP (400 mg/kg once daily) When compared to Indomethacin, extract showed the greatest inhibition of rat paw edema caused by carrageenan (74.07 percent), granuloma formation (62.63 percent), and adjuvant-induced arthritic edema (77.95 percent) in rats.

2.10 Justicia Gendarussa Burm F. (Family: Acanthaceae)

In traditional Chinese and Indian medicine, Justicia gendarussa (JG) is used to treat rheumatism, arthritis, headache, earache, respiratory problems, and digestive issues with its leaves. The freund's adjuvant-induced and collagen-induced arthritic rat models were used to evaluate the JG's anti-arthritic potential. The ethanolic extract of JG was given to the animals at a dose of 100 mg/kg, and the standard drug aspirin (360 mg/kg) had significant anti-arthritic effects on the animals.

2.11 Leucas aspera (Family: Labiatae)

Leucas aspera (LA) is generally utilized for pain relieving, antipyretic, antirheumatic, calming and antibacterial treatment and its glue is applied topically to aroused regions. Using a complete Freund's adjuvant arthritis model, the ethanolic extract of LA's chronic anti-inflammatory activity was examined. Significant anti-inflammatory activity was found at 100 and 200 mg/kg (p 0.001). Histopathological examinations of the treated joint revealed complete cartilage regeneration.

2.12 Mangifera Indica L. (Family: Anacardiaceae)

Mangifera indica (MI) is the biggest natural product tree, has been as of late announced for hindering lipid peroxidation, antifungal movement and hostile to ulcerogenic activity. The monosodium urate crystals-induced gouty arthritis model in rats was used to investigate the therapeutic effects of the ethanol extract from MI. The abnormalities in ankle swelling, synovial TNF-, IL-1 mRNA, and protein levels, and oral administration of ethanolic extract (100 and 200 mg/kg, p.o. for nine days) were significantly reduced, indicating that

MI has a beneficial effect on gouty arthritis.

2.13 Psidium Guajava Linn. (Family: Myrtaceae)

In traditional medicine, Psidium guajava (PG), more commonly known as Guava, is used to treat cholera, wounds, ulcers, and bowel problems (Begum et al., 2002). Rats were tested for adjuvant-induced arthritis on PG leaves. 250 and 500 mg/kg oral doses in the complete freund's adjuvant-induced arthritis model, significant dose-dependent anti-arthritic activity was observed in PG ethanolic extract.

2.14 Sida Rhombifolia (Family: Malvaceae)

Sida rhombifolia (SR) has been utilized from ancient times for the treatment of gout in Indonesia. Using an adjuvant-induced arthritis model, the effects of SR stem and root extracts on experimental rats were evaluated. It was discovered that extract normalized the varying levels of hematological parameters. Also significantly reduced was the elevated rate of erythrocyte sedimentation.

3 DISCUSSION AND CONCLUSION

Arthritis treatment can lessen pain and assist in more effectively overcoming functional limitations. Treatment and management of arthritis may include medication, physical or occupational therapy, patient education, weight loss, and surgery. Although conventional treatment for rheumatoid arthritis is getting better, remission is rare, so treatment is still inadequate. As a result, the search for effective alternative and additional treatments for this disease continues. Due to their anti-inflammatory and immunosuppressive properties, a number of inexpensive herbal medicines have been reported in the literature to be helpful for rheumatoid arthritis. The literature of Ayurveda talks about various plants that can be used to treat painful and inflammatory conditions like arthritis, either whole, in part, or as extracts. Chamomilla majus (Linn.), Linn., Cichorium intybus Plumbago rosea Linn., Rhododendron campanulatum, Acacia polyantha and Salvadora oleoides have been traditionally used to alleviate painful inflammatory conditions, but they have not yet been tested experimentally for arthritis treatment. Although individual plant compounds or plant extracts have great potential, the underlying molecular mechanism has not been fully understood. As potential chemotherapeutic agents, it is necessary to identify these medicines' active components and monitor their safety. As a result, a scientific approach to phytotherapeutics is needed to develop novel drug delivery systems that can sustainably deliver the components to patients and reduce the need for repeated administration.

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EXPLORING THE SYNERGIES OF HPLC-SPE-NMR: A COMPREHENSIVE GUIDE TO THE HYPHENATED TECHNIQUE

Sangu Jyothi
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
Roopani Madhu
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Post-column analyte trapping by solidphase extraction is the foundation of the novel and highly promising hyphenated method known as liquid chromatography–solid phase extraction–nuclear magnetic resonance (HPLC–SPE–NMR). Deuterated solvents are then used to elute the analytes from the SPE cartridges. This aberrant HPLC-NMR hyphenation offers various benefits contrasted with direct HPLCNMR techniques. With multiple trapping, the amount of analytes that can be used in NMR is dramatically increased, which makes it possible to quickly acquire high-quality 2D NMR data. Databases and spectra catalogues are necessary for spectra comparisons due to the well-defined NMR solvent conditions.
Keywords: Solid Phase Extraction (SPE), NMR, and HPLC.

1 INTRODUCTION

The term "hyphenated techniques" refers to online connections between chromatographs and spectrometers that have attracted attention in recent years as high-throughput analytical methods that simultaneously provide mixture separation and spectra of the various components. SPE is used for storage and post-column concentration, NMR is used for detection, and LC is used for separation in LC-SPE-NMR. The powerful method of NMR spectroscopy allows for the structural elucidation of organic molecules. As a result, analyte structure and complete assignments could be achieved through HPLC-NMR coupling. However, when the analyte's eluted concentration from an HPLC column is insufficient.

In order to collect the eluting compounds from the HPLC onto the SPE cartridges, a Solid-phase Extraction unit was inserted between the NMR Spectrometer and the HPLC. Using diluted solvent, each of the trapped compounds was eluted into the NMR probe. The elutropic power and hydrogen bonding capacity of the NMR solvent have a significant impact on analyzer release from the SPE, making acetonitrile and methanol prime candidates. The utilization of HPLC/SPE/NMR can yield results that are very interesting.

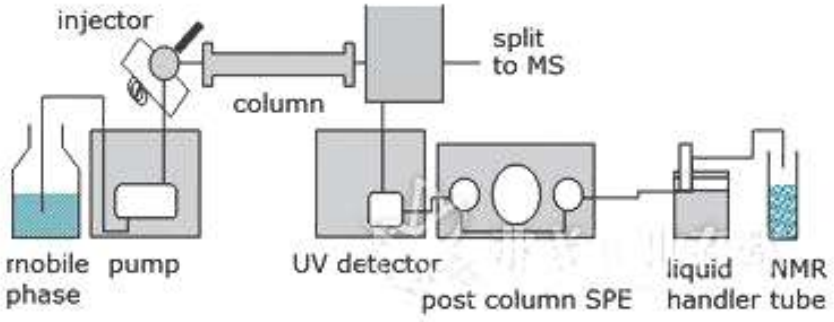

Figure (a1) Diagram of HPLC-SPE-NMR5

The offline combination of HPLC–SPE tube–NMR is typically used. This means that the flexibility of tube NMR and the advantage of automating, reproducible, and conserving the transfer of a chromatographic peak to a NMR solvent make it possible to use the best spectrometer available. It is possible to perform chromatographic separation using inexpensive nondeuterated solvents or even additives that are incompatible with NMR spectroscopy.

1.1 Features

- Since the eluent does not contain any D2O, there is no H-D exchange during the chromatographic procedure, resulting in accurate mass information.
- Very little amounts (around For the transfer, deuterated solvents (300 ml) are required.
- A small amount of liquid from the SPE cartridge (about 30 l) is used to elute the entire sample. Especially for broader peaks, this concentration effect results in a significant increase in sensitivity of 2 to 4.
- The amount and concentration of a sample can be further increased through multiple collections from subsequent chromatographic separations, increasing the sensitivity by ten or more.
- The deuterated solvent that is used for the elution and transfer is not affected by the chromatographic conditions and can be chosen to make exchangeable protons observable in the NMR and improve the spectral quality.

2 ADVANTAGES AND LIMITATIONS

- An improved NMR signal-to-noise ratio (S/N) and a number of other practical benefits accrue when an automated SPE unit is incorporated into an HPLC–NMR system for peak trapping. The catching productivity is displayed to rely upon compound extremity and is most elevated for compounds eluting late on switched stage HPLC frameworks. Again, for less polar compounds, multiple peak trapping yields the best results, further increasing the S/N.
- The method's independence from the NMR step makes it more adaptable than conventional

HPLC–NMR in terms of the HPLC and NMR solvents that can be utilized.

- The primary benefit of HPLC–SPE–NMR is the ability to focus analyte peaks after HPLC to match the volume of the NMR probe flow cell using a trapping device based on SPE. However, this necessitates optimal SPE trapping and elution conditions that must be optimized instance-by-instance at least for each analyte class.
- A promising candidate parameter for assay improvement is the quantity and composition of the post-HPLC added makeup flow used to promote analyte binding to the SPE stationary phase during the trapping procedure. Analyte discharge from the SPE is unequivocally affected by both the elutropic power and the hydrogen holding limit of the NMR dissolvable (like methanol and acetonitrile).
- As stationary SPE phases, divenylbenzene (DVB)-type polymers and RP–C18 silica are frequently utilized, with 1–2 mL/min H2O serving as the post-HPLC makeup solvent. Most published applications have been successful under these conditions. Alkaloids and organic acids, for example, are charged or polar analytes with potential drawbacks. These applications may benefit from the use of modified SPE phases, such as porous carbon materials or SAX or SCX materials. Although it is possible to trap multiple analytes in a single SPE cartridge, significant differences in the effectiveness of the various stationary phase materials have been observed.
- The analytes were eluted from the SPE cartridge using CD3CN (deuterated acetonitrile) and CD3OD (deuterated methanol). In addition, deuterated NMR solvents lessen the requirement for solvent signal suppression, which in LC–NMR typically results in the loss of useful spectral information in the vicinity of the solvent signals. 1 H NMR spectra can even be recorded without solvent suppression if multiple SPE trapping is used. Thus, a critical improvement in the nature of the NMR spectra got is noticed.
- HPLC–SPE–NMR can be performed with mobile phase systems that are typically utilized in high performance liquid chromatography diode array detection mass spectrometry (HPLC–DAD–MS/MS) setups. Any solvent mixture or buffer additive can be used, but volatile additives with few or no NMR signals, like formic acid, acetic acid, and their volatile ammonium salts, should be used to avoid precipitation in valves and capillaries. The use of semi-preparative HPLC equipment is typically not required due to the possibility of multiple SPE trappings.

3 APPLICATIONS

- For pharmaceutical impurity analysis, simple sample enrichment methods and HPLC–

SPE–NMR will also be useful. because the trapping step typically gets rid of impurities like salts and acids from previous preparative chromatographic operations.

- The black pepper petroleum ether extract was able to contain both piperine and two piperine analogues (MAO-A inhibitors) thanks to the HPLC–SPE–NMR/high-resolution MAO-A inhibition assay platform.
- Quantification and structural elucidation of the phenolic conjugates found in human urine following tea consumption.
- Reverberation spectroscopy for the examination of debasement results of V-class nerve specialists and nitrogen mustard.
- The primary use of HPLC–SPE–NMR right now is for the analysis of natural products. The confirmative HPLC–SPE–NMR analysis of known abundant secondary metabolites in unstudied plant species saves time and money by not requiring the preparation of milligram quantities for conventional tube NMR.

4 CONCLUSION

HPLC–SPE–NMR is a great technology for combining chromatography as the sample preparation and analyte separation step with NMR spectroscopy, which is necessary to characterize the structure of organic analytes. Concentrating chromatographic peaks to elution volumes that match NMR flow probes is possible with it. As a result, even chromatographic separation systems with less-than-ideal peak shapes (such as as a result of sample overload or other assay limitations, as is frequently the case with alkaloids), can be successfully transferred to the NMR instrument. Spectra can be compared using HPLC–SPE–NMR; Analyte identification can be done quickly using databases and spectra catalogues.

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REVOLUTIONIZING PHARMACEUTICAL SUPPLY CHAINS IN RESOURCE-LIMITED COUNTRIES: RETHINKING CAPACITY BUILDING STRATEGIES

Surendar Angothu
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Boggula Ratnakumari
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

1 INTRODUCTION

One of the Millennium Development Goals' targets is to ensure that all developing nations have easy access to essential medicines. Improved access to essential medicines, particularly those for HIV/AIDS, malaria, and tuberculosis, has been the result of international programs over the past decade, such as the World Bank, the United States Agency for International Development, and the Global Fund to Fight Aids, Tuberculosis, and Malaria. By the end of 2013, GAVI had received pledges totaling US$8.2 million for new and underused vaccines, while the Global Fund to Fight AIDS, Tuberculosis, and Malaria had received pledges totaling $30.5 billion and contributions totaling $25.6 billion. In addition, in their 2014 budgets, the President's Emergency Plan for AIDS Relief (pepfar) allocated US$3.2 billion exclusively for HIV and AIDS in Africa. Even though these efforts are admirable, the availability of commodities alone will not improve the impact on communities and livelihoods as a whole. The availability of essential medicines that are guaranteed to be of high quality must be linked to effective pharmaceutical supply chain management systems. Life-saving medications can be purchased, distributed, and used in a rational manner with the help of effective pharmaceutical supply management systems.

Pharmaceutical supply chain management systems remain weak in many resource-constrained nations, particularly in Africa, despite decades of significant investment in these systems in terms of resources and effort. Systems strengthening, capacity building, and technical assistance (TA) mechanisms have all been used to invest resources. The preceding raises the question of whether these initiatives have largely failed to improve supply chain efficiency. If the response is "no," then we ought to inquire about the reasons why systems continue to be weak; If the answer is "yes," then we ought to inquire as to the reasons behind the persistence of conventional, often ineffective, decades-long approaches to capacity building.

A combination of the negative and positive responses to the question above is the most appropriate response. Pharmaceutical supply chain management systems have seen some success in Sub-Saharan Africa, from Swaziland in the south to Sudan in the north, or from Zanzibar in the east to Liberia in the west. For instance, the Medical Stores Department (MSD) in Tanzania is said to function at an above-average level, while the MSDs in Rwanda and Zambia (to pick two randomly) are also said to be effective. These successes can be partially attributed to partners who have long supported supply chain programs at the central level as well as local efforts (political will). On the not-really sure side, the Focal Clinical Stores (CMS) in Malawi has kept on being tormented with difficulties. This is despite the fact that the Global Fund employed a full-time technical assistance agency for more than two years (from 2011 to 2013).

In addition, Ghana's procurement system is regarded as acceptable, whereas Liberia's National Drug Service is confronted with numerous difficulties. Direct technical assistance has occurred in all of the aforementioned instances, indicating that the various TA mechanisms have had varying degrees of success.

Not only medical stores face difficulties in Africa's supply chain systems. Systems for supply chain management continue to lack the necessary human resources in most nations. The majority of countries in the Sub-Saharan region, with the exception of South Africa, delegate to cadres who lack the necessary training or qualifications to manage medicines and other peripheral goods. In Liberia, some of the dispensers who manage medicines at service delivery points have less than seven years of formal education, while in Malawi, medicines are dispensed by assistants with no training in dispensing functions. Medical caretakers and birthing specialists shoulder the greater part of the store network capabilities at the wellbeing office level, yet most of them have not get any preparation on the best way to oversee medications. The majority of functions and skills are acquired on the job. Short training courses have frequently been offered by TA agencies over the years. It has been reported that these courses have little to no long-term impact.

Both technical and managerial skills are required for supply chain management. Technical skills are more concerned with product handling, whereas managerial skills are concerned with resource planning and management. To improve supply chain efficiency, comprehensive and trustworthy data are required for the proper management of activities and functions as well as for decision-making purposes. The absence of reliable data at all system levels, from the central to the smallest service delivery units, hinders supply chain efficiency in the majority of African nations. The quantification and forecasting of commodity requirements as well as the formulation of policies require such data. It is essential to accurately quantify needs if facilities are to have sufficient stock of medicines and other products related to them in a timely manner. Medicine shortages can result in fatalities or financial losses, both of which are undesirable events. The preparation of procurement and supply management plans—the primary instrument for monitoring the performance of grants—requires that commodity requirements be quantified based on consumption data for the Global Fund and other donor-funded programs. A quick look at some of the grants that have been awarded by the Global Fund reveals that a number of nations continue to use issues data as a proxy for consumption in order to quantify their requirements. This demonstrates that despite the number of TA agencies supporting the systems, data availability remains a challenge. To ensure that service delivery data, particularly for commodities, is readily available, technical assistance should invest more in capacitating programs.

In addition to the foregoing, irrational use of medicines persists across developing nation states. Worryingly, there is still a high rate of antimicrobial overuse. This has extreme ramifications for antimicrobials opposition, which would hit hardest the nations that can't bear more up to date age anti-toxins. Despite receiving prolonged technical assistance, these nations continue to face the aforementioned obstacles.

We advocate for a new strategy and contend that the time has come for a paradigm shift in the event that capacity building and technical assistance programs aimed at increasing supply chain efficiency have largely been ineffective. For a variety of reasons, we argue that the time is right now. First,

organizations like the People that Deliver Initiative (PtD, 2014) and the Reproductive Health Supply Coalition (RHSC, 2014) are actively working to promote supply chain efficiency worldwide. Furthermore, beneficiary nations are calling for change. They have been increasingly integrating approaches to addressing challenges in supply chain systems because they are aware that long-lasting solutions depend on their ability to solve their own problems. For instance, Rwanda has developed an integrated supply chain system that oversees the majority of goods from a single repository to service delivery points. With few to no shortages of essential medicines and other products, this strategy seems to be working well for the country. Finally, new approaches to capacity building for supply chain systems necessitate the involvement of the recipient nation and the assistance of capacity building services agencies in order to be acceptable and effective. This way, the process will continue even if the user exits the system. This argument is based on the fact that the majority of programs have a fixed duration and that developing nations frequently lack the capacity to manage program terminations. To expand on this argument, we provide a fundamental definition of what we mean by capacity building services.

2 WHAT IS CAPACITY BUILDING?

The process of improving an individual, group, or unit of an organization's capacity to carry out tasks in an efficient, sustainable, and effective manner is known as capacity building. Training is frequently mistakenly equated with capacity building. Workshops are currently viewed as a "magic bullet" for overcoming any obstacle in the current strategy for building pharmaceutical supply chain management capacity. Training is suggested as a solution in the event that there is a performance gap. Individual abilities, on the other hand, are just one component of a much larger set of factors that make up the capacity to consistently and effectively carry out particular tasks over time. Without adequate supplies and equipment, proper motivation, management support, and a positive relationship with the community they serve, individual health workers, regardless of their level of expertise, are unlikely to effectively deliver essential medicines or services. Limit building administrations are expected for these areas to guarantee execution objectives are accomplished.

2.1 Attributes of capacity building for pharmaceutical supply chain management systems

Managers and policymakers are concerned about capacity because it makes it possible for good performance and increases efficiency in the pharmaceutical supply chain. For instance, a healthcare facility that frequently experiences stock-outs of pharmaceuticals may require additional capacity for quantifying their requirements (such as interventions tailored to the particular performance objective of commodity supply). As a result, a method tailored to the underlying cause of the issue would be necessary for a capacity development strategy to enhance pharmaceutical supply. In this perspective limit can be seen as a moving objective. Capacity can rise or fall at any given time. It usually progresses over time in stages that show that you are more ready to influence performance. As a result, capacity building is an ongoing process whose stages are referred to as "development outcomes."

2.2 Why is Capacity Building Necessary?

In the context of systems for supply chain management, resources are invested with the ultimate objective of providing the populace with essential medicines and

goods related to them. The most money is spent on medicines in every hospital system. Additionally, proper medicine management is synonymous with thrifty financial management because medicines are costly commodities. It costs money to let medicines expire at a facility, while non-delivery, late delivery, or shortages cost lives. As a result, it is critical that those in charge of managing these necessary resources do so effectively and efficiently. For individuals and organizations alike, new or improved capabilities are often required to translate newly acquired skills into sustained performance. In this sense, capacity refers to the capacity to effectively utilize resources and maintain gains in performance despite gradually decreasing levels of external support. In this paper, we concentrate on the sustenance with less external support, i.e., the capacity of supply chain systems in developing nations to sustain performance in the face of gradually decreasing levels of support from TA agencies and donor contributions.

2.3 Capacity building for Procurement and Supply Management (PSM) systems: The Global Fund Model

Several nations have benefited from the Global Fund over the past ten years. In contrast, large commodity-based programs in developing nations have been supported by other programs like PEPFAR and PMI, which typically came packaged with ready-to-use TA. In contrast, the Global Fund model requires that the country or Principal Recipient (PR) be in charge of implementing the program. The achievement of particular programmatic benchmarks is used to measure success. The Worldwide Asset awards considers framework reinforcing mediations remembering effective money management for HR for wellbeing. Global Fund PRs are encouraged to identify challenges in procurement and supply management (PSM) and implement interventions to address these challenges in this context. However, despite the fact that the Global Fund has been in operation for a number of years, many recipient nations' PSM systems remain inadequate and continue to significantly increase grant signature lead time. This strengthens the need for a paradigm shift in how TA is provided and raises questions about why systems remain weak despite years of material and financial investments.

The Global Fund established the Procurement Support Service to facilitate grant recipients' access to Capacity Building Services/Supply Chain Management Assistance (CBS/SCMA) and Voluntary Pooled Procurement (VPP) in an effort to address the PR PSM challenges mentioned earlier. The procurement support service was a coordinated approach to assisting nations with supply chain management issues and bottlenecks in the procurement process in order to speed up access to medical supplies and pharmaceuticals. The CBS/SCMA component focused on both short-term and long-term interventions with the goal of making sure that in-country or programmatic PSM systems get better and stay around. The SCMA/CBS model claimed to give PRs the ability to identify their own TA requirements and hire them to boost grants' performance. However, only a few PRs appear to have benefited from the CBS program's speculation. It is unclear why a program with so much promise has not been strengthened or expanded to have a lasting effect.

With the goal of capacity-development interventions that result in a measurable improvement in performance, innovative approaches that build on success factors of assistance should be used in addition to focusing on the countries and programs that received the assistance. The progress of numerous limit improvement endeavors has been restricted by the way that they center

around specialized factors, while basic social and political obstructions are disregarded. The significance of top-level commitment and leadership, a favorable external environment, and the effective management of organizational change processes is highlighted by evidence as well as experiences in other areas. When thinking of a new way to make TA programs for supply chain management more effective, all of these factors would need to be taken into account.

Even though training programs are still important, they should not be viewed as the "magic bullet" for building capacity. However, for these to be effective, they need to address the underlying issues. Sustainable training should begin with the creation of appropriate curricula for various performance levels and the various cadres involved in supply chain functions in order to build capacity in supply chain management. These include programs for pharmacists, pharmacy technicians, and sometimes nurses. The majority of pharmacy curricula in Sub-Saharan Africa lack sufficient supply chain management content. However, pharmacists are increasingly being replaced by supply chain managers. Other cadres should take advantage of the opportunity to hold this crucial position for ensuring commodity security.

3 CONCLUSION

Throughout the long term there has been expanded financing endeavors pointed toward further developing production network frameworks of low-pay nations. It has been demonstrated that these efforts have little effect. How a country builds its capacity to manage medicines and related goods needs to change fundamentally.

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MUCOADHESIVE MICROSPHERES: A PROMISING APPROACH FOR TARGETED DRUG DELIVERY – A COMPREHENSIVE REVIEW

Usha Kondla
Assoc. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India
Dayyala Lavanya
Asst. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - This article's goal is to go over the fundamentals of making and evaluating mucoadhesive microspheres, as well as the research done on these systems. The design of drug delivery systems is currently focused on the issue of mucoadhesion. The adhesion of two materials, at least one of which is a mucosal surface, is commonly referred to as mucoadhesion. Mucoadhesive microspheres can be made to have a longer residence time at the site of application or absorption, make it easier to get close to the underlying absorption surface, and help drugs work better or better as a treatment. For either systemic or local effects, these mucoadhesive microspheres have recently been developed for oral, buccal, nasal, ocular, rectal, and vaginal routes. The purpose of this review article is to provide an overview of the various aspects of mucoadhesion, including mucoadhesive materials, factors that affect mucoadhesion, methods for evaluating them, and a variety of mucoadhesive drug delivery systems.

Keywords: Microspheres, site-specific, mucoadhesion, and the mechanism of mucoadhesion

1 INTRODUCTION

1.1 Microspheres

Microspheres are solid, roughly spherical particles with sizes ranging from 1 to 1000 m. During the manufacturing process or afterward through absorption, substances can be incorporated into microspheres in either a liquid or a solid state. Microparticles or microspheres are general wordings that include both microcapsule and micromatrix. Microcapsules, where the ensnared substance is totally encircled by an unmistakable container wall, and micromatrices, where the entangled substance is scattered all through the microsphere matrix.1

1.2 Mucoadhesive Microspheres

By developing novel delivery systems known as "mucoadhesive microspheres" and coupling microspheres with mucoadhesion characteristics, mucoadhesive microspheres can be made. “6 In general, microspheres have the potential to be utilized for controlled and targeted drug delivery; However, there are additional advantages to coupling mucoadhesive properties to microspheres, such as efficient absorption and improved bioavailability of drugs due to a high surface-to-volume ratio, a much closer contact with the mucus layer, and specific drug targeting to the absorption site achieved by anchoring plant lectins, bacterial adhesions, and antibodies,etc. on the microspheres' surface.

Over the past few years, there has been a lot of interest in the potential use of microspheres in pharmaceuticals. One method called microencapsulation is used to control and delay drug release, which prolongs therapeutic activity. Compared to conventional formulations, it offers greater effectiveness, lower toxicity, lower dosing, and longer-lasting stability. Mucoadhesive microspheres can be customized to stick to any mucosal tissue

remembering those found for eye, nasal depression, urinary and gastrointestinal lot, in this manner offering the potential outcomes of restricted as well as fundamental controlled arrival of medications.

1.3 Advantages of Mucoadhesive Microspheres

The formulation stays longer at the delivery site due to adhesion and intimate contact 8, which increases API bioavailability. It may be permitted to treat the disease with lower API concentrations for. It is a great way to deliver drugs to the whole body that have a high first-pass metabolism, increasing their bioavailability. By utilizing specific bioadhesive molecules, drug molecules can be targeted at specific sites or tissues, such as the gastrointestinal (GI) tract. It reduces administration frequency by increasing formulation residence time at the target site and controlling API release. Due to API localization at the disease site, significant cost reductions and dose-related side effects may also be achieved. As a result, patient compliance will rise and medication administration will become more convenient. It improves drug absorption by causing uniform and extensive drug distribution throughout the gastrointestinal tract. The drug is released over an extended period of time. It keeps the amount of drug in the therapeutic plasma constant. The disease condition is better controlled and the intensity of local or systemic side effects is reduced when steady state levels fluctuate less. The processability (more soluble, dispersible, and flowable) is improved. Because plasma levels are better controlled, high-potency drugs have a wider margin of safety. Drugs that break down in the acidic or alkaline environment of the intestine, such as buccal, sublingual, or vaginal, can be given through this route.

2 METHODS OF PREPARATION OF MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres can be prepared by using different techniques like:

2.1 Complex Coacervation

The coating material phase and the core material are continuously stirred into a coating polymer solution after being prepared by dissolving immiscible polymer in a suitable vehicle. One of the techniques for phase separation, namely altering the temperature of the polymer solution, was used to achieve microencapsulation; by altering the medium's pH, by adding a salt, an incompatible polymer, or a non-solvent to the polymer solution, or by changing the medium's pH. by creating an interaction between polymers. The principle of this method is that when solutions of two hydrophilic colloids are mixed under suitable conditions, it results in a separation of liquid precipitate. Typically, coatings are hardened by thermal cross linking or desolvation techniques to form a self-sustaining microsphere.

2.2 Hot Melt Microencapsulation

After melting the polymer, the solid drug particles are continuously mixed into it. After that, the prepared mixture is continuously stirred into a non-miscible solvent like silicone oil before being heated to a temperature above the polymer's melting point and stabilized as an emulsion. After cooling the emulsion to solidify the polymer particles, the microspheres are filtered and washed with petroleum ether.

2.3 Emulsion Solvent Evaporation Method

Polymer is dissolved in an organic solvent in this method, typically methylene chloride. The drug is then dispersed or dissolved within it. Droplets can be formed by dispersing the polymer and drug solution in an aqueous phase. The

more volatile organic solvent can be evaporated using constant mixing and high temperatures, leaving the solid polymer–drug particles suspended in an aqueous medium. The suspension is then filtered out of the particles. If the drug is water-soluble, an organic solvent immiscible solvent is used instead of an aqueous phase as the external phase

2.4 Solvent Removal

The method involves dissolving the drug and the polymer in a volatile organic solvent. This solution is then suspended in silicone oil containing span and methylene chloride and stirred until the solvent is extracted from the oil solution. Petroleum ether is then added and stirred until the solvent is removed. After that, the microspheres were dried in a vacuum, a non-aqueous microencapsulation technique that works best with water-labile polymers like polyanhydrides.

3 MATERIALS USED IN THE FORMULATION OF MUCOADHESIVE MICROSPHERES

Mucoadhesive microspheres are made up by using mucoadhesive polymers. Mucoadhesive delivery systems are being explored for the localization of the active agents to a particular site. Polymers have played a significant role in designing such systems so as to enhance the residence time of the active agent at the desired location. Polymers used in mucosal delivery system may be of natural or synthetic origin.

Mucoadhesive polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad classes:

- Polymers that become sticky on placing them in water and achieve their mucoadhesion due to stickiness.
- Polymers that adhere through nonspecific, noncovalent interactions that is primarily electrostatic in nature.
- Polymers that bind to specific receptor site on tile self surface.

3.1 Characteristics of an Ideal Mucoadhesive Polymer

- It should be nonirritant to the mucus membrane.
- The chain length of polymers must be long enough to promote the interpenetration and it should not be too long that diffusion becomes a problem, but as the cross linking increases, the chain mobility decreases which reduces the mucoadhesive strength.
- It should adhere quickly to most tissue and should possess some site specificity.
- The polymer and its degradation products should be nontoxic and should be no absorbable from the GI tract.
- It should preferably form a strong no covalent bond with the mucin epithelial cell surfaces.
- It should allow easy incorporation of the drug and should offer no hindrance to its release.
- It should posses sufficient high viscosity.
- The polymers must not decompose on storage or during the shelf life of the dosage form.
- The cost of polymer should not be high so that the prepared dosage form remains competitive.

4 CONCLUSION

In recent years, there has been a lot of interest in novel drug delivery systems in the field of modern pharmaceutical formulations. Due to their advantages of controlled and sustained release action as well as their versatility as a drug carrier, mucoadhesive microspheres have been attracting a lot of interest from various researchers and scholars. Mucoadhesive microspheres provide a novel pharmaceutical carrier system. With

these new specific targeting compounds (lectins, thiomers, etc.), mucoadhesion has undoubtedly entered a new domain. with scientists and medication organizations looking further into expected contribution of additional more modest complex particles, proteins and peptides, and DNA for future mechanical progression in the everevolving drug conveyance field. Mucoadhesive microspheres have been shown to be a promising method for delivering drugs to a specific site in a controlled or sustained manner. Because they deliver the drug to a specific site for a longer period of time, the drug's absorption increases, increasing its bioavailability. As a result, it can be said that mucoadhesive microspheres will also play a significant role in the future development of new pharmaceuticals made with more cutting-edge materials and methods.

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REVOLUTIONIZING CANCER TREATMENT: AN OVERVIEW OF NOVEL DRUG DELIVERY APPROACHES IN CANCER THERAPY

A Mallikarjun
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Anumula Narender Reddy
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Improved therapy by increasing the efficacy and duration of drug activity, as well as improved patient compliance through a reduction in dosing frequency, are just two of the many aspects of novel drug delivery systems (NDDS) that are of interest. In order to anticipate negative reactions, it provides appropriate management channels and enhanced targeting for a particular website. Bioactive and plant extracts have been used to express a variety of advanced drug delivery strategies, including polymeric nanocapsules, nanoparticles, liposomes, hydrogels, microspheres, and microcapsules. Improved solubility and bioavailability, low toxicity, maximum therapeutic effect, sustained and controlled drug delivery, improved stability, and batter security from physical and biochemical degradation are all significant advantages of NDDS over conventional cancer treatment. The fundamentals of novel drug delivery systems and their various types are discussed in this article.

Keywords: Hydrogel, new drug delivery system, drug release, dosage forms, cancer treatment, nanoparticles, liposomes, microspheres, and microemulsion.

1 INTRODUCTION

One of the major threats to global public health is cancer. The treatment of cancer still faces numerous difficulties. We are able to develop novel therapeutic and diagnostic strategies because of the great potential of NDDS in cancer therapy.

The specific method by which a drug is delivered can have a significant impact on its sufficiency. There are a few medications that have an ideal concentration range from which the greatest benefit can be derived; concentrations above or below this range can be harmful or do not result in therapeutic benefit.

There should be two main requirements for advanced drug delivery carriers. First and foremost, delivery must be rate-specific. Second, it should be unique to the location. None of these are possible for conventional dosage forms. There are different classes of NDDS. 1) A drug delivery system with sustained release. 2) Drug delivery system with controlled release. A drug delivery system is made to have a delayed therapeutic effect that lasts for a long time.

1.1 Advantages:

Less frequent drug administration leads to increased patient convenience and compliance; an increase in the safety margin; maximum drug

utilization; and less frequent dosing, among other benefits Negative aspects: Reduced systemic availability, inadequate in-vivo and in-vitro correlation, the possibility of dose dumping, and other factors Controlled drug delivery occurs after the drug is distributed locally or systemically at a predetermined rate. Zero-order drug release is excellent with controlled release. Lower dose concentration and frequency of administration, Lower GI toxicity; Greater patient acceptance

1.2 Disadvantages:

1) Portion unloading
2) Diminish potential for exact portion estimation
3) Need of additional patient guidance
4) Soundness issue.

The most preferred route of administration is defined as oral, parenteral, transdermal, and inhalation. Biodegradable and non-biodegradable polymers, such as polyethers, polyesters, polysaccharides, poly amino-acids, and proteins, have been used to investigate NDDS strategies for local medication, such as nanoparticles, microspheres, polymeric micelles, liposomes, and hydrogel systems, for targeting and controlled release. These polymers are mostly used as a parenteral drug delivery system in cancer treatment. These polymers are well-known for reducing harmful reactions and enhancing their anticancer drug effects.

2 DIFFERENT DRUG DELIVERY SYSTEM

2.1 Lipoprotein

A suitable amount of active therapeutic drug must be assimilated and transported to the site of action at the appropriate time and rate in any ideal drug delivery system.

Lipoprotein can be used as a targeted drug delivery system in cancer therapy, which helps to improve the therapeutic index of anticancer agents by either increasing the concentration of medication in tumor cells or decreasing the interaction in normal host tissues. Lipoprotein can be used as a medication system for cancer therapy. A potential transporter for chemotherapeutic mediators is low density lipoprotein. Because some types of cancer cells have a higher level of receptor-mediated uptake of low density lipoprotein, they are used for the targeted delivery of anticancer drugs. Liposomes and phospholipid vesicles have been identified as a potential drug delivery system for clinical cancer therapy. This system safeguards healthy cells from toxic effects and maintains their concentration in vulnerable tissues, such as the patient's kidneys and liver.

3 NANOPARTICLE

The solid state of nanoparticles is either amorphous or crystalline, and their sizes range from 10 to 200 nm. It protects the drug from chemical and enzymatic degradation. As carriers for gene therapy, biodegradable polymeric nanoparticles have limited applications in the controlled release of therapeutic medications that target specific organs or tissues.

Gold nanoparticles, nanotubes, nanowires, nanoshells, quantum dots, and other types of nanomaterial

3.1 Nanoemulsion

Nano emulsions can be described as oil-in-water (o/w) emulsions with a mean droplet size of 50 to 200 nm. The particles can also exist in oil-in-water and water-in-oil forms, with the core of the particle made of either oil or water. Microemulsion-like nano-emulsions may exhibit high kinetic constancy and optical transparency.

3.2 Microcapsules

Numerous cancer treatments, including paclitaxel and PCT; CPT, camptothecin; Additionally, the stumpy aqueous solubility of some porphyrins, such as meso tetraphenylporphine, TPP, which is used in photodynamic therapy (PDT), hinders their application and makes direct parenteral administration more difficult. To overcome their low solubility, low stability, and dangerous symptoms, novel drug delivery strategies based on drug carrier systems approaches have been recommended. PEG diacyllipid conjugates have received a lot of attention due to their excellent pharmacological properties and easy controllable properties.

3.3 Microemulsion

Microemulsions are defined as homogenous, transparent, or translucent liquid scatterings of water and oil that are made thermodynamically stable by adding a relatively high concentration of a surfactant and a surfactant. Microemulsion droplets have been extensively considered as a targeted drug delivery system to the brain, with diameters ranging from 10 to 100 nm. It is a cost-effective strategy that increases the bioavailability of medications that are difficult to dissolve.

3.4 Microspheres

The most recent innovation in cancer chemotherapy is microsphere technology. Particles that are solid and porous and have diameters ranging from 1 to 100m Through chemoembolization, or physical trapping in blood vessels, it can focus on their medication load and maintain therapeutic agent action through controlled release. By placing therapeutic drug in the vessels that lead to the end organ, biodegradable microspheres are utilized for the direct delivery of drugs to the organ(s). The microsphere's size and mode of administration—intravenous or intra-arterial—determine its effect.

3.5 Dendrimers

Dendrimers are monodisperse molecules with highly controlled structures that are highly branched in three dimensions. Its monodispersed, epitome capacity, water dissolvability and tremendous number of fringe practical gatherings, make them ideal possibility for appraisal as medicine conveyance framework. Dendrimers have recently been utilized in a variety of cancer therapies as a drug delivery system.

Dendrimers are primarily used for drug delivery in one of three ways: either by attaching the drug to the periphery of the dendrimers with a covalent bond to form dendrimers

pro-drugs, or by synchronizing the drug with the outer functional groups through ionic interactions or host-guest supramolecular assembly.

4 HYDROGELS

Polymers encased in water form three-dimensional networks called hydrogels. It typically consists of hydrophilic polymers that are crosslinked either by covalent bonds or by physical intramolecular and intermolecular attractions that cause them to swell readily and not dissolve in water. Hydrogels are an ideal class of materials for biomedical applications like drug delivery and tissue engineering due to their unique capacity to expand in biological conditions. Due to the presence of hydrophilic moieties like carboxyl, amide, amino, and hydroxyl groups, hydrogels are highly hydrophilic in nature.

5 CONCLUSION

For formulation researchers, novel drug delivery systems will present an opportunity to overcome numerous issues with conventional systems. Chemotherapy for cancer has long been recognized as an exceptional need for cutting-edge drug therapy. Microspheres, nanoparticles, lipoproteins, and others since advanced drug delivery systems have emerged as a promising area for new research and have been identified as excellent candidates with significant promise in the field of novel drug delivery systems.

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ENHANCING DISSOLUTION RATE OF PARACETAMOL THROUGH SOLID DISPERSION AND POLYMER COATING TECHNIQUES FOR RAPID DISPERSIBLE TABLET FORMULATION

Dr. Kadasi Sundeep
Assoc. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
Hariprasad Kadiyam
Assoc. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The fundamental requirement for achieving a rapid onset of action is the concept of dosage forms that are rapid dispersible or fast dissolving. Paracetamol is a common over-the-counter pain reliever and antipyretic that doesn't cause gastric irritation or ulcers. Due to its low solubility, the rate-limiting step in paracetamol absorption is its dissolution. In the case of pediatrics, developing a high-quality formulation is further complicated by the active's bitter taste. The goal of the current study was to develop rapid dispersible paracetamol tablets by coating them in polymer and using solid dispersion to improve solubility and flavor masking. Different carriers in solid dispersion were used to make different paracetamol formulations, and a thin layer of polymer coating was used to hide the taste. The detailing was than assessed for different physical and logical properties of fast dispersible tablets. Results got showed that there was a critical effect of transporters utilized during definition of Strong scattering of paracetamol. Using Mannitol and Betacyclodextrin as carriers in the solid dispersion phase of paracetamol, a rapid release was observed. The organoleptic properties of formulations A-5 and A-6 were also found to be superior to those of other formulations and acceptable.
Keywords: Tablets with Rapid Dispersibility, Solubility Enhancement, Solid Dispersion, Taste Masking, and a Dissolution Profile.

1 INTRODUCTION

The novel drug delivery system concept known as a rapid disintegrating drug delivery system was developed to address the fundamental flaws of conventional tablets. Based on recent advancements, dispersible tablets can be distinguished into two categories: one that dissolves or dissolves directly in the mouth without the need to drink water; the other, on the other hand, needs water to form a dispersion in a matter of seconds and is simple for the patient to take. In both the cases, bioavailability of medication is fundamentally more noteworthy because of moment scattering and solvency than those saw from ordinary dose form. Paracetamol (4'- hydroxyacetanilide, N-acetyl p-aminophenol, acetaminophen, Standard) is a broadly utilized over-the-counter pain relieving and antipyretic medication with practically no gastric disturbance and ulcerative effects. Paracetamol is white glasslike powder having unpleasant in taste. As per Biopharmaceutical Grouping framework, Paracetamol is a class IV, a low solvent and low porous drug. A medication with poor fluid dissolvability will commonly show disintegration rate restricted retention, and a medication with unfortunate layer porousness will regularly display saturation rate restricted absorption. Consequently, 'Definition researcher' centers around two regions for working on the oral bioavailability of medications include: (The Hydrotropy methods, the solid dispersion method, the use of carrier as co solvent, and the uses of surfactants, superdisintegrants, and polymers are some commonly used

approaches for enhancement of aqueous solubility of formulations that have been reported in literature. However, all these formulation concepts were focusing on the enhancement of solubility and release profile of active, and the organoleptic prosperities such as taste and patient compliance is still required to be developed with an effective release profile of formulation. The ebb and flow research work is plan to figure out a quick dispersible Tablets of Paracetamol with further developed disintegration qualities of medication with joined approach of strong scattering solvency upgrade and polymer covering on strong scattering granules of paracetamol to work on the organoleptic properties for definition. The two technologies work together to make paracetamol more soluble and better at masking the bitter taste. The study's intended release profile called for at least 85 percent of release to occur within 15 minutes. The formulation is primarily intended for use by children and the elderly.

2 MATERIALS AND METHODS

Elder Pharmaceuticals Ltd., Navi Mumbai, India, provided a sample of Paracetamol, Urea, and Eudragit-EPO. Cadila Pharmaceuticals Limited, based in Ahmadabad, India, provided a complimentary sample of Aspartame and Flavor Vanilla. From commercial sources, sodium starch glycolate, polyethylene glycol, lactose monohydrate, cyclodextrin, mannitol, methanol, hypromellose, talc, magnesium stearate, and ac-di-sol were obtained.

2.1 Solubility Enhancement of Paracetamol

Mayersohn and Gibaldi used the solid dispersion, also known as the solid-state dispersions, for the first time while researching various dispersion techniques. One of the most common methods for increasing the bioavailability and solubility of water-insoluble drugs is solid dispersion. The choice of the carrier ultimately has an impact on the dissolution characteristics of the dispersed drug because the second component mixture has an effect on the rate at which a component dissolves from a surface. Consequently a water solvent transporter brings about a quick arrival of medication from the blend.

3 EVALUATION OF SOLID DISPERSION

By Fourier Transform Infrared (FTIR) Spectrum Studies

In order to evaluate the impact of solid dispersion of Paracetamol with other excipients, the solid dispersion of Paracetamol with various carriers was placed using FTIR (Shimadzu). Using the KBR disc method, the FTIR spectra of each and every sample were recorded on Perkin Elmer instruments. The solid paracetamol dispersion was mixed with potassium bromide (KBr), triturated in a glass mortar, and finally placed in the sample holder for sample preparation. The sample was scanned in the range of 4000 to 400 cm-1 in frequency.

3.1 Drug content of Solid Dispersion of Paracetamol

The examination for drug content of definition was created in view of monograph of Paracetamol in English pharmacopeia. 1.0 grams of solid dispersion paracetamol were used to evaluate each formulation's assay. In a 200 ml volumetric flask, accurately weigh a quantity of powder equivalent to 150 mg of paracetamol. Add 50 milliliters of 0.1 M sodium hydroxide to 100 milliliters of water, shake for 15 minutes, and then dilute with water up to the limit. Mix, filter, and add 100 milliliters of water to 10 milliliters of the filtrate. Add 10 ml of 0.1M sodium hydroxide diluted to volume with water to the remaining 10 ml of the solution in a 100 ml volumetric flask, and then measure the maximum absorbance of the solution at 257 nm.

3.2 Dissolution Procedure

Disintegration of tablets was started by putting one tablet in every one of six vessels containing 900 ml disintegration medium, involving paddle at 50 rpm for an hour. Filter after removing 10 milliliters of the sample solution from each dissolution vessel at predetermined intervals. By adding dissolution media to the dissolution vessel, the same volume of the sample that was taken out is used to replace it. Dilute the filtrate to the mark with 0.1 M sodium hydroxide solution by transferring 5ml of it to a 100ml volumetric flask. Utilizing a UV Spectrophotometer (Shimadzu) at a wavelength of approximately 257nm and 0.1M Sodium Hydroxide as the blank solution, measure the absorbance of the sample solution.

3.3 Fourier Transform Infrared (FTIR)

Spectrum Studies FTIR studies were utilized to clarify the drug-carrier interaction. The drug's presence in the formulation was confirmed by comparing the IR spectra of solid dispersions and pure drug. Unsaturation absorbance bands at 1653 and 1610 cm-1, and aromatic absorbance bands at 1565 and 1502 cm-1. These bands shifted in a variety of solid dispersion formulations as a result of complexation between the drug and the carrier. This indicates that a complex was formed during solvent evaporation and that the drug was not degraded as a result of using solvent evaporation techniques for solid dispersion with various carriers. However, there was no additional peak observed in the paracetamol solid dispersion spectrum, indicating that there was no chemical interaction or degradation.

3.4 Drug content of Solid Dispersion of Paracetamol

In the observation, the drug contents of various solid dispersion paracetamol formulations were analyzed and tabulated. The assay values for paracetamol in various solid dispersion samples ranged from 97.50 percent to 101.2%. The process loss of the drug during the solid dispersion stage could be the cause of the lower Paracetamol assay.
The evaluation revealed a range of the mean value between 3.5 and 5. In terms of taste, the formulation with SSG, PEG, and lactose as a carrier was found to be less popular than the formulation with betacyclodextrin, urea, and mannitol. Among all of the taste mask formulations for rapid dispersible paracetamol tablets, it was observed that the formulation with betacyclodextrin and mannitol displayed the most pleasant flavor.

4 CONCLUSION

Based on a variety of physical and analytical evaluations of the formulation, the combination of solid dispersion technology and taste masking with a polymer coating of Paracetamol can easily achieve a rapid dispersion and prompt release profile with improved organoleptic properties. With better bioavailability of the active due to the active's rapid absorption into the systemic circulation, the combined approach demonstrated a promising effect in the formulation's acceptance by a targeted group of pediatrics and geriatric patients. The evaluation of formulations A-5 and A-6 in comparison to other formulations revealed a more favorable release profile and acceptable organoleptic properties, demonstrating the advantages of combined technology for formulation over single technologies.

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EXPLORING THE CORRELATION BETWEEN LAMOTRIGINE AND GABA LEVELS IN CEREBROSPINAL FLUID: A LINEAR RELATIONSHIP ANALYSIS

Dr. Kokkula Satyanarayana
Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Sunitha Chintala
Asso. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract- Anxiety, insomnia, depression, and bipolar disorder can all be treated with the assistance of GABA-mediated neurotransmission. The point of the current review is to supply proof about neurochemical impacts of intense lamotrigine therapy on GABA and L-glutamic corrosive levels in the cerebrospinal liquid of Wistar Pale skinned person rodents and the association of cholinergic framework. Microdialysis experiments were carried out in a conscious rat model one day after concentric microdialysis probes were inserted into the lateral ventricles of rats. Either 20 mg/kg lamotrigine or physiological saline were injected into the rats' peritoneum. For evaluating the cholinergic contribution 0.5 mg/kg physostigmine or 2 mg/kg atropine sulfate pre-medicines were given before lamotrigine infusion. High Performance Liquid Chromatography was used to analyze the dialysate concentrations of GABA, L-glutamic acid, and lamotrigine. While lamotrigine treatment significantly increased GABA concentrations (p 0.05), saline did not alter L-glutamic acid or GABA concentrations. Physostigmine or atropine sulfate pretreatment had no significant effect on GABA or L-glutamic acid levels. Lamotrigine-induced GABA levels were unaffected by pre-treatments with physostigmine or atropine sulfate. Given that there is a linear relationship between lamotrigine and GABA, the findings may suggest that lamotrigine-induced GABA contributes to the pharmacological effects of lamotrigine. However, lamotrigine-induced GABA release is unaffected by the central cholinergic system.
Keywords: Microdialysis, the cholinergic system, glutamate, and the conscious rat model.

1 INTRODUCTION

As a relatively new antiepileptic, lamotrigine is currently being widely used as the first-line treatment for certain types of seizures, such as Lennaux-Gestaut syndrome, grand or petit mal seizures, and myoclonus. Additionally, lamotrigine has been widely favored for bipolar disorders. As the brain's primary inhibitory neurotransmitter, amino butyric acid (GABA) has been linked to the pathophysiology of bipolar disorder. Lamotrigine's anti-glutamergic effects may also be influenced by GABAergic potentiation, according to previous research.

Lamotrigine blocks the exocytosis of these excitatory amino acids by inhibiting Na+ channels, thereby reducing the release of glutamate and aspartate. The job for restraint of Ca2+ channels was additionally illustrated. Lamotrigine has previously been shown to reduce glutamate and increase GABA release without affecting Na+ or Ca2+ channels in rat entorhinal cortex using whole patch clamped cells. In rat amygdale cells, it was also reported that lamotrigine affects presynaptic Ca2+ influx, which in turn suppresses GABAA-mediated neurotransmission. Lamotrigine likewise diminishes veratrineor electrically animated arrival of endogenous glutamate and [3H]-GABA, [3H]-5-hydroxytriptamine and [3H]-dopamine in rodent cortical cuts. Ex vivo studies, on the other hand, showed that while acute lamotrigine did not affect the amount of GABA or taurine

in hippocampal tissue, chronic treatment with the drug did.

The relationship between the cholinergic system and GABAergic transmission in the central nervous system has been studied for a long time. GABA and its analogues directly inhibit cortical acetylcholine release in freely moving guinea pigs and electrically stimulated slices, as previously demonstrated in vivo and in vitro. Some findings suggested that acetylcholine exerts direct inhibitory effects on GABA release at least in other brain areas and in the periphery. Although the excitation of GABAergic inter neurons in the cerebral cortex has been attributed to the local inhibitory effects of acetylcholine.

By measuring the amino acids dialyzed through microdialysis probes implanted into the lateral ventricles of conscious rats, this study aims to monitor the time-course changes of GABA and L-glutamic acid in rat cerebrospinal fluid produced by lamotrigine treatment and to demonstrate the potential modulatory effect of the cholinergic system on the lamotrigine-induced amino acid release.

2 MATERIALS AND METHODS

2.1. Animals and laboratory

Both sexes of Wistar albino rats weighing between 250 and 275 g were used, and they were obtained from Marmara University's Experimental Research and Animal Laboratory. Before the experiments took place (16.12.2005 - 63.2005.mar), the Ethical Committee for Experimental Animals at Marmara University granted their approval. The animals were fed standard animal food and water ad libitum in a temperature-controlled room with a 12-hour light and dark cycle.

2.2 Drugs used in the study

All medications were provided from Sigma Synthetic (USA) with the exception of lamotrigine (provided benevolent from GlaxoSmithKline, Turkey). Before being injected, physiological saline was used to dissolve lamotrigine.

2.3 Stereotaxic Surgery and Microdialysis

As previously mentioned, concentrated microdialysis probes were utilized. A stereotaxic frame (Stoelting, Model 51600, USA) and intraperitoneal ketamine (100 mg/kg) and chlorpromazine (1.0 mg/kg) mixture were used to anesthetize the rats. The periosteum and cranium were cut from the skin of the scalp. The right lateral ventricle was the site of the probe's placement (lateral ventricle coordinates; According to the Paxinos and Watson rat brain atlas, it is 3.8 mm ventral to the surface of the skull, 1.5 mm lateral to the midline, and 1.0 mm posterior to the bregma. Additionally, supporting screws were inserted, and dental acrylic cement was applied to the screws and the microdialysis probe. 24 hours after surgery, samples of intracerebral perfusion were collected.

Polyethylene tubings were attached to the microdialysis probes' inlet the day after they were placed to collect samples from a conscious rat model housed in a 42X42X20 cm plexiglass cage. Fake cerebrospinal liquid was conveyed consistently by means of 250 µl hamilton needle which was associated with a microinfusion siphon (KD Logical, USA). The pH of the artificial cerebrospinal fluid was set to 7, and its composition consisted of 2.5 mM KCl, 125 mM NaCl, 1.26 mM CaCl22H2O, 1.18 mM MgCl26H2O, and 0.2 mM NaH2PO42H2O. Filtering the artificial cerebrospinal fluid was done with nylon membrane filters of 0.4 m.

After an equilibration period of one hour, two basal samples were taken from Wistar rats in 0.25 ml ependorf tubes at a flow rate of 0.5 l/minute every 40 minutes. Five more samples were taken in succession after an intraperitoneal physiological saline injection was given. A similar convention was rehashed with

lamotrigine (20 mg/kg), physostigmine (0.5 mg/kg) or atropine sulfate (2 mg/kg). During the microdialysis procedure, the rats were observed and atypical behaviors were noted. The dialysates were divided into two equal ependorf tubes for various High Performance Liquid Chromatography analysis methods and kept at -80oC. Methylene blue was injected through the probe and the rats were decapitated after being anesthetized with ether. To verify the placement of the probe, the brains were sliced with a knife to observe the dye in the ventricles. In the data analysis, only the appropriate experiments were used.

3 DISCUSSION

The current study demonstrates that rats receiving 20 mg/kg of acute lamotrigine elevate GABA levels in their cerebrospinal fluid. In the past, Wheatley et al. reported that the rat tolerated this dose within the anticonvulsive range. We also used doses of 10 mg/kg in the initial experiments, but there was no change in GABA levels (data not shown), so we continued the study with the dose above. Morris and co. also demonstrated that the plasma concentrations of lamotrigine attained by rats at this dose were comparable to those suggested for epileptic patients. The difference in doses between rats and humans may be due to this.

We also looked at how much lamotrigine was in the dialysates from the lateral ventricles. Lamotrigine levels in the cerebrospinal fluid also went up in tandem with the rise in GABA concentrations. Similar observations have been made in the past. The plasma concentrations of lamotrigine and the doses administered appear to have a linear relationship. Lamotrigine, like all antiepileptics, must cross the blood–brain barrier to work as an anticonvulsant. As a result, determining the drug concentration at the neuronal sites of action is required for the interpretation of the plasma levels of lamotrigine. Lamotrigine rapidly appears in plasma after intraperitoneal administration (peak value at 0.25 hours), indicating rapid absorption from the peritoneal cavity. In addition, the rapid appearance of lamotrigine in the brain (peak value between 0.5 and 2 hours) suggests that it has already broken through the blood-brain barrier. Plasma and brain concentrations both show a monoexponential fall after peak values. The linear relationship that was established between the drug in the brain and plasma as well as the parallel patterns that were observed in the plasma and brain profiles suggested that the distribution of lamotrigine is dependent on blood flow, suggesting that lamotrigine simply diffuses across the blood–brain barrier. Lamotrigine also exhibits a pattern that is comparable to that previously demonstrated in our study.

The cholinergic system's role in lamotrigine treatment's GABA response was another goal of this study. GABA concentrations in the cerebrospinal fluid decreased by a non-significant amount following an injection of lamotrigine following physostigmine, a cholinesterase inhibitor, pretreatment. Atropine sulfate, a muscarinic antagonist, also caused GABA levels to rise in a non-significant manner.

4 CONCLUSION

In conclusion, our findings may imply that the drug's anticonvulsant and mood-stabilizing effects are mediated by the lamotrigine-induced GABA response, whereas the cholinergic system does not appear to be involved—at least in healthy rats—and this issue needs further investigation.

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EXPLORING THE EFFECT OF AGNIHOTRA ON THE ANTIBIOTIC PROPERTIES OF PIPER NIGRUM

Harikiran Lingabathula
Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Soorammagari Sunayana
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Piper nigrum L. is a significant medicinal plant with numerous antibiotic applications in Ayurveda. Piper nigrum extract has been shown to be effective in experiments on both gram-negative and gram-positive bacteria. Another important method for treating a variety of diseases is agnihotra therapy, in which a mixture of rice and the fume of a particular plant twig and clarified butter effectively inhibits the growth of bacteria that cause illness. The effects of Agnihotra and plant extract on the growth of some pathogenic bacteria were examined in this study together. It was demonstrated that Agnihotra had an effect on the growth of some pathogenic bacteria. It was demonstrated that Agnihotra aids in the effective control of pathogenic bacteria's growth. As a result, the treatment of Agnihotra and Piper nigrum extract is recommended.
Keywords: Agnihotra, Piper nigrum, antibacterial activity are key terms.

1 INTRODUCTION

Plants have proven to be an effective weapon in the fight against disease-causing microbes, which have plagued humanity since the beginning of time. Going back in time to the history of disease management, the tried-and-true therapy of Ayurveda contains numerous references to the antibiotic properties of plants. Synthetic antibiotics gained popularity in medical science over the past few decades, but their side effects quickly became apparent, and the entire world is now returning to herbal medicine and Ayurveda (Kumar et al.). 2013, Dandpat and others 2014). The plant Piper nigrum L., more commonly known as black pepper, is used to treat a wide range of conditions, including digestive problems, fever, sinusitis, asthma, and congestion, among others, according to Ravindran. Agnihotra Chikitsa is the name of another treatment method that is described in Ayurvedic literature. Agnihotra is a cycle during which fire is lit in a pyramid formed pot with the assistance of cow waste, twig of certain plants and explained spread. During sunrise and sunset, a pinch of rice is offered in flame. It has been demonstrated scientifically that the resulting fume has antibacterial properties. The debris has additionally incredible capability of killing microbe. Rao and Tewari (1987), Pathade and Abhang (2014), Mondkar (1987), Atul et al. (Agnihotra and the ash that is produced as a result have been shown to control the growth of bacteria, as Purandre and Prasad

(2012) and 2009 respectively reported. The purpose of this experiment was to investigate the combined effects of Piper nigrum and Agnihotra extracts on the growth of some bacteria.

2 MATERIAL AND METHODS

Before extraction, the Piper nigrum seeds were ground into a fine powder. The pepper powder (50 grams) was then extracted with methanol through continuous heat extraction for 24 hours using the Soxlet apparatus. By evaporating the solvent at a lower pressure, the obtained extracts were concentrated to dryness. The resultant concentration was dissolved in DSMO in such a way that the extract would have a final concentration of 1 g/ml of DSMO.

2.1 Disc Diffusion Method

The disc diffusion method was used to test the pepper methanol extracts' in vitro antibacterial activity. Plates were made by mixing log phase cultures that were actively growing in nutritional broth with 1% agar in soft agar. The extract was loaded onto a variety of filter paper discs made from Whatman's No: 1 filter cloth. After that, the discs were placed on the agar medium that contained the cultures and incubated for 24 hours at 370 degrees Celsius. The zone of growth's diameter was then measured.

2.2 Minimum inhibitory concentration (MIC)

Not entirely settled by sequential weakening technique. In the nutrient broth, the test compound was serially diluted by two folds. To each test tube 105 CFU/ml of effectively developing bacterial societies in log stage was immunized. For 24 hours, the culture tubes were kept at 370 degrees Celsius. The MIC of the extract was measured and expressed in ppm after the incubation, and the growth of bacteria in the tubes was checked.

2.3 Agnihotra Experiment

A pyramid molded copper pot, 14.5cmx14.5cm at top and 5.50cmx5.50 cm at the base and 6.50 cm in level was utilized for this examination. For seven days, dry twigs of Ficus benghalensis, Ficus glomerulata, Ficus religiosa, Butea monosperma, and Aegel marmelos, cow dung, and rice were offered for three minutes at sunrise and sunset. Experiments were conducted in parallel in two sets. In one set, only aqueous Piper nigrum extract was used, while Agnihotra practice and the same concentration of Piper nigrum extract were used in another set. In both situations, there was evidence of a zone of inhibition and a minimum concentration of inhibition. The Students t test was used to statistically test the obtained results.

3 RESULT AND DISCUSSION

Tables 1 and 2 show the obtained results. In the test plant extract, the ZOI for Staphylococcus aureus was 22 mm, but when plant extract and Agnihotra treatment were used together, it was 24 mm. Bacillus cereus settlement decreased to 14 mm when treated with plant just though Agnihotra and plant separate in mix hindered state to 26 mm. In the case of Streptococcus faecalis, the reduction in colony size was 10 mm, and in the combination condition, it

was 20 mm. Plant extract alone inhibited the Escherichia coli colony to 18 mm, whereas Agnihotra combined with plant extract reduced the zone to 26 mm. Treatment with test plant extract reduced the Salmonella typhi zone to 10 mm, while Agnihotra combined reduced the zone to 18 mm. Agnihotra's positive effect on Piper nigrum's antibacterial property was also seen at the minimum inhibition concentration (MIC). At 125ppm, the test plant extract reduced the size of Staphylococcus aureus, but at 80ppm, Agnihotra inhibited the bacteria. In a similar vein, both the combination condition and the test plant extract inhibited the colony of Bacillus cereus by 250ppm and 140ppm, respectively. The test plant's extract was effective against Streptococcus faecalis at a concentration of 140ppm; however, when Agnihotra was practiced in conjunction with the treatment with plant extract, it was later effective at a concentration of 90ppm. At 180ppm of plant extract, the Escherichia coli colony decreased, but Agnihotra further decreased the concentration to 100ppm. The effective concentration of plant extract against Salmonella typhi was 90ppm, but even 60ppm proved to be effective. Results plainly showed that Agnihotra rehearses altogether upgrade antibacterial adequacy of Flute player nigrum extricate. There is anyway variety up until this point effect of this training on various microbes are concerned. The highest increase in ZOI was observed in Bacillus cereus and Streptococcus faecalis, indicating that these two strains were the most affected by Agnihotra. There was also a significant effect on other bacteria that were tested. Bacillus cereus and Escherichia coli were found to be more susceptible to MIC. Other strains' MICs also changed significantly. Ulrich Berk (2016) of German Relationship of Homa Treatment has broadly returned to the study of Agnihotra which is otherwise called Homa and has directed a few tests which decisively lay out significance of this framework for treatment of different infections. Mishra (2016) has made sense of science of Agnihotra seethe and attempted to make sense of the component behind its activity. Agnihotra's ability to purify the air has been clearly demonstrated by recent research. Agnihotra is mostly made of cellulose and lignocelluloses. Water vapour is produced when the released hydrogen atoms combine with oxygen following combustion. Additionally, compounds like thymole, engomal, pinen, and terpenol can be found in this vapor. and travels in all directions for a considerable distance. Following these photochemical reactions, harmful gases are broken down into harmless components through oxidation-reduction and decomposition. After agnihotra, formaldehyde, one of the most common toxic gases, is converted into carbon dioxide, resulting in relatively clean air. As a result of its antiseptic properties, the formed formaldehyde kills pathogens in the air.

Table 1 Zone of inhibition (ZOI) in mm of Piper nigrum L. extract and extract in combination with Agnihotra on some pathogenic bacteria

Bacteria	Zone of Inhibition(mm)	
	Methanol extract (5µl) (M ± SD, n =6)	Methanol extract + Agnihotra (5µl) (M ± SD, n =6)
Staphylococcus aurens	22 ± 0.8	24 ± 0.9
Bacillus cereus	14 ± 25	26 ± 0.8
Streptococcus faecalis	10 ± 0.5	20 ± 0.7
Escherichia coli	18 ± 0.6	26 ± 0.8
Salmonella typhi	10 ± 0.5	18 ± 0.5

Level of significance: $p<0.001$

Table 2 Minimum inhibition concentration (MIC) in Piper nigrum L. extract and extract in combination with Agnihotra on some pathogenic bacteria

Bacteria	MIC in ppm	
	Methanol extract(M ± SD, n =6)	Methanol extract + Agnihotra(M ± SD, n =6)
Staphylococcus aurens	125± 15	80 ± 10
Bacillus cereus	250 ± 25	140 ± 15
Streptococcus faecalis	140 ± 20	90 ± 10
Escherichia coli	180 ± 10	100 ± 15
Salmonella typhi	90 ± 10	60 ± 5

Level of significance: $p<0.005$

4 CONCLUSION

Agnihotra Therapy, also known as homotherapy, has been proven to be a scientific and safe method for treating a wide range of conditions, particularly infections. Additionally, the method reduces medication doses by probably acting as a bioenhancer. It contributes to the preservation of medicinal plants in addition to increasing the efficacy of plant extracts. By conducting and supporting research in this area, this neglected area of Ayurveda must be strengthened. People will have a relatively safe choice thanks to this.

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ASSESSING THE ADAPTOGENIC PROPERTIES OF BORAGO OFFICINALIS, A HERB FROM THE UNANI SYSTEM OF MEDICINE

Thandu Rajini
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Malothu Suresh
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - This study was led to assess adaptogenic movement of ethanolic concentrate of leaves of Borago officinalis in rodents and mice. The purpose of this study was to determine whether or not rats' and mice's anoxic tolerance tests, as well as their swimming endurance times, organ weights, and biochemical parameters, were affected by forced swimming endurance stress. At oral doses of 50 and 100 mg/kg, these activities are tested. The stress-induced variations in these biochemical parameters—serum glucose, triglycerides, cholesterol, BUN and cortisol levels, and organ weights—were significantly reduced by pretreatment with extract in these stress models. In addition, rats' and mice's anoxic tolerance durations were longer in the extract-treated animals. Accordingly, adaptogenic properties are suggested by BO's capacity to improve stress-induced changes and extend swimming time in both stress models.
Keywords: Adaptogenic, tolerance to anoxic stress, Borago officinalis, and stress from forced swimming.

1 INTRODUCTION

Russians came up with the term "adaptogen" to describe a group of natural substances with unique properties. They are substances that assist the human body with adjusting to states of inward and outside pressure and oppose circumstances of stress that would ordinarily adversely affect its working. They additionally assist the body with accomplishing an ideal equilibrium of inner energies. An adaptogen's action must be nonspecific, or resistant to a wide range of harmful factors, whether they are biological, chemical, or physical. The body and mind react to changes in homeostasis as the root cause of stress. The useful pressure is called Eustress while destructive pressure is called Trouble. The organism's homeostatic mechanisms become compromised under extreme stress, putting its survival in jeopardy. Under these circumstances, stress sets off an extensive variety of body changes called General Transformation Condition (GAS). The annual herb Borago officinalis (B.officinalis L.), also known as "Star flower," is a member of the family Boraginaceae. It originated in Syria but spread naturally to Asia, Europe, North Africa, South America, and the Mediterranean. Gaozaban is the name given to the plant in the unani medical system. Borage is used by naturopathic doctors to control metabolism and the hormonal system. It was once used to treat depression, anxiety, and grief as a nerve tonic. To counteract the effects of steroid therapy, leaves have been used as an adrenal tonic. It also provides vitality during illness recovery. The antioxidant and free radical scavenging properties of an ethanolic extract of defatted B. officinalis seeds have been reported.

2 MATERIALS AND METHODS

2.1 Chemicals

The chemicals that were utilized were all of the analytical grade. SPAN Diagnostics Kits Ltd. provided the kits for measuring

glucose, BUN, corticosterone, serum cholesterol, and triglycerides.

2.2 Preparation of Extract

The pharmacist at S.U.C.P. college in Hyderabad verified the authenticity of the B. officinalis leaves, which were obtained from an Unani medical store in Hyderabad. In a soxhlet apparatus, the leaves were shade-dried, ground into a powder, and extracted for 48 hours with 95% ethanol. Using a rotary flash evaporator, the extracts were filtered and concentrated in a vacuum at lower pressure. Distilled water containing 1% (w/v) carboxymethyl cellulose (CMC) was used to make a suspension.

2.3 Acute Toxicity Studies

Using a specially designed oral needle attached to a polythene tube, B. officinalis ethanolic extract was administered orally to mice and rats at various doses (50-2000 mg/kg). The animals were observed for 48 hours during the study period (Short term toxicity) in accordance with CPCSEA guidelines for any mortality, and the extract was found to be safe up to 2000 mg/kg. The dosages chose were 50 and 100 mg/kg. The exploratory convention got endorsement from the Institutional Creature Morals Panel (IAEC/SUCP/05/2007).

2.4 Forced Swimming Endurance Test (Physical Stress)

For forced swim endurance stress, 200-250 g male and female rats were utilized. In saline, rats in Group I received 1% CMC; (vehicle management). Group II mice underwent stress and treatment with 1% CMC in saline; (negative influence). Bunch III rodents were treated with Withania Somnifera (100 mg/kg) and stress; (positive management). Ethanolic extract at doses of 50 and 100 mg/kg, p.o., was administered to rats in Groups IV and V. and anxiety. The rats were kept in a propylene tank with a diameter of 37 x 30 cm and a height of 25 cm that was filled with water to simulate swimming stress. For seven days, extracts were given to rats once daily. On the eighth day, the rats were allowed to swim until they were completely worn out, and the endpoint was taken when the animals began to drown. The mean swimming time for each gathering was determined. In order to estimate biochemical parameters like serum glucose, triglycerides, cholesterol, BUN, and cortisols, the animals were killed and their blood was collected through cardiac punctures. After alcohol washing, the weights of organs like the liver, adrenals, and spleen were recorded.

2.5 Anoxic Stress Tolerance Test in Mice

The male and female mice were randomly divided into five groups, each with six mice. The mice in Group I received 1% CMC in saline; (vehicle management). Withania somnifera (100 mg/kg, p.o.) was used to treat Group II mice; Ethanolic extract was given to rats in Groups III and IV at oral doses of 50 and 100 mg/kg, respectively and anxiety. For seven days, the drug treatment was done every day. The animals were subjected to anoxia stress at the conclusion of the drug treatment, and the duration of anoxia tolerance was recorded. To induce anoxia stress, a hermetic vessel with a capacity of one liter of air was used. Each animal was kept in the hermetic vessel, and the time it took for them to show the first signs of convulsion was recorded. The animals were then taken out of the vessel right away and resuscitated if necessary.

2.6 Statistical Analysis

Graphpad INSTAT was used to conduct a one-way ANOVA on the data, with all values expressed as mean standard error. Dunnet's multiple comparison tests were used to estimate the significance of the differences between the various groups during the post-hock analysis.

3 RESULTS AND DISCUSSION

The study found that the extract had adaptogenic properties because it significantly (p 0.05) increased swimming time. B. officinalis pretreatment at 50 and 100 mg/kg significantly (p 0.05) reversed the stress-induced increase in adrenal weight and spleen weight in the stress control group during swimming endurance stress. Plasma cortisol, triglycerides, glucose, BUN, and cholesterol were significantly restored after being pretreated with more than two doses (p 0.05) prior to forced swimming. In rats, the extract provided significant protection against anoxia-induced stress changes at various doses. The appearance of convulsion served as the end point for the Anoxia tolerance test. Both Dose I (50 mg/kg/b.w.) and Dose II (100 mg/kg/b.w.) of B. officinalis showed statistically significant (p 0) results. 001) an increase in the tolerance to stress time on day seven in comparison to the control group.

The substances known as adaptogens are intended to induce organs into a state of non-specific increased resistance in order to improve stress resistance and adaptability to extraordinary challenges. They normalize body functions, strengthen stressed-out systems and functions, and protect against a wide range of emotional and environmental stressors. The most common method for determining a novel compound's anti-stress property is forced swimming. This worldview depends on the perception that creatures when compelled to swim in water ultimately expected a trademark stationary stance, without any trace of any action. As a result, the mice's apparent immobility indicates a state of exhaustion, fatigue, and diminished stamina, culminating in their inability to swim any further and their beginnings of drowning. The expanded swimming time has been seen in mice, pre-treated with B.officinalis with improved actual execution fundamentally longer than untreated (control) gathering and in this manner affirming its adaptogenic nature. ACTH is released in response to stress, stimulating the synthesis and release of cortisol in the adrenal cortex.

The mobilization of stored fat and carbohydrate reserves is influenced by elevated plasma cortisol, which in turn raises blood glucose levels. Antistress medications reverse the elevated levels of cortisol. Stress-induced elevated levels of cortisol and blood glucose were significantly reduced by B. officinalis. The results of this study's reference drug, Withania somnifera, were also comparable. The stimulation of the hypothalamic-pituitary axis (HPA) and sympathetic system results in the liberation of catecholamines and glucocorticoids, which inhibit the immune system at multiple sites like the liver and kidney, causing the significant increase in serum cholesterol, triglycerides, and BUN levels in animals subjected to stress. The standard drug Withania somnifera and B. officinalis both significantly (p 0.05) reduced serum cholesterol, BUN, and triglycerides. This may be because B. officinalis inhibits sympathetic nervous system stimulation. The stress-induced adrenomedullary response, which results in increased production of corticotrophic hormone and an increase in adrenal weight, is the cause of the increased weight of adrenals in stressed animals. B. officinalis and Withania somnifera significantly (p 0.05) reduced the weight of the liver and adrenal glands. This could be because they reversed the stress-induced adrenomedullary response, which in turn decreased corticotropic hormone production. Squeezing the spleen can cause lymphocytes to move into the bloodstream and contribute to the spleen's weight loss. The spleen weight was significantly (p 0.05) increased when B. officinalis and the reference plant Withania somnifera were used as a pretreatment. This might be because of restraint of enlistment of lymphocytes to

blood from spleen. Anoxia is a very stressful condition. Oxygen is necessary for all body functions, including cellular respiration. Any lack of this essential component disrupts everybody mechanism. Any drug's primary antistress effect could be an increase in adaptation during this stress. The study showed that B. officinalis significantly extended the time before convulsion, confirming its antistress property. Due to its potent antioxidant and free radical scavenging properties, it may take longer to cause convulsions. Flavanoids, tannins, and phenolic glycosides were found to have a wide range of biological activities, including adaptogenic activity [18]. These constituents may be responsible for the adaptogenic activity, whereas the standard drug Withania somnifera contains glycosides, steroids, and flavanoids. Phytochemical constituents like Phytosterols, Saponins, Phenols, Tannins and Flavanoids have been accounted for to be available in the ethanolic leaf concentrates of B.Officinalis. These components with demonstrated antioxidant activity are primarily responsible for B. Officinalis' adaptogenic activity.

4 CONCLUSION

In conclusion, the restoration of a large number of parameters studied during various types of stress suggests that administration of B. officinalis is capable of increasing the capacity of experimental animals to tolerate non-specific stress and acts as an adaptogenic agent.

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ADVANCES IN HPLC METHOD DEVELOPMENT AND VALIDATION: A COMPREHENSIVE REVIEW

Thejovathi B
Assoc. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Zareena Begum shaik
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Portable stage conditions, such as the sort and synthesis of natural modifiers, have a significant impact on chromatographic divisions. As a result, a number of preparatory tests were conducted with various mixtures of various natural solvents and structures in order to acquire the appropriate maintenance component, determination, and other chromatographic parameters prior to selecting the appropriate chromatographic conditions. The formation, development, institutionalization, and quality control of medical products are all facilitated by expository methods. In studies of the medication digestion system and pharmacokinetics, they are just as important. In order to provide administrative entries with solid information, systematic system improvement must be accepted. Testing for quality control release, solidity tests, reference materials, and providing information to support decisions are just a few of the many uses for these strategies.
Keywords: HPLC, Method Development, and Validation.

1 INTRODUCTION

UV-Visible spectrophotometry is one of the methods that is used in pharmaceutical research the most frequently. It includes determining the amount of visible or bright radiation that a substance absorbs in a given arrangement. The HPLC procedure has its relative merits yet most of them are finished at lifted temperatures, delayed, use respectably expensive reagents, incorporate extraction, use of help system. In natural chemistry and scientific science, HPLC is a chromatographic system that can distinguish, measure, and refine individual components of a mixture. It is used to separate a mixture of mixes.

High-performance liquid chromatography formerly known as high-weight liquid chromatography), this method in science is used to separate the parts of a mixture, make each part distinct, and measure each part. A pressurized liquid dissolvable containing the example mixture is passed through a segment loaded with a powerful adsorbent material by means of pumps. The example's components interact slightly differently with the adsorbent material, resulting in distinct stream rates for the various sections and part separation as they exit the section.

The branch of science known as analytical chemistry makes use of cutting-edge technologies to analyze

substances to determine their composition. We are capable of producing results that are both qualitative and quantitative. The spectrophotometric method was found to be less sensitive than the HPLC and TLC methods. It was found that the HPLC method was more sensitive than the TLC method.

HPLC is distinct from conventional "low pressure" liquid chromatography due to its significantly higher operational pressures (50–350 bar), whereas conventional liquid chromatography typically uses gravity to move the mobile phase through the column. The typical analytical HPLC column dimensions are 2.1–4.6 mm in diameter and 30–250 mm in length due to the small sample amount separated. Additionally, the sorbent particles used in HPLC columns are typically of a smaller size, ranging from 2 to 50 micrometers. This makes HPLC a popular chromatographic method because it has better resolving power for separating mixtures.

The majority of HPLC methods required expensive equipment, the purchase of solvents for use and transfer, a method of concentrated specimen arrangement, and individual expertise in chromatographic strategies. In addition, the majority of the HPLC procedures that were investigated have the potential to be utilized in studies of collaborations, multi-drug pharmacokinetics, and clinical examination of medication blends.

A reversed phase HPLC (RP-HPLC) has a watery, tolerably polar portable phase and a stationary phase that is not polar. Silica that has been surface-adjusted with RMe2SiCl, where R is a straight chain alkyl group like C18H37 or C8H17, is one basic stationary phase. With such fixed stages, upkeep time is longer for iotas which are less polar, while polar particles elute even more speedily (early in the assessment). Increasing the amount of water in the portable phase can increase maintenance times for a specialist; thereby strengthening the hydrophobic analyte's natural preference for the hydrophobic stationary phase in comparison to the more hydrophilic versatile phase. By adding more natural dissolvable to the eluent, an examiner can cut down on maintenance time. RP-HPLC is so commonly used that it is routinely mistakenly implied as "HPLC" minus any additional detail. Before releasing sedatives, the pharmaceutical industry frequently uses RP-HPLC .

Multiple medications can now be measured using the delicate converse stage ultrafast liquid chromatography (RP-UFLC) method, which has gained widespread acceptance. The RP-HPLC method is delicate, precise, specific, reproducible. The system focuses on speedy research, a simple portable platform, fundamental specimen planning, and improved affectability. Because of this, the strategy is suitable for routine testing in labs used for quality control.

With varying pore sizes and hydrophobic coatings, various types of RP-HPLC segments are available, allowing the expert to control the quality of the analyte-stationary stage association. For smaller particles and

peptides, longer alkane chains are typically used to improve communication quality, while longer alkane chains are typically used for proteins and other larger analytes. Additionally, the portable stage's steepness of the natural dissolvable angle can be altered to alter the example tying qualities and analyte maintenance times.

Quality control laboratories employ a variety of investigational techniques, including HPLC, UV-spectroscopy, HPTLC, titration, and fluorescence spectroscopy, to guarantee the character, virtue, intensity, and performance of pharmaceutical products. Due to a few advantages like speed, specificity, precision, exactness, and simplicity of mechanization, the majority of medications in multicomponent dose structures can be examined using an HPLC system.

The proposed method's advantages include a fundamental test planning method and a short investigation time. The RP-HPLC method is useful for routine laboratory investigations with a high level of exactness and precision, and it can be used effectively for routine quantitative estimation. Any medication's test strategy is very important to pharmaceutical companies, so it's always tempting to choose and develop a simple, accurate, precise, and conservative method for determining medications in API pharmaceutical measurement structures and neurotic specimens like blood and serum.

In order to guarantee the accuracy and quality of the health data documented in support of investigational new medication applications (INDs), new medication applications (NDAs), abridged new medication applications (ANDAs), and supplements in creating bioanalytical system approval data utilized in human clinical pharmacology, bioavailability (BA), and bioequivalence (BE) studies obliging pharmacokinetic (PK) assessment, agreement with good laboratory practices (GLPs) for conveying test examination of non

2 ANALYTICAL METHOD DEVELOPMENT

The development and approval of expository strategies are essential components of any pharmaceutical improvement program. The HPLC investigation technique was developed to identify, quantify, or sanitize premium mixtures. This specific brief will focus on progress and endorsement practices as associated with drug things. The determination of chromatographic tops for dynamic medication fixings was the objective of the system improvement.

The instruments must be able to convert the divided part into meaningful full data and allow the flexible stage to follow the stationary stage. The HPLC framework is extremely muddled because it has many different components. A pump that moves the portable phase(s) and analyte through the section, as well as a locator that gives the analyte a trademark maintenance time, are common components of HPLC. It is a form of fluid chromatography that makes use of smaller section sizes, smaller media contained within the

segment, and more flexible stage weights.

Effective new approaches to examining the medication digestion system and mien have emerged as a result of recent advancements in mass spectrometers and ionization techniques. High-performance liquid chromatography/mass spectrometry (HPLC/MS) is gaining popularity as a result of the lack of a comprehensive finder for HPLC. Even though it isn't the best indicator, HPLC/MS has become a reliable method for xenobiotic testing. In three ranges, the most profitable application of HPLC/MS to studies of the pharmacology and toxicology of atoms with masses below 1,500 daltons is: improvement of specific procedures for follow-up research, including the identification and representation of metabolites, as well as research into collaborations between drug particles and peptides or proteins.

3 VALIDATION

The International Organization for Standardization (ISO) defines validation as "check, where the predetermined prerequisites are satisfactory for a proposed utilization," while confirmation is defined as "procurement of target proof that a given thing satisfies determined requirements." Linearity, precision, exactness, toughness, robustness, LOD, LOQ, and selectivity or specificity are among the various approval parameters.

- Linearity
- Accuracy
- Precision
- Robustness
- Range
- Limit on Quantity
- Specificity

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UNCOVERING THE COMPLEXITIES OF DRUG DISCOVERY: A TIME-INTENSIVE AND CHALLENGING ENDEAVOR

Dr. Durgaiah Gandamalla
Assoc. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Revuri Dinesh Reddy
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The process of discovering drugs is complicated and time-consuming. In the past, the process started with selecting a molecule that had been synthesized or extracted and screening it for various activities. Innovation does not imply abandoning traditional drug discovery. Every pharmaceutical industry in a developing nation like India cannot keep up with cutting-edge drug discovery technologies. By providing tax breaks and sponsoring projects through CSIR, DBT, and DST, the government is encouraging businesses to take on the task of drug discovery. In the next three to four years, global corporations will outsource approximately $1 billion to Indian CRO. China could eventually take our palace, just as we did with Europe. The pharmaceutical industries in India are known worldwide for producing generic drugs, but they are not known for their innovation.
Keywords: India, the global market, drug discovery, drug screening, R & D, and revenue.

1 INTRODUCTION

The process of discovering drugs is complicated and time-consuming. In the past, the process started with selecting a molecule that had been synthesized or extracted and screening it for various activities. It is actually an unwieldy occupation as the need might arise to be evaluated for movement (Old style drug revelation process). In this regard, India did not compete with other nations because of its lack of knowledge, economic status, and other factors. We have to rely on other nations, which increases the cost of the drugs. However, we worked hard to produce drugs by utilizing the process patent. Following 30 years, we are currently in a situation to make drugs without anyone else and involved third situation in the worldwide market by sending out our medications to different nations.

The development of cutting-edge methods like high throughput screening, robotics, and computer simulations to shorten the duration of the drug discovery process has resulted in a world-wide paradigm shift in the process. Neither chemistry nor technology drive the procedure. Instead, it is the combination of the two.

In the past, Indian companies invested 0.6% of their revenue in drug R & D. Currently, pharmaceutical companies spend 5% of turnover, a 70-fold increase from the 1960s and 1970s. Innovation is clearly the driving force behind R&D. Innovation

does not imply abandoning traditional drug discovery. Every pharmaceutical industry in a developing nation like India cannot keep up with cutting-edge drug discovery technologies. The expense of drug discovery can only be met by multinational corporations. Even medium-sized businesses in a region should use classical methods in the drug discovery process if industry and academics work well together. In addition, the Indian industry is primarily focusing on the creation of new dosage forms, or ANDAs, rather than the discovery of novel drug molecules, which necessitates significant investments and offers no assurance of profit.

By providing tax breaks and sponsoring projects through CSIR, DBT, and DST, the government is encouraging businesses to take on the task of drug discovery. Utilizing these advantages and moving further into the "omic" era (proteomics, genomics) with other nations is the responsibility of industries and academics. It is important to pay attention to new technologies like dendritic vaccines and research on stem cells, both of which are likely to be commercially available in the near future. Regulatory bodies should also respond as quickly as possible because biotechnology products will account for 30% of all R & D efforts globally. They should help the industries along the way and speed up the approval process for a drug molecule, like in Europe. In the United States, an IND approval takes one month, whereas in India, it can take any time at all. The H1N1 vaccine was first approved by China, but we haven't even started the trail. The DBT-sponsored DNA vaccine, on the other hand, has completed phase II in other nations and is not proceeding.

The process's complexity is somewhat reduced when industry, academics, and regulatory authorities work together well, and more and more businesses are taking on the responsibility. According to a report released by the organization Stratfer, no significant changes will occur in India's development over the next ten years. It also says that India has a lot of resources but hasn't used them all yet. In addition, there is a pressing need to establish a strong connection between pharmacy and traditional medical systems, particularly in the field of drug discovery.

Risorine (Rifampicin+ Isoniazid+ Piperine) by Cadila Pharmaceuticals is a classic example of a traditional medicine that uses the enhancing bioavailability property of piperine extracted from peppers to reduce the dose of rifampicin and isoniazid.

Due to its larger population and lower cost, India is likely to serve as a center for international clinical trials. In the next three to four years, global corporations will outsource approximately $1 billion to Indian CRO. China could eventually take our place, just as we did with Europe. The pharmaceutical industries in India are known worldwide for producing generic drugs, but they are not known for their innovation. We must also offer the world patented products. Let us demonstrate to the global market that we are not inferior to any other nation in the process of drug discovery by making use of our own

natural resources, which include our skilled workforce and traditional medical system.

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THE FACTORS INFLUENCING THE SELECTION OF COMMUNITY PHARMACIES IN THE CZECH REPUBLIC: A COMPREHENSIVE ANALYSIS

Gaddam Swetha Reddy
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
Ajmeera Rajya Laxmi
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - To find out what factors Czech citizens considered when choosing a community pharmacy and how their behavior changed between 2011 and 2013. A sociological survey of the Czech population over the age of 15 using a sample that is representative in terms of age, gender, and region (the sample deviation from the Czech Republic's population breakdowns is less than 0.2 percent). The anonymous questionnaires were administered in the Czech Republic (CR) from 21 November to 4 December 2011 and from 18 November to 6 December 2013, following preliminary research with 228 respondents in 2011 and 228 in 2013. SASD 1.4.5 (Statistical Analysis of Social Data) was used for all statistical analyses.

The information were gotten from an example of 1797 people haphazardly chosen utilizing shares. In 2011, the response rate was 90%, and in 2013, it was 86%. The location (proximity) is the primary factor in deciding which community pharmacy to choose in CR. Considerably less significance put on great help (individual involvement in proficient methodology) or great cost (lower costs and advancement on medications). It was observed that prices gradually increased in importance at the expense of other factors. P 0.001 is the statistical significance level for each result.

For the majority of citizens, choosing a pharmacy in a convenient location is a crucial factor. It was noted that more people are choosing their pharmacies primarily based on price. For them, the decision to buy medicine is becoming more and more important, and a lower price is more important than a good pharmacy service.

Keywords: Pharmaceutical care, pharmacy competition, the Czech Republic's health care system, and pharmacy choice.

1 INTRODUCTION

In addition to improving the competitiveness of individual pharmaceutical care providers, strengthening the relationship between pharmacists and patients is a long-term effort that will result in successful pharmacotherapy. In the Czech Republic (CR), the pharmaceutical care provider can be changed at will. There is no requirement to register in a particular pharmacy. Patients pay low participation (out-of-pocket payments, copayments) in addition to public health insurance in the Czech health care system, which covers the majority of costs. Among the 34 economically most developed

countries in the world that make up the Organization for Economic Cooperation and Development (OECD), CR allocates one of the largest portions of its national budget to funding public health. Patient behavior was affected by reforms that were implemented and funded in the Czech health care system over the past decade. However, private expenditures remain at just 16%, which is still among the lowest among OECD nations.

The competition between community pharmacies in CR is also growing as there are more of them. In CR, non-price competition (an effort to improve service quality) and price competition (lower drug prices and copays) are still present. Some functional enhancements of pharmacy services appear to be helped along by competition.

There is muddled framework in guideline by greatest costs and repayment of medications in CR. In the CR, EU states still do not have uniform copayments that are fixed, predictable, or consistent. In many instances, the pressure on non-price competition and the suppression of price competition were observed, as was the significance of price regulation in the health system. Community pharmacy deregulation and free market principles may have unintended consequences, according to surveys from other nations: Some vertically integrated pharmacy chains may form oligopolies that tend to dominate the market and align product offerings with those of their suppliers; The workload of pharmacists may increase as a result of increased competition and a shortage of pharmacy staff, which may result in a decrease in counseling quality; pharmacists may be more likely to focus on business goals than public health ones as a result of economic pressure.

The current state of competition among community pharmacies in CR as well as the factors that influence it can be learned from our investigation into the factors that Czech citizens consider when choosing a community pharmacy and the emergence of behavioral shifts between 2011 and 2013.

A sociological survey of the Czech population over the age of 15 using a sample that is representative in terms of age, gender, and region (the sample deviation from the Czech Republic's population breakdowns is less than 0.2 percent). The anonymous questionnaires were administered in the Czech Republic (CR) from 21 November to 4 December 2011 and from 18 November to 6 December 2013, following preliminary research with 228 respondents in 2011 and 228 in 2013. SASD 1.4.5 (Statistical Analysis of Social Data) was used for all statistical analyses, with a significance level of P 0.001.

A semi-open question was used in the research to investigate the factors that influence pharmacy selection: What factors do you consider most important when choosing a pharmacy? The choices for responses were "good location," "good service," "good price," "no preference," and "another factor," according to preliminary research. “Good service” refers to the staff's professional approach, a positive personal

experience, the opportunity to discuss personal health concerns, and additional pharmacy services. "Good location" refers to the closest pharmacy and its accessibility. "Good Price" omits discounts, medicine promotions, frequent-buyer programs, and cheaper prices. Only one response was available to respondents, and this was thought to be the most important factor in their decision. Table 1 contains comprehensive results.

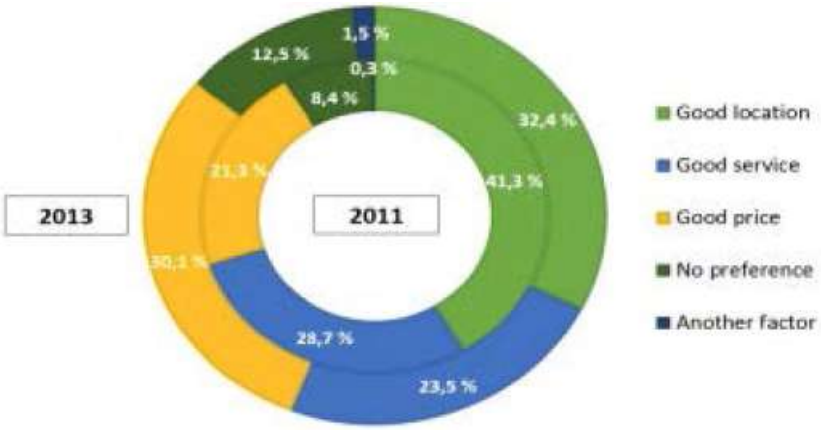
Table 1 Priority determinants of the choice of community pharmacy for different groups of the Czech population (in percentage, P<0.01 for each).

Priority determinant	Men		Women		15–24 years		25–64 years		65 years andover		Lower education[1]		Higher education[2]		TOTAL	
	2011 N=877	2013 N=874	2011 N=920	2013 N=923	2011 N=259	2013 N=245	2011 N=1222	2013 N=1211	2011 N=316	2013 N=341	2011 N=768	2013 N=721	2011 N=1029	2013 N=1076	2011 N=1797	2013 N=1797
Good Location	45,8	34,0	37,0	30,9	45,6	32,2	41,4	32,8	37,3	31,1	44,0	27,3	39,3	35,7	41,3	32,4
Good service	25,2	20,7	32,2	26,1	26,6	20,8	29,5	25,4	27,2	18,5	22,1	22,9	33,7	23,9	28,7	23,5
Good price	18,1	27,7	24,2	32,4	20,1	27,4	19,1	27,4	30,4	41,6	24,9	35,9	18,6	26,2	21,3	30,1
No preference	10,5	15,8	6,5	9,4	7,3	18,0	9,7	13,0	4,8	7,0	8,7	12,6	8,2	12,5	8,4	12,5
Another factor	0,4	1,8	0,1	1,2	0,4	1,6	0,3	1,4	0,3	1,8	0,3	1,3	0,2	1,7	0,3	1,5

Where a large number of significant differences between populations can be distinguished. See Chart 1 for changes in the graphical expression and differences between the dominant factors.

Area and accessibility of drug store is a vital determinant for the biggest piece of residents, albeit this strength diminished in the second review by practically 22%. As the primary cause of this decline, a notable increase in the number of citizens who choose their pharmacy primarily based on price and prefer a lower price over other factors was identified. The growth during that time was even higher than 41 percent, and the lower price became an increasingly important factor in their decision. Priority factor "personal experience," such as satisfaction with pharmacy services provided by staff, decreased by 18% over the course of the study, which cannot be considered a positive trend toward strengthening patient-pharmacist relationships. Long-term policy monitoring and evaluation should go along with the implementation of policy measures, and similar studies should continue in the following periods as well.

Chart 1 Priority determinants of the choice of community pharmacy in 2011 (inner circle) and 2013 (outer circle).



2 METHODS

A sociological survey of the Czech population over the age of 15 using a sample that is representative in terms of age, gender, and region (the sample deviation from the Czech Republic's population breakdowns is less than 0.2 percent). The anonymous questionnaires were administered in the Czech Republic (CR) from 21 November to 4 December 2011 and from 18 November to 6 December 2013, following preliminary research with 228 respondents in 2011 and 228 in 2013. SASD 1.4.5 (Statistical Analysis of Social Data) was used for all statistical analyses.

3 RESULTS

Using quotas, a sample of 1797 people was selected at random to obtain the data. In 2011, the response rate was 90%, and in 2013, it was 86%. The location (proximity) is the primary factor in deciding which community pharmacy to choose in CR. Considerably less significance put on great help (individual involvement in proficient methodology) or great cost (lower costs and advancement on medications). It was observed that prices gradually increased in importance at the expense of other factors. P 0.001 is the statistical significance level for each result.

4 CONCLUSION

For the majority of citizens, choosing a pharmacy in a convenient location is a crucial factor. It was noted that more people are choosing their pharmacies primarily based on price. For them, the decision to buy medicine is becoming more and more important, and a lower price is more important than a good pharmacy service.

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EXPLORING THE POTENTIAL OF ERDOSTEINE AS AN ANTIOXIDANT AGENT IN NAPHTHALENE-INDUCED OXIDATIVE STRESS: A RAT STUDY

Hariprasad Kadiyam
Asso. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
G Lavanya
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Erdosteine's protection from naphthalene-induced toxicity and the role of free radicals in this study are investigated. One single oral dose of corn oil containing 1100 mg naphthalene/kg was administered to female Sprague-Dawley rats. Before administering naphthalene, rats received 50 mg/kg/day of Erdosteine orally for three days. Twenty-four hours later, naphthalene was administered to the rats. Malondialdehyde (MDA), glutathione (GSH), Na+, K+-ATPase, and myeloperoxidase (MPO) activities were measured in liver and kidney tissue samples. The serum samples were tested for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) activity, blood urea nitrogen (BUN), creatinine, and TNF-, IL-1, IL-6, 8-hydroxy-2'-deoxyguanosine (OHdG), and total antioxidant capacity (AOC). Naphthalene administration led to significant increases in tissue MDA levels and MPO activity, as well as significant decreases in tissue GSH levels, Na+, K+-ATPase activity, and plasma AOC levels. In addition, the naphthalene group had significantly higher levels of pro-inflammatory mediators (TNF-, IL-6, and IL-6), 8-OHdG, LDH activity, AST, ALT, creatinine, and BUN. However, treatment with erdosteine stopped all of these naphthalene-induced biochemical changes. In conclusion, it appears likely that erdostein protects tissues by regulating the production of inflammatory mediators, balancing the oxidant–antioxidant status, and inhibiting neutrophil infiltration.
Keywords: Naphthalene; erdosteine; peroxidation of lipids; glutathione; myeloperoxidase.

1 INTRODUCTION

The majority of crude oils contain polycyclic aromatic hydrocarbons (PAHs), which are the most harmful and long-lasting compounds. PAHs are now widespread contaminants of aquatic ecosystems due to the release of petroleum oils into the sea. It has been demonstrated that some of these chemicals are mutagenic or carcinogenic, genotoxic, and cytotoxic. Naphthalene is a common name for the polycyclic aromatic hydrocarbons (PAHs) that are used in insect repellents, scent discs for bathrooms, and soil fumigants.

Naphthylamines, anthranilic and phthalic acids, and synthetic resins are all made with it. Naphthalene's toxic effects appear to be caused by the oxidative damage caused by hydroxylated products like 1-, 2-, and 1, 2-dihydroxynaphthalene, as well as the conversion of naphthalene to naphthoquinone. Naphthalene exposure has been shown to decrease hepatic selenium dependent glutathione peroxidase activity and raise serum and liver lipid peroxide levels. In both humans and rats, exposure to naphthalene is linked to the development of hemolytic anemia. The fact that female Sprague-Dawley rats received 1100 mg/kg of naphthalene and experienced 2.5-fold increases in lipid peroxidation in their mitochondria in the liver and brain 24 hours after treatment suggested that the toxicity of naphthalene is at least partially related to free radicals and free radical-mediated oxidative stress.

Erdosteine (N-(carboxymethylthioacetyl)-homocysteine

thiolactone) is a novel mucoactive agent with two blocked sulphydryl groups. One of the blocked sulphydryl groups is in an aliphatic side chain, and the other is in the heterocyclic ring. Following hepatic metabolism, these chemically blocked sulfhydryl groups are released, allowing the molecule to exercise its antioxidant and free radical scavenging capabilities. In various models of inflammation, its protective effects against oxidant-induced tissue damage have been demonstrated based on its free radical scavenging activity. In a similar vein, we have demonstrated that erdosteine protects against oxidative colonic tissue damage caused by colitis. Based on this background, we wanted to investigate the erdosteine's alleged protective effects on the hepatic and renal tissues of naphthelene-exposed rats through biochemical analysis.

2 MATERIALS AND METHODS

2.1 Animals

All trial conventions were supported by the Marmara College Institute of Medication Creature Care and Use Board of trustees. Standard rat food was fed to 200-250 g female Sprague-Dawlwey rats during 12 h of light and dark cycles at a constant temperature of 22 + 1o C.

2.2 Experimental Design

For three days, rats were given either saline (n=16) or erdosteine (50 mg/kg/ml) orally. On the fourth day, half of the saline- or erdosteine-treated rats were given 1100mg/kg/ml of naphthalene in corn oil by gavage (naphthelen groups) following an overnight fast in which they had free access to water. The other half of the saline- or erdosteine-treated rats were given corn oil orally (control groups). After being given naphthalene or corn oil for 24 hours, all of the rats had their heads cut off. Tissue malondialdehyde (MDA) and glutathione (GSH) levels, as well as the activities of Na+-K+ ATPase and myeloperoxidase (MPO), were measured by carefully dissecting and storing the liver and kidney at –70°C following the decapitation of the animals.

2.3 Biochemical Analysis

Using an automated analyzer, the concentrations of urea nitrogen, AST, ALT, creatinine and LDH in serum were measured spectrophotometrically. Using enzyme-linked immunosorbent assay (ELISA) kits designed specifically for the rat cytokines (Biosource International, Nivelles, Belgium), plasma levels of tumor necrosis factor alpha (TNF-), interleukin (IL)-1, and IL-6 were measured in accordance with the manufacturer's instructions and guidelines. The colorimetric test system (ImAnOx, catalog number) was used to measure the total antioxidant capacity of the plasma. KC5200, Immunodiagnostic AG, D-64625 Bensheim), following the manufacturer's instructions. The enzyme-linked immunosorbent assay (ELISA) method (Highly Sensitive 8-OHdG ELISA kit, Japan Institute for the Control of Aging, Shizuoka, Japan) was used to measure the amount of 8-OHdG present in the extracted DNA solution. The selection of these particular assay kits was based on their high level of sensitivity, specificity, precision both inter- and intra-assay, and the small amount of plasma sample required to carry out the assay.

2.4 Malondialdehyde and Glutathione Assays

For the purpose of determining the concentrations of malondialdehyde (MDA) and glutathione (GSH), tissue samples were homogenized with ice-cold 150 mM KCl. By keeping an eye on the formation of thiobarbituric acid reactive substances, the MDA levels were checked for lipid peroxidation products. Using an extinction coefficient of 1.56 x 105 M–1 cm–1, the results of lipid peroxidation were expressed in terms of MDA equivalents. The Ellman method was modified for the purpose of taking GSH

measurements. Briefly, 2 ml of 0.3 mol/l Na2HPO4.2H2O solution was mixed with 0.5 ml of supernatant after 10 minutes of centrifugation at 3000 rev/min. The absorbance at 412 nm was measured immediately following the addition of a 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml, 1% sodium citrate). Using an extinction coefficient of 1.36 x 104 M–1 cm–1, GSH levels were determined. The measurements are given in mol GSH/g tissue.

2.5 Myeloperoxidase Activity

An enzyme known as myeloperoxidase (MPO) is primarily found in the azurophilic granules of polymorphonu clear leukocytes (PMN). The number of PMN counted histologically in tissues is strongly correlated with tissue MPO activity, which is frequently used to estimate PMN accumulation in inflamed tissues. Similar to what was described by Hillegass et al., MPO activity was measured in tissues. After being homogenized in PB (50 mM potassium phosphate, pH 6.0), tissue samples were centrifuged at 41.400 g for ten minutes; In 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB), pellets were suspended. The samples were centrifuged for ten minutes at 41.400 g after three freeze-thaw cycles with sonication in between. Aliquots (0.3 ml) were added to 2.3 ml of response blend containing 50 mM PB, o-dianisidine, and 20 mM H2O2 arrangement. The amount of MPO present that caused a change in absorbance measured at 460 nm for three minutes was considered to be one unit of enzyme activity. U/g tissue was used to measure MPO activity.

2.6 Measurement of Na+, K+-ATPase Activity

The inorganic phosphate that is produced when 3 mM disodium adenosine triphosphate is added to the medium during the incubation period is used as the basis for measuring Na+,K+-ATPase activity. 100 mM NaCl, 5 mM KCl, 6 mM MgCl2, 0.1 mM EDTA, and 30 mM Tris HCl (pH 7.4) were added to the water bath to incubate the medium for five minutes. Each tube was incubated for 30 minutes at 37 degrees Celsius with a final concentration of 3 mM of Na2ATP following the preincubation period. After the hatching, the cylinders were set in an ice shower, and the response was halted. A spectrophotometer from Shimadzu, Japan, was used to measure the level of inorganic phosphate at an excitation wavelength of 690 nm. The enzyme's specific activity was represented as 1 Pi mg-1 protein h-1. The Lowry method was used to measure the supernatant's protein content.

3 DISCUSSION

The current data clearly demonstrate that treatment with erdosteine significantly reduced napthelene-induced lipid peroxidation and neutrophil infiltration in the hepatic and renal tissues due to its free radical scavenging properties, while restoring the depleted antioxidant GSH level and inhibited Na+-K+-ATPase activity to control levels. The severity of oxidative stress is also demonstrated by decreased AOC and elevated serum levels of LDH, AST, ALT, BUN creatinine, and 8-OHdG. The outcomes likewise exhibit that erdosteine treatment, as seen by the inversion of modifications in every one of the deliberate boundaries in tissues, mitigated the naphtelene-actuated plasma markers of oxidative pressure and worked on renal and hepatic capabilities.

The neutrophil-produced free radicals may be partly to blame for this rise in lipid peroxidation. Because; In addition to their direct harm to tissues, free radicals appear to cause the accumulation of leukocytes in the affected tissue, thereby indirectly causing tissue damage through activated neutrophils. Through the production and release of reactive oxygen metabolites and cytotoxic

proteins (like lactoferrin, proteases, and myeloperoxidase) into the extracellular fluid, activated neutrophils are known to cause tissue damage. At the point when neutrophils are invigorated by different energizers, MPO, as well as other tissue-harming substances are let out of the cells. In this manner, it is a list of neutrophil penetration. Naphthalene's increase in MPO activity may cause organ inflammation and damage because neutrophil infiltration is a crucial part of acute inflammation. In contrast, a significant increase in the proinflammatory cytokines TNF-alpha, IL-1, and IL-6 demonstrates that naphthalene toxicity is closely linked to inflammatory mechanisms and oxidative damage. Our findings suggest that the protective effects of erdosteine are partially mediated by blocking plasma cytokines and tissue neutrophil infiltration because treatment with erdosteine significantly reduced these cytokines and prevented the infiltration of neutrophils into the damaged tissue.

Testicular torsion and detorsion in rats have also been used to investigate the effect of erdosteine. Before rats were subjected to left unilateral testicular torsion and detorsion, Erdosteine was administered at a dose of 50 mg kg-1 per day for two days. By preventing the accumulation of free oxygen radicals, erdosteine administration reduced the histological damages associated with testicular torsion and detorsion.

4 CONCLUSION

In conclusion, this study clearly demonstrates that oxidative metabolism of naphthalene is one of the primary mechanisms that causes multiple organ damage. Erdosteine's protective effects can be attributed, at least in part, to its ability to inhibit neutrophil infiltration, balance oxidant-antioxidant status, and regulate the generation of inflammatory mediators. This suggests that erdosteine may play a role in the treatment of organ failures caused by chemical or drug toxicities.

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EXPLORING CAMPHOR SUBLIMATION FOR RAPID RELEASE OF ONDANSETRON IN FAST DISSOLVING TABLETS: A COMPREHENSIVE CHARACTERIZATION STUDY

Kasireddy Swetha Reddy
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Shireesha Bandirala
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Ondansetron Hydrochloride (OSH) is a drug that is only slightly soluble in water. Ondansetron fast dissolving tablets (FDTs) were made in this study by sublimating a variety of superdisintegrants. In vitro dissolution and physicochemical properties of FDTs were examined. The formulation batch that contained crospovidone (F2) had the shortest wetting time (10.5 s) and the tablets disintegrated the quickest (3.2 s). The formulation batch containing crospovidone (F2) was found to have the highest drug release (98 percent drug release after 30 minutes), making it a promising formulation. The drug release from FDTs containing superdisintegrants was higher than that from FDTs without superdisintegrants.

1 INTRODUCTION

OSH is a well-tolerated and effective antiemetic that is used to prevent nausea and vomiting caused by both radiotherapy and chemotherapy. Water soluble OSH is sporadic. The drug dissolves or disperses in the saliva when this fast-dissolving tablet is placed on the tongue and dissolves instantly. First-pass metabolism occurs and it is well absorbed from the gastrointestinal tract. Following oral administration of a single 8-mg tablet, the mean bioavailability in healthy subjects is approximately 56 percent.

Because it is convenient for self-administration, small, and simple to manufacture, the tablet form of administration is the most widely used among all dosage forms currently in use. However, poor patient compliance is frequently caused by hand tremors, dysphasia in elderly patients, underdeveloped muscular and nervous systems in young people, and the difficulty swallowing in uncooperative patients. The desire to provide patients with a conventional method of taking their medication led to the development of the concept of a fast dissolving drug delivery system as a means of overcoming these limitationas. Patients with persistent nausea, sudden episodes of allergic attack, or coughing for those who lead an active lifestyle—such as children, geriatrics, bedridden, or mentally disabled patients—may have difficulty swallowing conventional tablets or capsules, resulting in ineffective treatment. Fast dissolving tablets are useful in these patients.

Utilizing subliming agents like camphor, menthol, or thymol, among others, to maximize the tablet's pore structure is another method taken in the production of such tablets. in the method of sublimation. A permeable hydrophilic grid is produced which may effortlessly get the crumbling medium, break rapidly and subsequently give momentary deterioration.

The proposed work aimed to develop and characterize fast-dissolving ondansetron tablets using a sublimation method for rapid drug dissolution and absorption, potentially leading to a rapid onset of action.

2 MATERIALS AND METHODS

2.1 Materials

Zydus Cadila Ltd., Ahmedabad, provided OSH, Mannitol, aspartame, Crospovidone (CP), Sodium starch glycolate (SSG), and Low substituted hydroxy propyl cellulose (L-HPC). Relax Pharmaceuticals Ltd., Baroda, supplied the microcrystalline cellulose PH-101. Molychem Ltd., Samir Tech Chem Pvt., and Talc supplied the camphor, magnesium stearate, and talc. Ltd., Baroda respectively, and Allied Chemical Corporation Ltd. Analytical-grade chemicals and reagents were also used.

2.2 Preparation of Fast Dissolving Tablets

The sublimation method was used to prepare ten batches of OSH FDTs according to composition. Before use, a fine sieve was used to filter out mannitol, camphor, aspartame, and microcrystalline cellulose PH101. After that, the drug and excipients were combined by tumbling for ten minutes. Talc and one percent magnesium stearate were used to lubricate the mixture. Using a rotary tablet machine, the resulting blend was directly compressed into tablets. Rimek, India's RSB-4 mini press). The tablet weight was acclimated to ~150 mg. In a vacuum oven at 60 degrees Celsius for six hours, the sublimation process was performed on the compressed tablets.

3 EVALUATION OF FAST DISSOLVING TABLETS

3.1 Weight Variation

The average weight of twenty tablets was determined after they were each individually weighed. For weight variation, the percentage deviation was calculated and evaluated.

3.2 Hardness

The force required to break a tablet in a diametric compression of five tablets, or tablet hardness, was measured with a Monsanto hardness tester (Dolphin, Mumbai).

3.3 Friability

The Roche friabilator (Erection and Instrumentation Engineers, Ahmedabad) was used to spin a sample of ten tablets that had been pre-weighed. A soft muslin cloth was used to dedust the tablets, and they were reweighed. It is determined how much friability there is.

3.4 In vitro Disintegration Time

A modified disintegration method was used to measure the disintegration time of five tablets. A petridish with a diameter of 10 cm was filled with 10 mL of distilled water for this purpose.

The time it took for the tablet to completely break up into fine particles was recorded as the disintegration time. The tablet was placed with care in the middle of the petridish.

4 EVALUATION OF FAST DISSOLVING TABLETS

The tablets' hardness ranged from 2.5 to 4.3kg/cm^2 according to the evaluation parameters. The tablets' lower than one percent friability indicates their high mechanical resistance. The amount of drugs found was between 95.14 and 98.21%.

All formulations had disintegration times between 3.22 and 7.56 seconds, which was within FDA's official requirement of less than 30 seconds. In order to establish a correlation between oral cavity disintegration time and wetting time, Understanding the capacity of disintegrants to swell in the presence of little water is crucially dependent on this criterion. The measurement of wetting time can be used as an additional confirmative test for the evaluation of fast-dissolving tablets because the dissolution process of a tablet is dependent on the wetting and subsequent disintegration of the tablet. The results showed that FDTs containing CP as a superdisintegrant (F2) had the shortest wetting time (10.5 s) and the fastest disintegration (3.22 s) when compared to CCS, L-HPC, and SSG. The wetting time of the formulated tablets ranged from 10.5 to 14.12 s.

The results of the dissolution show that, in comparison to batch F1 without a superdisintegrant, more than 90% of the drug released within 30 minutes from all batches containing supedisintegrants (F2–F5). According to the findings, the batch containing CP (F2) had a higher rate of dissolution (98 percent release after 30 minutes) than the batches containing CCS, L-HPC, and SSG (F3–F5). The order in which the superdisintegrants increase the rate of dissolution could be CP> L-HPC> SSG> CCS. The comparison profile of the release. Due to its faster disintegration time, lower wetting time, and higher drug release, the F2 batch was chosen as a promising formulation.

Cross-sectional and surface scan electron micrographs of the promising batch (F2). The micrograph shows the exceptionally permeable nature of the pre-arranged sublimed tablet, which show up in both surface and the inward construction. These results indicate that the addition of camphor followed by sublimation had a significant impact on the inner structure of the tablet, which in turn had an impact on the wetting, disintegration, and dissolution of the final tablet. The rapid penetration of water into the tablet is explained by the tablet's highly porous nature.

After six months of storage at 25°C 2°C/50% 5 percent relative humidity, the tested parameters of the promising formulation did not significantly differ from the initial results, according to the stability studies. The selected formulation was found to be stable, as demonstrated by the stability study's findings.

5 CONCLUSION

In conclusion, the formulation of fast-dissolving Ondansetron tablets

prepared using crospovidone as a superdisintegrant through the camphor sublimation method appears to be promising, and additional in-vivo research may be conducted.

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IMPLEMENTING PHARMACEUTICAL QUALITY BY DESIGN FOR ENHANCED PRODUCT DEVELOPMENT

Korna Devamani
Asst. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India
Kurma Kirankumar
Asst. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The term "Quality by Design" (QbD) refers to a novel approach to product development that has the potential to boost productivity, provide flexibility and relief from regulations, and provide significant business advantages throughout the product life cycle. It encourages the pharmaceutical industry and FDA to adopt a risk-based, holistic, and proactive approach to drug development. A company must define the desired product performance profile (Target Product Profile (TPP) and Target Product Quality Profile (TPQP)) and identify critical quality attributed (CQA) during product design and development in QbD. The company then creates the product's formulation and manufacturing procedures to meet the product's characteristics. This lets you know how the CQAs are affected by the raw materials (Critical Material Attributes, or CMAs), as well as where variability comes from and how to control it. The traditional, heavily empirical approach to product development and manufacturing has greatly influenced this systematic approach. QbD is required by regulatory requirements as well as for the implementation of novel ideas like design space, the guidelines of the International Conference on Harmonization for Q8 pharmaceutical development, Q9 quality risk management, and the FDA's process analytical technology (PAT).

Keywords: Product Profile, Design, Quality, and Experimentation.

1 INTRODUCTION

A drug product of high quality is free of contamination and consistently delivers the therapeutic benefit promised to the consumer on the label. "The suitability of either a drug substance or drug product for its intended use" is the definition of quality in ICH Q8. The terms "identity," "strength," and "purity" are all included. "Delighting the customer by fully meeting their needs and expectations" is a common definition of quality. Performance, appearance, availability, delivery, dependability, maintainability, cost-effectiveness, price, and total customer satisfaction may all fall under this category. It is essential that quality be incorporated into the design. To accomplish the elevated degree of value there is need of Value by Plan.

1.1 Quality by Design

Joseph M. Juran, a well-known quality expert, first presented this idea in Quality by Design (J.M. : " Juran on "Designing for Quality" The internal FDA discussion began in the latter half of the 1990s, and in 2002, a concept paper on 21st-century Good Manufacturing Practice was published. Pilot programs to investigate the application and understandings of Quality by Design were initiated with the assistance of a number of biopharmaceutical companies.

1.2 Definition

"A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management," according to ICH Q8 (R2) Pharmaceutical Development 2009, is the

definition of QbD. It entails developing formulations and manufacturing procedures to meet predetermined quality goals. From the perspective of patients, quality by design (QbD) identifies characteristics that are essential to the quality of a drug product, translates these characteristics into the characteristics that the drug product ought to have, and establishes the key process parameters that can be varied to consistently produce a drug product with the desired characteristics.

1.3 Advantages of QbD

- It gives a more significant level of confirmation of medication item quality.
- The pharmaceutical industry can benefit from cost savings and increased efficiency.
- It makes the sponsor's understanding of the control strategy for the drug product's approval and eventual commercialization more transparent.
- It makes scaling up, validating, and commercializing transparent, logical, and predictable.
- It encourages new ideas to address unmet medical needs.
- It lowers manufacturing costs and product rejects while also improving the efficiency of pharmaceutical manufacturing processes.
- It reduces or eliminates drug recalls, costly penalties, and potential compliance actions.
- It provides opportunities for ongoing advancement.
- It improves regulatory oversight efficiency:
- It smoothes out post endorsement fabricating changes and administrative cycles.
- It makes it easier to get approval for the first cycle;
- It makes CGMP inspections after approval more focused.
- It works with consistent improvement and lessens the CMC supplement.
- It speeds up CMC review time and improves CMC quality.

2 ELEMENTS OF QUALITY BY DESIGN

a. Defining the target product quality profile

A "prospective and dynamic summary of the quality characteristics of a drug product that ideally will be achieved to ensure that the desired quality, and thus the safety and efficacy of a drug product are realized" is the definition of a Target Product Profile (TPP). This includes the dosage form, the route of administration, the strength of the dosage form, the release or delivery of the therapeutic moiety, and the pharmacokinetics characteristics (such as dissolution and aerodynamic performance) necessary to develop the drug dosage form and the drug product-quality criteria for the intended marketed product.

The term "TPQP" for "target product quality" is a natural extension of "TPP" for "product quality." The drug product ought to have the qualities necessary for reproducibly delivering the therapeutic benefit promised on the label. The TPQP directs formulation scientists in the development of formulation strategies and the maintenance of focused and effective formulation efforts. Identity, assay, dosage form, purity, and label stability are all related to TPQP. The physical, chemical, and biological properties of a drug are all biopharmaceutical properties.

b. Identifying critical quality attributes

The next step is to find the appropriate CQAs after TPP is found. According to ICH guidance Q9, a CQA is defined as "a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate

limit, range, or distributed to ensure the desired product quality." Risk assessment is used to identify CQAs. The key to these risk assessments is prior product knowledge, which can be gleaned from previous laboratory, nonclinical, or clinical experience with a particular product-quality attribute. Relevant data from similar molecules and data from literature references may also be included in this knowledge. The connection between the CQA and product safety and efficacy is supported by this data. The QbD paradigm is unique in that robust risk assessment methods are used to identify CQAs.

c. Performing Risk Assessment

It simply involves connecting CQAs to material attributes and process parameters. According to ICH Q9 Quality Risk Management, the production and use of a drug product always involve some risk. The link between the patient's therapeutic benefit and the risk of quality should be the basis for the assessment. The level of risk should be proportional to the level of effort, formality, and documentation of the quality risk management process. Before developing a pharmaceutical, manufacturers can choose which studies to conduct by conducting a risk assessment. Concentrate on results figure out which factors are basic and which are not, which then guide the foundation of control techniques for in-process, unrefined substance, and last testing.

3 TOOLS OF QUALITY BY DESIGN

a. Design of Experiments (DOE)

A structured and organized approach to determining the relationship between factors that influence a process's outputs is known as design of experiments (DOE). DOE may be able to provide returns four to eight times greater than the cost of running the experiments in a fraction of the time, it has been suggested. In his groundbreaking book The Design of Experiments (1935), Ronald A. Fisher proposed a method for designing experiments. When DOE is used in QbD, it helps to get the most information from the fewest number of experiments. When DOE is applied to a pharmaceutical process, the raw material characteristics (like particle size) and the process parameters (like speed and time) are the factors. On the other hand, the critical quality characteristics (like blend uniformity, tablet hardness, thickness, and friability) are the outputs. It is impossible to experimentally investigate all of the input and output variables and process parameters of each unit operation. The key input and output variables as well as the process parameters that will be investigated by DOE must be identified by scientists using risk management and prior knowledge. DOE results can assist with recognizing ideal circumstances, the basic factors that most impact CQAs and the individuals who don't, as well as subtleties, for example, the presence of collaborations and cooperative energies between factors, Each Calculate turn and Plan of analyses.

b. Process Analytical Technology (PAT)-

PAT has been described as "a system for designing, analyzing, and controlling manufacturing through measurements, during processing of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring the quality of the final product." This definition was provided by the definitions group. "Enhance understanding and control of the manufacturing process, which is consistent with our current drug quality system:" is the objective of PAT. Products cannot be tested for quality; It ought to be included by default or be built in. The key and critical process parameters, as determined by process characterization studies, along with their acceptable ranges, define the design space. On-line,

in-line, or at-line PAT applications focus primarily on these parameters. Real-time PAT assessments could theoretically serve as the foundation for ongoing feedback and enhance process robustness. Because it monitors the particle size, blend uniformity, granulation, content uniformity, polymorphism, dissolution, and the process online, at the line, and offline, NIR serves as a tool for PAT and is useful in RTRT (Real Time Release Testing). As a result, it reduces the product's release testing.

4 CHALLENGES OF IMPLEMENTING QBD WITH FDA PERSPECTIVE

Problems with implementation include:

- Implementing novel ideas and methods (Quality by Design, Design Space, Quality Risk Management)
- A wide range of products
- Expectation for QbD-based submission while meeting traditional requirements (dual process)
- Integration of ongoing review and inspection work, a broad range of approaches to development, manufacturing, and quality operations across the industry implementing, harmonizing
- Changes in culture, application information, and the role of an industry scientist in regulatory discussions are implementation challenges for the industry. Business challenges include removing budgeting silos across business units, removing silos across business units, and making additional investments during development to improve efficiency and reduce manufacturing costs over the lifecycle. Management support is also a challenge.

5 CONCLUSION

Quality by design is a common understanding of the ICH Q8, Q9, and Q10 concepts that will be crucial to the formulation process. The review clarifies the concept of critical process parameters, implements the control strategy, continues monitoring and updating the process, and explains the use of the target product profile, risk assessment, and critical material attributes. In addition, it explains how QbD principles and tools can be used in the development of drug products and processes. It is possible to draw the conclusion that the principles and tools of Quality by Design (QbD) play a significant role in facilitating a higher level of understanding of the process and creating opportunities for investigation and the development of control strategies for formulation and process development.

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DESIGN AND ANALYSIS OF GASTRO-RETENTIVE FLOATING TABLETS CONTAINING NORFLOXACIN: FORMULATION, CHARACTERIZATION, AND IN VITRO EVALUATION

Vaishnavi Munnangi
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Soorammagari Sunayana
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The research was conducted with the intention of developing a Norfloxacin drug delivery system that was gastro-retentive. Using sodium bicarbonate and citric acid anhydrous, like Hydroxypropyl Methylcellulose (HPMC K100M), Xanthum gum, and Microcrystalline Cellulose (MCC), the wet granulation method was used to make floating tablets. The prepared Norfloxacin floating tablets were found to be satisfactory, free of chipping, capping, and sticking. It was discovered that each tablet formulation contained the same amount of drug. The best formulation was determined to be Formulations F3 and F5, which demonstrated the desired drug release. In the end, it was determined that the optimized formulations F3 and F5, which adhered to all of the properties of floating tablets, were satisfactory in every way.
Keywords: Controlled release, floating drug delivery systems, and bioavailability.

1 INTRODUCTION

The most encouraging and most secure course of medication conveyance is the oral course and is considered as the most encouraging course of medication conveyance. Viable oral medication conveyance relies on the elements, for example, entrail discharging, travel season of measurements structure through the Gastrointestinal system, remedial conveyance from the dose structure and so on. Humans are affected in various ways by these factors. The variables, for example, balance by corrosive or postponed ingestion may likewise prompt accelerate arrangement of may cause balance of medication prompting non-uniform retention and makes the bioavailability erratic. Drug release is controlled by controlled-release drug delivery systems (CRDDS) at a predetermined, monitored, and predictable rate. So, a good delivery system should be able to control and extend the time it takes for the stomach to empty, as well as deliver drugs at higher concentrations to the absorption site (the upper part of the small intestine).

These gastro retentive systems keep drugs in the stomach for hours, significantly increasing their gastric residence time. As a result, prolonged retention increases bioavailability, thereby decreasing drug waste and increasing drug solubility. The floating drug delivery systems satisfy all of the aforementioned requirements, allowing for efficient drug delivery to

the absorption window for the treatment of gastric disorders like gastro-esophageal reflux.

Prostatitis, gonorrhea, and urinary tract infections are all treated with norfloxacin, a broad-spectrum fluoroquinolone antibiotic. The stomach absorbs norfloxacin better than the lower gastrointestinal tract, where it is least absorbed. This medication has a low bioavailability (30–40%), a short biological half-life (3–4 h), and a repetitive dose schedule (400 mg twice daily). As a result, Norfloxacin is a potential candidate for a gastro-retentive drug delivery system. The development and evaluation of floating Norfloxacin tablets' formulations are described in detail in this work.

2 MATERIALS AND METHODS

2.1 Materials Used

Solisto Pharmaceuticals Pvt Ltd. in India generously provided a sample of norfloxacin. The pharmaceuticals laboratory at Amity University in Lucknow provided the hydroxypropyl methylcellulose (HPMC), micro crystalline cellulose (MCC), polyvinylpyrrolidine (PVP), and magnesium stearate. Analytical-grade chemicals were all used.

3 METHODS

3.1 Preformulation Studies

The drug underwent appearance testing, solubility, and melting point analyses as part of the preformulation process.

3.2 Development of Tablets

Precisely gauged amounts of Norfloxacin, HPMC and MCC were gone through a 0.425 mm sifter to get uniform size particles, then, at that point, they were blended mathematically for 5 to 10 minutes and the combination was put in a polyethylene pack and further blended for 5 minutes to guarantee a homogeneous mass. The quantity of PVP added was accurately weighed. A 16-station punching machine made tablets from the powder mixture (Rimek, India).

Table 1 Ingredients for the Norfloxacin formulation.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Drug (Norfloxacin)	100	100	100	100	100	100	100	100
HPMC	100	80	100	100	100	100	100	100
Sodium Bicarbonate	100	50	25	-	50	-	-	-
Citric acid	100	50	25	-	25	-	-	-
Magnesium stearate	6	6	6	3	3	3	3	-
MCC	94	94	94	47	47	47	47	47
Xanthum gum	-	-	200	1000	250	120	-	-
Gum karaya	-	20	-	-	-	-	-	-
PVP	-	-	-	-	-	-	30	50
Isopropyl Alcohol (ml)	-	-	-	-	-	-	10	-

4 RESULT AND DISCUSSION

This work was intended to lead to the creation of floating Norfloxacin tablets, with the primary objective of increasing the buoyancy time of the delivery system to improve the drugs' retention time. The outcomes have shown that tablets were found to have

great properties as well as upgraded lightness time which gave great maintenance time to the medication in the body. A total of eight formulations of gastro-retentive floating Norfloxacin tablets were made in this study using a wet granulation technique with different polymers like HPMC K100M, Xanthan gum, and MCC as semi-synthetic and natural polymers. The gas-generating agents were sodium bicarbonate and citric acid, and the lubricants were magnesium stearate and talc. Different polymer ratios were used to make formulations work best. The drug was found to have a whitish appearance, no odor, and no taste. The drug's melting point was determined to be 220°C using a melting point apparatus, and the other parameters were found to be the same as those outlined in the preceding section.

4.1 Pre-compression Evaluations

Bulk Density, Tapped Density, Carr's Index and Angle of Repose

All of the observations were found to be within the prescribed IP limits after the drug and its components were evaluated for the aforementioned parameters. Based on the compressibility index and angle of repose, all of the formulations had good flow characteristics (Table 2).

Table 2 Precompression factors for Norfloxacin floating tablets.

Precompression parameters	A	B
Bulk Density	0.458	0.469
Tapped density	0.589	0.654
Carr's index	0.777	0.717
Angle of repose	26.8	29.78

5 CONCLUSION

Improved bioavailability of medications with a narrow absorption window is made possible by controlled release gastro-retentive dosage forms (CR-GRDF), which allow for prolonged and continuous drug delivery to the upper parts of the gastrointestinal (GI) tract. For these drugs, CR-GRDF makes it possible to make use of all the advantages of controlled release dosage forms in terms of pharmacokinetics (PK) and pharmacodynamics (PD). F3 and F5 were found to have the best parameters out of the eight tablet formulations. As a result, the study demonstrates that tablets containing the appropriate amount of Xanthum gum had adequate binding properties and a favorable buoyancy time due to citric acid. It was discovered that the tablet F3 had a delayed release over time, indicating that it provided greater retention than F1 and F2. Therefore, of the eight formulations, formulations F3 and F5 will offer the Norfloxacin with the longest retention time. According to the reviewed literature, drug absorption in the gastrointestinal tract is a highly variable process, and the dosage form's gastric retention prolongs drug absorption. Accordingly gastro retentive measurement structures give an extra benefit to drugs that are

assimilated essentially in the upper sections of gastrointestinal parcel, for example stomach, duodenum and jejunum. In order to determine the most effective dosage form for a given drug, in vivo studies are necessary due to the complexity of the pharmacokinetic and pharmacodynamics parameters. The advantages and disadvantages of controlled release gastro retentive forms in comparison to other dosage forms will be determined by the interaction of a drug's pharmacokinetic and pharmacodynamic parameters.

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EXPLORING THE POTENTIAL OF LIPOSOMES AS A TRANSDERMAL DRUG DELIVERY SYSTEM: A COMPREHENSIVE REVIEW OF PROGRESS, LIMITATIONS, AND NOVEL APPROACHES

Hariprasad Kadiyam
Assoc. Professor, Department of Pharmaceutical Chemistry, Princeton college of Pharmacy, Hyderabad, Telangana, india
Golla Lavanya
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton college of Pharmacy, Hyderabad, Telangana, india

Abstract - Because it circumvents a number of issues that are associated with the oral route of drug administration, the transdermal route of drug delivery has piqued the interest of pharmaceutical research. This kind of drug carrier system is one of a kind because it can carry hydrophilic, lipophilic, and amphiphilic drugs. Before being delivered to the skin, these medications are placed in a variety of locations within the vesicle. Liposomes are microscopic, lipoidal vesicles that are the subject of extensive research as potential drug carriers with the goal of enhancing the delivery of therapeutic agents. Several liposome-based drug formulations are currently in clinical trials as a result of recent advancements in liposome technology. Some of these formulations have recently received approval for clinical use. The reformulation of drugs into liposomes has provided an opportunity to alter the bio distribution of various agents, primarily in order to improve their therapeutic indices. With examples of formulations that have been approved for clinical use, this review discusses the potential uses of liposomes in drug delivery as well as the drawbacks of expanding their use.

Keywords: Liposome, Amphotericin B, Drug delivery system, Doxorubicin, Pharmacokinetic.

1 INTRODUCTION

The design and synthesis of hundreds of new agents with the potential to act in vitro against a wide range of therapeutic targets has been made possible by recent advancements in biomedical science and combinatorial chemistry. However, in the clinic, the majority of these new drugs do not live up to their potential. For instance, despite the fact that many anticancer agents are highly cytotoxic to tumor cells in vitro, they cannot be used in the clinic due to their lack of a selective antitumor effect in vivo. The low therapeutic index (TI) of antineoplastic drugs is one of their major drawbacks; this refers to the fact that the dose required to have an effect against tumors is toxic to healthy tissues. These drugs may have a low TI because:
(i) that they are unable to attain therapeutic concentrations at the target site (solid tumors); ii) nonspecific cytotoxicity to vital normal tissues like the heart, intestines, kidneys, and bone marrow; and/or (iii) issues with the drug's manufacturing, such as its low solubility in pharmaceutically appropriate containers, necessitating the use of organic cosolvents or surfactants, both of which have their own undesirable side effects. As a result, efficient delivery systems are required that not only facilitate drug formulation but also alter drug biodistribution so that a greater proportion of the dose reaches the intended site. Liposomes are colloidal or microparticulate carriers that spontaneously form when certain lipids are hydrated in an aqueous medium8. Their typical diameter ranges from 0.05 to 5.0 micrometers. Liposomes are made of a material that is relatively biocompatible and biodegradable. They are made up of an aqueous volume that is held in place

by one or more bilayers of lipids, either natural or synthetic.

2 CLASSIFICATION OF LIPOSOMES

2.1 On the Basis of Composition

Liposomes are composed of natural and/or synthetic lipids (phospho- and sphingo-lipids), andmay also contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The net physicochemical properties of the lipids composing the liposomes, such as membrane fluidity, charge density, steric hindrance, and permeability, determine liposomes' interactions with blood components and other tissues after systemic administration. The nature and extent of liposome-cell interaction in turn determines the mode of intracellular delivery of drugs. Thus, the predominant mechanism behind intracellular delivery of drugs by liposomes may mainly depend on their composition, as depicted**.** Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types as: (i) conventional liposomes (CL); (ii) pH-sensitive liposomes; (iii) cationic liposomes; (iv) immunoliposomes; and (v) long-circulating liposomes (LCL). The typical composition and characteristics for these types of liposomes**.**

2.2 On the Basis of Size

The liposome size can range from very small (0.025 /~m) to large (2.5/~m) vesicles. Furthermore, liposomes may have single or multiple bilayer membranes**.** The vesicle size is a critical parameter in determining circulation half-life of liposomes, and both size and number of bilayers influence the extent of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of three categories: (i) multilamellar vesicles (MLV); (ii) large unilamellar vesicles (LUV); and (iii) small unilamellar vesicles (SUV). The size and characteristics of these types of liposomes**.**

2.3 Applications of Liposomes in Drug Delivery

When an existing formulation is unsatisfactory and a reformulation offers superior therapeutic efficacy and safety over the existing formulation, new drug delivery systems like liposomes are developed. For sure, liposome plans of certain medications have shown a critical expansion in remedial viability or potentially helpful records in preclinical models and in people, contrasted with their non-liposomal details. There are a few general categories that liposomes can be used for in medicine, which are briefly described below.

Definition Help: For human systemic administration, hydrophobic medications like cyclosporin and paclitaxel typically consist of surfactants and organic co-solvents. At the dosages required to deliver the drug, these solubilizers may cause toxicity. Liposomes, on the other hand, can contain a wide variety of drugs that are water-insoluble (lipophilic) and are made up of lipids, which are relatively non-toxic, non-immunogenic, biocompatible, and biodegradable molecules. Preclinical studies have evaluated liposomes as a vehicle for the delivery of paclitaxel and its analogs as an alternative to the cremophor/ethanol vehicle. At the moment, liposomes or phospholipid mixtures are being used as excipients for the preparation of better-tolerated preclinical and clinical formulations of several lipophilic, poorly water soluble drugs, such as amphotericin B. These formulations are being prepared using liposomes Paclitaxel liposomes had the option to convey the medication foundationally and increment the restorative file of paclitaxel in human ovarian growth models.

Intracellular Medication Conveyance: For a drug to have pharmacological activity, it must cross the plasma

membrane and have targets or receptors within the cell. Liposomes can be used to improve the cytosolic delivery of some drugs, like N-(phosphonacetyl)-L-aspartate (PALA), which are typically not well absorbed by cells16,17. Through fluid-phase endocytosis (pinocytosis), PALA enters the tumor cells and diffuses into the cytoplasm as the endosome pH drops12. However, pinocytosis only works in a very limited way.

Drug Administration with Sustained Release: Drugs like cytosine arabinoside (Ara-C), which are rapidly cleared in vivo and require plasma concentrations at therapeutic levels for an extended period of time for optimal pharmacological effects4,9, necessitate sustained release systems. Now, formulations of sustained release liposomes with a longer half-life in circulation and an improved drug release rate in vivo can be designed. For instance, Ara-C embodied in LCL is viable as a drawn out discharge framework in the treatment of murine L1210/C2 leukemia3,5. By slowly leaking drugs from RES into the general circulation, conventional liposomes that localize by phagocytosis in the cells of RES may also serve as a sustained release depot.

Site-Evasion Conveyance: Drugs used to treat cancer typically have a low therapeutic index (TI) and can be extremely toxic to healthy tissues. By reducing their delivery to essential normal organs, these drugs may be less toxic. By encapsulating the drug in liposomes, it has been demonstrated that even a modest reduction in the amount of drug that reaches critical organs can significantly reduce its toxicity.

Site-Explicit Focusing on: The idea of site-specific delivery, which was first put forth by Paul Ehrlich15, entails delivering a larger portion of the drug to the target location, thereby minimizing exposure to normal tissues. Both passive and active drug targeting have been achieved with liposomes.

3 LIMITATIONS OF LIPOSOME TECHNOLOGY

As described above, liposomes have a great potential in the area of drug delivery. However Liposome-based drug formulations have not entered the market in great numbers so far. Some of the problems limiting the manufacture and development of liposomes have been stability issues, batch to batch reproducibility, sterilization method, low drug entrapment, particle size control, and production of large batch sizes and short circulation half-life of vesicles. Some of these issues such as short half-life have been resolved resulting in increased numbers of clinical trials and new approvals**.** Some of the remaining problems are discussed in detail below.

Stability: The physical and chemical stability of liposomes is one of the main barriers to their widespread use. The chemical and physical instability of the final liposome formulations may result in short shelf lives, depending on their composition. Ester bond hydrolysis and/or lipid unsaturated acyl chain oxidation can both lead to chemical instability. Drug leakage from the vesicles and/or vesicle aggregation or fusion to form larger particles can both result in physical instability.

Efficiency of Encapsulation: Because lipids in high doses may be toxic and also cause non-linear (saturable) pharmacokinetics of liposomal drug formulation, liposome formulation of a drug cannot be developed unless the encapsulation efficiency is such that therapeutic doses can be delivered in a reasonable amount of lipid. For hydrophilic drugs, some novel strategies with high encapsulation efficiencies have been developed. For example, dynamic stacking of the amphipathic frail acidic or fundamental medications in void liposomes can be utilized to build the embodiment productivity. Paclitaxel, a hydrophobic drug with an encapsulation efficiency of less than 3 mole percent, is not a good candidate for active loading

because of its low affinity for lipid bilayers.

Aiming at the Future: Due to RES's non-specific uptake by cells, active targeting with ligand-directed immunoliposomes has been limited by their rapid clearance. Since LCL are not as quickly cleared by RES, the creation of LCL that are conjugated with ligands has rekindled interest in this area. However, there are still numerous issues to be resolved. For instance, immunoliposome-conjugated foreign immunoglobulin ligands may increase clearance upon subsequent exposure and induce immunogenicity.

Degradation of Lysosomes: The efficacy of the liposome is determined not only by the quantity of drug associated with the cell but also by the quantity of drug reaching the "target molecule" within the cells once it reaches the target cell. Although immunooliposomes can selectively deliver the drug to cells, the drug's pharmacological activity is contingent on the drug's intact ability to diffuse sufficiently into the cytoplasm from the endosomes.

Liposome Formulations in Market

Several nations have granted clinical approval to liposome and lipid-complex formulations of doxorubicin, daunorubicin, and amphotericin B (AmB). Doxorubicin and daunorubicin, two examples of highly effective antineoplastic drugs, However, in humans, they can cause severe cardiac toxicity. The major dose-limiting factor for free doxorubicin is the potential for irreversible cardiomyopathy, which limits the human lifetime cumulative drug dose to 550 mg/m2. It has been demonstrated that long-circulating liposome formulations of anthracyclines improve the TI of the drugs against a variety of solid tumors by increasing drug accumulation in tumors and reducing cardiac toxicit. Doxorubicin is an excellent candidate for encapsulation in liposomes because it can be encapsulated into liposomes using an active loading technique with high efficiency. DoxilTM, a doxorubicin LCL formulation, was the first liposome product approved for use in the United States. Due to avoiding high peak concentrations of the free drug, Doxil TM had a circulation half-life that was up to 8 times longer than that of the free drug19. Additionally, the incidence of side effects was lower. Epitome of doxorubicin in liposomes has expanded the TI and made conceivable portion acceleration mostly by diminishing the portion restricting heart poisonousness. Amphotericin B (AmB) is an antifungal medication used to treat severe fungal infections of the body. However, serious side effects of AmB therapy include nephrotoxicity, thrombophlebitis, hypokalemia, and anemia. The primary reason for therapy failure or discontinuation is these side effects, which limit the dose levels that can be achieved (0.7-1.5 mg/kg of Fungizone TM). Due to a decrease in the dose-limiting nephrotoxicity, lipid- and liposome-based formulations of AraB have been shown to have superior TI to the deoxycholate-based formulation (FungizoneTM). Other adverse effects are also less common; in certain examinations these (basically hypokalemia) went from 10 to 20%. It's possible that the selective transfer of AmB from lipid bilayers or complexes to the fungus (the target site) has reduced toxicity by reducing the drug's interaction with human cell membranes. AmB doses have been increased because side effects like nephrotoxicity have decreased in severity and frequency. In conclusion, this article briefly discussed some formulation and development issues as well as a review of the potential uses for liposomes. The rising number of clinical trials involving liposome and lipid-based products is a positive sign. Several businesses are actively expanding and evaluating liposome products for use in anticancer and antifungal therapy as well as prophylaxis (vaccinations) against diseases in light of recent advancements in the field. The full development of

liposomes as drug carriers will be sparked by further technology refinements.

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ENHANCING THE ORAL BIOAVAILABILITY OF ALBENDAZOLE: DEVELOPMENT AND ASSESSMENT OF FAST-DISSOLVING TABLET FORMULATION

Dr. P.Raja Sridhar Rao
Assoc. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
G Satheesh
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Albendazole is a broad-spectrum anthelmintic that can be used to kill numerous helminths. Treatments for Tapeworm, Hookworm, and Threadworm are made with it. Due to its first-pass metabolism, it has a low bioavailability. In this study, a fast-dissolving Albendazole tablet was designed to provide a rapid onset of action. The study's primary objective was to develop Albendazole tablets with a faster dissolution rate in order to further enhance the drug's bioavailability. Tablets that are made to dissolve quickly, tested for the pre-compression parameters, and prepared through direct compression with various concentrations of super disintegrants. For the purpose of post-compressional evaluation, the prepared tablets were evaluated. The formulation F3 with 5% w/w superdisintegrant Crospovidone and 20% w/w microcrystalline cellulose was deemed to be the best of all, with a release rate of up to 99.097% in 40 minutes.

Keywords: Albendazole, superdisintegrants, *in vitro* disintegration time, *in vitro* dissolution test.

1 INTRODUCTION

By developing a dosage form that is intended for administration, recent advancements in novel drug delivery systems aim to enhance the drug molecule's safety and efficacy (Kuchekar et al., 2003). Patients such as children, geriatrics, bedridden, disabled, and mentally ill individuals experience difficulty swallowing (Seager et al., 1998). According to Shu et al., fast dissolving tablets are solid dosage forms containing medical substances that disintegrate rapidly upon being placed on the tongue, typically within a few seconds, requiring no additional water to facilitate swallowing. 2002; Bradoo and other, 2001). Albendazole (ABZ), also known as methyl [5-(propylthio)-1-H-benzimidazol-2yl] carba-mate, is a benzimidazol derivative that is effective against a variety of helminth parasites in both animals and humans (Cook et al., 1990). Echinococcosis, neurocysticercosis, and hydrated cysts can all be effectively treated with ABZ (Wen et al., 1993).

One method, direct compression, necessitates the inclusion of superdisintegrants in the formulation or highly. Superdi-sintegrants like cross-linked Croscarmellose Sodium, Polyvinyl Pyrrolidone K30, Microcrystalline Cellulose, and Crospovidone, among others, were the fundamental method utilized in the creation of FDT. Which provide instantaneous tablet disintegration upon tongue

placement, releasing the medication into saliva.

2 MATERIAL AND METHODS

Brasica Pvt. provided a sample of albendazole as a gift. Ltd. Boisar (India). A sample of crospovidone, croscarmellose sodium, and microcrystalline cellulose were presented as a gift by Curex Pharma, Jalgaon. Emcure Pharma, Pune, provided Polyvinyl Pyrrolidone K30 as a gift sample, and Merck Ltd, Mumbai, India, provided Manni-tol, Aspartame as a gift sample. All of the reagents and chemicals used were of analytical quality.

2.1 Preparation of Fast Dissolving Tablets

Using the direct compression method and superdisintegrants Crospovidone (CP), Croscarmellose Sodium (CCS), and Polyvinyl Pyrrolidone K30 (PVPK30), fast-dissolving Albendazole tablets were prepared beforehand. Using a pestle, the 200 mg of Albendazole, mannitol, and microcrystalline cellulose were thoroughly mixed in a glass mortar. In accordance with each tablet formulation, superdisintegrants were added to the powder mixture, and then Aspartame and magnesium stearate were added. Sieve No. 1 was used to process the entire mixture. The rotary tablet machine [Jaguar (JMD4-8)]'s 12mm round flat-faced punch was used to make the tablets.

3 PRECOMPRESSION PARAMETERS

3.1 Angle of Repose

The fixed funnel method was used to determine the angle of repose. A funnel that can be raised vertically to a maximum cone height (h) was used to pour the mixture through. The heap's radius (r) was measured, and a formula was used to determine the angle of repose (Rockville et al., 2007).

3.2 Bulk Density

Apparent bulk density (LBD) was determined by pouring blend into a graduated cylinder. The bulk volume (Vo) and weight of powder (M) was deter-mined.

3.3 Tapped Density

The measuring cylinder containing known mass of blend was tapped for a fixed time. The minimum volume (Vt) occupied in the cylinder and weight of powder blend (M) as measured.

3.4 Carr's Compressibility Index

The simplex way of measurement of the free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility index of the granules was determined by Carr's compressibility index (C) which is calculated.

3.5 Post compression parameters

All the batches of tablets were evaluated for various parameters like weight variation, friability, hardness, drug content, disintegration and dissolution and results.

3.6 Uniformity of weight

By randomly weighing 20 tablets and calculating their average weight, this test is carried out to maintain the uniform weight of each tablet within the prescribed range. The mean and standard deviation were calculated (Thahera et al.,) for no more than two of the individual weights that differ

from the average weight by more than the 2012).

3.7 Thickness

Using a Micrometer screw gauge, the tablets' thickness and diameter were determined. Average values were calculated using five tablets from each formulation. It is given in millimeters (Liberman et al., 1990).

3.8 Hardness Test

The hardness of the tablet was determined using Monsanto Hardness Tester (Rockville et al., 2007).

3.9 Friability Test

Using Roche Fribilator (Tropical Equip-ment Pvt.) and six tablets from each batch, the friability of the tablets was evaluated. Ltd. Mumbai, India), and the apparatus was turned on for four minutes at 25 RPM. The tablets were removed, dedusted, and reweighed, and the percentage of friability was determined (Rockville et al., 2007).

3.10 Water Absorption Ratio

A piece of tissue paper collapsed two times was kept in a Petri dish (inner breadth 5.5cm) containing 6ml of refined water. The tissue paper was placed over the tablet and allowed to completely absorb the liquid. The wet tablet was taken out and weighed again.

3.11 In Vitro Disintegration Time

At first, the Pharmacopoeia's standard test for tablets was used to measure the disintegration time of fast-dissolving tablets. Tablets were put in the crumbling cylinders and time expected for complete breaking down without leaving any deposits on the screen was recorded as deterioration time (EP, 1988).

4 CHARACTERIZATION OF ALBENDAZOLE TABLET FT-IR STUDIES

For the pure Albenda-zole, an infrared spectrum was taken. Computer-mediated Fourier transformed infrared spectroscopy (FTIR) (Shimadzu Model – IRAFFINITY-1, Serial No.) was used for the KBr disk method of FT-IR research. A21374600405).

Albendazole fast-dissolving tablets were prepared by direct compression using superdisintegrants like Crospovidone, Croscarmellose sodium, and Microcrystalline Cellulose in concentrations of 5 percent, 4 percent, and 15 percent, respectively. Position of repose: between 24.68 and 28.62 degrees indicate good flow. Tapped density and bulk density: between 0.61 and 0.72 g/ml, or 0.38 and 0.47 g/ml, respectively. The Hausner ratio and compressibility index, on the other hand, range from 1.13 to 1.58. The tablets were kept for 0 days, 30 days, 60 days, and 90 days at 45°C 2°C/75%. The hardness increased over time, but it remained within the limit in all cases. Time to decomposition: at various storage conditions rises, but not more than 40 seconds, or less than one minute (IP specification). According to studies on the dissolution of formulations, there was no significant difference between the dissolution data at the beginning and after a specific storage period.

5 CONCLUSION

Albendazole tablets that dissolve quickly can be made using direct compression techniques and certain

superdisintegrants for better patient compliance and effective treatment. Crospovidone was found to be superior to Croscarmellose sodium in terms of their ability to improve tablets' disintegration and dissolution rates.

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ADVANCES IN MATRIX TYPE DRUG DELIVERY SYSTEMS: A COMPREHENSIVE REVIEW

Korna Devamani
Asst. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India
Kurma Kirankumar
Asst. Professor, Department of Pharmaceutical Analysis, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - An effective controlled release drug delivery system can be a significant step toward resolving issues related to controlling the rate of drug delivery to the target tissue and directing a drug toward a specific organ or tissue. When developing a controlled release formulation for oral consumption, the matrix tablet is an intriguing option. The current study focuses on the various polymers used to make Matrix Tablets and oral controlled release dosage forms. In the process of making pharmaceuticals, the use of polymers to control the rate at which drugs are released has grown in importance.
Keywords: Matrices, Polymer, Therapeutic, Diffusion, Erosion, and Dissolution.

1 INTRODUCTION

Among the various routes (nasal, ophthalmic, rectal, transdermal, and parentral routes) that have been investigated for the systemic delivery of drugs through pharmaceutical products of various dosage forms, oral drug delivery is the mode of administration that is used the most frequently. Due to its ease of administration, patient acceptance, and cost-effective manufacturing process, the oral route is regarded as the most natural, straightforward, convenient, and safe [in comparison to the parenteral route]. The majority of pharmaceutical products that are intended for oral delivery are immediate release type or conventional drug delivery systems, which are intended for immediate release of the drug for rapid absorption. These prompt delivery measurement structures have a few impediments, for example: Drugs with a short half-life necessitate frequent administration, increasing the likelihood of missed doses and poor patient compliance. Because of the typical peak-valley plasma concentration-time profile, it is challenging to attain the steady state condition. The undeniable vacillations in the medication fixation might prompt under prescription or overmedication as the CSS values fall or ascend past the restorative reach.

When overmedication occurs, the fluctuating drug levels may precipitate adverse effects, particularly when the drug has a low therapeutic index.

A number of technical advancements have resulted in the development of controlled drug delivery systems, which have the potential to revolutionize the method of medication and provide a number of therapeutic benefits, in order to overcome the shortcomings of conventional drug delivery systems.

1.1 Controlled Drug Delivery Systems

Controlled drug delivery systems have been developed that are able to control the rate of drug delivery, maintain therapeutic activity for an extended period of time, or target drug delivery to a specific tissue.

1.2 Matrix Tablets

Direct compression of a mixture of drug, retardant, and additives to form a tablet in which the drug is embedded in a matrix of the retardant is one of the least complicated methods for manufacturing

controlled release dosage forms. Alternately, the drug and retardant mixture can be granulated prior to compression. These controlled drug delivery systems continuously release the drug through dissolution- and diffusion-controlled mechanisms. The drug is dispersed in swellable hydrophilic substances, an insoluble matrix of rigid non-swellable hydrophobic materials, or plastic materials to control its release. One of the simplest methods for manufacturing sustained release dosage forms is to directly compress a mixture of the drug, retardant, and additives to create a tablet in which the drug is embedded in a matrix of the retardant. Alternately, prior to compression, the drug and retardant blend can be granulated. Both hydrophilic and hydrophobic polymers are the materials that are most frequently used in the process of making matrix systems. Hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), hydroxyethylcellulose (HEC), Xanthan gum, sodium alginate, polyethylene oxide, and cross-linked homopolymers and copolymers of acrylic acid are examples of hydrophilic polymers that are readily available. Because a small particle size is necessary for the rapid formation of a gelatinous layer on the tablet surface, it is typically provided in micronized forms. 8-10 The introduction of matrix tablets as sustained release (SR) has resulted in a new breakthrough for pharmaceutical technology's novel drug delivery system (NDDS). The drug release rate from the dosage form is primarily controlled by the type and proportion of polymer used in the preparations, and complex production procedures like coating and pelletization are not included in this process. A lot of times, a hydrophilic polymer matrix is used to make an SR dosage form has increased its focus on the creation of sustained release or controlled release drug delivery systems due to the increased complexity and cost of marketing new drug entities.

2 OBJECTIVES

As of late, controlled discharge drug conveyance has turned into the norms in the advanced drug plan and serious exploration has been embraced in accomplishing much better medication item adequacy, dependability and security. The majority of drug delivery systems will continue to be administered orally via sustained release. As a result, this project focuses on developing tablets to reduce the risk of first-pass metabolism and improve bioavailability. As a result, the goal of this work was to develop a sustain release system that could produce a consistent plasma concentration profile for up to 24 hours. The selection of API as the model drug was based on its low water solubility and high permeability as a BCS class II drug. Additionally, it is required to maintain drug release.

- 20 percent bioavailability following oral administration are design features for sustain release tablets.
- Lower dose dumping danger.
- Less variation between and within subjects.
- A high degree of dispersion throughout the digestive system, reducing the likelihood of elevated drug concentrations in the local area.
- Due to reproducible bioavailability, the drug may reach the optimal absorption site.
- Drug transport is unaffected by emptying the stomach.

2.1 Advantages of Matrix Tablet

- Versatile, efficient, and inexpensive
- Capable of releasing compounds with a high molecular weight
- The sustained release formulations may maintain therapeutic concentrations for extended periods of time

- The high blood concentration is avoided when sustain release formulations are used.
- The patient's compliance may rise with sustain release formulations.
- Slow down the drug's absorption to reduce its toxicity.
- Protect the drug from hydrolysis or other derivative changes in the gastrointestinal tract to improve its stability.
- Reduce the local and systemic side effects to a minimum.

3 CLASSIFICATION OF MATRIX TABLETS

3.1 On the Basis of Retardant Material Used

Matrix tablets can be divided in to types.

1. Hydrophobic Matrices (Plastic matrices)

In 1959, the idea of using materials that are hydrophobic or inert as matrix materials was first presented. In this method, the drug is combined with an inert or hydrophobic polymer before being compressed into a tablet for sustained release. The dissolving drug has diffused through a network of channels between compacted polymer particles, resulting in sustained release. Polyethylene, polyvinyl chloride, ethyl cellulose, and acrylate polymers and their copolymers are examples of materials that have been utilized as inert or hydrophobic matrices. In these formulations, liquid penetration into the matrix is the rate-controlling step. Diffusion is one possible method by which such tablets could release drugs. Such kinds of framework tablets become dormant within the sight of water and gastrointestinal liquid.

2. Lipid Matrices

These prepared matrices made from materials related to lipid waxes Drug discharge from such networks happens through both pore dispersion and disintegration. As a result, digestive fluid composition has a greater impact on release characteristics than does an entirely insoluble polymer matrix. Carnauba wax in mix with stearyl liquor or stearic corrosive has been used for retardant base for some supported delivery plan.

3. Hydrophilic Matrices

The cost-effectiveness, flexibility, and widespread regulatory acceptance of hydrophilic polymer matrix systems make them a popular choice for oral controlled drug delivery. In the field of controlled release, hydrophilic polymers with high gelling capacities serve as base excipients for drug formulation in gelatinous capsules or, more frequently, tablets. A well-mixed composite of one or more drugs and a gelling agent (hydrophilic polymer) is known as an "infect a matrix."

3.2 On the Basis of Porosity of Matrix

Additionally, matrix systems can be categorized as macro porous based on their porosity; Systems with and without pores can be distinguished:

1. Macro Porous Systems: The drug diffuses in these systems through matrix pores that range in size from 0.1 to 1 m. The diffusant molecule's size is smaller than this pore size.

2. Micro Porous System: Dissemination in this sort of framework happens basically through pores. Pore sizes in microporous systems range from 50 to 200 A°, which is about the same size as diffusant molecules.

3. Non-porous System: Non-permeable frameworks have no pores and the atoms diffuse through the organization networks. There is only the polymeric phase and no pore phase in this instance.

3.3 Physicochemical Factors Influencing Release from Matrix Tablet

Dose size

The bulk size of the dose that can be administered is limited for systems that are administered orally. For a conventional dosage form, a single dose of 0.5-1.0 g is typically considered maximal. This is also true for the sustained release

form. Sometimes, compounds that need to be given in large doses can be given in multiple doses or made into liquid systems. The margin of safety involved in administering a large quantity of a drug with a restricted therapeutic range is another consideration.

3.4 Ionization, Pka and Aqueous Solubility

The majority of drugs are just acids or bases. It is essential to keep an eye on the connection between the compound's pka and the absorptive environment because the unchanged form of a drug preferentially penetrates lipid membranes. It is beneficial for drug penetration to present the drug unchanged. Sadly, the fact that the drug's aqueous solubility will typically decrease upon conversion to its unchanged form complicates the situation further. Diffusion or dissolution-based delivery systems will also be dependent on the drug's solubility in aqueous media. These measurements structures should work in a climate of evolving pH, the stomach being acidic and the small digestive system more nonpartisan, the impact of Telephone the delivery cycle should be characterized. Compounds with extremely low solvency (<0.01mg/ml) are intrinsically maintained, since their delivery throughout the time course of a dose structure in the GI parcel will be restricted by disintegration of the medication. Because the driving force for diffusion, which is the drug's concentration in solution, will be low, it is evident that the solubility of the compound will not be a good choice for drugs that are only slightly soluble.

3.5 Partition Coefficient

At the point when a medication is managed to the GI plot, it should cross various natural layers to deliver a remedial result in one more region of the body. These membranes are frequently thought to be lipidic; As a result, the efficiency with which oil-soluble drugs penetrate the membrane barrier depends on their partition coefficient. Compounds with a high partition coefficient that are lipophilic in nature are poorly soluble in water and remain longer in lipophilic tissue. Compounds with a low partition coefficient have a hard time getting through the membrane, which makes them less bioavailable. Furthermore, diffusion through polymer membranes is subject to the same partitioning effects. The drug's partitioning properties must heavily influence the choice of diffusion-limiting membranes.

3.6 Stability

Orally regulated medications can be dependent upon both corrosive base hydrolysis and enzymatic corruption. Drugs in their solid state will degrade at a slower rate; Consequently, this is the preferred delivery format for problem cases. Systems that extend delivery throughout the entire course of transit in the gastrointestinal tract are advantageous for dosage forms that are unstable in the stomach; This is also true for systems that wait for the dosage form to reach the small intestine before releasing it. When given in a sustained dosage form, compounds that are unstable in the small intestine may exhibit decreased bioavailability. This is due to the fact that more drugs pass through the small intestine and are subject to degradation there. Examples of such drugs include probanthine and propentheline.

4 CONCLUSION

Based on the above discussion, it is easy to draw the conclusion that sustained-release formulations improve patient compatibility while also increasing dose efficiency. Additionally, each of these is reasonably priced. When it comes to antibiotics, where irrational use may result in resistance, the dosage form is very helpful and simple to optimize.

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IMPROVING PATIENT COMPLIANCE: FORMULATING AND EVALUATING GLICLAZIDE FAST-DISSOLVING TABLETS

Thejovathi B
Assoc. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Nandru Ramya
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract- The development of a gliclazide tablet that dissolves quickly for rapid action was the goal of this study. The method of direct compression was modified to make the tablets that dissolve quickly. Post-compression parameters like hardness, disintegration time, weight variation, friability, wetting time, water absorption ratio, and mouth feel are evaluated for each formulation. Using a panel scale, human volunteers were recruited to test the mouth feel. The superdisintegrants were used to make a variety of formulations in three different concentrations: 3%, 5%, and 10%. In-vitro dissolution studies were used to select the formulations for various concentrations based on disintegration time, hardness, and wetting time. Superdisintegrants showed good hardness and less d.t at 10% concentration, according to the findings. This could be because of crosspovidone's secondary burst effect, high wicking, and capillary action, as well as the gelling tendency and slow water uptake of tablets containing sodium starch glycolate and crosscarmellose sodium. Hardness was found to be 5 kg/cm2, 5.14 kg/cm2, and 4.2 kg/cm2 for formulation at a 10% concentration, and disintegration time was found to be approximately 6 seconds, 11.5 seconds, and 8.9 seconds, respectively. Based on these data, the formulation sd9 with 10% crospovidone was chosen as the best one.
Keywords: Fast dissolving tablet, crosspovidone, sodium starch glycolate, crosscarmellose sodium, superdisintegrant.

1 INTRODUCTION

The desire to offer patients a more conventional method of taking their medications led to the development of the concept of the Mouth Dissolving Drug Delivery System. Many patients have trouble swallowing tablets and hard gelatin capsules. As a result, they don't follow prescriptions, which leads to a lot of non-compliance and ineffective treatment. Without the use of water, the Fast Dissolving Tablet rapidly dissolves or dissolves in saliva. Some tablets are truly fast-dissolving tablets 1-5 because they are designed to dissolve in saliva extremely quickly—within a few seconds. Others are referred to as "fast disintegrating" tablets because they contain ingredients that speed up the rate at which the tablet dissolves in the mouth. These tablets may take up to a minute to completely dissolve. This tablet dissolves instantly when swallowed, releasing the medication, which dissolves or disperses in saliva. As saliva descends into the stomach, some drugs are absorbed from the

mouth, pharynx, and oesophagus. The drug's bioavailability is significantly higher in these situations than in conventional tablet dosage forms. The Fast Dissolving Tablet is a novel dosage form that stands out from more conventional forms in several ways. Through pre-gastric absorption from the mouth, pharynx, and esophagus, the tablet's disintegration in the mouth may enhance the drug's clinical effect. Because of this, first-pass metabolism is avoided, resulting in an increase in bioavailability.

2 DESIRED CRITERIA FOR FAST DISSOLVING TABLET

The mouth-dissolving tablet should:

- Not necessitate swallowing water; rather, it should dissolve or disintegrate in the mouth in a matter of seconds.
- Compatibility with taste masking
- Portability without concern for fragility
- Feel good in the mouth.

3 EVALUATION OF FAST DISSOLVING TABLET 7-10

Variation in weight: The purpose of the weight variation test is to guarantee that all tablets in a batch weigh the same. The average and total weight of 20 tablets from each formulation were determined. Additionally, the weight variation and individual tablet weights were accurately determined.

Hardness: The strength of a tablet can be gauged by its hardness. The tablet is put to the test by measuring the force required to break it across. The force is expressed in kilograms, and for uncoated tablets, a hardness of 3-5 kg/cm2 is considered adequate. Using a Monsanto hardness tester, the hardness of ten tablets from each formulation was determined.

Test of friability: Friability is the weight loss of a tablet inside its container as a result of the surface's removal of fine particles. The tablet's ability to withstand abrasion during packaging, handling, and transport is assessed through a friability test. The tablets' friability was assessed using a Roche friabilator. Weighing 20 tablets from each formulation, we placed them in a Roche friabilator for four minutes at 25 rpm. The tablets were once more weighed and dedusted. Once more, the percentage of weight loss was determined.

Test for disintegration: Six glass tubes that were "3 long, open at the top, and held against 10" screens at the bottom end of the basket rack assembly served as the USP device for resting disintegration. Each tube contains one tablet, and the basket rack is poisoned in a 1 liter beaker of distilled water at 37 degrees Celsius so that the tablets don't touch the liquid as they move upward and don't touch the bottom of the beaker more than 2.5 centimeters.

Wetting time

A straightforward method was used to measure the tablets' wetting time. A petridish containing three milliliters of a solution containing 0.2% w/v methylene blue contained five 10 cm-diameter circular tissue papers. On the tissue paper's surface, a tablet was carefully placed. The wetting time was recorded as the amount of time it

took for the tablets' upper surface to turn blue.

4 MATERIALS AND METHODS

Sun Pharma, Baroda, India, presented a sample of gliclazide as a gift. A gift sample of Ac-Di-Sol, sodium starch glycolate, and crosspovidone was obtained from Signet Chemicals Mumbai. Aerosil, Magnesium Stearate, Sodium Saccharin, and tartaric acid were all commercially sourced analytical grade materials.

Cross carmellose, cross povidone, and sodium starch glycolate were used as disintegrants in the current study to make Gliclazide fast dissolving tablets at three, five, and ten percent concentrations, respectively. The direct compression method was used to prepare nine formulations in total. the information that was gathered regarding post-compression parameters like hardness, friability, variation in weight, and drug content; time for in-vitro wetting and disintegration. For each formulation, the hardness was found to be between 3.9 and 5.14 kg/cm2, which indicates that the material has good mechanical strength and can withstand physical and mechanical stress while being handled. The IP limits are met because the friability values in all formulations are less than one percent. The percentage weight variation of all the tablets was within the pharmacopoeial limits, so they all passed the weight variation test. All of the tablets had the same weight and low standard deviations, indicating that the drug, disintegrants, and excipients were mixed well. All of the tablets' in-vitro wetting and disintegration times met the requirements for fast-dissolving tablets and were found to be within the recommended ranges. The in-vitro disintegration time was found to be between 6 and 39 s, while the in-vitro wetting time was found to be between 4 and 33 s. Formulations containing SSG, SD9, and CCM, SD8 (both ten percent), on the other hand, produced formulations with minimum D.T. of 8.9 seconds and minimum DT of 11.5 seconds, respectively. As a result, superdisintegrants produced less D.T. and demonstrated good hardness at ten percent concentration. This could be because of the secondary burst effect and high wicking and capillary action of CPV, as well as the gelling tendency and slow water uptake of tablets with SSG and CCM rather than CPV. Super disintegrant's faster disintegration can be attributed to the increased hydrodynamic pressure and swelling that result from the increased rate and extent of water uptake.

5 CONCLUSION

Utilizing super disintegrants, Gliclazide's fast-dissolving tablets were developed. The mixture that was made through direct compression using a drug to crosspovidone ratio of 10 percent had ideal and repeatable characteristics, including a disintegration time of 6 seconds.

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EXPLORING THE POTENT ANTIMICROBIAL ACTIVITY OF 4-THIAZOLIDINONE DERIVATIVES: A COMPREHENSIVE REVIEW

Zareena Begum Shaik
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Chinnabathini Anilkumar
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The act of restorative science is dedicated to the disclosure and advancement of new specialists for treating infections. The development of a new drug is extremely difficult and necessitates the skills of experts in many different fields. Establishing a connection between chemical and biological activity has been an important part of medicinal chemistry. It has been established that heterocyclic compounds make up half of the therapeutic agents. The core of the active moiety, or pharmacophore, is the heterocyclic ring. Because they are utilized in a variety of pharmacy fields and possess a broad spectrum of biological activity, heterocyclic compounds containing sulphur and nitrogen receive particular attention. It is notable that various heterocyclic mixtures containing nitrogen, oxygen and sulfur show a wide assortment of organic movement. Compounds conveying the thiazolidinone ring have answered to exhibit many pharmacological exercises which incorporate enemy of microbial antifungal movement, antitubercular antitumor, antidiabetic action calming, anticonvulsant. Up until the 1970s, fungal infections could be easily treated, so there was little need for new antifungal medications. The fact that there is a limited selection of antifungal preparations, toxicity, a narrow range of action, and the possibility of resistant strains demonstrate the need for new, efficient treatments for systemic fungal diseases. As a result, it is necessary to look for new antifungal compounds that are less harmful. In hospitalized patients, fungemia is a significant cause of morbidity and mortality. Additionally, susceptibility testing of new antifungal agents has been initiated as the emergence of resistance to currently available antifungals is cause for serious concern. To examine the action profile of thiazolidinone subsidiaries bearing different substituent at 2-3 and 5 position have been arranged.

Keywords: Heterocyclic, antimicrobial, antifungal, and antibacterial properties of thiazolidinone.

1. INTRODUCTION

The act of restorative science is dedicated to the disclosure and advancement of new specialists for treating infections. The development of a new drug is extremely difficult and necessitates the skills of experts in many different fields. Establishing a connection between chemical and biological activity has been an important part of medicinal chemistry.

Despite the fact that many natural products are utilized in pharmaceuticals with their original chemical structures, successful structural modification efforts have been made to enhance their pharmaceutics and therapeutics properties. Identifying the portion of a natural molecule that is responsible for biological activity and synthesizing new molecules based on it is another strategy for improving therapeutic properties. Heterocyclic compounds have been among the most significant organic compounds for more than a century. They are the main substances (DNA, RNA) in living cells and participate in significant biochemical processes. It has been established that heterocyclic compounds make up half of the therapeutic agents. The core of the active moiety, or pharmacophore, is the heterocyclic ring. Because they are utilized in a variety of pharmacy fields and possess a broad spectrum of biological activity, heterocyclic compounds containing sulphur and nitrogen receive particular attention. In hospitalized patients, fungemia is a significant cause of morbidity and mortality. Additionally, susceptibility testing of new antifungal agents has been initiated as the emergence of resistance to currently available antifungals is cause for serious concern. Over the past two decades, fungal infections have increased, and candida species were the most common mycotic pathogen. These species cause a wide range of infections, from minor illnesses to life-threatening ones.

1.1 Heterocyclic Compounds:

Heterocyclic mixtures, or heterocycles, are cyclic mixtures in which at least one of the particles of the ring are heteroatoms. A heteroatom is a molecule other then carbon. The Greek word heteros, which means "different," is the source of the name. Ring structures can contain a variety of atoms, including As, N, O, S, Se, P, Si, and B, among others. Five- and six-member heterocyclic systems are by far the most prevalent and significant.

More than half of all known organic compounds are heterocyclic, making them an extremely important class. Heterocyclic compounds make up almost all of the substances that make up pharmaceuticals, vitamins, and other natural products.

1.2 4-Thiazolidinone:

The significance of heterocyclic mixtures has for some time been perceived in the field of engineered natural science. It is notable that various heterocyclic mixtures containing nitrogen, oxygen and sulfur show a wide assortment of organic movement. Compounds conveying the thiazolidinone ring have answered to show a large number of pharmacological exercises which incorporate enemy of microbial antifungal activity, antitubercular, antitumor, antidiabeticactivity, mitigating, anticonvulsant.

2. PREPRATION OF THIAZOLIDINONES

The activity profiles of thiazolidinone derivatives with distinct substituents at

the 2, 3, and 5 positions were investigated by preparing new thiazolidinones using the three methods listed below.

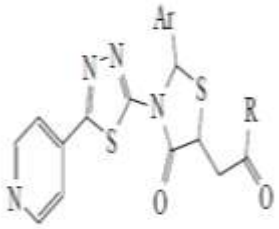
Nucleophilic addition of ethyl bromoacetate to thiosemi carbazides: Acylthiosemicarbazide got from the response of hydrazide and isothiocynates were responded with ethyl bromoacetate in outright C2H5OH within the sight of sodium acetic acid derivation to outfit 2-hydrazono-4-tiazolidinone subordinates.

Nucleophilic addition of thioglycolic/ thiolactic acid to C=N double bond: Using a Dean Stark water separator, hydrazide hydrazones made from the condensation of hydrazides and aldehydes were treated with thioglycolic and thiolactic acid in anhydrous benzene to produce 3-acylamino-2, 5-disubstituted-4-thiazolidinones and 3-acylamino-2, 4-thiazolidinones, respectively.

Reaction of α-halogenated amide with HN4SCN: Through a rearrangement reaction in EtOH with sodium acetate, halogenated amide and NH4SCN produced 2-imino-4-thiazolidinone.

3. ANTI-MICROBIAL ACTIVITY

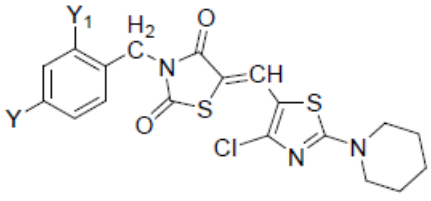
Ranjana Sharma et al. created phthalimido [2-aryl-3-(4'-pyridyl)-1',3', 4'-thiadiazol-2'-yl] ethanoates and tested their antimicrobial activity against Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas auregenosa, Salmonella typhi, and Bacillus subtilis In their comparison of how the substitution pattern of the aryl group affects antibacterial activity, they discovered that the donating group has less activity while the electron withdrawing group has more activity.



Ar (a-h) = 4-OCH_3.C_6H_4, 4-Cl.C_6H_4, 3,4,5-OCH_3.C_6H_2, 3-NO_2.C_6H_4, 4-NO_2.C_6H_4, 4-$(CH_3)_2NH$.C_6H_4, C_6H_5, C_4H_3O (2-furyl) R = phthalimidoxy.

Figure 1: Phthalimido[2-aryl-3-(5'-(4''-pyridyl)-1',3',4'-thiadiazol-2'-yl)-4-oxothiazolidin-5-yl] ethanoates

Meltem Ceylan et al. made 3-(substituted-benzyl)-5-(4-chloro-2-piperidin-1yl-thiazole-5-ylmethylene)-thiazolidine-2,4-dione derivatives and tested their antimicrobial activity against Staphylococcus aureus ATCC 250, Escherichia coli RSKK 313 and Candida albicans RSKK 628 using the disk difusion All orchestrated Mixtures were viewed as idle against Candida albicans3.



Y= H, Br, Cl, F, NO_2 Y_1= H,Cl

Figure 2 3-(substituted-benzyl)-5-(4-chloro-2-piperidin-1yl-thiazole-5-yl-methylene)-thiazolidine-2, 4- dione derivatives

Ameya A. Chavan, Nandini R. et al. created 2-[5-(arylidene)-2-imino-4-oxo-thiazolidin-3-yl] benzothiazole-6-carboxylic acid and tested it for antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli using the Cup plate According to their research, all of the newly synthesized compounds exhibit mild to moderate antifungal activity and antibacterial activity against S. aureus, B. subtilis, P. aeruginosa, and E. coli4.

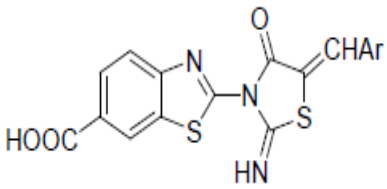


Figure 3 2-[5-(arylidene)-2-imino-4-oxo-thiazolidin-3-yl]benzo thiazole-6-carboxylic acid

Vagdevi H.M., Vaidya V.P., Latha K.P., and others created 2-[2-(2-Aryl-4-thiazolidinono)thiazol-4-yl] naphtha furans, which were found to have antimicrobial activity against Staphylococcus aureus, Klebsiella pneumonia, Aspergillus niger, and Candida albicans using the cup-plate method, antihelmintic

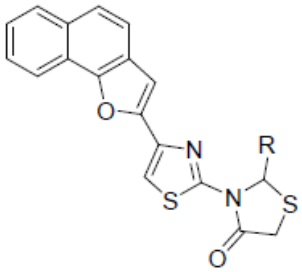


Figure 4 2-[2-(2-aryl-4-thiazolidinono) thiazol-4-yl] naphtho furans

4. FUTURE PROSPECTS

Up until the 1970s, fungal infections could be easily treated, so there was little need for new antifungal medications. The fact that there is a limited selection of antifungal preparations, toxicity, a narrow range of action, and the possibility of resistant strains demonstrate the need for new, efficient treatments for systemic fungal diseases. As a result, it is necessary to look for new antifungal compounds that are less harmful.

In hospitalized patients, fungemia is a significant cause of morbidity and mortality. Additionally, susceptibility testing of new antifungal agents has been conducted as a result of the emergence of resistance to existing antifungals1. Fungal infections have increased in frequency over the past two decades, with candida species being the most common mycotic pathogen. These species cause a wide range of infections, ranging from mild illness to life-threatening disease. As of late, there has been developing worry of quickly expanding microorganisms protection from the antibacterial arrangements in the business sectors. Despite the fact that numerous and diverse treatments for infections are currently in use, there are currently no safer or more effective options, and the infection mortality rate ranks second worldwide. Due to the emergence of a large number of antibiotic-resistant strains, the search for new high-effective antibiotics is a pressing issue.

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UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTIFICATION OF CEFIXIME AND LINEZOLID IN COMBINED DOSAGE FORM

Anumula Narender Reddy
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
A Mallikarjun
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Linezolid (LIN) belongs to the oxazolidinone class of antibiotics, while cefixime (CEF) belongs to the cephalosporin class. Numerous bacterial diseases can benefit from this drug combination. For the simultaneous estimation of Cefixime (CEF) and Linezolid (LIN) in tablet form, a method that is accurate, precise, quick, and inexpensive was developed. The method is based on simultaneous equations, and the wavelengths that were chosen for analysis in methanol were 289.0 nm (the maximum wavelength for cefixime) and 257.0 nm (the maximum wavelength for linezolid). Cefixime was found to be linear at concentrations of 5-40 g/ml, whereas Linezolid was found to be linear at concentrations of 10-30 g/ml. The relationship coefficient of Cefixime and Linezolid were viewed as 0.9998 and 0.9998 separately. The proposed strategy was effectively applied for the concurrent assurance of the two medications in business tablet readiness. The aftereffects of the investigation have been approved measurably and by recuperation studies have affirmed the exactness of proposed technique.

Keywords: Simultaneous equations, Cefixime (CEF), Linezolid (LIN), and the Ultra-Violet Spectrometric method.

1 INTRODUCTION

Cefixime (CEF) is an antibiotic taken orally of the third generation of cephalosporins. Chemically, it is (6R, 7R)-7-[2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxy-imino)acetyl]amino-3-ethenyl-8-oxo-5-thia-1 azabicyclo-[4.2.0]oct-2-ene-2 carboxylic acid1,3. Clinically, it is used to treat infections that are susceptible to infection, such as gon The first antibiotic in the oxazolidinone class is Linezolid (LIN), which is also useful as an antibacterial agent2,3. Chemically, Linezolid is N-[(5S)-3-[3-fluoro- 4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methylacetamide.

In the form of a combined dose tablet containing 200:600 mg CEF, both drugs are sold: LIN. According to the literature review, Linezolid can be estimated using spectrophoto-metrically22, HPLC1, 19, and HPTLC18 alone or in combination with other drugs in bulk drugs and human plasma. Cefixime can also be estimated using spectrophotometrically4-13 and HPLC13-17. However, no analytical method has been reported for estimating CEF and LIN in a formulation with a combined dosage. The Simultaneous equation method for the simultaneous estimation of

CEF and LIN in tablet formulation is described in this work.

2 MATERIALS AND METHODS

2.1 Instrument

A double-beam Shimadzu-1800 UV-visible spectrophotometer with a wavelength accuracy of less than 0.5 nm and a spectral bandwidth of 2 nm. It also measures the solution's absorbance in pairs.

2.2 Materials

Mepro Pharmaceuticals Ltd., Dholka, and Cadila Healthcare Pvt. provided the standard Cefixime and Linezolid samples that were included in the gift. Dabhasa, L. Tablets containing Cefixime and Linezolid in one dose were obtained from the local market. As a solvent, Loba Chemie, Mumbai, provided AR-grade methanol.

2.3 Stock Solutions

Stock solution The methanol-prepared standard stock solutions of CEF (100 g/ml) and AZI (100 g/ml) were utilized for the analysis.

2.4 Spectral Characteristics of CEF and LIN

A proper dilution of the standard stock solution was used to prepare the separate solutions of CEF and LIN (10 g/ml each). From 400 nm to 200 nm, both solutions were scanned in spectrum mode (fig. 1, 2).

2.5 Preparation of Calibration Curves

To obtain 1, 5, 10, 20, 30, and 40 g/ml of CEF and LIN, separate dilutions of the standard stock solution were performed. All of the solutions' absorption spectra were measured between 200 and 400 nm. At 289.0 nm (the maximum of CEF) and 257.0 nm (the maximum of LIN), the absorbances were measured. Working calibration curves for both CEF and LIN were plotted separately within the Beer's lamberts range.

An easy-to-use and accurate method for analyzing CEF and LIN simultaneously is presented in this work. The maximum wavelengths for analysis using the simultaneous equation method were 289.0 nm for Cefixime and 257.0 nm for Linezolid. Linearity in the detector response was observed in the concentration range of 10 to 30 g/ml for LIN and 10 to 40 g/ml for CEF using this method. For the purpose of determining the concentrations of CEF and LIN in tablet sample solution, absorptivities were calculated for both drugs at selected wavelengths and substituted into equations. In tablet analysis, this method revealed 99.03 percent label claim for CEF and 98.78 percent label claim for LIN. Low LOD and LOQ values indicated the proposed method's high sensitivity. Recovery studies verified the accuracy of the proposed methods. Using this approach, the percentages of recovery for CEF and LIN were found to be 100.51% and 100.23%, respectively. For routine quality control of Cefixime and Linezolid in combined dose tablet formulation, the proposed method could be used.

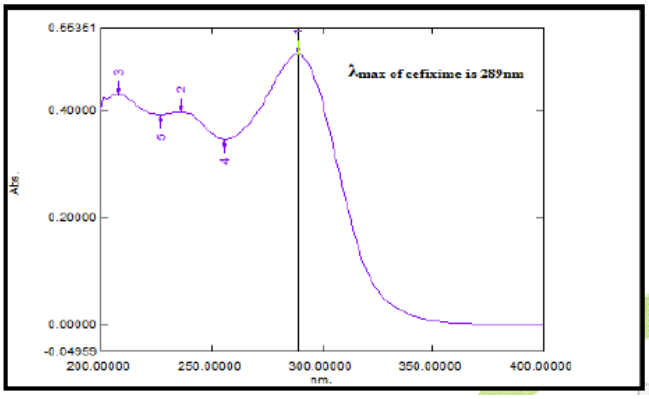

Figure 1 UV-Spectra of Cefixime (10µg/ml)

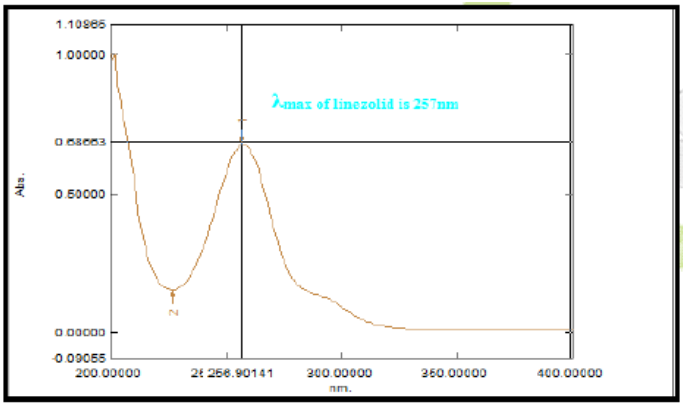

Figure 2 UV-Spectra of Linezolid (10µg/ml)

3 CONCLUSION

The developed methods were found to be within the specified limit after being validated in accordance with ICH guidelines. It comes to the conclusion that the developed methods are user-friendly, precise, sensitive, and suitable for both the real-world and tablet dosage forms.

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CHALLENGES AND OPPORTUNITIES IN PHARMACY EDUCATION IN INDIA: A COMPREHENSIVE OVERVIEW

Banuvari Sandeep
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Shireesha Bandirala
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - In India, pharmacy education has traditionally focused on industry and products. Graduate pharmacists, in contrast to the situation in developed nations, prefer placements in the pharmaceutical industry. In India, one must have at least a diploma in pharmacy to practice as a pharmacist. This diploma is awarded after only two years and three months of pharmacy studies. The foundation of pharmacy practice is made up of these pharmacists with diplomas. The curriculum for pharmacy practice has received little attention. In 2008, some private institutions began offering a practice-based doctor of pharmacy (PharmD) degree program in India. There has been a rise in the number of institutions offering pharmacy degrees at various levels. However, very little information about the current state of complex pharmacy education in India has been published. We highlight major issues in pharmacy practice, including curriculum deficiencies, in this paper, which describes pharmacy education in India. The establishment of the PharmD program and the profession's changing face are discussed. The information in this paper will be useful in further adapting pharmacy education to achieve desired educational outcomes and may encourage critical thinking, planning, and discussion.

Keywords: India, pharmacy education, and practice.

1 INTRODUCTION

India, an agricultural country that possesses most of South Asia, is a sovereign, mainstream republic comprising of 28 states and 7 association territories. With a populace of roughly 1.2 billion, India is the second most crowded country on earth. The establishment of a three-year bachelor of pharmacy (BPharm) program at Banaras Hindu University in 1937 marked the beginning of formal pharmacy education in India that culminated in a degree. At that time, the curriculum consisted of pharmaceutical chemistry, analytical chemistry, and pharmacy. This meant that graduates were prepared to work as specialists in pharmaceutical companies' quality control and standardization of drugs3, but not for pharmacy practice. There were three institutions that offered pharmacy degree programs before India became independent in 1947. When India became independent, the British rulers gave it an unorganized system for the pharmacy profession, and there were no legal restrictions on practicing pharmacy. After independence was achieved, the idea of pharmacy practice was not realized. In order to regulate the practice,

education, and profession of pharmacy, the Pharmacy Act was enacted in 1948 as the nation's first minimum educational requirement for pharmacy practice. Currently, a diploma in pharmacy is required to practice as a pharmacist. The Pharmacy Council of India (PCI) is responsible for carrying out Act provisions.6 The Act mandates that each state establish state pharmacy councils, which are in charge of regulating and registering pharmacists in their respective states. The term "institution" has been used to refer to both universities and colleges throughout this paper. For all pharmacy schools, English is the only language of instruction.

2 EDUCATIONAL PROGRAMS

India offers a variety of pharmacy degree programs: diploma in pharmacy (DPharm), bachelor's degree in pharmacy (BPharm), master's degree in pharmacy (MPharm), master's degree in science in pharmacy (MS(Pharm)), master's degree in technology in pharmacy (MTech (Pharm), doctor of pharmacy (PharmD), and doctor of philosophy in pharmacy (PhD) are all forms of pharmacy education. For DPharm, BPharm, and PharmD programs, formal education in the sciences for 12 years is the entry requirement. The DPharm program requires 500 hours of required practical training in a hospital or community setting within three months of completion of a minimum of two years of didactic coursework. The BPharm requires four years of study in a university department or a college affiliated with a university. An MPharm degree can be earned in two years by students with a BPharm degree. The second year is spent conducting research that will result in a dissertation in any pharmaceutical field, such as pharmaceutics, pharmacology, pharmaceutical chemistry, or pharmacognosy. MPharm programs on pharmaceutical biotechnology, quality assurance, and industrial pharmacy have recently been launched. The MPharm program in pharmacy practice was introduced at Jagadguru Sri Shivaratreeswara (JSS) College of Pharmacy in Mysore in 1996 and at Ooty in 1997 to train graduate pharmacists to provide clinical-oriented services.7 There are six National Institutes of Pharmaceutical Education and Research (NIPERs) in India that offer M S(Pharm), M.Tech (Pharm), and higher-level degrees. The goal of the NIPERs was to provide high-quality pharmacy and pharmacy-related education. With a minimum of three additional years of study and research, MPharm degree holders can pursue a PhD. The Pharm D program requires full-time study for six years. The Pharm D (post-baccalaureate degree) program lasts for three years. The Pharm Dprogram was presented in 2008 fully intent on creating drug specialists who had gone through broad preparation by and by destinations and could give drug care to patients.

2.1 Growth of Pharmacy Education

Up until the early 1980s, there were 11 universities and 26 colleges in India that offered bachelor's and master's degrees in pharmacy. In addition, there was at least one government school in each Indian state that offered the DPharm

program. However, the expansion of publicly funded higher education institutions—including pharmacy institutions—was extremely sluggish prior to the middle of the 1980s. India's pharmacy education has grown more quickly than anywhere else in the world since the late 1980s due to rapid pharmaceutical sector industrialization, privatization, and economic expansion. In 2007, there were 854 institutions that offered the BPharm degree to more than 52,000 students and 583 institutions that offered the DPharm degree to more than 34,000 students.9 The majority of these institutions, on the other hand, are privately funded colleges or universities. In the 1980s, about 10% of all admitted pharmacy students came from the private sector. Today, 91% of all admitted students come from the private sector.10

3 REGULATIONS AND QUALITY ISSUES

In India, there are two organizations that oversee pharmacy education: the Pharmacy Council of India (PCI), which was established by the Pharmacy Act of 1948, and the All India Council for Technical Education (AICTE), which was established by the AICTE Act of 1987. Both of these organizations are known as the PCI. As was mentioned earlier, the PCI sets standards for the minimum level of education required to become a pharmacist. It is in charge of registering individuals who meet the required eligibility requirements (minimum DPharm) and issuing licenses that allow them to practice in an Indian state. Enrollment action is decentralized and the state drug store committees are answerable for enlisting drug specialists in their particular states. As a result, the PharmD program and the DPharm program are governed by the PCI. The qualifications can only be accepted for registration purposes if the PCI recognizes the BPharm program. The MPharm and other higher-level degree programs are outside the purview of the PCI.

4 CURRICULUM

The DPharm curriculum, which was updated in 1991 and is the same for all colleges, can be changed by notifying the central government and amending the Pharmacy Act. The basic pharmacy courses in the program mostly teach old, out-of-date ideas, and they cover a lot of irrelevant, unimportant topics that don't really matter in practice. Preparations of aromatic waters, iodine and other simple solutions, tinctures, extracts, and spirits are among the topics covered in the Pharmaceutics I practical subject. The Pharmaceutics II useful commits 100 hours to advancing no less than 100 remedy items and their compounding and apportioning techniques, and covers combination, separated powders, liniments, and different contrary qualities in solution items. In a time when manufactured, ready-to-dispense medications are widely used and accepted, none of these subjects are particularly relevant.

5 EMPLOYMENT

The goal of the DPharm program was to prepare students to work as community and institutional pharmacists. With a DPharm degree, pharmacists can work in a government or private hospital or

community pharmacy (mostly private). The majority of pharmacists with a diploma prefer to work in government hospitals rather than private pharmacies or hospitals. Additionally, they are looked at for placements in the pharmaceutical sector. Pharmacists in government positions are paid less than nurses, diagnostic technicians, and radiographers, and pharmacists working in privately owned community pharmacies are always underpaid. The majority of pharmacists with a BPharm degree typically seek positions (such as production, quality control, and marketing) in the thriving pharmaceutical industries, where services are well defined and industrial pharmacists are well compensated, in accordance with the recently accepted recommendations of the sixth pay commission made by the Indian government. Additionally, the state or federal government may appoint them to quality control laboratories or drug regulatory agencies. Any of the aforementioned positions are open to applicants with an MPharm degree in any field, including clinical pharmacy. In the pharmaceutical industry, many graduates of the MPharm choose to work in areas like research, formulation development, and clinical trials. They also have the chance to work in the academic field, usually as researchers or professors. With the expansion of India's pharmaceutical industry, the demand for pharmacists continues to rise. Drug specialists with a PhDmainly work in scholarly community and in the innovative work segment of drug businesses.

6 SUMMARY

In India, pharmacy education is taught as an industry- and product-oriented profession with an emphasis on the fundamental sciences at the BPharm and MPharm levels. Over the past decade, the number of institutions offering pharmacy programs at various levels has significantly increased. However, one of the last options for students pursuing a university degree in India is pharmacy education. The majority of pharmacists with a BPharm or MPharm seek employment outside of pharmacy practice. These pharmacists would rather work in pharmaceutical production, regulatory affairs, management, quality assurance, or marketing. These graduates and postgraduates only choose to work in community and institutional pharmacies in small numbers. In India, pharmacists with a diploma (DPharm) engage in community or institutional pharmacy practice, making them global practitioners.

These postgraduates were not able to find employment in practice areas through the establishment of a specialized MPharm in pharmacy practice program in the 1990s. The introduction of the PharmD program in India is the primary change affecting pharmacy practice at the moment. One thousand four hundred and ten students have enrolled in 47 private colleges located in a small part of India (South India). Based on our country's socioeconomic status, this sharp increase in the required study period for producing practicing pharmacists—from a two-year DPharm to a six-year PharmD—raises concerns about PharmD-trained

pharmacists who appear "unavailable" to serve India.

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HOW INDIAN PHARMACIES CAN IMPROVE THEIR EMERGENCY PREPAREDNESS AND RESPONSE (EP & R) STRATEGIES: KEY LESSONS FROM COVID-19 PANDEMIC AND FUTURE DIRECTIONS

Chinnabathini Anilkumar
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Pasham Anusha
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - India's emergency preparedness and response (EP&R) education, training, capacity building, and infrastructure development are all highlighted by the COVID-19 pandemic. In India, pharmacy professionals (PPs) have continued to provide medications, supplies, and services throughout the pandemic. The public-private healthcare system in India is intricate and of varying quality. Providers face difficulties in providing patients with health services due to gaps in pharmacy education and training as well as a lack of consensus regarding pharmacist roles. Due to this lack of differentiation, it is difficult to define roles, place PPs in patient care, serve on EP&R task forces, or be represented at the policy level. The goal of this study was to quickly learn from PPs in India about their roles and how prepared they are for the COVID-19 pandemic. A web-based overview containing 20 inquiries in regards to EP&R and Tasks the board was created utilizing the Qualtrics® review programming and managed to an example of PPs. Despite their lack of EP&R training, the survey results show that pharmacists were actively involved in essential pharmacy services. In light of the lessons learned from COVID-19, gaps in knowledge, training, and regulations have been identified, and suggestions are made for expanding PP roles so that they can be better prepared for future emergencies and actively participate in EP & R.

Keywords: Coronavirus, Drug specialists, Drug store experts, India, Crisis readiness and reaction, Catastrophe the board.

1 INTRODUCTION

In December 2019, COVID-19, also known as the SARS Cov-2 virus, was discovered in Wuhan, China, and it quickly spread to 177 countries. Over 1.9 million confirmed cases of COVID-19 and 117,000 deaths had occurred worldwide as of April 14, 2020.1 While the majority of countries experienced a sudden rise in the number of cases, India, the second most populous nation with 1.38 billion people (or 17.7 percent of the world's population), reported 10,000 cases and 393 deaths as of April 14, 2020.2 The first COVID-19 case in India was discovered on January 30, 2020, in the southern state of Kerala. Community transmission is said to be slow and There were only a few testing facilities and a few hundred tests were conducted daily across the nation in the early days following the first COVID-19 case that was confirmed. The Indian Council of Medical Research has designated 146 government testing laboratories and 67 private laboratories for COVID-19 testing as of April 10, 2020. On March 24, 2020, an immediate nationwide lockdown was announced.

This sudden lockdown led to a massive exodus of 120 million people, most of whom were seasonal and migrant workers. This mass exodus has been compared to the partition of India, which resulted in the displacement of 14–15 million people. In terms of COVID-19 transmission or infections, this mass exodus in rural areas—where access to

healthcare services is typically much lower than in urban areas—may likely overwhelm the fragile and fragmented healthcare system and push them into poverty and starvation.

The public and private healthcare providers in India make up the intricate healthcare system. In 2018, India had 8.5 medical doctors, 17 nurses, 1.8 dentists, and 8.8 pharmaceutical personnel per 10,000 population.8 As of March 2019, there were 1,125,222 registered pharmacists and approximately 650,000 registered pharmacists9 of varied educational backgrounds reportedly working in 800,000 retail pharmacies.10 However, the public sector is perceived as being unreliable, of low quality, and generally not the first choice, unless one cannot afford private care.

2 METHODS

Using the Qualtrics® survey software, a 20-question online survey about emergency preparedness and operations management was created and distributed via WhatsApp to a select group of well-known Indian pharmacy networks.

The informal survey received responses from 24 PPs from seven states, five from the south, one from the north, and one from the west. The average age was 35, with a range of 24 to 58 years) and an average of 5.83 years of practice (range: 1–27 years). Of the 24 PPs, there were 13 Pharm. Ds, 5 M.Pharm, and 4 joint M.Pharm-Pharm. D. Two respondents work in clinics, eleven in community pharmacies, and eleven in academia.

The analysis did not include any surveys that were not completed. 87% of PPs who reported receiving COVID-19 training said it took between one and three hours. PPs requested instruction in mask selection and use. PPs reported increased demand for hydroxychloroquine, chloroquine, azithromycin, and antivirals but did not report price increases for these medications. They also emphasized social distancing and isolation as strategies for infection control. Additionally, PPs reported screening patients for fever (67%), cough (42%), emotional and anxiety issues (33%), and a variety of social distancing measures, including telephone consultations, patient education, and dissemination of information on hand washing and mask use.

3 DISCUSSION

Similar difficulties are beginning to confront PPs in India, as demonstrated by other RSAP journal articles. As discussed below, there are opportunities to reframe the roles of PPs and include them in EP&R planning beyond the policy level:

3.1 Emergency Preparedness and Response (EP & R)

In our review, PPs answered that they might want to be prepared and more ready for Coronavirus and different crises. The first step in addressing this gap in India's EP&R to COVID-19 and beyond is involving PPs in policymaking and training EP&R professionals to serve and respond. The Ministry of Health and Family Welfare's request to the Pharmacy Council of India, the Pharm's accrediting body, was a welcome first step. D training to set up a state-wise public rundown of drug specialists who can be selected as a component of the Coronavirus heroes wellbeing force and by making the State Drug store Committees as nodal workplaces. To encourage professional growth, PPs should be included in policymaking (for example, many states in the United States have implemented collaborative practice agreements that allow PPs to bill for services like point-of-care testing and vaccinations).

3.2 Operations Management

Logistics chain: India's supply chains are currently poorly designed, lack end-to-end visibility, and have been slow to adopt

digital technologies. Supply chain disruptions are likely to have an impact on procurement and distribution. During times of emergency and lockdowns, PPs may be useful in estimating supplies to ensure sufficient stocks of anti-infectives, PPE, and commonly used over-the-counter (OTC) medications for chronic conditions.

3.3 Inventory Management

Within days of the first COVID-19 case being discovered, India's pharmacies ran out of their limited supply of PPE. Small community pharmacies with limited capacity were unable to meet this surge in demand. Collaboration and cooperation among pharmacies may help ensure uninterrupted supplies and medications, particularly in difficult-to-reach locations, to ensure continuity of care and prevent infection. While prioritizing limited supplies and medications for patients based on their clinical needs, PPs may extend their involvement to include the recommendation of safe and effective therapeutic alternatives.

Patient Management: As essential healthcare providers, pharmacists have maintained accessibility and continued to supply their communities with medications and supplies. Currently, only government hospitals treat COVID-19 patients; however, if the number of cases rises, the private sector would be required to accept patients and provide the necessary treatments. To ensure optimal medication safety and utilization, major tertiary care hospitals have emergency preparedness plans, which should include hospital pharmacists in the planning and implementation stages (Box 1).

Workplace safety: India's 1.3 billion populace was unexpectedly positioned under lockdown for 21 days at first from Spring 24th onwards to forestall local area spread. The lockdown was extended by two more weeks when this paper was written. All healthcare workers should be protected, and measures need to be taken to ensure their physical safety and that they have adequate PPEs to protect themselves from contagion. There were reports of healthcare professionals being harassed and receiving violent and uncivil behavior from public and law enforcement officials.

Expanding PP Roles: Dispensing, stocking, manufacturing, quality assurance, clinical trial management, academia, and research are among the responsibilities of PPs in India since the Pharm's inception. D, these responsibilities could be further extended to include patient care and population health interventions like screenings for infectious and some noncommunicable diseases, addressing medication adherence, evaluating medication utilization, and overseeing antimicrobial resistance, which would provide additional assistance to the healthcare industry. PP would be able, for instance, to assess medication adherence, create patient care plans that include medication safety, and monitor health outcomes through collaborative practice agreements (CPPA) with physicians.

Ethics and Integrity: Following ICMR guidelines on their prophylaxis for COVID-19, media reports indicated that healthcare professionals and the general public faced difficulties hoarding hydroxychloroquine, chloroquine, azithromycin, and vitamin C26. In response, the Ministry of Health and Family Welfare granted Hydroxychloroquine Schedule H1 status, thereby reducing its demand and preventing its inappropriate use. In order to meet rising demand, the pharmaceutical manufacturing sector in India was instructed to increase production of these medications. Before exporting to needy nations, measures were taken to meet local requirements. Price increases and hoarding were not mentioned in our study. Nonetheless, pharmacy students and healthcare professionals require ongoing and

continuing education to reinforce rigorous training on professional ethics and integrity.

3.4 Research and Dissemination for Impact

Following the 2008 Pharmaceutical In India, as part of the D program, there has been an increase in the number of research projects and activities to raise awareness in hospitals and the community. Numerous schools of pharmacy regularly hold events to raise awareness and educate the general public on World Health Days like World TB Day and No Tobacco Day. However, EP&R programs like stockpiles, public awareness campaigns, and Point of Dispensing (POD) drills have not yet been implemented. To highlight and demonstrate the value of PPs in emergencies, additional research and evaluation are required, particularly in EP&R.

4 CONCLUSION

India's healthcare system, education, and training of PPs for EP&R have all been exposed by the COVID-19 pandemic. To create a PP EP&R framework that will allow for efficient emergency responses, a comprehensive analysis of the population's requirements is required. Through education, training, and research, these EP&R responses could be made more formal, which would help to expand the roles and recognition of PPs as healthcare professionals in India.

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ADVANCEMENTS IN DIAGNOSTIC IMMUNOLOGY: EXPLORING FUTURE AVENUES AND OPPORTUNITIES

Dr. A. Madhusudhan Reddy
Assoc. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Sunitha Chintala
Assoc. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - A variety of diagnostic methods based on the specificity of the bond between antibodies and antigens are referred to collectively as diagnostic immunology. The diagnosis of various diseases is made possible by a variety of different tests and methods. These cutting-edge methods aid in the early detection of numerous chronic diseases, including cancer, among others. furthermore, help in the fix of the illness right away. These tests, which are serological in nature, fall primarily into two categories: both direct and indirect ELISA is one of six methods that are utilized on a larger scale for the purpose of diagnostic immunology. Diagnostic immunology comes with its own set of benefits and drawbacks. In addition, these advanced methods are preferable to conventional ones for better diagnosis because they aid in the early detection of chronic diseases. There are a lot of different kinds of tests, and we can choose the ones that are most convenient, urgent, cost-effective, accurate, etc. All of these tests are reasonably priced and easily accessible worldwide. Because the devices are portable, many tests can be performed easily even at primary levels. Because many of these tests are computerized and automated, they offer quick and accurate results. Again, this aids in improved diagnosis.

Keywords: Diagnostic, Immunology, Antigen, Antibodies, ELISA, Cancer, Devices.

1 INTRODUCTION

A variety of diagnostic methods based on the specificity of the bond between antibodies and antigens are referred to as diagnostic immunology. It is excellent for detecting even the smallest quantities of biochemicals. Utilizing serological tests, antibodies that are specific for a desired antigen are used as a "probe" to detect it. These antibodies can be conjugated with a radiolabel, fluorescent label, or color-forming enzyme. These tests are fundamentally arranged in two following sorts:

Direct Exam: Distinguishes antigens from the patients' example.
Test Indirect: analyzes the patient's blood sample for antibodies.2

2 DIFFERENT TECHNIQUES USED

1) Precipitation Reactions
2) Agglutination Reactions
3) Complement Fixation Reaction
4) ELISA
5) Fluorescent antibody
6) Western blot.

2.1 Advanced Applications of Diagnostic Immunology

Using a variety of appropriate tests, diagnostic immunology is used to identify various chronic diseases. Typically, these tests are performed on patient blood and serum samples. Various antibodies are used to check these samples for specific antigens. This has helped us find cancerous cells in a patient's body, which can help us find other diseases like cancer and genetic disorders that need to be found early for better treatment. This is possible thanks to advanced immunology research. We will now see some tests that can be used to find these diseases.26

3 CANCER

The body's unchecked growth of abnormal cells is cancer. Malignant cells are another name for cancerous cells.

Risk factors, incidence, and causes Cells are what give life its structure. Cancer develops from healthy body cells. Normal cells divide and die when the body doesn't need them anymore. Cancer appears to develop when the body's cells divide too quickly and grow out of control. Additionally, cells may forget how to die. Cancer can take many different forms. Malignant growth can foster in practically any organ or tissue, like the lung, colon, bosom, skin, bones, or nerve tissue. However, many cancers have no known cause. Lung cancer is the leading cause of cancer-related death. The majority of cases involve the following cancers:

3.1 The Principle of the Test

This blood test measures antimalignan, a blood antibody. Early-stage cancer patients have higher levels of antimalignan antibodies. Antimalignan antibody levels are measured in this blood test. The naturally occurring antibody known as antimalignan antibodies in serum (AMAS) can be found in the serum of any individual, including children. Our body's natural defense against cancer is AMAS.

3.2 Benefits of the Test

This AMAS test is over 95% accurate in the initial test and over 99% accurate in the subsequent one. This test can detect any kind of cancer. Additionally, this test can be used to monitor the treatment of cancer. This test is superior to the marker test in terms of cancer detection and recovery screening. Patients can afford this option because it is a low-cost test. It is successful in locating cancer cells early in the disease.

3.3 It would be particularly valuable for

1. Those who have a strong family history of cancer, but are fit and well;
2. Those who have had suspicious test results from their doctors, but do not know whether they have cancer or not;
3. Those who have had cancer diagnosed and are about to embark upon a course of treatment; and those who have had cancer in the past, and want to make sure they are clear.
4. For fit and healthy people, over the age of 40, it could be argued that they should have the test every 2 years or so.

5. For fit and healthy people over the age of 55 – 60 it could be argued that they should have the test every 12 months.
6. For people with cancer, or a history of cancer, or a family history of cancer, it could be argued that the test should be carried out more frequently.

3.4 Procedure

A blood sample taken after fasting (doing without food) for at least 12 hours is used for the DR-70 test. When they haven't eaten since dinner the night before, patients are asked to give a blood sample in the morning. Enzyme Linked Immuno Sorbant Assay (ELISA) Based Serological Test is used to analyze it in the laboratory.

4 ADVANTAGES OF DIAGNOSTIC IMMUNOLOGY

Diagnostic immunology has recently reached new heights in the world of scientific research. Additionally, it can be utilized for cancer detection. Early cancer diagnosis and treatment have benefited from this. Adults who lead unhealthy lives are more likely to get these diseases. Lung cancer, pancreatic cancer, bladder cancer, esophageal cancer, and distal esophagitis are all caused by smoking and drinking. In these sicknesses, natural safe cells assume a fundamental part, perceiving focused cells or undigested cell contents when cells go through non-apoptotic or non-autophagic demise. Using Diagnostic Immunology, this aids in the detection or diagnosis of cancer. Because these are cutting-edge techniques, cancer is detected early. The primary factor in cancer diagnosis is early detection.

5 DISADVANTAGES OF DIAGNOSTIC IMMUNOLOGY

The fact that Diagnostic Immunology does not completely and completely succeed in the diagnosis, detection, or treatment of many more dangerous diseases, such as cancer and other genetic disorders like leukoderma, is the primary drawback of the method. The doctors will order additional tests, such as a biopsy, a whole-body CT scan, or X-rays, to confirm the disease after these ones are finished. Many physicians are unaware of these relatively new techniques. Therefore, promoting these tests is crucial. Because many of these tests don't give accurate results, doctors usually don't recommend them. Because it involves a lot of tests, like serum tests and blood tests, it's a very expensive and complicated procedure for the average person to go through. These tests utilize progressed gadgets and gear and consequently are not accessible wherever without any problem. In order to use these tools in the right way, technicians need to be well-trained. Because these tests are available in developed nations, they are only found in a small area.

6 CONCLUSION

Clinical and diagnostic immunology are undergoing an industrial revolution. We are already reaping the benefits of these new technologies, but in order to take full advantage of them and provide the direction needed to advance the field of clinical and diagnostic immunology, we need to keep up with the rapid changes and participate in their development. As a result, the various conventional methods and cutting-edge tests

utilized in diagnostic immunology have been discussed in the preceding summary. Additionally, we have observed that advanced tests are simpler and more accurate than traditional ones. The use of cutting-edge technology aids in the reduction of human error and the quicker delivery of results.

Many chronic diseases, like cancer and genetic disorders, can be found early, which aids in their early treatment. In the event that these sicknesses are analyzed in the beginning phase, it is advantageous to the patient as the therapy will be successful really at that time. If these diseases are not caught early, they can be fatal.

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EXPLORING THE POTENTIAL OF NASAL IN-SITU GEL FOR ENHANCED DRUG DELIVERY

Gaddam Swetha Reddy
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
Ajmeera Rajya Laxmi
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Numerous medical and biomedical applications, including controlled drug delivery, have developed as a result of advancements in in situ gel technologies over the past few decades. In order to meet the ever-increasing demands of the pharmaceutical and medical industries, numerous novel in situ gel-based delivery matrices have been developed and manufactured. In situ gel forming drug delivery is a type of mucoadhesive drug delivery system that is liquid at room temperature but gels when it comes into contact with body fluids or changes in pH. The drug is released in a controlled and sustained manner from the gel that forms as a result of temperature modulation, pH change, the presence of ions, and ultraviolet irradiation. When conventional drug administration methods, such as intravenous, intramuscular, or oral, are inapplicable, nasal delivery offers a promising alternative. Many drugs have recently been shown to be more bioavailable through the nasal route than through the oral route. This has been attributed to the nasal mucosa's dense vasculature and highly permeable structure, as well as avoiding hepatic first-pass elimination, gut wall metabolism, and/or gastrointestinal tract destruction. The nose's physiology has some challenges, but it offers a promising path for the noninvasive systemic delivery of many therapies and a questionable route for drug delivery to the brain. As a result, the focus of this review is on the delivery of drugs through the nose, various aspects of the anatomy and physiology of the nose, the mechanisms by which drugs are absorbed through the nose, and various nasal drug delivery systems and their uses in drug delivery.
Keywords: Nasal In Situ Gel, Absorption Enhancer, Nasal Formulation, Mucoadhesive Drug Delivery System, Microsphere Based Drug Delivery System.

1 INTRODUCTION

Due to their ease of administration, oral administration is the preferred and most convenient method of drug administration. Be that as it may, in many occurrences oral organization isn't attractive when the medication goes through huge corruption by means of first pass impact in liver. Subsequently, absence of fundamental ingestion through the gastrointestinal plot prompted research on backup courses of action of medication conveyance, for example, parenteral, intramuscular, subcutaneous, intranasal, transdermal etc.

Intranasal (IN) organization is a needle free and subsequently an optimal option in contrast to the parenteral course for fundamental medication conveyance. For systemic absorption, the nasal mucosa has a dense vasculature and a highly permeable structure. Nasal administration of drugs is simple and convenient. Evasion of first pass digestion is the fundamental benefit of nasal course of medication delivery.

Intranasal conveyance is harmless, basically easy, doesn't need sterile planning and it is effectively and promptly regulated by the patient or a doctor for example in a crisis setting. When developing new therapies, extending the life of an existing drug, or enhancing its profile, it makes sense to take into account intranasal

administration due to these advantageous properties.

2 ANATOMY AND PHYSIOLOGY OF NASAL CAVITY

It is essential to have a clear understanding of the nose's anatomy and physiology in relation to the characteristics of the delivery system when studying drug absorption from the nasal mucous membrane. The nasal passage that connects the nasal vestibule to the nasopharynx has a depth of approximately 12 to 14 centimeters. The mucosa is shielded from inspired air because the nasal cellular apparatus is in close contact with mucus in this passage. The nasal cavities are divided into three distinct functional zones, namely olfactory, respiratory, and vestibular regions.6 The zones are arranged in the order of order from anterior to posterior. The vestibular area is covered by a common pseudostratified epithelium that serves as a baffle system. The long hairs on this epithelium may filter airborne particles. The olfactory segment is lined with a specialized type of pseudostratified columnar epithelium known as olfactory epithelium, which contains receptors for the sense of smell. The respiratory area is normally covered by a dense layer of mucus that is constantly moving towards the posterior apertures of the nasal cavity by a powerful system of motile cilia.6 The respiratory area has a surface that is lined by a pseudostratified columnar epithelium. Along the nasal cavity's dorsal roof, you'll find this segment. Some types of cells in the olfactory mucosa are: basal cells, Bowman's glands, supporting (sustentacular) cells, and bipolar neurons The olfactory nerve (cranial nerve I) is made up of the axons of bipolar neurons.

2.1 Nasal Epithelium

Skin covers the nostrils, with a squamous and transitional epithelium covering the anterior third of the nasal cavity, an olfactory epithelium covering the upper portion, and a typical airway epithelium covering the remaining portion, which is ciliated, pseudostratified, and columnar5. The epithelial cells in the nasal vestibule are stratified, squamous, and keratinized with sebaceous glands. The nasal vestibule is extremely resistant to dehydration, can withstand noxious environmental substances, and prevents substances from permeating. The atrium is a transitional epithelial region with pseudostratified columnar cells with microvilli posterior and stratified squamous cells anterior.

2.2 Blood Supply to Nasal Cavity

Blood is abundantly supplied to the nasal vasculature to support the basic functions of the nasal cavity, including olfaction, mucociliary clearance, heating and humidification, and immunological functions. The internal and external carotid arteries, as well as the facial and maxillary arteries, supply blood to the body. The nose's named arteries are,

- **Sphenopalatine artery**, a branch of maxillary artery.
- **Anterior ethmoidal artery**, a branch of ophthalmic artery.
- **Branches of the facial artery** supplying the vestibule of the nasal cavity.

There are a lot of blood vessels in the nasal mucosa's lamina propria. There are three ways in which they differ from the vasculature in the tracheobronchial tree. The first is the nose's venous sinusoid. The second is a nose arteriovenous anastomosis. Third are the nasal vasculature shows repetitive changes of clog leading to the nasal cycle. One of the characteristics of nasal blood vessels has been described as the endothelial basement membrane's porosity. For rapid fluid movement through the vascular wall, the capillaries just below the surface epithelium and surrounding the glands are ideal.

3 NASAL DRUG DELIVERY SYSTEM

Diverse therapeutic compounds can be delivered locally and systemically using intranasal (IN) delivery. Nasal administration is one of the non-invasive routes that offers promising potential as a viable alternative for the delivery of some medications. Thus there has been a flood of revenue that has prompted numerous examinations including the nasal hole as a practical site for the organization of much remedial agents.

3.1 Advantages

- Compared to other epithelial surfaces, the nasal epithelium is thin, porous, and highly vascularized. For the purpose of initiating therapeutic action, this guarantees a high degree of absorption and rapid transport of absorbed substances into the systemic circulation.
- A basement membrane of porous endothelial cells that does not impede the drug's movement into the bloodstream.
- The substances that are absorbed are transported directly into the systemic circulation, avoiding the typical first-pass metabolic effect that occurs after oral drug administration.
- Drugs can sometimes bypass the blood-brain barrier and be absorbed directly into the central nervous system (CNS) following nasal administration.
- Drugs, particularly proteins and peptides, can be made more bioavailable thanks to the nasal epithelium's lower enzymatic activity than the GIT or liver. Likewise, catalyst inhibitors are more viable following nasal than oral application in view of a more serious level of weakening in the last option than in the previous.
- The realization of pulsatile drug delivery, such as insulin, human growth hormone, and others. is greater in NDD.
- Self-medication of the nose not only reduces therapy costs but also increases patient compliance. Nasal lavage can be used to get rid of any drug that hasn't been absorbed, and the risk of overdosage is low.
- Companies have the opportunity to extend the life cycle of their products by reformulating existing drugs into NDD products.

4 BIOLOGICAL FACTORS

First, physiological factors include mucociliary clearance, which involves the combined action of the mucus layer and cilia. The tips of the cilia are in contact with and transport the superficial viscoelastic mucus layer toward the nasopharynx, while the less viscous lower layer of mucus is relatively stationary. This is one of the major factors in drug clearance from the nasal cavity. Second, the nasal mucosa contains a wide variety of metabolic enzymes. However, this may limit the bioavailability of drugs given through the nose; These enzymes have a lower activity level than those in the GIT and liver. In addition, pathological conditions such as rhinitis and the common cold can affect drug absorption from the nasal cavity, and the pH of the nasal cavity also affects drug permeation. An adjustment of the pH of bodily fluid can influence the ionization and increment or decline the penetration of medication relying upon the idea of the drug.

5 PHYSICOCHEMICAL PROPERTIES OF DRUGS

Various physicochemical characteristics of drug can also affect nasal absorption of the drug.

Sub-atomic Weight and Size: The drug's absorption rate is influenced by its molecular weight, particularly in the case of hydrophilic compounds. The nasal route can effectively deliver drugs up to

1000 Daltons. Unless penetration enhancers are used, absorption significantly decreases when the molecular weight is greater than 1000 Daltons. The existence of a strong linear correlation between the log percentage of a drug absorbed through the nose and the log molecular weight of water-soluble compounds lends credence to the involvement of aqueous channels in the absorption of water-soluble molecules through the nose. The nasal cavity has been found to contain particles larger than 10 m, according to reports. Particles between 2 and 10 micrometers have the potential to remain in the lungs, while particles smaller than 1 micrometer are exhaled.

Dissolution and Solubility: A significant factor in determining drug absorption through biological membranes is drug solubility. If the drug is not sufficiently soluble in the desired vehicles, it can hinder a formulator's ability to formulate a product as well as the drug's absorption. Because nasal secretions are more watery, a medication should be able to dissolve more easily in water. Before being absorbed, particles that are deposited in the nostrils must be dissolved. Absorption may not be observed if the drug remains in the nostrils as particles or is removed from the nasal cavity.

Compound Structure: The chemical form in which a drug is delivered to the nasal mucosa can have a significant impact on how well it is absorbed. Changes in a drug's absorption, for instance, can occur when it is converted into a salt or ester form. The rise in lipophilicity that followed esterification, which increased the rate and extent of nasal absorption, is linked to this phenomenon.

6 NASAL FORMULATIONS

The particular drug molecule's therapeutic need, duration of action, and duration of therapy all play a role in the design of the nasal formulation. Nasal administration is an option for both controlled release and conventional release drug delivery. A wide variety of nasal formulations have been studied to date, including,

1. Nasal drops
2. Nasal powders
3. Nasal sprays (solution/suspension)
4. Nasal mucoadhesive particulate delivery (micro/nanoparticles, liposomes)
5. Nasal gel
6. Nasal ointments
7. Nasal microemulsions

7 EVALUATION OF NASAL IN SITU GEL SYSTEM

Clarity is one of the parameters that can be used to evaluate and characterize in situ gels: Under a black and white background, a visual inspection was used to determine the clarity of the formulated solution.

The study of texture: Using a texture analyzer, the formulation's firmness, consistency, and cohesiveness are evaluated. This primarily reveals the sol's syringeability, allowing the formulation to be easily administered in vivo.

Point of Gelation: At this temperature, the liquid phase transforms into a gel. Temperatures between 30°C and 36°C would be ideal for thermoreversible nasal gel's gelation. The temperature at which formulations would not flow when test tubes were tilted to a 90° angle as the temperature gradually increased was referred to as the gelation point. pH and ion dependent polymers change from sol to gel when their pH changes or they come into contact with nasal fluid.

The Gels' pH: Each batch's pH was measured with a pH meter that had been calibrated beforehand with buffers of pH 4 and pH.

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ADVANCING PHARMACEUTICAL MANUFACTURING WITH PROCESS ANALYTICAL TECHNOLOGY: A COMPREHENSIVE REVIEW

Golla Lavanya
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
B Venkatesham
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - To incorporate quality into products, process analytical technology (PAT) makes use of various technologies and tools. Science-based knowledge of the proposed drug product's physical, chemical, and mechanical properties are necessary for effective PAT implementation. An integrated systems approach to quality design, process analysis, understanding and control, continuous improvement, knowledge, and risk-based management looks promising for the PAT project as a whole. By acting on data in real time and eliminating sampling, early PAT devices increased process efficiency and safety. The more in-depth process knowledge gained by PAT applications leads to increased processing opportunities and robustness. For all kinds of chemical reactions and process monitoring, including drying, distillations, crystallizations, hydrogenations, and others, modern advancements in analytical technologies provide chemical and analytical insights.
Keywords: Process Analytical Technology, ICHQ10, cost control.

1 INTRODUCTION

The FDA defines Process Analytical Technology as a system for designing, analyzing, and controlling manufacturing processes by promptly measuring critical quality and performance attributes of raw materials, in-process materials, and processes with the intention of guaranteeing the quality of the finished product. PAT was introduced in 2001 by the Food and Drug Administration (FDA) to lessen the likelihood of producing a subpar product. Pharmaceutical companies now have the tools they need to design high-quality products and improve process efficiencies thanks to PAT. It looks at raw and in-process materials to make sure the final product is good. Utilizing conventional process sensors like pressure and analyzer technologies, PAT incorporates measurement science. PAT emphasizes in-line testing with near infrared, Raman, or other methods. The retrieved data would reveal the properties of blends, cores, and other process stages. Using tests all the while, consistency, drying, and blending endpoints, and other designated stages can be pinpointed to a serious level of conviction. In-line probes would be strategically placed throughout the production process to reduce sampling error. PAT is not a service or product. It's a concept, a principle, or a way of doing things that you have to put into action. As pharmaceutical companies strive to implement the framework established by regulators1, the PAT market is rapidly developing and evolving. It uses real-time information to reduce process variation and manufacturing capability. In industries like the chemical and pharmaceutical industries, the PAT improves quality while simultaneously cutting costs.

1.1 When to Introduce Process Analytical Technology (PAT):

A pharmaceutical product's quality must be taken into consideration from the very beginning. The equal participation of research and development and seamless communication between manufacturing

are essential preconditions. The introduction of quality into a product from the beginning is one goal of PAT. Therefore, its participation in the R&D phase is crucial. It will be much simpler to conduct root-cause analysis of quality or process failures from scale-up to commercial manufacturing if product quality requirements are understood and implemented from the beginning. To this end PAT could assume a considerably more significant part in the plan and examination of assembling processes, empowering execution control to be founded on ideal estimation of well - depicted basic handling information. To meet emerging requirements for the speed and volume of data collection, data processing requirements should also be taken into consideration in the context of the overall strategy for process analysis. Knowledge management and real-time analysis necessitate the collection of all production batch data, such as through data warehousing. As a result, large sets of measurement data can be generated long before a PAT data management strategy based on online process analysis or data mining can be established.

1.2 Basis for Process Analytical Technology:

It is common knowledge that several of the core concepts were pioneered decades ago by other manufacturing industries such as fine chemicals, semiconductors, petroleum, and consumer products. This is the case despite the fact that the FDA's PAT framework began to take shape just prior to the creation of the twenty-first century cGMPs initiative in 2001. Process analytical chemistry (PAC) and advanced manufacturing science are the primary concepts that set PAT apart from the traditional industrial pharmacy skill set (which also includes pharmaceutical and materials science, chemistry, and engineering).

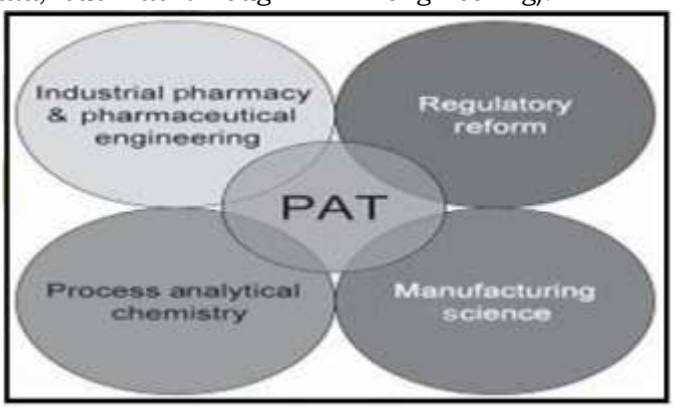

Figure 1 PAT

The science and technology of displacing laboratory-based measurements with sensors and instruments closer to the operation site is typically referred to as process analytical chemistry. The more recent initiative “Quality by Design” (QbD) is also aligned with the PAT concepts.5 The ICH Q8 guideline defines Quality by Design as “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.”6 The objective of PAC is to “supply quantitative and qualitative information about a chemical process for monitoring, control, and optimization.”

2 DEVELOPMENT OF PAT

Process/method/equipment validation, process controls in accordance with standard operating procedures (SOPs), process instructions/master recipes, and off-line sampling at the conclusion of each batch are all examples of traditional

pharmaceutical quality systems. Manufacturing process enhancements are not innately encouraged by this kind of system. Process logical science (PAC) has been acted in the petrochemical business for quite a long time. This method, which makes use of analytical and process chemistry in addition to multivariate tools, has recently been referred to as process analytical technology (PAT). PAT tools are used to make sure that products have quality built in, understand processes better, work more efficiently, and cost less. In 2002, the Food and Medication Organization (FDA) declared another drive, Drug Current Great Assembling Practices (cGMPs) for the 21st 100 years, to improve and modernize the guideline of drug assembling and item quality. The pharmaceutical industry's early adoption of new technological advancements was part of this initiative. Following the cGMPs initiative, the industry received more in-depth PAT guidance (FDA, 2004). The International Conference on Harmonization's guidelines later included similar components of risk analysis, real-time quality control, and continuous improvement (ICH Q8, 2005; ICH Q9, 2005). The most recent initiative, ICH Q10 "Quality Systems," completed the harmonization process on June 5, 2008. The outcome was that the US, Europe, and Japan were all parties to the ICH, and they agreed to fully implement the guideline through their respective regulatory bodies. The ideas behind ICH Q8 and "Pharmaceutical Development"7 are incorporated into ICH Q10.

2.1 Types of Process Measurement:

1] Off-line testing
2] On-line testing
3] At-line testing
4] In-line testing

3 EXAMPLES OF APPLICATION OF PROCESS ANALYTICAL TECHNOLOGY

3.1 Particle Size

Since active pharmaceutical ingredients (APIs) and excipients have a significant impact on the majority of solid dosage products, theft sampling and laboratory analysis are typically used to monitor their particle sizes. This method of control has a few drawbacks for systems where particle size is important. It's possible that a small sample isn't as accurate as the whole thing. To sample, transport, measure, and report results takes time. There is probability of openness to administrators and lab faculty. Lots that are rejected can be expensive. Traditional control strategies are impractical for real-time quality assurance due to all of these limitations. PAT methods for measuring particle size take samples of larger, more accurate portions of the bulk product and provide quick analysis with immediate feedback to the control system. Personnel are not exposed because PAT is a closed system.

3.2 Content Uniformity

The production of a uniform mixture of API and excipients in the final dosage form is a primary objective of blending. Due to the fact that measurements of component identity and concentration are frequently not specific to distribution, it is difficult to identify incomplete mixing in the final dosage form. Blend uniformity is typically assumed by blending for a predetermined amount of time, which is actually monitored by release testing. There are a few drawbacks to this strategy: It's possible that release testing on small samples doesn't accurately reflect the bulk. Cycle time is shortened when you blend for longer than necessary. Blend time can be affected by feed material variations from batch to batch. "Demixing" may occur when there is too much mixing. Bombed discharge tests risk the whole bunch. In order to control blending more precisely, a PAT strategy provides process comprehension and feedback.

3.3 Drying

Product drying is a necessary step that can have a significant impact on the final product's solid form, either after synthesis or during processing. A drying step can be made to just get rid of too much solvent for later processing, or it can be an essential part of manipulating the solid-state form through dehydration or desolvation. Under most situations, drying is completed temporarily, which can prompt exorbitant process duration as well as unwanted structure change if drying go on past the endpoint. Uncompensated variations in drying time may result from variations in feed materials from batch to batch; The entire batch may be in danger if release tests fail.

3.4 Crystallization

The molecule spectroscopic method of Raman, NIR, ATR, and FT-IR spectroscopy is used as the sensor in a recent review of the study of the crystallization process using Process analytical technology. Ranitidine HCL Tablet's various polymorphic forms were identified and quantified with the help of Raman spectroscopy and chemometric data analysis. Due to the rise in structure-based drug design fueled by recent advances in genomics and proteomics, protein crystallization has also been of some interest for PAT applications.

3.5 Pellet Manufacturing by Extrusion-Spheronization

To gain a better understanding of the solid-state behavior of the active pharmaceutical ingredients (APIs) during pelletization, an at-line process analytical technology (PAT) approach was utilized. During the process, the characterization of polymorphic changes was carried out using X-ray powder diffraction (XRPD), near-infrared (NIR), and Raman spectroscopy. At the conclusion of each stage of processing—blending, granulation, extrusion, spheronization, and drying—samples were collected. Batches were dried at 60°C, 100°C, and 135°C temperatures. During processing, water caused both model formulations to form hydrates. Due to saturation of the water signal, NIR spectroscopy was unable to detect hydrate formation in theophylline and nitrofurantoin formulations during the granulation, extrusion, and spheronization stages. However, it did provide valuable real-time information about the system's water state. The expected pseudopolymorphic changes of the APIs during the wet process stages were confirmed by Raman and XRPD measurements.

4 CONCLUSION

PAT can be thought of as a constellation that places more or less emphasis on a particular activity depending on the problem or situation at hand. There is no written rule or clear path through PAT. In addition to having a solid understanding of the pharmaceutical industry, experience and expertise are essential. Continuous management support for the development and maintenance of PAT-related activities is essential once a pharmaceutical company decides to implement PAT. For PAT's future success, encouraging, stimulating, and initiating scientific collaboration and interaction, as well as the necessary education and training, is a strategic and necessary step.

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EXPLORING INNOVATIVE STRATEGIES FOR DEVELOPING FLOATING DRUG DELIVERY SYSTEMS: AN EXTENSIVE REVIEW

Sangu Jyothi
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India
Roopani Madhu
Asst. Professor, Department of Pharmaceutical Chemistry, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Currently, floating drug delivery systems (FDDS), swelling and expanding systems, polymeric bioadhesive systems, highdensity systems, modified-shape systems, and other delayed gastric emptying devices are used to extend the GRT. The most recent and current developments in stomach-specific FDDS are discussed in this review. Poor absorption occurs when drugs have a limited window of absorption in the gastrointestinal tract. As a result, gastroretentive drug delivery systems (GRDDS) have been developed to extend the time it takes for the stomach to empty. Floating drug delivery systems, raft systems, mucoadhesive systems, high density systems, superporous hydrogels, and magnetic systems are some of the methods that have been used. This audit additionally sums up the in vitro procedures, in vivo examinations to assess the presentation and use of drifting frameworks, and utilizations of these frameworks. The primary objective of this review of floating drug delivery systems (FDDS) was to compile the most recent research, with a particular focus on the principal mechanism by which floatation is used to achieve gastric retention.
Keywords: Floating drug delivery systems, gastrointestinal tract, mucoadhesive systems.

1 INTRODUCTION

These new drug delivery systems' most important goals are: In the beginning, it would be a single dose, which spreads out the active ingredient over a longer time period. Second, it ought to deliver the active substance directly to the site of action, thereby minimizing or eliminating any adverse effects. Floating tablets have been developed to overcome the limitations of the conventional drug delivery system. Poor absorption will occur for medications that have a limited window of absorption in the gastrointestinal tract (GIT). Gastroretentive drug delivery systems have the advantage of extending the gastric emptying time for these medications. Floating drug delivery systems are one of the current methods used to create a successful stomach-specific or gastroretentive drug delivery system1. It has been frequently observed that drugs that are easily absorbed from the gastrointestinal tract have short halflives and are quickly eliminated from the systemic circulation, resulting in drug absorption from the upper small intestine being incomplete. The development of oral sustained-controlled release formulations is an attempt to slowly release the drug into the GI tract, which helps to maintain an effective drug concentration in the systemic circulation for a longer period of time. A controlled-release drug delivery system is capable of achieving the benefits of maintaining the optimal therapeutic drug concentration in blood with predictable and reproducible release rates for an extended period of time; this is necessary to achieve appropriate therapeutic activity. enhancement of long-term activity for drugs with short half-lives; the absence of side effects; reducing drug waste and dosage frequency; enhanced treatment and enhanced patient compliance, Understanding the three components of the system is

necessary for the successful development of oral controlled drug delivery systems.

1. The drug's physiological and chemical properties
2. The GIT's anatomy and physiology, as well as the characteristics of the dosage forms

2 FLOATING DRUG DELIVERY SYSTEMS AND ITS MECHANISM

Because FDDS have a bulk density that is lower than that of gastric fluids, they can remain buoyant in the stomach for an extended period of time without affecting the rate at which the stomach empty. While the framework is drifting on the gastric items the medication is delivered gradually at the ideal rate from the framework. The stomach expels the remaining system following the drug's release. As a result, GRT rises and plasma drug concentration fluctuations are better managed. However, in order for the dosage form to remain consistently buoyant on the surface of the meal, a minimal level of floating force (F) is also required, in addition to a minimal gastric content. A novel apparatus for determining the resultant weight has been described in the literature for measuring the floating force kinetics. The apparatus works by continuously measuring the force F, which is the force necessary to maintain the submerged object over time. If F is higher on the positive side, the object will float better. In order to avoid the negative effects of unanticipated intragastric buoyancy capability variations, this apparatus contributes to the optimization of FDDS with regard to the stability and durability of the produced floating forces.

2.1 Approaches to Gastroretention

Several techniques are reported in the literature to increase the gastric retention of drugs.

Systems with a High Density: These systems, which have a density of less than 3 g/cm3, are able to withstand the peristaltic movements of the stomach and remain in the rogue18, 20. The main significant downside with these frameworks is that it is in fact challenging to fabricate them with a lot of medication (>50%) and accomplish required thickness of 2.4-2.8g/cm. For the production of such a high-density formulation8, diluents like zinc oxide, titanium oxide, barium sulfate (density=4.9), and iron powder are required.

Systems of swelling and expansion: Due to their propensity to remain plugged in the pyloric sphincters, these systems are also referred to as "Plug type systems." Even when fed, these polymeric matrices remain in the gastric cavity for several hours12.

Including Excipients with a Delay in Time: Feeding digestible polymers or fatty acid salts that charge the motility pattern of the stomach to a fed stage, thereby reducing the gastric emptying rate and permitting significant prolongation of the drug release, is another approach of interest for delayed gastric emptying. Prolongation of GRT of medication conveyance framework comprises of consolidating deferring excipients like trietanolamine myristate in a conveyance system14.

Systems that Float: Because FDDS have a bulk density that is lower than that of gastric fluids, they can remain buoyant in the stomach for an extended period of time without affecting the rate at which the stomach empty. The drug is slowly released from the system at the desired rate while the system is floating on the contents of the stomach. The stomach expels the remaining system after the drug has been released18. A floating chamber filled with vacuum, air, or inert gas can be used to float a drug delivery system in the stomach.

3 CLASSIFICATION OF FLOATING DRUG DELIVERY SYSTEMS (FDDS)

Floating drug delivery systems are classified depending on the use of 2

formulation variables: effervescent and noneffervescent systems.

Forms of effervescent, floating dosage: Swellable polymers like methylcellulose and chitosan and various effervescent compounds like sodium bicarbonate, tartaric acid, and citric acid are used to create these matrix-type systems. They are made so that when they come into contact with the acidic contents of the stomach, CO2 is released and gets stuck in swollen hydrocolloids, giving the dosage forms buoyancy.

Ichikawa et al. came up with a novel floating dosage system that can be used for a variety of conditions. It is made up of effervescent layers and swellable membrane layers that are coated on sustained release pills. To keep the tartaric acid and sodium bicarbonate from coming into direct contact with one another, the inner layer of effervescent agents was divided into two sublayers. A swellable polymer membrane made of purified shellac and polyvinyl acetate surrounded these sublayers. This system settled down after being immersed in the buffer at 37 degrees Celsius, and the outer swellable membrane allowed the solution to enter the effervescent layer. The neutralization reaction between the two effervescent agents resulted in the production of CO2 and balloon-like swollen pills with a density of less than 1.0 g/mL. It was discovered that the system had good floating ability regardless of pH or viscosity and that the drug (para-amino benzoic acid) released continuously.

Choi et al. used gasforming agents (calcium carbonate and sodium bicarbonate) to make floating alginate beads and studied how CO2 changed the physical properties, morphology, and release rates. The size, floating ability, pore structure, morphology, release rate, and mechanical strength of the floating beads were all significantly influenced by the type and quantity of gasforming agent used, according to the study. It was determined that calcium carbonate produced beads that were both smaller and more durable than sodium bicarbonate. While sodium bicarbonate outperformed calcium carbonate as a gas-forming agent, calcium carbonate produced superior floating beads that allowed for better rate control of drug release.

3.1 Non-Effervescent Floating Dosage Forms

Polysaccharides, gel-forming or swellable cellulose hydrocolloids, and matrix-forming polymers like polycarbonate, polyacrylate, polymethacrylate, and polystyrene are utilized in non-effervescent floating dosage forms. The drug and the gel-forming hydrocolloid are thoroughly mixed in a straightforward manner as part of the formulation method. This dosage form expands when it comes into contact with gastric fluids after oral administration and reaches a bulk density of G 1. The dosage form is buoyant because of the air that is trapped within the swollen matrix. The gelatinous mass's swelling gel-like structure serves as a reservoir and allows for sustained drug release.

Nur and Zhang et al created drifting tablets of captopril utilizing HPMC (4000 and 15000 cps) and carbopol 934P. In vitro lightness concentrates on uncovered that tablets of 2 kg/cm2 hardness after drenching into the drifting media drifted right away and tablets with hardness 4 kg/cm2 sank for 3 to 4 minutes and afterward rose to the top. Tablets in the two cases stayed drifting for 24 hours. The tablet with a hardness of 8 kg/cm2 was unable to float. It was determined that the presence of internal voids (porosity) in the tablet's center and the swelling of the hydrocolloid particles on the tablet's surface when it comes into contact with gastric fluids control the tablet's buoyancy. When compared to conventional tablets, these floating tablets had a longer release time, and the controlled release of captopril from the dosage form lasted for 24 hours.

4 ADVANTAGES & DISADVANTAGE OF FLOATING DRUG DELIVERY SYSTEM

4.1 Advantages of Floating Drug Delivery System

1. When it comes to drugs that are absorbed through the stomach, the gastroretensive systems are helpful. Such as antacids and ferrous salts.
2. When they come into contact with the stomach wall, acidic substances like aspirin irritate it. As a result, the HBS formulation might be useful for administering aspirin and other similar medications.
3. When a prolongs release floating dosage form, such as a tablet or capsule, is taken, the drug dissolves in the gastric fluid. They break down in the gastric liquid would be accessible for retention in the small digestive system after expected that a medication will be completely retained from drifting measurements structures assuming it stays in the arrangement structure even at the soluble pH of the digestive tract.
4. Drugs that are intended to have a local effect in the stomach benefit from the gastroretensive systems. such as antacids
5. Poor absorption is to be expected when there is a rapid transit time and a vigorous intestinal movement, as in certain types of diarrhea. In such instances, it may be advantageous to keep the medication floating in the stomach for a more favorable response.

4.2 Disadvantages of Floating Drug Delivery System

1. For medications that have a problem with solubility or stability in the GI tract, a floating system is not an option.
2. In order for these systems to float and effectively coat water, the stomach must contain a significant amount of fluid.
3. Only desirable candidates are those drugs that undergo significant first-pass metabolism and are significantly absorbed throughout the gastrointestinal tract.
4. A few medications present in the drifting framework makes disturbance gastric mucosa.

5 CONCLUSION

Due to the fact that absorption is restricted to the upper GI tract, previous studies suggest that gastro retentive drug delivery offers a number of potential benefits for drugs with low bioavailability. Additionally, they can be delivered effectively, maximizing absorption and increasing absolute bioavailability. Moreover, the ID of new sicknesses and the opposition displayed towards the current medications thought about the requirement for the presenting new remedial particles. As a result, a wide variety of chemical entities have been introduced that are absorbed throughout the digestive system and A specialized delivery system is required for the drugs that need to show local action in absorption sites, and FDDS has achieved this. Numerous FDDS methods, including hollow microspheres, raft forming systems, single and multiple unit HBS, and single and multiple unit gas generating systems, have been developed. Because each of these gastroretentive drug delivery systems has distinct benefits and drawbacks, they are all intriguing and have prompted a significant amount of research and development effort. In addition, it is anticipated that additional research will be conducted in the near future, which will ultimately result in enhanced efficacy of various pharmacotherapies.

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EXPLORING THE VERSATILITY OF MULTIPLE EMULSIONS: A COMPREHENSIVE REVIEW OF PREPARATION TECHNIQUES, EVALUATION METHODS, AND DIVERSE APPLICATIONS

Sunitha Chintala
Assoc. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Thejovathi B
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract- Emulsions of emulsions, liquid membrane system, and double emulsion are all other names for multiple emulsions. Polydispersed systems known as multiple emulsions are those in which oil in water and oil in water emulsions exist simultaneously. Preparation, characterization, and potential applications of multiple emulsions are the primary topics of this review. Water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions are two types of multiple emulsions. In this review, five approaches to the preparation of multiple emulsions, namely two-step emulsification, modified two-step emulsification, phase inversion, membrane emulsification, and micro channel emulsification methods are some of the more common approaches. Average globule size and distribution, interface area, number of globules, rheological evaluation, zeta potential, percentage of drug entrapment, and in-vitro drug release are all characteristics of the Multiple emulsion. It has been suggested that multiple emulsions can be used for a variety of purposes, including as a prolonged drug delivery system.
Keywords: Multiple Emulsions, Emulsifying agent, Membrane emulsification.

1 INTRODUCTION

Multiple emulsions are complex systems that are referred to as "emulsions of emulsions," which means that the droplets in the dispersed phase are made up of even smaller droplets in the dispersed phase. Multiple emulsions are also known as emulsions of emulsions, liquid membrane system, or double emulsion. In the double emulsion, each dispersed globule forms a vesicular structure with single or multiple aqueous compartments separated from the aqueous phase by a layer of oil phase compartments. The fact that the drug contained in the innermost phase is compelled to partition itself through several phases prior to release at the absorption site is the fundamental reason for the utilization of W/O/W and O/W/O type multiple emulsions as a means of prolonged drug delivery. Consequently the parcel and dissemination coefficient of the medication and the strength of the center film stage, which is a multimolecular layer of oil, water and emulsifier particles at both the points of interaction of numerous emulsion framework, controls the medication discharge from these framework.

2 TYPES OF MULTIPLE EMULSIONS

The two major types of multiple emulsions are the water-oil-water (w/o/w) and oil-water-oil (o/w/o) double emulsions.

Water-in-Oil-in-Water (W/O/W) Emulsion System: In W/O/W system, an organic phase (hydrophobic) separates internal and external aqueous phase. In other words, W/O/W is a system in which oil droplets may be surrounded by an aqueous phase, which in turn encloses one or several water droplets.

Oil-in-Water-in-Oil (O/W/O) Emulsion System: In O/W/O systems, an aqueous phase (hydrophilic) separates internal and external oil phase. In other words, O/W/O is a system in which water droplets may be surrounded in oil phase,

which in turn encloses one or more oil droplets.

2.1 Formulation of Multiple Emulsions

Type a multiple emulsions were those in which the secondary emulsion droplet contained only one large internal drop. Florence and Whitehil referred to these three distinct types as Types A, B, and C. There were a few small internal droplets in the secondary emulsion droplet in type B emulsions, and there were a lot of internal droplets in type C emulsions. Drug delivery and drug targeting are only possible with type C systems.

2.2 Oil Phase

A nontoxic oil phase must be used in a pharmaceutical emulsion. The various oils derived from plants (such as soybean, sesame, peanut, safflower, and others) are acceptable if properly purified. Double emulsions 1 have also utilized refined hydrocarbons like light liquid paraffin, squalane, and fatty acid esters like ethyl oleate and isopropyl myristate. Oils made from vegetable sources break down in the body, whereas oils made from mineral oils are only slowly eliminated by the body.

Emulsifying agents' nature and quantity Two distinct emulsifiers—lipohilic and hydrophilic—are required to produce a stable emulsion. The ideal HLB value for a w/o/w emulsion will typically fall somewhere between 2 and 7 for the primary surfactant and between 6 and 16 for the secondary surfactant. Variation is also possible in the emulsifier concentration. Systems that have too little emulsifier may be unstable, while systems that have too much emulsifier may have toxic effects or even destabilize. The inversion of a w/o/w emulsion into a simple o/w emulsion can be caused by an excessive amount of lipophilic surfactant.

Phase Volume for the formulation of a stable multiple emulsion, the order in which the dispersed phase is added to the continuous phase must be carefully followed by the dispersed phase. The emulsion formulation can be carried out with an ideal internal phase volume (22-50%). A stable multiple emulsion had also been reported to be produced by a very high phase volume ratio (70-90 percent).

3 METHODS OF PREPARATION

3.1 Two-Step Emulsification Method (Double Emulsification)

It is the most common method because it is very easy and gives high yield with reproducibility. Multiple emulsions prepared by reemulsification of a primary emulsion. In this method two stages involved.

- Obtaining an ordinary W/O or O/W primary emulsion wherein appropriate emulsifier system is utilised.
- The freshly prepared W/O or O/W primary emulsion is re-emulsified with an excess of aqueous phase or oil phase. The final prepared emulsion could be W/O/W or O/W/O respectively.

3.2 Modified Two-Step Emulsification Technique

This method is different from the conventional two-step technique in two points.

- Sonication & stirring are used to obtain fine, homogenous & stable W/O emulsion.
- A continuous phase is poured into a dispersed phase for preparing W/O/W emulsion.

3.3 Evaluation of Multiple Emulsions

The Size Distribution and Average Size of Globules: The globule size of both internal dispersed phase and multiple emulsion droplets can be measured using the optical microscopy technique with a calibrated ocular and stage micrometer. Multiple emulsions' internal droplets have been characterized using bright field micrographs with differential interference contrast optics. Coulter counter, freezefracture electron microscopy, and scanning electron microscopy, among

other methods used to characterize colloidal carriers, are also utilized to ascertain the average globule size and size distribution of multiple emulsions. Recently, multiple emulsion characterization has been made possible by adapting NMR self-diffusion methods.

Rheological Assessment: In terms of emulsion stability and clinical performance, a crucial parameter is the rheology of multiple emulsions. Product rheology is influenced by two primary parameters: viscosity and interfacial elasticity.

Percentage of Drug Capture: Percent capture of medication or dynamic moiety in the various emulsions is by and large resolved utilizing dialysis, centrifugation, filtration and conductivity estimations. An internal tracer/marker was recently used to evaluate the entrapment of an impermeable marker molecule in the inner aqueous phase of a W/O/W emulsion. The amount of the unentrapped marker was calculated, and the amount of the entrapped marker was deducted from the amount that was initially added.

3.4 Applications of Multiple Emulsions

Sustained and Controlled Drug Administration: The controlled, prolonged release of drugs is the fundamental potential of multiple emulsions (both w/o/w and o/w/o) in clinical therapeutics. Before a drug in its innermost phase can be absorbed by the system, it must pass through several phases in both systems. Due to their lower viscosity, W/O/W emulsions for parenteral administration are easier to handle, use, and inject.

Targeting inversely: Talegaonkar and Vyas examined the effect of poloxmer 403 on surface modification for inverse targeting to rich organs of the reticuloendothelial system by preparing poloxamer 403 with spherein oil-in-water (s/o/w) multiple emulsion of diclofenac sodium and gelatinizing the inner aqueous phase. According to the findings, this poloxamer-containing multiple emulsion system can slow down the RES uptake of drugs, primarily to the liver and brain, as well as targeting non-RES tissues like the lungs and inflammatory tissue. Vaccine Adjuvant Herbert first reported the use of w/o/w multiple emulsion as a novel adjuvant for antigen. Compared to antigen alone, these emulsions elicited a stronger immune response. A multiple-emulsion vaccine against cattle infections caused by Pasteurella multocida was developed by Rishendra and Jaiswal14. This vaccine protected against the infection through both humoral and cell-mediated immune responses. It was determined that this vaccine based on multiple emulsions could effectively control hemorrhagic septicemia.

3.5 Oxygen Substitute

A multiple emulsion of oil in the outer aqueous phase that contains an oxygen-carrying aqueous material is suitable for oxygen supply in oxygen transfer processes. In an outer aqueous saline solution, a hemoglobin multiple emulsion in physiologically compatible oil is provided in sufficiently small droplet sizes to provide a blood substitute by allowing oxygen to flow through blood vessels to the desired body tissues or organs. A method for forming water-in-oil-in-water multiple emulsions with a high hemoglobin content while maintaining high yields and high oxygen exchange activity is provided. Hemoglobin is a fragile material.

Impairment of immunity: A potential strategy for enhancing immunosuppressive agents locally to the site of the target organs while avoiding the complications of systemic immunosuppressant. The immunosuppressant can now be delivered via the W/O/W multiple emulsion.

Booster of Bioavailability: In order to increase the bioavailability of lipophilic drugs, which have a high first pass metabolism, multiple emulsions have also been used. Multiple emulsion increases a

drug's bioavailability by either protecting the drug in a physiological, ionic, or enzymatic environment in the GIT, where it would otherwise be degraded like proteins or peptides, or by passing through the first pass metabolism in the liver.

Immobilization of a Enzyme: A multiple emulsion can be used for the enzymatic conversion of water-insoluble, highly lipophilic substrates like steroids. The substrate solution is in the organic phase, while the enzyme is in a microdroplet called a "water pool." To immobilize Urease, for instance, liquid surfactant membranes based on hydrocarbons have been utilized.

Treatment with drugs at high doses: By utilizing the pH difference, this system could be used to treat overdosage, such as with barbiturates. These emulsions have a basic buffer in the inner aqueous phase, and when taken orally, the stomach's acidic pH serves as an external aqueous phase. Barbiturate stays mostly in unionized form in the acidic phase, where it moves through the oil membrane into the inner aqueous phase and becomes ionized. Ionized drugs are less likely to cross the oil membrane and become stuck there. Thus, overdosage can be treated by encapsulating excess drug in multiple emulsions.

Taste Masking: Multiple chloroquine emulsions, an antimalarial drug, have been successfully prepared and found to effectively mask the bitter taste. Multiple emulsions have also reported that chlorpromazine, an antipsychotic, masks the taste.

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MICROEMULSION: A PROMISING STRATEGY FOR DRUG DELIVERY

Thandu Rajini
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India
Malothu Suresh
Asst. Professor, Department of Pharmaceutics, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - Even though oral drug delivery is still the most common method today, the ideal transdermal drug delivery system is still needed. A micro emulsion is a single optically isotropic and thermodynamically stable liquid solution made up of water, oil, and an amphiphile. As drug delivery systems, microemulsions have a number of advantages, including their thermodynamic stability, which enables the system to self-emulsify by acting as a supersolvent for drugs that are poorly or insoluble in water. They are favored more when contrasted with regular emulsions due strength. The water-insoluble drug is primarily dissolved in the dispersed phase. When compared to conventional vehicles, it has been demonstrated that the cutaneous absorption of lipophilic and hydrophilic APIs is enhanced by microemulsions.
Keywords: Microemulsion, Amphiphile, Thermodynamic stability.

1 INTRODUCTION

Hoar and Schulman introduced the microemulsion for the first time in 1943; By dispersing oil in an aqueous surfactant solution and adding alcohol as a co-surfactant, they created the first microemulsions that were transparent and stable. Using a variety of technologies, the theoretical structure's existence was later confirmed, and we can now use Attwood7's definition. A microemulsion is an optically clear pre-concentrate that dissolves poor water-soluble drugs by combining an oil, hydrophilic surfactant, and hydrophilic solvent. The formulations spontaneously disperse upon contact with water, forming an extremely clear emulsion of extremely small, uniform oil droplets containing the solubilized, poorly soluble drug. This process is referred to as "self emulsifying." because drug loading, bioavailability, and penetration through various biological membranes are all enhanced by microemulsion.

The kinetic stability of microemulsions is what sets them apart from emulsions. The utilization of different mixes of surfactant and co surfactant makes microemulsion more dynamically subterranean insect thermodynamically steady. Compared to emulsions, these are clear and less viscous. The system has very low interfacial tension due to the presence of surfactant and cosurfactant. Consequently, the microemulsion is thermodynamically steady and structures precipitously, with a typical bead width of 1 to 100 µm. 7-9. Preparation for microemulsion requires less energy than that of emulsion.

The optimal concentration of surfactant and cosurfactant decreases oil droplet surface and interfacial tension. On the other hand, a microemulsion is a highly complex system with a microstructure that can be altered by a small change in component concentration. The pseudoternary diagram and studying the system's phase behavior can be used to optimize these characters. Using three components, pseudoternary phase diagrams were created to examine the formation of oil in water microemulsions and the existing zone of microemulsions. The concentration of one component was kept constant while the concentration of the

remaining two components was varied in pseudoternary phase diagrams. The titration method is typically used to figure out how a microemulsion is made. If turbidity followed by phase separation was seen, the samples were said to be biphasic. After being stirred, the samples were considered monophasic if they appeared to be clear and transparent. The phase diagram depicted the samples as points. It was thought that these points covered the microemulsion's existence region. Phase diagrams were also created in the presence of the drug using drug-enriched oil as the hydrophobic component to determine the effect of the drug on the existing zone of the microemulsion.

`The drug delivery applications of the most recent advancements in microemulsion technology are the primary focus of this review. The article provides a synopsis of the most recent patents on microemulsion systems and investigates their potential, in addition to their novel applications, for the delivery of drug compounds that are poorly soluble. The field continues to be of sufficient importance, in addition to its traditional applications in lubrication and detergency, to continue attracting a number of scientists. From a fundamental research perspective, microemulsion properties have advanced significantly over the past two decades, highlighting their significance and need for investigation.

2 MERITS OF MICROEMULSION-BASED SYSTEMS

This is one of the advantages that microemulsions offer over conventional dosage forms.

- Microemulsions are thermodynamically stable systems that are identical to emulsions, allowing for self-emulsification of the system whose properties are not dependent on the process that is followed.
- Microemulsions function as drug "supersolvents." Microemulsions can be used to deliver drugs that are lipophilic or hydrophilic.
- Because microemulsion droplets have a mean diameter of less than 0.22 m, they can be sterilized through filtration rather than emulsion or suspension.
- Microemulsions require little energy to prepare because of their thermodynamic stability, making them simple to prepare.
- Compared to other emulsions, microemulsions have a low viscosity.
- Using microemulsions as delivery systems can increase drug efficacy and reduce side effects by lowering the total dose.
- Microemulsion formation can be reversed. Microemulsions are formed when the temperature reaches a stable range and these may become unstable.

3 COMPONENTS OF MICROEMULSION

1. Oil Phase the drug's solubility is the primary consideration when selecting an oil. Since most drugs dissolve in O/W microemulsions, the drug should be highly soluble in the oil-surfactant system. The solubility criteria may improve drug penetration through various biological membranes, the concentration gradient, and the drug's release from the system.
2. Surfactant Molecules that have a polar head group and an apolar tail are known as surfactants. They have a strong chemical dipole and are molecules that form microstructures and are active on the surface. Surfactant molecules can take on a variety of configurations, with the most common types being ionic (cationic or anionic) and non-ionic (zwitterionic). They can form reverse micelles, hexagonal reverse micelles, lamellar (sheet) phases, spherical micelles, rod-shaped micelles, and a

hexagonal phase made up of rod-shaped micelles.

3. Cosurfactant in some cases, using surfactant alone may not result in the successful formation of microemulsions and regions that form microemulsions. To set up an ideal microemulsion, once in a while there is need of expansion of second surfactant with low sub-atomic weight amphiphile like liquor subsidiaries. Cosurfactants make the hydrocarbon chain of primary surfactants more fluid.

Cosurfactants contribute to the system's thermodynamic stability by increasing its entropy and fluidizing the surfactant film, both of which reduce surface tension further. Co-surfactants make the surfactant film around the microemulsion droplet more flexible. Cosurfactants typically include alcohols with short and medium chains, such as butanol, pentanol, ethanol, isopropanol, or propylene glycol.

3.1 Types of Microemulsion

Wisnor defined the four general types of microemulsion,

- Wisnor Type I (O/W): With two phases, the lower (o/w) microemulsion phases in equilibrium with the upper excess oil.
- Wisnor Type II (W/O): With two phases, the upper microemulsion phase (w/o) microemulsion phases in equilibrium with lower excess water
- Wisnor Type III (B.C.): With three phases, middle microemulsion phase (o/w plus w/o, called bicontinous) in equilibrium with upper excess oil and lower excess water.
- Wisnor Type IV (Isotropic micellar solution): In single phase, with oil, water and surfactant homogenously mixed.

3.2 Advantages

Potential advantages of these systems (SMEDDS) include,

1. Enhanced oral bioavailability enabling reduction in dose.
2. More consistent temporal profiles of drug absorption.
3. Selective targeting of drug(s) toward specific absorption window in GIT
4. Protection of drug(s) from the hostile environment in gut.
5. Control of delivery profiles.
6. Reduced variability including food effects.
7. Protection of sensitive drug substances.
8. High drug payloads.
9. Liquid or solid dosage forms.

3.3 Microemulsion Characterisation

1. Viscosity

The viscosity of microemulsions is lower than that of standard emulsions. The Brookfield viscometer is used to measure the viscosity. When determining the structure of micells, such as rod-shaped or reversed micells, a viscosity measurement is helpful. Whether the system is w/o or o/w, this determination of viscosities is consistent. If the system has a low viscosity, it is of the o/w type, and if it has a high viscosity, it is of the w/o type.

2. Conductivity

Dielectric measurements are a potent tool for probing both the structural and dynamic characteristics of microemulsion systems. They can be used to determine whether a microemulsion is oil-continuous or water-continuous, as well as to monitor percolation or phase inversion phenomena.

3. Measurement of Droplet Size

Using a Zetasizer and photon-correlation spectroscopy (PSC), the size of a droplet can be determined. At 25°C and a scattering angle of 90°, all measurements are taken. Before being measured, the microemulsion is diluted in pure water in two steps before being added to the cuvette by filtering it through a 0.22um

filter. It is diluted with the same amount of water in the first step. In second step the combination is additionally weakened to fitting fixation for the estimation. That depends on the size of the droplets (typically diluted 100-200 times).

4 RECENT DEVELOPMENTS IN MICROEMULSION

A recent pharmacokinetic study with the Sand immune cyclosporine A Neoral microemulsion concentrate demonstrated improvements in bioavailability and inter/intra-patient variability, facilitating the efficient management of psoriasis. This was done in regards to the issue of oral microemulsion administration.

A number of recent reports provide information on microemulsion formulations intended for transdermal or topical use. In a hairless mouse model, both o/w and a w/o microemulsions have been tested for prostaglandin E1 delivery. Oleic acid or Gelucire 44/14 served as the oil phase in the microemulsions, which were stabilized with a mixture of Labrasol (C and C polyglycolyzed glycerides) and Plurol Oleique CC 497 as a surfactant.

Phase changes that occur following administration and are triggered by changes in temperature, pH, or ionic strength are particularly useful in environmental-responsive drug delivery systems. The phase change from a reverse micellar lecithin solution in IPM to a lamellar liquid crystal is one example of this behavior. For this situation the progress was set off by contact of the converse micellar arrangement with an organic watery stage, bringing about the controlled arrival of the calming fenoprofen. The patent is likewise given for the creation of microemulsion containing alkanolammonium salts of alkysulfates and alkypolyyalkyleneglycole the rsulfates, UV channels and antidandruff substances. The invention is about using emulsion for dermatological and cosmetic purposes.

Test microorganisms such as pseudomonas aeruginosa, cadida albicans, staphylococcus aureus, and aspergillus niger have recently been used to investigate the oil-in-water microemulsion's antimicrobial activity. The results clearly show that the microemulsion system has good biocidal activity against the bacterial species (more than 6 log cycles of reduced P. aeruginosa viability when exposed to Formula 1 microemulsion in less than 15 seconds).

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ASSESSING THE IMPACT OF E-COMMERCE ON SUPPLY CHAIN MANAGEMENT IN INDIAN ONLINE PHARMACY INDUSTRY: AN EXPERIMENTAL STUDY

Vaishnavi Munnangi
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Soorammagari Sunayana
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract- Store network The board is an outer point of interaction of strategies frameworks of the associations with its clients, partners and any remaining reevaluating parties with the intend to offer best types of assistance to every one of the clients. The supply chain system is very important in India when it comes to getting products to customers. The majority of consumers now make their product purchases online as a result of digitalization; consequently, the significance of e-commerce in supply chain management cannot be overstated. This research paper describes India's online pharmacy system. The goal of this research paper is to demonstrate India's cutting-edge pharmacy e-commerce logistics system. In this exploration paper, the scientists approach regular web based business organizations which are bargain in web-based drug store in India. This research paper's practical and social implications include raising awareness of online pharmacies among Indian citizens so that they can save time and money by purchasing medications from home.

Keywords: E-Commerce, Logistics System of Pharmacy, Online Pharmacy and Supply Chain Management.

1 INTRODUCTION

The administration of all standard business functions between at least three legitimately connected businesses is referred to as supply chain management. It involves at least one of the upstream and downstream progressions of goods, services, funds, and data from a source to a customer across any part of the framework. According to Christopher (1992), a Supply Chain is the system of associations that participate, through upstream and downstream links, in the various procedures and activities that result in incentives such as products and services in the hands of extreme customers.

1.1 Characteristics of Supply Chain Management

The most common characteristics of Supply Chain Management are as follows:

- Superior Value to Customers
- Single Entity
- Inventory Perspective
- Strategic Focus
- Relationships in the Supply Chain
- Flexible Approach

1.2 Supply Chain Management and Information Technology

An efficient connection and interface between supply chain management and information technology can be attributed to the following factors:

- A high level of inter-function integration and coordination within the company is made possible by information technology.
- Inter-firm integration and coordination were made possible by firms thanks to information technology.
- By making the supply chain more adaptable to meet the needs of competitive markets in the most effective manner, IT improves businesses' market responsiveness.

- Data Advancements and frameworks, for example, Undertaking Asset Arranging (ERP), Bar Coding, RFID Frameworks, and so on., are crucial tools for enhancing the flow of information among businesses and enabling resource optimization across the supply chain network.
- Supply chain confusion, ambiguity, uncertainty, and risks are reduced when information is made available in real time through information technology, resulting in improved relationships, trust, and comprehension.

1.3 Online Pharmacy

A drug store that operates over the internet and communicates with customers via messenger with the assistance of transportation companies is known as an online pharmacy. Examples of these kinds of pharmacies include:

- Pharmacy Director - Corporate professionally prescribed drug organizer.
- Veritable online pharmacy present in that country where the individual puts in the request.
- A legitimate online pharmacy that is located in the nation from which the customer places their order. In these pharmacies, the license is issued by the nation from which the customer is originally from, and customers are only required to abide by the laws of that nation. They are not required to adhere to the regulations of the nation from which the order is issued.

2 REVIEW OF LITERATURE

Sahet. al. (2018) describes in the research paper how easy it is to buy a wide range of medications from online pharmacies. These provide a variety of preferences, such as lower prices, straightforward accessibility, doorstep delivery, and so on. Additionally, online drug stores carry flaws like the inability to collaborate with a doctor, the administration of medications without solutions, the sale of inadequate medications, and so on. In India, there is a lack of information regarding online drug store customers' concerns. Hence study was planned to review purchaser's care and direct towards the use of online pharmacy benefits in the Delhi, India. A cross-sectional, poll-based study was conducted to inquire about customers' attitudes and actions regarding the use of online pharmacy services. Even though 85.8% of respondents to this survey were aware of online drug stores, only 6% of them purchased prescriptions from them. 85 percent and 75 percent of members, respectively, expressed their most pressing concerns regarding online drug stores in terms of the accessibility of prescriptions and the availability of counterfeit medications. Variable preferences for distributing drugs without the need for a prescription (91 percent) and illicit sites (57 percent) were identified as typical for dubious online drug stores. 68% and 60.8% of members, respectively, identified doorstep delivery and update services as appealing features of online pharmacies. The respondents did not typically purchase drugs from online drug stores. It is necessary to inform customers about the advantages and disadvantages of online drug shopping. Instructive measures should be used in conjunction with administrative specialists performing satisfactory checks on online drug stores.

According to Uttarwar and Riat (2018), the idea of a lean supply chain is becoming increasingly commonplace in Indian industry. They argued that, thanks to advancements in communication and information technology, businesses now have a great opportunity to lower their operating costs through improved coordination and effective management. They emphasized the fact that the store network of the future will unquestionably be more global and adaptable than at any

other time in recent history. They showed that Indian businesses need to change their mindset quickly and be adaptable because many of the old practices, like vertical incorporation and trading services, are seen as out of date in the new economy. That was featured very quickly; Green Supply Chain will be of primary interest due to the observed effect of assembling on condition. Finally, they demonstrated a cutting-edge supply chain model that takes into account emerging key patterns and will benefit Indian industry.

2.1 Indian Online Pharmacy

In India, there are several organizations which deals in online pharmacy such as Netmeds.com, Medlife.com, etc. The details are as follows:

2.2 Netmeds.com - the trusted Online Medicine Partner

Netmeds.com, India Ki Pharmacy is brought to you by Dadha & Company, one of India's most trusted pharmacies that has been distributing high-quality prescriptions for more than a century. At netmeds.com, we make it simple for you to take care of your own health and communicate with friends and family anywhere in India. With just a few clicks of the mouse, you can buy and send medications from anywhere in the country.

3 CONVENIENCE

Overwhelming traffic, a lack of rest stops, a rainstorm, a closed store, neglect—these are just a few of the factors that could lead to missing important prescriptions. It is best not to run out of basic prescriptions because taking medications on a regular basis is an essential part of managing persistent illnesses. Simply log in to netmeds.com, make a request online, and your medications will be shipped to you without you having to leave the comfort of your own home.

3.1 One-Stop Shop

At netmeds.com, we not just furnish you with a wide scope of drugs recorded under different classifications; we additionally offer a wide decision of OTC items including health items, nutrients, diet/ wellness supplements, herbals items, torment relievers, diabetic consideration units, child/mother care items, beauty care items and surgical supplies.

3.2 Trust

Netmeds.com proceeds with a heritage of 100 years of achievement in the pharmaceutical business. We are committed to provide reliable, safe and affordable prescriptions just as a client support way of thinking that is deserving of our esteemed clients' loyalty. We offer a predominant web based shopping background, which incorporates simplicity of route and outright value-based security.

4 RESEARCH METHODOLOGY

The pursuit of knowledge is similarly referred to as research. In addition, inquire about can be described as an effective and logical search for relevant data on a particular topic. To tell you the truth, research is an art form of scientific investigation. The process of defining the research's system, action, and components in relation to logically accepted models or methodologies, plans, and tools is known as a research methodology.

The researchers used an analytical and descriptive approach in this study. The western region of Uttar Pradesh served as the primary population for this study, which collected 80 samples. In this study, a questionnaire was used, and the researchers used convenient random sampling.

5 OUTCOME OF THE STUDY

The outcome of the study is explain in the following points:

- This study explain several features of supply chain management and

information technology infrastructure which are related to online pharmacy system of India.
- This research also emphasizes on risks and concerns of online pharmacy.
- This article depicts about the online pharmacies laws in India and its' recent developments.
- This study also describe relevant information regarding Indian online pharmacy companies.
- This study explain the benefits and procedure of online purchasing medicines.

6 SUGGESTIONS

In the light of foregoing analysis, findings and observations, some broad suggestions have been recommended. The present research has indicated some of the facts about online pharmacy services. So based on these observations, some specific suggestions are as follows:

- The filling of questionnaire form is simple to some extent.
- The file size of the prescription will increase.
- The information related to the salt of the medicine should be more specific.
- There is an option related to medical advice/ online doctor is available.
- The rules and regulations related to online pharmacy system must be available on the website.

6.1 Contributions of Online Pharmacy System to our Society

The contributions of Online Pharmacy System to our society are as follows:

- The awareness of medicine information will increase.
- Customers are getting discounted price.
- In case of patient, who is not able to reach the medical store for purchasing the necessary medicines, door step delivery is significantly appreciable.
- It is helpful in personalize medicine reminder services.
- This system is quite beneficial in case of access to the drug information.

6.2 Limitations of Research

The limitations of research are as follows:

- The research area of the study is the entire western part of Uttar Pradesh but it is very difficult to cover each and every district of the specified region.
- The research topic is very wide and the researcher has limitation of time to cover each and every aspect related to online pharmacy system. Thus time factor can be considered as one of the limitations.
- This research is completely based on the information provided by the respondents. Therefore, the accuracy of findings and results of this research are not fool proof.
- The results of this study may change due to demographic characteristics and time of the study.
- The present research has focused only on the opinions of those respondents, which are involved in online pharmacy.

The above points explain the limitations of the research.

7 CONCLUSION

This study demonstrates how Indian online pharmacies use e-commerce in Supply Chain Management. The IT infrastructure that is necessary for the Supply Chain Management System is also described in this study, as are the various aspects of Supply Chain Management. The Indian internet based drug store framework is developing step by step. a number of businesses, including Medlife.com, Netmeds.com, and others. are contributing significantly to the distribution of medicines to customers. This article discusses a variety of

strategies, including Indian online pharmacy laws and recent developments. According to this study, the majority of Indians are aware of online pharmacies. People in India are also aware of the appealing features of online pharmacies, such as lower prices, door-to-door delivery, drug information, and so on. The majority of respondents, according to Atlast, are pleased with the current community pharmacy services.

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ADVANCEMENTS IN DRUG DELIVERY: A COMPREHENSIVE REVIEW OF BILAYER TABLETS VIA MICROSPHERE TECHNOLOGY

Dr. Harikiran Lingabathula
Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India
Soorammagari Sunayana
Asst. Professor, Department of Pharmacology, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The development of bilayer tablets with immediate release and sustained release microspheres on one layer is the goal of this work. The purpose of the proposed dosage form is to reduce the frequency with which an anti-diabetic medication is administered at the same time. For a variety of reasons, several pharmaceutical companies are currently developing bi-layer tablets: marketing, therapeutics, and patent extension, to name a few. When developing and manufacturing such tablets, existing but modified tablet presses are frequently utilized to reduce capital expenditure. Utilizing microspheres, or microparticles, as drug carriers is one such strategy. It is the reliable method for maintaining the desired concentration at the site of interest and delivering the drug to the target site with specificity in the event that it is altered. Microspheres received a lot of attention for their extended release as well as their ability to target anti-diabetic medications. The microsphere-based bilayer tablet represents a new era in the successful development of controlled release formulations and includes a number of features that make it an effective drug delivery system. especially when a high output of production is also required. This review article aims to educate society about the most recent technological advancements in bilayer and floating drug delivery systems.

Keywords: Microsphere, anti-diabetic bilayer tablet, Floating drug delivery system.

1 INTRODUCTION

Diabetes mellitus, also known as diabetes, is a group of metabolic diseases in which a person has high blood sugar levels either because the body does not produce enough insulin or because the cells in the body do not respond to the insulin that is produced1. This condition is commonly referred to as diabetes mellitus. Diabetes is one of the significant reasons for death and handicap on the planet. In 2000, the World Health Organization estimated that 171 million people worldwide had diabetes. By 20302, that number is expected to rise to at least 366 million. Type 2 (non-insulin dependent) diabetes is a multifaceted condition marked by an underlying insulin deficiency.

A low dose combination of two different agents reduces the dose-related risk, the addition of one agent may counteract some deleterious effects of the other, and using low dosage of two different agents minimizes the clinical and metabolic effects that occur with maximal dosage of individual component of the combined tablet4. This insufficiency results from defective insulin utilization and can be corrected by administration of one or more of the currently available oral hypoglycemic agents.

Sidney Walter Fox was a biochemist from Los Angeles who was born on March 24, 1912, and died on August 10, 1998. He was the one who discovered how life started. Fox created what he believed to be the first "protocells" out of proteinoids and water and investigated the synthesis of amino acids from inorganic molecules, proteinous amino acids, and amino acid polymers known as "proteinoids" from

inorganic molecules and thermal energy. He called these protocells "microspheres" and they have now been named "protobionts." Fox suggested that the conditions in his experiments were comparable to those found in the Earth's early days and that life could have formed naturally. He demonstrated in his experiments that using thermal energy and inorganic molecules, protein-like structures can be made. After creating microspheres that, according to Dr. Fox, were very similar to bacterial cells, he came to the conclusion that these could be the earliest forms of life.

Microspheres are tiny, spherical particles with diameters ranging from one micrometer to one thousand micrometers (1 mm). Microparticles are another name for microspheres. Natural and synthetic materials can be used to make microspheres. Ceramic, polymer, and glass microspheres can all be purchased commercially. The density of solid and hollow microspheres is very different, so they are used for different things. Typically, hollow microspheres are added to materials to reduce their density. Depending on the material they are made of and how big they are, solid microspheres can be used for a variety of things.

The most common kinds of polymer microspheres are polyethylene, polystyrene, and expandable microspheres. Due to their ability to facilitate processes like cell sorting and immunoprecipitation, polystyrene microspheres are typically utilized in biomedical applications. Polystyrene microspheres are suitable for use in biological laboratory experiments as well as medical research due to their ability to adsorb proteins and ligands on it quickly and permanently.

Polyethylene microspheres are frequently utilized as filler, either permanently or temporarily. Polyethylene microspheres can create porous structures in ceramics and other materials thanks to their lower melting point. Polyethylene microspheres are highly desirable for numerous research applications, including flow visualization and fluid flow analysis, microscopy techniques, health sciences, process troubleshooting, and the availability of colored and fluorescent microspheres. Electronic paper digital displays also use charged polyethylene microspheres.

Polymer microspheres called expandable microspheres are used as a blowing agent in things like puff ink, automotive underbody coatings, and thermoplastic injection molding. They can also be used as a light filler in waterborne paints, crack fillers, and joint compound, for example. When heated, expandable polymer microspheres can grow to more than 50 times their original size.

Each sphere has a thermoplastic shell on the outside that houses a hydrocarbon with a low boiling point inside. The hydrocarbon exerts pressure on the internal shell wall as the outside shell expands and softens when heated.

Glass microspheres are primarily utilized as a filler and volumizer for the purpose of reducing weight, a retroreflector for the purpose of improving highway safety, and an additive for cosmetics and adhesives. There are only a few applications for them in medical technology. Fired microspheres are utilized essentially as crushing media. Quality, sphericity, uniformity, particle size, and distribution of particle size all vary greatly in microspheres. Each individual application necessitates the selection of the appropriate microsphere.

1.1 Advantages of Bi-layer Tablets

1. Execution with two layers and an optional conversion kit for one layer
2. Low price when compared to other dosage forms.
3. Compared to other oral dosage forms, it has the highest chemical and microbial stability.

4. Coating technologies can cover up unpleasant tastes and odors.
5. Flexible idea.
6. provide the lowest content uniformity while the highest precision.
7. The least amount of hangup issues and easy to swallow.
8. Suitable for mass production.
9. Bi-layer tablet is reasonable for forestalling direct contact of two medications and hence to amplify the adequacy of blend of two medications.
10. Because one of the layers can be kept as extended and the other as immediate release, bi-layer tablets can be designed to modify release.
11. Expansion of an established technology.
12. The use of feed granules with only one entity as a potential option.
13. Separation of components that are incompatible.
14. Patient consistence is worked on prompting further develop drug routine effectiveness.

1.2 Disadvantages of Bi-layer Tablets

1. Adds complexity and costs a lot for bi-layer rotary presses.
2. Reduced yield, layer separation, and insufficient hardness
3. Individual layer weight control that is not precise.
4. There is cross-contamination among the layers.
5. Difficult to swallow in children and patients who are unconscious.
6. Due to their amorphous and low-density nature, some drugs resist being compacted.
7. Drugs with unfortunate wetting, slow disintegration properties, ideal retention high in GIT may challenging to fabricate as a tablet that will in any case give adequate medication bio accessibility.

2 VARIOUS TECHNIQUES FOR BILAYER TABLETS

Oros ® Push Pull Technology: This system is mostly made up of two or three layers, one or more of which are necessary for the drug and the other is the push layer. The drug and two or more distinct agents make up the majority of the drug layer. As a result, the drugs in this drug layer are poorly soluble. Osmotic and suspending agents are additionally added. The tablet's core is surrounded by a semi-permeable membrane.

L-OROS Technology : Alza invented the L-OROS system, which addresses the issue of solubility. It begins with the production of a lipid soft gel product containing the drug in a dissolved state before coating it with a barrier membrane, an osmotic push layer, a semi-permeable membrane, and an exit orifice.

DUROS Technology: An outer cylindrical titanium alloy reservoir makes up the system. This reservoir protects the drug molecules from enzymes and has a high impact strength. The minuscule drug dispensing system known as DUROS technology resembles a miniature syringe and delivers a minute amount of concentrated form.

2.1 Evaluation of Bilayer Tablets

Thickness of Tablets: Using a dial caliper that has been calibrated, thickness and diameter are measured. The formulation's three tablets are selected at random, and each one's thickness is measured.

Hardness of Tablets: The Monsanto hardness tester is used to measure hardness. Three tablets from each batch are tested.

Friability: After weighing twenty tablets, the Roche friabilator is turned for four minutes at 25 rpm. The tablets are deducted and weighed once more after each revolution. The formula will be used to determine the percentage of friability,

$$\% F = \{1-(Wt. /W)\} \times 100$$

Where, % F = Friability in percentage
W = Initial weight of tablet
Wt. = Weight of tablets after revolution

Weight Variation: Each batch contains twenty tablets, each of which is individually weighed. Twenty tablets' average weight and standard deviation are calculated. If not more than two of the individual tablet weights deviate from the average weight by more than the percentage shown in, and if none deviate by more than twice the percentage, then the batch has passed the weight variation test.

Drug Content: The assay of the drug content is carried by weighing ten tablets and calculated the average weight. Then the tablets are triturated to get a fine powder. From the resulting weighed accurately about 155 mg of the powder (equivalent to 100 mg) of metformin hcl is taken, shake with 70 ml of water for 15 minutes, dilute to 100 ml with water and filter. Dilute 10 ml of the filtrate to 100 ml with water. Further dilute 10 ml to 100 ml with water and measure the absorbance at the maximum at about 233nm.

Buoyancy Determination: One tablet from each formulation batch is placed in a USP type II dissolution apparatus containing 900 ml 0.1 N HCl dissolution medium using a paddle at a rotational speed of 75 rpm. The time it takes for the dosage form to emerge on the surface of the medium is referred to as floating lag time, and the time it takes for the dosage form to continuously emerge on the surface of the medium is referred to as total floating time (TFT). The medium is kept at a temperature of 37° 2°C. The time taken for tablet to arise on surface of medium and the term of time by which the tablet continually stay on surface of medium will be noted.

Swelling Study: The singular tablets are weighed precisely and kept in 50ml of water. After 60 minutes, the tablets are carefully removed, blotted with filter paper to remove any water on the surface, and accurately weighed. Rate enlarging is determined by utilizing equation; The swelling study is as follows: 100 x wet weight x dry weight

In-Vitro Drug Release Study: Each batch's tablet is broken down using a paddle-operated USP type II apparatus. A dissolution vessel contains 900 milliliters of dissolution media at a temperature of 37° 2°C. Each dissolution vessel contains one tablet, and the paddle's rotational speed is set at 50 rpm. The 10 ml of test is removed at foreordained time span for 12 hours and same volume of new medium is supplanted. Using a double beam UV visible spectrophotometer, the drug content of the samples is compared to dissolution media as a blank at 233 nm.

3 CONCLUSION

The quality of bilayer tablets and GMPrerequisites can vary significantly. Metformin and pioglitazone bilayer tablets were made in this study using modified direct compression techniques, and the drug release study yielded significant results. As a result, the formulations might be a good candidate for multiple unit administration, opening up new therapeutic options that would be extremely beneficial to the patient. These bilayer tablets are mostly made to reduce lag time, but they may also increase the drug's bioavailability by making full use of the drug and avoiding excessive plasma levels. This explains why bi-layer tablets are produced using a wide variety of presses, from straightforward single-sided presses to highly sophisticated machines. The use of an "air compensator" in conjunction with displacement control appears to be the best option whenever high-speed production of high-quality bi-layer tablets is required.

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EXPLORING THE ADVANTAGES AND CHALLENGES OF MOUTH DISSOLVING TABLETS: A COMPREHENSIVE REVIEW

Surendar Angothu
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India
Boggula Ratnakumari
Asst. Professor, Department of Pharmacognosy, Princeton College of Pharmacy, Hyderabad, Telangana, India

Abstract - The oral route is regarded as the most convenient, safest, and most cost-effective method of drug administration due to its high rate of patient compliance. One of the most advantageous examples of oral drug delivery is fast-dissolving tablets. Without the use of water, these tablets easily dissolve or break down in saliva in less than 60 seconds. They have been made for bedridden, elderly, and pediatric patients. Additionally, these dosage forms are ideal for patients who are active, busy, and may not have access to water. FDTs have attracted a lot of attention for patients with dysphagia and hand tremors who have trouble swallowing. They also help unconscious, young patients whose nervous and muscular systems aren't fully developed. This audit portrays the different benefits, limits, wanted attributes, detailing viewpoints, superdisintegrants utilized; technologies made for FDTs, evaluation tests, and formulations that are sold.
Keywords: Thymoquinone, antitubercular medications, and renal toxicity.

1 INTRODUCTION

Because of their convenience, compactness, ease of manufacturing, and self-administration capabilities, solid dosage forms like tablets and capsules are the most widely used among all of the dosage forms that are currently available. When there is no water available, tablets and hard gelatin capsules can be difficult to swallow, as can motion sickness, allergic attacks of coughing during the common cold, and bronchitis. As a result, tablets are referred to as "fast dissolving tablets" because of their ability to rapidly dissolve or break down in the mouth. When swallowed, these tablets instantly break down, releasing the medication, which, in the absence of water, dissolves or disperses in saliva within 60 seconds. FDTs are ideal not only for active people but also for people who have difficulty swallowing.

Other names for fast-dissolving tablets include mouth-dissolving tablets, melt-in-mouth tablets, orodispersible tablets, rapidly melts tablets, porous tablets, and so on. As saliva descends into the stomach, some drugs are absorbed from the mouth, pharynx, and oesophagus. The drug's bioavailability is significantly higher in these situations than in conventional tablet dosage forms. The drug's absorption and onset of clinical effect are accelerated the faster it is dissolved in the solution. The fundamental strategy for developing FDTs is to make use of superdisintegrants like

croscarmellose, sodium starch glycolate, polyvinylpyrollidone, crosspovidone, and others. These superdisintegrants help break down tablets quickly and release drugs into saliva. Additionally, compared to standard tablets, the amount of drug subjected to first pass metabolism is reduced. FDTs are simple to use for patients whose chewing is difficult and painful. Children who have lost their teeth but still lack full use of their permanent teeth can also easily use fast-dissolving tablets. Frost-drying, tablet molding, spray-drying, sugar-based excipients, sublimation, tablet compression, disintegration addition, and numerous other pat ended technologies are the technologies utilized in the production of fast-dissolving tablets. According to a recent market study, FDTs are preferred by more than half of the world's population to other dosage forms.

1.1 Advantages of Fast Disintegrating Tablets

Technology that dissolves quickly offers:

- Ease of administration for patients whose swallowing difficulties are a problem.
- The dosage form does not require water to be swallowed.
- Useful for patients with mental health, geriatric, and pediatric conditions.
- Possess adequate taste-masking properties.
- Increase bioavailability by allowing drugs to pass through the mouth, pharynx, and oesophagus before entering the stomach.
- Feel good in the mouth and leave little or no residue in the mouth after being taken.
- The drug should dissolve and be absorbed quickly, resulting in a rapid onset of action.
- It combines the stability and bioavailability of a liquid dosage form with those of a solid dosage form.

Drug Properties: Drug properties can have an impact on FDT performance. The solubility, crystal morphology, particle size, hygroscopicity, compressibility, bioavailability, flow property, bulk density, and final tablet characteristics like disintegration and strength of a drug, for instance, can all have a significant impact.

Taste of Active Ingredients: The drug will partially dissolve in close proximity to the taste buds when FDTs dissolve or disintegrate in the patient's mouth. There should be little or no residue left in the mouth after swallowing. The ideal taste-masking technology ought to deliver drugs with a pleasant taste and no grittiness.

Moisture Sensitivity: There should be little humidity sensitivity in these tablets. This issue can be particularly challenging due to the use of numerous highly water-soluble excipients in formulation to improve fast dissolving properties and mouthfeel. Moisture can damage those highly water-soluble excipients; some will try and deliquesce at high mugginess.

1.2 Drug Selection Criteria

- Have a better soluble nature. Promethazine, for instance For

instance, terazosin HCL is more readily available to penetrate oral mucosal tissue.
- Taste less or not as bitter.
- Excellent stability in saliva and water. For example rizatriptine benzoate

Super-Disintegrants: Superdisinteg-rants are substances added to tablet formulations to increase the available surface area and encourage the breakup of the tablets into smaller pieces in an aqueous environment.

Mechanism of Superdisintegrants: There are four major mechanisms for tablet disintegration as follows:

Swelling The most widely accepted general mechanism of action for tablet disintegration is swelling. Tablets that lack sufficient swelling force and have high porosity exhibit poor disintegration. The tablet has low porosity and exerts sufficient swelling force. Fluid cannot penetrate the tablet if the packing fraction is very high, and disintegration again slows down.

Porosity and Capillary Action (Wicking): It is believed that effective disintegrants that do not swell impart their dissolving action through capillary and porosity action. The porosity of a tablet makes it possible to form fluid into tablets through the use of channels. The disintegrant particles themselves make the tablet more porous and provide entryways. Through capillary action, liquid is drawn up or "wicked" into these pathways, breaking the terparticulate bonds and causing the tablet to break apart.

Due to disintegrating particle/ particle repulsive forces Another disintegration mechanism tries to explain why tablets made with "nonswellable" disintegrants swell. Based on Guyot-Hermann's observation that tablets also break apart when nonswelling particles are present, he proposed the particle repulsion theory. Water is required for the disintegration mechanism, which is the electric repulsive forces between particles.

2 CONVENTIONAL TECHNIQUES USED FOR PREPARATION OF FDTS

1) Disintegration Addition: The principle involved in formulating FDTs by disintegrant addition technique is the addition of superdisintegrants in optimal concentration in order to achieve rapid disintegration and a pleasant mouthfeel. This makes the disintegrant addition technique one of the most popular methods for creating FDTs.

2) Freeze drying: a method in which the product's frozen water is sublimated. Lyophilization is a pharmaceutical technique that enables heat-sensitive drugs and biologicals to be dried at a low temperature under conditions that permit the sublimation of water. Preparations made by lyophilization are highly porous, have a very high specific surface area, dissolve quickly, and have improved bioavailability and absorption.

3) Moulding: Using water-soluble ingredients, these molded tablets are prepared so that they dissolve completely and quickly. A hydro-alcoholic solvent is used to moisten the powder mixture before molding it into tablets under a pressure that is

lower than that used for traditional tablet compression.

4) Spray- Drying: Spray drying can result in powders that are extremely porous and fine, and they dissolve quickly. As supporting agents, the formulations incorporate mannitol as a bulking agent, sodium starch glycolate or crosscarmellose sodium as a disintegrating agent, and an acidic material (such as citric acid) and/or alkali substance (such as sodium bicarbonate) to boost dissolution and disintegration.

5) Mass- Extrusion: In order to cut a cylinder of the product into even segments using heated blades to form tabets, this technology involves softening the active blend with a solvent mixture of water-soluble polyethylene glycol and methanol, then extruding the softened mass through an extruder or syringe.

3 EVALUATION TESTS FOR FAST DISSOLVING TABLETS

3.1 In Vitro Evaluation Methods

General Appearance: Consumer acceptance of a tablet is dependent on its overall "elegance," visual identity, and general appearance. The tablet's size, shape, color, physical flaws, odour, taste, surface texture, consistency, and legibility of any identifying marking are all included.

Tablet Thickness: The thickness of the tablet is an important property for reproducing appearance and counting with filling equipment. The uniform thickness of the tablets is used as a counting mechanism in some filling equipment. The thickness of ten tablets was measured with a micrometer.

Uniformity of Weight: Twenty tablets were taken, and their individual and collective weights were measured on a digital weighing balance in accordance with the I.P. procedure for uniform weight.

Tablet Hardness: Hardness of tablet is defined as the force applied across the diameter of the tablet in the order to break the tablet. Hardness of the tablet of each formulation was determined using Monsanto Hardness tester and many other testers like the Strong-Cobb tester, the Pfizer tester, the Erweka tester, and the Schleuniger tester available for determining hardness of particular tablet.

Disintegration Test: There are a number of limitations to the standard disintegration test for FDTs, and it does not adequately measure very short disintegration times. Because ODT requires disintegration without water, the test should mimic disintegration in salivary contents, necessitating a modification to the disintegration time. For this reason, a petridish (10cm width) was loaded up with 10 ml of water. The time it took for the tablet to completely disintegrate into fine particles was recorded after it was carefully placed in the petridish's center.

Wetting Time: The approach that yunixia et al. describe, was followed to gauge tablet wetting time. A small dish with an ID of 6.5 cm and 6 ml of Sorenson's buffer at pH 6.8 was filled with a piece of tissue paper that measured 12 cm x 10.75 cm and had been folded twice. The time required to completely wet the paper was measured by placing a tablet on it. Each batch underwent three trials,

and the standard deviation was also calculated.

4 CONCLUSION

Some of the issues with administering medications to children and the elderly, who make up a large portion of the world's population, have been resolved by the introduction of dosage forms that dissolve quickly. As a result, patient demand and the availability of various technologies have increased the acceptance of tablets that dissolve quickly, which extends a drug's useful life. Maintaining considering the upsides of the conveyance framework, quick breaking down measurement structures have been effectively popularized and these dose shapes very much acknowledged at specialists as well as quiet level.

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