RESEARCH ARTICLE

Formulation, characterization and *in vitro* evaluation of floating microspheres of famotidine as a gastro retentive dosage form

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The present study involves preparation and evaluation of floating microspheres using famotidine (FM) as a model drug for prolongation of the gastric retention time. The microspheres were prepared by the solvent evaporation method using different polymers, i.e. acrycoat S100 and cellulose acetate. The size or average diameter (d_{avg}) and surface morphology of the prepared microspheres were recognized and characterized by the optical and scanning electron microscopic methods, respectively. *In vitro* drug release studies were performed and the drug release kinetics were evaluated using the linear regression method. Effects of the stirring rate during preparation, polymer concentration on the size of the microspheres and drug release were also observed. The prepared microspheres exhibited prolonged drug release (18 h) and remained buoyant for more than 12 h. The mean particle size increased and the drug release rate decreased at a higher polymer concentration. No significant effect of the stirring rate during preparation on drug release was observed. *In vitro* studies demonstrated a diffusion-controlled drug release from the microspheres. The objective of the present study was to develop floating microspheres of FM in order to achieve an extended retention in the upper gastrointestinal tract, which may result in enhanced absorption and thereby improved bioavailability. The prepared microspheres were evaluated for particle size, *in vitro* release and buoyancy and incorporation efficiency. The effect of various formulation variables on the size and drug release was investigated. *In vitro* drug release studies were performed and the drug release kinetics were evaluated using the linear regression method. FM was obtained as a gift sample from Intas Pharmaceuticals, Ahmedabad, India. Polyvinyl alcohol was obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Dichloromethane, acrycoat S100, cellulose acetate and Tween 80 were obtained from Central Drug House (P) Ltd., Delhi, India. All other chemicals/reagents used were of analytical grade. A UV/visible spectrophotometer was used for drug analysis. Experimental results were expressed as mean ± SD. Chi-square test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant at $P = 3.23$, DF = 1, i.e. $P < 0.05$. The prepared floating microspheres exhibited prolonged drug release, i.e. <18 h, and the floating time was <12 h in 0.1 N HCl. The mean particle size of the prepared floating microspheres increased but the drug release rate from the microspheric-coated layer decreased as the polymer concentration increased. No significant effect of the stirring rate during preparation on drug release was observed. *In vitro* data obtained for floating microspheres of FM showed excellent floatability, good buoyancy and prolonged drug release. Microspheres of different size and drug content could be obtained by varying the formulation variables. Diffusion was found to be the main release mechanism. Thus, the prepared floating microspheres may prove to be potential candidates for multiple-unit delivery devices adaptable to any intragastric condition.

Key words: *Bioavailability, famotidine, floating microspheres, in vitro release*

INTRODUCTION

Floating drug delivery systems are among the several approaches that have been developed in order to increase the gastric residence time of the dosage forms.[1-3] The multipleunit system has been developed to identify the merit over a singleunit dosage form because the singleunit floating systems are more popular but have a disadvantage owing to their "all-or-nothing" emptying process, leading to high variability of the gastrointestinal transit time.[4,5] Still, the multipleunit dosage forms may be better suited because they are claimed to reduce the intersubject variability in absorption and lower the probability of dose dumping.[6] Such a dosage form can be widely distributed

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throughout the gastrointestinal tract (GIT), which afforded the possibility of a longer lasting retention and more reliable release of the drug from the dosage form.[7] The synthetic polymers have been used to prepare floating microspheres. Kawashima *et al.* prepared hollow microspheres or microballoons of ibuprofen by the emulsion–solvent diffusion method using acrylic polymers.[8] These microspheres exhibited good *in vitro* floatability but showed drastically decreased drug release with increasing polymer concentration. Floating microspheres of cellulose acetate loaded with three different model drugs were prepared by the solvent diffusion–evaporation method.[9] Those prepared microspheres remained buoyant for more than 12 h, but methylcellulose and chitosan micropellets loaded with lansoprazole as a model drug have a lower density than gastric contents and exhibited better encapsulation efficiencies.[10] Other polymer solution systems have been used to prepare floating microspheres such as polycarbonate/dichloromethane,[11,12] cellulose acetate butyrate/eudragit RL100 mixture in acetone[13] and eudragit S100/i-propanol.[14] The present study was based on famotidine (FM) used as a model drug. It is a histaminic drug that has been widely used in treating gastric and duodenal ulceration and also in Zollinger Ellison syndrome and reflux esophagitis.[15] It is poorly absorbed from the lower GIT and has a short elimination half life (3 h).

MATERIALS AND METHODS

Famotidine was received as gift sample from Intas Pharmaceutical Pvt. Ltd., Ahmedabad, India. Cellulose acetate and Acrycoat S100 was purchased from Corel Pharmaceutical Chemicals, Ahmedabad, India. Cellulose Acetate was procured from Ottokemi, Mumbai, India. Polyvinyl alcohol, Hydrochloric acid and Tween 80 were procured from Central Drug House Ltd., Delhi, India. Dichloromethane and Ethanol were purchased from E. Merck (India) Ltd., Mumbai. All the other chemicals used were of analytical grade.

Preparation of floating microspheres

Microspheres were prepared by the solvent evaporation technique as employed by Struebel.[16] FM and acrycoat S100/cellulose acetate were dissolved in a mixture of the solvent system [Table 1] at room temperature. This was poured into a 150 ml 0.1 M acidic solution containing polyvinyl alcohol maintained at a temperature of 30-40°C and subsequently stirred at speed [Table 1] for 2 h to allow the volatile solvent to evaporate completely. The microspheres formed were collected by filtration using a nylon cloth, washed repeatedly with distilled water and dried in vacuum or for 1 h at room temperature and subsequently stored in a desiccator over fused calcium chloride.

Table 1: Different formulations of the floating microspheres

Code	Drug: polymer ratio	Organic solvent system		Polyvinyl alcohol (% w/v)
C1*	1:1	Ethyl acetate:acetone	1:1	0.05
C2*	1:2	Ethyl acetate: acetone	1:1	0.05
C3*	1:3	Ethyl acetate:acetone	1:1	0.05
C4**	1:2	Ethyl acetate:acetone	1:1	0.05
C5***	1:2	Ethyl acetate:acetone	1:1	0.05
A1*	1:1	Dichloromethane:ethanol	1:1	2.0
A2*	1:2	Dichloromethane:ethanol	1:1	2.0
A3*	1:3	Dichloromethane:ethanol	1:1	2.0

Stirring rate = *300 rpm, **500 rpm and ***1000 rpm

Size and shape of the microspheres

The size distribution in terms of average diameter (d_{avg}) of the microspheres was determined by an optical microscopic method. A compound microscope fitted with a calibrated ocular micrometer and a stage micrometer slide was used to count at least 100 particles (Olympus NWF 10x; Educational Scientific Stores, India). Scanning electron microscopy (SEM; Department of geology, M. L. S. university, Udaipur) was performed to characterize the surface morphology of the formed microspheres (Philips-XL-20; the Netherland). The parameters of SEM were an acceleration voltage of 20 kV, a chamber pressure of 0.6 mmHg and an original magnification of X800.

Flow properties

Flow properties were determined in terms of Carr's index (I_c) and Hausner's ratio (H_R) using the following equations:

$$H_R = \rho_t / \rho_b$$

$$I_c = \rho_t - \rho_b / \rho_t$$

where, ρ_t = tapped density

ρ_b = bulk density

The angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by the fixed funnel method, using the following equation:

$$\tan \theta = 2H/D$$

where, 2H/D is the surface area of the free-standing height of the heap that formed after making the microspheres flow from the glass funnel.

Table 2 shows the flow properties of the prepared floating microspheres.

Table 2: Angle of repose, Carr's index and Hausner's ratio as an indication of powder flow properties

Angle of repose (°)	Carr's index (%)	Hausner's ratio	Type of flow
>20	5-15	-	Excellent
20-30	12-16	<1.25	Good
30-40	18-21	-	Fair to passable
-	23-35	>1.25	Poor
-	33-38	1.25-1.5	Very poor
>40	>40	-	Extremely poor

Incorporation efficiency

To determine the IE, microspheres (100 mg) were taken, thoroughly crushed by trituration and suspended in a minimal amount of dichloromethane for dissolving the coat shell of the microspheres. The suspension was suitably diluted with water and filtered to separate the shell fragments. Drug content was analyzed after suitable dilution by spectrophotometry at 265 nm (Thermospectronic, UV 1-103909; England). The amount of drug incorporation in the microspheres was calculated by the following formula:

IE = (amount of drug actually present/theoretical drug load expected) × 100

Buoyancy percentage

An *in vitro* buoyancy study was carried out using an USP XXIV dissolution apparatus (type II) (DA-6DR USP standards; Veego-Scientific, Mumbai, India) filled with 900 ml 0.1 M acidic solution (HCl) containing 0.02% Tween 80[17] as a dispersing medium. The medium was agitated with a paddle rotating at a speed of 100 rpm for 12 h. After each time interval, two fractions of the microspheres were observed, one was floating on the surface of the medium and the other was the settled portion. The settled portion of the microspheres was collected and recovered separately at a pre-determined time interval, dried in vacuum and weighed. The buoyancy percentage was calculated by the following formula:

% buoyancy of microspheres = (weight of floating microspheres/initial weight of floating microspheres) × 100

***In vivo* floating behavior**

In vivo floating behavior of the prepared microspheres was investigated by microspheres loaded with barium sulfate used as a diagnostic agent or as a core material for the justification of floating behavior. Microspheres in gelatin capsules were administered with 100 cm^3 of water to a dog after a light meal. After a 10-h period, the dog's stomach was X-rayed. This study was performed in the Government Veterinary Hospital, Udaipur, Rajasthan.

***In vitro* release**

A USP basket apparatus has been used to study drug release from the prepared floating microspheres.[18-20] In the present study, drug release was studied using a modified USP XXIV[17] dissolution apparatus type I (basket mesh # 120, equals 125 μm) (DA-6DR USP standards; Veego-Scientific) at 100 rpm in 0.1 mol/l hydrochloric acid (pH 1.2) as the dissolution fluid (900 ml) maintained at 37 ± 0.5°C. The withdrawn samples (5 ml) were analyzed spectrophotometrically as stated above. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Floating microspheres were prepared by the solvent evaporation method using various proportions of drug and polymer, such as cellulose acetate (batch C1-C5) and acrycoat S100 (batch A1-A3), by variation of the stirring rate for qualitative and quantitative determination of the microspheric characteristics [Table 1]. It was found that cellulose acetate-containing microspheres showed a desirable high drug content, good flow properties, buoyancy and adequate release characteristics; hence, formulations prepared by such a polymer are suitable for the development of gastric retention dosage forms. To precisely understand and quantify the effect of the drug–polymer ratio, the effect of process variables such as stirring speed was used.

The surface morphology was observed by scanning electron microscopic photographs, which showed that the fabricated microspheres were spherical with a smooth surface [Figure 1]. The presence of pores was detected on the microspheric surface, which indicated leaching of the drug during the dissolution without gelation of the polymeric surface.

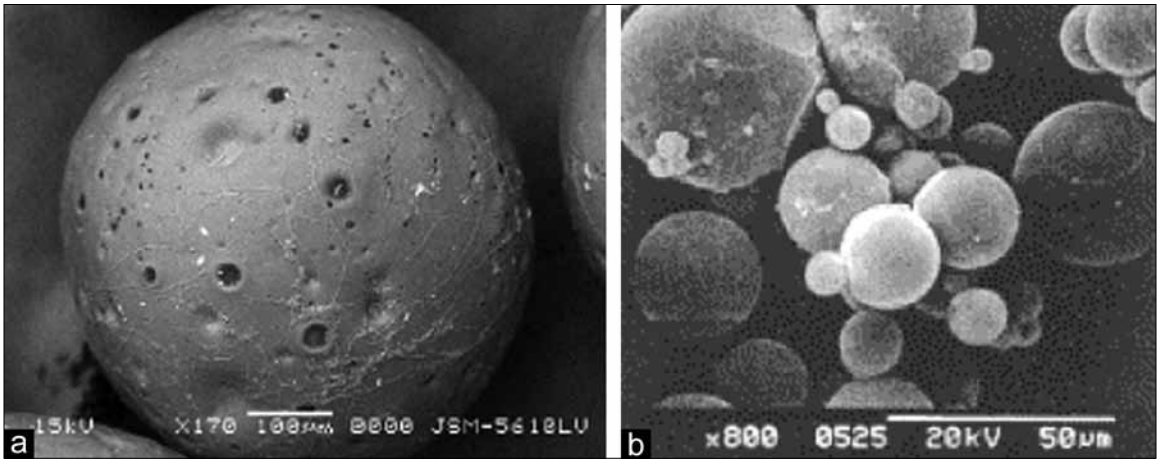

Figure 1: Surface morphology of the floating microspheres by scanning electron microscopic photographs (the parameters of scanning electron microscopy were acceleration voltage of 20 kV, chamber pressure of 0.6 mmHg and original magnification ×800)

Microspheres were prepared using a gradually increasing polymer concentration in combination with a fixed concentration of the drug to assess the effect of polymer concentration on the size of the microspheres. The mean particle size or average diameter (d_{avg}) of the microspheres significantly increased with increasing cellulose acetate concentration ($P = 4.5$, DF = 2, i.e. $P < 0.05$) and was in the range 368.2 ± 2.128-389.3 ± 1.984 μm [Table 1]. Larger particles developed due to increased viscosity of the medium with an increasing higher polymeric concentration. This is because at higher viscosities there is enhanced interfacial tension and diminished shearing efficiency. Thus, as expected, the higher polymeric concentrated microspheres influence particle size, drug IE and drug release of the microspheres. Cellulose acetate polymer-containing microspheres were smaller in size and had a smooth surface than the acrycoat S 100 polymer-coated microspheres. Microspheres prepared by a higher stirring rate showed smaller sized particles and lower drug content but the size of the microspheres reduced and thus changed the flow properties of the microspheres than the other formulations [Tables 1 and 2]. Those particles or microspheres having a smaller size showed good flow properties [Table 3].

All formulations floated for more than 12 h over the surface of the dissolution medium without any apparent gelation. The microspheres showing lower densities (having a hollow core; Figure 1), influenced buoyancy, and they were to be retained for longer than 12 h, which helped in improving the bioavailability of the drugs used for gastric ulcerative treatment. Buoyancy percentage of the microspheres is shown in Table 1 and the *in vivo* buoyancy photograph of the dog's stomach is shown in Figure 2.

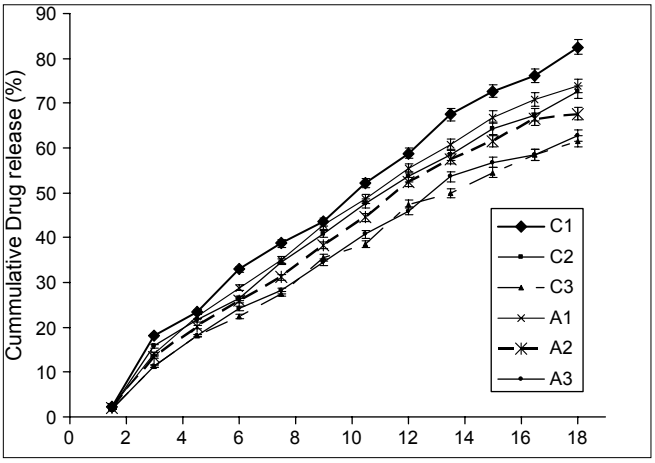

Figure 3: Effect of cellulose acetate (batches C1-C3) and acrycoat S100 (batches A1-A3) polymeric concentrations on the *in vitro* release of famotidine from the floating microspheres (bars represent mean ± SD, *n* = 3, codes in Table 2)

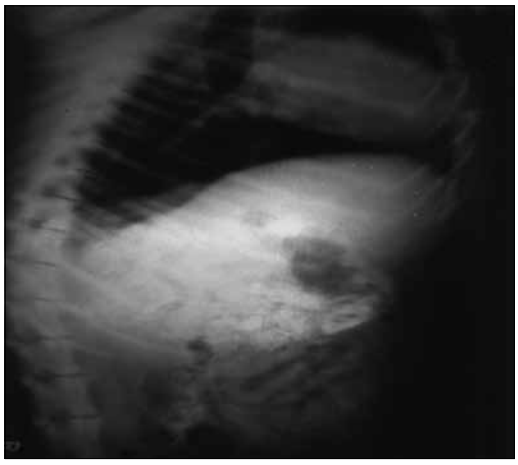

Figure 2: X-ray photograph of the dog's stomach showing buoyancy behavior of cellulose acetate floating microspheres after 10 h

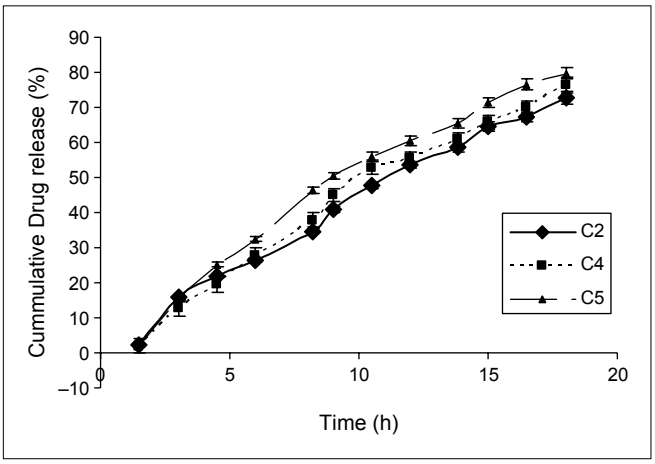

Figure 4: Effect of stirring rate during microsphere preparation on the *in vitro* release of famotidine from the floating microspheres (bars represent mean ± SD, *n* = 3, codes in Table 2)

Table 3: Various parameters of characterization of the formulations

Code	Mean particle size[a]	Angle of repose[a] (θ)	Carr's index[a] (%)	Hausner's ratio[a]	Incorporation efficiency (%)[b]	Buoyancy percentage[b]
C1*	375.1 ± 1.818	21.390 + 0.671	13.253 + 0.624	1.152 + 0.013	86.15 ± 0.044	70.6 ± 2.1
C2	378.7 ± 2.914	23.410 + 0.035	14.285 + 0.345	1.66 + 0.023	83.15 ± 0.011	69.2 ± 3.3
C3	389.3 ± 1.984	22.820 + 0.553	13.496 + 0.413	1.156 + 0.072	84.15 ± 0.012	62.6 ± 3.6
C4*	372.2 ± 1.154	25.390 + 0.308	13.812 + 0.823	1.160 + 0.062	85.23 ± 0.025	61.25 ± 4.1
C5*	368.2 ± 2.128	29.810 + 0.071	12.972 + 0.316	1.149 + 0.012	83.56 ± 0.035	63.17 ± 3.6
A1	380.8 ± 2.164	22.520 + 0.351	12.359 + 0.749	1.166 + 0.039	83.15 ± 0.032	62.2 ± 2.1
A2	383.6 ± 1.854	23.460 + 0.421	10.054 + 0.613	1.141 + 0.013	83.51 ± 0.034	58.1 ± 1.8
A3	390.6 ± 1.931	24.310 + 0.312	11.053 + 0.931	1.148 + 0.027	81.35 ± 0.016	60.6 ± 1.9

[a]Mean ± SD, n = 10; [b]Mean ± SD, n = 3; *Statistically significant ($P < 0.05$)

In vitro FM release studies were performed in 0.1 mol/l hydrochloric acid for 18 h. The cumulative release of FM significantly decreased with increasing polymer concentration ($P = 3.2$, DF = 1; i.e., $P < 0.05$; Figure 3). The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres formed at a lower polymer concentration and having a larger surface area when exposed to the dissolution medium showed a faster drug release [Figure 4]. The data obtained for *in vitro* release were fitted into equations for the zero-order, first-order and Higuchi-release models. The interpretation of the data was based on the value of the resulting regression coefficients. The *in vitro* drug release showed the highest regression coefficient values for the Higuchi's model, indicating diffusion to be the predominant mechanism of drug release.

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