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PREPARATION AND *IN-VITRO* CHARACTERIZATION OF ORAL SUSTAINED RELEASE CHITOSAN COATED CEFEPIME HYDROCHLORIDE MICROSPHERES

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ABSTRACT: Chitosan is a cationic hydrophilic polysaccharide widely used as mucoadhesive polysaccharides. Chitosan microspheres have been reported to provide controlled release of many drugs and these microspheres are being investigated for both for parenteral and oral drug delivery. In the present study chitosan microspheres of cefepime hydrochloride as a model drug were prepared using glutarldehyde as crosslinkng agent and effect of concentration of chitosan was studied on entrapment efficiency, mucoadhesion, water sorption and drug release. Prepared microspheres were whitish and spherical shape. The obtained microspheres were whitish with spherical in shape, surface is smooth and showed cross linking by physical observation. The results showed that as concentration of chitosan increases entrapment efficiency, percent water sorption, mucoadhesion increases and drug release decreases which may be due to increases in drug holding capacity with increases in chitosan concentration.
KEYWORDS: Chitosan, Microspheres, Cefepime hydrochloride.

INTRODUCTION

Chitosan is a widely used mucoadhesive polymer. It is a cationic hydrophilic polysaccharide comprising copolymers of glycosamine and N-acetylglucosamine and can be derived by partial deacetylation of chitin from crustacean shells.[1] Chitosan microspheres have been reported to provide controlled release of many drugs and these microspheres are being investigated for both for parenteral and oral drug delivery. [2] Chitosan is a polymer of choice, because it enhances the nasal absorption of low molecular weight molecules as well as peptides and proteins.[3] It is a mucoadhesive polymer having advantages as nontoxicity, biocompatibility and biodegradability.[4] Chitosan were studied as a new class of polymers for vaccination and proved to be promising candidates.[5] Mucoadhesive polymers may fulfill the desirable features of a prolonged residence time at the site of drug absorption owing to increased contact with the absorbing mucosa, resulting in a steep concentration gradient to favor drug absorption, and localization in specified regions to improve and enhance the bioavailability of the drug.[6] Chitosan obtained by deacetylation of chitin (a naturally occurring polymer) has been shown to possess mucoadhesive properties owing to the molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces.[7] Chitosan has 1primary amino and 2 free hydroxyl groups for each C6 building unit. Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus, in turn, reacts with many negatively charged surfaces/polymers.[8] Chitosan microspheres can be prepared by various methods such as cross-linking with anions,[9] precipitation,[10] complex-coacervation,[11] modified emulsification and ionotropic

gelation,[12] precipitation- chemical cross-linking,[13] glutaraldehyde cross-linking,[14] thermal cross-linking,[15] and more. The cross-linking of polymers affects the mucoadhesive strength of the microspheres. In the present study chitosan microspheres of cefepime hydrochloride as a model drug were prepared using glutarldehyde as crosslinkng agent and effect of concentration of chitosan was studied on entrapment efficiency, mucoadhesion, water sorption and drug release.

MATERIALS AND METHODS

Materials:

Chitosan was obtained as gift sample from Quingdao Jiaonan Bright Moon Seaweed Industrial Co. Ltd. Cefepime hydrochloride was obtained as gift sample from Dr. Reddy's Laboratory Ltd., Hyderabad (India). All other chemicals used of analytical grade were purchased from Loba Chemicals Pvt. Ltd., Mumbai (India).

Methods:

1. Preparation of chitosan microspheres:

The desired amount of chitosan was dissolved in 25 ml 2 % acetic acid at room temperature with stirring. Then required amount of cefepime hydrochloride were dissolved in 2 ml of distilled water separately. The drug solution was added to the chitosan solution under stirring and mixed to get an emulsion. The prepared emulsion was added drop wise to 500 ml light liquid paraffin containing 2 % w/v glutaraldehyde and stirred at 600 rpm. The emulsion was stirred at room temperature for 1 hour, at 35 °C for 5 hours and finally 57° C for 1 hour during which microsphere were formed. Prepared microspheres were added slowly to 250 ml freshly prepared double solvent layers of n-hexane/2 M sodium hydroxide (2:3 v/v). The residue was washed with n-hexane warmed at 55°C to yield chitosan coated cefepime hydrochloride microspheres. After 1 hour solution was filtered using whatman's No. 1 filter paper. The formulation quantities were as given in table 1. Diameters of the dried microspheres were measured by optical microscopy.

2. Evaluation of microspheres:

1. Percent yield and entrapment efficiency:

Prepared microspheres were weighed after drying, and percent yield was calculated using following formula

Percentage Yield = (Actual weight x 100)/ Theoretical Weight (1)

Microspheres of known weights were stopper tightly in a flask containing 50 ml of 6.8 pH phosphate buffer. The flasks were shaken using orbital shaker for 48 hours to break the beads completely. After 48 hours the solution was filtered using whatman's No. 1 filter paper and the filtrate was centrifuged using a tabletop centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was then analyzed for cefepime hydrochloride content by a UV spectrophotometer (JASCO-V500, Japan) at the λ max value of 258.4 nm. The complete extraction of drug was confirmed by repeating the extraction process on the already extracted polymeric debris. The % entrapment efficiency of the matrix was then calculated as

% Entrapment efficiency = (Drug loading / Theoretical drug loading) x 100. ……(2)

2. Water sorption study:

The microspheres were put on 10 cm diameter wet filter paper disc soaked in purified water in a petri dish at 25 ± 1°C for 48 hours during this interval the microparticles reached equilibrium and exhibited swelling. The swollen microparticles were quickly removed and weighted. The percentage water sorption at the end of 1 hour, 24 hours and 48 hours was calculated as per following formula

% water sorption = [(W1 – W2) / W2] x 100…..(3)

Where Ws = Weight of swollen microspheres and Wd = Weight of dry microspheres.

3. Scanning Electron Microscopy (SEM):

Beads were coated with a thin gold-palladium layer by sputter coater unit (VG- Microtech, United Kingdom), and the surface topography was analyzed with a Cambridge Stereoscan S120 scanning electron microscope (SEM; Cambridge, United Kingdom) operated at an acceleration voltage of 10 kV.

4. Mucoadhesion study:

The mucoadhesive microspheres were placed on goat intestine, collected from local market, which was cleaned using phosphate buffer pH 6.8. Then the intestine was mounted on glass slide. The entire system was attached to disintegration apparatus so that it can move up and down in the beaker of disintegration apparatus containing 500 ml phosphate buffer pH 6.8. After different time intervals the microspheres adhere to the goat small intestine was counted.

5. In vitro dissolution study:

Preparation of calibration curve: Different concentrations of cefepime hydrochloride (1 to 100 µg/ml) were prepared from stock solution and absorbance is measured by UV- visible spectrophotometer (JASCO-V500, Japan) at the λ max value of 258.4 nm. Calibration curve was plotted to determine R^2 and the equation of straight line which is used to calculate drug release. The dissolution studies were performed by using USP 26 type II dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). Dissolution medium used were phosphate buffer (pH

6.8), each 900 mL, temperature was maintained at 37 ± 2°C and 100 rpm stirring was provided for each dissolution study. Drug-loaded chitosan microspheres equivalent to 100 mg of pure drug were used for each dissolution study. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through Whatman filter paper 41, concentration of cefepime hydrochloride was determined spectrophotometrically at 258.4 nm. Dissolution study of pure cefepime hydrochloride was determined in the same manner as given above.

RESULTS AND DISCUSSION

1. Preparation of chitosan microspheres:

The Chitosan coated cefepime hydrochloride microspheres prepared by emulsion dry-in-oil method. During optimization of the process various parameters were tried. It was found that for 0-1% w/v of glutaraldehyde concentration there were no formation of microspheres. For more than 2% w/v glutaraldehyde concentration it was observed that drug particles were precipitated and aggregated masses of particles were formed. At 2% w/v glutaraldehyde concentration desired microspheres were formed. For less than 600 rpm speed drug got the tendency to settle down on the bottom and at more than 600 rpm speed high turbulence was observed with adhesion of particles to the surface of container. The preparations were stirred at room temperature for 1 hour, at 35 °C for 5 hours and for 57°C for 1 hour. If the temperature is more than 60°C the products were precipitated and separated from liquid paraffin. Also it was observed that 2M sodium hydroxide is required to give solidity for the chitosan coated microspheres. It was necessary to wash off excess glutarldehyed from the surface of the microspheres or neutralized it by using 2M sodium hydroxide. But the higher concentration of sodium hydroxide has hardened the surface of microspheres. The prepared microspheres were spherical with smooth surface as given in Fig. 2. Sizes of microspheres were as given in Table 2. It was observed that as concentration of chitosan increases size of microspheres also increases, it may be due to increase in adhesion of cellulose in formed emulsion.

2. Evaluation of microspheres:

1. Percent yield and entrapment efficiency:

The percentage yield and percentage entrapment efficiency of chitosan coated cefepime hydrochloride microspheres were as given in Table 2. It was observed that there was no significant difference in the percent yield for all formulations but as concentration of chitosan increases percentage entrapment efficiency of cefepime hydrochloride increases. It may be because with increase in chitosan concentration drug holding capacity increases which leads to decrease in drug leaking from microspheres.

2. Water sorption study:

Swelling is a consequence of the affinity of polymeric components for water. Percentage water sorption was as given in table 3. It was observed that as time and concentration increases percentage water sorption increases. It may be due to increase in swelling capacity of the polymer with time and concentration.

3. Scanning Electron Microscopy (SEM):

SEM of microspheres was as given in Fig. 1. It showed that the microspheres were whitish with spherical in shape. The surface is smooth and showed cross linking by physical observation. The SEM after dissolution showed that microspheres were porous and the spot indicates the drug leaking from the microspheres.

4. Mucoadhesion study:

In mucoadhesion study at different time intervals the microspheres adhere to the goat small intestine was counted and the values were as given in Fig. 2. It was observed that as time increases mucoadhesion capacity decrease which was shown by decrease in adhered particles. Also as concentration of chitosan increases mucoadhesion increases it may be due to increase in mucoadhesion capacity.

5. In vitro dissolution study:

The calibration curve generated using standard solution gives R2 value 0.998, the linearity was observed in the range of 5 to 60 µg/ml and the equation of the line is $y = 0.0248 x + 0.0029$. The percentage drug release was as given in Fig. 3. It was observed that for pure cefepime hydrochloride within 15 minutes 95 % drug was released. The microspheres showed sustained drug release. The sustaining order was F3>F2> F1. It showed that as concentration of chitosan increases drug release decreases. It revealed that increase in concentration retards the drug leaking from microsphere.

CONCLUSION

The desired chitosan coated cefepime hydrochloride microspheres prepared by emulsion dry-in-oil method were optimized at 2% w/v glutaraldehyde concentration at 600 rpm stirred at room temperature for 1 hour, at 35 °C for 5 hours and for 57°C for 1 hour. It showed that the microspheres were whitish with spherical in shape. The surface is smooth and showed cross linking by physical observation. It was revealed that as concentration of chitosan increases entrapment efficiency, percent water sorption, mucoadhesion increases and drug release decreases which may be due to increases in drug holding capacity with increases in chitosan concentration.

Table 1: Formulation codes with quantities.

Formulation Code	Ratio	Drug (g)	Chitosan (g)
F1	1 : 1	0.5	0.5
F2	1 : 2	0.5	1.0
F3	1 : 3	0.5	1.5

Table 2: Mean particle diameter (MPD), percentage yield and percentage entrapment efficiency (EE) of chitosan coated cefepime hydrochloride microspheres.

Formulation Code	MPD (µm)	Yield (%)	EE (%)
F1	10.4 ± 1.2	91 ± 2	83 ± 2.8
F2	15.4 ± 2.1	91 ± 1	90 ± 1.6
F3	20.3 ± 1.7	92 ± 2	93 ± 2.3

Table 3: Percentage water sorption of chitosan coated cefepime hydrochloride microspheres at 1, 24 and 48 hours.

Formulation Code	Percentage water sorption		
	After 1 hour	After 24 hours	After 48 hours
F1	106 ± 9	228 ± 4	250 ± 5
F2	138 ± 8	232 ± 6	360 ± 7
F3	165 ± 6	269 ± 5	390 ± 4

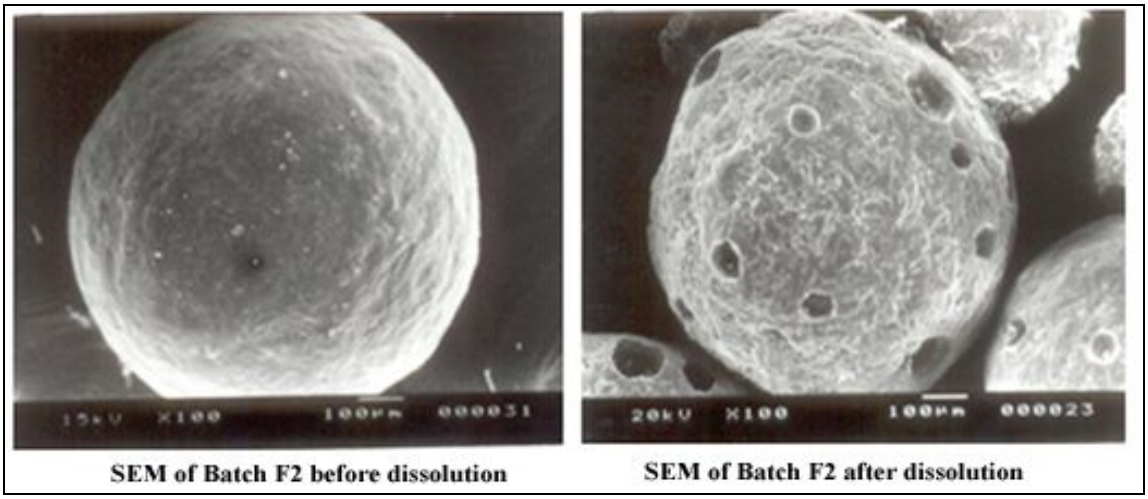

Fig. 1: Scanning Electron Microscopy of chitosan coated cefepime hydrochloride microspheres Batch F2.

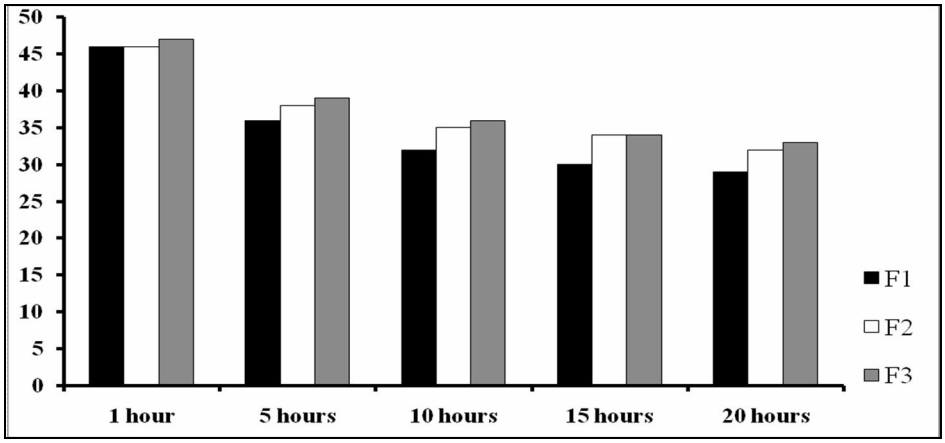

Fig. 2: Mucoadhesion study of chitosan coated cefepime hydrochloride microspheres.

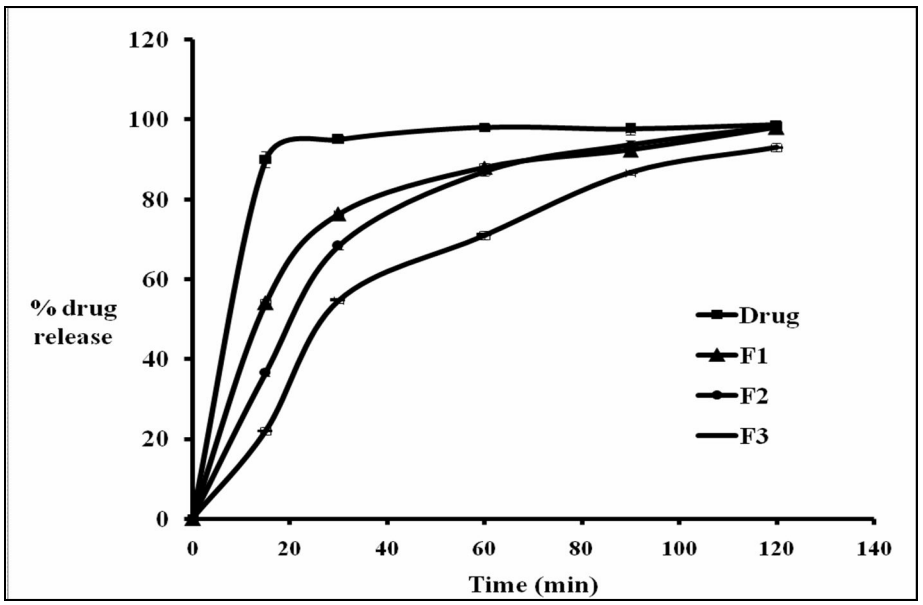

Fig. 3: Percentage drug release of cefepime hydrochloride through chitosan coated microspheres.

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