Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2010, 2 (3): 131-142

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Enhancement of intestinal absorption of poorly absorbed Ceftriaxone Sodium by using mixed micelles of Polyoxy Ethylene (20) Cetyl Ether & Oleic Acid as peroral absorption enhancers

Singh Neelam*[1], Gupta Puneet[2], Bhattacharyya Arundhati[1]

[1] *RKGIT College of pharmaceutical sciences,*
Delhi-Meerut road Ghaziabad, India
[2]*KIET School of Pharmacy, Ghaziabad-Meerut Road, Ghaziabad, India*

Abstract

Ceftriaxone sodium is a third generation cephalosporin. This antibiotic cannot be absorbed orally owing to its very less permeation through GI epithelia. Other problem associated with the drug is its acid labile nature. The present study attempts to increase the intestinal permeability of BCS Class III drug ceftriaxone sodium by using certain intestinal absorption enhancers. The blend of permeation enhancer, drug, and other excipients were incorporated into Beads to formulate the final dosage form. To enhance the permeation, intestinal permeation enhancers were used in various molar ratios with the drug. The effect of absorption enhancers on the lipophilicity of ceftriaxone sodium was determined by means of the n-octanol/water system. The changes in partition coefficient by the octanol/water system were confirmed using an in vitro transport model with excised animal intestinal membrane. The results indicated that there is significant improvement in the permeability of the drug and the extent of enhancement was highly dependent on the type of used absorption enhancer. Permeation enhancer and drug were formulated into beads further evaluated for permeability by using biological membrane. The release profile of ceftriaxone from beads was observed in both gastric and intestinal pH (7.4 buffer). Release of drug from the beads in both the media was found to occur predominantly by diffusion following non Fickian transport mechanism and was higher and more rapid in intestinal pH than in gastric pH. The results obtained from this study indicate that ceftriaxone sodium could be successfully delivered orally when formulated with permeation enhancers.

Keywords: Intestinal permeability, permeation enhancers, beads, ceftriaxone sodium.

INTRODUCTION

A great number of currently available drugs fall under the class III of the biopharmaceutical classification system, possess high therapeutic potential but cannot be delivered by oral route

because of its poor permeation across the GIT epithelia. Drugs have low intrinsic membrane permeability, probably because of their low lipophilicity and zwitterionic character at physiological pH or substrate to drug efflux pumps like p-glycoprotein, ionic charge and high molecular weight. Ceftriaxone sodium, one of the third generation cephalosporin, capable of inhibiting the biosynthesis of bacterial cell wall belongs to a group of most effective drug used as antibacterial agent. Ceftriaxone is normally poorly absorbed through the mucosal membrane of the intestine and is thus ineffective when administered by the oral route. One method to improve the permeability of BCS Class III drugs to use permeation enhancers. The need of high doses of enhancer is closely related to the fact that most permeation enhancers deliver drug into the blood stream not by increasing the permeability of the drug itself but by perturbing the biological membrane and consequently allowing drug absorption nonspecifically. Permeation enhancers reversibly and specifically or non specifically increases permeation of drug across the GIT epithelia.[1,2] Polyoxyethylene ether, mixed micelles containing sodium taurocholate and glycerol monooleate or oleic acid were reported to enhance the absorption of cephalosporins. The effect of selected permeation enhancers on the lipophilicity of the drug was investigated using the octanol/buffer system. The experimental results on the partition coefficient were confirmed using in vitro transport model (static Franze diffusion cell) with excised intestinal membrane of animal [3]. Blend of permeation enhancers and drug was incorporated into beads. The objective of this study was to prepare sodium alginate beads of ceftriaxone sodium incorporating some permeation enhancers and *in vitro* evaluation of the beads.

MATERIALS AND METHODS

Materials

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

Determination of partition coefficients

The partition coefficients of ceftriaxone sodium with or without absorption enhancers in different molar ratios were determined between pH-7.4 phosphate buffer and n-octanol. These two phases were saturated with each other. The compounds were dissolved in aqueous phase (5 mg/ml). The buffer/octanol solutions were shaken for 8 hr at room temperature. After separation of the samples, into two phases, the drug content was analyzed spectrophotometrically at λ_{max} 241.0 nm. The partitioning coefficient was calculated using the following equation:

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

Where a_o and a_b are the concentrations of the drug in n-octanol and buffer respectively [3].
Ceftriaxone sodium alone shows a small $P_{o/w}$ of 0.026

Ceftriaxone sodium, a hydrophilic drug containing many polar groups, exhibited very small $P_o/_{buffer}$ pH 7.4 value (0.026). However, the combination with absorption enhancers led to improvement in the $P_o/_{buffer}$ pH 7.4 values. It was observed that the partition coefficient value did not increased significantly with all above used absorption enhancers. Hence Batch P14 and P15 were selected for further studies.

Table1. Partition coefficient of drug with Polyoxyethylene (20) cetyl ether

Batch code	Drug: Brij 58 (mM)	$P_{o/buffer}$
P1	1:0.03	0.024
P2	1:0.04	0.035
P3	1:0.05	0.039
P4	1:0.06	0.042
P5	1:0.07	0.078
P6	1:0.08	0.081
P7	1:0.09	0.052
P8	1:0.1	0.040
P9	1:0.2	0.032
P10	1:0.3	0.030
P11	1:0.4	0.039
P12	1:0.5	0.039

❖ CMC of Brij 58 is 0.077 mM

Table2. Partition coefficient of drug with mixed micelles of Brij 58 & Oleic acid.

Batch code	Drug: Brij 58 : OA	$P_{o/buffer}$
P13	1 : 0.08 : 0.5	0.107
P14	1 : 0.08 : 1	0.121
P15	1 : 0.08 : 1.5	0.130
P16	1 : 0.08 : 2	0.093
P17	1 : 0.08 : 2.5	0.099

***In vitro* permeation studies using excised animal intestinal tissue**

The permeability studies were conducted using the static Franz cell system. The Franz cell is a diffusion chamber made of glass comprising an upper donor and lower acceptor compartment between which the tissue is clamped, with the mucosal side oriented upwards [4]. The effective permeation area of the intestinal epithelium was 1.54 cm^2. Transport medium was Hank's Balanced Salt Solution (HBSS) buffer (pH-7.4). 2.5 ml of sample solution (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^oC. Samples were periodically removed from the acceptor compartment over 4 h. The volume of the acceptor compartment was kept constant by adding fresh HBSS after each withdrawal. The samples were appropriately diluted and their 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 (0.34mM), D-Glucose (5.55mM). Adjust the pH by NaOH.

Calculation of permeability coefficient / apparent permeability coefficient

The apparent permeability (P_{app}), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

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

Where V_A is the volume (in mL) in the acceptor well, Area is the surface area of the intestinal membrane, and time is the total transport time in seconds [4].

Preparation of beads

The method used is ionotropic gelation method.[5] In which, sodium alginate was dispersed in water by gently stirring with glass rod at room temperature and left to stand for 24 hr in order to attain maximum hydration. Drug was similarly mixed with the sodium alginate dispersion. This alginate-drug dispersion was extruded drop wise in to zinc chloride solution by constant stirring using magnetic stirrer to prepare beads. The beads were collected, washed with water and dried in air for 24 hrs.

Optimization of the formulation was carried out in the given manner. 2%, 3%, 4%, 5%, 6% wt/v Sodium alginate solutions in distilled water were prepared. 10 ml of each of these solutions was thoroughly mixed in different ratios with the drug. Beads were prepared by adding the dispersion drop wise in the solution of curing agent i.e. zinc chloride (in different molarities). Beads were cured for different duration of time, washed with water and dried in air for 24 hr. The parameters, which were to be optimized, are Sodium alginate concentration (w/v), Drug – polymer ratio, Con. of zinc chloride (mM), Curing time (min)

Table-3 Formulation of beads without permeation enhancer (Optimized batch)

Batch	Concentration of polymer	Drug: polymer	$ZnCl_2$ (mM)	Curing time (min)
B1	5	0.5:1	0.4	2

Table-4 Formulation of beads with permeation enhancer (Optimized batch)

Batch	Concentration of polymer	Drug: PE	Drug: polymer	$ZnCl_2$ (mM)	Curing time (min)
B2	5	1 : 0.08 : 1.5 (Drug: Brij 58: OA)	0.5 : 1	0.4	2

PE-Permeation enhancer; Drug-Ceftriaxone sodium (mM); Polymer- Sodium alginate % (w/v) ST- Sodium Taurocholate (mM); OA- Oleic acid % (v/v); Brij 58- Polyoxy ethylene (20) cetyl ether (mM)

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. [6, 7]

Determination of drug content and entrapment efficiency:
The drug content in the beads was determined by dispersing accurately weighed beads (10 mg) into 100 ml phosphate buffer (pH7.4) and stirred for 24 hr. The dispersion was filtered and the drug concentration was determined spectrophotometrically, at absorption maxima 241.0 nm. Furthermore, the amount of the drug that diffused in to the zinc chloride solution during the hardening of beads was determined. Immediately after isolation of beads aliquots of hardening solution were filtered, diluted, and assayed spectrophotometrically at the absorption maxima [8].

The encapsulation efficiency was calculated according to the following relationship:

E.E = % drug content× amt of dried beads produced/ amt of drug added amt of drug remaining in apparatus

Swelling studies
Beads were studied for swelling characteristics; only those batches were selected which have drug content and entrapment efficiency more than 50%. Dry ionicaly cross linked beads increase their volume after few minutes in water or in buffers with different pH, due to matrix rehydration in accordance with the degree of crosslinking [9].

The initial weight of beads was recorded and placed in 100 ml of phosphate buffer pH 7.4, then shaken and allowed to swell. The temp of medium was maintained at 37^{o}C. The agitation ensured that water penetration and swelling occurred three dimensionally after 8 hr (After 10 h total breakdown of gel structure was take place). The swollen beads were carefully removed blotted dry and weighed. Water sorption was calculated from the difference between the initial weight and the weight at the time of determination. 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 [8].

Swelling ratio= wt of wet beads/ wt of dried beads

In vitro drug release
The USP paddle method was adopted in this study. The release medium consisted of 900 ml of pH-7.4 buffer. 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, 1 ml of the release medium was withdrawn, appropriately diluted and absorbance determined at a wavelength of 241 nm using UV spectrophotometer. The volume of the release medium was kept constant by replacing it with 1 ml of fresh pH-7.4 buffer after each withdrawal. The release study was repeated using pH 1.2 buffer as a release medium and the absorbance was determined at a wavelength of 265 nm [10].

Permeability study of 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) buffer (pH-7.4). Beads were crushed and dispersed in HBSS pH 7.4. 2.5 ml of the beads dispersion (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^{o}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. The samples were appropriately diluted and their absorbance determined at a wavelength of 241.0 nm. [2]

RESULTS AND DISCUSSION

Permeability study

Table-5 Permeability study of Selected batches

Time(min)	Cum amt permeated (µg/cm^2)		
	CTZ	Batch P14	Batch P15
0	0	0	0
30	71.419	136.57	190.45
60	150.35	310.73	349.58
90	220.52	444.80	541.28
120	302.21	562.33	706.68
150	389.67	722.96	864.55
180	451.07	855.78	1022.43
210	516.22	1002.38	1182.81
240	599.92	1174.29	1347.20

To measure the hydrophobic/hydrophilic properties of Ceftriaxone sodium, partition coefficients in the n-octanol/water system were investigated. Ceftriaxone sodium alone shows a small Po/w of 0.026, with permeation enhancers it exceeds to 0.130. Ceftriaxone sodium alone exhibited limited absorption via the lipid membranes (biological membranes). The combination with permeation enhancers leads to a permeation rate of Ceftriaxone from 599.92µg/cm^2 to 1347.20µg/cm^2 and a permeation coefficient ranging from 1.55×10^{-4} to 3.45×10^{-4} cm/sec (tables 5 & 6).

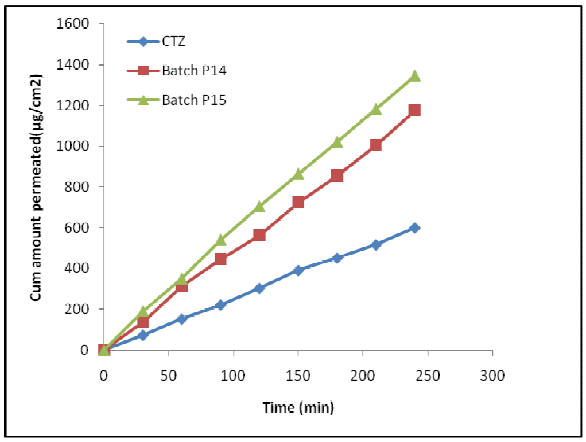

Figure-1 Permeation rate of ceftriaxone sodium (µg/cm^2) using biological membrane
CTZ: Ceftriaxone Sodium

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 Brij58 and oleic acid after 4 h.

Table-6 Permeability coefficient of selected batches

Batch code	P_{app}(cm/sec)
CTZ	1.55×10^{-4}
P 14	3.01×10^{-4}
P 15	3.45×10^{-4}

The influence of enhancers on the absorption of ceftriaxone sodium was studied. It was observed that the partition coefficient (in the n-octanol/ pH-7.4 system) was increased significantly. The experiment using *in vitro* model with biological membrane shows enhanced penetration of ceftriaxone in the membrane through the combination of Brij 58-oleic acid. The results show that the optimal effect was obtained through the combination rather than using Brij 58 alone.

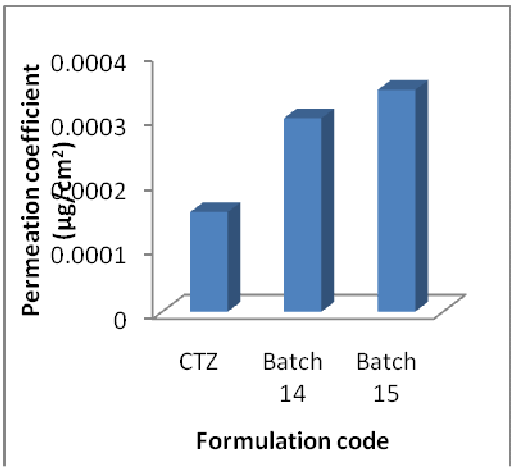

Figure-2 Permeation coefficient of ceftriaxone sodium using biological membrane

Morphology and size of beads

The surface morphology of the alginate beads were investigated by scanning electron microscopy. Prior to examination samples were gold coated under vacuum to render them electrically conductive**.** The beads formed are, spherical to disc shape with changing concentration and ratio of polymers. As the results show, the spherical shape of beads were obtained 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.

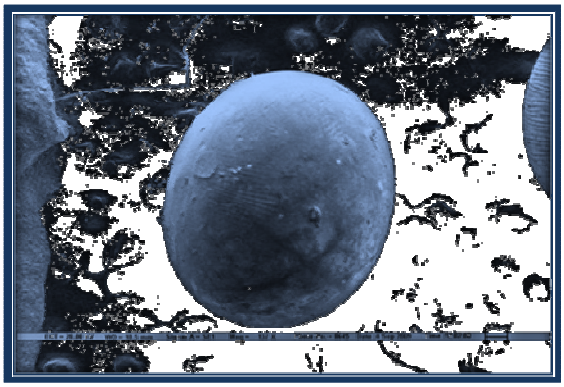

Figure-3 SEM of bead

Drug content and entrapment efficiency

Table-7 Drug content and entrapment efficiency of beads

Batch Code	Drug content (%)	Entrapment efficiency (%)
B1	39.99	75.33
B2	39.00	77.10

There were various factors, which were responsible for this variation in drug content and entrapment efficiency of each batch. These factors include curing time, drug: polymer ratio, Zinc Chloride concentration.

Optimized curing time is 2 min, drug polymer ratio is 0.5:1.0, and Zinc Chloride concentration is 0.4 mM,

Swelling studies

Table-8 Swelling behavior of beads in pH 7.4

Batch code	Swelling ratio in pH 7.4			
	2h	4h	6h	8h
B1	2.21	2.71	3.34	3.90
B2	2.31	2.88	3.21	3.56

Table-9 Swelling behavior of beads in pH 1.2

Batch code	Swelling ratio in pH 1.2			
	2h	4h	6h	8h
B1	0.98	1.0	1.0	1.10
B2	0.95	0.98	1.01	1.08

***In vitro* drug release**

The release profile of ceftriaxone sodium from the beads in two different release media (pH-1.2 and pH-7.4) is shown in table 3.24 and 3.25. There was rapid release of ceftriaxone from the beads at pH-7.4 within 30 min. The highest release ranged between 82.05-94.52%. Less release of ceftriaxone sodium in pH-1.2 could possibly be a result of the limited swelling of the beads in the acidic medium. This situation may have interesting implications in the protection of ceftriaxone sodium from the acidic environment of the stomach when administered in vivo via the oral route. A characteristic feature of the release profile of drug from the beads is the biphasic pattern of release. 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. The high release of ceftriaxone sodium in phosphate buffer pH-7.4 could give an indication of the rate and extent of in vitro bioavailability, which may suggest that ceftriaxone, an acid labile drug, could be successfully delivered orally when embedded in polymeric beads.

Table 10.Drug release study in pH 7.4 buffer

	Cum drug release (%)	
Time(min)	Batch B1	BatchB2
0	0	0
15	29.59	30.12
30	37.82	34.85
45	43.54	38.03
60	49.52	42.40
90	52.73	46.51
120	58.81	49.95
180	65.10	57.77
240	73.34	64.004
360	81.90	76.06
480	87.80	83.08
720	93.86	91.30
1440	94.52	92.09

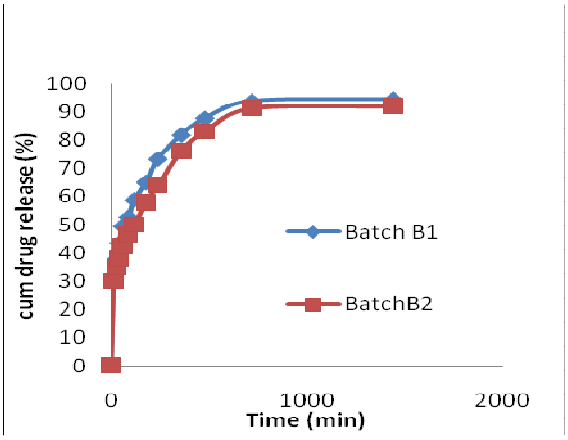

Figure.4. Drug release profile of ceftriaxone sodium from the beads in pH-7.4 buffer

Table 11.drug release study in pH1.2buffer

Time(min)	Cum drug release (%) Batch B1	Batch B2
0	0	0
15	2.60	2.70
30	4.82	4.43
45	7.17	6.39
60	9.52	8.21
90	14.21	12.26
120	17.86	15.78

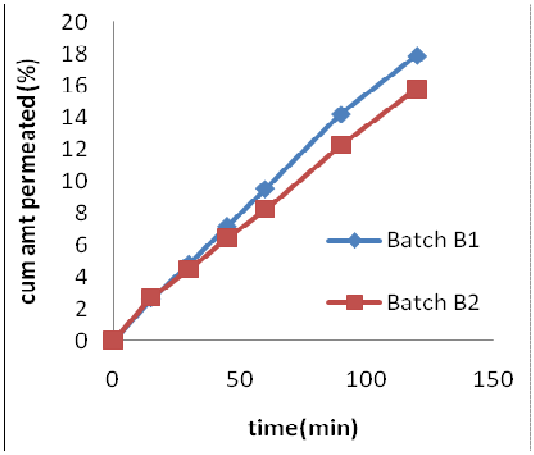

Figure.5 Drug release profile of drug from the beads in pH-1.2buffer

Permeability study of beads

Table12. Permeability profile of beads

Time(min)	Cum amt permeated(µg/cm^2) Batch B1	Batch B2
0	0	0
30	70.10	187.45
60	147.91	346.64
90	217.54	538.71
120	300.21	701.60
150	376.72	862.20
180	449.61	1018.90
210	523.10	1178.61
240	594.91	1340.20

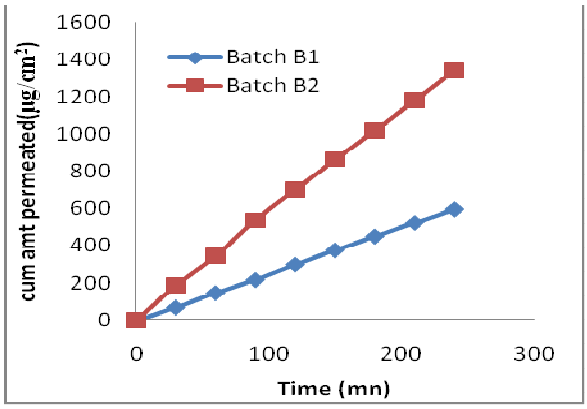

Figure6. Drug Permeation profile of ceftriaxone sodium from the beads.

More amount of ceftriaxone sodium was permeated from formulation B2, in formulation B2 mixed micelle of Brij 58 and oleic acid was used as permeation enhancer.

Alginate beads were successfully prepared by using combination of polyoxyethylene 20 cetyl ether (Brij 58) and oleic acid as a absorption enhancer and evaluated in vitro. Sodium alginate is practically insoluble in pH less than 3; therefore prevent the degradation of drug in acidic environment of stomach.

Drug release kinetics

After studying the drug release kinetics for optimized batches i.e. B1 and B2, it was found that release of ceftriaxone sodium from beads occur by Higuchi model following non-Fickian transport mechanism [11, 12].

CONCLUSION

Mixed micelles of Brij 58-oleic acid combination significantly influences the permeation of drug across the biological membranes. Although the drug permeation was lower when Brij 58 was used alone. Hence it can be concluded that the use of combination of permeation enhancers is more effective for enhancing intestinal permeation of ceftriaxone sodium.

The extent of enhancement was found to be highly dependent on the absorption enhancers species used. Absorption enhancer with other excipients and drugs were used in formulation of beads.

Considering ceftriaxone sodium as a class III drug, results of pharmacokinetics studies shows that the use of absorption enhancers to enhance bioavailability of ceftriaxone offers viable method to deliver them by oral route.

Acknowledgement

The author is grateful to Aurobindo Pharma Pvt Ltd Hyderabad, India, for providing a gift sample of ceftriaxone sodium.

REFERENCES

[1] Seulki Lee, Sang Kyoon Kim. *Journal of pharmaceutical sciences*, **2005**; 94:2541-2548.
[2] Panchagula R, Sharma P, Verma VS, Chawla HPS. *IL Farmaco*.**2005**; 60: 874-883.
[3] Neubert R, Mrestani Y, Mrestani-Klaus C. *European Journal of Pharmaceutics and Biopharmaceutics.* **2004**; 58: 653-657.
[4] Udo Bock, Christian Hiller, Sigrid Balser. *European journal of pharmaceutics and biopharmaceutics*, **2008**; 68: 390-399.
[5] Smrdel P, Bogataj M, Podlogar F, Planinsek O, Zajc M, Mazaj M, Kaucic V. *Drug Development and Industrial Pharmacy*. **2006**; 32: 623-633.
[6] Murata Y., Sasaki N, Miyamoto E, Kawashima S, *Eur.J.Pharm Biopharm* **2001**; 50: 221-226.
[7] Kulkarni AR., Soppimath KS., Aminabhavi TM., *Pharma Acta Helve* **1999**; 74 (1): 29-36.
[8] Yagnesh L.Patel, Praveen Sher, Atmaram P. Pawar. *AAPS Pharma Sci Tech*. **2006**; 7. E1-E7.
[9] Simonoska M, Goracinova K. *European Journal of Pharmaceutics and Biopharmaceutics*. **2008**; 68:565-578.
[10] Ofokansi KC, Adikwu and Okore V.C. *Drug Development and Industrial Pharmacy*. **2007**; 33: 691-700.
[11] M. Harrish Shoaib, Jaweria Tazeen, Hamid A.Merchant. *Pakistan Journal of Pharmaceutical Sciences*.**2006**; 19: 119-124.
[12] Mario Grassi, Gabriele Grassi. *Current drug delivery*.**2005**; 2:97-116.