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Colon specific drug delivery of mesalamine using eudragit S100-coated chitosan microspheres for the treatment of ulcerative colitis

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ABSTRACT

The purpose of the present study was to prepare, characterize and evaluate the colon-targeted microspheres of mesalamine for the treatment and management of ulcerative colitis (UC). Microspheres were prepared by the ionic-gelation emulsification method using tripolyphosphate (TPP) as cross linking agent. The microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach. The prepared microspheres were evaluated for surface morphology, entrapment efficiency, drug loading, micromeritic properties and in-vitro drug release. The microspheres formed had rough surface as observed in scanning electron microscopy. The entrapment efficiency of microspheres ranged from 43.72%-82.27%, drug loading from 20.28%-33.26%. The size of the prepared microspheres ranged between 61.22-90.41µm which was found to increase with increase in polymer concentration. All values are statistically significant as $p<0.05$. Micromeritic properties showed good flow properties and packability of prepared microspheres. The drug release of mesalamine from microspheres was found to decrease as the polymer concentration increases. The release profile of mesalamine from eudragit-coated chitosan microspheres was found to be pH dependent. It was observed that Eudragit S100 coated chitosan microspheres gave no release in the simulated gastric fluid, negligible release in the simulated intestinal fluid and maximum release in the colonic environment. It was concluded from the study that Eudragit-coated chitosan microspheres were promising carriers for colon-targeted delivery of Mesalamine.

***Key Words*:** Ionic-gelation emulsification method, cross-linking, drug release, delivery, particle size, pH dependent.

INTRODUCTION

Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Oral drug delivery system represents one of the frontier areas of drug delivery systems. Such a dosage form manages common concern which exists in area of cost-efficient treatment, patient compliance, optimum drug delivery and bioavailability (Kumar *et al.*, 2012). The last two decades there has been a remarkable improvement in the field of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc, which modulates the release and absorption characteristics of the drug (Dehghan *et al.*, 2010).

Microspheres constitute an important part of this particulate drug delivery system by virtue of their small size and efficient carrier characteristics. However, the success of this novel drug delivery system is limited due to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing gastric mucosal membranes (Lohani and Gangwar, 2012). Microspheres are characteristically free powders consisting of proteins or synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200µm (Alagusundaram *et al.*, 2009).

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Colon specific drug delivery systems have gained increasing attention for the treatment of diseases such as Chrohn's disease, ulcerative colitis and irritable bowel syndrome (Patel *et al.*, 2010). Ulcerative colitis is a type of inflammatory bowel disease (IBD) that affects the lining of the large intestine (colon) and rectum. Repeated swelling (inflammation) leads to thickening of the intestinal wall and rectum with scar tissue. Death of colon tissue or severe infection (sepsis) may occur with severe disease (Burger and Travis, 2011). Mesalamine (5-ASA) is an anti-inflammatory drug used to treat crohn's disease and ulcerative colitis. Since Mesalamine (5-ASA) is largely absorbed from the upper intestine, selective delivery of drugs into the colon may be regarded as a better method of drug delivery with fewer side effects and a higher efficacy (Swapna *et al.*, 2011).

In the present study, an attempt has been made to prepare mesalamine microspheres prepared by ionotropic gelation method using chitosan as polymer and sodium tripolyphosphate (TPP) as the cross-linking agent. Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications owing to its biocompatibility, non-toxicity and mucoadhesive properties. TPP is an extensively researched well established, charged, non-toxic, multivalent, anionic cross-linking agent with five bonding sites on the molecules.

MATERIALS AND METHODS

Materials

Mesalamine was obtained from Zydus Cadila, Ahmedabad, India. Chitosan was a gift sample from Central Institute of Fisheries Technology, Cochin. Eudragit S100 was obtained from Ranbaxy Laboratories Limited, New Delhi, India. TPP was purchased from Loba Chemicals. All other chemicals used in experiment were of analytical grade and used as such.

Preparation of microspheres

Cross linked chitosan microspheres were prepared using ionic-gelation emulsion method. Chitosan solution (4% w/v) was prepared in 5% aqueous acetic acid solution in which the drug was previously dissolved and dispersed in liquid paraffin containing span 80 (1%w/v) (Gawde and Agrawal, 2012). The dispersion was stirred using a specially fabricated stainless steel half-moon paddle stirrer and saturated aqueous solution of TPP (1 ml to 3 ml), a cross-linking agent was added with continuous stirring. The stirring was continued for 4 h, prepared microspheres were centrifuged, washed twice with hexane to remove oily phase from the solution and acetone and were then dried in vacuum desiccators for 48 hrs.

Coating of chitosan microspheres

Chitosan microspheres were coated with Eudragit S-100 solvent evaporation method (Vasir *et al.*, 2003). Chitosan microspheres (50 mg) were dispersed in 10 ml of coating solution prepared by dissolution of 500 mg of Eudragit S-100 in ethanol: acetone (2:1). This organic phase was then poured in 70 ml of light liquid paraffin containing 1% w/v Span 80. The system was maintained under agitation with speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and dried in desiccators (Jain *et al.*, 2012).

Identification by FT-IR spectrophotometer

FTIR studies of mesalamine and formulation was carried out to find any possible interactions between the drug and the polymers during formulation (Garud *et al.*, 2011a). FTIR spectra of drug and drug-polymer in formulation were obtained in KBr pellets using a Perkin Elmer model spectrum BX-FTIR spectrophotometer in the ranges, 4000- 400 cm^{-1}.

Morphology and particle size

Shape and surface morphology of microspheres were studied using Scanning Electron Microscope (SEM LEO 430, Leo Electron Microscopy Ltd., England). For determination of surface characteristics all the microspheres were coated uniformly with gold palladium by using sputter coater for 5 to 7 minutes, after fixing the sample in individual steps. All samples of microspheres were then randomly examined for surface morphology at different magnification ranges. Particle size of the microcapsules was evaluated using optical microscopy method (Lachman and Lieberman, 1991). Approximately 100 microspheres were counted for particle size determination using a calibrated optical microscope (Magnus MLX-DX). The experiments were performed in triplicate (n=3).

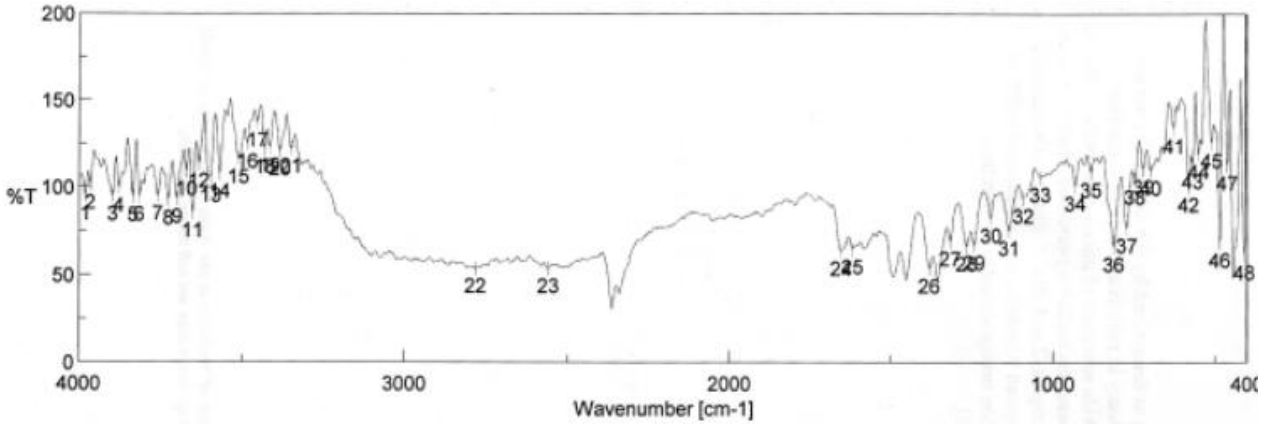

Figure 1: FT-IR Spectra of mesalamine.

Micromeretic properties

Accurately weighed microspheres were poured gently through a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped density were noted using tapping method using 10 ml measuring cylinder. Angle of repose (θ), Hausner's ratio (H) and Carr's index (% C) were calculated to study the flow properties of microspheres by using following formulas (Kancharla *et al.*, 2011):

$$\theta = \tan^{-1}\frac{h}{r}$$

where, h is height and r is radius of the pile, respectively.

$$H = \frac{Dt}{Db}$$

$$\%\,C = \frac{Dt - Db}{Dt} \times 100$$

where, Dt is tapped and Db is bulk density, respectively.

Entrapment efficiency, drug loading and % yield of microspheres

50 mg of microspheres were dispersed in 10 ml PBS pH 6.8 for 10 min with occasional shaking. The suspension was then centrifuged for 5 min and the supernatant was kept aside. The sediment microspheres were then incubated for 48 hrs with PBS pH 6.8 and the drug concentration was determined spectrophotometrically by UV at 334 nm (Shimadzu Pharmspec UV-1700, Japan). The entrapment efficiency, drug loading and % yield of microspheres (n=3) were calculated by using following formulas (Garud and Garud, 2011b):

$$\%\,EE = \frac{Dcal}{Dth} \times 100$$

where, Dcal is the calculated drug content and Dth is the theoretical drug content, respectively.

$$\%\,DL = \frac{Wd}{Wm} \times 100$$

where, Wd and Wm represents weight of drug and weight of microspheres, respectively.

$$\%\,Y = \frac{Wm}{Wt} \times 100$$

where, Wt represents total expected weight of drug and polymer

***In-vitro* release studies**

The drug release rate from the microspheres was studied in a medium of changing pH using the dissolution apparatus II at 37±0.5 °C with a rotation speed of 100 rpm. A weighed amount of mesalamine microspheres (equivalent to 50 mg of drug) were added to dissolution medium (350 ml of 0.1N HCl, pH 1.2) for the first two hours. At the end of second hour, the pH of the dissolution medium was raised to 4.5 by the addition of 250 ml solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH. At the end of fourth hour pH was raised to 7.4 by adding 300 ml of phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) (El-Bary *et al.*, 2012). At predetermined time intervals, 5 ml sample was withdrawn, passed through a 0.45 μm membrane filter (Millipore). After appropriate dilutions, the concentration of drug in samples was analysed spectrophotometrically at predetermined $\lambda_{max(s)}$. The initial volume of dissolution medium was maintained by adding 5 ml of fresh dissolution medium after each withdrawal.

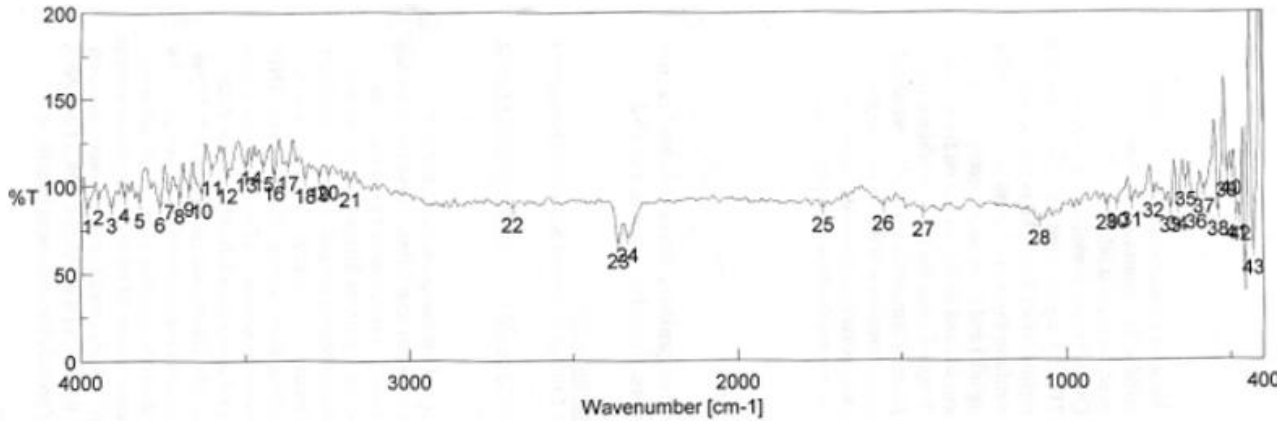

Figure 2: FT-IR Spectra of formulation.

Perfect sink conditions prevailed during the drug release studies.

Statistical analysis

The results were expressed in mean ± S.D. One way ANOVA (Analysis of Variance) was performed for studying the statistical significance using Minitab 15 software. Values of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Identification by FT-IR spectrophotometer

FTIR studies of mesalamine and prepared formulation is shown in Figure 1 and 2. It is clear from the FTIR that the characteristic peaks of the drug are also present in the formulation depicting no incompatibility between the drug and polymers in the formulation.

Morphology and particle size

Visual examination of the SEM indicated that the microspheres of mesalamine were spherical with varied surface roughness (Figure 3). The particle size of microspheres ranged from 61.22-90.41 µm and were found to increase with increasing polymer content ($p<0.05$) (Table 2). As the emulsifier concentration was increased from 0.5 to 1.5 ml, the particle size was found to increase in the prepared formulations ($p<0.05$).

Table 1: Formulation composition of cross linked chitosan polymer.

Sl. No.	Formulation Code	Drug:Polymer	Emulsifier concentration (ml)
1	M1	1:1	0.5
2	M2	1:1	1.0
3	M3	1:1	1.5
4	M4	1:2	0.5
5	M5	1:2	1.0
6	M6	1:2	1.5
7	M7	1:3	0.5
8	M8	1:3	1.0
9	M9	1:3	1.5

Micromeretic properties

For the prepared formulations angle of repose (11.65-16.29°), Carr's index (6.72-22.16%) and Hausner's ratio (1.10-1.29), confirmed good flow properties of the microspheres (Table 2).

Entrapment efficiency, drug loading and % yield of microspheres

The microencapsulation efficiency for the different formulations was high (ranged from 43.72% to 82.27%) and significantly increased with increasing chitosan content ($p<0.05$). Drug loading of microspheres was found to be ranging from 33.26±1.04 to 20.28±0.96 and it significantly decreased with increasing chitosan content ($p<0.05$). The % yield of microsphere significantly increased with increasing chitosan content ($p<0.05$) and ranged from 63.99% to 84.94% for the prepared formulations (Figure 4). An increase in polymer concentration resulted in formation of larger microspheres entrapping greater amount of drug (Swapna *et al.*, 2011).

***In vitro* release studies**

In the *in-vitro* release studies, changing the pH conditions was attempted in lieu to mimic the GI conditions without enzymes. The pH condition used was pH 1.2 for a period of 2 h (stomach), pH 4.5

Table 2: Angle of repose, Carr's index, Hausner's ratio and particle size (μm).

Formulation codes	Angle of repose Mean±SEM	Carr's index Mean±SEM	Hausner's Ratio Mean±SEM	Particle Size(μm) Mean±SEM
M1	16.29±0.68	6.72±0.43	1.29±0.30	72.21±1.93
M2	16.16±0.65	8.75±0.42	1.10±0.28	65.22±0.94
M3	15.52±0.63	19.25±0.39	1.23±0.31	61.22±1.28
M4	13.65±0.54	20.45±0.53	1.22±0.27	80.02±1.80
M5	13.85±0.59	20.34±0.56	1.25±0.27	74.15±0.84
M6	14.34±0.53	20.28±0.39	1.31±0.26	72.05±1.12
M7	11.65±0.61	20.17±0.41	1.34±0.30	98.91±1.20
M8	11.98±0.59	21.64±0.41	1.27±0.29	93.41±1.43
M9	12.34±0.52	22.16±0.55	1.28±0.27	90.41±1.83

(duodenum) for 2 h followed by pH 7.4 (distal ileum and colon) for the remaining duration of the study. A successful colon targeted drug delivery should have minimum drug release during its transit in the stomach and upper intestine to ensure maximum drug release in the colon (Chandran *et al.*, 2009).

Eudragit S100 is an anionic copolymer of methacrylic acid and methyl methacrylate, the ratio of free carboxyl groups to the ester groups is approximately 1:2. It exhibits a dissolution threshold pH slightly above 7.2 (Sinha and Kumria, 2003). Due to the pH-sensitive property of this polymer, it was selected to avoid the rapid dissolution of mesalamine during the initial transit of the microspheres through the gastric cavity and the upper small intestine.

The retardation in drug release was found to be significant with increasing polymer concentration ($p<0.05$). The increased density of polymer matrix at higher concentration resulted in an increased diffusion pathlength. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release (Srivastava *et al.*, 2005). However, increase in emulsifier concentration in the formulations showed insignificant results in the drug release rate ($p>0.05$). Eudragit coating of chitosan microspheres prevented the release of drug in stomach and targeted the delivery of drug to colon.

It was found that formulations with drug-polymer ratio of 1:1 (M1 to M3) released complete drug at 12 hours. A comparison of percentage release of drug from cross-linked chitosan microspheres vs time without coating is shown in Figure 5. A comparative % drug release of chitosan microspheres (M3, M6 and

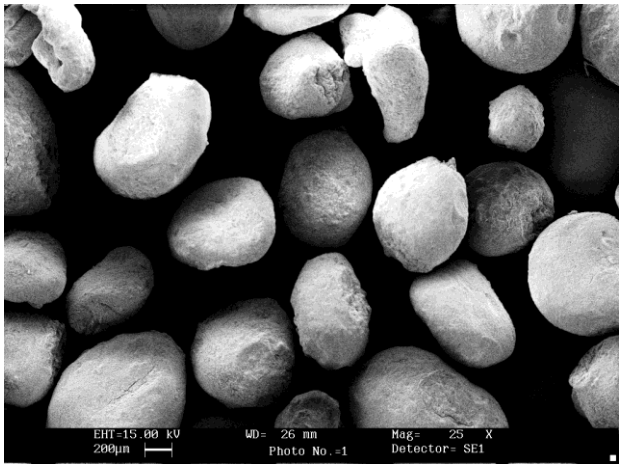

Figure 3: SEM of mesalamine-loaded chitosan microspheres.

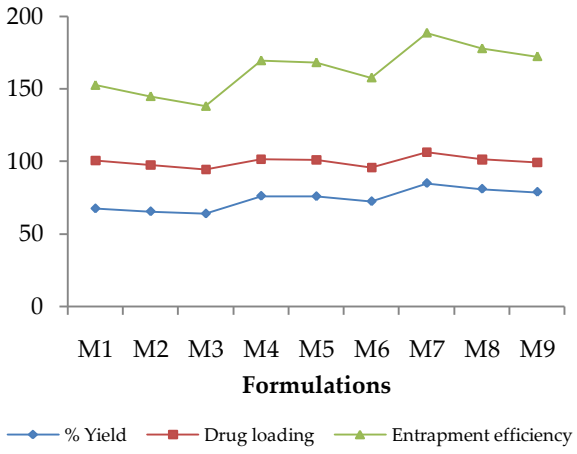

Figure 4: Percent yield, drug loading and entrapment efficiency of formulations.

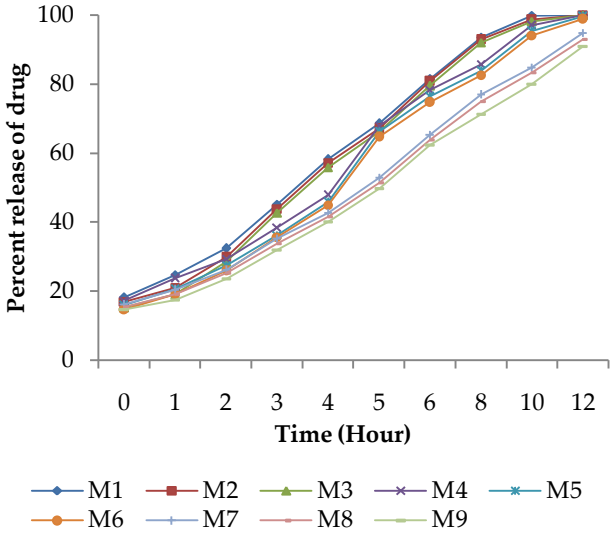

Figure 5: A comparison of percentage release of drug from cross-linked chitosan microspheres vs time (in hours) without coating.

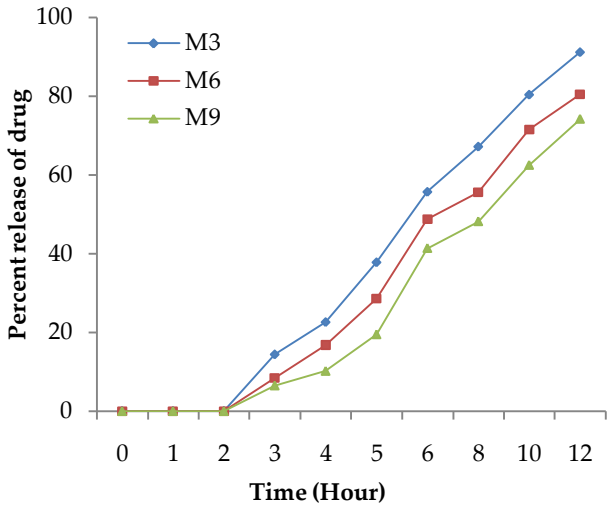

Figure 6: A comparative % drug release of chitosan microspheres (M3, M6 and M9) coated with Eudragit S-100 polymer.

M9) coated with Eudragit S-100 with drug-polymer ratio 1:1, 1:2 and 1:3, respectively at 1.5 ml emulsifier concentration is depicted in Figure 6. It was observed that Eudragit S100 coated chitosan microspheres gave no release in the simulated gastric fluid, negligible release in the simulated intestinal fluid and maximum release in the colonic environment.

CONCLUSION

Mesalamine microspheres were prepared successfully by using the ionic-gelation emulsification method. Prepared microspheres showed good % yield and drug loading. Encapsulation efficiency of microspheres was good for all formulations. The prepared microspheres with 1:3 ratio of drug-polymer coated with Eudragit S100 (M9) was found suitable for colonic release of mesalamine resisting drug release in gastric medium, minimizing release in the upper intestinal region and showing maximum release in the colonic region. Therefore, the developed formulation proves to be promising for the colon targeted drug delivery of mesalamine and thereby facilitating in the management of ulcerative colitis.

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