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**FORMULATION AND EVALUATION OF DICLOFENAC SODIUM LOADED ALBUMIN  
MICROSPHERES**

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**ABSTRACT**

The aim of present work is to develop Diclofenac sodium loaded biocompatible microspheres to reduce the dosing frequency, Gastro intestinal side effects and hence to improve patient compliance. The microspheres were prepared by emulsion –thermal cross –linking process at two different drug: polymer ratios Diclofenac sodium (DS), is a potent drug in the NSAID group having non-steroidal, anti-inflammatory properties and is widely used in the treatment of rheumatoid arthritis. Diclofenac sodium with its low oral bioavailability and short plasma half-life is an ideal candidate for formulation as a sustained release drug delivery system. The prepared microspheres were evaluated for particle size, drug entrapment efficiency (EE) and *in vitro* drug release study. The mean particle sizes were found to be 27.71µm and 32.30 µm. The result reveals that the particle size and EE increases as the polymer content in the formulation increases. The values of cumulative percentage of drug released during 7 hours for F1, drug: polymer ratio – 1:10 was 75% and for F2, drug:polymer ratio – 1:15 was 58%. The increase in polymer concentration prolonged the release of drug from the microspheres with a sustained release pattern.

**KEYWORDS**

Diclofenac sodium, Microspheres, Albumin, Arthritis and Emulsion Thermal Cross Linking Process.

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**INTRODUCTION**

The maximum therapeutic efficacy can be achieved by delivering the therapeutic agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering therapeutic substance to the target site in a sustained controlled release fashion<sup>1</sup>. One such approach is using microspheres as carriers for drugs. Microspheres are

characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200  $\mu\text{m}^2$ . Among the various polymeric carrier systems, albumin microspheres have been shown to be very suitable systems for drug targeting and drug delivery, due to their biodegradability, lack of toxicity and low antigenicity<sup>3</sup>.

Controlled drug delivery system (CDDS) based in microspheres show several advantages in comparison to single unit forms, because the former can: (i) provide more uniform drug dispersion in the gastrointestinal (GI) tract and more homogeneous drug absorption, (ii) decrease the inter- and intra-individual variability, (iii) allow predicting the gastric emptying and (iv) decrease the local irritation. Furthermore, when compared to conventional dosage forms, microspheres-based CDDS provide more consistent and reproducible transit through the GI tract<sup>4,5</sup>.

Diclofenac sodium is a Non-Steroidal Anti-inflammatory Drug used in the treatment of arthritis. It has a short half-life of about 1 to 2 hours and requires multiple dosing. Generally 100 to 200 mg of drug in divided doses is prescribed to be administered twice or thrice a day for chronic pain associated with arthritis and this leads to fluctuation in drug blood levels and causes dose related side effects. To overcome the limitations of conventional therapy, sustained/controlled release dosage forms are designed which are able to maintain steady state drug plasma levels for extended periods of time as a result of which the variations of the drug levels in the blood and drug related side effects are minimized<sup>3</sup>. Sustained release preparations are useful to reduce the dosage frequency to improve patient convenience<sup>5</sup>. Diclofenac sodium with its low oral bioavailability and short plasma half-life is an ideal candidate for formulation as a sustained release drug delivery system. The most frequent adverse side effects of Diclofenac sodium on long term administration are gastro-intestinal disturbances, peptic ulceration and perforation<sup>6</sup>. In order to eliminate the gastrointestinal adverse effects of this drug, several swellable controlled-release pharmaceutical dosage forms have been developed<sup>7</sup>.

The objective of the present study was to develop microspheres of Diclofenac sodium by emulsion thermal cross linking method at two different drug:polymer ratio i.e 1:10 (F1), 1:15 (F2) and to study the effect of drug:polymer ratio on particle size, encapsulation efficiency and *invitro* drug release .

## MATERIAL AND METHOD

### Material

Diclofenac sodium (DS) was kindly supplied as a gift sample from Sangrose Laboratories Pvt Ltd (Kerala, India). Bovine serum albumin and span 60 were purchased from Spectrum chemicals and reagents (Cochin, Kerala, India). All other reagents and chemicals used were of analytical grade, supplied by Nice Chemicals (Cochin, Kerala, India), which includes methanol, petroleum ether and light liquid paraffin.

### Method

#### Preparation of Microspheres

Principle used here is emulsion heat stabilization<sup>1</sup>. First step is the formation of w/o emulsion. Then it is subjected to a temperature above 95° C with stirring. This results in denaturation and aggregation of protein accompanied by evaporation of aqueous pool surrounding the protein. Subsequent washing with organic solvent results in the formation of free flowing microsphere<sup>8</sup>.

Briefly, DS was dissolved in aqueous albumin solution. The resulting solution (10 ml) was added dropwise using a 22 – gauge hypodermic syringe into 100 ml of oil phase containing 0.4% w/v span 60 in light liquid paraffin. Stirring was performed with a mechanical stirrer (Remi Motors, Mumbai, India) at 1000rpm to form a w/o emulsion. The temperature of oil bath was raised to 95 °C and stirred until microspheres get completely dehydrated. The formed microspheres were centrifuged at 3000rpm and the sediment washed with petroleum ether. After drying a fine yellow free flowing powder of microspheres were obtained that was stored in desiccators at room temperature. The drug polymer ratio was varied to prepare two different batches, as shown in Table 1.

## Evaluation of Microspheres

### Particle Size

The particle size of the albumin microspheres were first evaluated using an optical microscope fitted with a calibrated eyepiece micrometer under a magnification of 40<sup>3</sup>. The particle diameters of about 100 microspheres were measured randomly and the average particle size was determined.

### Drug Loading Efficiency (DL)

Drug loading capacity (DL) was calculated according to the following equation<sup>3,8</sup>.

$$DL (\%) = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \times 100$$

100mg of microspheres were taken and extracted with 5ml of methanol. Shaken well for 15minutes and then allowed to centrifuge for 10 minutes. From the supernatant liquid take 1ml and dilute upto 100ml with water. Measure the absorbance at 276nm in UV-visible spectrophotometer (Systronics) using reference.

### Entrapment Efficiency (EE)

Entrapment efficiency is defined as the percentage of drug incorporated into microspheres relative to the total drug added<sup>8,9,10</sup>. The amount of drug loaded into the microspheres was determined as follows: 5 ml of methanol was added to 100 mg of microspheres and the mixture was stirred at 37 °C for 30 minutes. The resulting mixture was centrifuged to separate the undissolved components, and the supernatant containing the drug extracted from microspheres and analyzed using UV spectrophotometer at 276 nm. The EE was determined using the following equation.

$$EE (\% w/v) = \frac{\text{weight of drug in microspheres}}{\text{weight of drug used in the preparation of microspheres}} \times 100$$

### Drug Release Studies

The microspheres were tested for the in vitro release of DS in simulated GI fluids<sup>3,8,11,12</sup>. An accurately weighed amount of microspheres, were added to 500 ml of dissolution medium and the drug release from microspheres was processed using USP rotating paddle dissolution apparatus at 100rpm and at 37±0.5 °C. Perfect sink condition was maintained during the drug dissolution study period. The simulation of GI pH

variations was accomplished by modifying the pH of the dissolution at various time intervals. The pH of the dissolution medium was kept at 1.2 for 2 h with 0.1NHCl. Then, 1.7 g of KH<sub>2</sub>PO<sub>4</sub> and 2.225 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O were added, adjusting the pH to 4.5 with 1.0 M NaOH. The release rate analysis was run for another 5 h. A sample volume of 2ml was withdrawn from the medium at various time intervals and replaced with fresh dissolution medium. The samples collected were filtered & analyzed at 276 nm. All release tests were performed in triplicate. The effects of drug-polymer ratio on in vitro drug release of DS loaded albumin microspheres were also evaluated.

## RESULTS AND DISCUSSION

DS-loaded albumin microspheres prepared by emulsion thermal cross-linking method. Two batches of formulations F1 and F2 in Table No.1 were made by varying the polymer concentration and obtained mean particle size is given in Table No.2. When varying the drug-polymer ratio from 1:10 to 1:15, the mean diameter of microspheres was in the range between 27.71µm and 32.30 µm. The size increased with increasing polymer concentration. Optical microscopic analysis revealed the spherical shape of microspheres with more or less uniform size distribution Figure No.1.

The values of LC and EE of two batches of DS-loaded albumin microspheres are also listed in Table 2. The LC values varied between 6.55% (F1) and 4.56% (F2). The result in Table No.2 reveals that the EE increases as the polymer content in the formulation increases. The EE for formulation F1 and F2 were found to be 65.5% and 68.4% respectively. The increase in matrix content is expected to raise the EE by providing more space to incorporate the drug. Increment of the polymer content also reduces the escaping of drug into the external phase, which accounts for an increase in EE.

DS-loaded albumin microspheres were subjected to *in vitro* drug release studies in the presence of simulated GI fluids using USP dissolution test apparatus I. The studies were carried out in 500 ml of the dissolution medium, stirred at 100rpm at 37°C. The dissolution

profiles for both batches were studied using acid buffer solution of pH 1.2 for 2 h (simulated gastric fluid), pH 5 for remaining 5 h. The *in vitro* release profiles of DS from the albumin microspheres in simulated GI fluids are depicted in Figure No.2. The result in Table No.3 shows that 5.4 - 11.6% of drug was released from the formulations in the initial 15 minutes. This may be due

to the drug desorption and release from the surface of microspheres. A sustained release of 75.32% and 58.86% respectively were found for formulations F1 and F2 over the entire period of study in Figure No.2. The increase in polymer concentration prolonged the release of drug from the microspheres.

**Table No.1: Composition of prepared formulations**

| S.No | Formulation code | Drug:polymer | Drug (mg) | polymer (mg) | Surfactant (% w/v) |
|------|------------------|--------------|-----------|--------------|--------------------|
| 1    | F1               | 1:10         | 100       | 1000         | 0.4                |
| 2    | F2               | 1:15         | 100       | 1500         | 0.4                |

**Table No.2: Average particle size, Loading efficiency, Encapsulation efficiency of formulations**

| S.No | Formulation Code | Drug-polymer ratio | Particle size (µm) | DL (%)    | EE (%)    |
|------|------------------|--------------------|--------------------|-----------|-----------|
| 1    | F1               | 1:10               | 27.71±1.04         | 6.55±1.28 | 65.5±0.68 |
| 2    | F2               | 1:15               | 32.30±2.94         | 4.56±0.64 | 68.4±1.22 |

**Table No.3: Cumulative percentage release of F1 and F2 during 7 h in simulated GI fluid**

| S.No | Time (hrs) | % release (F1) | % release (F2) |
|------|------------|----------------|----------------|
| 1    | 0          | 0              | 0              |
| 2    | 0.25       | 11.63±3.50     | 5.40±0.92      |
| 3    | 0.3        | 12.73±2.92     | 8.10±2.26      |
| 4    | 0.45       | 19.93±3.12     | 11.88±1.18     |
| 5    | 1          | 22.15±1.54     | 15.66±1.94     |
| 6    | 2          | 26.58±1.96     | 18.90±2.28     |
| 7    | 3          | 31.01±2.34     | 20.52±1.32     |
| 8    | 4          | 38.76±2.06     | 26.46±3.24     |
| 9    | 5          | 49.29±1.02     | 35.64±0.84     |
| 10   | 6          | 61.47±3.58     | 46.44±1.22     |
| 11   | 7          | 75.32±2.74     | 58.86±2.08     |



**Figure No.1: Optical microscopic image of DS loaded microspheres**

### CONCLUSION

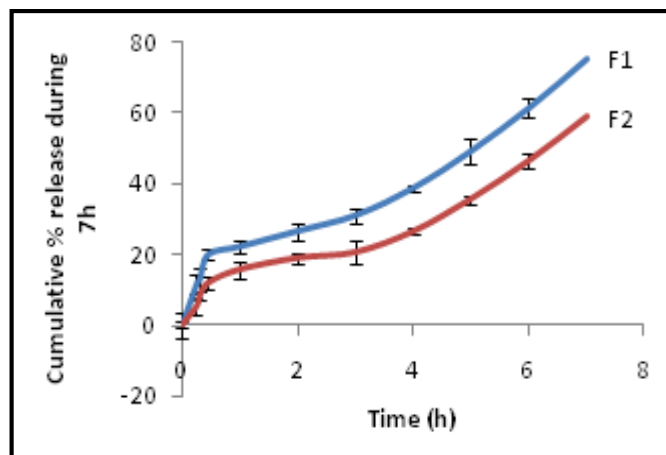
Diclofenac sodium loaded albumin microspheres were prepared by emulsion thermal cross linking method. Two batches of formulations, of drug: polymer ratio i.e. 1:10 and 1:15, were prepared and evaluated for particle size, drug loading capacity, drug entrapment efficiency and *in vitro* drug release. The microspheres were spherical with more or less uniform size distribution and an increase in particle size was observed as the drug-polymer ratio decreased from 1:10 to 1:15. The encapsulation efficiency increased as the polymer content in the formulation increased. *In vitro* release studies demonstrated a sustained release pattern with an initial burst release. In our study, it was observed that an increase in polymer content delayed the release of drug from the formulation.

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### CONFLICT OF INTEREST

We declare that we have no conflict of interest.



**Figure No.2: Dissolution profile of Formulation F1 and F2**

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