Preparation and *in vitro* Evaluation of Bioadhesive Microparticulate System

*Nagda C. D.¹, Chotai N. P.², Patel S. B.¹, Soni T. J.³, Patel U. L.⁴, Nagda

¹Indukaka Ipcowala College of Pharmacy, New VV Nagar,
²A. R. College of Pharmacy and G.H. Patel Institute of Pharmacy, VV Nagar,
³Anand Pharmacy College, Anand,
⁴Arihant College of Pharmacy, Ahmedabad, Gujarat, India.

**ABSTRACT:** Aceclofenac (ACE) is NSAIDs of a phenyl acetic acid class. It is indicated in arthritis and osteoarthritis, rheumatoid arthritis, ankylosing spondylitis. It has short elimination half life of 4 hours. The objective of the study is to design, characterize and evaluate bioadhesive microspheres of ACE employing carbopol (CP) as bioadhesive polymer. Bioadhesive microspheres of ACE were prepared by solvent evaporation method. The prepared microspheres were free flowing and spherical in shape and characterized for drug loading, mucoadhesion test, infrared spectroscopy (IR), differential scanning colorimetry (DSC) and scanning electron microscopy (SEM). The *in-vitro* release studies were performed using pH 6.8 phosphate buffer. The drug loaded microspheres in a ratio of 1:5 showed 47% of drug entrapment; percentage mucoadhesion was 81% and 89% release in 10 h. The infrared spectra and DSC showed stable character of aceclofenac in the drug loaded microspheres and revealed the absence of drug-polymer interactions. SEM studies showed that the microspheres are spherical and porous in nature. The *in vitro* release profiles from microspheres of different polymer-drug ratios followed Higuchi model.

**KEYWORDS:** Aceclofenac, bioadhesive, Microspheres, Carbopol, Solvent evaporation method

**Introduction**

Controlled release multiple unit dosage forms have advantages over single unit ones as they can spread out in a more uniform manner over a large surface area in the gastrointestinal tract (Lee et al., 2000). This can reduce a local irritation of the gastrointestinal tract by some drugs and can provide a large area for drug absorption. Microspheres form an important part of novel drug delivery system which can precisely control release rates and target drugs to a specific body sites. Bioadhesion is a topic of interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with underlying absorption surface to improve and enhance the bioavailability of drugs.

Aceclofenac (ACE), phenyl acetic acid derivative 2-[(2,6-Dichlorophenyl)amino] phenyl acetoxy acetic acid, is a novel NSAID indicated in the symptomatic treatment of pain and inflammation with a reduced side effect profile especially regarding gastrointestinal complications (Parfitt, K, 1999, Brogden RN et al., 1996). Recommended dose is 200 mg daily in divided doses. The successful treatment of arthritis depends on the maintenance of effective drug concentration in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time and achieve this objective. The mean plasma elimination half life of aceclofenac is 4 hours (Maryeule JO et al., 1996). To reduce the dosing frequency and adverse effects during prolong treatment it is needed to formulate in long acting dosage form. Different workers have attempted to prepare sustained release oral formulations of aceclofenac like sustained release tablet and microparticulate system (Mutalik S et al., 2007; Dashora K et al., 2006). Preparation of bioadhesive microspheres would be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes (Patel JK et al, 2004). This can be achieved by coupling bioadhesion characteristic to microspheres and developing bioadhesive microsphere. Carbopol 974CP selected as polymer in the production of bioadhesive microspheres due to its excellent bioadhesive properties (Harikarnapakdee S et al., 2006). The purpose of present work was to design, characterize and evaluate bioadhesive microspheres of ACE employing carbopol as bioadhesive polymer.

**Materials and Methods**

**Materials**

Aceclofenac(ACE) was obtained as a gift sample from Comed Chemicals Limited (Vadodara, India). Carbopol 974P NF (CP) was obtained as a gift sample from Lubrizol Advanced Materials Inc (Mumbai, India). All other reagents and solvent used were of analytical grade.
Preparation of Mucoadhesive Microspheres

Bioadhesive microspheres were prepared by an oil-in-water-in-oil (O/W/O) double-emulsion method (Sandra K. et al., 2005). Aqueous polymer solution was prepared and subsequently stored in sealed containers at 48 ºC for 24 h prior to use. Carbopol (0.50 g) was dispersed in 50.0 g of deionized water and mixed by rapid vortexing; the pH was adjusted to 7 using dilute aqueous sodium hydroxide. Aceclofenac (ACE) was dissolved in dichloromethane. For the first emulsion, ACE dissolved in dichloromethane was emulsified into 50.0 g of aqueous polymer solution. The concentrations and amounts applied are summarised in Table 1. The addition of 0.15 ml of Tween 80 aided the emulsification process. A Silverson homogenizer was used for rapid mixing of the emulsions for 15 min. The first emulsion (25 ml) was added drop wise to 250 ml light liquid paraffin containing 1% Span 80. The resulting double emulsions were stirred at 800 rpm. The samples were heated to 60-70 ºC to promote evaporation of water. Solid polymer microspheres were subsequently separated from the oil by centrifugation, washed in hexane and dried in a vacuum oven at 40 ºC for 24 hr. For each polymer to drug ratio, three batches of microspheres were prepared for the purpose of assessing the reproducibility of drug loading by this method.

Encapsulation Efficiency

To determine Encapsulation efficiency, 100 mg of accurately weighed drug loaded bioadhesive microspheres were added to 100 ml of methanol. The resulting mixture was kept shaking on a mechanical shaker for 24 h. Then, after the solution was filtered and 1 ml of this solution was appropriately diluted with methanol and analyzed with spectrophotometrically at 275 nm using a Shimazu UV-1700 (UV/VIS double beam spectrophotometer, Kyoto, Japan). The drug encapsulation efficiency was calculated using the following formula: (Practical drug content/Theoretical Drug content) × 100.

Particle Size

A microscopical imaging analysis technique for determination of particle size distribution was used (Filipovic G. et al, 1996). Microsphere size and distribution were determined with an AXIOPALN microscope (Zeiss MPM400, Germany), equipped with a computer-controlled image analysis system (Zeiss, KS300, Germany).

Swelling Index

The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium (Jain S.K. et al, 2004). Accurately weighted amounts of microspheres were immersed in a little excess of Phosphate buffer (pH 6.8) and kept for 24 hr. The following formula was used for calculation of degree of swelling:

\[ \alpha = \frac{W_s - W_0}{W_s} \]

Where, \( \alpha \) = degree of swelling, \( W_0 \) = initial weight of microspheres, and \( W_s \) = weight of microspheres after swelling.

Mucoadhesion

Mucoadhesion of different microspheres system was assessed using the method reported by Jain SK et al (2004) with little modification. A strip of rat intestinal mucosa was mounted on a glass slide and accurately weighed bioadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45°. Phosphate buffer saline (pH 6.8), previously warmed to 37 ± 0.5 ºC, was circulated to the cell over the microspheres and membrane at the rate of 1 ml/min with the help of pump. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50 ºC. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated by the following formula:

\[ \text{Percentage mucoadhesion} = \frac{W_o - W_t}{W_o} \times 100 \]

Where \( W_o \) = weight of microspheres applied; \( W_t \) = weight of microspheres leached out.

Table 1. Composition of mucoadhesive microspheres formulations.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Drug (g)</th>
<th>CP (g)</th>
<th>Dichloromethane (ml)</th>
<th>Span-80 (%)</th>
<th>Liquid paraffin light (ml)</th>
<th>n-Hexane (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECP1</td>
<td>0.500</td>
<td>0.500</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>DECP2</td>
<td>0.500</td>
<td>1.000</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>DECP3</td>
<td>0.500</td>
<td>1.500</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>DECP4</td>
<td>0.500</td>
<td>2.000</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>DECP5</td>
<td>0.500</td>
<td>2.500</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>DECP6</td>
<td>0.500</td>
<td>3.000</td>
<td>10</td>
<td>1.0</td>
<td>250</td>
<td>50</td>
</tr>
</tbody>
</table>
Scanning Electron Microscope (SEM)
A scanning electron microscope (ESEM TMP with EDAX, Philips, Holland) was used to characterize the surface topography of the microscope. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum. The surface was scanned and photographs were taken at 30kV accelerating voltage for the drug loaded microspheres.

Fourier Transform Infrared Spectroscopy (FTIR)
The spectra were recorded for pure drug, drug loaded microspheres and blank microspheres using FTIR (Perkin-Elmer, Spectrum GX, USA). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400 – 4000 cm⁻¹ and the resolution was 2 cm⁻¹.

Differential Scanning Calorimetry (DSC)
Differential scanning calorimetry (DSC) scans of aceclofenac, blank microspheres and drug loaded microspheres were performed using DSC-PYRIS-1(Perkin-Elmer, USA). The analysis was performed with a heating range of 50-300°C and a rate of 10 °C min⁻¹.

Drug Release Study
The drug release study was performed using USP XXIV basket apparatus (Electrolab, TDT-06T, Mumbai, India) at 37°C and at 50 rpm using 900 mL of phosphate buffer (pH 6.8) as a dissolution medium up to 10 hr (Mutalik S et al., 2007). Microspheres equivalent to 100 mg of Aceclofenac were used for the test. Five ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 mm membrane filter, diluted suitably, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation.

Release kinetics
In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations like zero order’s release vs t, first order (log% unrelease vs t), Higuchi matrix (% release vs square root of time), (Yadav K.S. et al., 2007). In order to define a model which will represent a better fit for the formulation, drug release data further analysed by Peppas equation, Mt/M∞=ktⁿ, where Mt is the amount of drug released at time t and M∞ is the amount released at time ∞, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. r² values were calculated for the linear curves obtained by regression analysis of the above plots.

Results and Discussion
Effect of Experimental Variables on Particle Size
The processing variables like drug to polymer ratio, stirring speed, stabilizer concentration affect the particle size of microspheres. The drug to polymer ratio appeared to influence on particle size distribution of microspheres (Table 2). When drug to polymer ratio was increased from 1:1 to 1:6, the proportion of larger particles formed became higher, because this may be due to increase in viscosity of the solvent with increase in polymer to drug ratio. The mean particle size ranged from 28 to 53 μm. The minimum concentration of span 80 required to form stable emulsion was found to be 1%. Changing the stirring speed during emulsification process seems to influence the mean particle size of the microspheres. When the stirring speed was kept below 800 rpm, the mean particle size of the microspheres was increased and they became large and aggregated. We found optimal temperature at 60-70 °C as at higher temperature; it might affect polymer stability and increased aggregation at lower temperature.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Theoretical drug content</th>
<th>Practical drug content</th>
<th>Encapsulation efficiency (%)</th>
<th>Mean Particle size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECP1</td>
<td>50</td>
<td>16.28</td>
<td>32.57 ± 1.35</td>
<td>28.50 ± 1.10</td>
</tr>
<tr>
<td>DECP2</td>
<td>33.5</td>
<td>12.98</td>
<td>38.75 ± 0.98</td>
<td>33.44 ± 2.54</td>
</tr>
<tr>
<td>DECP3</td>
<td>25</td>
<td>8.57</td>
<td>34.30 ± 2.13</td>
<td>39.22 ± 1.76</td>
</tr>
<tr>
<td>DECP4</td>
<td>20</td>
<td>8.15</td>
<td>40.76 ± 1.15</td>
<td>46.24 ± 1.87</td>
</tr>
<tr>
<td>DECP5</td>
<td>16.5</td>
<td>7.81</td>
<td>47.35 ± 1.75</td>
<td>52.70 ± 0.85</td>
</tr>
<tr>
<td>DECP6</td>
<td>14.4</td>
<td>5.44</td>
<td>37.78 ± 2.04</td>
<td>51.80 ± 1.92</td>
</tr>
</tbody>
</table>

*Each observation is the mean (± SD) of three determinations
Encapsulation Efficiency

The drug entrapment efficiency within microspheres produced using the solvent evaporation method is of fundamental importance as failure to achieve acceptable drug loadings may preclude the use of this method for economic reasons (Jones et al., 1995). The entrapment efficiency of various formulations was found to be in the range of 32 to 47% as shown in Table 2. The entrapment efficiency less than expected may be due to solubility of the drug in the solvent. Because of its solubility, the drug may be migrated to the processing medium during extraction and evaporation process of dichloromethane.

Swelling Index

The most promising approach to achieving gastro retention is that of creating a swelling or expanding system in situ (Davis SS, 2005). Figure 1 depicts the swelling index of microspheres. It is evident from the figure that all obtained microspheres rapidly swelled in phosphate buffer pH 6.8. The high swelling property of CP could be attributed to high molecular weight and their ionized ability to uncoil polymer into an extended structure.

Mucoadhesion

In the mucoadhesion process, it is necessary for swelling and expansion of the polymer chain since interpenetration and entanglement of the polymers and the mucous networks are considered to be responsible for adhesion (Ponchel G et al., 1997). Therefore, bioadhesives should swell and expand rapidly when they come in contact with water. A high percentage of adhesion indicates that microspheres have excellent mucoadhesion to mucosal tissue. Percentages of mucoadhesion are given in Figure 2. It can be seen that the microspheres had good mucoadhesive properties and could adequately adhere to intestinal mucosa. The results also showed that with change in polymer to drug ratio, the % mucoadhesion also varies. The maximum and prolonged mucoadhesion (85.46%) was observed with the product DECP5.

Fig. 1 The Profiles of percentage swelling index with time of microspheres.

Fig. 2 Percentage mucoadhesion of microspheres.
Scanning Electron Microscopy

Surface morphology of microspheres and the morphological changes produced through polymer degradation can be investigated and documented using scanning electron microscopy (SEM). The morphological surfaces changes occurring due to the hydrolytic degradation of the polymers. From Scanning Electron Microscopy (SEM) study, it was found that microspheres were spherical as shown in Figure 3. The surface of microspheres was rough. The study of drug loaded microspheres shows the presence of drug particles on the surface, which may be responsible for an initial burst release of the drug during dissolution.

![Fig. 3 SEM photographs of microspheres (a) Different size of microspheres (b) Single microsphere.](image-url)
Infrared Spectroscopy

The IR spectra of pure aceclofenac, drug loaded microsphere and blank microsphere are shown in the Figure 4. The peak at 3319 nm indicating the –NH stretching, two peaks at 1771 nm and 1717 nm for the –C=O stretching of –COO and –COOH group respectively. The peaks at 1589 nm, 1281 nm, and 749 nm show as major peaks for drug. All the above are peaks presents in drug loaded microspheres that confirms the presence of drug in the polymer without any interaction.

Fig. 4 FT-IR spectra of pure Aceclofenac (a), Drug loded microspheres (b), Blank microspheres (c).
Differential Scanning Colorimetry Study

The results of DSC study are given in Figure 5. DSC thermograms showed endothermic peak of ACE at 153°C, which corresponded to its melting point. Thermograms of drug loaded microspheres showed peak at 153°C and blank microspheres showed at 78°C. No interaction was observed between drug and polymers.

Fig. 5 DSC thermograms of pure Aceclofenac (a), Drug loaded microspheres (b), Blank microspheres (c).
In-Vitro Release Study

in vitro release profiles of ACE microspheres are shown in Figure 6. The release profiles of the formulations appear to be slow release with negligible burst effect. The burst effect corresponds to the release of the drug located on or near surface of the microspheres or release of poorly entrapped drug. The rate of release of drug from the bioadhesive microspheres was slow and found to further decrease with increase in drug to polymer ratio. The slow release may be due to the medium being diffused in the polymer matrix and the drug diffusing out of the microspheres.

Release Kinetics

The in vitro release profile was analyzed by various kinetic models. The kinetic models used were Higuchi, zero order, first order and Korsmeyer Peppas equations (Table 4). The release constants were calculated from the slope of the respective plots. Higher correlation was observed in the Higuchi equation. For planer geometry, the value of $n=0.5$ indicates a Fickian diffusion mechanism, for $0.5<n<1.0$, indicates anomalous (non-fickian) transport, and $n=1$ implies case II (relaxation controlled) transport. In the present systems, the value for $n$ was found to be in the range of 0.457 to 0.624 indicating that the release mechanisms followed fickian diffusion and anomalous (non-fickian) transport. The optimized batch DECP5 was having $n=0.457$, indicating that the release mechanism followed is fickian diffusion controlled mechanism.

![Fig. 6 Cumulative percent release of aceclofenac (n=3) from different mucoadhesive microspheres prepared with different drug: polymer ratio.](image-url)
Table 3. Various parameters of the model equations on the in vitro release kinetics.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Higuchi model</th>
<th>Zero order</th>
<th>First order</th>
<th>Krosemeyer Peppes model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>$n_0$</td>
<td>$R^2$</td>
<td>$K_0$</td>
</tr>
<tr>
<td>DECP1</td>
<td>0.9738</td>
<td>34.72</td>
<td>0.9795</td>
<td>9.67</td>
</tr>
<tr>
<td>DECP2</td>
<td>0.9771</td>
<td>32.16</td>
<td>0.9899</td>
<td>8.54</td>
</tr>
<tr>
<td>DECP3</td>
<td>0.9910</td>
<td>30.01</td>
<td>0.9763</td>
<td>7.50</td>
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<td>DECP4</td>
<td>0.9844</td>
<td>29.07</td>
<td>0.9905</td>
<td>7.34</td>
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<td>DECP5</td>
<td>0.9939</td>
<td>26.04</td>
<td>0.9856</td>
<td>6.53</td>
</tr>
<tr>
<td>DECP6</td>
<td>0.9844</td>
<td>27.75</td>
<td>0.9922</td>
<td>7.09</td>
</tr>
</tbody>
</table>

Conclusions

In attempt to prepare bioadhesive microspheres of Aceclofenac using solvent evaporation method, the microspheres were at a suitable size and had good mucoadhesive property. The entrapment efficiency was in the range of 32 to 47 %. Aceclofenac release from these bioadhesive microspheres was slow and extended over longer periods of time and depended on composition of the coat. Drug release was fickian diffusion controlled. Further, the desired goals can be obtained by systemic evaluation of bioadhesive microspheres in animals and/or human volunteers.

References


