

## Comparative Studies on Diclofenac Sodium Loaded Albumin/Gelatin Magnetic Microspheres for Intra-arterial Administration.

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### Abstract

Magnetite was prepared by oxidation of ferrous sulphate by sodium hydroxide. Diclofenac sodium loaded gelatin and albumin magnetic microspheres were prepared by emulsification/cross-linking by glutaraldehyde using ethylcellulose in chloroform and sesame oil with/without span80 as stabilizing medium. The microspheres were characterized by drug loading, entrapment/encapsulation efficiency, particle size, optical microscopy, magnetite content and *in vitro* release studies. Sesame oil with span80 was found to yield spherical and compact microspheres. Gelatin magnetic microspheres showed better loading, entrapment, encapsulation, release profile and stability than albumin magnetic microspheres.

**Key Words:** Gelatin, albumin, magnetic microspheres, magnetite, diclofenac sodium.

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### Introduction

Targeting the drug with magnetic microspheres was described by Widder (Widder *et al.* 1979) who used magnetically responsive biodegradable drug carrier with the capacity to localize both carrier and therapeutic agent, by magnetic means to a specified *in vivo* target site. Many authors attempted to develop magnetic microspheres to target drugs (Widder *et al.* 1979, Tetsuro 1983, Lalla and Ahuja 1991). In general, magnetic microspheres are infused into an artery supplying a given *in vivo* target site. A magnet of sufficient field strength to retard the microspheres solely at the capillary level vasculature is placed externally over the target area. Because of arterial administration with subsequent magnetic localization, the majority of infused microspheres does not circulate systemically and are therefore not cleared by the macrophages/reticuloendothelial system. This targeting system allows therapeutic levels of drugs to be attained at a desired target with smaller doses and thus avoiding side effects due to accumulation of the drug at non-target area.

Albumin and gelatin are well known biocompatible and biodegradable natural carriers widely used for drug targeting and controlled release. Though various authors reported particles of these polymers for drug delivery, relatively no information is available regarding the better choice among them. Hence we attempted a comparative study to find out best carrier based on physicochemical properties and stability.

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In the present study diclofenac sodium loaded magnetic microspheres were formulated by using albumin/gelatin and were characterized by various physical and chemical parameters to find out the suitable carrier which can deliver the drug for prolonged period with stable physicochemical characters.

## Materials and Methods

### *Materials*

Gelatin-Type B 300 bloom strength was purchased from Sigma Chemicals, U.S.A. and albumin from Qualigens Fine Chemicals, India. Diclofenac sodium is a gift Sample obtained from MARAL, Chennai. Ethyl cellulose was purchased from Loba chemie, Mumbai. Chloroform and Glutaraldehyde were purchased from S.D. Fine Chemicals Ltd, Boisar, India.

### *Preparation of magnetite*

Magnetite was prepared by the oxidation of ferrous hydroxide (Sada *et al.* 1990, Leun and Sengupta 2000) as follows: 13.9 g of ferrous sulphate was dissolved in 70 ml of water and this solution was aerated. 4.1 g of sodium hydroxide was dissolved in 30 ml of water and added to the first solution. The temperature of the ferrous sulphate and sodium hydroxide mixture was increased to 50°C, in 20 min in a temperature controlled water bath and maintained for 5 h along with aeration to give cubic magnetite powders. The pH of the reaction system was maintained at 4.5. Magnetite particles were allowed to sediment by placing a magnet at the bottom of the beaker. The supernatant fluid was removed. Then the particles were washed with 3 x 100 ml of distilled water and dried at room temperature.

### *Preparation of magnetic microspheres using ethylcellulose as stabilizing medium*

2 g of gelatin was dissolved in 10 ml of phosphate buffer (pH 7.4) by heating. 2 g of drug were dissolved separately in 10 ml of phosphate buffer (pH 7.4) and added to gelatin solution. 2 g of magnetite was dispersed in 2 ml of 50 % w/v alcohol in a mortar. To this, gelatin - diclofenac solution was added drop by drop with trituration. The mixture was made up to 25 ml with phosphate buffer. Then the mixture was added drop wise to 100 ml of 10% ethyl cellulose in chloroform and emulsified by stirring with help of hand blender (10,000 rpm/2 min). The stabilized emulsion was stirred with help of a stirrer attached to a motor. 1 ml of glutaraldehyde-saturated toluene solution (Yan *et al.* 1991) was added successively each hour and stirring was continued for five hour at room temperature. The microspheres formed were collected by filtration using Whatman filter paper (No: 41). After filtration, the microspheres were washed with chloroform to remove ethylcellulose. Then it was washed with 10 ml of 1% sodium metabisulphite to terminate cross-linking, the washing was done with 3 x 10 ml of phosphate buffer (pH 7.4.) in order to remove the unencapsulated diclofenac sodium. Finally, it was washed with 2 x 10 ml of water and dried at room temperature. Albumin magnetic microspheres loaded with diclofenac sodium were also prepared by the same procedure.

### *Preparation of magnetic microspheres using sesame oil as stabilizing medium.*

Gelatin/albumin magnetic microspheres were formulated by using sesame oil with and without 1% w/v span80 as stabilizing medium with the same procedure as explained above, but after cross-linking, the microspheres were filtered and washed with petroleum ether. The resulting microspheres were washed with 10 ml of 1% sodiummetabisulphite to neutralize unreacted glutaraldehyde. Then it was washed with phosphate buffer (pH 7.4.) in order to remove the unencapsulated drug. Finally, it was washed with water and dried at room temperature.

### *Characterization of Microspheres*

#### *Drug loading, entrapment and encapsulation efficiency*

Drug loaded microspheres (100 mg) were digested (Saravanan *et al.* 2002a) with 10 ml of 1N sodium hydroxide at room temperature for 24 h. The solution was filtered and analyzed at 277 nm (Shimadzu UV-Vis 1601 spectrophotometer), to determine the amount of diclofenac sodium present in the microspheres.

### Determination of magnetite content

The magnetite content in microspheres were estimated quantitatively (Gupta *et al.* 1988) by hydrolyzing an aliquot of the microspheres in concentrated hydrochloric acid and assaying the resultant hydrolysate for iron by atomic absorption spectroscopy at 248 nm using AAS/127 instrument, electronic corporation, India.

### Particle size analysis

Microspheres were dispersed in water. A smear was made on glass slide, size of about 200 microspheres were determined using an optical microscope fitted with a micrometer scale.

### Optical microscopy

The morphological characteristics of microspheres were observed using a radical phase contrast and dark field microscope with photographic attachment. Photomicrographs were taken using Pentax K1000 camera and 35 mm ASA/ISO black and white film.

### In vitro release study

The *in vitro* release studies of drug-loaded microspheres were carried out at 37°C using phosphate buffer pH 7.4. Each batch of microspheres containing 50 mg of diclofenac sodium was individually added to 200 ml of phosphate buffer pH 7.4 in flasks. The flasks were shaken (Saravanan *et al.* 2002a) in an incubator at 37°C. 1 ml of samples was withdrawn at regular time intervals and same volume of phosphate buffer was replaced. After suitable dilution, diclofenac sodium content in phosphate buffer (pH 7.4) was estimated at 277 nm by using UV visible spectrophotometer.

### Stability studies

The magnetic microspheres prepared by using sesame oil and span80 were kept for a short term accelerated stability study (Saravanan *et al.* 2002b) in high density polyethylene sealed cover at 40±2°C and 75±5% RH for one month as per the "International Conference on Harmonization States" (ICH) guidelines. Similarly the microspheres were also kept in refrigerator to find out the stability in refrigerated conditions. After one month the microsphere were analyzed for drug content, change of shape and drug release.

## Results and Discussions

During the formulation of microspheres, the aqueous phase containing drug, polymer and magnetite were stabilized by using ethylcellulose in chloroform, sesame oil and sesame oil with span80. The recovery of microspheres from ethylcellulose solution was found to be difficult and the recovered microspheres were sticky due to presence of residual ethylcellulose, which needs more number of washing for complete removal. The recovery from sesame oil was easy and fast. The oil was removed with help of petroleum ether.

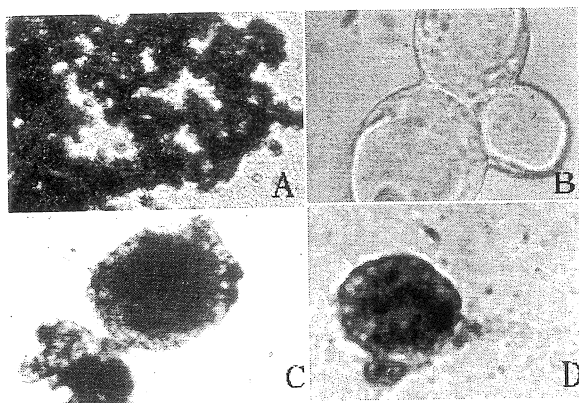
**Table 1.** Physicochemical properties of prepared magnetic microspheres.

| Microsphere/<br>Stabilizing<br>Medium | % of loading |                 | %<br>Entrapment | %<br>Encapsulation<br>n=3 | Average<br>Particle<br>size (µm)<br>n=200 | Magnetite<br>% w/w<br>n=3 |
|---------------------------------------|--------------|-----------------|-----------------|---------------------------|---|---------------------------|
|                                       | Theoretical  | Measured<br>n=3 |                 |                           |   |                           |
| GM-EC                                 | 33.3         | 31.8±1.4        | 95.4±4.2        | 69.7±3.8                  | 36.3                                      | 30.1±1.3                  |
| GM-SO                                 | 33.3         | 30.7±2.1        | 92.1±6.3        | 74.8±6.4                  | 40.8                                      | 29.5±0.8                  |
| GM-SOS <sub>80</sub>                  | 33.3         | 31.3±1.8        | 94±5.4          | 75.1±4.3                  | 38.1                                      | 31.5±0.6                  |
| AM-EC                                 | 33.3         | 29.3±1.2        | 87.9±3.6        | 68.5±5.8                  | 25.4                                      | 28.3±1.2                  |
| AM-SO                                 | 33.3         | 27.5±1.6        | 82.5±4.8        | 72.4±3.2                  | 30.7                                      | 27.5±2.1                  |
| AM-SOS <sub>80</sub>                  | 33.3         | 28.0±2.3        | 84±6.9          | 73.2±4.5                  | 28.2                                      | 29.2±1.6                  |

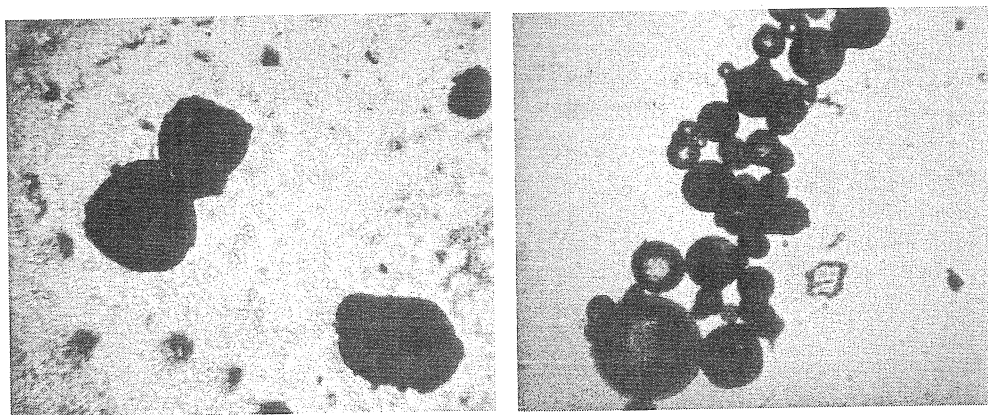
GM-Gelatin microspheres, AM-Albumin microspheres, EC-Ethylcellulose, SO-Sesame oil, SOS<sub>80</sub>-Sesame oil with span80.

The loading in gelatin microspheres was comparatively high and was found to be between 31.8 and 30.7%. The entrapment and encapsulation of gelatin microspheres were better than with albumin microspheres. Though microspheres prepared with ethylcellulose as stabilizing medium showed good loading and entrapment but showed less encapsulation efficiency and thus indicating wastage of drug during encapsulation process. This is because of adhesion of microspheres to beaker and filter paper due to sticky ethylcellulose residual film, which is formed during evaporation of chloroform. The microspheres prepared with sesame oil showed comparatively better encapsulation efficiency as shown in Table 1. The magnetite content of microspheres was found to be between 27.5 and 31.5% w/w as given in Table 1. The particle sizes of formulated microspheres were found to be between 10 and 100  $\mu\text{m}$ . Depending on the stabilizing medium used, the average size of gelatin microspheres was 36 to 40  $\mu\text{m}$  and for albumin micro sphere was 25 to 30  $\mu\text{m}$ . Ethylcellulose in chloroform has produced smaller microspheres than sesame oil. Addition of span80 produced smaller spherical particles with comparatively narrow particle size distribution.

**Figure 1.** Photomicrograph of magnetite (A), gelatin microspheres without (B) and with magnetite (C and D) prepared by ethylcellulose as stabilizing agent (600 x magnification).



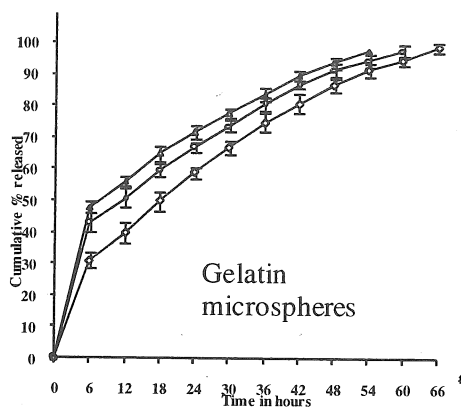
**Figure 2.** Photomicrograph showing gelatin magnetic microspheres prepared by sesame oil without (left) and with (right) span 80 as stabilizing agent (600 x magnifications).



The photograph shown in Fig. 1 evidenced the presence of magnetite particles in the microspheres. The photograph shows the magnetite particles (Fig. 1A) and gelatin microspheres without (Fig. 1B) and with magnetite prepared by using ethylcellulose as stabilizing agent (Fig. 1C and D). The spheres were aggregated as shown in Fig. 1B. The gelatin magnetic microspheres prepared with sesame oil alone showed irregular shapes and aggregation as shown in Fig. 2. The shape of the microspheres prepared by sesame oil containing span80 as evidenced by photomicrograph (Fig. 2) was spherical, compact and less aggregated. Albumin microspheres also showed similar character.

But the size of gelatin microspheres was larger than albumin microspheres.

**Figure 3.** *In vitro* release of diclofenac sodium from albumin (left) and gelatin (right) microspheres prepared by using ethylcellulose ( $\diamond$ ), sesame oil (o) and sesame oil with span80



( $\Delta$ ) as stabilizing agent. Each data indicates average  $\pm$  S.D (n=3).

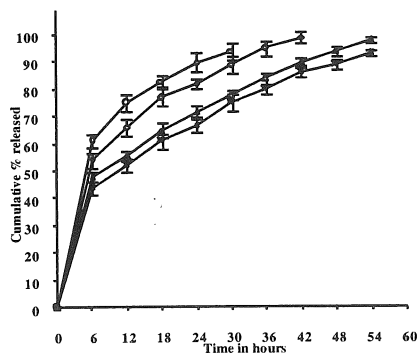
Albumin magnetic microspheres as shown in Fig. 3, has released the drug for a period of 42 to 48 h. Gelatin magnetic microspheres showed release (Fig. 4) for a period of 54 to 66 h, which is comparatively longer period than albumin microspheres. The microspheres formulated with ethylcellulose in chloroform has released the drug slowly, may be due to residual ethylcellulose on the surface of microspheres. The microspheres prepared by using sesame oil as stabilizing medium released the drug faster than that formulated with ethylcellulose medium. The inclusion of span80 in sesame oil slightly increased the rate of drug release from the microspheres may be due to small size and less aggregation of spheres, which might have enhanced the contact between the microsphere and release medium.

**Table 2.** Physicochemical parameters of microspheres during stability studies.

| Batch                           | Drug content %w/w (n=3) |                 | Particle shape |  |
|---------------------------------|-------------------------|-----------------|----------------|--|
|                                 | Initial                 | After one month | Initial        | After one month                                  |
| GM-SOS80<br>Stored at<br>0-6°C  | 31.3±1.8                | 30.5±2.3        | Spherical      | No change  |
| GM-SOS80<br>Stored at<br>40±2°C | 31.3±1.8                | 29.4±3.4        | Spherical      | Spherical-<br>some<br>particles<br>disintegrated |
| AM-SOS80<br>Stored at<br>0-6°C  | 28.0±2.3                | 27.7±3.9        | Spherical      | Some<br>particles are<br>disintegrated           |
| AM-SOS80<br>Stored at<br>40±2°C | 28.0±2.3                | 25.4±4.1        | Spherical      | Many<br>particles are<br>disintegrated           |

Storage of microspheres under refrigerated conditions for one month has not produced much change in drug content, shape and *in vitro* release pattern. The storage of microspheres under accelerated conditions as per the guidelines of ICH, showed changes in physicochemical parameters as shown in Table 2 and Fig. 5. Albumin microspheres showed more stability problem than gelatin microspheres. The release of drug from the albumin microspheres was very fast after one month of stability study, may be due to breakdown of microspheres. Gelatin microspheres released the drug more or less similarly but slightly at a slower rate.

**Figure 4.** *In vitro* release of diclofenac sodium from albumin/gelatin microspheres during short-term stability studies. The figure shows release of diclofenac sodium initially from albumin ( $\diamond$ ), gelatin ( $\Delta$ ) microspheres and after one month from albumin (o), gelatin (x) microspheres. Each data indicates average  $\pm$  S.D (n=3).



## Conclusion

In the present study we attempted to develop magnetic microspheres loaded with diclofenac sodium intended for targeted drug delivery. The microspheres were prepared using albumin /gelatin as carrier and studied to select the suitable one based on formulation, physicochemical and stability parameters. Under our experimental conditions gelatin microspheres showed better stability and prolonged drug release than albumin microspheres. Sesame oil with span80 was found to be the effective stabilizing medium to prepare microspheres.

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Received: 19.12.2007

Accepted : 23.03.2008