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Formulation Characterization and Evaluation of Controlled Nasal Drug Delivery Systems for Cefuroxime Axetil

Shubham Sharma,Research Scholar, IIMT College of Medical Sciences, IIMT University Meerut U.P

Dr. T.S Easwari, GuideIIMT College of Medical Sciences, IIMT University Meerut U.P.

Mr. Atul Pratap Singh,Co-guide, IIMT College of Medical Sciences, IIMT University Meerut U.P.

Email:-Shubhamsharma967566@gmail.com

Abstract

The present study has been a satisfactory attempt to formulate a CA microsphere for nasal drug delivery. From the experiments, it can be concluded that nasal microspheres of CA were prepared using Pectin and βCD. The FTIR was no interaction between polymers; they are compatible with each other. PDI and zeta potential were measured and the mean particle size and distribution of microspheres were in the range, Mucoadhesion, drug release, and entrapment efficiency were found to be fairly acceptable range. SEM studies indicate surface topography having spherical slightly rough surface of the formulation, DSC and XRD were recorded to see the drug status. In vitro show a significant effect on drug release. Stability studies revealed that optimized formulation was stable. Finally, it was concluded that the prepared nasal microspheres of CA may prove to be potential enough for effective drug delivery.

Keywords:- *Potentially Enough, Bioavailability, Entrapment Efficiency, Cefuroxime Axetil.*

1.Introduction:

Nasal drug delivery received a great attention as alternative way for systemically acting drugs that are difficult to deliver through other routes other than injections. The nasal mucosa provides rapid, non-invasive route for drug administration due to its highly perused tissues; permeable epithelial surface and rapid absorption resulting directly reach to the systemic circulation and due to possibility for by passing the blood–brain barrier and targeting the brain directly through drug absorption through olfactory mucosa Nanoparticles may provide improved targeting and transport through the nasal mucosa [1].

Nanoparticles are particles usually made from biocompatible, biodegradable polymers such as poly-D, L-lactic-co-glycolic acid (PLGA) with diameter <100 nm that can be used as drug carriers and they have abilities to bypass the various bio barriers due to their small sizes [2, 3,4].

PLGA degrades by the hydrolysis and converted to CO2 & H2O in the Krebs cycle and can deliver drug without causing long term damage or toxicity [5].

Nanotechnology is one of the effective methods used to improve solubility and dissolution behaviour of poorly soluble drugs and the same time mucosal routes of administration offer advantages as improved bioavailability of active pharmaceutical ingredients, possibility of targeting particular organs. Cefuroxime Axetil (CA) is selected as a model drug, a poor soluble, broad-spectrum, beta-lactamase stable cephalosporin antibiotic which undergoes enzymatic degradation in GI tract. It is used orally for the treatment of respiratory tract infections, pharyngitis, tonsillitis, skin infections, and many more diseases. In humans, GI absorption of CA is negligible and average bioavailability about 37%. When given orally, it goes hepatic first-pass metabolism, thereby reducing the bioavailability drastically. Therefore, CA missing the absorption site causing high concentration of antibiotic entering colon leading to colitis [6]. Administering CA through nasal route will avoid such undesirable functions such as first-pass metabolism and increase the bioavailability. Reaching efficacious site concentrations of antibiotics are essential and suppress the progressive resistance. The majority of lung infections and the site of infection are the epithelial lining fluid (ELF). Thus, reach to the ELF, antibiotics need to pass lung capillary into the interstitial space and move across the alveolar wall epithelium. The quantification of antibiotic concentrations in ELF during development of antibiotic agents for bronchial infection is considerable importance [7].

Well-designed drug delivery system can overcome some to the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. There are several such approaches are there but one such approach is using microspheres as a carrier for drugs. Microspheres based formulations can be formulated to provide a constant drug concentration in the systemic circulation or to efficacy site of cells or organs. There for by developing a model that can be used to provide effective alternative drug delivery of nasal drug targeting for upper and lower respiratory tract infections this study describes the intranasal mucosal drug delivery systems to achieve desired release profile, complete dissolution and also highlights the bioavailability to improve the effectiveness. The main objective of the present work is to formulation development, optimization, characterization, and evaluation of the nasal drug delivery for CA.

Nasal drug delivery, which is in the focus of this study, has received a significant attention in recent years as a convenient and reliable route, not only for local but also for the systemic administration of drugs [8].

2.Materials and Methods

2.1 Materials:

Cefuroxime Axetil (CA) was obtained from Ajanta Pharma Ltd. (Mumbai, India)Pectin (87% deactivated), Beta-Cyclodextrin, and liquid paraffin (light) purchased from Fisher scientific, Mumbai, India.Span 80, Glacial acetic acid, diethyl ether, and Isopropyl alcohol purchased from Merck; all other chemicals used were in analytical grade.

2.2 Methods:

2.2.1 Preparation of Microspheres by Modified:

2.2.1.1 Emulsion-Lyophilization Method:

CA containing microspheres were prepared by adding into previously water dispersed with beta-Cyclodextrin (βCD) of appropriate concentrations kept aside for 24h. Then, the solutions of drug (100mg) with βCD were added into Pectin solutions of different concentrations which were prepared accordingly. Then, the complete mix of drug, βCD, and Pectin solutions was drop wise poured into appropriate mixing speed using T 25 digital Ultra-Turrax dispersing instrument as per design experimentation of trials containing light liquid paraffin and surfactant. After stirring with design specification, resulting solutions were separated by repeated wash with solvents and filtered to remove insoluble ingredients followed by freeze-drying appropriately [9].

2.2.1.2 Experimental Design:

A 24-factorial design with four factors at two levels with centre point value was considered in this model which was selected to optimize the various response variables. Statistical design of experiments implemented by software design expert version 9.0.2.0 (Trial Version of Stat-Ease Inc., Minneapolis, USA). Experimental trials performed for nine formulations of possible combinations. In this model, four factors at two levels in coded with low and high settings (−1, & +1 one to- one) were considered for dependent variables; polymer concentration (F1), enhancer concentration (F2), mixing speed (F3), and freeze-drying temperature (F4) were selected as independent variables, and four responses as particle size (F1), % entrapment efficiency (F2), Muco-adhesion (F3), maximum drug release (F4) were measured for each trial and taken as dependent variables. 3D response surface graph is utilized to study of factor's interaction between the factor and responses. The factorial design parameters with respective formulations are drawn in Table 1. All the formulations variables and processing variables were kept constant during this model. In this model, analysis was carried out ANOVA calculation with parameters of analysis results of R2: Coefficient of regression, SD: Standard deviation, CV: Co-efficient of variation, SS: Sum of squares, DG: Degree of freedom, MS: Mean sum of squares, and f: Fisher's ratio. These results of variance of these observations pooled over all to get an estimate of pure error of variance [10].

2.2.1.3 Microsphere Characterization:

2.2.1.3.1 Percentage Yield:

The percentage yield of microspheres was determined as the percentage weight of dried final product (practical weight) with respect to theoretical weight of CA microspheres used [11].

2.2.1.3.2 Particle Size & Size Distribution & Polydispersity index (PDI):

The mean particle size was determined by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, and U.K). Samples after appropriate dilutions in Milli-Q water were taken for analysis. Particle size analysis for the formulations was carried out following proper dilutions in Milli-Q water at 25.1°C with equilibration time is 70s & eight attenuation PDI used to measure of broadness with M.W distribution [12].

2.2.1.3.3 Drug Entrapment Efficiency:

The microspheres (100 mg) loaded with CA were added in a mixture of 10 mL of phosphate buffer pH 6.2 & methanol (9:1) under stirring. The mixture was filtered and the amount of CA was determined spectrophotometrically at 277nm on UV spectrophotometer (Shimadzu UV 1800, Japan). Preliminary UV studies of system suitability adjustment for polymers present in the formulation were controlled to study the drug absorbance interference [13].

The percentage entrapment efficiency was calculated using Equation (1).

Entrapment efficiency = (Practical drug content/Theoretical drug content) x 100

2.2.1.3.4 *Ex-vivo* Mucoadhesion Studies:

The falling liquid film technique was followed to carry out Mucoadhesive property. A freshly cut piece, 5cm long, of sheep nasal mucosa obtained from a local abattoir within 2h of killing the animal was prepared by washing with isotonic saline solution. Accurately weighed number of microspheres was sprinkled on the nasal mucosa, which was attached over a glass slide. This glass slide was kept aside for 15min in a desiccator at 90% relative humidity to permit the polymer complex of microspheres to interact with the membrane and then position of stand changed to 45o angle. Previously heated (37±0.5°C) phosphate buffer pH 6.2 was allowed to flow over the microspheres present in membrane content concentration was determined Spectrophotometric method. The amount of microspheres equivalent of drug amount in perforate was calculated. The amount of retained microspheres drug amount was calculated from the difference among the applied microspheres and surged microspheres amount with percentage of Mucoadhesion strength [14].

2.2.1.3.5 Drug Diffusion Studies:

In vitro drug permeation test of the microspheres was performed using Franz diffusion cell of 140ml capacity. The semi permeable membrane molecular weight cut of 12–14 kDa was placed on mouth of the diffusion cell. The microsphere drug equivalent to 100mg taken in the donor compartment was incorporated in simulated nasal fluid (8.77g NaCl, 2.98g KCl & 0.59g CaCl2 with1 L). The receptor compartment was made with full volume capacity of phosphate buffer of pH 6.2, similarly to that of pH range of nasal cavity and maintained at 37±0.5°C. A magnetic stirrer was placed in the receptor compartment. One millilitre sample was periodically withdrawn and replaced with same amount of buffer solution during 6 h study. Appropriately diluted drug sample solutions were determine using UV–VIS Spectrophotometric method and 277nm as λmax. *Ex vivo* drug permeation was performed using afresh slice (~2.5 cm2) of goat nasal mucosa which was obtained from local slaughterhouse as membrane to place on the mouth of the Franz diffusion cell instead of semi permeable membrane as like experimentation of *in-vitro* drug diffusion study. The drug retained in goat nasal mucosa during drug release was adjusted for the calculation for optimal drug concentration determination.

2.2.1.3.6 Zeta Potential:

Electrophoresis light scattering was performed to attain the electrophoresis mobility of microspheres in using a Zetasizer Nano ZS (Mumbai). Measurements were carried out in eight runs at 25.1°C using water as a dispersant (refractive index: 1.59) in a clear disposable zeta cell [15].

2.2.1.3.7 Thermal Analysis:

2.2.1.3.7.1 FT-IR and Differential Scanning Calorimetric (DSC):

Pure drug and optimized microspheres were subjected to FTIR analysis (Model used to analysis, Bruker). Using a DSC Shimadzu DSC 60 Thermo grams concealed the range of 0°C-300°C with heating and cooling rates of 10°C/min. The melting point was observed on from endothermic peak of the DSC curve documented in the first Heating scan the glass transition temperatures were recorded from the second heating scan [16].

2.2.1.3.7.2 X-Ray diffraction (XRD) Studies:

X-ray diffraction studies were verified to Analyse the crystalline of pure drug and optimized formulation by DY 1042-Empyrean diffract meter/furnished with a Ni-filtered Cu Kα radiation (k=1.54060) with Gonio scan axis in the angle range of 100–500 at a speed of 50/min.

2.2.1.3.7.3 Surface Morphology:

2.2.1.3.7.3.1 SEM:

Surface morphology scanning electron microscopy was performed for pure drug and optimized formulation. By carry out at low accelerating voltage of about 15 kV with load current about 80 mA and working distance WD=9.1 mm using a standard error mean (SEM) (Model JSM 840 A, Joel, Japan).

2.2.1.3.8 Kinetics of Drug Release:

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s. t), first order [Log (Q0- Q) v/s. t], Higuchi's square root of time (Q v/s. t 1/2) and Koresmeyer Peppas double log plot (log Q v/s. log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0- Q) is the cumulative percentage of drug remaining after time t. In short, the results obtained from in vitro release studies were plotted in four kinetics models of data treatment as follows:-

- Cumulative percentage drug release Vs. Time (zero order rate kinetics)
- Cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation)
- Log cumulative percentage drug release Vs. log time (Korsmeyer Peppas equation)
- Log cumulative percentage drug remaining Vs. time (First order rate kinetics)

Kinetic analysis was performed and the data was evaluated after fitting to Zero order, First order, Higuchi, Peppas values observed where Regression co-efficient (R) and Diffusion exponent (n) value in case of Peppas model. Criteria for selecting most appropriate model were based on best reliability of fit indicated by 'R' value nearer to one. When drug release is concentration dependent, first order model is an indicator. Zero order models are independent of concentration of drug. Matrix model is applicable when matrix polymer is used and Peppas model is used when release mechanism is not well known Fickian diffusion exists when $n<0.5$, but at $n>0.5$ Non-fickian diffusion mechanism was observed

2.2.1.3.9 Accelerated Stability Studies:

The stability testing will assist the robustness of prepared microspheres to evident the quality of a drug encapsulated in microspheres with quality attributes drug product varies with age under the

various environmental factors influence such as temperature, humidity, and light as per the approved ICH guidelines. Stability studies were carried out on optimized microspheres according to ICH guidelines to ensure their shelf life. The optimized formulation was packed in amber colure glass vials closed with airtight closures and stored in a programmable environmental test chamber at 40°C and 75% RH for 6 months and evaluated at 1, 2 & 3months interval [17].

3. Result and Discussion

3.1 Preparation of Microspheres by Modified:

Table.3.1 Modified Microspheres

Ingredients (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Cefuroxime Axetil	100	100	100	100	100	100
Pectin	5	5	4	10	5	5
Beta-Cyclodextrin	6	5	7	5	8	7
Liquid Paraffin	4	2	4	5	6	4
Span 80	7	8	6	5	6	5
Isopropyl Alcohol	5	-	4	-	-	4
Glacial Acetic Acid	qs	-	-	-	-	-

3.2 Obtained Microsphere Characterization Data:

Table.3.2 Summary of Regression Analysis and ANOVA for Microspheres

Formulation	**Model**	**R^2**	**Standard Deviation**	**Coefficient Variation**	**Sum Square**	**Degree Freedom**	**Mean some Sq**	**Fisher's Ratio**	**P Value**	**MS**
F1	Linear	0.9862	0.135	0.9398	18.23	5	4.89	248.2	≤0.001	SG
F2	-	0.9175	1.39	1.65	101.52	5	24.59	16.42	0.0106	-
F3	-	0.9436	.7823	1.01	64.01	5	19.41	30.26	0.0032	-
F4	-	0.9572	1.19	1.52	131.29	5	29.35	21.15	0.0139	-
F5	-	0.9654	0.342	1.43	129.17	5	31.28	23.45	0.0134	-
F6	-	0.9432	1.23	1.11	130.23	5	32.24	20.54	0.0130	-

*MS – Model Significance, SG- Significant.

3.3 Drug Diffusion Studies:

In vitro drug permeation test of the microspheres was performed using Franz diffusion cell of 140ml capacity. The semi permeable membrane molecular weight cut of 12-14 kDa was placed on mouth of the diffusion cell. The microsphere drug equivalent to 100mg taken in the donor compartment was incorporated in simulated nasal fluid (8.77g NaCl, 2.98g KCl & 0.59g CaCl2 with 1L). The receptor compartment was made with full volume capacity of phosphate buffer of pH 6.2, similarly to that of pH range of nasal cavity and maintained at 37±0.5°C. A magnetic stirrer was placed in the receptor compartment. One millilitre sample was periodically withdrawn and replaced with same amount of buffer solution during 6hrs study. Appropriately diluted drug sample solutions were determine using UV-VIS Spectrophotometric method and 277nm as λmax. *Ex-vivo* drug permeation was performed using afresh slice (~2.5cm2) of goat nasal mucosa which was obtained from local slaughterhouse as membrane to place on the mouth of the Franz diffusion cell instead of semi permeable membrane as like experimentation of *in-vitro* drug diffusion study. The drug retained in goat nasal mucosa during drug release was adjusted for the calculation for optimal drug conc. determination.

3.4 DSC Study:

The pure drug and optimized formulation were investigated using a DSC Shimadzu DSC 60. Thermograms concealed the range of 0°C–160°C with heating and cooling rates of 10°C/min. The melting point was observed on from endothermic peak of the DSC curve documented in the first heating scan. The glass transition temperatures (Tg) were recorded from the second heating scan.

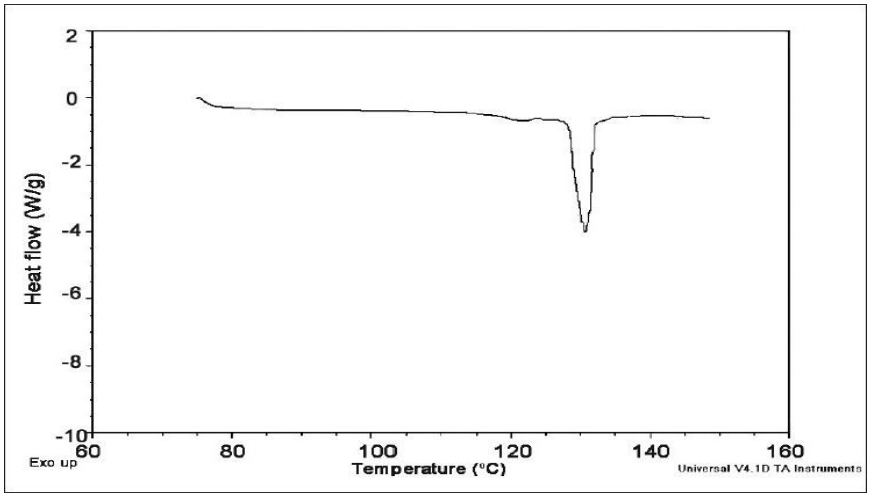

Fig.3.1 DSC Study Cefuroxime Axetil Microspheres

3.5 FTIR Study:

Pure drug and optimized microspheres were subjected to FTIR analysis (Model used to analysis, Bruker).The FTIR spectra of Cefuroxime Axetil, Pectin &Beta-Cyclodextrin. The objective behind studying the FTIR spectra of CA and excipients was to investigate any possible interactions between drug and lipid carriers. CA showed two absorption bands corresponding to carbonyl groups at 1678 cm-1 and 1680 cm1 assigned to amide and carbonyl group stretching.The peak at 1760 cm-1 is characterized for carbonyl group stretching in the vinyl ester group, and the absorption bands for NH and NH2 complex were seen from 3260 cm1 to 3480 cm-1. All major peaks of carbonyl stretching vibrations were present with less intensity. This is an indicative of presence of an interaction between

CA and excipients. Possibly the C=O group of CA must have formed hydrogen bonding with -OH groups of Gelucire lipids.

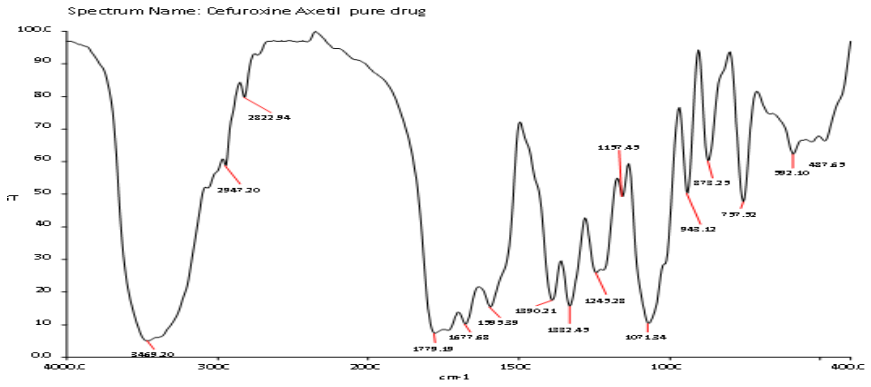

Fig.3.2 FTIR StudyCefuroxime Axetil

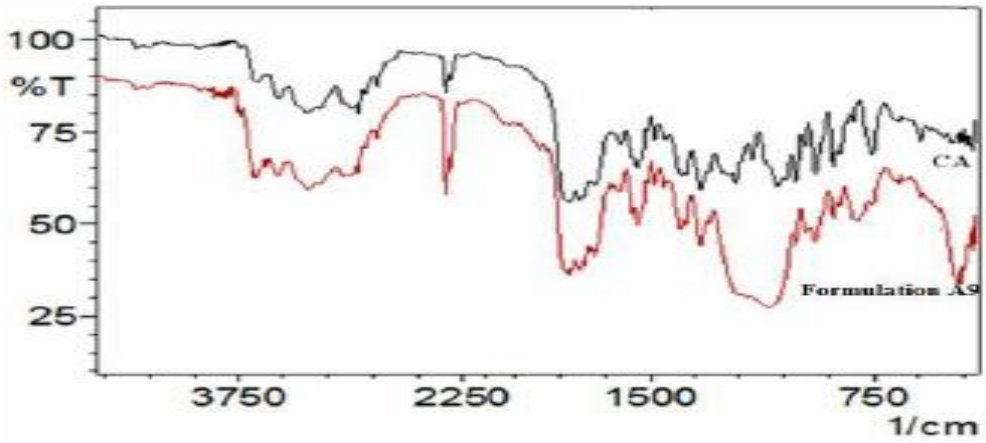

Fig.3.3 FTIR StudyCefuroxime Axetil & Pectin

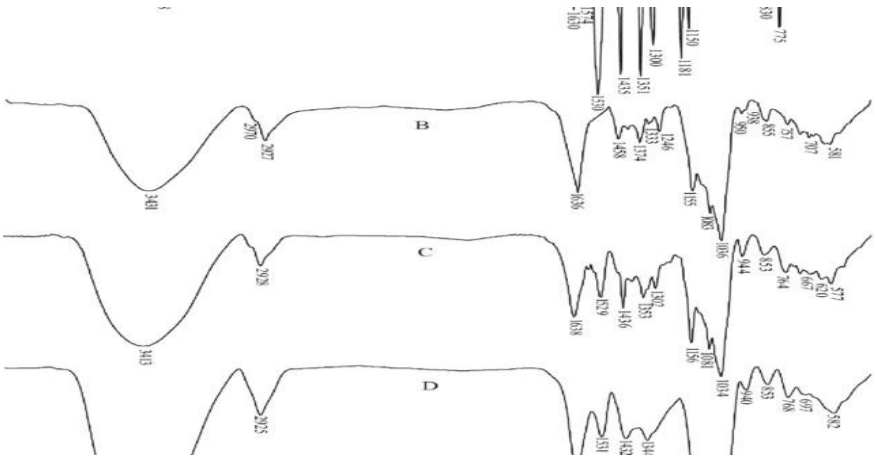

Fig.3.4 FTIR StudyCefuroxime Axetil & Beta- Cyclodextrin

3.6 Zeta potential

Electrophoretic light scattering was performed toattain the electrophoretic mobility of microspheres inusing a Zetasizer nano ZS (Malvern Instruments, UK).Measurements were carried out in eight runs at 25.1°Cusing water as a dispersant (refractive index: 1.59) in a
Clear disposable zeta cell.

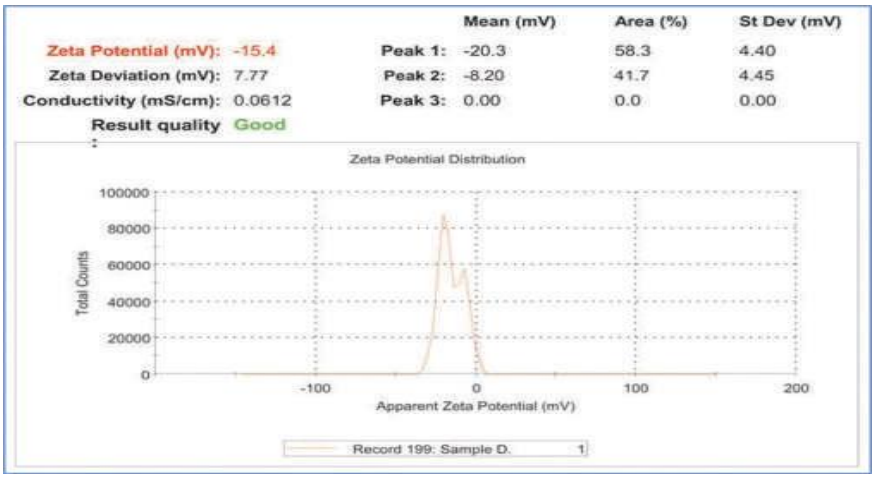

Fig.3.5 Zeta potential

3.7 X-Ray Diffraction Studies:

The diffraction spectra of Cefuroxime Axetil and urea show numerous distinct peaks indicating that both are present in a highly crystalline state. The XRD pattern of the solid dispersion of sample SD5 exhibits all the characteristic diffraction peaks of urea and crystalline Cefuroxime Axetil, although of lower intensity. This study reveals that some Cefuroxime Axetil still exists in the crystalline state in the solid dispersion.

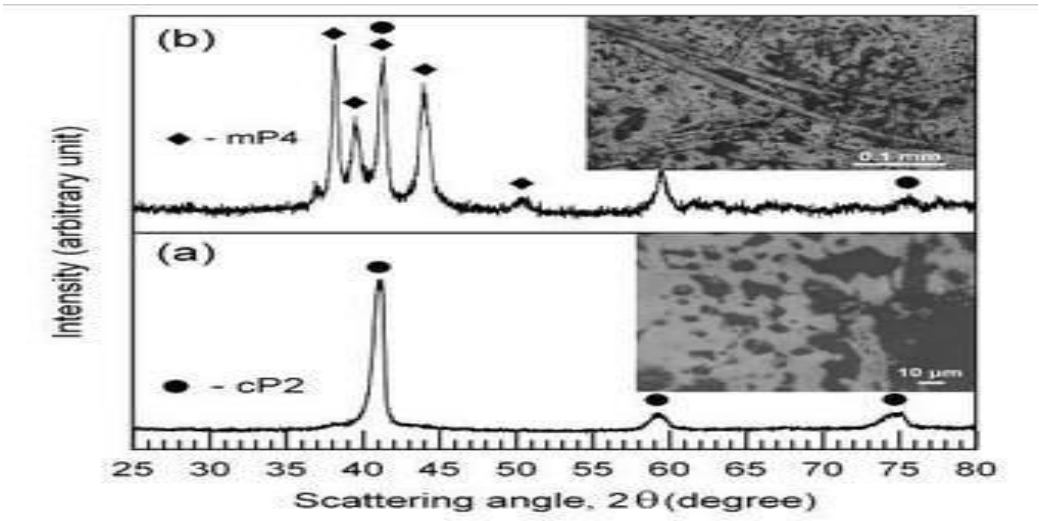

Figure. 3.6 XRD graph of (b) Cefuroxime Axetil pure drug &(a) optimized formulation of Cefuroxime Axetil

3.8 Surface morphology:
3.8.1 Scanning electron microscope (SEM):

Surface morphology scanning electron microscopy was performed for pure drug and optimized formulation. By carry out at low accelerating voltage of about 15 kV with load currentabout 80 mA and working distance WD = 9.1mm using a standard error mean (SEM) (Model JSM 840 A, Jeol, Japan).

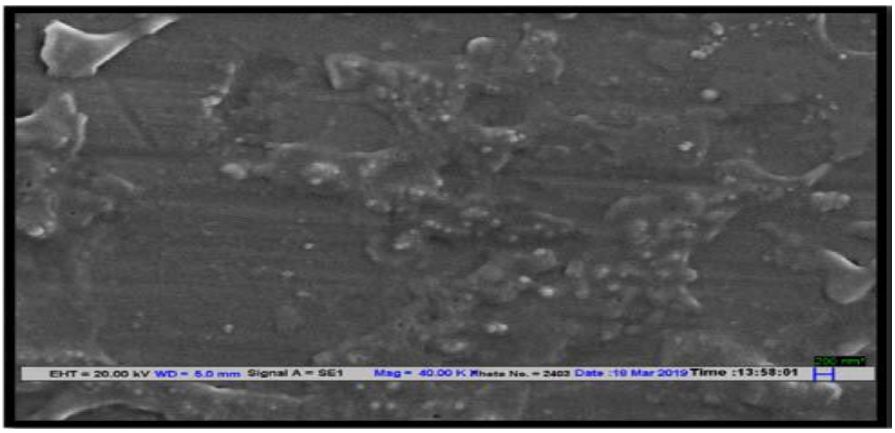

Figure.3.7 Surface morphology SEM of pure drug Cefuroxime Axetil formulation.

The SEM photomicrograph ofthe optimized formulation is shown in table. The surface ofthe microspheres is shown spherical in shape which representsdistinct pores in polymeric wall surface of microspheres, thismay enhance drug release from the microspheres in better wayand even this also indicate effect of lyophilisation process inpreparation of microspheres.

3.9 Optimized Characterization Data of Cefuroxime Axetil:

Table. 3.3 Characterization Data of Cefuroxime Axetil

Formulation Code	Particle size (µm)	Entrapment Efficiency (%)	Mucoadhesion (%)	Percentage Yield (%)
F1	16.33±0.13	81.21±0.12	81.71±0.17	77.87±0.19
F2	17.17±0.08	75.15±0.20	66.27±0.24	70.98±0.14
F3	15.41±0.17	82.83±0.19	70.13±0.16	68.89±0.23
F4	17.94±0.19	82.55±0.13	80.78±0.23	83.71±0.32
F5	13.08±0.15	79.29±0.27	71.16±0.19	69.49±0.34
F6	11.75±0.07	59.77±0.29	67.45±0.15	62.24±0.28

With increase in polymer concentration in the microspheres from F1 to F6, the particle size of microspheresincreased. This is because the viscosity and mucoadhesiveproperty complement the polymer solution increases withincreasing polymer concentration. The effect was elucidated. The entrapment efficiency was in the range of 59.77–82.83% [Table.3.3], increased efficiencywas observed with increased concentration of polymer and enhancerhence, the above result indicates that the factors play animportant role in the formulation of microspheres containingCA. By this optimization, factorial design model consists ofmaterial and process parameters as variable with responses, itwas observed that desirability conclusive report accordingly with control strategic parameters was suggested Particle size 11.75 to17.94µm, entrapment efficiency 59.77 to 82.55%, Mucoadhesion 66.27 to 80.78 %.

3.10 Percentage Drug Release of optimized formulation

Table.3.4 % Drug Release Formulation

Time	F1	F2	F3	F4	F5	F6
0.5	34.2±0.12	31.5±0.11	27.1±0.12	37.3±0.12	32.4±0.09	19.5±0.09
1	62.3±0.12	55.1±0.09	47.4±0.14	63.3±0.12	53.2±0.07	30.6±0.29
1.5	63.7±0.14	60.5±0.14	50.6±0.11	69.4±0.09	61.3±0.08	35.2±0.19
2	77.4±0.17	68.4±0.17	58.8 ±0.15	75.6±0.11	69.1±0.05	43.4±0.08
2.5	85.3±0.16	81.5±0.13	69.9±0.14	76.9±0.12	76.3±0.06	56.8±0.15
3	87.4±0.18	84.7±0.16	76.2±0.12	78.5±0.13	78.4±0.07	62.7±0.16
3.5	87.5±0.5	86.8±0.15	82.5±0.13	79.2±0.06	80.2±0.24	71.9±0.06
4	87.6±0.3	86.9±0.6	85.9±0.14	80.7±0.09	83.3±0.07	88.3±0.14
4.5	87.7±0.4	87.1±0.9	86.4±0.5	83.4±0.8	84.4±0.8	90.1±0.03
5	88.2±0.6	90.3±0.10	91.4±0.17	88.7±0.14	87.5±0.12	91.3±0.6
5.5	91.7±0.10	93.9±0.09	94.3±0.11	95.6±0.10	89.4±0.5	93.5±0.7
6	93.4±0.09	94.3±0.17	96.7±0.4	97.9±0.6	91.5±0.15	93.7±0.3

As the releasestudy indicates a little effect of enhancer on drug release, thedecrease in crystallinity may be attributed to the formation ofsmall amounts of complexes.

3.11 Stability Studies:

Table.3.5 Stability Studies of F4

	F4			
Time in months	Particle size (μm)	Entrapment efficiency (%)	Mucoadhesion (%)	Maximum drug release (%)
Initial	14.31±0.05	78.41±0.21	77.15±0.18	80.62±0.28
1	14.33±0.08	78.39±0.24	77.14±0.09	80.58±0.24
2	14.29±0.20	78.40±0.23	77.14±0.12	80.55±0.30
3	14.26±0.15	78.48±0.29	77.12±0.21	80.53±0.26

Discussion: -

The duration of stability studies of the Formulation 4, there is change in Particle Size, but the variation of an Entrapment Efficiency, Mucoadhesion and Maximum Drug Release.

Conclusion

The present study has been a satisfactory attempt to formulate a CA microsphere for nasal drug delivery. From the experiments, it can be concluded that nasal microspheres of CA were prepared using Pectin and βCD. The FTIR was no interaction between polymers; they are compatible with each other. PDI and zeta potential were measured and the mean particle size and distribution of microspheres were in the range, Mucoadhesion, drug release, and entrapment efficiency were found to be fairly acceptable range. SEM studies indicate surface topography having spherical slightly rough surface of the formulation, DSC and XRD were recorded to see the drug status. In vitro show a significant effect on drug release. Stability studies revealed that optimized formulation was stable. Finally, it was concluded that the prepared nasal microspheres of CA may prove to be potential enough for effective drug delivery.

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