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Preparation and *in vitro* evaluation of altered density gastro retentive microspheres of Famotidine with synthetic and natural polymers

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ABSTRACT

The main aim of the present work was to fabricate and evaluate gastro retentive high density microspheres with both synthetic and natural polymers for the sustained release of Famotidine to treat gastric ulcers. The microspheres were prepared by the coacervation phase separation technique. Famotidine was checked for its compatibility with polymers used by using Fourier Transform Infrared spectroscopic (FTIR) analysis. The surface morphology was studied by scanning electron microscopic (SEM) studies. The percentage of yield, surface associated drug content, drug entrapment efficiency and in vitro dissolution studies were performed and the dissolution data was treated with mathematical kinetic models. Accelerated stability studies were also carried out to the optimized formulation (F-6). The FTIR spectrum of pure drug and drug-polymer blend showed the stable character of Famotidine in the micro capsules. The microspheres were found to be spherical. The microspheres had good entrapment efficiency and percentage yield. The release of drug from the microspheres extended up to 12 h. The release kinetics data and characterization studies indicate that drug release from microspheres was diffusion sustained and that the microspheres were stable. The study revealed that Gellan gum and Karaya gum in combinations with Povidone found to be effective combination for microspheres and the gastric retention was aided with Iron oxide.

Key words: Famotidine, Microspheres, Gellan gum, Karaya gum, Iron oxide.

INTRODUCTION

Microspheres drug delivery systems made from the natural, biodegradable polymers have been attracted by several researchers in recent years for sustaining the drug release [1]. Microspheres have varied applications and are prepared using various polymers. However, the success of

microspheres is limited due to their short residence time at the site of absorption/action [2]. High density microspheres provide an increase residence time by making them to sink in gastric fluid. This can be achieved by coupling high density materials which has higher density then gastric fluid [3]. High density systems have advantages like increased gastric residence time and specific targeting of drugs in the absorption site, efficient absorption and enhanced bioavailability [4, 5]. Iron oxide was selected as high density material in the present study [6]. Gellan gum was obtained from *Pseudomonas elodea,* which is chemically D-glucose, D-glucuronic acid and rhamnose in ß-1, 4 linkages whereas Karaya gum was obtained from the plant *Sterculia urens*, which is chemically Mixture of D-galactose, L- rhamnose and D-galacturonic acid [7]. Both Gellan gum and Karaya gumwere proved their ability as encapsulating polymers.

Famotidine is a histamine H_2-receptor antagonist. It is widely used in the treatment of gastric ulcers, duodenal ulcers, Zollinger- Ellison syndrome and gastro esophageal reflux disease. In the management of benign gastric and duodenal ulceration the dose is 40 mg daily by mouth at bedtime, for 4 to 8 weeks. In gastro esophageal reflux disease the recommended dose is 20 mg by mouth twice daily for 6 to 12 weeks; where gastroesophageal reflux disease is associated with esophageal ulceration, the recommended dosage is 40 mg twice daily for a similar period. For the short term symptomatic relief of heartburn or non-ulcer dyspepsia a dose of 10 mg up to twice daily is suggested. In the Zollinger-Ellision syndrome the initial dose by mouth is 20 mg every 6 h, increased as necessary; dose up to 80 mg daily have been employed. The low bioavailability (40-45%) and short biological half-life (2.5-4.0 h) of famotidine favors development of a sustained release formulation [8]. In contest of the above principle, a strong need was recognized for the development of a dosage form to deliver sustained release gastro retentive delivery system of Famotidine.

MATERIALS AND METHODS

Materials

Famotidine was obtained as a gift sample from Waksman Selman Pharmaceuticals, Anantapur, India (Batch # F 01645), Gellan gum, Karaya gum, Povidone, Formaldehyde, Iron oxide and sodium hydroxide were procured from SD Fine Chemicals, Mumbai, India. Sunflower oil was procured from MORE super market, Anantapur, India. All the chemicals and reagents were of analytical reagent grade and double distilled water was used throughout the experiment.

Preformulation Studies

Solubility analysis

Preformulation solubility analysis was done to select a suitable solvent system to dissolve the drug and also to test its solubility in the dissolution medium which was to be used.

Melting Point determination

Melting point determination of the obtained sample was done because it is a good first indication of purity of the sample since the presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range.

Compatibility Studies

Fourier Transform Infrared Spectroscopic (FTIR) analysis

The FTIR spectrums of Famotidine and Formulation (F-6) blend were studied by using Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, spectrum-100, Japan) using the KBr disk method (5.2510 mg sample in 300.2502 mg KBr). The scanning range was 500 to 4000

cm^{-1} and the resolution was 1 cm^{-1}. This spectral analysis was employed to check the compatibility of drugs with the polymers used.

Preparation of microspheres

Famotidine microspheres were prepared by coacervation phase separation technique utilizing temperature change [9, 10]. Gellan gum, Karaya gum and Iron oxide and Povidone were dissolved in 10 ml of water which was previously heated to 50^0 C, to this Famotidine was added and stirred at 300 rpm with the help of magnetic stirrer for 15 min to get a stable dispersion. The dispersion was poured drop wise into the 10 ml of sunflower oil which was also previously heated to 50^0 C on a water bath. The mixture was stirred with a help of magnetic stirrer for 2 h at 300 rpm at room temperature. At the end of 2^{nd} h crosslinking agent formaldehyde 0.5 ml was added to the dispersion medium and stirring was continued for next 30 min. Finally it was kept in refrigerator for 24 h to ensure the rigidness of microspheres. This Procedure was followed to prepare 6 batches of Famotidine microspheres with different ratios of Gellan gum and Karaya gum mixtures. The core: coat ratio, amount of drug and polymers used were given in Table 1.

Table 1: Composition of Famotidine Micro spheres

Ingredients	Formulation					
	F-1	F-2	F-3	F-4	F-5	F-6
Famotidine	40	40	40	40	400	400
Gellan gum (g)	0.5	1.0	-	-	0.5	1.0
Karaya gum (g)	-	-	0.5	1.0	0.5	1.0
Povidone (g)	5	5	5	5	5	5
Iron oxide (g)	0.1	0.1	0.1	0.1	0.1	0.1

Flow Properties

Angle of repose

This was determined by using funnel method. Powder was poured from a funnel that can be raised vertically until a maximum cone height (h), was obtained. Diameter of heap, (D), was measured [11]. The angle of repose (Θ) was calculated by the following equations.

$$\tan \Theta = h / r$$
$$\Theta = \tan^{-1} (h / r)$$

Where, Θ = Angle of repose, h = height of the pile (cm) and r = radius of the pile.

Bulk Density:

A quantity of 2g of granules from each formula, previously lightly shaken (to break any agglomerates formed) was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 s intervals. The tapping was continued until no further change in volume was noted [11]. Loose bulk density (LBD) and tapped bulk density were calculated using the following equations.

LBD = Weight of the Powder/ Volume of the packing
TBD = Weight of the powder/ Tapped volume of the packing

Compressibility Index

The loose bulk density and tapped bulk density values were considered for calculating compressibility index [11]. The compressibility index was calculated by the following equation.

I_C = TBD - LBD / TBD
Where, TBD = Tapped density of the granules, LBD = Loose Bulk density of the granules

Hausner ratio
The ratio of Tapped density and bulk density gives the Hausner ratio [11] and it was calculated using the following equation.

$$H_R = TBD / LBD$$

Where, TBD = Tapped density of the granules, LBD = Loose bulk density of the granules

Particle Size Analysis
Particle size distribution was analyzed by placing 5 g of the formulated microspheres in a set of standard test sieves and shaken for a particular time interval using Indian Standard Sieves # 16, #20, #30, #40, #60 and #80 respectively. The particles collected in each sieve were weighed and the percentage particles retained was calculated [12].

Percentage yield
The percent yield [12] of each batch of formulation was calculated using the following equation.
% yield = (weight of microspheres)/weight of solid starting material ×100

Surface associated drug content
The Famotidine encapsulated microspheres prepared were evaluated for surface associated drug content on the surface of microspheres. From each batch, 100 mg of microspheres were shaken in 20 ml of 0.1N HCl for 5 min and then filtered through whatman filter paper 41. The amount of drug present in filtrate was determined by spectroscopic method and calculated as a percentage of total drug content. All the experiments were conducted in triplicate (n=3).

Estimation of drug loading/incorporation efficiency
Drug loaded microspheres equivalent to 40 mg were powdered and suspended in water and then sonicated (Power sonic 505, Hwashin technology co, Korea) for about 20 min. It was shaken for another 20 min in mechanical shaker (Orbitex, Scigenics biotech, India) for the complete extraction of drug from the microspheres. The mixture was filtered through a 0.45 µm membrane filter (Millipore, Bangalore, India). Drug content was determined by UV- visible double beam spectrophotometer (Ellico SL210, India) at 288 nm. The percent entrapment was calculated using the following equation [12].

Total incorporation efficiency =surface associated drug + entrapped drug

Determination of wall thickness
Wall thickness of microspheres was determined by the following equation [12].

$$h = [r (1-P) d_1/3\{Pd_2 + (1-P) d_1\}] \times 100$$

Where, h= wall thickness, r = arithmetic mean radius of microspheres,
d_1 and d_2 = densities of core and coat material respectively,
P = proportion of medicament in microspheres.

Estimation of Famotidine

The content of Famotidine in the microspheres was estimated by a double beam UV spectrophotometer based on the measurement of absorbance at 288 nm in phosphate buffer (pH 7.4). The method obeyed Beer's law (at 1 to 10 mg/ml). The mean error and precision were found to be 0.9% and 1.0% respectively. These experiments were conducted for six times.

***In vitro* drug release study**

In vitro drug dissolution studies were performed using USP type I dissolution apparatus (DR-3, Campbell Electronics, Mumbai, India) at 75 rpm. The microspheres were weighed and filled in the empty capsule shells and placed in the basket. The dissolution medium (900ml) consisted of 0.1M HCl for first 2 h and then changed to phosphate buffer pH 7.4 from 3^{rd} to 12^{th} h; Temperature was maintained at 37 ± 0.5^0C. A 5 ml sample was withdrawn at specific time intervals and replaced with an equivalent volume of dissolution fluid. Drug content was determined by UV – visible double beam spectrophotometer at 288 nm. The release studies were conducted in triplicate.

***In vitro* drug release kinetic studies**

Kinetic model had described drug dissolution from solid dosage form where the dissolved amount of drug is a function of test time. The exact mechanism of Famotidine release from the microsphere was further studied by kinetic models. The drug release data was analyzed by zero order [13], first order [13], Higuchi [14], Korsmeyer Peppa's [15] and Hixson Crowell [16] models. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.

Scanning Electron Microscopy (SEM) studies

The surface morphology of selected microspheres (F-6) was studied by scanning electron microscopy (SEM) (FE-SEM, Carl Zeiss, Germany). The samples were coated to $200A^0$ thickness with gold palladium prior to microscopy.

Accelerated Stability studies

The promising formulation (F-6) was tested for accelerated stability studies by storing at stressed storage conditions for the period of 3 months at a temperature of 40^0C with 75% RH. The physicochemical properties of formulation F-6 were observed before and after accelerated stability studies [17].

RESULTS AND DISCUSSION

The Famotidine sample was found to be freely soluble in water and in methanol, sparingly soluble in ethanol and very slightly soluble in methylene chloride. The melting point of the obtained drug sample was found to be 163^0C which is within the reported limit (163-164^0C). It complies with IP standards thus indicating the purity of the drug sample. The FTIR spectrum of the pure drug was found to be similar to the standard spectrum of Famotidine. It was observed that all the characteristic peaks of Famotidine were present in the pure drug spectrum were present in combination spectra which indicates the compatibility of the drug with the polymers used. The FTIR spectrums were shown in Figures 1 and 2.

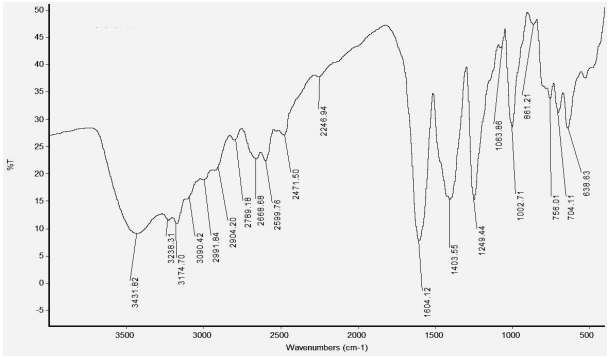

Fig. 1: FTIR spectrum of Famotidine

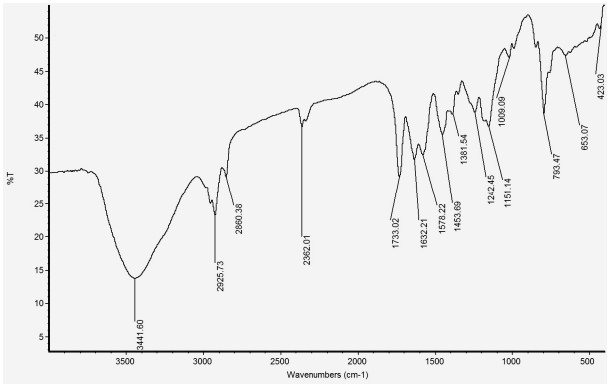

Fig. 2: FTIR spectrum of F-6 blend

The angle of repose of formulated microspheres was ranged from 22.26±0.18 to 28.12±0.25^0 which indicates that the microspheres have excellent flow properties. The Loose Bulk density of formulations was ranged from to 0.419±0.02 to 0.741±0.05 g/cm^3 and the tapped Bulk density of formulations were ranged from 0.584±0.08 to 0.875±0.05 g/cm^3. The Loose Bulk density and the tapped Bulk density values were utilized for determining the compressibility Index which was ranged from 15.55±0.12 to 28.34±1.15% and the Hausner ratio was ranged from 0.010±0.001 to 1.176±0.001. These studies revealed the granules have good flow properties. All these values were represented in Table 2.

Table 2: Flow Properties of Famotidine Microspheres

Formulation	Angle of repose (^{0})	Loose Bulk Density (g/cm^3)	Tapped Bulk Density (g/cm^3)	Carr's Index (%)	Hausner's ratio
Pure drug	32.12±0.45	0.299±0.06	0.358±0.01	17.46±0.44	1.211±0.021
F-1	22.26±0.18	0.541±0.04	0.639±0.01	18.43±1.29	0.010±0.001
F-2	24.20±0.26	0.561±0.05	0.629±0.02	21.74±0.98	0.139±0.002
F-3	28.12±0.25	0.419±0.02	0.621±0.04	28.34±1.15	0.060±0.001
F-4	25.27±0.15	0.457±0.06	0.584±0.08	26.06±0.11	0.081±0.001
F-5	24.21±0.06	0.438±0.01	0.626±0.04	28.04±2.22	0.119±0.011
F-6	25.31±0.14	0.741±0.05	0.875±0.05	15.55±0.12	1.176±0.001
Values were mentioned in mean ± SD; Number of exper iments (n) =6					

The average particle sizes of F-1 to F-6 formulations were 615.00, 594.00, 662.00, 562.00, 704.00 and 630.00 µm respectively. The percentage yields of among formulated micro capsules, F-6 showed highest percentage yield of 86.75±0.24%. The surface associated drug content was least for F-6 (10.41±0.09). High drug entrapment efficiency was observed to the formulation F-6

and it was 92.58±2.39%. The wall thickness of formulated microspheres was ranged from 15.54±0.02 to 24.16±0.54 µm. The wall thickness of formulated microspheres was found to be increased from F-1 to F-6. All these values were shown in Table 3.

Table 3: Particle size, Percentage of yield, Surface associated drug content, Drug entrapment efficiency, Wall thickness of Famotidine Microspheres

Parameters	F-1	F-2	F-3	F-4	F-5	F-6
Particle size (µm)	615.00	594.00	662.00	562.00	704.00	630.00
Percentage yield (%)	83.65±0.15	81.18±0.25	81.95±0.16	85.19±0.23	84.27±0.25	86.75±0.24
Surface associated drug content (%)	15.56±0.15	14.22±0.15	14.15±0.11	12.25±0.18	11.36±0.19	10.41±0.09
Drug entrapment efficiency (%)	82.16±2.56	89.13±0.15	79.16±2.47	81.29±0.25	85.54±2.56	92.58±2.39
Wall thickness (µm)	15.54±0.02	19.25±0.35	21.54±0.27	22.17±0.14	22.42±0.23	24.16±0.54
Values were mentioned in mean ± SD; Number of experiments (n) =6						

The *in vitro* dissolution data was treated with mathematical models viz., zero order, first order, Higuchi, Korsmeyer Peppa's and Hixson Crowell's models and shown in Figures 3, 4, 5, 6 and 7. The *in vitro* drug release kinetics data studies indicate that the formulations either followed zero order release or the Higuchi release model. Famotidine release from the microspheres was found to be by diffusion controlled.

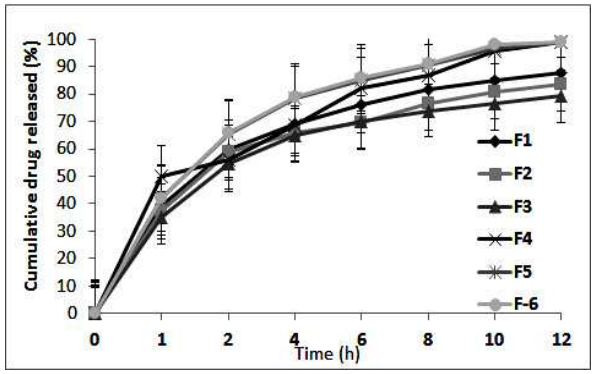

Fig. 3: Zero order plots

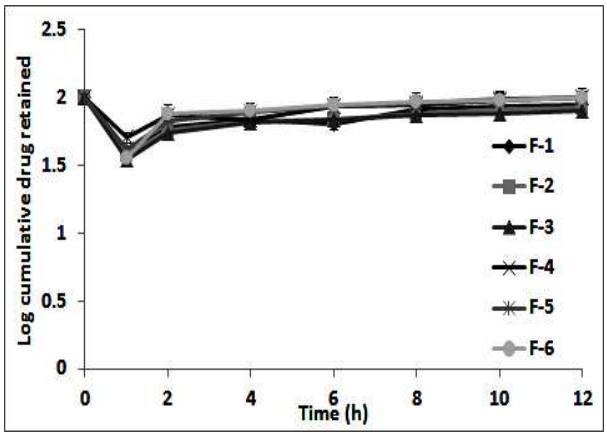

Fig. 4: First order plots

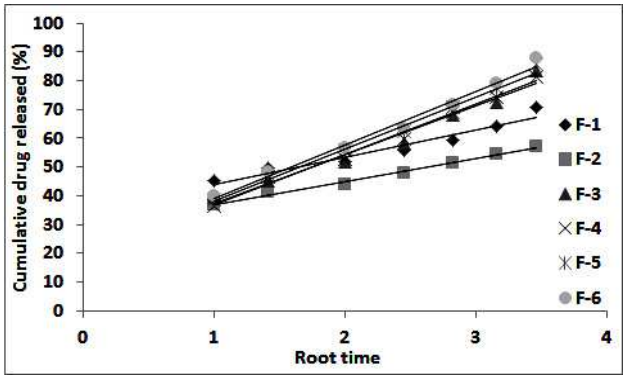

Fig. 5: Higuchi plots

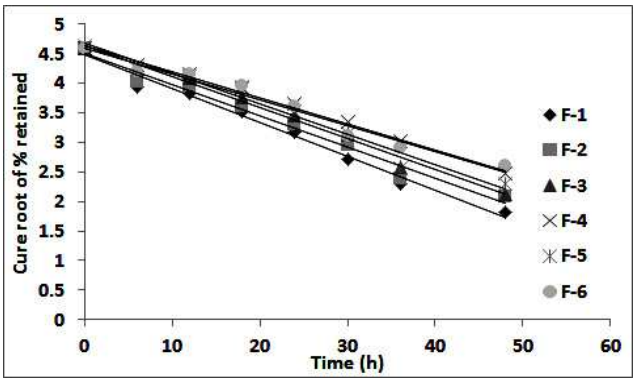

Fig. 6: Korsmeyer- Peppas plots

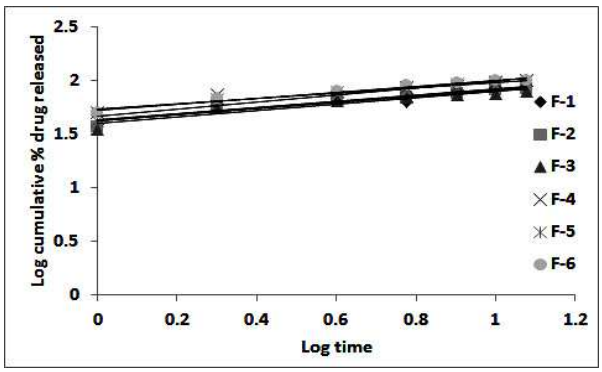

Fig. 7: Hixson Crowell plots

The SEM results shows that the microspheres were spherical and with a smooth surface. The SEM photographs were shown in Figure 8. The results indicate that F-6 formulation showed the slowest release rate.

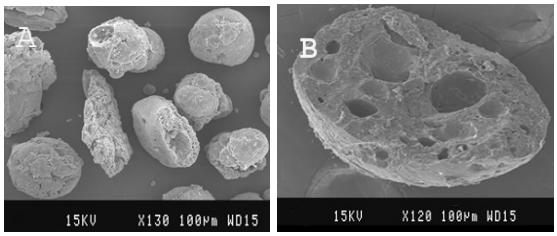

Fig. 8: SEM photographs of microspheres (F-6); A) whole micro capsules, B) Cross section of microsphere

The accelerated stability revealed that the formulated Famotidine microspheres were stable even at accelerated environmental conditions.

CONCLUSION

The Famotidine microspheres prolonged drug release for 12 h or longer. The formulated Famotidine microspheres reduced the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional Famotidine tablets. This study concluded that Famotidine was found to be compatible with Gellan gum, Karaya gum, Povidone and Iron oxide. The formulated microspheres were found to retain in the stomach for prolonged period followed by its release.

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