Journal of Pharmaceutical Research Vol. 9, No. 4, October 2010 : 153-158.

DEVELOPMENT OF ENTERIC-COATED POLYSACCHARIDE BEADS INTENDED FOR COLONIC DRUG DELIVERY

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Received on : 28.07.2010 Revised : 22.09.10 Accepted : 25.09.10

ABSTRACT
Crohn's disease is a type of inflammatory bowel disease (IBD) that frequently affects the ileo-cecal region. For effective treatment, a site-targeting delivery system in the ileo-cecal region is essential. Ciprofloxacin, a broad spectrum antibiotic is widely used for the treatment of Crohn's disease. The present study is an attempt to develop enteric coated polysaccharide beads which targets the drug to the colon. Polysaccharide beads were prepared using different polymers like sodium alginate, gellan gum and chitosan by dispersing the drug loaded solution into calcium chloride and sodium tripolyphosphate solution respectively. The prepared beads were then coated with Eudragit S 100 using pan coating method. Characterization done using SEM revealed that, eudragit coated beads had uniform surface texture. Swelling study suggested that, alginate beads alone or in combination with gellan gum showed good swelling behavior in phosphate buffer (pH 7.4), whereas chitosan beads showed better swelling in pH 1.2. The drug release showed up to 10 % in acidic environment and sustained under neutral condition for a period of 10 h. Release kinetics indicated non-Fickian transport controlled by diffusion and relaxation of the polymer. *In-vivo* studies in rabbits indicated that the formulation was targeted to the colon region.

Keywords: *Ciprofloxacin; Crohn's disease; polysaccharide beads; colon specific.*

INTRODUCTION

Site-specific drug delivery refers to the targeting directly to a certain biological location, where the target is adjacent to or in the diseased organ or tissue[1]. Colonic drug delivery has gained increased importance not only for the delivery of drugs in the treatment of local diseases associated with the colon like Crohn's disease (CD), ulcerative colitis, irritable bowel syndrome, colorectal cancer, but also for the systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by per oral delivery, is still deemed to be the ideal site for the delivery of agents to cure the local diseases of the colon. The most critical challenge in such drug delivery approach is to protect the formulation during its passage through the stomach and about first six meters of the small intestine and finally releasing the drug in the large intestine. Due to the distal location of the colon in the GIT, a colon specific drug delivery should prevent drug release in the stomach and small intestine, and produce an abrupt onset of drug release upon entry into the colon. Various approaches have been used for the delivery of drugs to colon which includes the use of prodrugs, hydrogels, biodegradable azo polymers, pH sensitive polymer coatings, time dependent formulations, bacterial degradable coatings. Despite intensive investigation, the etiological factors of Crohn's disease remain unclear. Bacteria are included on the list of possible causative factors, although their precise role is disputed. Animal models of inflammatory bowel disease lend support to the theory that normal enteric bacteria may contribute to or perpetuate the chronic inflammatory process in CD[2, 3]. Loss of normal mucosal integrity because of active bacterial contamination may contribute to the inflammatory reaction in the gut wall. Ciprofloxacin is a fluoroquinolone anti-infective agent, which can be widely used. The oral bioavailability is 50 – 85 % and has an apparent volume of distribution of 2 – 3 L/kg. It is 20–40 % bound to plasma proteins. It is eliminated by renal and non-renal mechanisms and its plasma half life is 2–6 h. In recent years, several reports[4–7] have appeared in the medical literature suggesting that antibiotic treatment of CD might be beneficial. Ciprofloxacin has excellent *in vitro* response against a variety of organisms, including enteric pathogens. It was chosen because of its extensive coverage for intestinal flora, relatively favorable side-effect profile and preliminary data suggesting its efficacy in the treatment of active CD[3]. Srinatha et al., had investigated ionic cross linked chitosan beads for extended release of ciprofloxacin and studied its behavior in acidic and alkaline medium[4]. Hence, its necessity as colon target formulation was attempted in the present study as beads with natural polysaccharides, so that the drug can be available in maximum concentration at the site of action.

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MATERIALS AND METHODS

Materials

Ciprofloxacin was a gift sample from M/s Dr. Reddy's Laboratories (Hyderabad, India). Chitosan (82% deacetylation) was purchased from CIFT, Cochin. Sodium alginate was purchased from Suvidhinath Laboratories, Borada, India. Gellan was obtained from Himedia Pvt. Ltd. Mumbai, India. All the other ingredients used in the study were analytical grade.

Methods

Preparation of uncoated calcium alginate beads

Different formulations of polysaccharide beads were prepared as shown in Table 1. Required quantity of sodium alginate was dispersed in distilled water with continuous stirring. The drug then dissolved in the above solution and was allowed to stand till the entrapped air was removed. Simultaneously, aqueous solution of calcium chloride (2 %w/v) was prepared. The sodium alginate solution containing drug was added to the calcium chloride solution, drop by drop using 22 G needle fitted with a syringe, with continuous agitation. The prepared beads were left under stirring for 20 – 30 min and then removed by filtration. The excess calcium chloride was removed by washing with distilled water and dried at 50 °C overnight[3].

Table 1: *Formulations of uncoated beads*

Formulation Code	Alginate : $CaCl_2$ (w/w)	(Alginate + Gellan gum) : $CaCl_2$ (w/w)	Chitosan : Na TPP	Coated beads Formulation codes
F1	1 : 2	-	-	F1C
F2	1.5 : 2	-	-	F2C
F3	-	1.5 : 2	-	F3C
F4	-	1 : 2	-	F4C
F5	-	-	1 : 2	F5C

Preparation of uncoated chitosan beads

100 mg of chitosan was dissolved in 1 % acetic acid solution. Then weighed amount of drug was added to this dispersion, stirred and allowed to stand till the removal of entrapped air. Chitosan solution containing drug was then added drop wise using 22 G needle fitted with a syringe into sodium tripolyphosphate solution (3 %w/v) at 50°C. Beads were left in the above solution for 20 – 30 min, collected by filtration, washed with distilled water and dried at 50°C overnight[5].

Enteric coating of core beads

The drug loaded beads were then coated with an enteric polymer, eudragit S 100 (10 %w/v) using pan coating procedure. The coating solution was prepared by dissolving known quantity of eudragit S 100 in a mixture of dichloromethane and methanol in the ratio 4:1 and polyethylene glycol 400 was added as plasticizer and stirred to obtain a homogenous solution. The coating solution was then sprayed on to the prepared beads in pan coating machine (PSM Scientific Instrument PVT LTD). The coated beads were then dried[6].The formulations which are coded are denoted as F1C, F2C, F3C, F4C, and F5C.

CHARACTERIZATION OF POLYSACCHARIDE BEADS

Particle size determination

The particle size and the surface morphology of the beads were measured by using scanning electron microscope (SEM), JEOL JSM-6380LA. The surface characteristics of core and coated beads were analyzed by using scanning electron microscope. Before being observed by SEM, the beads were fixed on brass stub with an adhesive and sputter coated with platinum at 20 mA current for 90 sec using auto fine coater. Samples were then scanned using SEM under vacuum at an accelerating voltage of 15 kV[3].

Sieve analysis

The particle size and size distribution were determined by sieve analysis method. The average particle size was calculated by the equation[6]:

$$d_{av} = \frac{\Sigma \varphi d}{\Sigma \varphi}$$

φ is the weight fraction retained on each sieve.

d is the mean aperture size.

Drug content estimation

100 mg of the beads of each formulations containing drug were weighed, dispersed in 100 ml phosphate buffer (pH 7.4) and allowed to stand for 24 h with intermittent shaking to ensure complete extraction of drug. The solution was diluted suitably, filtered and absorbance was measured at 274 nm using UV/Visible spectrophotometer. The drug content was estimated using calibration curve[3,5].

Swelling studies

Water uptake by the uncoated beads was determined by suspending the beads in simulated gastric and intestinal fluid. This was done by placing 100 mg (W_1) of beads separately in 100 ml of 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). At regular intervals of time, the beads were carefully removed and excess of moisture was removed using tissue paper. The swollen beads were reweighed (W_2). The percentage swelling index of the beads was determined using the following equation[7],

$$\% \text{ Swelling Index} = \frac{(W_2 - W_1)}{W_1} \times 100$$

In vitro drug release studies

The drug release study was carried out using USP dissolution test apparatus (XXIII), paddle type. Study was conducted in 900 ml of dissolution medium maintained at 37 ± 0.5 °C with a paddle rotation speed of 100 rpm[8]. The pH of the medium was varied over the course of the experiment: 0.1 N hydrochloric acid (pH 1.2) was used for the first 2 h and of phosphate buffer pH 7.4, till the complete release of drug took place[9]. Samples of 5 ml volume were withdrawn at predetermined time intervals and were replaced with fresh dissolution medium to maintain sink conditions.

Samples withdrawn were later filtered and assayed spectrophotometrically at 274 nm using corresponding buffers as blank**.**

In order to assess the susceptibility of polysaccharide, being acted upon by colonic bacteria, drug release studies were carried out in presence of rat cecal contents because of the similarity with human intestinal flora. In order to mimic intestinal environment, especially enzymes glycosidase specially acting on alginate gum and chitosan gum in the cecum, male albino rats weighing between 150 – 200 gm maintained on normal diet were kept with teflon tubing and 4% w/v dispersion of alginate/ chitosan gum in water were administered for 7 days. All the rats were killed by spinal traction, 30 min before the commencement of drug release studies. The abdomens were opened, cecal bags were isolated, ligated at both ends and cut loose and immediately transferred into phosphate buffer pH 7.4 previously bubbled with nitrogen gas. As the caecum is naturally anaerobic, all these operations were carried out under nitrogen gas. *In vitro* drug release studies in the presence of rat cecal contents was carried out using USP dissolution test apparatus (XXIII), paddle type using the above said dissolution media. The paddle was rotated at 100 rpm and the medium was maintained at a constant temperature of 37±0.5 °C. Samples were analyzed spectrophotometrically. Dissolution studies were performed in triplets and mean values were reported. The percentage drug release was then graphed against time and the release profiles were studied.

In vivo targeting efficiency

In vivo targeting efficiency study was carried out to check the efficiency of the formulation to target to colon after obtaining ethical clearance (KSHEMA/AEC/092/2009 dated 17/06/2009). The coated beads of ciprofloxacin containing radio opaque material such as barium sulphate (15 %w/v) were given to the fasting rabbits with water. After the administration of the formulation, X–ray images were taken under the supervision of a radiologist, to follow the movement, location and the integrity of the beads in different parts of GIT at different time intervals[4].

Stability studies

The selected formulations were wrapped in butter paper and placed in vials and sealed. They were then stored at different temperatures of 4 ± 2 °C, 25 ± 2 °C and 40 ± 2 °C for 3 months and evaluated for their physical appearance and drug content at specified intervals of time.

RESULTS AND DISCUSSIONS

Polysaccharide beads of ciprofloxacin were prepared using different concentrations of polymers like chitosan, gellan gum, sodium alginate and coated with eudragit S 100. The percentage yield of polysaccharide beads was found be maximum of 86%. Dissolution and biodegradation of alginate under normal physiological conditions allows it to be used as a matrix for the entrapment of proteins and drugs. This copolymer, consisting of mannuronic acid and glucuronic acid residues which undergo cross linking in presence of divalent cations, thus leads to the formation of beads. Chitosan is a cationic polysaccharide. The amine groups present in this undergoes protonation in acidic environment which interact with phosphate ions provided by tripolyphosphate and forms beads either by intermolecular or intramolecular linkage. Similarly, gellan gum, an anionic microbial polysaccharide containing glucose, glucuronic acid and rhamnose undergo cross linking in presence of multivalent ions. The beads were coated with eudragit S 100 which is a copolymer of methacrylic acid and methyl methacrylic acid containing about 30 % of methacrylic acid units that tend to dissolve at around pH 7. For this reason, it was considered a suitable coating material for colonic delivery, able to adequately protect the drug core system during its passage through the stomach and small intestine.

Particle size of beads

The particle size was found to be in the range of 800 – 1600 µm and by sieve analysis method the average particle size was found to be 1196.97 ± 50 µm.

Surface morphology

It was found that, coated beads have uniform and smooth surface when compared to uncoated beads. The SEM images of uncoated and coated beads indicating surface morphology at different magnifications are shown in the Figure 1.

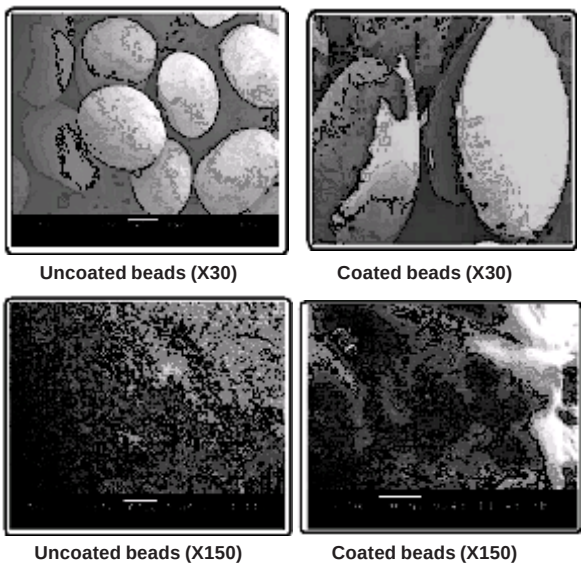


Fig. 1: *SEM images of beads indicating surface morphology at different magnifications*

Drug content estimation

The percentage drug content in all the formulations was found to be in the range of 85 % to 90 %. The decrease in drug entrapment efficiency may be due to the presence of pores on the surface of the beads and the higher water solubility of the drug.

Swelling studies

Water absorption is dependent on the type and concentration of polymer used and also on the type of medium in which the beads are suspended. Formulations containing sodium alginate alone or in combination of gellan gum showed better swelling in phosphate buffer (pH 7.4) when compared to that in HCl (pH 1.2) but formulations containing chitosan showed better swelling in HCl when compared to that in buffer. This increased swelling of chitosan beads in acidic pH was due to the pH dependent solubility of the polymer. Swelling index of all formulations is given in Table 2.

Table 2: *Swelling index of different formulations at pH 1.2 and 7.4*

Time (min)	% Swelling index									
	F1		F2		F3		F4		F5	
	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4
0	0	0	0	0	0	0	0	0	0	0
10	28	27	25	28	9	20	5	17	36	22
30	–	35	30	53	–	31	–	35	56	–
60	–	63	–	72	–	66	–	78	–	–
90	–	168	–	143	–	135	–	150	–	–
120	–	294	–	318	–	194	–	218	–	–

In vitro drug release studies

The release of drug from the formulation depends on the type and concentration of polymer used, and the pH of the dissolution medium. The release of ciprofloxacin from uncoated and coated beads was carried out in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4). The drug release profiles of uncoated and coated beads are shown in Figure 2 and 3. In order to investigate the extent to which these polymers succeed in targeting the drug to the colon, release studies have been conducted in the 2 different pH range. Further to mimic the colon environment, the colonic microflora was also taken into consideration for the *in vitro* release study, as polysaccharide polymers release the drug faster in the presence of colonic microflora as they release glycosidase (Fig. 2). The drug release was faster in uncoated alginate beads. Dissolution studies revealed formulation containing alginates, gellan gum showed only 52.20 % of drug release in 5 h, whereas, other formulations F1, F2, and F5 showed up to a maximum of 90.40% drug release in 5 h. In this, the dissolution medium entered through the pores created by the burst release of the water soluble drug causing faster diffusion of dissolved drug. The formulation containing gellan gum in combination with sodium alginate showed pH independent release in comparison to those formulations which contains only alginate. This might be due to similar swelling nature of gellan gum at pH 1.2 and 7.4. Thus the presence of gellan gum

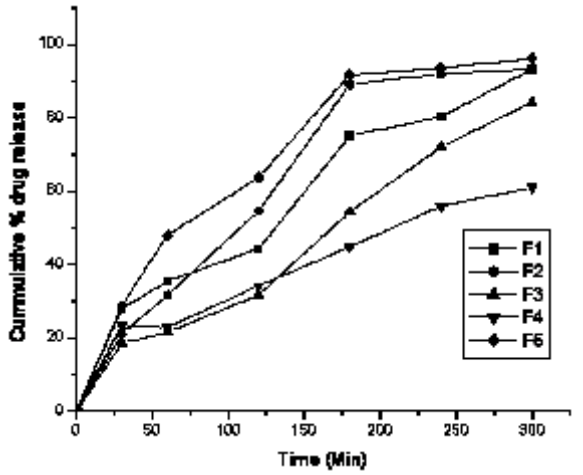


Fig. 2: *Cumulative percentage drug release of uncoated beads in absence of rat cecal matter*

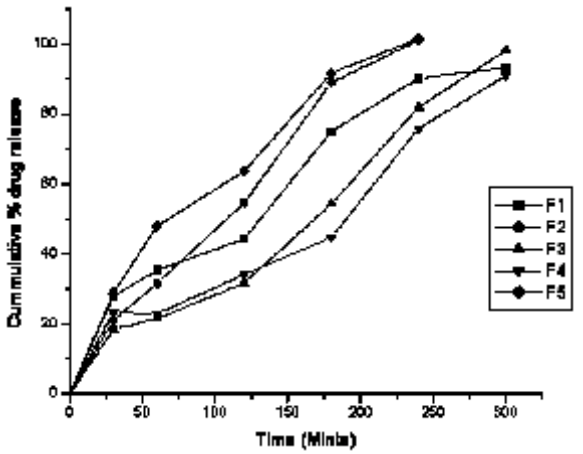


Fig. 3: *Dissolution of uncoated polysaccharide beads in presence of rat cecal matter*

decreased the burst release and showed better ability to sustain the drug release from the beads. Chitosan beads showed faster drug release in acidic pH. This was due to the higher solubility of chitosan in acidic media. At pH 7.4 it was found that, the drug release showed sustained release profile up to 5 h due to the slow erosion of the polymer at this pH and subsequent slow release of drug from the beads in this medium. Since, all the uncoated beads showed maximum drug release at acidic pH and hence these would not constitute an efficient colon drug delivery system. In order to prevent or minimize the drug release in the stomach and small intestine, the beads should be coated with pH sensitive polymers. The present investigation has revealed that, in spite of use of these natural polymers, the hydrophilic nature of these polymers make them vulnerable to release the drug to some extent in the upper digestive tract. Hence, they have less efficiency in site specific drug delivery. As a

result, the use of these polymers alone may not successfully target the drug to the colon. Hence there is a need of further coating of the tablet with pH dependent enteric polymer. Coated beads prevent the drug release at pH 1.2 and maximum amount of the drug was released at pH 7.4. The observed weight loss and drug release (up to 5 %) at lower pH may be due to the leaching of water soluble plasticizer, polyethylene glycol 400 into the aqueous medium and subsequent erosion of the polymer and subsequent release of water soluble drug in to the medium. At lower pH, eudragit S 100 is not soluble and hence no appreciable amount of drug was released[9]. At higher pH, the leaching was more rapid, explaining the high drug release at pH 7.4 due to higher solubility of eudragit S 100 at this pH. But due to the presence of sodium alginate matrix, subsequent sustained release was seen after erosion of film coat surrounding the bead. The dissolution profile of coated beads is shown in Figure 4 and 5. Once the coating dissolves, further drug release was controlled by the polysaccharides in the target area. When the drug release of formulations was carried out in the presence of rat cecal content (Fig. 3) there was an increase (10-23 %) in the release when compared to that of the release studies performed in absence of rat cecal content. The rat cecal content in the release study was considered to mimic the human colonic environment as it contains micro flora which releases many glycosidases and degrade the polysaccharide polymers. Hence, combination of enteric polymer coated formulations containing polysaccharides can be ideal for targeting the drug to colon[9,10].

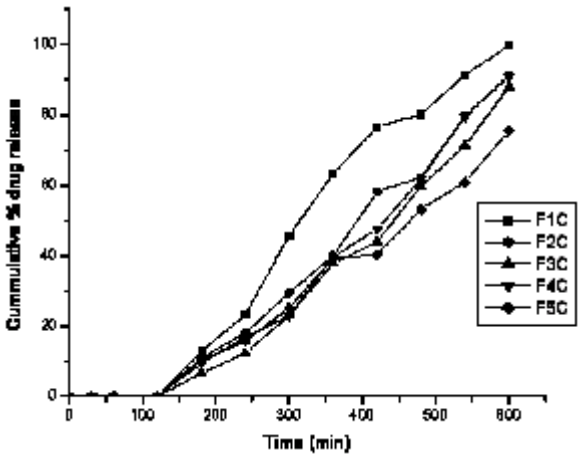


Fig. 4: *Dissolution profile of Coated beads in absence of rat cecal contents.*

In vivo targeting efficiency

In vivo study was carried out in order to know whether the developed coated beads reach the colon. From the X–ray images (Figure 6), it was concluded that, the coated beads have remained intact in the upper parts of GIT. The images of swollen beads in the colon indicate that drug release takes place mainly in the colon.

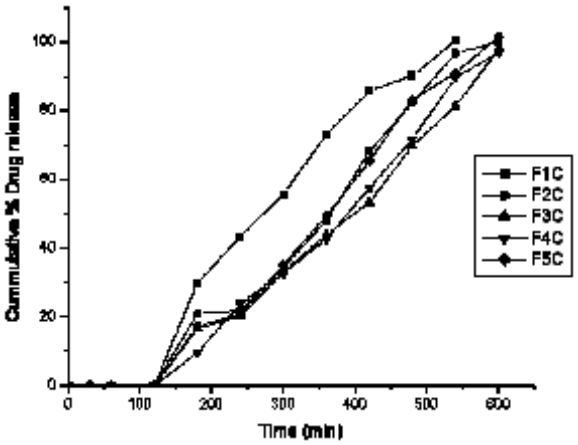
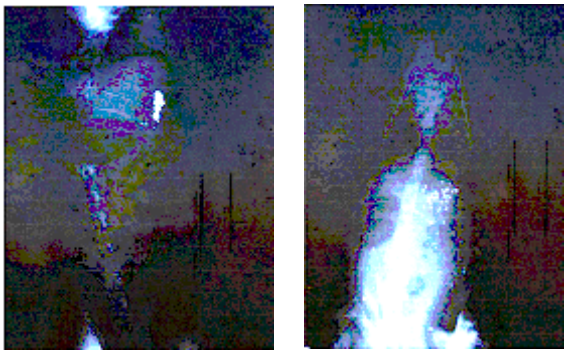


Fig. 5: *Dissolution profile of Coated beads in presence of rat cecal content*



After 1 h of administration **After 7 h of administration**

Fig. 6: *X – ray images showing the location of beads in different parts of GIT in rabbits at different time intervals*

Stability studies

All the formulations were observed for any change in color and appearance for a period of 3 weeks. The stability of the drug in the formulation was further confirmed by FTIR spectra and there was no spectral changes observed. Drug content estimation was also carried out as a part of stability studies. The results indicated that, there was no significant change with respect to drug content at the end of 3 weeks at, all the 3 temperatures.

Release kinetics

In order to determine the mechanism of drug release from the formulations, different models were examined which includes, zero order, first order, Higuchi's plot and Korsmeyer-Peppas model. Using the least square procedure the values of 'n' for all the systems under study were estimated. The values of 'R^2' and 'n' depend upon the extent of cross linking and the percentage loading of drug. For all the formulations, the values of the exponent 'n' were found to be between 0.55–0.8, indicated non-Fickian transport. This suggests that, the

transport is possibly controlled by diffusion and/or relaxation of the polymer chains.

The drug release from the polymeric systems was mostly by diffusion and is best described by Fickian diffusion. But in case of the formulations containing swelling polymers, as gellan gum and/or sodium alginate, other processes take place, like relaxation of polymer chains, imbibitions of water causing polymer swelling and considerable volume expansion. Korsmeyer and Peppas equation superposes two apparently independent mechanisms of drug transport, Fickian diffusion and a case-II transport, for the description of drug release from a swelling polymer. The relative complexity of the prepared formulations may indicate that, the drug release was controlled by more than one process; a coupling of diffusion and erosion. Also it was understood that, the formulations which were coated showed zero order release, whereas the uncoated beads showed more of first order release[11].

CONCLUSION

It is concluded that the formulation prepared in our laboratory is good to produce targeting as well as sustained drug release. The *in vitro* release study data treated with various kinetic equations, reveals that drug release follows fickian diffusion mechanism. The formulations were stable at accelerated stability study conditions for a period of three months.

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