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Research Article

FORMULATION AND EVALUATION OF ORAL MULTI DRUG DELIVERY SYSTEM OF CEFTRIAXONE SODIUM BY USING INTESTINAL ABSORPTION ENHANCERS

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ABSTRACT

A great number of currently available drugs and those being developed that fall under the class III of the BCS (biopharmaceutical classification system) possess high therapeutic potential but suffers from serious drawback of very low bioavailability on peroral administration because of poor permeation across GIT epithelia. The present study attempts to increase the intestinal permeability of ceftriaxone sodium (as a model drug) by using intestinal absorption enhancers viz., sodium taurocholate, sodium deoxycholate, Polyoxyethylene ether and oleic acid. Blend of drug, permeation enhancer and other excipients were incorporated into beads. The results indicated that there was significant improvement in the permeability of the drug and the extent of enhancement was highly dependent on the absorption enhancer's species used. The beads were evaluated in both, gastric and intestinal pH. Release of drug from the beads in both the release media was found to occur predominantly by diffusion mechanism and was higher and more rapid in intestinal pH than in gastric pH.

Keywords: Intestinal permeability, Permeation enhancers, Beads, Ceftriaxone sodium, Bile salts, Polyoxyethylene ether

INTRODUCTION

Most of the cephalosporins can not be administered orally. The absorption limiting barriers of these drugs are likely to be located in the mucous layer, the apical cell membrane and the tight junctions. Surfactants like Polyoxyethylene ethers were reported to enhance gastric or rectal absorption of Penicillins and cephalosporins. In general, the non ionic surfactants exert benign effects on the membrane structure, in comparison with cationic surfactants and anionic surfactants. Beskid et al demonstrated an enhancing effect of glyceryl-1-monooctanoate on cephalosporin absorption after oral, intraduodenal and rectal delivery in various animal species. In the large intestine of rat, mixed micelles containing sodium taurocholate and glyceryl monooleate / oleic acid promoted the absorption of cephalosporins[1-5]. In the present study, ceftriaxone was selected as a hydrophilic model drug. One possible way to solve the permeability problem is to formulate the compound with membrane absorption enhancer excipients[6]. The effect of absorption enhancers on the lipophilicity of the model drug was investigated using the n-octanol/buffer system.

MATERIALS AND METHODS

Materials

Ceftriaxone sodium (Aurobindo Pharma Pvt. Ltd Hyderabad), polyoxyethylene 20 cetyl ether (Sigma Aldrich, Germany), sodium taurocholate and sodium deoxycholate (Loba Chemie Pvt, Ltd, Mumbai) were used in this study. All other reagents were of analytical grade and used as such.

Determination of partition coefficients of drug with & without absorption enhancers

The partition coefficient (n-octanol/pH-7.4 phosphate buffer) for ceftriaxone sodium was determined in different molar ratios with and without absorption enhancers. The drug content was analyzed spectrophotometrically at λ_{max} 241.0 nm. The partition coefficient was calculated using the following equation[6, 7].

$$P_{o/w} = a_o / a_b$$

Where a_o and a_b are the concentrations of drug in n-octanol and buffer respectively.

Ceftriaxone sodium being a hydrophilic drug exhibited very small $P_{o/w}$ value (0.026). However, the combination with absorption enhancers leads to improvement in the $P_{o/w}$. It was observed that the partition coefficient values, owing to many polar groups did not increase significantly with all used absorption enhancers. Hence FP23, formulations P2 and P6 were selected for further studies. The partition coefficient values are given in Table 1 & Table 2.

Table 1: Partition coefficient of drug with mixed micelles of sodium taurocholate & oleic acid

Drug : S.T : OA	$P_{o/w}$	Formulation code
1:3:0.5	0.177	P1
1:3:1	0.507	P2
1:3:1.5	0.390	P3
1:3:2	0.125	P4

Table 2: Partition coefficient of drug with mixed micelles of brij 58 & oleic acid

Drug : Brij 58 : OA	$P_{o/w}$	Formulation code
1 : 0.08 : 0.5	0.107	P5
1 : 0.08 : 1	0.121	P6
1 : 0.08 : 1.5	0.130	P7
1 : 0.08 : 2	0.093	P8
1 : 0.08 : 2.5	0.099	P9

Preparation of beads

Ionotropic gelation method was used for the preparation of beads, in which sodium alginate is dispersed in water with gentle stirring at room temperature and left to stand for 24 hrs in order to attain maximum hydration [8,9,10]. Then mixture of the drug and absorption enhancer is mixed with the sodium alginate dispersion. This dispersion is extruded drop wise in to zinc chloride solution with constant stirring by a magnetic stirrer to prepare beads. The beads are finally washed with water and dried in air for 24 hr. The parameters to be optimized are Sodium alginate concentration (w/v), Drug-polymer ratio (mM), Concentration of zinc chloride (mM), and Curing time (min). The formula for beads is given in Table 3.

Size and morphology of beads

The diameter of beads was determined by screw gauge. For this purpose, 20 dried beads were randomly selected from each batch and then mean diameter was determined. Color and shape of beads of each batch was noted[11,12].

Determination of drug content and entrapment efficiency of beads

The drug content was determined by dispersing beads (equivalent to 10 mg of drug) into 100 ml phosphate buffer (pH

7.4) with stirring for 24 hrs. The dispersion was filtered and the drug content was determined spectrophotometrically at absorption maxima of 241.0 nm[12]. Similarly the amount of the drug that diffused into the zinc chloride solution during hardening of beads was also determined, immediately after isolation of beads.

Table 3: Formulation of beads with permeation enhancer

Formulation code	Concentration of polymer	Drug : P.E.	Drug : polymer	$Zncl_2$ (mM)	Curing time (min)
J_1	5	*1:3:1	0.5:1	0.4	2
J_2	5	€1:0.08:1.5	0.5:1	0.4	2

*Drug : Sodium taurocholate : Oleic acid, €Drug : Brij 58 : Oleic acid

The entrapment efficiency was calculated according to the following relationship[13]

$$E.E = \frac{\text{Percentage drug content} \times \text{amount of dried beads produced}}{\text{Amount of drug added - Amount of drug remaining in apparatus}}$$

Swelling studies

Beads were studied for swelling characteristics, only those batches were selected which have good drug content and entrapment efficiency more than 50%. Dry, ionically cross linked beads increases their volume after minutes in water or in buffers with different pH and composition, due to matrix rehydration in accordance with the degree of crosslinking[14,15].

The initial weight of beads was recorded and placed in 100 ml phosphate buffer pH 7.4, shaken and allowed to swell for 8 hrs (After 10 h total breakdown of gel structure takes place). The temperature of medium was maintained at 37±2°C. The agitation ensures three dimensional water penetration and swelling. The swollen beads were carefully removed blotted dry and weighed. Water sorption was calculated from the difference between the initial and final weight of the beads. The swelling experiment was further repeated using HCl buffer pH 1.2 as a swelling medium. Swelling ratio was calculated as per the following formula[13].

$$\text{Swelling ratio} = \frac{\text{Weight of wet beads}}{\text{Weight of dried beads}}$$

In vitro drug release of beads

The USP dissolution apparatus was used in this study. The release medium consisted of 900 ml of pH-7.4 buffer maintained at 37±0.5 °C. A known quantity from each batch of the ceftriaxone sodium loaded beads were placed in appropriate chamber of the release apparatus and agitated at 100 rpm. At predetermined time intervals, 1ml of the release medium was withdrawn, appropriately diluted and absorbance determined at a wavelength of 241 nm using digital UV spectrophotometer. The volume of the release medium was kept constant by replacing it with 1 ml of fresh buffer after each withdrawal. The release study was repeated using pH 1.2 buffer as a release medium and the absorbance determined at a wavelength of 265 nm[16].

Permeability studies for the beads

Permeation study was performed using excised animal intestinal tissue in the Franz diffusion cell. The effective permeation area of the intestinal epithelium was 1.54 cm^2. Transport medium was Hank's Balanced Salt Solution (HBSS-pH-7.4). Beads were crushed and dispersed in HBSS (pH 7.4). 2.5 ml of the dispersion (approx. 2mg/ml) was placed in donor compartment and 18.5 ml of the buffer were filled into the acceptor compartment. The acceptor medium was continuously stirred and the experiment was performed at 37±2°C. Samples were periodically removed from the acceptor compartment over 4 h. The volume of the acceptor compartment kept constant by adding fresh HBSS after each withdrawal [17,18,19] Samples were appropriately diluted and absorbance determined at a wavelength of 241.0 nm.

HBSS Buffer pH-7.4: Calcium chloride (1.67 mM), Magnesium sulphate (0.812 mM), Potassium chloride (5.37 mM), Potassium phosphate monobasic (0.44 mM), Sodium bicarbonate (0.42 mM), Sodium chloride (136.89 mM), Sodium phosphate dibasic (0.34 mM), D-Glucose (5.55 mM). Adjust the pH by Sodium hydroxide solution.

Calculation of permeability coefficient / apparent permeability coefficient:

The apparent permeability (P_{app}) (cm/sec) was calculated by using the following relation[20]

$$P_{app} = \left(\frac{V_A}{\text{Area} \times \text{time}}\right) \times \left(\frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{Initial, donor}}}\right)$$

Where V_A is the volume (in mL) in the acceptor well (18.5ml), Area is the surface area of the intestinal membrane (1.54 cm^2), and time is the total transport time in seconds.

RESULTS AND DISCUSSION

Ceftriaxone sodium alone shows a small $P_{o/w}$ of 0.026, but with permeation enhancers it exceeds to 0.507. Ceftriaxone sodium (CTZ) alone exhibited limited absorption via the lipid membranes (biological membranes). The combination with permeation enhancers leads to a permeation rate of Ceftriaxone from 599.92 to 1970.68 µg/cm^2 and a permeation coefficient ranging from $1.55\times10^{-4}$to 5.05×10^{-4}cm/sec (Table 4).

Table 4: Permeability coefficient of selected batches

Formulation code	P_{app}(cm/sec)
CTZ	1.55×10^{-4}
P 1	3.86×10^{-4}
P 2	5.05×10^{-4}
P 3	4.14×10^{-4}
P 4	3.08×10^{-4}
P 6	3.01×10^{-4}
P 7	3.45×10^{-4}

CTZ-Ceftriaxone Sodium

In the in vitro transport model with intestinal epithelium, it turns out that the largest permeation rate of drug was reached using mixed micelle of sodium taurocholate and oleic acid after 4 h (Fig. 1).

The experiment using the in vitro model with biological membrane shows an enriching of ceftriaxone in the membrane through mixed micelles of sodium taurocholate-oleic acid and Brij58-oleic acid. The results with sodium taurocholate-oleic acid confirmed that the optimal effect was obtained through the combination with sodium taurocholate and oleic acid in the ratio of 3:1.5. The other absorption enhancers did not significantly influence the partition coefficient and permeability of ceftriaxone sodium.

The surface morphology of the alginate beads was investigated by scanning electron microscopy. [Preeti V. Kulkarni , Jathi Keshavayya, Chitosin- Sodium alginate biodegradable interpenetrating Polymer network (IPN) Beads for delivery Ofloxacin Hydrochloride. International Journal of Pharmacy and Pharmaceutical Sciences, 2010;2 (2):77-82.] Spherical shape of beads were obtained (Fig. 2) when the polymer concentration was high i.e. 5% w/v. Size of beads varies from 1.212 mm to 1.398 mm. Color of beads in solution was white, but after drying it changed in to yellowish brown.

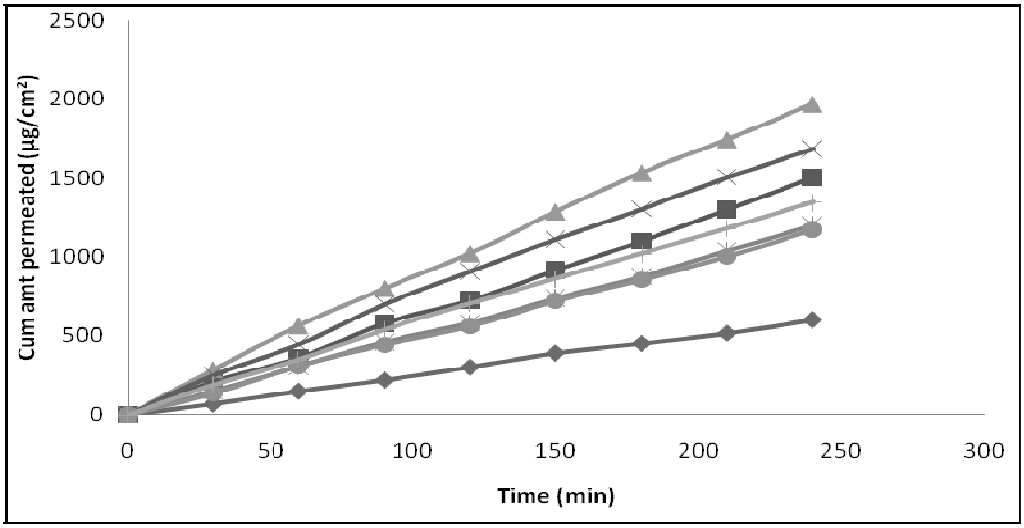

Fig. 1: Permeation rate of ceftriaxone sodium (µg/cm²) using biological membrane

Ceftriaxone sodium alone ( ); Formulation P1 ( ); Formulation P2 ( ); Formulation P3 ( ); Formulation P4 ( ); Formulation P6 ( ); & Formulation P7 ( )

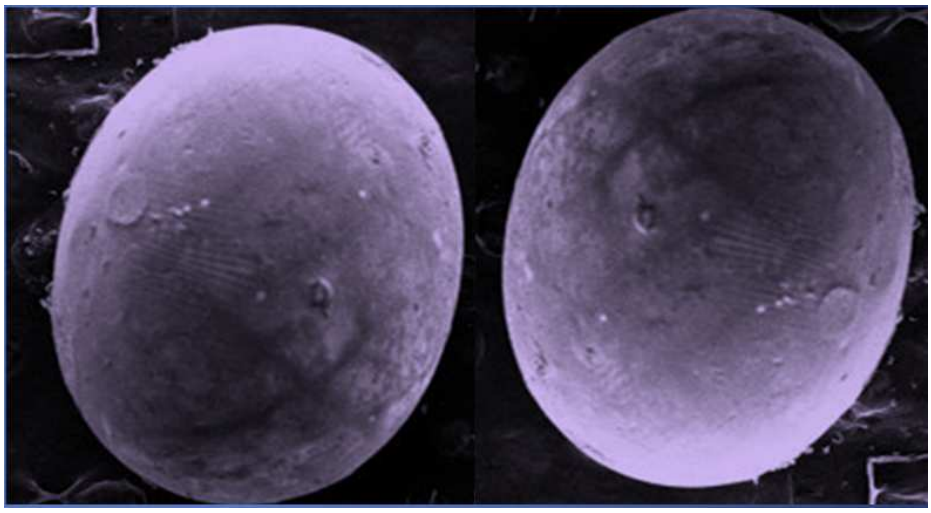

Fig. 2: Surface Morphology of bead

There were various factors, which were responsible for the variation in drug content and entrapment efficiency of each batch .These factors include curing time, drug: polymer ratio, Zinc chloride concentration. Drug content and entrapment efficiency of optimized formulations J1and J2 were 39.9%, 76.02% and 39.0%, 77.9% respectively.

Optimized curing time was 2 min, drug polymer ratio is 0.5:1.0, and Zinc chloride concentration was 0.4 mM. Swelling behavior of beads in buffer pH 7.4 and pH 1.2 were shown in table 5.

Being electrolyte, alginate exhibits swelling behavior that is sensitive to the pH, ionic strength and specific ionic concentration of the medium. The dried beads swelled after being placed in the medium (HCl buffer pH-1.2 and phosphate buffer pH-7.4). Low swelling of alginate coats in acidic media was probably due to Hydrogen–Zinc ion exchange forming insoluble alginic acid regions and followed by solvent penetration into the gel network. In phosphate buffer pH-7.4 beads swelled more extensively than in acidic media. Zinc was remaining in the swollen beads till 8 h which prevent total breakdown of the gel structure. Maximum swelling takes place at 8 h. Swollen beads could maintain its integrity for 10 h, after 10 h complete breakdown of beads structure was occurred.

The release profile of ceftriaxone sodium from the beads in two different release media (pH-1.2 and pH-7.4) is shown in fig 3. Low release of ceftriaxone sodium in pH-1.2 could possibly be a result of the limited swelling of the beads in the acidic medium.

Table 5: Swelling behavior of beads in buffer ph 7.4 and ph 1.2

Formulation code	Swelling ratio in pH 7.4				Swelling ratio in pH 1.2			
	2h	4h	6h	8h	2h	4h	6h	8h
J1	2.1	2.62	3.34	3.96	0.98	1.0	1.01	1.1
J2	2.31	2.88	3.21	3.56	0.95	0.98	1.01	1.08

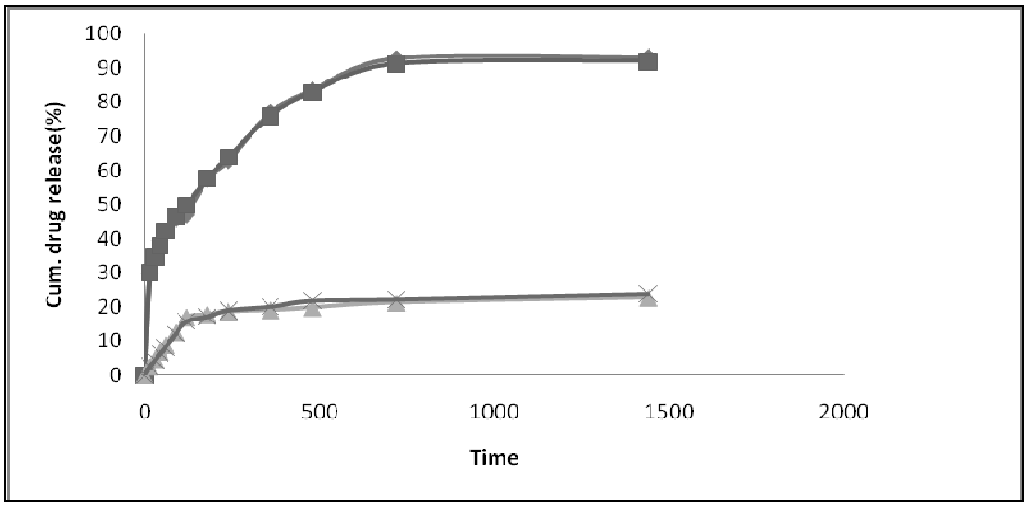

Fig. 3: In vitro drug release from Ceftriaxone beads in pH-1.2 and pH-7.4 buffer.

Cum drug release (%)of Formulation J1at pH 7.4 ( ); & at pH 1.2( ); Cum drug release (%) pH 7.4 of Formulation J2( );& Cum drug release (%) pH 1.2 of2( )

There was an initial rapid release within 15 min, referred to as 'burst' effect, followed by a slower first-order release. This rapid release of ceftriaxone sodium may also be attributed to its high aqueous solubility since water soluble molecules are generally known to be released quicker than hydrophobic and less soluble molecules Permeability study of optimized batches of Ceftriaxone beads were shown in table 6.

Table 6: Permeability study of formulations D1, J1 and J2

Time(min)	Cum amt permeated (µg/cm²) Formulation D1	Formulation J1	Formulation J2
0	0	0	0
30	70.10	283.42	187.45
60	147.91	563.98	346.64
90	217.54	798.89	538.71
120	300.21	1020.20	701.60
150	376.72	1286.1	862.20
180	449.61	1532.99	1018.90
210	523.10	1743.20	1178.61
240	594.91	1964.67	1340.20

*Formulation D1 is beads without permeation enhancer

The experiment using the in vitro model with biological membrane shows (fig 4) an enriching of ceftriaxone in the membrane through the combination with sodium taurocholate-oleic acid (Formulation J1) and Brij58-oleic acid (Formulation J2).

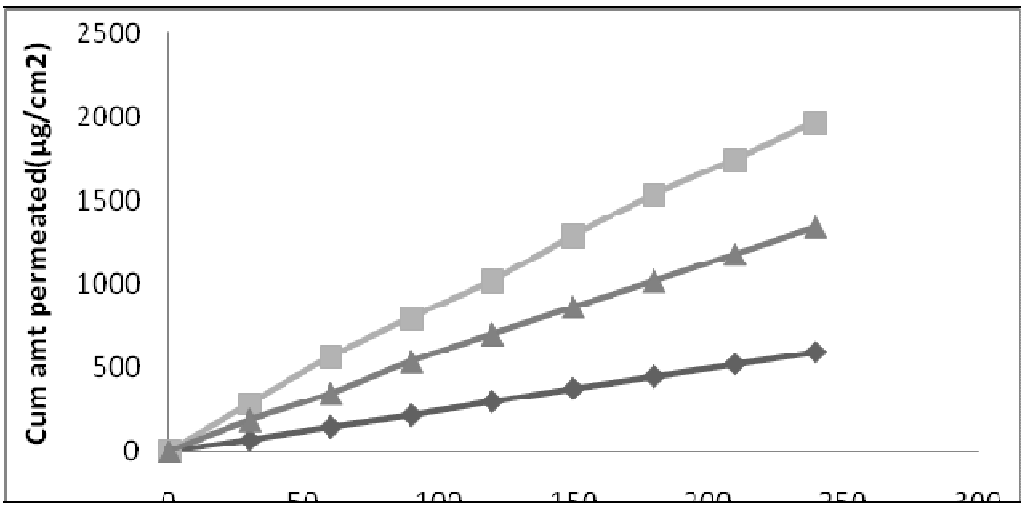

Fig. 4: Drug permeation profile of ceftriaxone sodium beads.

*D1 Formulation without permeation enhancer

Drug permeation profile of Formulation D1 ( ); Formulation J1 ( ); & Formulation J2 ( )

Kinetic models were fitted to dissolution data of optimized formulation using linear regression analysis [21]. Drug release kinetics of the formulation (table 7) correspond best to Hinguchi's model and drug release mechanism as per n value of Korsmeyer & Peppas can not be predicted clearly as it appears to be complex mechanism of swelling and diffusion.

Table 7: Release parameters of Ceftriaxone Sodium beads

Zero order		First order		Higuchi		Korsmeyer-Peppas	
r^2	K_0 (h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H ($h^{-1/2}$)	r^2	n value
0.715	2.793	0.844	0.048	0.964	15.0	0.796	0.344

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