INTRODUCTION

Chronopharmacotherapy, the drug regime based on circadian rhythm is gaining attention worldwide. Various diseases like asthma, hypertension and arthritis show circadian variation that demand time-scheduled drug release for effective drug action, for example, inflammations associated with morning body stiffness, heart attack and asthma in the early hours of the day. In this principle, an "ideal" dosage form ought to be taken at a convenient time before sleep, providing maximum drug release in the morning hours.

A pulsatile drug delivery system that can be administered at night before sleep but that release drug early morning would be a promising chronopharmacological system. The combinations of floating-pulsatile principle are very well suitable for above mentioned diseases. Site and time specific oral drug-delivery have recently been of greater interest in pharmaceutical field to achieve improved therapeutic efficacy. Gastro retentive drug delivery system is an approach to prolong gastric residence time, thereby targeting site specific drug release in the upper gastrointestinal tract. Pulsatile drug delivery system (PDDS) is characterized by a time period of no release (lag time) followed by a rapid and complete drug release. The combination of floating-pulsatile delivery provides various advantages such as nearly constant drug level at the site of action, avoidance of undesirable side effects, reduced dose, improved patient compliance and increased gastric residence of the dosage form. Additionally, multiple unit dosage forms provides many relative advantages over single unit dosage forms such as predictable GI transit time, maximum drug absorption with reduced inter and intra subject variability due to difference in gastric emptying rate, thus giving greater product safety. Various approaches like pulsatile release displaying rapid as well as transit release and specific technologies like OROS®, CONTINU®, CEFORM®, TIMERR®, etc. based on various principles and mechanisms have been developed for chronotherapy. The major disadvantage of these systems is that they do not have long residence time which is desired for diseases needing morning medication. To overcome this, novel approach termed as "floating pulsatile drug delivery system" is to be developed. The main objective of the present work is to develop multiple unit, floating pulsatile drug delivery system for Aceclofenac to provide relief for rheumatoid arthritis.

Computer-aided optimization technique, using a 3² factorial design, was employed to investigate the effect of 2 independent variables (factors) (i.e., the drug-to-polymer ratio and stirring speed) on particle size and drug entrapment efficiency.

MATERIALS AND METHODS:

Aceclofenac was purchased from Amoli Organics Pvt. Ltd. Mumbai India. Eudragit-L100 and Eudragit S100 were obtained as a gift sample from Evonik Degussa India Pvt. Ltd. Mumbai. All other chemicals/reagents used were of analytical grade.

Preparation of microspheres

Microspheres containing aceclofenac as a core material were prepared by emulsion solvent diffusion technique. Drug, Eudragit L100 and Eudragit S100 were mixed in dichloromethane and ethanol at 1:1 ratio at room temperature. The resulting drug-polymer solutions were poured gradually into 200ml of water containing 0.50%/w/v polyvinyl alcohol, maintained between 30-40°C and the preparation was stirred at 500 rpm for one hour using a mechanical stirrer equipped with three bladed propellers. The microspheres obtained were washed repeatedly with water until it was free from polyvinyl alcohol. The collected microspheres were dried overnight at 60°C.

Experimental Design

Factorial design was employed during the construction of batches. It was applied for two factors with three levels for each. Thus 3² factorial design was employed to assess the effect of independent variable on the constructed batches to obtain the desired batch for acceptable particle size and high drug entrapment efficiency in a suitable microspheres formulation. In the formulation of microspheres two factors were varied as shown in Table 1. Here, a commercially available software program was used (Design Expert, Version 7.0.2). The experimental design chosen was Response Surface, 2 factor, 3 level factorial; 9 formulations were formulated as shown in Table 2.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low (-1)</th>
<th>Medium (0)</th>
<th>High (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug to polymer ratio</td>
<td>1:1</td>
<td>1:2</td>
<td>1:3</td>
</tr>
<tr>
<td>Stirring speed (rpm)</td>
<td>500</td>
<td>700</td>
<td>1000</td>
</tr>
</tbody>
</table>
Table 2: Formulation of floating microspheres of aceclofenac F1-F9

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Polymers Endragit S100 (mg)</th>
<th>Endragit L100 (mg)</th>
<th>Stirring Rate (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F1</td>
<td>500</td>
<td>250</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>2.</td>
<td>F2</td>
<td>500</td>
<td>250</td>
<td>250</td>
<td>700</td>
</tr>
<tr>
<td>3.</td>
<td>F3</td>
<td>500</td>
<td>250</td>
<td>250</td>
<td>1000</td>
</tr>
<tr>
<td>4.</td>
<td>F4</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>5.</td>
<td>F5</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>700</td>
</tr>
<tr>
<td>6.</td>
<td>F6</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>7.</td>
<td>F7</td>
<td>500</td>
<td>750</td>
<td>750</td>
<td>500</td>
</tr>
<tr>
<td>8.</td>
<td>F8</td>
<td>500</td>
<td>750</td>
<td>750</td>
<td>700</td>
</tr>
<tr>
<td>9.</td>
<td>F9</td>
<td>500</td>
<td>750</td>
<td>750</td>
<td>1000</td>
</tr>
</tbody>
</table>

Evaluation of Microspheres

Drug-Excipients compatibility studies

Drug-excipients compatibility was studied using differential scanning calorimetry. Thermal analysis of different samples namely drug, physical mixture, drug loaded microspheres and blank microspheres were obtained. Accurate amount of samples were weighed into aluminium pans and sealed. All samples were run at a heating rate of 10°C/min over a temperature range of 30-300 °C in atmosphere of nitrogen.

FTIR measurements of different samples of drug and physical mixture were obtained. The spectra were scanned at ambient temperature. The FT-IR spectrums of pure aceclofenac and physical mixture of aceclofenac were analyzed for compatibility studies.

Percentage yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield of different formulation F1-F9 were calculated as follows11.

\[
\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100
\]

Drug entrapment efficiency

About 10 mg of accurately weighed drug loaded microspheres were added into 10 ml of methanol and the drug concentrations were determined spectrophotometrically at 275 nm in UV-visible double beam spectrophotometer11.

\[
\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug load expected}} \times 100
\]

Particle size analysis

The particle size of microsphere was determined using optical microscopy method. Approximately 100 microspheres were counted for particle size determination using calibrated optical microscope11,12.

Angle of repose

The angle of repose of floating microspheres was determined by fixed funnel method. The floating microspheres were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel11.

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

Where,

- \( h \) = height of pile
- \( r \) = radius of the pile formed by the floating microspheres

Determination of bulk density and tapped density

It is the ratio between a given mass of floating microspheres and its volume after tapping. The bulk density and tapped density of floating microspheres were determined by the tapping method.

Accurately weighed quantity of floating microspheres was transferred into a 10 ml measuring cylinder. After observing the initial volume of the floating microspheres, the tapping was continued on a hard surface until no further change in volume was noted. The bulk density and tapped density were calculated according to following formula11.

\[
\text{Tapped density} = \frac{\text{mass of floating microspheres}}{\text{volume of floating microspheres after tapping}}
\]

Percentage compressibility index / Carr’s index

The same tapping method was used to determine percentage compressibility index. The percentage compressibility index was calculated according to following formula11.

\[
\% \text{ Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100
\]

Hausner’s ratio

Hausner’s ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation11.

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

Floating ability of microspheres

Floating microspheres (100 mg) were placed in 0.1 N HCl (100 ml) containing 0.02% Tween 80. The mixture was stirred at 100 rpm using a magnetic stirrer and the floating times were recorded13-16.

In-vitro release studies

The dissolution studies of the microspheres equivalent to 100 mg of aceclofenac were performed using USP Type II dissolution test apparatus. Volume of the dissolution medium was 900 ml with a stirring speed of 100 rpm and the temperature was maintained at 37°C ± 0.5°C. These conditions were kept constant for all dissolution studies. The drug release study was carried out in 0.1 N HCl (pH 1.2) for a time period equivalent to floating time which varied for each batches of microspheres, followed by dissolution in phosphate buffer, pH 7.4 till complete release of drug. Periodically samples were withdrawn and filtered through Whatman filter paper and the concentration of aceclofenac was measured spectrophotometrically at 275 nm17.

RESULTS AND DISCUSSION

Effect of drug polymer ratio on responses

Response plots (Fig. 1 and Fig. 2) indicates that on increasing the concentration of drug-polymer ratio, the particle size was increased. The viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles. As the ratio of drug-to-polymer increases, encapsulation efficiency increased; this is due to higher ratio of drug-to-polymer, which would produce large size droplets with decrease surface area, here diffusion of drug from such microspheres will be slow, resulting in higher encapsulation efficiency.

Effect of stirring speed on responses

Response plots (Fig. 1 and Fig. 2) indicates that on increasing stirring speed, particle size was decreased and formations of
Microspheres were irregular in shape. The fact that high shearing rate required for emulsification caused the breakdown of the viscous drug polymer solution into fine globules resulting in small microspheres. The stirring speed has a negative effect on % drug entrapment i.e. as the stirring speed was increased the % drug entrapment was decreased.

Design Expert tool was used for selection of optimized formulation. The tool offers the possibility to vary each variable simultaneously and presents possible optimum selections with their respective value. According to the desired criteria of higher drug entrapment efficiency, F7 was selected as the optimized formulation.

Drug-Excipients compatibility studies

The possible interaction between the drug and the excipients was studied by DSC and IR spectroscopy. Drug-excipients compatibility studies using differential scanning calorimetry revealed that pure aceclofenac had a sharp melting endothermic peak of 154.11°C. There was no significant change in the endothermic values of aceclofenac after mixing with other excipients compared with those of pure aceclofenac (Fig. 3).

These observations indicate the absence of any interaction between aceclofenac and the excipients, and therefore suggested compatibility between them. Hence, the excipients selected in this study were found to be suitable for development of the formulation.

![Fig. 1: Response Surface Plot for Particle size](image1)

![Fig. 2: Response Surface Plot for Drug Entrapment Efficiency](image2)

![Fig. 3: DSC thermograms of drug (A), physical mixture (B), drug loaded microsphere (C) and blank microspheres (D)](image3)
FTIR spectrum of drug and physical mixture were taken. No additional peaks were found in the spectrum, which indicates absence of any incompatibility between aceclofenac and other excipients as shown in Fig. 4 and 5.

**Percentage yield and percentage drug entrapment efficiency**

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 loss during the washing process. Percentage yield of all formulations varied from 62.33% to 78.33%. Drug entrapment efficiency was found to be in the range of 78.15% to 90.1%.

The mean particle size of the microspheres significantly increased with increasing quantity of Eudragit L100 and Eudragit S100 concentration and was in the range of 80 μm to 118.66 μm as shown in Table 3.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Percentage Yield</th>
<th>Particle size μm</th>
<th>% Drug Entrapment Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>70.33 ± 1.5</td>
<td>109±0.5</td>
<td>84.89 ± 0.005</td>
</tr>
<tr>
<td>F2</td>
<td>68.33 ± 1.52</td>
<td>86±2.5</td>
<td>80.88 ± 0.01</td>
</tr>
<tr>
<td>F3</td>
<td>68.66 ± 1.15</td>
<td>90±1</td>
<td>78.15 ± 0.02</td>
</tr>
<tr>
<td>F4</td>
<td>72.33 ± 3.51</td>
<td>112.66±0.57</td>
<td>89.20 ± 0.01</td>
</tr>
<tr>
<td>F5</td>
<td>67.33 ± 0.57</td>
<td>104±0.57</td>
<td>87.60 ± 0.04</td>
</tr>
<tr>
<td>F6</td>
<td>62.33 ± 1.52</td>
<td>89±1</td>
<td>80.7 ± 0.04</td>
</tr>
<tr>
<td>F7</td>
<td>78 ± 1</td>
<td>118.66±1.15</td>
<td>90.1 ± 0.04</td>
</tr>
<tr>
<td>F8</td>
<td>78.33 ± 0.57</td>
<td>106.33±1.52</td>
<td>86.28 ± 0.01</td>
</tr>
<tr>
<td>F9</td>
<td>77.33 ± 0.57</td>
<td>98.33±1.52</td>
<td>85.11 ± 0.02</td>
</tr>
</tbody>
</table>

Values expressed as Mean±SD, n= 3

**Angle of repose**

All the formulation showed angle of repose value in the range of 20.58-28.36, i.e. less than 30, which indicates free-flowing nature of the formed microspheres as shown in Table 4.

**Bulk and tapped density**

The bulk density value of different microspheres ranged from 0.30 - 0.36 gm/ cm³ and the tapped density value of microspheres ranged from 0.36 - 0.43 gm/cm³ as shown in Table 4. The density value of microspheres were less than the density of gastric fluid (~1.004 g/cm³) thereby, it will have good buoyancy in the stomach.

**Carr’s index (CI)**

Carr’s index ranges from 13.05% to 15.92%, i.e. all the preparation showed that they had good flow properties as shown in Table 4.

**Hausner’s ratio**

It ranged from 1.15 to 1.18 i.e. all the preparation showed good flow properties as shown in Table 4.
Table 4: Micromeritic properties of floating microspheres of aceclofenac: F1–F9

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Angle of repose</th>
<th>Bulk Density gm/cm³</th>
<th>Tapped Density gm/cm³</th>
<th>carr’s index</th>
<th>Hausner’s ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>20.6±0.1</td>
<td>0.33 ± 0.02</td>
<td>0.39 ± 0.030</td>
<td>14.34 ± 2.11</td>
<td>1.16 ± 0.03</td>
</tr>
<tr>
<td>F2</td>
<td>20.58±0.21</td>
<td>0.32 ± 0.005</td>
<td>0.38 ± 0.05</td>
<td>15.92 ± 0.24</td>
<td>1.18 ± 0.015</td>
</tr>
<tr>
<td>F3</td>
<td>24.65±0.66</td>
<td>0.30 ± 0.011</td>
<td>0.36 ± 0.017</td>
<td>14.78 ± 0.86</td>
<td>1.16 ± 0.011</td>
</tr>
<tr>
<td>F4</td>
<td>24.54±0.50</td>
<td>0.34 ± 0.017</td>
<td>0.40 ± 0.02</td>
<td>14.98 ± 2.19</td>
<td>1.17 ± 0.030</td>
</tr>
<tr>
<td>F5</td>
<td>23.72±0.63</td>
<td>0.30 ± 0.005</td>
<td>0.36 ± 0.01</td>
<td>14.79 ± 1.24</td>
<td>1.17 ± 0.017</td>
</tr>
<tr>
<td>F6</td>
<td>23.73±0.64</td>
<td>0.32 ± 0.005</td>
<td>0.38 ± 0.05</td>
<td>15.92 ± 0.24</td>
<td>1.18 ± 0.015</td>
</tr>
<tr>
<td>F7</td>
<td>28.36±0.66</td>
<td>0.35 ± 0.005</td>
<td>0.41 ± 0.11</td>
<td>13.05 ± 3.36</td>
<td>1.15 ± 0.045</td>
</tr>
<tr>
<td>F8</td>
<td>27.39±0.18</td>
<td>0.36 ± 0.017</td>
<td>0.42 ± 0.02</td>
<td>14.26 ± 2.15</td>
<td>1.16 ± 0.032</td>
</tr>
<tr>
<td>F9</td>
<td>25.64±0.11</td>
<td>0.36 ± 0.017</td>
<td>0.43 ± 0.011</td>
<td>15.65 ± 1.74</td>
<td>1.18 ± 0.028</td>
</tr>
</tbody>
</table>

* Values expressed as Mean±SD, n= 3

Floating ability of microspheres

The floating test was carried out to investigate the floating ability of the prepared microspheres.

Floating Microspheres were dispersed in 0.1 N HCl containing 0.02% w/v Tween 80. Tween 80 was added to counteract the downward pulling at the liquid surface by lowering surface tension. Floating ability of different formulations was found to be different depending on the polymer ratios. Microspheres of Batches F1, F2, F4 and F7 remained floating for more than 6 hrs as shown in Table 5. The floating properties of microspheres may be attributed to their low density.

Table 5: Floating time of microspheres: F1–F9

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Floating time(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>7.5±0.25</td>
</tr>
<tr>
<td>F2</td>
<td>6.5±0.5</td>
</tr>
<tr>
<td>F3</td>
<td>4.5±0.28</td>
</tr>
<tr>
<td>F4</td>
<td>7.5±0.25</td>
</tr>
<tr>
<td>F5</td>
<td>5.5±0.25</td>
</tr>
<tr>
<td>F6</td>
<td>4±0.25</td>
</tr>
<tr>
<td>F7</td>
<td>6±0.5</td>
</tr>
<tr>
<td>F8</td>
<td>4±0.25</td>
</tr>
<tr>
<td>F9</td>
<td>3.5±0.25</td>
</tr>
</tbody>
</table>

* Values expressed as Mean±SD, n= 3

In-vitro release

To simulate the pH variation of GI tract dissolution studies were performed first at pH 1.2 for time equivalent to floating time (round figure-hours) and then subsequently medium was replaced with fresh pH 7.4 having maintained temperature of 37±0.2°C. In pH 1.2 all the formulations showed 3-4% cumulative drug release. The low amount of drug release at the gastric pH is advantageous to reduce gastric irritation caused by NSAIDs. After this lag time, complete drug was released within 1 hour in phosphate buffer pH 7.4 in which Eudragit L100 and Eudragit S100 got dissolved.

The microspheres showed excellent lag at acidic pH, which may be due to insolubility of the drug and polymer as shown in Fig. 6.

Fig. 6: In vitro drug release profile of floating aceclofenac microspheres, formulation F1 to F9

CONCLUSION

Novel floating-pulsatile microspheres containing aceclofenac were prepared by emulsion solvent diffusion technique. A 3² factorial design was employed to assess the effect of independent variables, drug to polymer ratio and stirring speed on the constructed batches. Acceptable particle size and high drug entrapment efficiency were selected as the response variables for the optimized formulations. Drug to polymer ratio of 1:3 and stirring speed of 500 rpm yielded the desired responses for the optimized batch, F7.

Overall, the buoyant microspheres provided lag phase while showing gastroretention in the acidic medium, while a pulsatile drug release in the alkaline pH would be beneficial for chronotherapy of rheumatoid arthritis. The developed system offers a simple and novel technique for pulsed release of drugs in the small intestine.
Such work can be further extended using various excipients for a variety of drugs suitable for chronopharmaceutical drug delivery. This approach suggested the use of floating pulsatile microspheres as promising drug delivery for site and time specific release of Aceclofenac acting as per chronotherapy of rheumatoid arthritis.

Above technology employed for the preparation of floating microspheres is relatively simple and the manufacturing process can be easily adopted in an industrial setup and on commercial scale.

ACKNOWLEDGEMENT

Authors are thankful to Evonik Degussa India Pvt. Ltd. Mumbai, for providing gift samples of Eudragit L100 and Eudragit S100.

REFERENCES