Comparison of Emulsification and Ionic Gelation Method of Preparation of Mucoadhesive Microsphere

Swati Chaturvedi1*, Prof. P. K. Sharma1, Mr. Sharad Visht1, Shanu Tyagi1

1. Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat Bypass Crossing, Delhi-Haridwar Highway, Meerut-250005 (UP).
[E-mail: swatichaturvedi99@yahoo.com]

The purpose of this study was to prepare and characterize microspheres loaded by Aceclofenac. To achieve this goal, Chitosan and Sodium alginate microspheres loaded by Aceclofenac were prepared by emulsification and ionic gelation methods. Morphology, size, encapsulation efficiency and drug release from these microspheres were evaluated. Microscopic evaluation of microspheres showed that microspheres were spherical in shape. The size analysis results indicated that size range varied from 1 to 13 µm. Encapsulation efficiency of microspheres was increased by increasing drug to polymer ratio. Drug release was found to be Zero order. In conclusion, microspheres loaded with Aceclofenac were prepared that could be used for control delivery of Aceclofenac.

Keyword: Emulsification, Ionic Gelation Method, Mucoadhesive Microsphere

1. Introduction

Gastric residence time (GRT) is an important parameter which may affect drug bioavailability of dosage forms. Many Drug Suffer from low bioavailability because of short gastric emptying time. Gastro retentive systems are the current approach to overcome the above problem of GRT. Among the number of approaches mucoadhesive drug delivery system (FDDS) is one of the promising delivery system which adhere to the mucous layer of the stomach and thus remains in the stomach for long period of time1].

Aceclofenac; a potent non-steroidal anti-inflammatory drug [NSAID] has been indicated for various conditions like post-traumatic pain, rheumatoid arthritis, ankylosing spondylitis. Aceclofenac is practically insoluble in water, but almost totally absorbed from gastrointestinal tract, its biological half-life is 4hrs and administered twice daily with single dose of 100mg. When administered orally, it is showing slow and incomplete absorption from the gastrointestinal tract1,2]. To overcome the side effects associated with conventional administration of NSAIDS and increase the patient compliance controlled release dosage forms. So that a delivery system that adhere to mucus membrane of gastric delivering aceclofenac in sustained manner to the proximal part of gastrointestinal tract (absorption window) may improve its bioavailability3]. Due to relatively very high lipophilicity and poor solubility in aqueous media, oral absorption of Aceclofenac is usually low. These properties have limited the design of Aceclofenac dosage forms for various administration routes4]. To overcome the problems associated with
conventional delivery systems and to improve the efficiency of Aceclofenac, alternative dosage forms such as microemulsions, microspheres, nanospheres and liposomes have been suggested\[5\]. Mucoadhesion has been 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 the underlying absorption surface to improve the bioavailability of drugs\[13,5\]. The use of biodegradable mucoadhesive microspheres as drug delivery systems offer several important advantages including controlled and continuous drug release, easy administration, biodegradability, biocompatibility, protection of drug against acid and enzymes in the GI tract and no need for daily use of the drug\[6\]. These microspheres are suitable alternatives for conventional dosage forms. Concerning the use of different biodegradable polymers, several synthetic polymers have been widely used for preparation of microspheres due to its safety and physicochemical properties, as it is nontoxic, biodegradable and biocompatible\[4,6\]. Several synthetic polymers have been used to formulate multiunit dosage forms. Recently, much research efforts have been concentrated to develop drug-loaded microspheres using synthetic polymer like chitosan and sodium alginate because of simple, mild and eco-friendly preparative condition\[7\]. The two most important factors in choosing the mostsuitable polymer are the biodegradability and the toxicityof the polymer. The polymer Chitosan and Sodium alginate are biodegradable and non-toxic and it was chosen for the preparation of the microspheres\[5,8\]. The aim of this study was to reduce the side effects of Aceclofenac and to extend the release time using synthetic polymers. Different microsphere formulations loaded by Aceclofenac were obtained and fully characterized for morphology, size, encapsulation efficiency and release rate of Aceclofenac\[9\].

2. Materials and Methods
2.1 Materials
Chitosan (RM9358) was obtained from Hi media, Mumbai. Aceclofenac was given by Fine Lab as a free gift sample, DCM (dichloromethane), gelatine, liquid paraffine was obtained from CDH (Delhi, India). Sodium alginate, Petroleum ether and all other reagents were of analytical grade.

2.2 Methods
2.2.1 Preparation of Microspheres
Aceclofenacmucoadhesive microspheres prepared by emulsification and emulsion gelation techniques using chitosan and sodium alginate as polymer. Both the process differ on the basis of the cross-linking agent in emulsification process glutaraldehyde is used and emulsion gelation technique calcium chloride is used. Microspheres were prepared by water in oil emulsification solvent evaporation technique. A 1% polymeric aqueous solution was made with drug and then the solution poured into 100 ml of light liquid paraffin containing 0.5% span-60 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker at constant stirring speed 1000 rpm was carried out using magnetic stirrer. The emulsion was cross linked by dropping through a spray gun into the emulsion. After cross linking was allowed for varying time, microspheres were washed with petroleum ether repeatedly and dried at room temperature. Three different formulations with drug: polymer ratios (1:1, 1:2, 2:1) are prepared and coded as F1, F2 and F3. The whole procedure was repeated in triplicates.

2.3 Evaluation of Microsphere
2.3.1 Drug Polymer Interaction (FTIR) Study
FTIR spectra of pure drug, pure polymers and formulations containing both drug and polymers were performed to study the drug polymer interaction. FTIR study was performed by using Fourier transformed infrared spectrophotometer (Prestige-21, Shimadzu, Japan).
2.3.2 Microsphere morphology and size analysis

Particle size
All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope. The average particle size was determined using the Edmondson's equation:

\[ \text{Average particle size} = \frac{\sum nd}{\sum n} \]

Where \( n \) = No. of microspheres observed, \( d \) = mean size range

2.4 Determination of shape and surface morphology
The shape and surface morphology of the microspheres was studied by using scanning electron microscope (Zeiss EVO 50). Microspheres were discrete, uniform and spherical with a smooth surface. The smooth surface of the microspheres can be attributed to the slow solvent evaporation and slow precipitation of polymers during formation of w/o type of emulsion.

2.5 Production yield (% w/w)
The percentage yield of each batch was calculated on weight basis with respect to the weight of starting material. All experiments were carried out in triplicate.

\[ \% \text{ yield} = \frac{\text{Amount of microsphere prepared}}{\text{Amount of Drug + Polymer taken}} \times 100 \]

2.6 Drug content and Entrapment efficiency
The drug content and entrapment efficiency of prepared microsphere was determined by method of extraction of drug present in microsphere. The dried microspheres (100mg) were taken and extracted in 100 mL of 0.1N HCl (pH 1.2) for 24 hours. Then the dispersion of microspheres was sonicated for 30 min and the solution was filtered through a 0.45 μm filter. The concentration of drug present in filtrate determined spectrophotometrically at 272 nm (UV-2450, Shimadzu, Japan). Each determination was made in triplicate. The drug content and entrapment efficiency of prepared microsphere was determined by putting value in given below formula.

\[ \text{Drug Content} = \frac{\text{Calculated drug content}}{\text{Total amount of microsphere}} \times 100 \]

\[ \text{Encapsulation efficiency} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100 \]

2.7 Micromeritic properties of microspheres
The flow properties of microspheres were studied by determining various parameters like the bulk density, tapped density and hausner ratio. The Bulk and tapped density was determined by cylinder method and were obtained.-

2.8 Swelling Study
A weighed amount of microsphere was placed in 100 ml of 0.1N HCl (pH 1.2) and allowed to swell. At each time intervals, the swollen microspheres were removed from the media and weighed. Fluid sorption was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination.

\[ \% \text{ Swelling Index} = \frac{W_f - W_i}{W_i} \times 100 \]

Where
\( W_f \) = final weight of microsphere,
\( W_i \) = Initial weight of microsphere

2.9 Ex-vivo mucoadhesion study
The mucoadhesive property of the microspheres was evaluated by using 0.1N HCl (pH 1.2). The freshly excised pieces of intestinal mucosa (2x3 cm) from hen were mounted onto glass slides with cyanoacrylate glue. About 100 mg of microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the
slides with suitable support were hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid at 37°C contained in a one L vessel. At different time intervals up to 24 h the machine was stopped and the number of microspheres still adhering to the tissue was counted and % mucoadhesion was calculated.

\[
\text{% Mucoadhesion} = \frac{\text{Mi} - \text{Mt}}{\text{Mi}} \times 100
\]

Where \( \text{Mi} \) = Initial weight of adhere microsphere (t=0), \( \text{Mt} \) = Amount of microsphere adhered after particular time period.

2.10 In vitro drug release study
In vitro release profile of aceclofenac from the microspheres was examined in 0.1N HCl (pH 1.2) using USP (XXI) six stage dissolution rate test apparatus (Electrolab TDT-08L). Microspheres equivalent to 100 mg of drug packed in filter paper and was suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. An aliquot of 1 ml was withdrawn periodically at intervals of one hour and same volume of fresh medium was replaced. The samples were filtered through Whatman filter paper and analysed spectrophotometrically at 272 nm for amount of drug released.

2.11 Analysis of release profiles
The rate and mechanism of release of both drugs from the prepared were analyzed by fitting the dissolution data into the zero-order equation:

\[
\text{Q} = k_0 \times t
\]

Where \( Q \) is the amount of drug released at time \( t \) and \( k_0 \) is the release rate constant, first order equation:

\[
\ln(100-Q) = \ln100 - k_1 \times t
\]

Where \( k_1 \) is the release rate constant and Higuchi’s equation:

\[
\text{Q} = k_2 t^{1/2}
\]

Where \( k_2 \) is the diffusion rate constant. Drug release data was further analyzed by the Peppas Equation:

\[
\frac{\text{Mt}}{\text{M}^{\infty}} = k t^n
\]

Where \( n \) is the release exponent indicative of the mechanism of release, \( \frac{\text{Mt}}{\text{M}^{\infty}} \) is the fractional release of the drug, \( t \) is the release time, and \( k \) is the kinetic constant.

3. Results and Discussion
Microsphere of aceclofenac could be prepared by ionotropic gelation process and emulsification gelation process employing chitosan and sodium alginate as the polymer. The microspheres of Chitosan were found to be discrete spherical and free flowing. The size analysis of different batches of microsphere showed that microspheres of chitosan were in the size range of 5-7µm and microsphere of sodium alginate in the size range of 7-10 µm. The size distribution of microsphere was found to be good in both batches with drug – polymer ratio 1:2. The microsphere of chitosan imparted good flowability in comparison with sodium alginate microsphere as indicated by

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Method</th>
<th>Core:c Roth ratio</th>
<th>Carr’s index</th>
<th>Hausner’s ratio</th>
<th>Drug content</th>
<th>Entrapment efficiency</th>
<th>Average particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1</td>
<td>Emulsification</td>
<td>1:1</td>
<td>10.98±0.85</td>
<td>1.12±0.01</td>
<td>35.5%</td>
<td>71.12%</td>
<td>5.437±0.10</td>
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<tr>
<td>CF2</td>
<td>Emulsification</td>
<td>1:2</td>
<td>10.22±0.76</td>
<td>1.11±0.04</td>
<td>31%</td>
<td>93%</td>
<td>6.873±0.01</td>
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<tr>
<td>CF3</td>
<td>Emulsification</td>
<td>2:1</td>
<td>11.18±0.24</td>
<td>1.13±0.02</td>
<td>50.3%</td>
<td>76.30%</td>
<td>5.858±0.00</td>
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</tr>
<tr>
<td>SF1</td>
<td>Solvent Evaporation</td>
<td>1:1</td>
<td>19.55±0.78</td>
<td>1.24±0.01</td>
<td>35.48%</td>
<td>70.96%</td>
<td>7.359±0.03</td>
</tr>
<tr>
<td>SF2</td>
<td>Solvent Evaporation</td>
<td>1:2</td>
<td>18.99±0.70</td>
<td>1.24±0.01</td>
<td>25.04%</td>
<td>75.87%</td>
<td>9.505±0.32</td>
</tr>
<tr>
<td>SF3</td>
<td>Solvent Evaporation</td>
<td>2:1</td>
<td>14.28±0.81</td>
<td>1.17±0.01</td>
<td>26.2%</td>
<td>39.69%</td>
<td>8.055±0.01</td>
</tr>
</tbody>
</table>

angle of repose, the Carr’s index (10 – 11 for chitosan microsphere and 14- 20 for sodium alginate microsphere) and the Hausner Ratio (1.11-1.13±0.035 for chitosan microsphere and 1.17– 1.24±0.01 for sodium alginate). The Microencapsulation efficiency was in the range of 73% to 93% for chitosan microsphere 39% to 75% for sodium alginate microsphere. The surface morphology was also studied by SEM and found that chitosan microsphere were discrete, uniform and spherical with a smooth surface as comparison to sodium alginate microsphere depicted in Figure 1&2. Selected DSC thermogram of the drug and microcapsule were shown in Fig 3 respectively. The DSC analysis (Fig.3) of pure Aceclofenac showed a characteristic, sharp endotherm peak at 151°C corresponding to its melting point and indicates the crystalline nature of the drug. The DSC analysis of drug and polymer revealed negligible change in the melting point of Aceclofenac indicating no modification or interaction between the drug and polymer.

Compatibility study of drug and polymer were conducted by employing I.R. Spectral studies. The IR spectrum of Aceclofenac, chitosan, sodium alginate & its formulation is shown in figure: 4-7. The following characteristic peaks were observed with Aceclofenac as well as the formulations containing Aceclofenac. C=N - (stretching) 1629.55, 1655.59, 1669 cm⁻¹, C-N- (stretching) 1061.62, 1029.48, 1030.77 cm⁻¹, N-H - (stretching) 3397.96, 3378.67, 3394.1 cm⁻¹. As the identical principle peaks were observed in all the cases, hence it shall be confirmed that interactions do not exist between the drug and polymer.

Aceclofenac release from the microcapsules was studied in 0.1 HCl (pH1.2) for 12 hours as prescribed for Aceclofenac tablets in USP XXIV. Aceclofenac release from chitosan microsphere was slow, spread over extended period of time and depended on the composition of the coat and method employed for the preparation of microsphere.
3.1 I.R Spectra of Sodium alginate

3.2 I.R Spectra of Chitosan

Fig a: Thermogram of pure Aceclofenac

Fig b: Thermogram of Aceclofenac Containing Microsphere

Fig. 4: IR Spectra of Aceclofenac

Fig.5: IR Spectra of chitosan and sodium alginate

Fig.6: I.R Spectra of Aceclofenac microsphere using Chitosan as polymer
The model that best fits the release data was evaluated by correlation coefficient (r). The correlation coefficient (r) value was used as criteria to choose the best model to describe the drug release from the microcapsules. The r value in various models is given in Table 2. In most of the formulated microcapsules the r values were higher in zero order models than that of first order model indicating the drug release from the most of the microcapsules was according to zero order kinetics. To analyze the mechanism of release of drug from the microcapsules the equation, \( Q = K't^n \) was used, where \( Q \) is the percentage of drug released; \( t \) is the release time; \( K' \) is a constant incorporating structural and geometric characteristics of the release device, \( n \) is the release exponent indicative of mechanism of release.

The drug release mechanism from the microcapsules was supercase 2 transport as \( n \) value is more than 0.82. The drug release from the selected formulation followed zero order kinetics and controlled by Korsmeyer-Peppas mechanism. The wash-off test was conducted upto 8hrs at pH 1.2. The wash-off was slow in the case of microsphere containing chitosan as coat increases when compared to that of Sodium alginate microsphere (Table 3). Hence the results indicate that the microsphere with a coat consisting of chitosan and prepared by emulsification process exhibited good mucoadhesive properties in the in ex-vivo wash-off test when compared to sodium alginate microsphere.

4. Conclusion

The microsphere prepared with a coat consisting of chitosan by emulsification process exhibits spherical, free flowing, good entrapment and exhibit good mucoadhesive property hence these microsphere was slow and extended release over prolonged periods of time and depended on composition of the coat. Drug release was diffusion controlled and followed zero-order kinetics. These mucoadhesive microspheres are thus suitable for oral controlled release of Aceclofenac.
Table 3: In vitro Wash off test data of Aceclofenac microsphere formulated with by employing different techniques

<table>
<thead>
<tr>
<th>Formulation</th>
<th>%mucoadhesion of chitosan after 8 hr</th>
<th>%mucoadhesion of sodium alginate after 8 hr</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>60%</td>
<td>57%</td>
</tr>
<tr>
<td>2</td>
<td>81%</td>
<td>77%</td>
</tr>
<tr>
<td>3</td>
<td>75.3%</td>
<td>71%</td>
</tr>
</tbody>
</table>

Table 2: Correlation coefficient (R) values in various kinetic models tested to describe drug release from the mucoadhesive microsphere of Aceclofenac

<table>
<thead>
<tr>
<th>formulation</th>
<th>Zero order</th>
<th>1st order</th>
<th>Higuchi Matrix</th>
<th>Korsmeyer-Peppas</th>
<th>Best fit model</th>
<th>Mechanism of drug release</th>
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<tbody>
<tr>
<td></td>
<td>R² k</td>
<td>R² k</td>
<td>R² k</td>
<td>R² k</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>SF1</td>
<td>0.990 3</td>
<td>7.7822 0</td>
<td>0.950 4</td>
<td>0.925 9</td>
<td>9.6787</td>
<td>0.974 8</td>
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<tr>
<td>SF2</td>
<td>0.983 9</td>
<td>7.7304 0</td>
<td>0.981 9</td>
<td>0.930 1</td>
<td>9.5737</td>
<td>0.949 7</td>
</tr>
<tr>
<td>SF3</td>
<td>0.987 1</td>
<td>8.1562 0</td>
<td>0.881 5</td>
<td>0.916 5</td>
<td>10.012 1</td>
<td>0.975 2</td>
</tr>
<tr>
<td>CF1</td>
<td>0.993</td>
<td>8.2898</td>
<td>0.926</td>
<td>-0.179 9</td>
<td>0.924 2</td>
<td>9.9827</td>
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<tr>
<td>CF2</td>
<td>0.990</td>
<td>7.5235</td>
<td>0.972</td>
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<tr>
<td>CF3</td>
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<td>0.972</td>
<td>-0.142 3</td>
<td>0.929 4</td>
<td>9.5212</td>
</tr>
</tbody>
</table>

5. Reference