In vitro evaluation of floating microspheres of Ketoprofen

Abdul Hafeez, Arun Maurya, Jagpal Singh, Lakhan Rana

Abstract

Purpose: Floating microspheres were designed to increase the residence time in stomach with lesser direct contact with gastric mucosa. Method: Floating microspheres of Ethyl cellulose (EC), blend of Ethyl cellulose and Hydroxypropyl methyl cellulose (HPMC) (in a ratio of 1:1) loaded with ketoprofen were prepared using emulsion solvent diffusion method. Results: Shape and surface characteristics were analyzed using optical and scanning electron microscopy respectively. They found to be sufficiently buoyant over simulated gastric fluid for more than 4 hrs. Entrapment of the drug in polymer matrix was found to be increased with an increase in amount of polymer. In vitro release study was done in phosphate buffer (pH 7.4, 6.8) for 6 hrs and the prepared microspheres exhibit prolonged drug release. The mean particle size increased and the drug release rate decreased at higher polymer concentration.

Keywords: Floating microspheres, Ketoprofen, In-vitro release

Introduction

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half life are eliminated quickly from the blood circulation. To avoid this problem, the oral controlled release (CR) formulation has been developed, these are proposed to release the drug slowly into the GIT and maintain a constant drug concentration in the serum for a longer period of time.1 The gastrointestinal (GI) residence time determines the period available for release of bioactives from oral controlled release delivery system within the gastrointestinal tract.2 Various approaches have been pursued to increase the retention of an oral dosage form in the stomach, including floating systems, swelling and expending systems, bioadhesive systems, modified shape systems, high density systems and other delayed gastric emptying devices.

Floating systems can be classified in to two categories: (A) Single unit dosage systems and (B) Multiple unit dosage systems.3

The single unit floating systems are more popular but have a disadvantage owing to their ‘all-or-nothing’ emptying process leading to high variability of the gastrointestinal transit time.4 However, the multiple unit particulate dosage forms pass through the GIT to avoid the vagaries of gastric emptying and thus release the drugs in an uniform fashion. The uniform distribution of these multiunit dosage forms along the GIT could result in the more reproducible drug absorption and reduced risk of local irritation than the use of single unit dosage forms.5
Floating systems first described by Davis in 1968, are low density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at a desired rate, which results in increased GI residence time (GRT) and reduced fluctuation in plasma drug concentration. Both natural and synthetic polymers have been used to prepare floating microspheres. Jain et al, (2006) developed porous carrier based floating microspheres of Orlistat, an antiobesity agent using calcium silicate as porous carrier and Eudragit S as polymer by solvent evaporation method. Yang et al, (2004) prepared microspheres with microbubbles inside the microspheres using xanthan gum and gelatin by water in oil method using Theophylline as model drug. Umamaheshwari et al, (2003) developed floating microspheres of Acetohydroxyamic acid for the treatment of Helicobacter pylori infection. Ketoprofen, a nonsteroidal anti-inflammatory drug is readily absorbed from the GI tract and possesses a bitter taste and after taste. The half life of the drug in plasma is about 2-3 hours. It is a II drug to BCS. Ketoprofen has low water solubility and exposure of the stomach to high levels of ketoprofen can cause severe gastric damage such as ulceration or bleeding. To improve these disadvantages sustained release or enteric coated dosage forms have been developed, resulting in less frequent dosing and less GI disturbances.

Experimental

Materials

Ketoprofen was obtained as a gift sample from Torrent laboratories, Ahmedabad, India. Ethyl cellulose, ethanol, sodium lauryl sulfate and tween-80 were received from S.D. Fine Chemicals Ltd. India. HPMC was obtained from Central drug house (P) Ltd. India and dichloromethane from Qualigens Fine Chemicals, Mumbai, India.

Preparation of floating microspheres

Microspheres of ethyl cellulose, blend of ethyl cellulose and hydroxypropyl methyl cellulose (HPMC) (1:1) loaded with ketoprofen were prepared using emulsion solvent diffusion method. Three batches were prepared for both (EC) and blend of polymers with drug polymer ratio 1:1, 1:2 and 1:4. The mixture of drug and polymer was dissolved in 10 ml of dichloromethane and ethanol (1:1). The resulting mixture was added drop wise in 0.02% w/v aqueous sodium lauryl sulfate. The solution was stirred using a propeller type agitator for 2 hrs at 500 rpm. The microparticles so formed were then filtered, washed with water and dried at room temperature in a desiccator. Microspheres were weighed and stored in storage vials.

Characterization of microspheres

Particle size analysis

Particle size was measured using an optical microscope and the mean particle size was calculated by measuring 300 particles with the help of a calibrated ocular micrometer.

Scanning electron microscopic study

Scanning electron microscopy was performed to study the morphology of the prepared microspheres. Ketoprofen loaded microspheres were sprayed on double sided carbon tabs an aluminium stub. The stubs were then coated with gold using a polagon sputter coater Scanning electron photomicrographs were taken on a JSM 6100 scanning electron microscope (Japan), at an acceleration voltage of 15 KV.

Drug content

The drug content in floating microspheres was determined by dispersing 20 mg microspheres in 20 ml methanol and agitating using a magnetic stirrer for 8 hrs. Stirring facilitated the polymer to dissolve and the drug extract out. The resulting solution was filtered and aliquots of samples. These were then adequately diluted with methanol and analyzed using UV visible spectrophotometer dissolve the polymer and to extract the drug. After filtration, samples were withdrawn, diluted with methanol and analyzed with UV spectrophotometer at 254 nm (Varian Cary-5000 UV-visible spectrophotometer). Each of the determination were made in triplicate.

The percentage drug entrapment calculated using the formula

\[
\text{Calculated drug concentration} = \frac{\text{Percentage drug entrapment}}{100}
\]

Floating behavior (buoyancy)

Floating microspheres 50mg dispersed were in gastric fluid (pH 1.2, 100ml) containing 0.02% w/v Tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer.
(Remi Ltd. Mumbai) After 4 hrs, the layer of buoyant microspheres were pipetted and separated by filtration; particles in the sinking particulate layer were also separated by filtration. Microspheres of both types viz floating and sinking were dried in a desiccator until they acquired a constant weight. Each of the the fractions of microspheres were weighed. Buoyancy of the microspheres was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

\[
\text{Buoyancy (\%) = } \frac{W_f}{W_f + W_s} \times 100
\]

\(W_f\) and \(W_s\) are the weights of the floating and settled microparticles, respectively.

**In vitro drug release studies**

The drug release rate of floating microspheres was determined at a rotation speed of 100rpm using USP XXIII basket type dissolution apparatus. Weighed amount (100mg) of floating microspheres was filled into a capsule and placed in the basket. Phosphate buffer (pH 6.8) was used as the dissolution medium and maintained at 37 ± 1°C at a rotation speed of 100 rpm. Volume of dissolution medium was 250 ml. Five samples were withdrawn every time. First two samples were withdrawn at an interval of 30 minutes. And next five samples were withdrawn at 1 hr interval. Five ml of the sample was withdrawn every time these were analyzed spectrophotometrically at 258 nm to determine the concentration of drug present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. The dissolution studies were repeated using phosphate buffer saline (pH 7.4). All the experiments were conducted in triplicate.

**Results and Discussion**

**Formation of microspheres**

Floating microspheres were prepared by the emulsion solvent diffusion method using EC. Blend of EC and HPMC. Three batches (A1 to A3) were prepared using only EC and three batches (B1 to B3) prepared by using a combination of EC + HPMC (1:1). Microspheres were prepared by gradually increasing the concentration of polymer in combination with a fixed concentration of ketoprofen.

**Particle size**

The mean particle size of the microspheres was found to be increased with increasing polymer concentration and was in the range 25±1.505 μm to 37±1.651 μm (Table 1). Similar results of an increase in particle size with increasing polymer have been reported by Schlicher et al., (1997). also reported that increasing the concentration of the polymer in the second emulsion increases size of microspheres.16

**Percentage drug entrapment**

The drug entrapment efficiency of ketoprofen in all the formulations was found to be at all level of drug loading. It was found to be more than 50% for all batches and increased with an increase in polymer concentration. Ghaderi et al.also found that increasing the concentration of polymer in the organic phase increased the entrapment efficiency.15 Batch B3 revealed drug entrapment of more than 80 % (Table 1).

**Buoyancy**

The floating test was carried out to investigate the floatability of the floating microspheres. The microspheres were found to float floated for 4 hrs over the surface of simulated gastric fluid (pH 1.2). Percentage buoyancy of the microspheres was in the range of 58% (batch A1) to 72% (batch B3) (Table 1). The good floating behavior of the microspheres may be attributed to hollow nature of the microspheres.
Table 1

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug - polymer ratio</th>
<th>Yield (%)</th>
<th>Mean particle size $^a$ ($\mu$m)</th>
<th>Entrapment efficiency (%)</th>
<th>Buoyancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1</td>
<td>1:1</td>
<td>62.5</td>
<td>32.107±1.754</td>
<td>54.18</td>
<td>58</td>
</tr>
<tr>
<td>A_2</td>
<td>1:2</td>
<td>69</td>
<td>35.771±1.916</td>
<td>66.83</td>
<td>62</td>
</tr>
<tr>
<td>A_3</td>
<td>1:4</td>
<td>75.7</td>
<td>37.107±1.651</td>
<td>72.83</td>
<td>69</td>
</tr>
<tr>
<td>B_1</td>
<td>1:1</td>
<td>60</td>
<td>23.551±1.306</td>
<td>63.64</td>
<td>60</td>
</tr>
<tr>
<td>B_2</td>
<td>1:2</td>
<td>67</td>
<td>24.871±1.220</td>
<td>73.68</td>
<td>68</td>
</tr>
<tr>
<td>B_3</td>
<td>1:4</td>
<td>73.4</td>
<td>25.935±1.505</td>
<td>82.44</td>
<td>72</td>
</tr>
</tbody>
</table>

$^a$ mean ± SEM

Morphology

Morphology of the prepared floating microspheres was examined by scanning electron microscopy (SEM). Results show that Ethyl cellulose microspheres were predominantly spherical in appearance. The porous nature and spherical shape of the microspheres are evident from their SEM photomicrographs. Ethyl cellulose and HPMC based floating microspheres were found to be somewhat elongated in nature. A cavity was found to be in EC and HPMC based microspheres. The cavity was supposedly formed as follows according to the previous report (Kawashima et al., 1992). Eudragit based microspheres were spherical in nature and a cavity was also formed in the microsphere.

(A) Scanning electron photomicrographs of (a) Surface view of Ethyl cellulose microsphere (2,000X); (B) Ethyl cellulose based microsphere (1,100X)

Figure: 1 Scanning electron photomicrographs of (a) Surface view of Ethyl cellulose microsphere (2,000X); (B) Ethyl cellulose based microsphere (1,100X)
**Figure:** 2 Scanning electron photomicrographs of (a) Ethyl cellulose and HPMC based microsphere (3,300X); (B) Cavity formed in EC and HPMC based floating microsphere (3,300X)

**In vitro drug release study**

In vitro release study was performed in phosphate buffer (pH 7.4, 6.8) for 6 h. The cumulative release of ketoprofen was decreased with increasing polymer concentration. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.⁴

**The data of release rate studies of microspheres in phosphate buffer (pH 6.8)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A₁</th>
<th>A₂</th>
<th>A₃</th>
<th>B₁</th>
<th>B₂</th>
<th>B₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>180</td>
<td>27.553</td>
<td>20.551</td>
<td>18.125</td>
<td>23.014</td>
<td>19.639</td>
<td>16.381</td>
</tr>
</tbody>
</table>
The data of release rate studies of microspheres in phosphate buffer (pH 7.4)

Table 2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cumulative release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A₁</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>11.474</td>
</tr>
<tr>
<td>120</td>
<td>23.302</td>
</tr>
<tr>
<td>180</td>
<td>28.949</td>
</tr>
<tr>
<td>240</td>
<td>31.375</td>
</tr>
<tr>
<td>300</td>
<td>34.209</td>
</tr>
<tr>
<td>360</td>
<td>38.194</td>
</tr>
</tbody>
</table>
Figure: 3 Percentage cumulative release of ketoprofen loaded microspheres (Batch A₁)

Figure: 4 Percentage cumulative release of ketoprofen loaded microspheres (Batch A₂)

Figure: 5 Percentage cumulative release of ketoprofen loaded microspheres (Batch A₃)
Results show that drug release decreased with increasing polymer concentration. Drug release was found to be more in case of ethyl cellulose based microspheres as compared with EC and HPMC based microspheres.
Conclusion

The present study includes development of floating microspheres of EC and HPMC using solvents like ethanol, dichloromethane. These prepared microspheres of ketoprofen showed better encapsulation efficiencies and prolonged drug release. The microspheres exhibited good floating capability and may be retained in the stomach for 4 hrs. Thus these floating microspheres may prove to be good candidates for gastro retentive dosage systems.

Reference


