Development and Evaluation of Floating Microspheres of cephalexin as Gastroretentive Dosage Form

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MATERIALS AND METHOD

Chemicals

Cephalexin was a gift sample from aurobindu pharmaceuticals,Hyderabad.Ethyle cellulose & HPMC purchase from SD Fine Che Ltd., Mumbai, Cellulose acetate from NR Chem, Mumbai, Eudragit S 100 fromEvonik industries. All the chemicals used in the present study were of AR Grade.

Preparation of cephalexin loaded floating microspheres

The floating microspheres loaded with
cephalexin were prepared by emulsion-solvent evaporation/solvent diffusion method using different polymers as follows:

1. Using EC polymer 46: The drug and ethyl cellulose were mixed in dichloromethane at various ratio and triethyl citrate (10%) was added as plasticizer. The slurry was slowly introduced into the solution of polyvinyl alcohol (0.25%, 200ml). The resultant solution was stirred at 500 rpm for 1 hrs. This formed microsphere was filtered and dried at room temperature.

2. Using HPMC and EC polymers: Drug, HPMC and EC were dissolved in a mixture of ethanol and dichloromethane at room temperature. This was poured into 250 ml water containing 0.01%. Tween-80 maintained at 30-40ºC and subsequently stirred for 45 min to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried overnight at room temperature.

3. Using cellulose acetate polymer: Floating microsphere loaded with cephalexin were prepared by using solvent diffusion/evaporation method. The polymer, cellulose acetate and drug was dissolved in ethyl acetate and acetone in different proportion. The resulting solution was added slowly to 150 ml of 0.1 M acidic solution containing 0.05% polyvinyl alcohol maintaining at 30-40ºC. The emulsion was continuously stirred for 2 hours at the speed of 500 rpm. The floating microspheres were collected by decantation and then dried over night.

4. Using Eudragit S-100 polymer: Eudragit S-100 and a drug were dissolved in 8 ml ethanol, followed by the addition of 2 ml isopropanol and 5 ml dichloromethane. The polymer solution was slowly introduced into 1000 ml of 0.4% poly (vinyl alcohol) aqueous solution with stirring at 250 rpm using a mechanical stirrer equipped with a 3 blade propeller. The solution was stirred for 10 min and the microspheres were collected by filtration. The collected microspheres were dried for 12h at 50ºC.

Characterization of floating microspheres:
The prepared floating microspheres were evaluated for drug carrier interaction using scanning electron microscopy (SEM) and FTIR (Perkin Elmer 1600 series) spectral studies. For SEM studies The sample was prepared by lightly sprinkling the powder on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300A under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Joel JSM-1600, Tokyo, Japan). : Fourier Transformation Infrared spectra of pure drug, polymer and drug loaded floating microspheres were obtained in KBr pellets at moderate scanning speed between 4000-200 cm⁻¹ in a Perkin-Elmer Fourier Transformation Infrared spectroscope.

Evaluation of floating microspheres

1. Yield of microspheres:
The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the microspheres.

\[
\text{Practical yield} = \frac{\text{Actual weight of product}}{\text{Weight of excipient & drug}} \times 100
\]

2. Particle size:
The size was measured using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

3. Drug entrapment efficiency:
Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl and small quantity of methanol repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution with 0.1N HCl spectrophotometrically (UV-1700, Shimadzu, Japan) at 262 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by using the following formula:

\[
\text{Percentage drug entrapment} = \frac{\text{Amount of drug actually present}}{\text{Theoretically expected drug loaded}} \times 100
\]
4. Micrometric properties

The microspheres were characterized by their micrometric properties such as angle of repose, tapped density, compressibility index and true density.

a) Angle of repose

The angle of repose $\Theta$ of the microspheres, which measures the resistance to particle flow was calculated as:

$$\tan \Theta = \frac{2H}{D}$$

where $2H/D$ is the surface area of the free-standing height of the microsphere heap that is formed after making the microspheres flow from the glass funnel.

b) Tapped density and compressibility index

The tapping method was used to calculate tapped densities and compressibility index using tapped density:

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}$$

$$\% \text{ compressibility index} = \left(1 - \frac{V}{V_o}\right) \times 100$$

Here, $V$ and $V_o$ are the volumes of the sample after and before the standard tapping respectively.

c) True density

Density of hollow microspheres is determined by immersing the microspheres in 0.02% tween-80 solution in a metal mesh basket. The microspheres that are sunk after this process are used for density measurement as carried out by the displacement method.

5. In vitro buoyancy

An in vitro floating study was carried out using 0.1N HCl containing 0.02% tween-80 as a dispersing medium. Microspheres were spread over the surface of 900 ml of dispersing medium at 37±0.5°C. A paddle rotating at 100 rpm agitated the medium. Each fraction of microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighed after drying.

$$\% \text{ floating microspheres} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100$$

6. In vitro dissolution studies

The in vitro release of drug from floating microspheres was carried out using paddle type Electrolab tablet dissolution tester USP XXIII. Drug loaded microspheres equivalent to 250 mg of drug was introduced into 900 ml of the dissolution medium (0.1N HCl) maintained at 37±0.5°C with paddle rotating at 100 rpm. Aliquots were withdrawn at regular interval and analyzed spectrophotometrically using Shimadzu-1700 UV-visible spectrophotometer. The dissolution studies were carried out in triplicate in 0.1N HCl for 12 hours. The volume of the dissolution medium was adjusted to 900 ml at every sampling time by replacing 5 ml with same dissolution medium.

RESULTS AND DISCUSSION

All the floating Microspheres were prepared by solvent evaporation/ diffusion methods. Ethylcellulose floating microspheres were prepared by solvent evaporation method as shown in table-4 In this method, o/w emulsion was formed stabilize by ethylcellulose and after evaporation of organic solvent cephalaxin get encapsulated by ethylcellulose. EC-HPMC floating microspheres were prepared by solvent evaporation method as shown in table-5. In this method, the emulsion was stabilized by tween-80 and the volatile solvent get evaporated leaving a solidified thin film at the interface between aqueous phase and organic phase, where cephalaxin gets encapsulated in the core-coat of polymers. Cellulose acetate floating microspheres were prepared by solvent diffusion/ evaporation method as shown in table-6. When the polymer solution pour into aqueous phase, all the methanol or acetone will diffused along with small amount of ethyl acetate resulting in an interfacial polymer deposition leading to the formation of floating microspheres. Eudragit S-100 floating microspheres were prepared by solvent diffusion method as shown in table-1. In this method, the organic solvent ethanol gets diffused out of dispersed droplets with controlled rate in presence of dichloromethane or propanol. The acrylic polymer eudragit instantly solidified as thin film at the interface between aqueous and organic phase where cephalaxin get encapsulated in core-coat of polymer. The ratio of mixture of solvent was kept constant and the concentration of respective polymer was varied in all the preparation methods.

Physical characteristics of cephalaxin floating microspheres were measured according to the
The invitro dissolution studies were performed over a period of 12 hours and results are shown in figure 1 & 2. in vitro release was significantly decreased with increase in polymer concentration. Release obeys first order kinetics model and the drug release was diffusion controlled with Fickian or non Fickian transport depending upon the polymers. scanning electron microscopy shows that spherical microsphere with porous surface were formed

IR studies indicated that during the process of formulation no chemical reaction has been taken place. At the end of 12 hours all the formulation were found to be satisfactory in terms of excellent micrometric properties, percent yield, percent drug entrapment efficiency, in vitro buoyancy and highest in vitro drug release of 99.75% in sustained manner with constant fashion over a extended period of time of 12 hours. The results proved that the cephalexin loaded floating microspheres Were better in releasing the drug in the sustained fasion for extended period of 12 hours.

CONCLUSION

The in vitro evaluation study of cephalexin loaded floating microspheres was greatly improved when compared with those of conventional tablet dosageform. Among all formulations C1 with the drug: polymer ratio of (1:2) was found to be satisfactory in terms of excellent micrometric properties, percent yield (87.28%), percent drug entrapment efficiency (81.66%), in vitro buoyancy (88.59%), and highest in vitro drug release of 99.75% in sustained manner with constant fashion over a extended period of time of 12 hours. The study reveals satisfactory results with a further scope of pharmacokinetic and pharmacodynamic evaluation.

Table1: Formulations of cephalexin loaded floating microspheres prepared using ethyl cellulose, EC and HPMC, cellulose acetate & Eudragit S-100

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>Drug (mg)</th>
<th>EC (gm)</th>
<th>DICLORO METHANE (ml)/Triethyl citrate (%)</th>
<th>CONTINUOUS PHASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>500</td>
<td>0.50</td>
<td>15/10</td>
<td>0.25% PVA aqueous solution</td>
</tr>
<tr>
<td>E2</td>
<td>500</td>
<td>1</td>
<td>15/10</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>500</td>
<td>1.5</td>
<td>15/10</td>
<td></td>
</tr>
<tr>
<td>HE1</td>
<td>500</td>
<td>0.5/1.0</td>
<td>1:2</td>
<td>250 ml water containing 0.01% w/v tween-80</td>
</tr>
<tr>
<td>HE2</td>
<td>500</td>
<td>0.5/2.0</td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td>HE3</td>
<td>500</td>
<td>0.5/3.0</td>
<td>1:2</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>500</td>
<td>1</td>
<td>1:1</td>
<td>250 ml 0.1 M acidic solution containing 0.05% w/v polyvinyl alcohol</td>
</tr>
<tr>
<td>C2</td>
<td>500</td>
<td>2</td>
<td>1:1</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>500</td>
<td>3</td>
<td>1:1</td>
<td></td>
</tr>
<tr>
<td>EU1</td>
<td>500</td>
<td>0.5</td>
<td>8:5:2</td>
<td>250 ml water containing 0.4% w/v polyvinyl alcohol</td>
</tr>
<tr>
<td>EU2</td>
<td>500</td>
<td>1</td>
<td>8:5:2</td>
<td></td>
</tr>
<tr>
<td>EU3</td>
<td>500</td>
<td>1.5</td>
<td>8:5:2</td>
<td></td>
</tr>
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</table>
Table 2: mean particle size, Practical yield, in vitro buoyancy and Drug entrapment efficiency of floating microspheres of Cephalexin

<table>
<thead>
<tr>
<th></th>
<th>Mean particle size (mean±SD) (m)</th>
<th>Practical yield (mean±SD) (%)</th>
<th>Drug entrapment efficiency (mean±SD) (%)</th>
<th>In vitro buoyancy (mean±SD) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>316.15±22.25</td>
<td>72.56±0.67</td>
<td>78.33±1.52</td>
<td>81.42±2.45</td>
</tr>
<tr>
<td>E2</td>
<td>342.89±24.45</td>
<td>80.83±0.63</td>
<td>83.11±2.07</td>
<td>85.23±2.80</td>
</tr>
<tr>
<td>E3</td>
<td>371.28±18.14</td>
<td>88.28±0.57</td>
<td>86.31±1.05</td>
<td>88.33±3.14</td>
</tr>
<tr>
<td>HE1</td>
<td>262.50±23.81</td>
<td>81.26±0.69</td>
<td>68.17±2.05</td>
<td>72.05±2.58</td>
</tr>
<tr>
<td>HE2</td>
<td>297.70±16.21</td>
<td>86.67±0.45</td>
<td>74.11±1.07</td>
<td>76.66±2.60</td>
</tr>
<tr>
<td>HE3</td>
<td>335.39±21.00</td>
<td>88.59±0.38</td>
<td>78.22±1.82</td>
<td>80.83±3.18</td>
</tr>
<tr>
<td>C1</td>
<td>328.49±19.80</td>
<td>87.28±0.76</td>
<td>81.66±2.08</td>
<td>88.59±1.52</td>
</tr>
<tr>
<td>C2</td>
<td>357.21±20.26</td>
<td>91.53±2.02</td>
<td>84.00±1.26</td>
<td>89.70±2.02</td>
</tr>
<tr>
<td>C3</td>
<td>389.72±17.50</td>
<td>94.39±1.50</td>
<td>88.66±1.52</td>
<td>91.24±2.28</td>
</tr>
<tr>
<td>EU1</td>
<td>128.23±15.65</td>
<td>67.48±2.07</td>
<td>65.74±4.04</td>
<td>78.07±0.98</td>
</tr>
<tr>
<td>EU2</td>
<td>185.15±22.25</td>
<td>74.73±1.54</td>
<td>69.24±1.52</td>
<td>86.78±2.30</td>
</tr>
<tr>
<td>EU3</td>
<td>248.63±21.53</td>
<td>79.18±1.97</td>
<td>74.57±3.05</td>
<td>89.32±2.06</td>
</tr>
</tbody>
</table>

Fig. 1: In vitro release data of cephalexin loaded floating microspheres of ethyl cellulose, HPME & EC

Fig. 2: In vitro release data of cephalexin loaded floating microspheres of cellulose acetate & eudragit S-100
REFERENCES