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Preparation and evaluation of magnetic microspheres of mesalamine (5-aminosalicylic acid) for colon drug delivery

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ABSTRACT

Objective: To study magnetic microspheres of mesalamine (5-aminosalicylic acid) for colon drug delivery. **Methods:** Magnetic microspheres were prepared by solvent evaporation technique for use in the application of magnetic carrier technology. An attempt was made to target mesalamine (5-aminosalicylic acid) to its site of action i.e. to colon. Eudragit S-100, ethylcellulose and chitosan were used in three different drug: polymer ratios i.e. 1:1, 1:2 and 1:3. The microspheres were characterized in terms of particle size, percentage yield, drug content, encapsulation efficiency, *in vitro* release pattern and *ex vivo* study. The microspheres were uniform in size and shape. The *in vitro* release profile was studied in pH 7.4 phosphate buffer medium using USP dissolution apparatus. **Results:** Chitosan microspheres were found to be better retained in terms of percentage release of the drug. Thus chitosan microspheres could be better retained at their target site. **Conclusion:** Flow characteristics are also better in case of chitosan magnetic microspheres. Thus reticuloendothelial clearance can be minimized and site specificity can be increased

1. Introduction

Pharmaceutical inventions is singly stressing 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[1]. The site specific delivery of the drugs to the target sites has the potential to reduce the side effects and improved pharmacological response[1,2]. Targeting by magnetic microspheres i.e. incorporation of magnetic particles in to drug carriers (Polymers) and using an externally applied magnetic field is one way to physically direct these magnetic drug carriers to a desired site[3]. Drug targeting is the delivery of drugs to receptors or organ or any other specific part of the body to which one wishes to deliver the drug exclusively. Various nonmagnetic micro carriers (nanoparticles, microspheres and micro particles etc.) are successfully utilized for drug targeting but they show poor site specificity and are rapidly cleared off by RES (reticuloendothelial system) under normal circumstances. Magnetism play an important role in these case, magnetic particles composed of magnetite which are well tolerated by the body, magnetic fields are believed to be harmless to biological systems and adaptable to any part of the body[4]. Colon specific drug delivery systems have gained increasing attention for the treatment of diseases such as Crohn's disease, ulcerative colitis and inflammatory bowel syndrome[5]. Magnetic microspheres will be formulated with an intension to produce a depot near the target organ, by placing a suitable magnet near it. From the depot, drug will be released slowly & carried to the target organ through blood. By localizing the drug carrier near the target organ, unwanted distribution of drug to non target organ can be avoided. This approach will localize the drug only at target site & minimize the drug-induced toxicity[6-9]. A major problem associated with all red blood cells (i.e. 7-8 mm), have a proper size range, and contain high concentrations of the magnetic material. However, the hydrophilic surface properties of magnetite compounds make it challenging to attain high magnetite content in hydrophobic biodegradable polymers such as Eudragit (S-100), ethyl

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cellulose and chitosan[10]. 5-aminosalicylic acid is one of the drugs of choice to treat ulcerative colitis because of its potential activity. Mesalamine has been shown to block the production of interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a)[11,12]. Mesalamine is a potent inhibitor of the cyclo-oxygenase pathway, inhibiting the production of prostaglandin E2 in inflamed intestinal specimens. In the present study, ethyl cellulose, Eudragit and chitosan magnetic microspheres loaded with mesalamine are formulated to target the drug at its site of action. 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. 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. Chitosan is a high molecular weight polycationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation. Chitosan has favorable biological properties such as non toxicity, biocompatibility and biodegradability. Magnetic chitosan microspheres have the ability to localize drugs by both biochemical and physical means

2. Materials and methods

2.1. Materials used

Mesalamine was obtained as a gift sample from Ipca Laboratories Ltd .Ethyl cellulose (Central Drug House lab. New Delhi). All other chemicals and reagents used were of analytical grade.

2.2. Method

2.2.1. Preparation of magnetite

The nitrogen gas was flushed through a 500 mL, two-necked round-bottom flask fitted with a condenser. The flask was charged with 8.9 g (0.1 mol) of FeO, 9.94 g (0.05 mol) of $FeCl_2 \cdot 4H_2O$ along with 250 mL deionized water and then 50 mL of 2 M NaOH was added while stirring vigorously. The reaction mixture was heated to reflux for 1-2 h. During the transformation of the pH, its pH fell from 14 to orange 8-9 and a black precipitate was formed. After precipitation was completed, the Fe_3O_4 particles were washed with distilled water, filtered and dried under vacuum at room temperature[13].

2.2.2. Formulation of magnetic microspheres

Microspheres were prepared by solvent evaporation technique. Accurately weighed but varying amounts of Eudragit S-100, ethylcellulose and chitosan were dissolved individually in 10 mL each of acetone over a cyclo-mixer, and accurately weighed drug was added to each of the polymer solution. 10 mg of magnesium stearate was then added to the solution of polymer and drug in acetone. Finally specified amount of magnetite was added to the drug-polymer solution. The organic phase was poured drop-wise to 25 mL of 1:1 mixture of light and heavy liquid paraffin with vigorous stirring over a mechanical stirrer. High stirring rates of approximately 4 000 rpm were employed to obtain microspheres of smaller size. Stirring was continued for eight hours. 20 mL of hexane was added to the stirred contents. The batch was filtered and washed thrice with hexane, 10 mL each, to remove any adhering liquid paraffin from the surface of microspheres. Then, several washings with distilled water were given to remove any un-entrapped drug on the surface of the microspheres. Several batches of microspheres were prepared by varying drug-polymer ratio, keeping all other formulation factors constant (Table 1)[14].

2.3. Characterization of magnetic microspheres of 5-aminosalicylic acid

2.3.1. Determination of percentage yield of microspheres

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was calculated using formula[15]

Percentage yield = (Practical yield/Theoretical yield) × 100.

Table 2 shows the percentage yield of the microspheres recovered.

Table 1
Formulation of magnetic microspheres.

Formulation code	Magnetite (mg)	Polymer	Drug (mg)	Drug: Polymer ratio	Method
F1	50	Chitosan (125mg)	125	1:1	Solvent evaporation
F2	50	Chitosan (166mg)	84	1:2	Solvent evaporation
F3	50	Chitosan (187mg)	63	1:3	Solvent evaporation
F4	50	Ethyl cellulose (125 mg)	125	1:1	Solvent evaporation
F5	50	Ethyl cellulose (166mg)	84	1:2	Solvent evaporation
F6	50	Ethyl cellulose (187 mg)	63	1:3	Solvent evaporation
F7	50	Eudragit (125mg)	125	1:1	Solvent evaporation
F8	50	Eudragit (166 mg)	84	1:2	Solvent evaporation
F9	50	Eudragit (187 mg)	63	1:3	Solvent evaporation

Table 2

Percentage yield of formulations of magnetic microspheres.

S.no.	Formulation code	Percentage yield
1	F1	70.96
2	F2	74.19
3	F3	80.64
4	F4	64.50
5	F5	72.58
6	F6	74.10
7	F7	67.74
8	F8	73.54
9	F9	77.41

2.3.2. Micromeritic 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: $\theta = \tan^{-1}h/r$; Where, h is height and r is radius of the pile, respectively.

$H=Dt/Db$

$\%\ C = Dt-Db/Dt \times 100$

Where, Dt is tapped and Db is bulk density, respectively[16]. Table 3 shows the flow characteristics of the prepared microspheres

Table 3

Depiction of flow properties of magnetic microspheres.

Formulation code	Carr's index (%)	Hausner ratio	Angle of repose	Flow character
F1	9.09	1.10	28.20	Excellent
F2	14.47	1.16	32.00	Good
F3	10.00	1.10	26.50	Excellent
F4	24.80	1.33	40.59	Passable
F5	25.30	1.33	41.18	Passable
F6	29.10	1.42	43.36	Poor
F7	10.00	1.10	28.20	Excellent
F8	20.00	1.20	26.50	Fair
F9	20.10	1.20	30.10	Fair

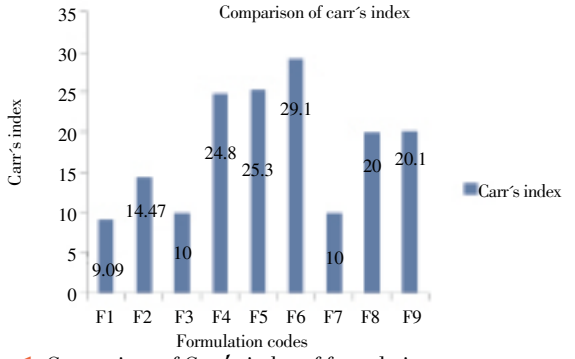

Figure 1. Comparison of Carr's index of formulations.

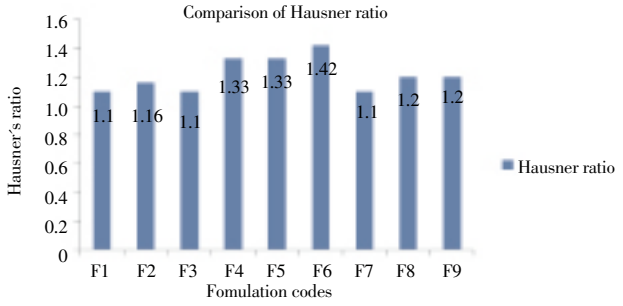

Figure 2. Comparison of Hausner ratio of formulations.

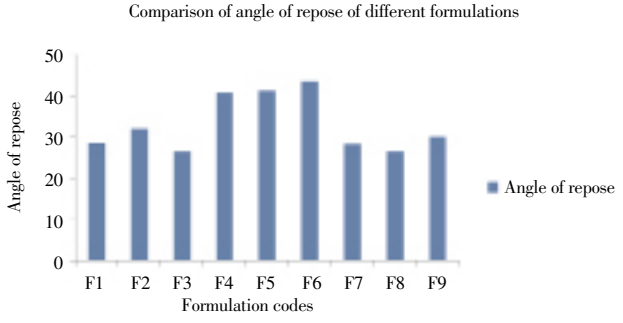

Figure 3. Comparison of angle of repose of formulations.

2.3.3. Drug entrapment efficiency

Magnetic microspheres equivalent to 10 mg were weighed and suspended in 10 mL solution (0.5 mL 0.1 N HCl + 9.5 mL PBS) for 5 min. The suspension was then filtered.

Table 4

Entrapment efficiency of different drug: polymer ratio magnetic microspheres of 5-aminosalicylic acid.

Formulation code	Drug content (in 10 mg microspheres)	Drug content (in total microspheres recovered)	Drug content (in 100 mg microspheres)	Entrapment efficiency (%)
F1	5.00	110.00	50.00	88.00
F2	3.23	76.59	33.30	80.30
F3	2.83	70.80	28.30	94.40
F4	4.50	90.00	45.00	72.00
F5	3.16	71.20	31.66	84.70
F6	2.83	65.00	28.30	86.60
F7	4.60	97.80	46.60	78.20
F8	3.33	75.90	33.30	90.30
F9	2.83	67.90	30.00	90.56

The digested homogenate was centrifuged for and the supernatant was analyzed for drug content by measuring the absorbance at 230 nm by UV-Vis spectrophotometer (UV1800 Shimadzu) after appropriate dilutions with PBS[17].

Entrapment efficiency = Experimental drug content / Theoretical drug content × 100

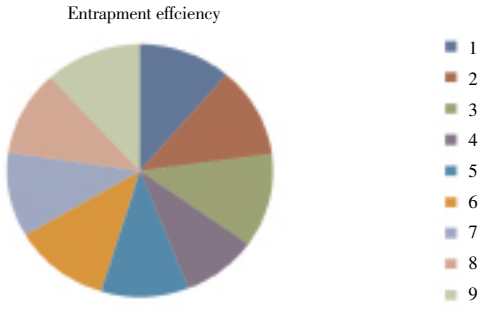

Figure 4. Comparison of entrapment efficiency.

2.3.4. Particle size analysis

It was carried out by using compound microscope. Dried magnetic microspheres were firstly redispersed in distilled water. These were then placed on a glass slide. The number of divisions of the calibrated eyepiece was counted by a micrometer using the stage micrometer[18]. Table 5 shows the particle size of the prepared formulations with different drug: polymer ratios.

Table 6

Particle size of formulations with codes F1 to F9.

S.no.	Formulation code	Particle size (μ m)
1	F1	153
2	F2	169
3	F3	184
4	F4	200
5	F5	215
6	F6	230
7	F7	153
8	F8	184
9	F9	200

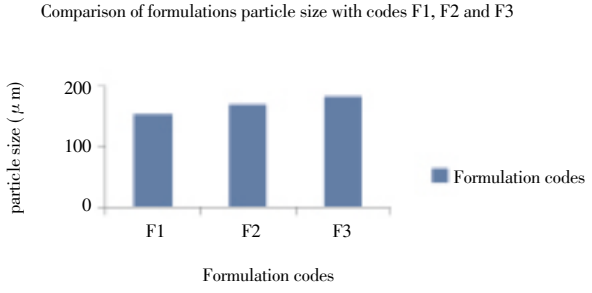

Figure 5. Particle size of formulations with formulation codes F1, F2, F3.

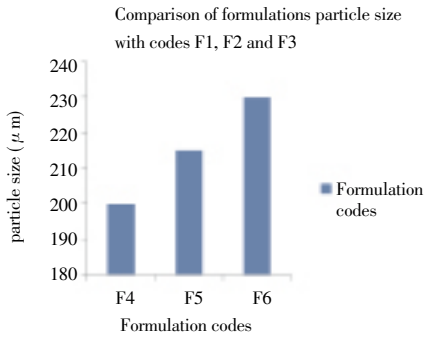

Figure 6. Particle size of formulations with formulation codes F4, F5, F6.

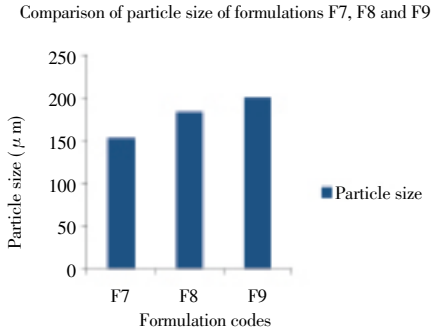

Figure 7. Particle size of formulations with codes F7, F8, F9.

2.3.5. Dissolution studies.

Drug release tests were performed according to USP XXIV paddle method for each size fraction separately. Accurately weighed amounts (100 mg) of microspheres were introduced into 900 mL of PBS (phosphate buffer saline, pH 7.4) and stirred with 100 rpm at (37.0±0.5) ℃. Five milliliters samples were withdrawn and filtered at selected time intervals. The concentration of mesalamine was determined spectrophotometrically at 230 nm[19].

Dissolution studies of formulations

Table 7

In vitro release study of different formulations.

Formulation codes	Drug : Polymer ratio	Percentage release
F1	1:1	93.00
F2	1:2	82.40
F3	1:3	74.70
F4	1:1	80.66
F5	1:2	74.83
F6	1:3	72.05
F7	1:1	84.97
F8	1:2	78.37
F9	1:3	74.57

Table 8
Swelling ratio of magnetic microspheres with respect to number of days.

Formulation code	Weight of microspheres In dry state (Wd) (mg)	Weight of adsorbed water (WS)			Swelling ratio (WS+Wd)/Wd		
		Day1	Day2	Day3	Day1	Day2	Day3
F1	10	1.0	2.0	2.0	1.1	1.2	1.2
F2	10	1.0	2.0	3.0	1.1	1.2	1.3
F3	10	1.0	2.0	5.0	1.1	1.2	1.5
F4	10	1.0	3.0	3.0	1.1	1.2	1.3
F5	10	1.0	2.0	3.0	1.1	1.3	1.3
F6	10	1.0	3.0	4.0	1.1	1.2	1.4
F7	10	1.0	1.0	2.0	1.1	1.1	1.2
F8	10	1.0	2.0	3.0	1.1	1.2	1.3
F9	10	1.0	2.0	3.0	1.1	1.3	1.3

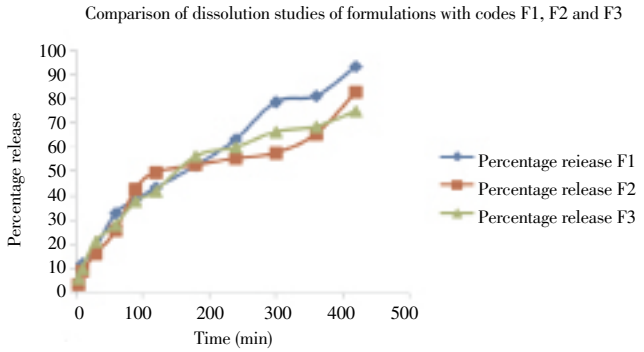

Figure 8. Percentage release of formulations with codes F1, F2, F3.

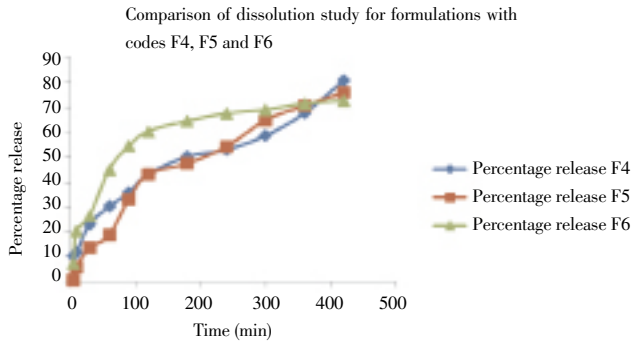

Figure 9. Percentage release of formulations with codes F4, F5, F6.

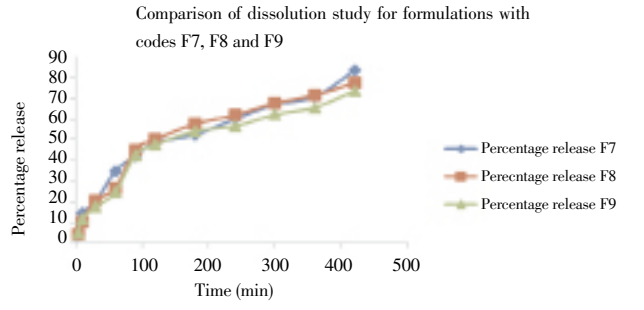

Figure 10. Percentage release of formulations F7, F8, F9.

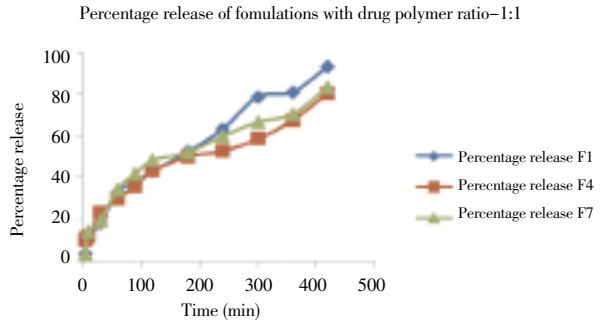

Figure 11. Percentage release of formulations with drug: polymer ratio -1:1.

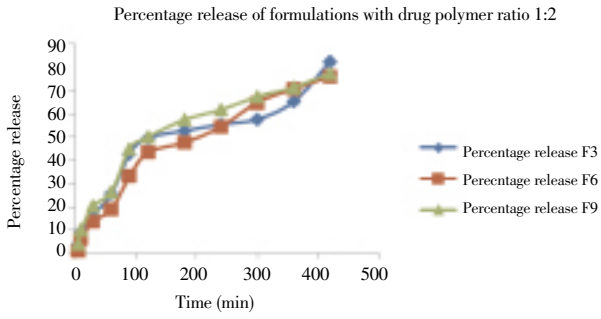

Figure 12. Percentage release of formulations with drug: polymer ratio-1:2.

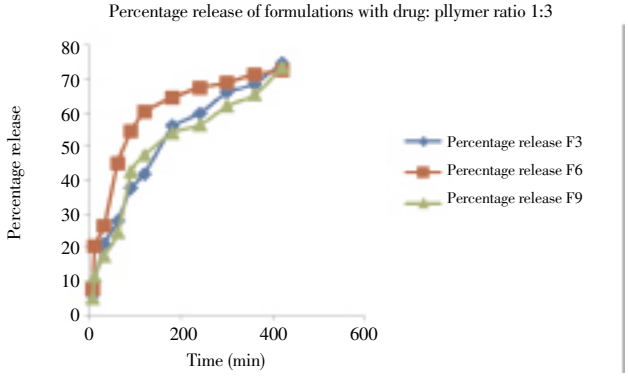

Figure 13. Percentage release of formulations with drug: polymer ratio 1:3.

2.3.6. Measurement of swelling kinetics of magnetic microspheres

Swelling kinetics of the magnetic microspheres was determined by swelling ratio (SR) at a given time. Dried microspheres were immersed in distilled water at each predetermined time at room temperature. Then, the sample was removed from distilled water and was frequently weighed after it was trapped with a filter paper to remove excess water on the surface. Thus, the wet weight of the microspheres was recorded during the swelling period at regular time intervals. The swelling ratio (SR), (Ws + Wd)/Wd, is defined as the ratio of the total weight of water in swollen microspheres to the weight of the dried microspheres, where Ws is the weight of adsorbed water and Wd is the weight of the microspheres at the dry state[20-22].

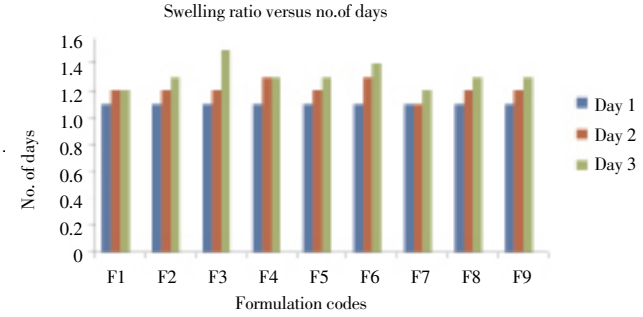

Figure 14. Swelling ratio of microspheres with respect to number of days.

3. Result

Chitosan magnetic microspheres were found to be best in terms of *in vitro* release characteristics. Drug encapsulation efficiency is also better in chitosan microspheres. However swelling ratio varies with drug content also. As the more drugs are entrapped thus water molecules cannot acquire much space and thus results in low swelling ratio.

4. Discussion

Flow characteristics are also better in case of chitosan magnetic microspheres. Thus reticuloendothelial clearance can be minimized and site specificity can be increased.

Conflict of interest statement

We declare that we have no conflict of interest.

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