ABSTRACT
The purpose of this study was to evaluate the potential of floating microballoons. Famotidine was chosen as a model drug because of its poor absorption and short half-life in the lower gastrointestinal tract. A solvent diffusion technique was adopted to prepare famotidine loaded microballoons (FMD-microballoons) using Eudragit S 100 and Hyaluronic acid. The effect of various formulations and process variables including drug content, polymer amount, surfactant concentration, stirring speed and temperature were investigated in detail. The final formulation attributes of FMD-microballoons viz. 121 ± 3 µm size and 79 ± 5 % drug content. Drug release of FMD-microballoons was studied using paddle type dissolution apparatus demonstrated the burst followed by slow sustained release profile. The buoyancy study revealed that FMD-microballoons had excellent floating ability in simulated biological fluids, while stability study confirmed that 4±1°C temperature was suitable without any detrimental effect on formulation. A radiographical study was conducted in male rabbits after oral administration of barium sulphate loaded microballoons, it was found that microballoons remained buoyant even after 4 h satisfactory for a gastroretentive formulation. Therefore, FMD-microballoons exhibited excellent floatability, high buoyancy and prolonged drug release necessary for oral administration.

KEYWORDS: Famotidine, Microballoons, Release, Buoyancy, Stability, Radiography

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INTRODUCTION

Famotidine is a histamine H2-receptor antagonist which inhibits stomach acid production and widely accepted as anti-ulcer along with NSAID. It is also used to prevent postoperative nausea and to reduce the risk of aspiration pneumonitis. However, poor oral bioavailability and short biological half-life proven it unsuccessful. Numerous drug delivery approaches were tried to decode this challenge, however, every delivery systems suffer certain merits and demerits.

Microballons, refer to hollow microsphere is gastro-retentive drug delivery based non-effervescent approach. Microballoons are spherical empty particles without core made up of synthetic polymers or natural proteins, ideally having a size less than 200 µm. Apart from sophistication of microballoons technology, it can be option for various gastro-retentive drug delivery systems for various active pharmaceutical ingredients which exhibit absorption window, low bioavailability, and extensive first pass metabolism. Microballons have successfully explored to enhance the bioavailability and controlled delivery of numerous drugs.

The present study deals with the potential of famotidine loaded hollow microballoons (FMD-microballoons) for oral delivery. FMD-microballoons were prepared by solvent diffusion evaporation method using Eudragit S 100 and Hyaluronic acid. The formulations and process parameters were investigated to obtain minimum size and high drug encapsulation. The behavior mechanism including buoyant properties and drug release from FMD-microballoons were also discussed. Stability study of FMD-microballoons was evaluated to check the suitable storage temperature. Radiographical study in animals demonstrates the in vivo behavior in biological milieu of FMD-microballoons. In future formulation can be further used as medicated product for several active constituents.

MATERIALS AND METHODS

Materials

Famotidine was obtained as a gift sample from Aristo Pharmaceuticals, Mumbai. Eudragit S 100 was purchased from Rohm GMBH, Germany. Hyaluronic acid and polyvinyl alcohol (PVA) was procured from Sigma, USA. In house ultrapure water was used. All other reagents were of HPLC or analytical grade.

Preparation of microballoons by solvent diffusion-evaporation method

Famotidine loaded Microballoons (FMD-microballoons) with an internal hollow structure were prepared by solvent diffusion evaporation method. An accurate quantity of polymer mixture viz.
Eudragit S 100 and Hyaluronic acid was dissolved in 1:1 mixture of ethanol and dichloromethane. The 100 mg of famotidine was homogeneously mixed in polymer solution. The solution was slowly introduced into 200 mL of aqueous polyvinyl alcohol solution with stirring at 400 rpm for 3 h using a mechanical overhead stirrer (Remi, India) equipped with a blade propeller. The FMD-microballoons were collected by filtration, washed three times with distilled water and dried at room temperature for 24 h.

**Optimization of formulation and process variables**

Various formulation and process variables including drug concentration, polymer ratio, surfactant concentration, stirring speed and effect of temperature which could affect the preparation and properties of microballoons were identified and studied. The optimization was done on the basis of particle size and entrapment efficiency (%EE).

**Optimization of drug concentration**

The amount of drug concentration was optimized after varying percentage of drug to polymer ratio (10 to 50 %w/w), while keeping other parameters constant. Optimization was done on the basis of particle size and %EE.

**Optimization of polymer mixture concentration**

For optimization of polymers content, the earlier optimized formulations of FMD-microballoons was selected and the effect of ratio of polymer (20 to 70 %w/w) on the particle size and %EE were studied, albeit other parameter kept constant.

**Optimization of surfactant concentration**

The FMD-microballoons were prepared by using different surfactant (PVA) concentrations (0.50 to 1.25 %w/v) in external aqueous phase. Other formulations and process parameter kept constant.

**Optimization of stirring speed**

Stirring speed of the stirrer was varied from 300 to 500 rpm for FMD-microballoons preparation and their effect on particle size and %EE was recorded. Other parameters *viz.* drug concentration (40%w/w), quantity of polymer (60%w/w) and PVA concentration (0.75% w/v).

**Optimization of temperature**

The FMD-microballoons were prepared at different temperatures *viz.* 25°C, 37°C and 45°C. Other formulations parameters were kept constant.
Characterization of FMD-microballoons

Particle size
The FMD-microballoons were studied microscopically for particle size using calibrated ocular micrometer. Least count of the ocular micrometer was calculated. Each time around 100 particles per formulation were detected and particle sizes were recorded.

Shape and surface morphology
The FMD-microballoons were examined by optical and scanning electron microscopy (SEM). FMD-Microballoons were suspended in water; a drop was placed on a glass slide, covered with a cover slip and visualized using optical microscopy (Leitz-Biomed, Germany) to examine their shape. In order to examine the surface morphology, the formulations were viewed using scanning electron microscope (SEM, Hitachi, Japan). The samples for SEM were prepared by sprinkling of FMD-microballoons powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were coated with gold using a sputter. The samples were scanned to observe surface morphology.

Drug content
For drug content determination, a 100 mg of FMD-microballoons was dispersed in 100 mL of PBS (pH 7.4), shaken vigorously for 10 min and 1\textsuperscript{st} supernatant was collected at 3000 rpm for 5 min. The sediment was again treated in the same manner and 2\textsuperscript{nd} supernatant was obtained. The FMD-microballoons obtained after two washings were dissolved in 20 mL of PBS (pH 7.4) for 2 h and centrifuged at 3000 rpm for 5 min.\textsuperscript{5,6} The solution was filtered through 0.45µm syringe filter (Millipore Millex HN, USA) and filtrate was assayed for FMD spectrophotometrically. The percent drug entrapped was calculated.

\textit{In vitro drug release study in simulated biological media}

The FMD-microballoons were evaluated for the \textit{in vitro} drug release profile. The dissolution test of FMD-microballoons was carried out by the paddle type dissolution apparatus specified in USP XXIII.\textsuperscript{7,8} Briefly, 50 mg of FMD-microballoons was weighed accurately and gently spread over the surface of 500 mL of dissolution medium. The content was rotated at 100 rpm and thermostatically controlled at 37±1°C. Perfect sink condition was prevailed during the drug dissolution. The release of FMD was checked in biological media including simulated gastric fluid (SGF, pH 1.2) and phosphate buffer saline (PBS, pH 7.4). An aliquot of the release medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the release medium. The collected
samples were filtered through 0.45µm-syringe filter (Millipore millex HN) and amount of released FMD was analyzed by spectrophotometrically.

\[
\% \text{ released of FMD} = \frac{\text{Amount of FMD released at time } t}{\text{Amount of FMD released at time } t_{100}} \times 100
\]

**In vitro** buoyancy test of FMD-microballoons formulation

The floating test of the FMD-microballoons was carried out using dissolution test apparatus II as specified in USP XXII. The FMD-microballoons weighing around 500 mg were immersed in 900 ml SGF 37 ± 1°C and agitated by a paddle at 100 rpm. The paddle blades were positioned at the surface of dissolution medium. The FMD-microballoons floating on the surface of SGF and PBS were recovered with a sieve No 60 (100 µm sieve opening) at defined time intervals. The FMD-microballoons were dried and weighed. The floating percentage of the microballoons was defined as the weight ratio of the floating granules against the total granule weight in the floating test. The buoyancy (%) of FMD-microballoons in SGF and PBS was calculated by the following equation:

\[
\text{Bouyancy (\%)} = \frac{Q_f}{Q_f + Q_s} \times 100
\]

Where, Qf and Qs are the weights of the floating and settled microballoons respectively.

**Stability studies**

The stability of FMD-microballoons is major concern for successful development of stable marketed formulation. The prepared FMD-microballoons was stored in amber colored glass bottles at 4°C, 25°C and 40°C for 45 days and their effect on particle size and surface morphology and residual drug content was estimated. For the determination of residual drug content, FMD-microballoons were dissolved in PBS and amount of FMD in supernatant was estimated spectrophotometrically using UV-visible spectrophotometer.

**In vivo radiographical Study**

In order to assess the gastro-retentive efficacy of floating formulations, the % buoyancy in a biological system was determined by using barium sulphate X-ray contrast medium (10% w/v). The microballoons containing 15%w/w of barium sulphate (BS-microballoons) as a contrast agent were prepared for radiographical study. The study was carried out with six healthy male rabbits free of detectable gastrointestinal diseases or disorders. The study was carried out under the guidelines compiled by Committee for the Purpose of Control and Supervision of Experiments on Animal, Ministry of Culture, Government of India and all the study protocols were approved by Institutional
Animal Ethics Committee. The rabbits were fasted overnight before start of the experiment. The rabbits were administered 25 mL of BS-microballoons and intragastric behavior of BS-microballoons in gastrointestinal track (GIT) were studied by a series of X-ray photographs at different time intervals.  

RESULTS AND DISCUSSIONS

Preparation and optimization of FMD-microballoons

The polymer and surfactant complexed FMD-microballoons were prepared by solvent diffusion method. The water insoluble polymer including Eudragit S100 and hyaluronic acid were molded into inter-polymer complex in presence of PVA solution. The droplets of Eudragit S100-Hyaluronic acid-PVA complex gradually solidified and hardened as ethanol and DCM diffused out of the internal phase. It was found that the intensity of inter-polymer hydrogen bonding between Eudragit S100-Hyaluronic acid and PVA is strong enough for the formation of FMD-microballoons.

The effect of formulation variables (Figure 1) such as drug concentration, polymer concentration, surfactant concentration and process variables (Figure 2) viz. stirring speed and temperature were studied in order to optimize the formulation. The results suggested that these variables influence the shape, size and %EE. These parameters were optimized to prepare FMD-microballoons of smaller size with acceptable drug loading efficiency.

Initially, the effect of drug concentration on particle size and %EE was calculated as shown in Figure 1(A). When the concentration of drug increased (>40% w/w), the matrix of polymer become fully saturated. It resulted in drug leaching from the polymer matrix owing to poor %EE (75% w/w). 14,15 Therefore, optimal concentration of drug was found to be 40% w/w with respect to the polymer weight.

The effect of polymer concentration was found to influence the particle size and %EE as shown in Figure 1(B). The amount of polymer exponentially increases the particle size of FMD-microballoons. It have similar exponential effect on %EE such as low polymer concentration resulted in low %EE and high polymer weight results in higher %EE. Though %EE was decrease at very high polymer ratio (>60% w/w) might be due to the increase in matrix density with the increase in polymer concentration. Another reason could be high amount of polymer may arrange themselves into own particulate matrix results in decreases in actual amount of polymer required for encapsulation of drug moieties. 14,16 Therefore, drug moieties was remain un-encapsulated and %EE was low.

The surfactant concentration was optimized on the basis of particle size and %EE of FMD-microballoons (Figure 1(C)). In case of low surfactant concentration (≤ 0.5% w/v), the amount of
surfactant molecules are insufficient to stabilize the inter-polymer matrix resulting in larger sized particles. While increase in further surfactant amount, increases the polymer-PVA interaction and particle size was decrease. Although amount of surfactant have negative effect on %EE, later was decreases with the increase in surfactant amount. High surfactant solubilizes the FMD in water affect in decreases in amount of FMD require for encapsulation. While increase in further surfactant amount, increases the polymer-PVA interaction and particle size was decrease. Although amount of surfactant have negative effect on %EE, later was decreases with the increase in surfactant amount. High surfactant solubilizes the FMD in water affect in decreases in amount of FMD require for encapsulation. While increase in further surfactant amount, increases the polymer-PVA interaction and particle size was decrease. Although amount of surfactant have negative effect on %EE, later was decreases with the increase in surfactant amount. High surfactant solubilizes the FMD in water affect in decreases in amount of FMD require for encapsulation.

**Figure 1:** Effect of formulation variables namely (A) drug concentration, (B) Polymer concentration and (C) surfactant concentration on particle size and %EE of FMD-microballoons

Stirring speed was optimized to get optimum particle size and %EE as evidenced in Figure 2(A). The results confirmed that stirring speed exponentially decreases the particle size, albeit >400 rpm speed had insignificant effect on both particle size and %EE. Application of external energy in the form of stirring, broken down the inter-polymer complexes. At low rpm (<300 rpm), the shearing force was notably low resulting in larger particle size, whereas lower %EE may be attributed to lesser volume of larger particles than smaller particles. Therefore, stirring speed of 400 rpm was considered as optimum for preparation of FMD-microballoons.

As evident from Figure 2(B), 37°C was found to be optimum temperature may be attributed to formation of stronger inter-polymer hydrogen bond at 37°C. On the contrary, lower (25°C) or higher (45°C) temperature resulted in temperature dependent aggregation of particle. The %EE remain
unaffected at all temperatures, albeit %EE might be decreased at higher temperature owing to temperature dependent solubilization of FMD.

![Graph](A)  ![Graph](B)  

**Figure 2:** Effect of process variables viz. (A) stirring speed and (B) temperature on particle size and %EE of FMD-microballoons

The microscopy evaluation of particles confirmed the shape morphology and size of FMD-microballoons. The particle was found to have spherical surface morphology and particle size was ~100 µm as depicted in Figure 3.

![Microscopy Images](A)  ![Microscopy Images](B)  

**Figure 3:** Microscopic evaluation of FMD-microballoons using (A) optical microscopy and (B) scanning electron microscopy

**In vitro** drug release study in simulated biological media

The *in vitro* drug release study confirmed that floating FMD-microballoons resulted in slow sustained release of FMD in the simulated biological fluids. It was found that more than 80 % of entrapped drug was released within 24 h as initial burst followed by controlled release fashion (Figure 4). The data obtained for *in vitro* release were fitted into mathematical models viz. zero order, first order and Higuchi type. Highest regression coefficient values for Higuchi’s model revealed that the FMD-
microballoons was matrix type delivery system executing diffusion followed by erosion of matrix is predominant mechanism of drug release.\textsuperscript{7}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Release profile of FMD-microballoons in simulated biological fluids}
\end{figure}

\textit{In vitro} buoyancy test of FMD-microballoons

The % buoyancy of FMD-microballoons was found to be 47\% and 69\% in PBS (pH 7.4) and SGF (pH 1.2), respectively as shown in Figure 5. The solubility of polymer or hydration was negligible in SGF (acidic) condition than PBS (neutral or basic) which can be evidenced by high floating capacity in SGF over PBS.\textsuperscript{9}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{\textit{In vitro} buoyancy analysis of FMD-microballoons}
\end{figure}

\textbf{Stability studies}

A stable drug delivery system not only maintains the integrity and morphology, but also preserve the nature and content of the entrapped drug. In most of the stability studies, the major emphasis has been
directed towards the accelerated stability studies. The stability of FMD-microballoons during storage is undoubtedly important prerequisite and hence they were subjected to accelerated stability testing at 4±1°C, 25±1°C and 40±1°C for 45 days as depicted in Table 1. The particle size of the microballoons was found to be increased at higher temperature viz. 25±1°C and 40±1°C may be attributed to the aggregation of microballoons, albeit unaffected at 4±1°C.\textsuperscript{10} The change in surface morphology owing to higher temperature was observed using optical microscopy. The percent residual drug content demonstrated that FMD-microballoons releases very less amount of drug at 4±1°C owing to slow or little degradation. On the contrary, higher temperature (>25°C) not only makes them leaky but also pulls out maximum amount drug from microballoons complexes. Collectively, such temperature-dependent change revealed that inter-hydrogen bonding between complexes was labile to temperature and product must be store at 4±1°C without any detrimental effect on formulation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial observation (0 day)</th>
<th>Final Observation (45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Particle size (µm)</td>
<td>148 ± 3</td>
<td>148 ± 3</td>
</tr>
<tr>
<td>Residual drug content (%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Surface morphology</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

\textit{In vivo} radiographical study

No study can be accomplished conclusively without the help of animals, therefore \textit{in vivo} radiographical study was investigated using BS-microballoons to determine floating performance. X-ray images depicted the intragastric behavior of the BS-microballoons as evidenced in Figure 6. As evident in Figure 6, BS-microballoons remained buoyant even after 4 h satisfactory for a gastroretentive formulation. The floating time is sufficient enough for widespread distribution of FMD during the course of the gastrointestinal tract which affords the possibility of a longer lasting and more reliable release of the drug from the FMD-microballoons.\textsuperscript{18-22} Prolonged gastric retention not only
improves the bioavailability, reduces drug waste but also suitable for local drug delivery drugs with narrow absorption windows in the small intestinal region.9

![Figure 6: In vivo floating performance of BS-microballoons](image)

**CONCLUSION**

The present study explained the preparation and exhaustive optimization FMD-microballoons by solvent diffusion method. The importance of various formulation and process parameters for formulation of FMD-microballoons were also investigated. Famotidine loaded Microballoons (FMD-microballoons) with an internal hollow structure were prepared by solvent diffusion evaporation method. The formulation attributes of FMD-microballoons exhibited excellent floatability, high buoyancy and prolonged drug release. An oral radiographical study showed that microballoons improved the residence time in gastric environment. It appears that FMD-microballoons offer a promising delivery system for the intragastric delivery. However, the potential of FMD-microballoons is confirmed after extensive pre-clinical evaluation.

**REFERENCES**


