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Microspheres For Colonic Delivery Of Betamethasone In Inflammatory Bowel Disease

HARISH K. KUNJWANI[1*], DINESH M. SAKARKAR[2]

[1] S.G.S.P.S. Institute of Pharmacy, Akola, MS, India

[2] S. N. Institute of Pharmacy, Pusad, MS, India

ABSTRACT

For treating colonic diseases, conventional oral drug delivery systems are not effective, as they fail to reach the appropriate site of action. Thus, there is a need to develop effective and safe therapy for the treatment of colonic disorders. The aim of the present study was to design a colon-specific delivery system for an anti-inflammatory synthetic glucorticoid, Betamethasone, with minimal degradation and optimum delivery of the drug with relatively higher local concentration, which may provide more effective therapy for inflammatory bowel disease including Crohn disease and ulcerative colitis. A multiparticulate system having pH sensitive property and specific enzyme biodegradability for colon targeted delivery of Betamethasone was developed. Tamarind gum microspheres were prepared by emulsion dehydration technique using different ratio of polymer. These microspheres were coated with Eudragit S-100 by oil in oil solvent evaporation method using core : coat ration (5:1) . Tamarind gum microspheres and Eudragit coated tamarind gum microspheres were evaluated for surface morphology, particle size and size distribution, percentage drug entrapment, surface accumulation studies, in vitro drug release in simulated gastrointestinal fluids. The prepared microspheres were spherical in shape in the size range of 53 µm to 190 µm, the encapsulation efficiency was in range of 64-80 % depending upon the concentration of gum. The drug release was about 10-12% in first four hours of study gradually rises in 5th hour and 80% drug release occurs in 8-10 hr thus showing desirable drug release in the colonic simulated environment.

KEYWORDS : Inflammatory bowel disease, Betamethasone, Colon, Microspheres.

INTRODUCTION

Inflammatory bowel disease (IBD) is a relapsing, debilitating, chronic, inflammatory disorder

of the gastrointestinal tract (GIT), having two major forms: ulcerative colitis (UC) and Crohn's disease (CD). The difference between UC and CD is the fact that in UC, the inflammation occurs in the colon, while CD could affect any part of the GIT, commonly the terminal ileum or the perianal region [1].Colon delivery of a therapeutic agent could reduce the systemic side effects and provide effective and safe therapy that may reduce the dose and duration of therapy when compared with the conventional treatment.

Presently various strategies have been used for targeting colon, like use of pH-sensitive polymers, coating with biodegradable polymers, synthesis of pro-drugs, timed release systems, embedding in biodegradable matrices and hydrogel system [2,3]. Polysaccharides, such as pectin, guar gum, chitosan and amylose, have extensively been studied and widely accepted for colon targeting [4]. The matrices of polysaccharides are used to remain intact in the physiological environment of stomach and small intestine, but after reaching the colon, they are acted upon by bacterial polysaccharases enzyme [5]. The combination of resistant core containing the drug coated with the pH sensitive polymer could result in better control over drug release from formulation. Also microspheres perform better in vivo than single unit systems as they spread thorough out the length of intestine causing less irritation , enjoy slower transit through the colon and give more reproducible drug release.[6] Tamarind gum matrices have been examined and tested for controlled drug delivery. Tamarind gum is degraded in colon by the enzymes released from colonic microbial flora and hence it can serve as an effective polysaccharide for colon-specific drug delivery [7]. Eudragit S 100 coating will provide pH sensitivity to the formulation.

Materials

Betamethasone was obtained as gift sample from Kremoint Pharma Pvt Ltd Mumbai. Tamarind gum was obtained from Mangalwedhe tamarind industry, Bijapur, Eudragit S-100 , Span 80, n Hexane, Ethanol, Acetone etc where of analytical grade.

Preparation of Microspheres

The Tamarind Gum microspheres were prepared by emulsion dehydration technique. The drug-polymer dispersion in distilled water (10ml) was dispersed in 35 ml coconut oil containing span 80 (1.0%w/v) and dispersion was continuously stirred at varied speed to obtain stable w/o emulsion. Then 50 ml of acetone was added, in order to dehydrate the tamarind gum droplets. The formulation was continuously stirred at 1000 rpm for 30 min at 30º C for complete solvent evaporation. Microspheres were dried

and washed with acetone. Similarly, the tamarind gum microspheres with varying compositions were prepared as shown in Table

Table 1. Formulation of Microspheres

Sr. No	Formulation Code	Drug : Tamarind Gum Ratio	Core : Coat Ratio
1	F1	1 :2	
2	F2	1 :3	
3	F3	1 :4	
4	F4	1 :5	
5	F5	1 :6	
6	EC1	1 :2	1:5
7	EC2	1 :3	1:5
8	EC3	1 :4	1:5
9	EC 4	1 :5	1:5
10	EC 5	1 :6	1:5

Coating of Microspheres

Tamarind gum microspheres were coated with Eudragit S-100 using oil-in-oil solvent evaporation method. Tamarind gum microspheres (50 mg) were dispersed in 10 ml of organic solvents mixture (acetone: ethanol, 2:1) containing Eudragit S-100 to give 1:5 core: coat ratio. This organic phase was added into 40 ml of light liquid paraffin containing 1% w/v span 80. The system was continuously agitated for 3 hr at 1000 rpm to evaporate the solvent at room temperature. Finally, the coated microspheres were filtered, washed with n-hexane and dried, stored in tightly capped container.

Evaluation of Microspheres

Scanning Electron Microscopy[8]:

The shape and surface morphology of tamarind gum microspheres and Eudragit- coated microspheres were investigated using scanning electron microscopy (SEM). The dried microspheres were coated with gold foil (100Aº) under an argon atmosphere in a gold coating unit and scanning

electron microscopy in both higher and lower resolution were observed. (Scanning Electron Microscope coupled EDAX model-JEOL-SEM- 6360).

Particle Size Distribution[9]:

The particle size distribution was done by the optical microscopy using a calibrated stage micrometer around 100 particles were calculated and mean diameter was calculated.

Percentage Yield[10]:

The percentage yield of all the batches were calculated on dry weight basis with respect to the solid materials added at the initial stage was calculated by using the following equation.

Percentage Yield = Amt of microspheres **/** Theoretical amount X 100

Drug Entrapment Efficiency[11]:

The drug entrapment efficiency was determined by dissolving 500 mg of microspheres in 100 ml of 0.2 M phosphate buffer pH 7.2 under sonication. After 24 hrs the filtrate was assayed spectrophotometrically at 242 nm. The drug content in the sample were calculated from the calibration plot and drug entrapment efficiency was calculated

$$\% \text{ Drug Entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Drug Loading[12]:

Drug loading was calculated according to formula:

$$\text{Drug Loading (\%)} = \frac{Qm}{Wm} \times 100$$

Where, Wm = Weight of the microsphere and Qm = Quantity of drug present in the microsphere

Surface Accumulation Study[12]:

This study was conducted to estimate the amount of drug present on the surface of the microspheres which may show immediate release in the dissolution media. 100 mg of microspheres (# 22 sizes) were suspended in 100 ml of phosphate buffer (pH 7.2), simulating the dissolution media. The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed by UV spectrophotometer Shimadzu- 1800, Japan at 242nm. Percentage of drug released with respect to entrapped drug in the sample was recorded.

In Vitro Drug Release Studies[13]

In vitro drug release studies were carried out using US Pharmacopoeia paddle type-II dissolution apparatus at 37 $\pm$ 0.5ºC with constant stirring rate of 100 rpm. Microspheres equivalent to 10 mg of Betamethasone valerate were used for the test. An accurately weighed sample was responded in dissolution media consisting 900 ml 0f 0.1 N (pH1.2) HCl and dissolution was done for 1hrs. The dissolution medium was then replaced with pH 4.5 phosphate buffer (900 ml) and drug release study was carried out for further 3 hr. After then the dissolution medium was replaced with phosphate buffer pH 7.2 (900 ml) and dissolution was continued for a further period of 5 hrs. Finally the dissolution medium was replaced with phosphate buffer 6.8 (900 ml) for a further period of 24 hrs as the average residence time for intestine A sample volume of 1 ml was withdrawn from each dissolution vessel at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and analyzed spectrophotometrically at 242 nm.

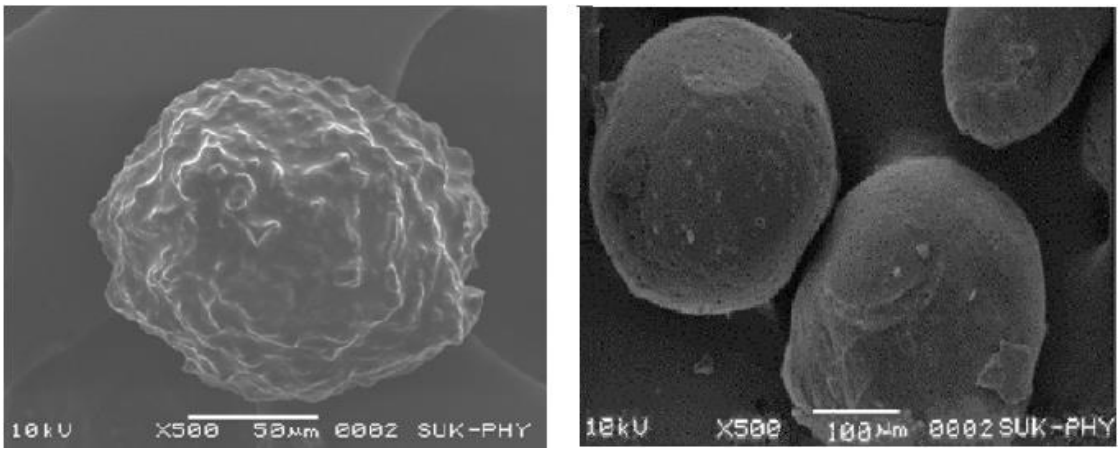
Kinetics of Drug Release [14]:

Data obtained from dissolution studies was fitted to various kinetic equations. The kinetic models were used zero order equation ($Q = Q_o - k_o t$), first order equation ($\ln Q = \ln Q_o - k_1 t$), Higuchi's equation ($Q = k_h t_{1/2}$) and Korsmeyer-Peppas equation, Log Q vs. Log t, where Q_t is cumulative amount of drug release at time t and Q_o is the initial amount of drug present in microspheres. k_o is the zero order release rate constant, k_1 is the first order release rate constant, and k_h is the diffusion rate constant. The coefficient of regression and release rate constant values for zero, first and Higuchi's and Korsmeyer- Peppas models were computed.

Result and Discussion

Tamarind gum microspheres were prepared by emulsion-dehydration technique without the use of chemical cross linking agents: to avoid the toxic and undesirable effects such as neurotoxicity, loss of protein bioactivity by chemical cross-linking agents (usually glutaraldehyde).

Surface Morphology of the Microspheres:



(a) (b)

SEM micrograph of microparticle prepared 1:3 drug: TKP ratio (a) uncoated microspheres (F3) (b) Eudragit S-100 coated microspheres (EC3)

The SEM analysis revealed that the shape and surface of uncoated microspheres prepared in this study were found to be spherical and rough in surface while Eudragit coated microspheres were found to be smooth in surface and spherical in shape.

Particle Size Distribution analysis: As the drug to polymer ratio was increased, the mean particle size of tamarind gum microspheres was also increased (Table). Mean particle size was found to be 73.25 µm with microspheres having 1:2 drug: polymer ratio while it was significantly increased to 161.37 µm up to the formulation F4. The significant increase may be because of the increase in viscosity of the droplets (due to increase in concentration of polymer solution). But further increase in polymer concentration results in difficult dispersion and thus subdivision of droplets may takes place, thus decreased in particle size (F5). The % yield increases with increase in polymer concentration. The increase in tamarind gum concentration increases encapsulation efficiency but further increase leads to increased viscosity with decreased encapsulation.. Eudragit coating decreases surface accumulation.

Table 2. Mean Particle Size, % Yield, % Drug Loading and % Drug Entrapment Efficiency

Formulation No.	Mean Particle Size (µm)	Percentage yield	Drug Loading Percent	Drug Entrapment Efficiency (%)	Surface accumulation w.r.t. Entrapped drug (%)
F1	73.25	68.13	48.92	69.54	11.24
F2	94.25	72.06	34.69	73.84	8.63
F3	138.4	74.12	26.98	78.45	6.59
F4	161.37	85.71	19.44	68.41	9.46
F5	53.37	87.23	16.37	64.79	4.35
EC 1	144.25	76.45		72.44	2.11
EC2	157.22	78.33		75.68	1.47
EC 3	162.19	69.44		79.88	1.43
EC 4	190.31	71.23		70.81	2.06
EC 5	127.47	81.21		74.12	1.88

The In vitro drug release of uncoated microspheres was carried out in different pH of gastrointestinal fluids. The effect of tamarind gum concentration was observed on in vitro drug release. Betamethasone valerate release from tamarind gum microspheres in SGF followed the order F1 > F2 > F3 > F4 > F5. The initial higher release of betamethasone valerate from microspheres might have resulted from the dissolution of drug on the surface of microspheres.

In case of batches with varying polymer concentrations, batch F1 released13.45% in pH 1.2, 25.69% in pH 4.5, 39.61% in pH 6.8 and 76.28% of drug in pH 7.2 which was higher than all the batches with varying polymer concentrations. It may be due to lower tamarind gum content.

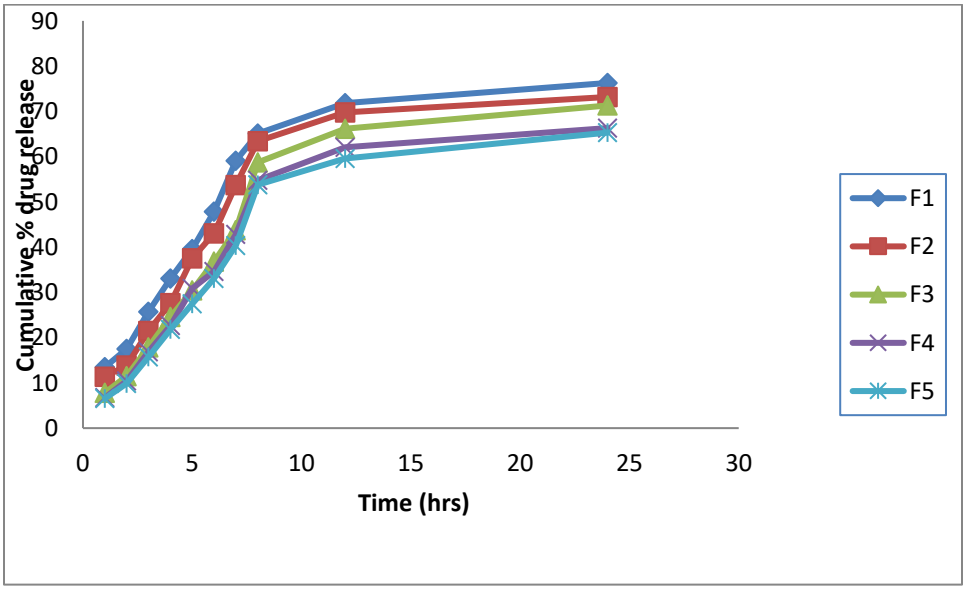
In case of F5 tamarind gum concentration was too high that it did release 6.56% in pH 1.2, 15.71% in pH 4.5, 27.49% in pH 6.8 and 65.31% in pH 7.2.

The coating of Eudragit S-100 on tamarind gum microspheres was done using oil-in- oil solvent evaporation method.

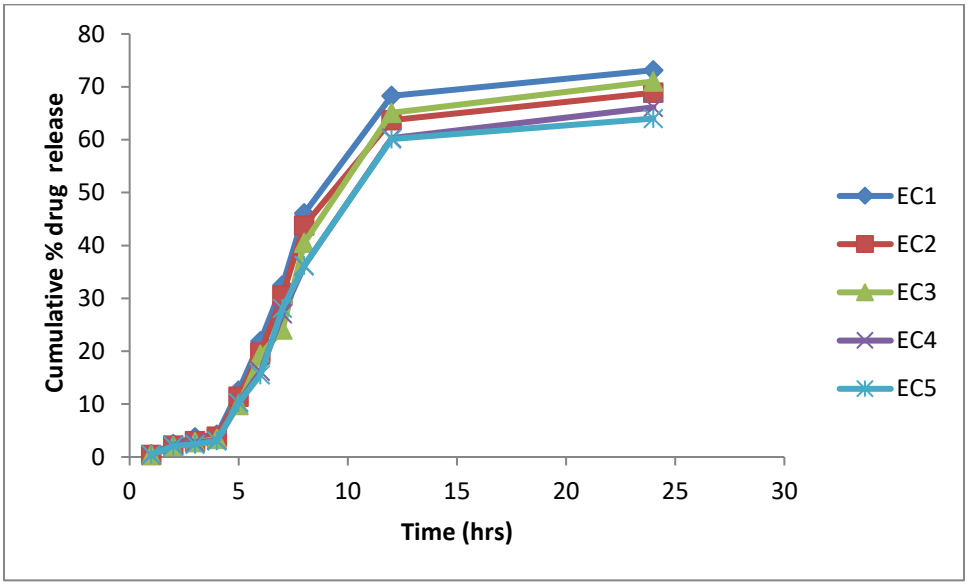
The results of in vitro drug release of multiparticulate system clearly revealed that Eudragit coated microspheres exhibited slow release, that is, only 10 – 12% drug releases in 5hrs. No measurable drug release was observed in simulated stomach fluid (pH 1.2 in 1 hr); and only 3 – 4% drug release was observed in a mixture of simulated gastric and intestinal fluid (pH 4.5). While in colonic fluid, 46.06% drug release was observed within 8 hrs, this could be due to dissolution of the Eudragit coat at pH 6.8 and on exposure of the tamarind gum microspheres were degraded and results higher percentage of drug release.

Thus, highest drug release from uncoated tamarind gum microspheres could be due to the fact that they are not able to maintain their integrity in upper part of GIT and show maximum release, while Eudragit coated tamarind gum microspheres maintain their integrity in upper part of GIT hence drug release was slow in comparison to uncoated microspheres.

Thus Eudragit coated tamarind gum microspheres has the potential for targeting the drug to colon.



Time Vs Cumulative % Drug Release Of Uncoated Microspheres



Time Vs Cumulative % Drug Release Of Eudragit coated Microspheres

Conclusion

Eudragit S-100 coated tamarind gum microsphere containing Betamethasone, maintain its integrity in the stomach and small intestine and release of drug in colon. Hence Eudragit S-100 tamarind gum microsphere can be utilized and are having potential for the site specific delivery

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