Research Article

FORMULATION, OPTIMIZATION AND EVALUATION OF A GASTROBUOYANT MULTIPARTICULATE SYSTEM OF FAMOTIDINE USING HYPROMELLOSE PHTHALATE

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ABSTRACT

A multiparticulate gastro-retentive floating microballoons of famotidine for improving the bioavailability by prolongation of gastric residence time was prepared by emulsion solvent diffusion method using hydroxypropyl methylcellulose phthalate in ethyl alcohol and dichloromethane organic solvent system. Central composite design was applied to study the influence of rate of stirring, polymer concentration and temperature on the drug entrapment and drug release features. Better entrapment and drug release was attained at lower possible polymer concentration and stirring rate at 40°C. The drug encapsulation was found to be 91 % against the predicted 92 %. The micromeritic properties indicated better flowability and packability of the spheres. The *in vitro* percentage buoyancy was around 94 ± 0.12 with good floatability up to 12 h. *In vitro* dissolution profile showed prolonged release of drug up to 94 % over 12 h demonstrating non-Fickian diffusion mechanism of drug release. Acute oral toxicity studies as per OECD guidelines performed on wistar rats showed no mortality with normal haematological and biochemical values. Histopathogical studies also ruled out prevalence of any toxicity. The mean volume of gastric juice for control, pure drug famotidine and FHPMCP-D1 was found to be 6.51 ± 0.199, 4.01 ± 0.133 and 4.2 ± 0.081 ml respectively. Free acidity and total acidity for the optimized formulation by pylorus ligation method was found to be 40.33 ± 1.145 mEq/l/100g and 131.33 ± 1.33 mEq/l/100g respectively compared to 57.66 ± 2.27 and 180.33 ± 1.14 of control animal, 44.83 ± 1.66 and 134.83 ± 1.424 mEq/l/100g of pure drug. An appreciable rise in the pH 5.11 ± 0.094 of FHPMCP-D1 substantiated the antiulcer activity of the formulation. Long term and accelerated stability studies showed the intactness of the drug without any significant change in the physical properties. Thus microballoons of famotidine with HPMCP prove to be a novel formulation for controlled drug delivery with enhanced bioavailability and patient compliance.

Keywords: HPMCP, Microballoons, optimization, Antiulcer activity, Residual Solvent analysis.

INTRODUCTION

The oral route offers multiple advantages like ease of administration and enormous surface area for passive diffusion of drugs. Another great advantage that the oral route offers for formulation design is it has variable and versatile physiological conditions at different parts starting from mouth thus enabling developing formulations that can selectively release the medicament for optimal absorption and therapeutic advantage. However, it is a well accepted fact that it is difficult to predict the real in vivo time of release with solid, oral controlled release dosage forms. Thus, drug absorption in gastrointestinal tract may be very short and highly variable in certain circumstances[1]. Single unit oral formulations have no control over drug delivery leading to fluctuations in plasma drug level. These have a disadvantage of release all or nothing emptying process, while the multiple unit particulate system pass through the gastrointestinal tract to avoid the vagaries of gastric emptying and thus release the drug more uniformly. Various approaches have been worked out to improve retention of oral dosage form in the stomach e.g. floating systems, swelling an expanding system, bioadhesive systems and high density systems.

One such approach is floating microspheres (hollow microspheres). Floating microspheres are low density gastro-retentive drug delivery systems based on non-

effervescent approach with sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. The drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration. Indeed, the gastric emptying of a multiparticulate floating system would occur in consistent manner with small individual variations. On each subsequent gastric emptying, sunk particles will spread over a large area of absorption sites, increases the opportunity for drug release profile and absorption in a more or less predictable way. Since, each dose consists of many subunits; the risk of dose dumping is reduced[2, 3].

Hypromellosephthalate (hydroxypropylmethylcellulose phthalate) is a monophthalic acid ester of hypromellose.It contains methoxy and 2-hydroxypropoxy (-OCH$_2$CHOHCH$_3$) groups and not less than 21.0 per cent and not more than 35.0 per cent of phthaloyl groups. It is a white or slightly off-white, free-flowing flakes or a granular powder and practically insoluble in water. HPMCP was developed and used originally as an enteric coating agent, its favorable properties have led to extension of its range of applications into other fields, including sustained release preparations, binders and microcapsule bases HPMCP is usually used alone, but can be used in combination with other polymers, as in the case of the sustained release preparations[4].

Famotidine, a H_2 receptor antagonist is widely used for the short term treatment of acute duodenal ulcer, gastric ulcer and gastro-oesophagal reflux. It is also indicated for maintenance therapy of duodenal ulcer and management of Zollinger-Ellison syndrome and multiple endocrine adenomas. Famotidine is rapidly but incompletely absorbed with low bioavailability (20 to 60 %) from the gastrointestinal tract. The poor bioavailability and short biological half-life of 2.5 to 4 hours suffice the development of controlled release formulation as floating microballoon[5, 6].

Objective

To formulate, optimize and characterize floating hollow microspheres of famotidine using hypromellose phthalate for controlled drug delivery by prolonging the gastric residence time for increased bioavailability and patient compliance.

MATERIALS AND METHODS

Preparation of Microballoons of Famotidine with Eudragit S – 100

Microballoons were prepared by emulsion solvent diffusion method as follows:

Famotidine and hydroxypropyl methylcellulose phthalate were incorporated into a mixture of dichloromethane and ethanol at room temperature separately. The polymeric suspension of famotidine was added into an aqueous solution of polyvinyl alcohol (0.75 % w/v, 15 cps, and 200ml) that was thermally controlled at 40°C. The above resultant suspension was stirred with a propeller type agitator at 300 rpm. The finely dispersed droplets of the polymer solution of drug were solidified in the aqueous phase via diffusion of the solvent. The dichloromethane that evaporated from the solidified droplet was removed by a fabricated aspirator flask, leaving the cavity of the microspheres filed with water. After agitating the system for one hour, the microspheres were filtered, washed repeatedly with distilled water and dried in an oven at 40°C[6]. The entire preparation was carried out in a chamber with minimum exposure to light.

Optimization

Preliminary runs were conducted to assess the impact of independent variables on the physical characteristics of microballoons. The three independent variables stirring rate, polymer concentration and temperature were maintained constant. The dependent response variables measured was the drug entrapment and drug release. Experiments were conducted in random sequence in a face-centered manner in order to evaluate the interaction[7-11].

X1 - Rate of Stirring (RPM) – 200 (-1) & 800 (+1), X2 - Concentration of polymer (mgs) – 500 (-1) & 2000 (+1), X3- Temperature 25℃ (-1) & 5 ℃ (+1).*Response*- Drug entrapment (Y1) and Drug release (Y2) - (dependent variables)

Statistical analysis

The effect of formulation variables on the response variables were statically evaluated by applying one-way ANOVA at 0.05 level using Design-Expert® 6.05 (Stat Ease, USA).

PHYSICO-CHEMICAL PROPERTIES OF MICROBALLOONS

Roundness or sphericity

The morphology (outer surface and sphericity) of microballoons was examined using a scanning electron microscope (GEOL 5400, USA). Completely dried microballoons were coated with gold-palladium alloy for 45 Sec under an argon atmosphere in an ion sputter before observation.

X-Ray diffraction patterns

The drug polymer compatibility and any change in the physical form of the drug in the formulation were studied by XRD (Philips). The diffraction patters were obtained separately for pure drug, polymer and formulation

Differential Scanning Calorimetry

Thermal analysis of famotidine, Eudragit S-100 and famotidine loaded microballoons were studied by differential scanning calorimeter (Mettler Toledo DSC, USA). Accurate amount of samples were weighed into aluminium pans and sealed. All samples were run at a heating rate of 10 °C/min over a temperature range of 25-300ºC in atmosphere of nitrogen.

Micromeritic Studies

The size of microspheres was determined using optical microscope (Olympus NWF 40X, Educational Scientific Stores, India) fitted with an ocular micrometer and stage micrometer. The images were taken in an optical microscopy to characterize the surface and for the confirmation of formation of hollow microspheres. The arithmetic mean diameter was determined with MicroLite Image software attached to optical microscope. The flow properties of microspheres were characterized in terms of angle of repose, Carr's index and hausner's ratio. Accurately weighed microspheres were poured gently through a glass funnel into a graduated cylinder cut exactly to 10 ml mark. Initial volume was noted. Bulk density (ρ_b) and tapped density (ρ_t) were calculated by tapping method using 10 ml measuring cylinder. Hausner's ratio (H_R) and Carr's index ($IC_)$ were calculated according to the two equations given below:

$$H_{R\,=}\,(\rho_t)\,/\,(\rho_b) \quad \text{and} \quad I_{c\,=}\,(\rho_t \times \rho_b)\,/\,(\rho_t)$$

In-Vitro Buoyancy

Microspheres (100 mg) were spread over the surface of a USP dissolution apparatus type Π filled with 900 ml of 0.1 N hydrochloric acid containing 0.02%v/v tween 80. The use of tween 80 was to account for the wetting effect of the natural surface-active agents in the GIT. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portions of microspheres

were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the microspheres that remained floating and the total mass of the microspheres[12].

Buoyancy (%) = W_f / (W_f + W_s) X 100

Where W_f and W_s are the weights of the floating and settled microspheres. All the determinations are made in triplicate.

In Vitro Drug Release Studies

The release rate of famotidine from the optimized formulation FHPMCP-D1 was determined in a United States Pharmacopoeia XX111 basket apparatus (Electrolab, Mumbai) in simulated gastric fluid (300 ml) pH 1.2 hrs containing Tween 20 (0.02% w/v) for 2 hrs and phosphate buffer (900 ml) pH 6.8 containing Tween 80 (0.5% w/v). The extent drug released was determined spectrophotometrically at 265 nm using Shimadzu UV-VIS 1601 in triplicate[13].

ANTIULCER ACTIVITY OF OPTIMIZED FORMULATIONS

The animal experiments were carried out with prior permission from the Institutional Animal ethics Committee approval (IAEC NO: MSRCP/P-08/2008).

Pyloric ligation model: The ulcer protective effect of the optimized formulations were studied as per the method of Shay et al., The ulceration is caused by accumulation of acidic gastric juice in the stomach and by this method several parameters can be estimated[14-16]. In this method albino rats were fasted in individual cages for 24 h. Care was being taken to avoid Coprophagy. Control vehicle, three doses of optimized formulations and reference drug (Ranitidine 30 mg/kg) were administered by oral route. The pyloric ligation was carried out 30 minutes and 4h after the drug administration in each group animals. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. They are deprived of both food and water during the post-operative period and are sacrificed at the end of 19 hours with excess of anesthetic ether. Stomach was dissected out and gastric contents subjected to analysis for volume, pH, free acidity and total acidity. The glandular portion of the stomach was opened along the greater curvature, and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0 to 5. Ulcer index and percentage ulcer protection was determined for all the five groups.

Mean ulcer score for each animal is expressed as Ulcer Index. The percentage protection was calculated using the formula –

$$Percentage\ of\ ulcer\ protection = \frac{Ut}{Uc} \times 100$$

Where   Ut = Ulcer index of treated group

        Uc = Ulcer index of the control group

Determination of free and total acidity

One ml of supernatant liquid was pipetted into a 100 ml conical flask and diluted to 10 ml with freshly prepared distilled water. Added 2 or 3 drops of Topfer's reagent and titrated against 0.01N Sodium hydroxide until all traces of red colour disappears and the colour of the solution turns yellowish orange. The volume of alkali (0.01N Sodium hydroxide) added was noted, which corresponds to free acidity of the gastric juice. Titration was further continued with 2 to 3 drops of freshly prepared phenolphthalein solution (1% in 50% absolute ethanol) till the solution regained pink colour. Again the total volume of alkali added was noted and was taken as corresponding to the total acidity[17-23].

Acidity was expressed as:

$$Acidity\ mEq/l/100\ g = \frac{Volume\ of\ NaoH\ x\ Normality\ of\ NaoH\ x\ 100}{0.1}$$

RESIDUAL SOLVENT ANALYSIS

Residual solvents present in traces in the optimized formulations were determined as per ICH Harmonised Tripartite Guideline on Impurities: Guideline for Residual Solvents Q3C (R4). Since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extent possible to meet product specifications or other quality based requirements. The presence of volatile solvents ethanol (Class 3) and dichloromethane (Class 2) in all the three optimized formulations was determined by Gas Chromatography; concentration limit was expressed in terms of ppm.

STABILITY STUDIES

The stability studies of the finalized formulations were designed and carried out as per ICH 'Q1AR2' guidelines. The optimized formulations filled in capsule were stored in vial covered with aluminium foil in order to minimize the accidental exposure of the sample to the light. The packed formulations were stored at 25 ± 2°C and 65±5% RH in a stability chamber for a period of 12 months (long term storage conditions) and at 40±2°C and 75±5% RH for a period for 6 months (accelerated storage condition). Periodical testing of the stored samples for drug content and for any physical change was done at 3 month intervals for the both the studies to ascertain the physical integrity of the drug product.

RESULTS AND DISCUSSION

The scanning electron microphotographs and optical photographs of microspheres have proved the spherical shape with hollow cavity in the sphere (Fig 1). The outer surface was also found to be porous in nature. The XRD pattern of the formulation showed physical integrity of famotidine without any signs of drug polymer interaction (Figure 2) and no change in the physical nature of the drug. DSC thermograms of famotidine, HPMCP and FHPMCP-D1 are shown in the Figure 3. It was clearly evident from the thermogram of FHPMCP-D1 that the

drug has not undergone any polymorphic change and very much being in its natural form. The drug entrapment and drug release optimization data for famotidine-HPMCP was fitted to quadratic model for drug release and linear model for drug entrapment as it showed the maximum values of R^2 and model sum of squares for drug entrapment.
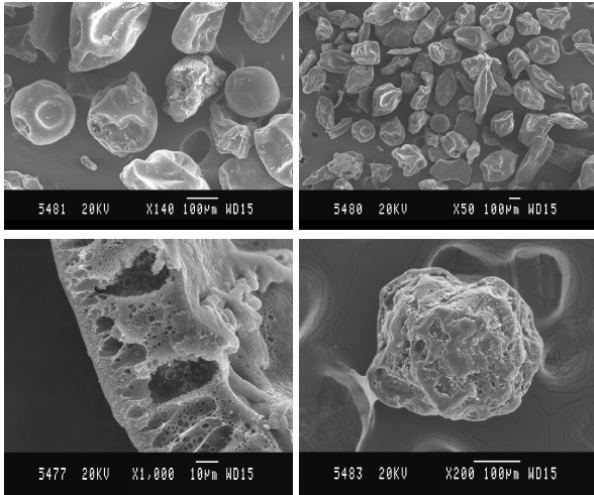
Figure 1: Optical (Panel A) and SEM photographs Panel B) of optimized formulation FHPMCP-D1 with porous outer surface and hollow cavity.
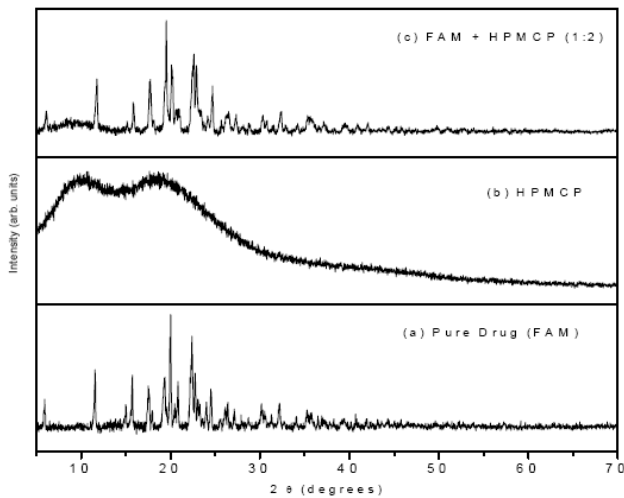
Figure 2: X Ray diffraction patterns of famotidine, HPMCP and Formulation FHPMCP-D1
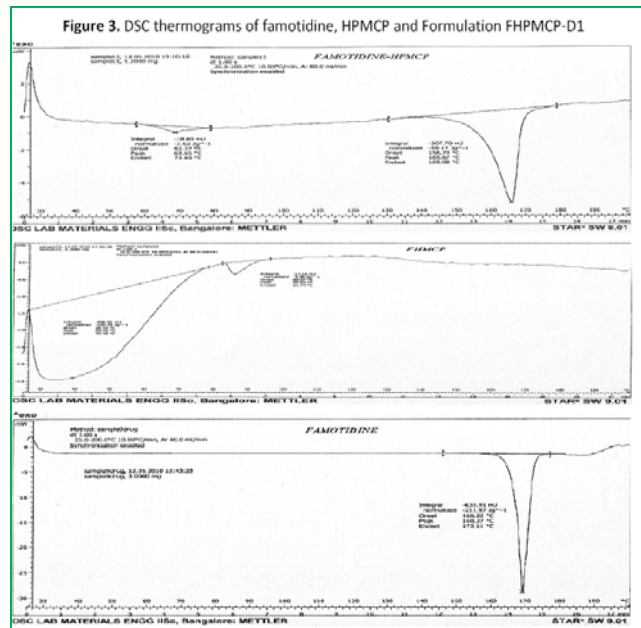
Figure 3: DSC thermograms of famotidine, HPMCP and Formulation FHPMCP-D1.

The result of DOE (design of experiment) is shown in the Table 1 with 16 batch runs. Numerical optimization solutions of famotidine HPMCP hollow microspheres is indicated in Table 2, out of which FHPMCP-D1 was selected for further studies as the desirability value was around 0.954. Summary of ANOVA results in the analysis of lack of fit and pure error of quadratic model for drug entrapment and quadratic model for drug release are summarized in Table 3. ANOVA proved that the model was significant (with a probability F value of <0.0001) and polymer concentration most significantly affected the drug entrapment as indicated by a probability F value of <0.0001 and obeyed linear model. The three-dimensional response surface graph along with the contour graph indicated that with the lower polymer concentration maximum drug release and drug entrapment could be achieved.

Table 1: Results for DOE for famotidine HPMCP hollow microspheres

Std	Run	Block	Factor 1 A: Stirring rate (rpm)	Factor 2 B: Conc. of polymer (mgs)	Factor 3 C: Temp (degrees)	Response 1 Entrapment (%)	Response 2 Drug release (%)
5	1	Block 1	200.00	500.00	50.00	85	96
8	2	Block 1	600.00	2500.00	50.00	72	74
12	3	Block 1	400.00	3181.79	37.50	69	74
9	4	Block 1	150.00	1500.00	37.50	75	78
6	5	Block 1	600.00	500.00	50.00	84	85
10	6	Block 1	736.36	1500.00	37.50	77	80
1	7	Block 1	200.00	500.00	25.00	86	88
11	8	Block 1	400.00	200.00	37.50	88	96
15	9	Block 1	400.00	1500.00	37.50	75	76
17	10	Block 1	400.00	1500.00	37.50	75	80
3	11	Block 1	200.00	2500.00	25.00	71	79
19	12	Block 1	400.00	1500.00	37.50	75	80
2	13	Block 1	600.00	500.00	25.00	86	84
4	14	Block 1	600.00	2500.00	25.00	70	76
7	15	Block 1	200.00	2500.00	50.00	71	77
14	16	Block 1	400.00	1500.00	58.52	76	80

Table 2: Numerical optimization solutions of famotidine HPMCP-D1 hollow microspheres

Number	Stirring rate (rpm)	Polymer Conc (mgs)	Temp (℃)	Drug release (%)	Drug Entrapment (%)	Desirability
1	300.00	500.00	40.00	84.32	91.31	0.954 FHPMCP-D1
2	334.27	500.00	40.00	84.24	90.69	0.923 FHPMCP-D2

Table 3: Summary of ANOVA results in the analysis of lack of fit and pure error of Quadratic model for drug entrapment and Quadratic model for drug release

Response	Model	Sum of Squares	F Value	Prob > F
Drug Entrapment	Quadratic	56.25	85.35	< 0.0001
Drug Release	Quadratic	82.58	3.52	< 0.0001

Table 4: Model validation chart of predicted and actual values for optimized formulations of HPMCP

Observations	Polymer	RPM	Polymer conc. (mgs)	Temp (°C)	Drug entrapment (%)	Drug release (%)
Predicted values	HPMCP	500	500	40	91.31	84.32
Actual values	HPMCP	500	500	40	91.20	94.98

Table 5: Micromeritic properties of coded optimized formulations

Formulation Code	Mean particle size (µm)	Angle of repose (θ)	Tapped density (gm/cm^3)	Porosity %	Hausner ratio (H$_R$)*	Carr index (I$_C$)*
Pure Drug (Famotidine)	255.84 ± 4.56	53^0 13'	0.816	34.8	1.28 ± 0.020	0.168 ± 0.014
FHPMCP-D1	345 ± 32	43˚55′± 0.45	0.736	36.88	1.140±0.011	0.130±0.014

* Each value is ± of three independent determinations

Table 6. Comparison of various calculated parameters of the formulations with the control

Treatment	% Ulcer protection	Ulcer Index	Mean volume of gastric juice (ml)	pH	Free acidity (mEq/l/100 g)	Total acidity (mEq/l/100 g)
Control	------	4.28 ± 0..137**	6.516 ± 0.199**	1.83± 0.088**	57.66 ± 2.275**	180.33 ± 1.145**
Famotidine	70.86	3.033 ± 0.08 **	4.016 ± 0.130**	3.83 ± 0.071**	44.83 ± 1.662**	134.83 ± 1.424**
FHPMCP--D1	64.48	2.764 ± 0.0 8**	4.2 ± 0.081**	5.11 ± 0.094**	40.33 ± 1.145**	131.33 ± 1.33**

Values are mean ± SEM of 6 animals (n=6); Statistical comparision was performed by using ANOVA

Table 7: Residual solvent limits for optimized formulations

Sl.No.	Formulation code	Prescribed limit (ppm)		Detected limit (ppm)	
		Ethanol	Dichloromethane	Ethanol	Dichloromethane
1	FHPMCP-D1	5000	600	787.30	406.64

Table 8: Observations of stability test studies in real time storage conditions (25 ± 2° C and 65 ± 5 % RH)

Formulation code	Drug content in %, after months					Particle size (µm)					Physical Change* (after 12 months)
	0	3	6	9	12	0	3	6	9	12	
FHPMCP- D1	99.33	98.25	97.55	95.88	95.29	342	341	342	642	340	-No-

* No significant physical change

Table 9: Observations of stability test studies in accelerated storage conditions (40 ± 2^{O}C and 75 ± 5 % RH)

| Formulation code	Drug content in %, after months				Particle size (µm)				Physical change (after 6 months)*
	0	2	4	6	0	2	4	6	
FHPMCP- D1	98.56	96.56	95.89	93.72	340	340	340	341	-No-

* No significant physical change

It was observed that an increase in the ratio of drug to polymer concentration resulted in a decrease in the entrapment efficiency (Figure 4 and 5) due to the influence of the process variables. From the numerical optimization results (Table 2), solution 1 was selected randomly as the optimized formula for the preparation of famotidine-HPMCP microspheres as it showed maximum drug entrapment and drug release efficiency. The influences of stirring rate, polymer concentration and temperature on drug entrapment and drug release from microspheres are shown in the Table 4. There was a considerable increase in drug entrapment and drug release from the optimized formulation over the predicted values.

The micromeritic property of optimized formulations projects improved flowability, packability, porosity and density lesser than unity for floatation in the gastric contents (Table 5). The tapped density values were 0.816 and 0.736 g/cc respectively for drug and formulation. Optimized formulations showed better buoyancy (up to 94 %) till 12 hrs over. HPMCP (94.98 %) showed a better drug release up to 12 hours (Table 4, Figure 6). Regression
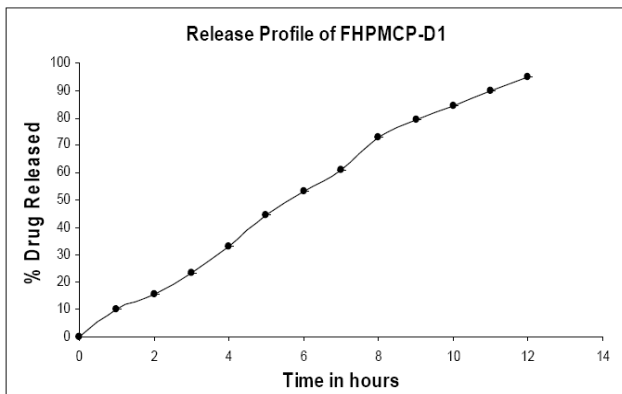
analysis suggests that the release of famotidine from microballoons followed zero order with non-Fickian diffusion mechanism (Figure 7).
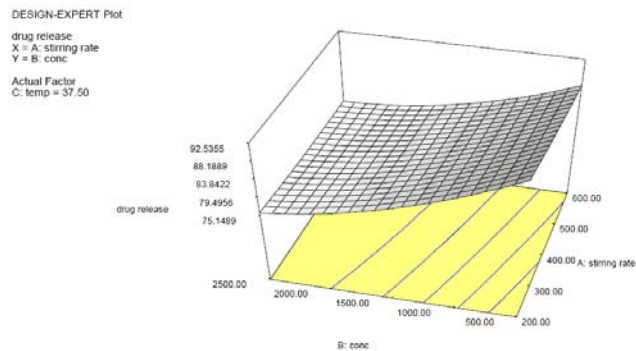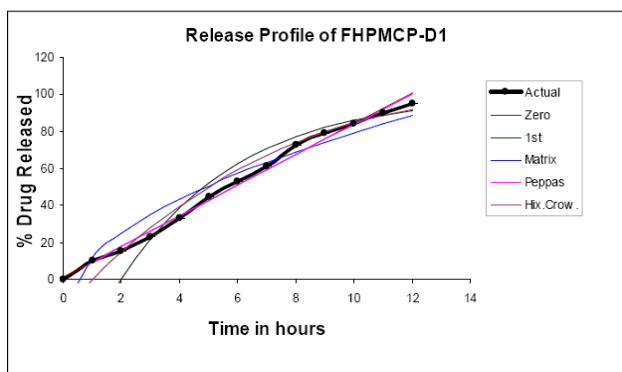

Figure 4: 3D RSM graph of famotidine hollow microsphere (FHPMCP-D1, Drug release).
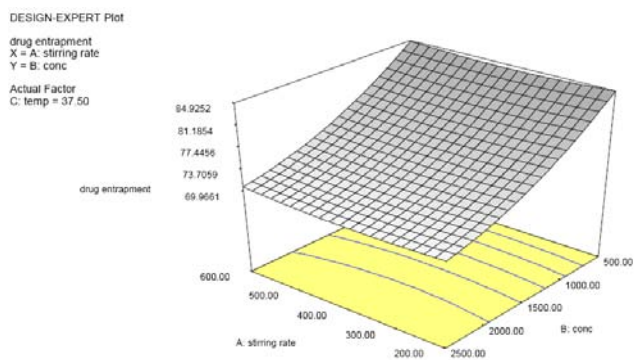
Figure 5: 3D RSM graph of famotidine hollow microsphere (FHPMCP-D1 – Drug entrapment).


**Figure 6:** *In-vitro* drug release profile of FHPMCP-D1.

Figure 7: Model fitting *In vitro* dissolution profiles of Famotidine for FAS-D1 hollow microspheres in phosphate buffer pH 6.8.

The percent ulcer protection of the formulations was found to be 64.48 % which is statistically significant as there was a significant reduction in the ulcer index of the formulations over the pure drug and control. The values of various pathological parameters are shown in the Table 6. The mean pH of the control was very high (1.833 ± 0.088) compared to pure drug famotidine (3.833 ± 0.071) and found to be decreasing in the acidic pH with the mean pH of 5.11 ± 0.094 for the optimized formulations (Table 6). The mean free and total acidity of FHPMCP-D1 was found to be 40.33 ± 1.145 and 131.33 ± 1.33 mEq/l/ 100 g respectively. Appreciable decline in the mean gastric juice (4.2 ± 0.081 ml) of the optimized formulation over the control and pure drug (6.51 ± 0.199 and 4.016 ± 0.130 ml) confirms the ulcer protective activity of FHPMCP-D1.

The amount of ethanol and dichloromethane in the formulations were found be within the limits as prescribed by the ICH guideline for impurities 'Q3C for the residual solvents. It can be inferred that the formulations were safe for oral administration (Table 7). The related chromatograms of the standard and FHPMCP-D1 are shown in Figures 8 and 9.
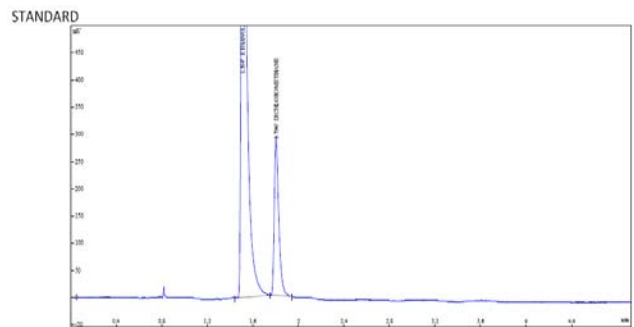
Figure 8: Gas chromatogram of standard (ethanol and dichloromethane).
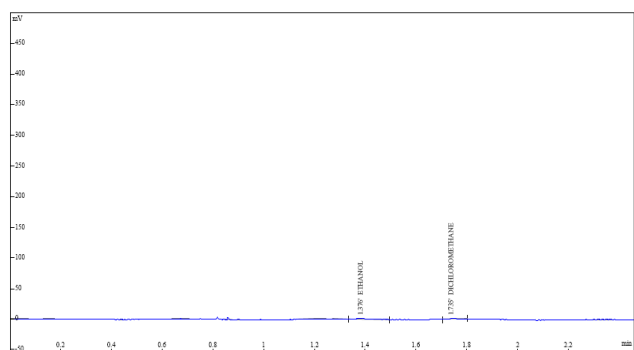
Figure 9: Gas chromatogram of FHPMCP-D1for residual solvents

Long term and accelerated stability studies carried out as per ICH guidelines showed that there was no drastic changes in the drug content (not more than 5 %) as well as in the particle size. The values are presented in the Tables 8 and 9 for both the studies.

CONCLUSION

The microballoons of famotidine prepared by emulsion-solvent diffusion method exhibited excellent *in vitro* buoyancy and zero order drug release with non-Fickian transport mechanism. The surface response central composite design methodology could be successfully employed for assessing the influence of formulation parameters on the desired response. The drug polymer concentration and stirring rate played a vital role in achieving the desirable designed product. Ulcer protection activity of the formulation was found to be superior with significant reduction in the gastric juice volume, free and total acidity and gastric pH towards alkalinity. Hence the floating hollow microspheres of famotidine prepared with HPMCP may provide a convenient delivery system for achieving better floatation and drug release.

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