ABSTRACT

PURPOSE: The present study involves the preparation of floating microspheres (1) of Famotidine with Ethyl cellulose in the different ratio of drug and polymer. The aimed of floating microspheres were to achieve an extended retention in the upper GIT (2) which may result in enhanced absorption & thereby improved bioavailability. Famotidine is a non- imidazole blocker of histamine H2 receptors that mediate gastric secretion. METHODS: Microspheres were prepared using solvent evaporation method (3). RESULTS: The average particle size was found to be 59.85µm. The maximum % yield of the microspheres was found to be 86.05±2.74. Powder had good flow property. In-vitro drug release was satisfactory presenting sustained release effect and it is showing good floating ability (>12hrs). CONCLUSION: The concept of floating microspheres can be utilized the minimized the irritant effect of drug in the stomach by avoiding direct contact with mucosa and increasing gastric residence time (4).

Keywords: Floating microspheres, Famotidine, GIT dosage form.

INTRODUCTION

Drug delivery is the method of process of administering pharmaceutical compound to achieve the therapeutic effect in human beings or animals (5). Despite of the advances in the inhalable, injectable, transdermal, nasal, and other routes of administration, we can't deny the point that oral drug delivery system remains well ahead as the preferred route of drug delivery. The oral route is the most convenient route of drug administration because of ease of administration, patient compliance and flexibility in formulation, etc. but the limitations of the conventional oral drug delivery system is that they don't have control over the drug release pattern from the dosage form and they may result in the fluctuation of plasma drug concentration. One of the reasons for its limitation is the unique GI physiology. In these areas even small improvements in the transit time of our formulation by formulating gastroretentive dosage forms (GRDFs) greatly improve the gastric residence time. Dosage forms with a prolonged GRT, i.e. predictable drug delivery profile in the GI tract is to control the absorption, distribution for the benefit of improving product efficacy and safety as well as patient convenience and compliance.

One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GI tract is to control the gastric residence time. Dosage forms with a prolonged GRT, i.e. gastroretentive dosage forms (GRDFs), will provide us with new and important therapeutic options.

GRDFs not only prolong dosing intervals, but also increase patient compliance beyond the level of existing controlled release dosage forms. This application is especially effective in delivery of sparingly soluble and insoluble drugs. It is known that, as the solubility of a drug decreases, the time available for drug dissolution becomes less adequate and thus the transit time becomes a significant factor affecting drug absorption. To address this, oral administration (7-8) of sparingly soluble drugs is carried out frequently; here we can increase the transit time of our formulation by formulating gastroretentive drug delivery system. GRDFs greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentrations at the gastric mucosa (eradicating Helicobacter pylori (9) from the submucosal tissue of the stomach), making it possible to treat stomach (10-11) and duodenal ulcers, gastritis and eosinophilitis, reduce the risk of gastric carcinoma and administer non-systemic, controlled release antacid formulations (calcium carbonate). Drugs reported to be used in the formulation of floating dosage forms are floating microspheres (griseofulvin, p-nitroaniline, ibuprofen (12), cinetidine (13), piroxicam (14), metformin Hydrochloride (15) and repaglinide (16)). Floating granules (diltiazem hydrochloride-Gelucire (17), diclofenac sodium, indomethacin and prednicarone), films (cinnarizine), floating capsules (Nicardpine hydrochloride (18), chloridiazepoxide hydrogen chloride, diazepam, furosemide, misnoprostol, L-Dopa, benserazide, ursodeoxycholic acid and peptide) and floating tablets and pills (acetaminophen, acetylsalicylic acid, amoxicillin, amoxycillin trihydrate, atenolol, diltiazem, ranitidine (19), fluoroouracil, isosorbide mononitrate, para-aminobenzoic acid, piretamine, theophylline and verapamil hydrochloride, etc.). Excipients used most commonly in these systems include HPMC, ethyl cellulose, polyacrylate polymers, polyvinyl acetate, ethyle cellulose, polyacrylate polymers, polyvinyl acetate, carbopol, agar, sodium alginate, calcium chloride, polyethylene oxide and polycarbonates etc.

MATERIALS AND METHODS

Materials

Famotidine was obtained as gift sample from Sun Pharma, Jammu, India. Ethyl Cellulose Procured as gift sample from Evonik Degussa India Pvt. Ltd. Dichloromethane Selected as a solvent and procured from CDH(New Delhi) Ethanol Selected as a solvent and procured from CDH, New Delhi. All other chemical were analytical grade.

Preparation of Microspheres

Floating Microspheres were prepared solvent diffusion evaporation method. Various formulations of microspheres (Table-1) were prepared using gradually increase EC concentration. In this method the polymer is dissolved in the polymer solution. This drug polymer solution is dispersed in an external medium of 0.75%aq. Solution of PVA hot (polyvinyl alcohol) in a 500 ml of beaker. The whole system was stirred at a 800-1000 rpm using mechanical stirrer equipped with three blade propellers for 3-4 hrs at 25-40°C to ensure the evaporation of the solvent. The solvent removal leads to precipitation at o/w interphase of droplets forming cavity and thus making them hollow to impart floating property. The prepared microspheres then filtered off, washed with n- hexane, air-dried and stored in a desiccator.

Physicochemical Evaluation of Floating Microspheres

The prepared Microspheres were evaluated for % yield, particle size, flow property, density (bulk and tapped density), and In-vitro drug release.

Calculation of % yield

The prepared microspheres were collected and weighed. The ratio of the actual weight of obtained microspheres and weight of the
material that was used for the preparation of the microspheres multiplied by 100 gives the % yield of the microspheres (Equation-1)

\[
\% \text{ Yield} = \frac{\text{Actual weight of product obtained} \times 100}{\text{Total weight of recipient and drug}} \quad (1)
\]

% Yield = Actual weight of product obtained \times 100 \quad (1)

**Particle size analysis**

**Optical microscopy**

The size of microspheres was determined using an optical microscope magnification 10X (Magnus MLX-DX) fitted with an ocular micrometer and stage micrometer. At least 200 particles must be counted to obtain a good size distribution analysis of data.

**Morphology**

**Scanning electron microscopy**

Scanning electron microscopy (LEO, 430 surface controlled digital SEM) was performed to characterize the surface of formed microspheres. A small amount of microspheres were spread on glass stub, Gold palladium coating on the prepared stub was carried out by using sputter coater. Afterwards, the stub containing the sample was placed in the electron microscope. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg.

**Angle of Repose**

It is used to determine the flow property of powder. This is determined by fixed funnel method (21). This is calculated by the between the surface of a pile of the powder and the horizontal plane (radius of pile) (Equation-2).

\[
\tan \theta = \frac{h}{r} \quad (2)
\]

Where, \( h \) = height of pile
\( r \) = radius of the base of the pile.

**Tapped density**

The prepared micro spheres were weighted, collect, and poured in to a 5 ml of graduate cylinder. This system was tapped 100 times and then measured the volume of filled microspheres. Tapped density was calculated by using the following formula (Equation-3).

\[
\text{Tapped Density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \quad (3)
\]

% Compressibility index

The prepared microspheres were weighted, collect, and poured into a 5 ml of graduate cylinder. This system was tapped 100 times and then measured the volume of filled microspheres. It is the ratio of the volume before tapping which was filled in the graduate cylinder and after tapped volume (Equation-4).

\[
\% \text{ Compressibility index} = \frac{1 - V}{V_0} \times 100 \quad (4)
\]

Where \( V \) and \( V_0 \) are the volume of the samples after tapping and before tapping.

**Partition Coefficient**

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. For a drug delivery system, Lipophilic/ Hydrophilic balance has been shown to be contributing factors for rate and extent of drug absorption. Partition coefficient provides a means of characterizing, Lipophilic/ Hydrophilic nature of drug. The measurement of drug lipophilicity and indication of its ability to cross the lipoidal cell membrane is the oil/water partition coefficient in system such as octanol/water and octanol / buffer.

Partition coefficient of famotidine was determined by using shake flask method. This relies on the equilibrium distribution of a drug between an oil and aqueous phase. In this study 10 mg of drug was taken in a 60 ml vial and then 20 ml of Simulated Gastric Fluid pH 1.2 was added to it and shaken it, then 20 ml of n-octanol was added. n-octanol layer was less dense than water, so the n-octanol layer was on the top of the water. The system was then shaken for 30 minutes and then it was left to reach equilibrium for 24 hrs in a separating funnel. The two phases were then separated. Then the concentration of drug was measured in each phase using UV spectrophotometry (Shimadzu 1800) at 264 nm. The partition coefficient was calculated by the following equation (Equation-5).

\[
\frac{C_{\text{organic}}}{C_{\text{aqueous}}} = \text{Partition coefficient} \quad (5)
\]

**Buoyancy Percentage**

Microspheres 50 mg were spread over the surface of a USP xxiv dissolution apparatus (type II) filled with 900 ml of simulated gastric fluid containing 0.02% tween 80. This medium was agitated with paddle rotation speed 100 rpm for 12 hours. After 12 hours the floated and settled microspheres were collected separately. The microspheres were dried and weight, then calculate by the following formula (Equation-6).

\[
\frac{\text{Mass of floating microspheres}}{\text{Total mass of microspheres}} \times 100 \quad (6)
\]

**Drug Entrapment Efficiency**

The 50 mg of prepared microspheres were crushed in a glass mortar and the powder microspheres were suspended in a 10 ml of methanol after 24 hours, the solution was filtered and the filtrate was analyzed after suitable dilutions using UV spectrophotometer (Shimadzu UV-1800 series) at Amax 227 nm. The amount of drug entrapped in the microspheres was calculated by the following formula (Equation-7).

\[
\frac{\text{Entrapment efficiency}}{\text{Drug in microspheres}} \times 100 \quad (7)
\]

**In-vitro Dissolution Study**

The drug release study was performed using USP xxiv dissolution apparatus at 37°C±0.5°C at 50 rpm using 900 ml of simulated gastric fluid containing 0.02% tween 80. Microspheres were taken and tied in muslin cloth and introduced in 900ml of 0.1N HCl (PH 1.2). Aliquots (5ml) were withdrawn at 1 hr interval for 12 hrs and an overnight reading was also taken at 24 hrs. Their volume was making up to 10 ml with SGF. Sink condition was maintained throughout the study by replacing with equal amounts of fresh dissolution medium i.e. SGF. These aliquots were then analyzed under UV spectrophotometer (Shimadzu 1800) and their absorbance is recorded. With the help of the absorbance obtained, the concentration of drug released in particular time is also calculated and a graph is plotted between time and drug release.

**RESULT AND DISCUSSION**

Now let us discuss the effect of another variable that is effect of stirring speed during the formulation on the mean particle size of the microspheres. Consider the batch formulated using Ethyl cellulose. The average mean particle size of the microspheres formulated under the stirring speed of 700 rpm was found to be 74.21±20.96µm whereas those formed under the stirring speed of 1000 rpm was 70.58±17.50 µm. In Figure-1 the graph showed the significant effect of the ratio of the polymer Ethyl cellulose with drug on the physical properties of the microspheres such as mean particle size, % compressibility and angle of repose. The graph is representing three series of variables, first series denotes the mean particle size, second series denotes the % compressibility and the third series is denoting angle of repose. As we increased the polymer ratio a increased in particle size has been observed and Second series represents also increasing the % compressibility and third series angle of repose found to be little increased of the microspheres on increasing the polymer ratio (Figure-1). The Figure-2 showed the Comparative Study of Particle Size, Incorporation Study and Buoyancy. As increased the particle size the incorporated efficiency has been observed increased this may be due to the polymer had greater drug loading capacity and poor solubility of drug in water and when increased the particle size buoyancy had also increased because poor solubility of polymer in simulated
gastric fluid and floating tendency also depend on amount of dichloromethane used during the formulation of floating microspheres. When greater volume of dichloromethane was used that formulation showed good buoyancy because of greater volume of dichloromethane formed the larger air core. When prepared the floating microspheres with solvent ratio of ethanol: dichloromethane and acetone, ethanol and acetone present in oil droplet diffuse to water thus drug and polymer mixture solidified on dichloromethane droplet, which was core of microspheres so when increased the temperature more than 50°C the dichloromethane evaporated and larger air was created in core of microspheres and due to this air core microspheres showed hollowness.

The maximum percentage yield were found to be 86.05±2.74 (Batch FE-7) (Table-2A). The range found between 71.51±2.84 (Batch FE-5) to 95.02±1.25 (Batch FE-6). The mean particle size of the microspheres significantly increased with increasing polymer concentration and was in the range of 30.90±12.10μm to 74.21±20.96μm (Table-1). It was observed that, on increasing the polymer amount the average particle size increased. This may be due to higher viscosity.

The SEM photograph showed that the floating microspheres were spherical, smooth and hollow with a perforated smooth rough surface (Figure-3). The microspheres surface was also found smooth and shape is roundness (Figure-3, Batch FE-1). The perforation may be due to evaporation of dichloromethane from embryonic microspheres. These images also confirm that rapid evaporation of dichloromethane causes capture of microspheres. The tapped and bulk density was in range between 0.115±0.07 (Batch FE-7) to 0.182±0.07 (Batch FE-1) and 0.099±0.12 (Batch FE-7) to 0.201±0.65 (Batch FE-1) g/cm³ respectively (Table-1). Density is decreases as polymer size of microspheres is increases. The pellets of KBr and drug were prepared and examined under spectrum RX1, Perkin Elmer, FTIR system, UK. The drug sample peak is similar to reference standard (Figure-4). Compressibility index ranged from 12.33±3.02 (Batch FE-1) to 21.66±1.03 (Batch FE-7) (Table-1). The percentage compressibility is increases as particle size is increases. The drug entrapment efficiency was found in the range of 52.28±7.75 (Batch FE-1) 73.03±2.83 (Batch FE-7) (Table-2A). Maximum drug entrapment efficiency was found 73.03±2.83 (Batch FE-1). The cause of good drug entrapment efficiency may be the insolubility of drug in water. The microspheres were floating for prolong time (more than 12 hrs.) over the surface of simulated gastric fluid with 0.02% tween 80 (pH 1.2). Buoyancy percentage of microspheres was in the range 66.17±3.65 (Batch FE-1) to 80.76±1.46 (Batch FE-7) (Table-2A). It was observed that floating ability increased with increasing average particle size.

In-vitro Famotidine release study was performed in simulated gastric fluid (pH 1.2) with 0.02% tween 80 for 24 hours (Graph-1). The cumulative release of Famotidine significantly decreases with increasing polymer concentration. The cause of low drug release in the acidic medium was the weak acid is nature of drug and it will remain unionized in the same medium. No significant increase in cumulative drug release was observed on increasing the ratio of polymer ethyl cellulose. Solvent ratio was not affected (ethanol: dichloromethane: acetone) on cumulative drug release profile in simulated gastric fluid pH 1.2 (Graph-2). All release kinetics model were applied on all seven formulation. The best fit model was found to be Higuchi for all formulation. The best model was based on residual sum of squares. If the release of drug from the matrix, when plotted against square root of time, shows a straight line, having highest R value and lowest Residual Sum of Square (RSS), it indicates that the release pattern is obeying Higuchi’s kinetics (Equation-8). Where Qt is the amount of drug released at time t; in the formulation; kH, is release rate constants for, Higuchi model rate equations.

\[ Qt = kHt^{1/2} \] 

\[ (8) \]

**In-vivo Study**

Two groups containing three animals in each group were used for performing the experiment. New-Zealand white rabbit species for performing this study weighing 2.0-2.5 kg. Animals were kept fasting for overnight. Water was given during fasting and throughout experiment. Microspheres were swallowed easily without any difficulties. The procedures employed in this study were approved by Institutional Ethical Committee, Bundelkhand University, Jhansi UP, India by using Registration No: BU/Pharm/IAC/10/031.

One group was fed with pure drug (Famotidine) at a dose of 10 mg/kg, other groups were fed with prepared floating microspheres of 100 mg, which is equivalent to 10 mg/kg of drug (FE-1 having the drug polymer ratio 1:1). One animal of each group was kept as control.

Blood samples (3ml) were collected from marginal ear vein of control animals using heparin into centrifuge tubes. Blood sample was centrifuged at 2000rpm and plasma separated. In 1 ml plasma sample added 4 ml of acetonitrile, now the tubes were centrifuged at 3900 rpm in ultracentrifuges for 10 minutes. 2ml of the supernatant was pipette out to which 0.3 ml of 1.47M perchloric acid was added and the concentration was determined by UV spectrophotometer (Shimadzu-1800) at 260nm. The same method was followed in all cases at an interval of 1, 2, 4, 6, 8, 10, 12, 18 and 24th hr during study.

The graph - 3 showing the blood plasma concentration of the drug vs time for the Pure drug (famotidine) and batch FE-1 of floating microspheres. The maximum plasma drug concentration was found at the fourth hour and then the rapidly decreases drug concentration of the pure drug. While in case of floating microspheres (FE-1) the maximum plasma drug concentration was found at 8th hour and slowly decreased the concentration as showed controlled release profile (Table-3). The controlled effect was observed for a longer period of time in the case of floating microspheres due to the slow release and extended absorption. This sustained release formulation was more effective than the immediate release famotidine suspension.

<table>
<thead>
<tr>
<th>Table 1: Batch Specification of the Prepared Microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation Code</strong></td>
</tr>
<tr>
<td><strong>(in mg.)</strong></td>
</tr>
<tr>
<td><strong>ETN: DCM: ACT</strong></td>
</tr>
<tr>
<td>FE-1</td>
</tr>
<tr>
<td>FE-2</td>
</tr>
<tr>
<td>FE-3</td>
</tr>
<tr>
<td>FE-4</td>
</tr>
<tr>
<td>FE-5</td>
</tr>
<tr>
<td>FE-6</td>
</tr>
<tr>
<td>FE-7</td>
</tr>
</tbody>
</table>

ETN: ethanol, DCM, Dichloromethane, ACT: Acetone

<table>
<thead>
<tr>
<th>Table 2: Micromeritics Properties of Optimized Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation Code</strong></td>
</tr>
<tr>
<td><strong>(μm)</strong></td>
</tr>
<tr>
<td>FE-1</td>
</tr>
<tr>
<td>FE-2</td>
</tr>
<tr>
<td>FE-3</td>
</tr>
<tr>
<td>FE-4</td>
</tr>
<tr>
<td>FE-5</td>
</tr>
<tr>
<td>FE-6</td>
</tr>
<tr>
<td>FE-7</td>
</tr>
</tbody>
</table>

*Mean±SD, n=3
Table 2A: Others Properties of Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Angle of Repose*</th>
<th>% age Yield*</th>
<th>Incorporation Efficiency*</th>
<th>Buoyancy % age*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE-1</td>
<td>14.23±0.89</td>
<td>72.03±2.10</td>
<td>52.28±3.75</td>
<td>66.17±3.65</td>
</tr>
<tr>
<td>FE-2</td>
<td>18.89±1.23</td>
<td>74.26±2.80</td>
<td>54.23±3.05</td>
<td>68.2±0.71</td>
</tr>
<tr>
<td>FE-3</td>
<td>22.78±2.45</td>
<td>76.11±1.45</td>
<td>59.67±2.39</td>
<td>71.6±0.18</td>
</tr>
<tr>
<td>FE-4</td>
<td>25.7±0.85</td>
<td>78.31±0.68</td>
<td>64.26±1.51</td>
<td>73.34±1.43</td>
</tr>
<tr>
<td>FE-5</td>
<td>28.65±1.45</td>
<td>71.51±2.84</td>
<td>69.08±3.61</td>
<td>76.8±1.85</td>
</tr>
<tr>
<td>FE-6</td>
<td>29.45±0.12</td>
<td>85.02±1.25</td>
<td>72.51±0.88</td>
<td>79.9±3.85</td>
</tr>
<tr>
<td>FE-7</td>
<td>31.45±2.87</td>
<td>86.05±2.74</td>
<td>73.03±2.83</td>
<td>80.7±1.46</td>
</tr>
</tbody>
</table>

*Mean±SD, n=3

Table 3: In-vivo comparative study of Famotidine and floating microspheres (Batch FE-1)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (hour)</th>
<th>Pure Drug Concentration (µg/ml)</th>
<th>Floating Microspheres (Batch FE-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pure Drug (Famotidine)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.58</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.82</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1.63</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0.94</td>
<td>1.28</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0.72</td>
<td>1.67</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>-</td>
<td>1.18</td>
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<tr>
<td>8</td>
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<td>1.02</td>
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<td>9</td>
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<td>-</td>
<td>0.63</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>-</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Fig. 1: Effect of Polymer Ratio on Micrometrics properties

Fig. 2: Comparative Study of Particle Size, Incorporation Study and Buoyancy
Fig. 3: (a) showed the single microsphere with spherical smooth surface (b) it was showed the Hollowness and rough surface of microsphere.

Fig. 4: FTIR Spectra of Drug Famotidine and Polymer Ethyl Cellulose

Graph 1: Release Profile of Different Floating Formulation
CONCLUSION

Drug sample gives almost straight line for graph between concentration vs absorption in methanol and buffer, which follows Beer’s law; it indicates that drug sample was pure. The yield of microballoons was good. Microspheres showed good floating for more than 12 hours. SEM confirmed their spherical size, perforated smooth surface and a hollow cavity in them hours. In-vitro drug studies were performed in Simulated Gastric Fluid (pH 1.2). Different drug release kinetics models were applied for selected batches. It was concluded that for kinetic drug release Hugichi was the best fit model.

The hollow floating microspheres were prepared by solvent evaporation diffusion method. The floating microspheres are free flowing powder having particle size less than 100 μm. Different batches have been prepared on which the effect of different variables has been studied. The mean particle size of the microspheres has been obtained by optical microscopy. In the batches, mean particle size of the microspheres found to increase on increasing the drug polymer ratio. When we have decreased the volume of internal phase, there is significant increase in the mean particle size of the microspheres. We have also concluded that on increasing the stirring rate during the formulation, the particle sizes have decreased. Tapped density refers to the bulk density of the powder after a specified compaction process. Tapped density is determined because bulk density is not an intrinsic property of the material as it can be changed depending how material is handled. After studying the effect of such variables on the physical characteristics of the microspheres, we can optimize the processing parameters to design required characteristics in our formulation and since it is known that physical characteristics of the dosage form effect the pharmacokinetic properties of the drug, so, this approach may play a role in designing a formulation with desirable pharmacokinetic patterns.

ACKNOWLEDGMENT

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