Preparation and characterization of aceclofenac microspheres

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The objective of the present study was to microencapsulate the anti-inflammatory drug (aceclofenac) to provide controlled release and minimizing or eliminating local side effect by avoiding the drug release in the upper gastrointestinal track. The drug was targeted to the colon and their aligned area for their local effect. Aceclofenac was microencapsulated with Eudragit (S 100, RL 100, and RS 100), using an O/W emulsion-solvent evaporation technique. Aceclofenac microspheres were subjected to micromeritic properties including angle of repose, bulk density, tapped density, Carr’s index, Hausner’s ratio, and particle size determination. Microspheres were subjected to drug loading, in vitro drug release as well as for scanning electron microscopy. The prepared microspheres were white, free-flowing, and almost spherical in shape. The drug-loaded microspheres show 60-82% drug entrapment, angle of repose was in the range of 16.13 ± 0.621-24 ± 0.590, bulk and tapped densities respectively were in the range of 0.311 ± 0.006-0.562 ± 0.012 and 0.373 ± 0.01-0.735 ± 0.02, Carr’s index ranges from 14.04 ± 0.026 to 27.25 ± 1.405, Hausner’s ratio was 1.14 ± 0.026-1.37 ± 0.03, and particle size was in the range of 79.7016-144.840 µm. In vitro drug release studies were carried out up to 24 h in three different pH media, i.e., 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8), and phosphate buffer (pH 7.4). The drug-polymer concentration of dispersed phase influences the particle size and drug release properties. All the formulations at higher pH were followed by the Matrix-Higuchi model.

Key words: Aceclofenac, Eudragit (S100, RL100, RS 100), microsphere, release kinetics, solvent evaporation

INTRODUCTION

Aceclofenac is non-steroidal anti-inflammatory drug used extensively in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac is one of the emerging NSAID molecules. [1-3] Aceclofenac is a new derivative of diclofenac and has less GIT complications, the short biological half-life 4 h, and dosing frequency more than an average makes it an ideal candidate for modified release multiple unit preparation[1-4]. To reduce the frequency of administrations and to improve patient compliance, aceclofenac is suitable for making sustained release dosage form.[4-5]

Microencapsulation is useful method for prolonging a drug release from dosage forms and reducing adverse effect[6-8] among various microencapsulation methods. The emulsion-solvent evaporation can be used to prepare microcapsules of water-insoluble drug with water-insoluble polymer[6-8] for sustained release system; the oral rate of drug administered has by for received the most attention as it is natural, uncomplicated, convenient, and safer route.[4,7,9]

In this present study aceclofenac microspheres were prepared by solvent evaporation technique using Eudragit (S100, RL 100, and RS100) as a release retarded material. [6-7,9-13] Poly vinyl alcohol, chloroform were used for the preparation of microsphere. The prepared microsphere were evaluated for drug content,[10-15] particle size,[16-18] micromeritic properties,[14,11,15,18] SEM,[9] and for in vitro drug release studies.[6-7,9,19]

MATERIALS AND METHODS

Materials
Aceclofenac (Ind-Swift Ltd., Chandigarh), Eudragit RS 100, Eudragit RL 100, Eudragit S100 (Ranbaxy Laboratories Ltd., New Delhi), sodium lauryl sulfate (SLS; CDH, New Delhi), disodium hydrogen phosphate (CDH, New Delhi), chloroform (Merck Ltd., Mumbai), potassium dihydrogen phosphate (CDH, New Delhi), NaOH (Merck Ltd., Mumbai), polyvinyl alcohol (MW = 12500; CDH, New Delhi), methanol (Merck Ltd., Mumbai), HCl (Merck Ltd., Mumbai), sodium chloride (Merck Ltd., Mumbai), and deionized water. All chemicals used in the experiment were of analytical
grade and purchased from their respective commercial sources.

**Methods**

Aceclofenac microspheres were prepared by dissolving the drug in polymer, which was previously dissolved in the chloroform. The resulting solution was added to the aqueous phase containing 0.2% sodium of PVA as an emulsifying agent and the mixture was then agitated using a propeller with the rotation speed 500 rpm. The dispersed drug and Eudragit (S100, RS100, and RL100) were immediately transformed into fine droplets, which subsequently solidified into rigid microspheres due to solvent evaporation. The particles were collected by filtration, washed with dematerialized water, and desiccated at room temperature for 24 h.\(^4\)

**Determination of drug entrapment efficiency, drug loading, and yield**

Microspheres (25 mg) were suspended in 25 ml of methanol. After 24 hrs, the solution was filtered and the filtrate was analyzed for drug content; this filtrate was diluted up to appropriate dilution; and for the determination of drug entrapment efficiency, the following formulas were used:

\[
\text{Encapsulation efficiency} (\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]

\[
\text{Drug loading} (\%) = \frac{\text{Weight of drug}}{\text{Weight of microparticle}} \times 100
\]

Yield (\%) = \frac{\text{Weight of microparticle}}{\text{Total expected weight of drug and polymer}} \times 100

Drug loading, yield, and the drug encapsulation efficiency, for batch A1, A2, A3; B1, B2, B3; and C1, C2, C3, are respectively reported in Table 1. Each determination was performed in triplicate manner.

**Particle size analysis**

The particle size of microsphere was determined using optical microscopy method.\(^1\) Approximately 100 microspheres were counted for particle size using a calibrated optical microscope (Magnus MLX-DX). Particle size for each formulation (n = 3) was reported in Table 2 and Figure 1.

**Micrometric properties**

**Angle of repose**

Angle of repose of different formulations was measured according to fixed funnel standing method\(^1\) (n = 3) [Table 2].

\[\theta = \tan^{-1} \frac{h}{r}\]

where \(\theta\) is the angle of repose, \(r\) is the radius, and \(h\) is the height.

**Bulk density**

Bulk and tapped densities were measured by using 10 ml of

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**Table 1:** Percentage yield, percentage loading and encapsulation efficiency of aceclofenac microsphere

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug:polymer</th>
<th>Theoretical loading (%)</th>
<th>Actual drug loading (%)</th>
<th>Encapsulation efficiency (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1:1</td>
<td>50.00</td>
<td>33.00</td>
<td>66.00</td>
<td>84</td>
</tr>
<tr>
<td>A2</td>
<td>1:1.5</td>
<td>40.00</td>
<td>28.00</td>
<td>70.00</td>
<td>78</td>
</tr>
<tr>
<td>A3</td>
<td>1:2</td>
<td>33.33</td>
<td>26.33</td>
<td>79.48</td>
<td>84</td>
</tr>
<tr>
<td>B1</td>
<td>1:1</td>
<td>50.00</td>
<td>30.00</td>
<td>60.00</td>
<td>61</td>
</tr>
<tr>
<td>B2</td>
<td>1:1.5</td>
<td>40.00</td>
<td>26.80</td>
<td>67.00</td>
<td>85.3</td>
</tr>
<tr>
<td>B3</td>
<td>1:2</td>
<td>33.33</td>
<td>22.93</td>
<td>68.78</td>
<td>81.4</td>
</tr>
<tr>
<td>C1</td>
<td>1:1</td>
<td>50.00</td>
<td>31.50</td>
<td>66.30</td>
<td>86</td>
</tr>
<tr>
<td>C2</td>
<td>1:1.5</td>
<td>40.00</td>
<td>26.88</td>
<td>67.21</td>
<td>89</td>
</tr>
<tr>
<td>C3</td>
<td>1:2</td>
<td>33.33</td>
<td>27.33</td>
<td>82.00</td>
<td>91</td>
</tr>
</tbody>
</table>

\(n = 3\)

**Table 2:** Micrometric properties

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Angle of repose ((\theta))</th>
<th>Bulk density (g/ml)</th>
<th>Tapped density (g/ml)</th>
<th>Carr’s index (Ci) (%)</th>
<th>Hausner’s ratio</th>
<th>Particle size ((\mu)m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean* SD* ((\pm))</td>
<td>Mean SD ((\pm))</td>
<td>Mean SD ((\pm))</td>
<td>Mean SD ((\pm))</td>
<td>Mean SD ((\pm))</td>
<td>Mean SD ((\pm))</td>
</tr>
<tr>
<td>A1</td>
<td>16.79 0.681 ((\pm))</td>
<td>0.555 0.007 ((\pm))</td>
<td>0.649 0.009 ((\pm))</td>
<td>14.46 1.385 ((\pm))</td>
<td>1.16 0.020 ((\pm))</td>
<td>105.985 23.375 ((\pm))</td>
</tr>
<tr>
<td>A2</td>
<td>16.16 0.621 ((\pm))</td>
<td>0.429 0.009 ((\pm))</td>
<td>0.517 0.007 ((\pm))</td>
<td>17.34 0.697 ((\pm))</td>
<td>1.20 0.010 ((\pm))</td>
<td>132.790 22.112 ((\pm))</td>
</tr>
<tr>
<td>A3</td>
<td>17.52 0.164 ((\pm))</td>
<td>0.516 0.005 ((\pm))</td>
<td>0.614 0.004 ((\pm))</td>
<td>15.89 0.576 ((\pm))</td>
<td>1.18 0.011 ((\pm))</td>
<td>144.840 35.529 ((\pm))</td>
</tr>
<tr>
<td>B1</td>
<td>18.65 0.202 ((\pm))</td>
<td>0.311 0.006 ((\pm))</td>
<td>0.373 0.010 ((\pm))</td>
<td>16.48 0.610 ((\pm))</td>
<td>1.19 0.005 ((\pm))</td>
<td>91.638 22.302 ((\pm))</td>
</tr>
<tr>
<td>B2</td>
<td>20.25 0.017 ((\pm))</td>
<td>0.426 0.008 ((\pm))</td>
<td>0.497 0.001 ((\pm))</td>
<td>14.04 1.716 ((\pm))</td>
<td>1.14 0.026 ((\pm))</td>
<td>117.869 29.750 ((\pm))</td>
</tr>
<tr>
<td>B3</td>
<td>16.13 0.621 ((\pm))</td>
<td>0.507 0.013 ((\pm))</td>
<td>0.606 0.008 ((\pm))</td>
<td>16.16 1.965 ((\pm))</td>
<td>1.19 0.035 ((\pm))</td>
<td>127.468 26.263 ((\pm))</td>
</tr>
<tr>
<td>C1</td>
<td>19.81 0.543 ((\pm))</td>
<td>0.511 0.019 ((\pm))</td>
<td>0.703 0.039 ((\pm))</td>
<td>27.25 1.405 ((\pm))</td>
<td>1.37 0.030 ((\pm))</td>
<td>79.7016 17.768 ((\pm))</td>
</tr>
<tr>
<td>C2</td>
<td>20.78 0.586 ((\pm))</td>
<td>0.494 0.005 ((\pm))</td>
<td>0.673 0.014 ((\pm))</td>
<td>26.58 0.829 ((\pm))</td>
<td>1.35 0.015 ((\pm))</td>
<td>93.404 29.546 ((\pm))</td>
</tr>
<tr>
<td>C3</td>
<td>24.08 0.590 ((\pm))</td>
<td>0.562 0.012 ((\pm))</td>
<td>0.735 0.020 ((\pm))</td>
<td>22.47 1.248 ((\pm))</td>
<td>1.28 0.020 ((\pm))</td>
<td>112.096 46.280 ((\pm))</td>
</tr>
</tbody>
</table>

\(*\text{Mean} \pm \text{SD}; n = 3\)
graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated. Each experiment for micromeritic properties was performed in triplicate manner and reported in Table 2.

**Carr’s index**

Compressibility index (Ci) or Carr’s index value of microparticles was computed according to the following equation:

\[
\text{Carr (\%)} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100
\]

**Hausner’s ratio**

Hausner’s ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation:

\[
\text{Hausner’s ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}
\]

**Scanning electron microscopy**

The microparticles were coated uniformly with gold-palladium by using Sputter coater (POLARON SC-76430), after fixing the sample in individual stabs. All samples were randomly examined for surface morphology of microspheres by using scanning electron microscope (SEM; LIO-430) [Figures 2 and 3].

**In vitro dissolution analysis**

In vitro dissolution studies were carried out on the microsphere at 37°C ± (0.5°C) at 100 rpm with USP dissolution apparatus II; 100-mg aceclofenac microsphere was place into the dissolution apparatus.

The in vitro dissolution studies were performed at three different pH values: (i) 1.2 pH, i.e., simulated gastric fluid pH; (ii) 6.8 pH, and (iii) 7.4 pH, which is simulated intestinal fluid pH. An accurately weight sample was responded in dissolution media consisting 900 ml of 0.1 N (pH 1.2) HCl containing 0.01% SLS and the dissolution was done for 2 h. At the end of 2 h, the 25.92 g of disodium hydrogen phosphate and 10.305 g potassium dihydrogen phosphate were added to make pH 6.8; the dissolution was done for 4 h. After the total 6 h, add 2.142 g disodium hydrogen phosphate and 0.171 g sodium chloride and change the pH up to 7.4 and after that the study was performed up to 24 h. The sample (5 ml) was withdrawn at each hour interval and replaced with the same volume of test medium and the withdrawn samples were diluted if required and then estimated for aceclofenac concentration at 275 nm spectrophotometrically (Shimadzu Pharmspec UV-1700 series, Japan). Finally, the drug content in all fluid was determined from the calibration curve of aceclofenac to determine the release pattern (n = 3) [Table 3; Figure 4].

Kinetic parameters were also obtained by mathematical processing of drug release data. Evaluation of the influence of formulation variables on release rate constant k values, obtained for different groups of microsphere preparation, was determined by using the PCP Disso v2.08 (Program developed by - Anant Ketkar, Vinay Patil and A.R. Paradkar, Bhartiya Vidyapeeth Deemed University; Pune).
RESULT AND DISCUSSION

Percentage yield, percentage loading, and encapsulation efficiency of aceclofenac microsphere

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulation may be due to microspheres lost during the washing process. Percentage yield of all formulations varies from 61% to 91%, the best one is C3 as given in Table 1. Theoretical concentration of drug in microsphere was evaluated to be 33.34%, 40%, 50% w/v, respectively in different formulations. Actual drug loading increases as the theoretical drug loading increases, which is shown in Table 1. The amount of drug remaining and available for encapsulation increased as the theoretical drug loading increased. Consequently, the actual drug loading increased. As the molecular weight of the polymer increased, its hydrophobicity increased, leading to better precipitation of polymer at the boundary phase of the droplets.

Morphology

The sustained release microspheres of aceclofenac prepared by solvent evaporation were found to be almost spherical, free-flowing, white or almost white in color.

SEM was performed on the prepared aceclofenac microspheres to access their surface and morphological characteristics as shown in Figures 2 and 3. The presence of uncovered drug crystal on the surface could be attributed to the formation of drug nucleus in the non-stirred layer surrounding the emulsified droplets during solvent evaporation.

Particle size

Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity, which influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. If there was increase in the amount of polymer concentration, there was increase in relative viscosity so as a result increases in mean particle size [Table 2; Figure 1].

Micromeritic properties of aceclofenac microspheres

Angle of repose

All the formulations show angle of repose value in the range of 16.130-24.080, i.e., less than 30 (n = 3), which shows free-flowing nature of the formed microspheres [Table 2].

Bulk density and tapped density

Bulk and tapped densities showed good packability of the microspheres [Table 2].

Carr’s index (Ci)

Carr’s index ranges from 14.04% to 27.25%, B2 had lowest Ci index, indicating excellent compressibility [Table 2].
Hausner's ratio
It was ranging from 1.14 to 1.37, i.e., all the preparation showed that they had good flow properties. Upon considering the micromeritic properties of all the formulations, B2 had the best flow property, as it had high angle of repose value (20.250), the lowest Carr's index (14.04%), and Hausner's ratio (1.14) [Table 2].

Drug release behavior
Aceclofenac release from the microsphere was studied for 24 h, i.e., in simulated gastric fluid (pH 1.2, 0.1 N HCl) containing 0.01% SLS for 2 h; in this duration, all the preparation showed negligible drug release. But changing the pH from 1.2 to 6.8 (mixed phosphate buffer), burst release was observed after 4 h. On further changing pH from 6.8 to 7.4 till 24 h, the drug released at constant rate in all these preparation and showed constant release from 12 h.

All the preparation having ratio 1:1 showed good release properties. As the amount of polymer increased, drug release was decreased. This is because smaller the particle size, larger the surface area available for drug release. The drug release from formulation containing Eudragit RS 100 was slow as compared from the formulation containing polymers Eudragit RL 100 and S 100. Eudragit RS 100 is less permeable, because it possesses less quaternary compound than Eudragit RL 100. Both Eudragit RL 100 and Eudragit RS 100 are pH-independent polymers, whereas Eudragit S 100 is pH-dependent polymer, which dissolves above pH 7, but the drug release from Eudragit S 100 was also found at pH 6.8 due to formation of pore and channels and due to swelling of polymer up to some extent [Table 3, Figure 4].

Release kinetics
The release mechanism of aceclofenac from various formulations was determined by comparing their respective correlation coefficient. It was found that the mechanism of drug release from microspheres was diffusion controlled.

Figure 4: Comparative release studies of aceclofenac from various Eudragit formulation (A-F)
The release patterns of various formulations at pH 7.4:

C1>A1>B1>C2>A3>C3>B2>A2>B3

The drug release from all formulations at higher pH is described by the Matrix-Higuchi model.

CONCLUSION

Aceclofenac microspheres were prepared successfully by using the solvent evaporation method. Polymer-drug ratio influences the particle size as well as drug release pattern of microsphere.

The yield was high and encapsulation efficiency was good for all the preparation, but was highest for C3 formulation. As the polymer concentration increases, the particle size increases.

The assessment of release kinetic showed that drug release from aceclofenac microspheres followed the Matrix-Higuchi model (diffusion-controlled drug release mechanism).

Initially at gastric medium (pH 1.2), very less release of drug (aceclofenac) from microspheres was found, but at pH 6.8 and pH 7.4, all formulations showed burst release initially and then tend to release at constant rate. As per our aim, formulation does not show release in gastric medium for desired period of time and releases the drug at pH 6.8 and 7.4, which is the pH of colon and their allied areas; the prepared microspheres proved to be good candidate for site-specific drug delivery.

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