PREPARATION AND EVALUATION OF OFLOXACIN MICROSPHERES USING NATURAL GELATIN POLYMER

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ABSTRACT: In the present study, gelatin microspheres containing ofloxacin were prepared by coacervation phase separation method and characterized by optical microscopy and scanning electron microscopy. The microspheres were analyzed for drug entrapment, bulk density, angle of repose, particle size and In-vitro release pattern. The effect of process variables on microsphere size was studied and based on these preliminary studies, different batches of microspheres were prepared by altering the drug: polymer ratio and cross-linking with glutaraldehyde. The size of microspheres was in range of 42-45µm. They were spherical in shape as evidenced by photomicrographs and scanning electron microscopy. The percent drug entrapment was in the range of 78-90 % and they could sustain drug release over a period of 8 hrs.

Keywords: Microspheres, Ofloxacin, Gelatin, Coacervation phase separation method. Sustain release
INTRODUCTION

The goals of sustained drug (Arthur.H.Kibbe, 2003, Goodman & Gilmans, 2005) delivery are to conserve and maintain effective drug concentration, eliminate night time dosage, improve compliance and decrease side effects thus, optimizing drug therapy. Sustained release dosage forms provide a prolonged dosing of the drug from the product by supplying an initial amount of loading dose, perhaps one-half of the total dose release, followed by a gradual and uniform release of the reminder of the drug over the desired time period. Ofloxacin \(^{[7,9]}\) is a fluoroquinolones antibacterial agent. It is used in various urinary and respiratory tract infections, gonorrhea, and skin. Normal dosage regimen varies from 200-600 mg administered twice or thrice a day, depending on severity of infection. In severe cases, long-term therapy may also be required. Biological half-life of drug is from 5-6 hrs. As it requires frequent dosing to maintain the therapeutic effect, it was chosen as a model drug for the present study. These particles consist of core material, which is the drug, and a coating material. The coat material can be of various types ranging from natural polymers, such as albumin, gelatin\(^{[8,12]}\), chitosan and synthetics such poly(vinyl alcohol), poly(lactide-co-glycolide) and a combination of two polymers such as chitosan-sodium CMC, alginate-chitosan etc Also literature survey revealed that not much work has been done on sustained release drug release of ofloxacin, except for few workers.

MATERIALS AND METHODS

Gelatin (Reachem Laboratory chemicals, pvt ltd, Chennai) and ofloxacin were obtained as gift sample from Karnataka antibiotics and pharmaceuticals limited, Bangalore. Glutaraldehyde (Paxmy, Chennai) and various oils (Sunflower, Castor and Groundnut oil) of food grade were purchased from local market. All other reagents used were of analytical grade.


Gelatin microspheres containing ofloxacin (GMC) were prepared by coacervation phase separation technique utilizing temperature change. Gelatin was dissolved in 10ml water previously heated to 50°C. The drug was dispersed with stirring in this solution and the dispersion was then poured drop wise into the oil phase, which was also heated to 50°C on a water bath. The oil phase contained 0.5 ml of Span 20, which acted as an emulsifier. The mixture was stirred for 5 min to ensure uniform dispersion. The temperature of the entire system was lowered down to 10°C to effect phase-separation. The dispersion was stirred for 2 hrs in ice bath at 10°C. At the end of first hour, 1.5 ml of glutaraldehyde was added to the dispersion and stirring continued for next one hour.
After two hours, stirring was stopped; beaker was covered and refrigerated at -5°C for 24 hrs to ensure rigidisation of microspheres. After 24 hrs, beaker was removed and slurry was filtered. Microspheres collected were washed with ice-cold isopropyl alcohol to make them free of oil. The solvent also acted as hardening agent. Microspheres so obtained were air dried for 24 hrs.

**Characterization of Prepared Microspheres** (S.P.Vyas, R.K.Khar, 2006)

**Particle size analysis**

The sample of prepared microspheres was randomly selected and their size was determined using an optical microscope (Olympus, India).

**Scanning electron microscopy**

Scanning electron microscopy (JEOL JSM-6701F.Japan) was carried out to study their morphological characteristics of GMC.

![Fig. 1](image1)

![Fig. 2](image2)

**Micromeretic properties**
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The angle of repose was determined (Subramanyam, C.V.S, 2000) by funnel method, where known quantity of microspheres was passed through the funnel and heap formed on the paper was encircled. From the radius of circle and height of conical heap, angle of repose was calculated. Bulk density was determined (Subramanyam, C.V.S, 2000) by transferring known quantity of microspheres to 100 ml measuring cylinder and tapping it 3 times from 1 inch at 2 seconds interval. The bulk density was obtained by dividing weight of sample by final volume of sample.

**In-vitro release study**

The *In-vitro* drug release studies were conducted in 0.1N HCl for 2 hours and in pH 7.4 buffer (M. Shahar yar, et al, 2006) for 6 hours using USP XXIII, type-II dissolution apparatus under sink conditions. Accurately weighed samples of the microspheres were added to dissolution medium and temperature was maintained at 37°C ±1°C and fluid was agitated at 100 rpm. One ml of dissolution media was with drawn every one hour and volume withdrawn and replaced with equal quantity of the fluid and maintain constant volume. After suitable dilution, the samples withdrawn were analyzed spectrophotometrically at 294 nm (Henry A, et al, 2008).

**Percentage entrapment of ofloxacin in GMC** (S.P.Vyas, R.K.Khar, 2006)

GMC (100 mg) was digested in 100 ml distilled water. The suspension was then warmed for a few minutes, filtered & 1 ml of filtrate was made up to 10 ml with distilled water. The solution was analyzed at 294 nm (ELICO limited, Hyderabad) to determine amount of ofloxacin entrapped in microspheres.

**Table: 1. Comparative dissolution study of different batches with various ratios of polymer**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Time in hours</th>
<th>% of drug release (Ratio 1:1)</th>
<th>% of drug release (Ratio 1:2)</th>
<th>% of drug release (Ratio 1:3)</th>
<th>% of drug release (Ratio 1:4)</th>
<th>% of drug release (Ratio 1:5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
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<td>1</td>
<td>9.476</td>
<td>8.151</td>
<td>7.233</td>
<td>6.046</td>
<td>5.733</td>
</tr>
<tr>
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<td>2</td>
<td>16.752</td>
<td>16.380</td>
<td>15.673</td>
<td>14.584</td>
<td>13.500</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>27.431</td>
<td>26.434</td>
<td>25.505</td>
<td>25.096</td>
<td>24.055</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>38.760</td>
<td>36.450</td>
<td>35.032</td>
<td>34.211</td>
<td>32.833</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>55.255</td>
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<td>51.062</td>
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<td>70.684</td>
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<td>8</td>
<td>91.09</td>
<td>85.551</td>
<td>83.632</td>
<td>81.069</td>
<td>78.530</td>
</tr>
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</table>
Fig. 3 Comparative Dissolution profiles of Different batches with various ratios of polymer.

Stability studies

The microspheres was taken in a crucible and placed at 45 °C and 75%RH for 45 days, the microspheres were analyzed for their drug content and dissolution studies.

Table: 2 Stability data for formulation (For-1, Ratio1:1) at 45 ±1°C

<table>
<thead>
<tr>
<th>S.No</th>
<th>Time in days</th>
<th>Physical changes</th>
<th>Percentage drug content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>----</td>
<td>87.58 ± 0.93</td>
</tr>
<tr>
<td>2.</td>
<td>15</td>
<td>No change</td>
<td>87.49 ± 0.96</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>No change</td>
<td>87.36 ± 0.98</td>
</tr>
<tr>
<td>4.</td>
<td>45</td>
<td>No change</td>
<td>87.27 ± 0.90</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSIONS

The particle size is formulated batches of microspheres were found to be in the range of 42.68 – 43.60 µm. The particle size distribution was uniform and narrow. From the above result it can be observed that the microspheres with core coat ratio of 1:1 gave highest percent encapsulation of the drug. Angle of repose less than or equal to 40° indicates free flowing properties of the microspheres. However angle of repose greater than 40° indicates poor flow of material. It can be observed from above table that the angle of repose for various batches of the microspheres is found to be less than 40°, it indicates good flow properties of the microspheres. The packing properties of the drugs and their formulations widely depend upon bulk density. It has been stated that bulk density values less than 1.2gm/cm$^3$ indicate good flow and values greater than 1.5 gm/cm$^3$ indicate poor flow. From the above result it can be seen that the bulk density values are less than 1.2gm/cm$^3$. This indicates good flow characteristics of the microspheres. The microspheres prepared by coacervation phase separation technique utilizing temperature change showed a good specificity, with smooth surface and the particles are distributed uniformly without any lumps. Scanning electron microscopy for the optimized formulation FOR-I (Ratio 1:1) was carried out and the photographs are present in Fig 1,2. The release profile of five batches of microspheres was studied for first two hours in stimulated gastric pH using 0.1N hydrochloric acid followed by stimulated Intestinal pH 7.4 (phosphate buffer). The percent of drug release was calculated by adding the amount of drug released at the end of 2$^{nd}$ hour in stimulated gastric pH to the amount of drug released in stimulated intestinal pH. The formulations FOR- I microspheres with core: coat ratio of 1:1 (ofloxacin: gelatin) has given(Table :1,Fig.3) the best sustained effect over a period of 8 hours and 91.09 % of the drug was released at the end of 8 hours. The microspheres was taken in a crucible and placed at 45 °C and 75RH for 45 days, the microspheres were analyzed for their drug content and dissolution studies. The results of stability studies shows (Table .2) that there is about 87% of drug is present in the formulation after storage at 45 °C for 45 days, it indicates good stability of the ofloxacin microspheres.

CONCLUSION

The technique chosen microencapsulation with gelatin by coacervation phase separation with temperature change and cross-linking with glutaraldehyde was able to sustain the release effectively. Because of good results of microspheres having core: coat ratio of 1:1 and size range of 42-45µm gave the best-sustained release effect.
REFERENCES


