Formulation and Evaluation of Floating Microspheres of Ranolazine for the Treatment of Chronic Angina

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ABSTRACT
Ranolazine is indicated for the treatment of chronic angina alone or in combination with other cardiovascular agents. The objective of present investigation was to formulate floating microspheres of ranolazine to increase gastric residence time, increased bioavailability and reduce dose frequency of drug therapy. Novel o/w emulsion solvent diffusion technique was used for preparation of microspheres of ranolazine by using various polymers such as HPMC (hydroxyl propyl methyl cellulose), ethyl cellulose and Eudragit L 100. Entrapment efficiency of drug was up to 80.16%. Eudragit L100 based microspheres were found to be hollow cavity, spherical and porous nature from the results of scanning electron microscopy. Micromeritic profile of these microspheres was found satisfactory. From the results of FTIR spectroscopy it was revealed that there was no drug–polymer interaction. Eudragit L100 based microspheres shows good in vitro buoyancy and sustained release profile for longer period of time (> 14 hours), suggesting the viability of floating microspheres of ranolazine for improved pharmacokinetics for treatment of chronic angina.

KEYWORDS: Ethyl cellulose; Ranolazine; Microspheres; Eudragit L100 and HPMC.

Introduction
Oral controlled release dosage forms have been developed over the past few decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation (Woo BH et al., 2001). Gastric emptying is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms (Shweta et al., 2005).

Several techniques are used to design gastro retentive dosage forms. These include floating, swelling, inflation, adhesion, high-density and low density systems that increase the gastric residence time. Gastric retention is useful for drugs which (i) act locally (ii) have a narrow absorption window in the small intestinal region; (iii) unstable in the intestinal environment (iv) low solubility at high pH environment (Sheth et al., 1984). Various dosage forms developed for gastric retention include, floating tablets, floating beads, pellets, floating granules, floating microspheres (Chien, 1993).

Microspheres are considered to be one of the most promising floating systems, because they combine the advantages of multiple unit systems, good floating properties and are prepared using assorted polymers (Curatolo et al., 1995). However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. The floating microspheres have been developed in order to overcome frequent dosing to release the drug slowly into the GIT.

The objective of present study was to fabricate microsphere by using o/w emulsion–solvent diffusion technique in order to prolong the gastric residence time, in addition to enhance bioavailability and decrease the dose frequency of Ranolazine. The influence of several factors such as the particle size, drug entrapment efficiency, floating properties and dissolution of the resulting microspheres were investigated.

Materials and Methods
Drugs and Chemicals
Ranolazine was obtained as a gift sample from MSN Laboratories Ltd. Hyderabad; Eudragit L 100 was supplied by Degussa Pharmaceutical Ltd. Mumbai. Ethyl cellulose and HPMC was supplied by Colorcon Asia Pvt. Ltd. Goa. All ingredients and solvents used were of analytical grade Supplied by Loba chem. Mumbai.
Method of preparation for Floating Microsphere

Floating microsphere with of Ranolazine was prepared by using Emulsion solvent diffusion–evaporation technique (Ichikawa et al., 1991). Accurately weighed quantities of drug, Eudragit L100 were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio). Above prepared solution was poured into 150 ml distilled water containing 0.75% w/v Polyvinyl alcohol (PVA) and maintained at a temperature of 30–40°C. The resultant emulsion was stirred with a propeller type agitator at 400 rpm for 1 hour to allow the volatile solvent to evaporate.

Characterization of Microspheres

Entrapment Efficiency

Microspheres (50 mg) were crushed by using mortar and pestle, and then the crushed powder was transferred into 100ml volumetric flask. Add some quantity of 0.1N HCl to the volumetric flask and sonicate the resulting solution for 30 min. on ultrasonicator. Further make up volume with 0.1N HCl. And make up the suitable dilutions of resulting solution so that to obtained the solution of desired drug concentration. The absorbance was measured spectrophotometrically at 272 nm for Ranolazine.

\[
\text{Drug Entrapment Efficiency (\%) = } \left( \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \right) \times 10 \quad \ldots(1)
\]

Percentage Yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows (Ramachandran S et al., 2010).

\[
\text{Percentage Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100 \quad \ldots(2)
\]

Buoyancy percentage

Microspheres (50 mg) were spread over the surface of a USP XXIV dissolution apparatus (type II) filled with 900 ml 0.1M HCl containing 0.02% Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 hrs. The floating and the settled portion of microspheres were measured spectrophotometrically at 272 nm for Ranolazine.

\[
\text{Buoyancy percentage} = \left( \frac{\text{Weight of Floating Microspheres}}{\text{Initial Weight of Microspheres}} \right) \times 100 \quad \ldots(3)
\]

Micromeritic Properties

Particle size: The particle size of the microsphere was measured by using an optical microscope and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer (Yasunori et al., 2004).

Angle of Repose ($\theta$): It is the maximum angle possible between the surface of pile of powder and the horizontal plane. It was calculated using the following equation (Lachman et al., 1991).

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

\[
\theta = \tan^{-1}(\frac{h}{r}) \quad \ldots(4)
\]

Where, $h$- Height of the powder cone and $r$- Radius of powder cone.

Density: The Bulk Density (BD) and Tapped Density (TD) of microsphere were determined. Two grams of microspheres was introduced into a 10 ml calibrated measuring cylinder. After noting down the initial volume, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 inch at 2 seconds intervals. The tapping was continued until no further change in volume was noted. BD and TD were calculated using following equations (Lachman L et al., 1991).

\[
\text{BD} = \left( \frac{\text{Weight of Powder}}{\text{Volume of the packaging}} \right) \quad \ldots(5)
\]

\[
\text{TD} = \left( \frac{\text{Weight of powder}}{\text{Volume of Packaging after tapping}} \right) \quad \ldots(6)
\]

Hausner’s Ratio: Hausner’s ratio of the microsphere was calculated by using following formula (Lachman L et al., 1991).

\[
\text{Hausner's ratio} = \left( \frac{\text{Tapped Density}}{\text{Bulk Density}} \right) \quad \ldots(7)
\]

Carr’s Index: The Carr’s index of microsphere was determined by following equation (Lachman L et al., 1991).

\[
\text{Carr's Index} = \left( \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \right) \times 100 \quad \ldots(8)
\]

In-vitro % Drug Release

In-vitro dissolution studies in 0.1N HCl

The United States Pharmacopoeia basket-type dissolution rate test apparatus was used for the in vitro release studies at 100 rpm in 0.1N HCl solution as dissolution medium (900 ml) maintained at 37±5°C. A sample (5 ml) of the solution was withdrawn up to 12 hour from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered and diluted to a suitable concentration with 0.1N HCl solution. Absorbance of these solutions was measured at 272 nm using UV spectrophotometer. Percentage drug release was calculated using an equation obtained from a standard calibration curve.

In-vitro dissolution studies in phosphate buffer (pH 6.8)

The in-vitro dissolution study was repeated for all the batches of formulated microspheres using phosphate buffer pH 6.8 to investigate the influence of enteric polymers on the release of ranolazine at gastric pH and at intestinal pH from formulated floating microspheres.
**Results and Discussion**

**Infrared Spectroscopic Study:** FT-IR spectra of pure Ranolazine, Eudragit L100 and drug-loaded microsphere were obtained to verify the chemical interaction between drug and polymer. It is reported that the peaks for functional group of active ingredient were remain same in both spectra of the drug as well as the formulation. Hence, it indicates that there will be no interaction takes place between drug and polymer. In FT-IR spectra of ranolazine loaded microsphere, it was found that there was no significant spectral shift, as shown in Figure 1 and Figure 2.

![Fig. 1 IR spectrum of pure drug ranolazine.](image1)

![Fig. 2 IR spectrum of optimized formulation B2.](image2)
Preparation of floating microspheres

Floating microspheres containing ranolazine were prepared by emulsion solvent diffusion-evaporation technique using EC, HPMC, and Eudragit L100 polymer in ratio 1:1, 1:2, 1:3. Composition of all batches of floating microspheres of ranolazine was shown in Table 1.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Formulation code</th>
<th>Ranolazine (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>Eudragit L100 (mg)</th>
<th>HPMC (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>500</td>
<td>500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>500</td>
<td>1000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>A3</td>
<td>500</td>
<td>1500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>B1</td>
<td>500</td>
<td>-</td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>B2</td>
<td>500</td>
<td>-</td>
<td>1000</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>B3</td>
<td>500</td>
<td>-</td>
<td>1500</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>C1</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>500</td>
</tr>
<tr>
<td>8</td>
<td>C2</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1000</td>
</tr>
<tr>
<td>9</td>
<td>C3</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1500</td>
</tr>
</tbody>
</table>

By emulsion solvent diffusion-evaporation technique was implemented by using organic solvents such as dichloromethane and ethanol (Lee et al., 1991). Excellent buoyancy was shown by prepared microspheres because of their hollow nature, which can be retained for a longer period of time in the upper part of gastrointestinal tract (GIT) in order to increase gastric residence time of the drug. The emulsion of ranolazine and Eudragit L100 in dichloromethane and ethanol was poured into aqueous poly-vinyl alcohol solution. The ethanol rapidly partitioned into external aqueous phase and the polymer precipitated around the dichloromethane droplets, subsequently the evaporation of the dichloromethane led to formation of hollow cavities in the micro balloons.

Micromeritic Properties

The particle size of floating microspheres varied somewhat among the formulation due to variation in the composition of formulations. The average particle size of the microsphere formulations was found to be in range of 182.37 ± 5.90 to 322.72 ± 5.66 µm (as shown in Table 2). From all the formulations C1 showed lower mean particle size (182.37 ± 5.90) and B3 showed higher mean particle size (322.72 ± 5.66). As the concentration of the polymer increase particle size of the microspheres also increased.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Mean Particle size (µm)</th>
<th>Angle of repose</th>
<th>Bulk density (gm/cm³)</th>
<th>Tapped density (gm/cm³)</th>
<th>Hausner’s ratio</th>
<th>Carr’s Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>190.96 ± 4.74</td>
<td>18.54</td>
<td>0.333</td>
<td>0.392</td>
<td>1.21</td>
<td>15.05</td>
</tr>
<tr>
<td>A2</td>
<td>192.13 ± 5.94</td>
<td>23.66</td>
<td>0.344</td>
<td>0.400</td>
<td>1.16</td>
<td>14.00</td>
</tr>
<tr>
<td>A3</td>
<td>210.49 ± 5.86</td>
<td>21.91</td>
<td>0.327</td>
<td>0.370</td>
<td>1.13</td>
<td>11.62</td>
</tr>
<tr>
<td>B1</td>
<td>300.46 ± 5.45</td>
<td>12.41</td>
<td>0.350</td>
<td>0.392</td>
<td>1.12</td>
<td>10.71</td>
</tr>
<tr>
<td>B2</td>
<td>319.57 ± 6.65</td>
<td>13.66</td>
<td>0.312</td>
<td>0.344</td>
<td>1.10</td>
<td>9.32</td>
</tr>
<tr>
<td>B3</td>
<td>322.72 ± 5.66</td>
<td>10.51</td>
<td>0.322</td>
<td>0.357</td>
<td>1.10</td>
<td>9.80</td>
</tr>
<tr>
<td>C1</td>
<td>182.37 ± 5.90</td>
<td>20.42</td>
<td>0.327</td>
<td>0.363</td>
<td>1.11</td>
<td>9.91</td>
</tr>
<tr>
<td>C2</td>
<td>191.79 ± 5.51</td>
<td>19.67</td>
<td>0.341</td>
<td>0.382</td>
<td>1.12</td>
<td>10.73</td>
</tr>
<tr>
<td>C3</td>
<td>212.93 ± 4.41</td>
<td>17.58</td>
<td>0.347</td>
<td>0.381</td>
<td>1.09</td>
<td>8.92</td>
</tr>
</tbody>
</table>

Values are mean ±SD (n=3)

The bulk density, tapped density and Hausner’s ratio of all the formulations was in range of 0.312-0.350, 0.344-0.400 and 1.09-1.21 respectively. The Carr’s index was in range of 8.92 to 15.05 and Angle of Repose was between 10.51-23.66 (as shown in Table 2). The values of carr’s index and angle of repose indicate excellent flow properties.

**Scanning Electron Microscopy:** To examine morphology of floating microspheres Scanning electron microscopic studies was performed. As shown in Figure 1; it was observed that Microspheres were observed as a hollow structure with the outer surface of microsphere was smooth, while the internal surface was porous. Some pores were seen at the surface of microsphere may be due to evaporation of dichloromethane entrapped within the matrix of microsphere after forming smooth and dense layer as presented in Figure 3.

Fig. 3 SEM image of optimized formulation (B2).

**Drug Entrapment Efficiency.** The drug entrapment efficiency of all the formulation was shown in Table 3. All batches show percent entrapment more than 50% and it is found that entrapment of drug increases with an increase in the amount of the polymer. Formulation B3 shows maximum entrapment (80.16±1.37%) whereas formulation A1 shows minimum entrapment (58.56 ± 2.21%) of the Ranolazine in the polymer.

**Percentage Yield:** Percentage yield of floating microspheres was depending on concentration of polymer. As the polymer concentration increases the percentage yield of floating microsphere decreases, for all formulations are shown in Table 3.

**% Buoyancy test:** Buoyancy of prepared microspheres were investigated by in-vitro buoyancy test. The buoyancy percentage for all batches was almost above 70%, which was studied for 12 h. Average buoyancy in percentage was found to be 70.36±1.74% to 94.97±1.45%. The highest percentage was obtained with formulation B2. Results of buoyancy test for all formulations are shown in Table 3.
TABLE 3
Percent yield, % entrapment efficiency and % buoyancy after 12 hours of all the formulations.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>% Yield</th>
<th>% Entrapment efficiency</th>
<th>% Buoyancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>73.12±2.13</td>
<td>58.56±2.21</td>
<td>79.63±1.45</td>
</tr>
<tr>
<td>A2</td>
<td>72.52±1.22</td>
<td>62.18±1.98</td>
<td>77.21±2.25</td>
</tr>
<tr>
<td>A3</td>
<td>70.46±1.12</td>
<td>66.96±1.58</td>
<td>74.56±2.64</td>
</tr>
<tr>
<td>B1</td>
<td>90.02±1.19</td>
<td>75.12±1.63</td>
<td>90.49±1.19</td>
</tr>
<tr>
<td>B2</td>
<td>88.26±1.56</td>
<td>78.15±2.52</td>
<td>94.97±1.45</td>
</tr>
<tr>
<td>B3</td>
<td>86.10±2.10</td>
<td>80.16±1.37</td>
<td>92.29±1.89</td>
</tr>
<tr>
<td>C1</td>
<td>81.23±1.52</td>
<td>72.36±1.59</td>
<td>70.56±2.15</td>
</tr>
<tr>
<td>C2</td>
<td>79.59±2.16</td>
<td>75.10±2.46</td>
<td>71.35±1.53</td>
</tr>
<tr>
<td>C3</td>
<td>77.89±3.22</td>
<td>79.74±1.10</td>
<td>70.36±1.79</td>
</tr>
</tbody>
</table>

In-vitro Drug Release

% Drug release for all formulations was determined as mentioned in Figures 4-9. Out of all the formulations, the B1-B3 (Eudragit L100) formulation was found to be the best formulation, as it released Ranolazine in sustained manner. It was observed as the concentration of Eudragit L 100 was increased percent release of Ranolazine decreases. The increase in Eudragit L 100 concentration leads to the increased density of polymer matrix into the microspheres which result in an increased diffusional path length. This may decrease the overall drug release from polymer matrix. Furthermore, 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.

In the present study effect of the enteric coated polymers on drug release was observed by comparing the release of drug in two different pH conditions (at pH 1.2 HCl and pH 6.8 phosphate buffer) as showed in Table 4 and Table 5. Eudragit L100 microspheres are showed slow release in 0.1N HCL compared with pH 6.8 phosphate buffer.
Fig. 6. Comparative drug release profile of HPMC based microspheres in 0.1N HCl.

Fig. 7. Comparative drug release profile of ethyl cellulose based microspheres in pH 6.8 phosphate buffer.

Fig. 8. Comparative drug release profile of Eudragit L100 based microspheres in pH 6.8 phosphate buffer.
Conclusions

Floating microsphere of ranolazine were prepared by o/w emulsion solvent diffusion technique, using various biodegradable polymers such as Eudragit L100, HPMC and ethyl cellulose in order retain drug in body for longer period of time to increased bioavailability. Eudragit L100 based microspheres show there buoyancy for more than 14 hours, required for sustained therapeutic activity, in comparison to HPMC and ethyl cellulose based microsphere due to their more hollow structure and porous nature. B2 formulation showed good result among all other formulations. The property of polymer and its quantity in the formulation played crucial role on particle size of microspheres, their floating time and release profile of drug molecule. From the present work it was concluded that ranolazine microspheres based on Eudragit L100 offer a most suitable floating dosage form to improve bioavailability of ranolazine.

References


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