ORIGINAL PAPERS

Polim. Med. 2015, **45**, 2, 67–72
DOI: 10.17219/pim/59137

ISSN 0370-0747

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Effect of Calcium Chloride on Release Behavior of Babul (*Acacia nilotica*) gum Microbeads

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of the article

Abstract

Background. Oral delivery of drugs is the most common method, but due to the inability of drugs to restrain and localize in the gastro-intestinal tract, oral administration of drugs in conventional dosage forms have short-term limitations. Carrier technology may provide many approaches for the delivery of drugs by coupling the drug to a carrier particle, such as Microspheres, nanoparticles and liposomes, which modulate the release and absorption characteristics of the drug.
Objectives. The aim of this study was to prepare Diclofenac sodium microspheres using a natural polymer and show the effect of calcium chloride on the release behavior of microspheres. The microspheres of Diclofenac sodium were successfully developed by ionic gelation technique using natural polymer babul gum with sodium alginate.
Material and Methods. Diclofenac Sodium was received as a gift sample from Aegis Pharmaceuticals Pvt. Ltd., Roorkee. *Acacia nilotica* gum was purchased from Ghaziabad and purification was done in the laboratory. All other excipients used analytical grade method. The microspheres of diclofenac sodium were prepared by Ionic gelation method using a natural polimer, i.e. *Acacia nilotica*. Calcium chloride (5% solution) was used as a cross-linking agent. In this research article all the data was presented as averages and standard deviations.
Results. Five formulations were successfully prepared, i.e. F1, F2, F3, F4 and F5. All the formulations were evaluated for micromeritic properties, particle size analysis, percentage yield, drug content, drug entrapment efficacy, percent moisture loss, swelling index and *in vitro* dissolution studies. The size of the microspheres varied between 14.55 ± 0.29 to 20.18 ± 0.15 μm and as high as 81.51 ± 0.14% entrapment efficiency for babul gum was obtained.
Conclusions. Batch F1 and F5 was found to release the drug 91.35% and 75.48% respectively for 6 hrs. The formulations were found to be effective in providing controlled release of drug for a prolonged period of time **(Polim. Med. 2015, 45, 2, 67–72).**

Key words: microspheres, *Acacia nilotica*, Diclofenac sodium, Ionic gelation technique, *in-vitro* drug release.

Oral route has for decades been one of the best means of administering drugs. The delivery of drugs orally is the most common and widely acceptable route all over the world. Due to their inability to restrain and localize at gastro-intestinal tract, oral administration of drugs in conventional dosage forms have short-term limitations [1]. Some times it also leads to a decrease in patient compliance. In order to overcome these problems various types of controlled release formulations have been formulated [2]. Microparticulate drug delivery systems are considered as one of the more effective systems of delivering the drug to the specific biological site, maintaining the desired concentration, without any side-effects. It is one of the methods of providing controlled and sustained delivery of drugs for a prolonged period of time [3]. Carrier technology may provide many approaches for the delivery of drugs by coupling the drug to a carrier particle such as Microspheres, nanoparticles and liposomes, which modulate the release and absorption characteristics of the drug [4].

Microspheres are spherically small particles with a diameter of 1–1000 μm. Microspheres are also called as micro particles. Microspheres vary widely in quality, sphericity, uniformity of particle and particle size distribution [5]. Characteristically, microspheres are free flowing particles which can be prepared from various synthetic and natural materials [6]. Microspheres are the multi-particulate delivery system and are prepared

to control the release of drugs from the dosage form, which helps to improve the bioavailability and to reduce the adverse effect and prolong the action of drug. During prolonged treatment, microspheres also reduces the dosing frequency, absorption difference, and adverse effects in the patients. To reach the effective biological site rapidly, long acting dosage form needs to be formulated [7]. Microsphere-based drug delivery system has received wide appreciation due to its flexibility, cost effectiveness and broad regulatory acceptance. Microspheres help in providing constant and prolonged therapeutic effects which reduce the dosing frequency and toxic effects of GIT and thereby improve patient compliance [8].

Advantage of microsphere [9–10]:

– Reduces the frequency of administered drug.

– Improved patient compliance.

– Administration of drug can be more convenient.

– Reduces the blood level oscillation characteristics of multiple dosing of conventional dosage form, because a more even blood level can be maintained.

– Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.

– Microspheres received much attention for targeting anticancer drugs to the tumor.

– Reliable means of site-specific drug targeting by maintaining the desired concentration at the site of interest without any untoward effect.

Disadvantages of Microsphere [11]:

– Administration of drug through sustained release does not permit prompt termination of therapy.

– Limitation in the flexibility of dosage regimen adjustment.

– On the basis of biological half-lives, controlled release forms are designed for normal population.

– Economy factors may also be assessed.

Diclofenac Sodium: Diclofenac Sodium or Sodium 2-[(2, 6-dichlorophenyl) amino] phenyl acetate is a broadly used non-steroidal anti-inflammatory drug for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosing spondilytis [12].

Babul gum: *Acacia nilotica*, commonly known as Babul, is indigenous to India and is one of the most useful medicinal plants in India. Its gum, bark, pods, leaves and flowers have medicinal value [13].

Material and Method

Material: Diclofenac Sodium was received as a gift sample from Aegis Pharmaceuticals Pvt. Ltd., Roorkee. *Acacia nilotica* gum was purchased from Ghaziabad and purification was done in the laboratory. All other excipients were used analytical grade method.

Purification of *Acacia nilotica* gum: As described by the author elsewhere, the crude plant material was soaked in warm water for 4 h, boiled for 2 h and kept aside for 2 h in order to release the gum in water. After that, the material was squeezed in a muslin bag to remove the mark from the filtrate. For isolation of gum, equal volume of ethyl alcohol was added in the filtrate to separate the gum. After separation, gum was dried in the oven at 45°C, powdered and passed through sieve #80. The powdered gum was stored in the desiccator until further use [14].

Method: The microspheres of diclofenac sodium were prepared by Ionic gelation method. Sodium alginate was dissolved in sufficient amount of water by maintaining the temperature between 40–50°C. Then, the required amount of polymer was added. When the polymer dissolved, the drug was added into it and dispersed in the polymeric solution. A 5% Calcium chloride solution was prepared as a cross-linking agent and placed on the magnetic stirrer. The drug and polymers solution were filled into the syringe and drop wise added into the calcium chloride solution by using needle size 24#. The prepared microspheres were allowed to stand in the calcium chloride solution for 2 hrs for curing. After that the prepared microspheres were filtered by using Whatman filter paper and dried using hot air oven at 50°C temperature and stored carefully. Compositions of the prepared microspheres are represented in Table 1 [15]. In this research article all the data was presented as averages and standard deviations.

Table 1. Different Concentration of Drug and Polymer

Batch No.	Diclofenac Sodium (mg)	Sodium Alginate (% w/w)	*Acacia nilotica* polymer (% w/w)	Calcium chloride (% w/w)
F1	200	2.5	2.5	5
F2	200	2.5	3.5	5
F3	200	2.5	4.5	5
F4	200	2.5	5.5	5
F5	200	2.5	6.5	5

Evaluation of Microspheres

Micromeritic Properties: The prepared microspheres were evaluated for their flow properties by determining various parameters like the Bulk density, Tapped density, Angle of repose, Carr's Index, Hausner's ratio. These parameters were calculated by using the following formula [16]:

$$\text{Bulk density} = \frac{\text{Weight of Powder}}{\text{Bulk Volume}} \quad \text{(Equation 1)},$$

$$\text{Tapped density} = \frac{\text{Weight of Powder}}{\text{Tapped Volume}} \quad \text{(Equation 2)},$$

$$\text{Carr's index} = \frac{\text{Tapped density-bulk density}}{\text{Tapped density}} \times 100 \quad \text{(Equation 3)},$$

$$\text{Hausner's ratio} = \frac{\text{Bulk Density}}{\text{Tapped Density}} \quad \text{(Equation 4)},$$

$$\text{Angle of repose, } \tan\theta = h/r \quad \text{(Equation 5)}.$$

Particle Size Determination: Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope. A small amount of dry microspheres was suspended in n-hexane (10 mL). A small drop of the suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 50 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using equation 6 [17].

$$\text{Particle Size Determination} = \frac{\text{Stage reading}}{\text{(Occular reading)}} \times 0.01 \quad \text{(Equation 6)}.$$

Percentage yield: The percentage yield of each batch was calculated on the basis of weight with respect to the weight of starting material. All experiments were carried out in triplicate. The percent yield of prepared microsphere was calculated by using equation 7 [18].

$$\% \text{ Yield} = \frac{\text{Weight of dried microsphere recovered}}{\text{Weight of drug + + Weight of polymer}} \times 100 \text{ (Equation 7)}.$$

Swelling Index: Swelling index helps to examine the ability of the microspheres swell at the absorbing surface by absorbing fluids available at the site of absorption. Microspheres (100 mg) were weighed and placed in a Petri-dish containing 100 ml of phosphate buffer pH 6.8 and kept aside and swelling was allowed at 37°C and readings were taken at different time intervals and changes in weight variation between initial weight of microspheres and weight due to swelling was measured by taking weight periodically after soaking with filter paper. The swelling index of the microsphere is calculated by using the equation 8 [19].

$$\text{Swelling index} = \frac{\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}}{\text{Mass of dry microsphere}} \times 100 \quad \text{(Equation 8)}.$$

Drug entrapment efficiency: 100 mg drug equivalent microspheres of each batch were finely powdered in a glass mortar. From that 50 mg powder was accurately weighed and taken in a volumetric flask. A clear solution was made using phosphate buffer after vigorous shaking. Then the solution was filtered through filter paper and analyzed spectrophotometrically at 276 nm for drug content. The weight of diclofenac sodium theoretically contained in the microspheres was compared with the weight actually obtained from the drug content studies, i.e., the quantity loaded into the microspheres formulated, to get the diclofenac sodium loading efficiency. The drug entrapment efficiency was calculated by using Equation 9 [18].

$$\% \text{ Entrapment efficiency} = \frac{\text{Drug loading}}{\text{Theoretical drug loading}} \times 100 \quad \text{(Equation 9)}.$$

Drug content estimation: As described elsewhere, the drug content of the prepared microspheres was determined by the method of extraction of drug present in microspheres. Drug loaded microspheres (100 mg) were powdered and extracted in 100 ml Phosphate buffer 6.8 PH for 24 hrs. Then the resultant dispersion of microspheres was sonicated for 30 minutes for complete mixing and filtered through a Whatman filter paper. The concentration of drug present in filtrate was determined spectrophotometrically at 276 nm using 6.8 PH phosphate buffer as blank. Each determination was made in triplicate. The drug content of prepared microsphere was calculated by using equation 10 [18].

$$\text{Drug Content} = \frac{\text{Drug content}}{\text{Total amount of microspheres}} \times 100 \quad \text{(Equation 10)}.$$

Percent of moisture loss: As described elsewhere, the Diclofenac Sodium loaded microspheres of different polymers were evaluated for percentage moisture loss which gives an idea about its hydrophilic nature. The microspheres were weighed initially and kept in desiccators containing calcium chloride at 37°C for 24 hrs. The final weight was noted when there was no further change in the weight of sample. The percent of moisture loss was calculated by using equation 11 [20].

$$\% \text{ Moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \text{ (Equation 11)}.$$

In vitro drug release study: *In vitro* release of Diclofenac Sodium from the microspheres was examined in Phosphate buffer at pH 6.8 using USP (XXI) one stage dissolution rate test apparatus. Microspheres equivalent to 100 mg of drug were taken and packed in capsule suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. And 5 ml was withdrawn periodically at different intervals for the next 6 hrs. Same volume of fresh medium is replaced. The samples were filtered through Whatman filter paper and analyzed at 276 nm for amount of drug released [21].

SEM analysis: Scanning electron microscopy (SEM) of the polymer powder *Acacia nilotica was* done. The powder was evaporated with carbon and then sputtered with gold to make the samples electrically connected. The SEM was taken in Hitachi S-2400 electron microscope [23].

Result and Disscussion

The Diclofenac sodium microspheres were prepared by ionic gelation technique by using Sodium alginate and babul gum as natural polymers. Five formulations were prepared, i.e. F1 to F5.

The result of the micromeritic properties of the prepared Babul gum loaded microspheres was shown in Table 2. The bulk density and tapped density of babul gum loaded microspheres were in ranged from 0.68 ± 0.004 to 1.27 ± 0.004 g/cm^3 and 0.80 ± 0.004 to 1.49 ± 0.004 g/cm^3 respectively. The angle of repose was < 25° showed excellent flow property of the prepared microspheres. The Carr's index of all batches was in the ranged of 12.50 ± 0.11% to 16.31 ± 0.005%, which indicate good packability of microspheres, whereas the Hausner's ratio of the maximum batches was less than 1.19 indicate good flow.

The particle size, percentage yield, swelling index, entrapment efficiency, drug content and moisture loss of formulated microspheres were determined and the results are shown in table 3. The particle size of the prepared microspheres ranged from 14.55 ± 0.29 µm to 20.18 ± 0.15 µm. It was observed that the microspheres prepared from babul gum have small particle size. They have moderate size range and show spherical, oval and irregular shape of microspheres at different batches.

The percentage yield of microspheres ranged from 51.70 ± 0.02% to 85.27 ± 1.16%. The maximum percentage yield was found of F1 formulation. The average percentage yield of all batches was greater than 50%. Thus, this shows that ionic gelation technique is acceptable for microspheres preparation.

The drug entrapment efficiency of the microspheres prepared from babul gum ranged from 41.45 ± 0.11% to 81.51 ± 0.14%. It was observed that the drug entrapment efficiency is increased as the concentration of babul gum decreased. The % drug content recovered from the microspheres containing babul gum ranged from 69.68 ± 0.24% to 89.67 ± 0.04%. The percent moisture loss of microspheres containing babul gum ranges from 2.37 ± 0.01% to 3.96 ± 0.01%. The swelling index of microspheres prepared from babul gum ranges from 0.79 ± 0.02% to 1.40 ± 0.06%. From the data we concluded that the formulations shows swelling index in respective order: F1 < F2 < F3 < F4 < F5. Hence, we said that the formulation F5 possess higher swelling index and F1 shows low swelling index. Swelling index studies showed that there was an increase in swelling with increase in polymer concentration.

The *in vitro* drug release studies of babul gum containing microspheres ranged from 8.99% to 91.35%. The maximum in *vitro* drug release was found to be 91.35% for the formulation F1 at 6 hrs. Formulation F2, F3, F4 and F5 showed maximum release of 90.42, 84.74, 86.38 and 75.48 respectively at 6th hrs. The result of % drug release of all formulation was shown in table 4. The percent drug release data of all the formulation shows that the release time of the drug decreases as the concentration of the polymer increases.

SEM analysis of the polymer showed that the purified polymers *Acacia nilotica* had a rough surface. SEM analysis of the gum is shown in Figure 1.

Microspheres of Diclofenac sodium were successfully prepared by ionic gelation method using babul

Table 2. Micromeritic properties of babul gum loaded microspheres

Parameter	Formulation				
	F1	F2	F3	F4	F5
Bulk density (g/cm^3)	1.27 ± 0.004	0.68 ± 0.004	1.23 ± 0.002	1.18 ± 0.010	0.84 ± 0.002
Tapped density (g/cm^3)	1.49 ± 0.004	0.80 ± 0.004	1.46 ± 0.040	1.41 ± 0.020	0.96 ± 0.020
Angle of repose (°)	24.85 ± 0.30	20.23 ± 0.10	24.32 ± 0.100	21.40 ± 0.040	23.26 ± 0.10
Carr's index (%)	14.76 ± 0.30	15.00 ± 0.015	15.75 ± 0.016	16.31 ± 0.005	12.50 ± 0.11
Hausner's ratio	1.14 ± 0.004	1.17 ± 0.004	1.18 ± 1.011	1.19 ± 0.040	1.14 ± 0.004

Table 3. Particle size analysis, percentage yield, swelling index, entrapment efficiency, drug content and moisture loss of babul gum loaded microspheres

Formulation	Particle size (µm)	Yield (%)	Swelling index (%)	Entrapment efficiency (%)	Drug content (%)	Moisture Loss (%)
F1	19.46 ± 0.45	85.27 ± 1.16	0.79 ± 0.02	81.51 ± 0.14	69.68 ± 0.24	2.95 ± 0.01
F2	14.55 ± 0.29	64.23 ± 0.19	0.85 ± 0.13	79.37 ± 0.25	85.29 ± 0.02	3.96 ± 0.01
F3	16.89 ± 0.12	51.70 ± 0.02	0.88 ± 0.03	63.91 ± 0.24	72.38 ± 0.01	2.37 ± 0.01
F4	20.18 ± 0.15	75.00 ± 0.86	1.06 ± 0.10	41.45 ± 0.11	90.25 ± 0.24	3.38 ± 0.02
F5	17.98 ± 0.21	67.56 ± 0.38	1.40 ± 0.06	59.19 ± 0.13	89.67 ± 0.04	2.88 ± 0.01

Table 4. *In vitro* drug release data of babul gum microsphere

Time (min)	Drug release (%)				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
15	10.26	9.86	11.61	8.99	10.62
30	23.64	22.54	21.14	24.32	20.96
45	35.82	34.57	36.38	39.13	35.11
60	47.18	49.31	46.15	71.73	49.26
120	69.32	65.28	59.90	64.35	59.01
180	76.19	74.23	70.60	68.17	65.43
240	81.48	79.79	78.14	73.22	68.71
300	87.35	83.98	81.26	79.43	72.13
360	91.35	90.42	84.74	86.38	75.48

gum and sodium alginate. The microspheres thus obtained were subjected to different tests such as drug content, particle size analysis, percent drug release, swelling index etc. From these tests, it was concluded that batch F1 shows the highest drug release for 6 hrs. The concentration of the polymer affected the particle size as well as the *in vitro* release. The *in vitro* release studies showed that the drug release was prolonged for more than 6 hrs. From the data, we also concluded that the formulations shows swelling index in order: F1 < F2 < F3 < F4 < F5. Hence, we said that the formulation F5 possess higher swelling index and F1 shows low swelling index. Swelling index studies showed that there was an increase in swelling with an increase in polymer concentration. So from the above data, we concluded that the prepared microspheres can be used for the controlled delivery of the drug for a prolonged period of time.

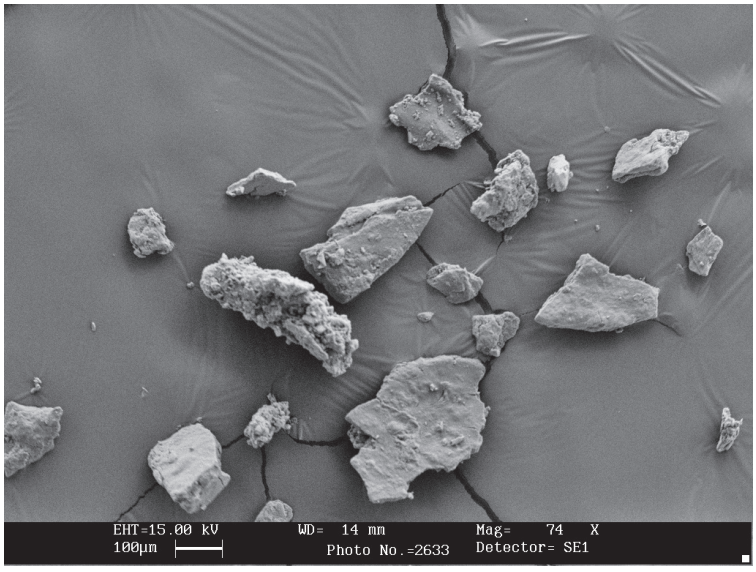

Fig. 1. SEM analysis of *Acacia nilotica* gum

Acknowledgment. Authors would like to thank the Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University and NISCAIR (National Institute of Science Communications and Information Resources), New Delhi for providing library facilities.

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Conflict of Interest: None declared

Received: 29.05.2015
Revised: 3.08.2015
Accepted: 18.08.2015