

**PREPARATION AND CHARACTERIZATION OF MAGNETIC ALBUMIN
MICROSPHERES ENCAPSULATED WITH DICLOFENAC SODIUM**

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Summary

In the present work, magnetic albumin microspheres were prepared by emulsification / heat stabilization technique. The formulated microspheres were characterized by SEM, DSC and were studied for particle size distribution, release characteristics and their in-vitro accumulation (magnetic responsiveness) in presence and absence of external magnetic field. The formulated microspheres were below 5µm and spherical in nature as evidenced from SEM. DSC revealed that, there was no drug-polymer interaction. The in-vitro release profile was studied in normal saline medium up to 7 hours using USP XXII dissolution apparatus. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 30% of the total drug content from the microspheres within 7 hours. A third order equation for the drug release was also calculated. Microspheres showed greater retention time under the influence of magnetic field created by an electromagnet with field strength 8000 G, when compared to the retention in the absence of magnetic field. From this study, it could be suggested that magnetic albumin microspheres could be retained at their target site in-vivo, following the application of the magnetic field and are being capable of releasing the drug for an extended period of time, thus making them a suitable depot for delivering chemotherapeutic agent in-vivo

Keywords: magnetite, albumin, microspheres, magnetic responsiveness, Diclofenac sodium.

Introduction

The activity of most drugs against disease suffers from their inability to accumulate selectively at the site of action. Drug targeting is the delivery of drugs to receptors or organs or any other specific part of the body to which one wishes to deliver the drug. Drug targeting via magnetism can be achieved by magnetic microspheres where magnetically responsive biodegradable drug carrier with the capacity to localize both carrier and therapeutic agent, by magnetic means was used [1]. These magnetic microspheres consist of magnetite (Fe₃O₄) particles, which are well tolerated by the body [2] and are responsible for magnetic property. A biodegradable polymer such as gelatin or albumin is used to encapsulate or entrap the therapeutic agent.

In recent years, considerable interest has been shown in the use of albumin microspheres as platforms for active drug targeting as well as producing a sustained and controlled rate of drug release [3,4,5]. In the previous studies, a phase separation emulsion technique was developed for the preparation of the microspheres. Stabilization of the albumin microspheres matrix was accomplished by either heat denaturation at various temperatures (110-190°C) or crosslinking with carbonyl compounds in an ether phase reaction. The degree of stabilization controls the rate of drug diffusion out of the carrier as well as the extent of carrier degradation [6].

Magnetic albumin microspheres are capable of being retained in the capillaries by using extracorporeal magnets. Electron microscopic studies of rat tail skin perfused with microspheres in a 8000 G magnetic field of 30 min duration showed that microspheres were internalized by endothelial cells and trapped between the plasma membranes of two adjacent endothelial cells and hence were not cleared by the reticuloendothelial system [7]. The studies confirm second order drug targeting (targeting to specific organs or tissues [8]) in the target tissue of healthy animals [9].

Targeting by magnetically responsive albumin microspheres has a high efficiency. For example, doxorubicin hydrochloride, entrapped in magnetic albumin microspheres, has been shown to cause enhanced drug concentration in the target tissue compared with the administration of free drug [10]. In addition, the amount of drug reaching the heart and liver was reduced.

Diclofenac sodium is one of the drugs of choice to treat arthritis because of its potential anti-inflammatory and analgesic activity and this is the only approved NSAID available for parenteral delivery. Because of shorter biological half-life, diclofenac sodium should be given frequently to maintain its therapeutic activity and is often associated with Gastric ulcers, gastrointestinal bleeding, blood dyscrasias and anaphylaxis. [11]

In the present study, albumin magnetic microspheres are formulated to overcome the toxicity produced by the Diclofenac sodium and to achieve prolonged therapeutic effect and to target the drug at its site of action. The albumin magnetic microspheres were prepared by emulsification / heat stabilization technique. The formulated microspheres were characterized by particle size distribution, scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and evaluated for their in vitro magnetic responsiveness and the in vitro drug release characteristics.

Materials

Human serum albumin (Sigma-Aldrich, USA), Magnetite (Lobe-chemie, Mumbai), Diclofenac sodium (KAPL, Bangalore), Neodymium magnet, 8000 G field strength (ABY systems, Chennai), Cottonseed oil (Thomas baker chemicals, Mumbai), all other chemicals used was of analytical grade.

Experiment

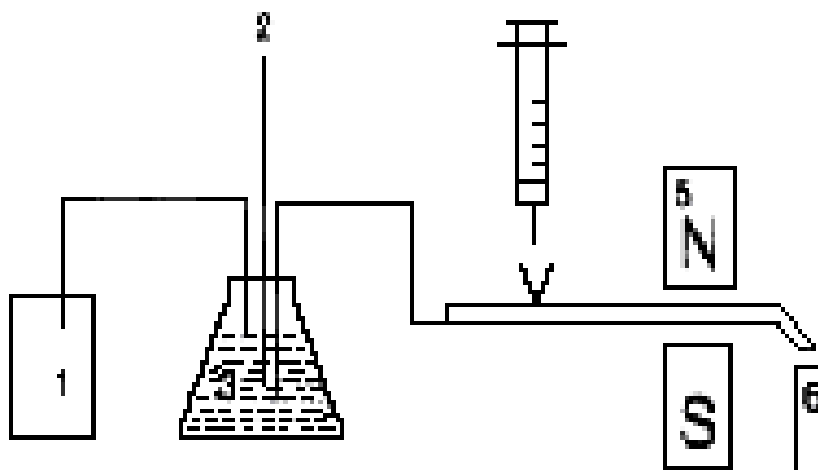
Magnetic microspheres were prepared by a modified method, similar to that described by Widder et al [12]. Human serum albumin (150 mg), Diclofenac sodium (40 mg) and magnetite (40 mg) were added to 0.5 mL of distilled water. The resulting dispersion was then added dropwise (20 drops/min) into 100 mL of a continuously stirring cottonseed oil and stirred at a constant rate of 2200 rpm for 30 min on electric stirrer. The emulsion was then homogenized using homogenizer for 5 min at 25°C. This procedure generated small, non cross- linked microspheres, containing both magnetite and the entrapped drug. Heating the homogenate at 120°C for 10 min then denatured the resulting microspheres. The suspension was then cooled to 25°C, washed 4 times with anhydrous diethyl ether, stored for 48 h at - 10°C and finally dried in a desiccator at 4°C. The prepared microspheres were then stored until further use.

Characterization

FT-IR spectroscopy was employed to ascertain the compatibility of Diclofenac with the excipients. The individual drug and drug with excipients were separately scanned. Both the spectra were compared for confirmation of common peaks. The surface and surface characteristics of the microcapsules was studied using scanning electron microscopy (SEM, S-500, and HITACHI). Prior to observation, samples were mounted on metal grids, using double-sided adhesive tape and coated by gold under vacuum before observation. To investigate the cross-section, the wet beads were cut in to two halves with blade and examined in wet condition. DSC of Diclofenac sodium, magnetite and microspheres were performed using Perkin-Elmer DSC-7 model to determine the possible drug-polymer interaction and the physical state of the drug in the microspheres.

Evaluation

Magnetic responsivity of the drug-loaded magnetic microspheres [13]
The apparatus shown in Fig. 1 was designed for this study.



(Fig. 1) 1 - Pump, 2 – Thermometer, 3 – Normal saline, 4 – Injection site, 5 – Magnet, 6 – collecting sample.

The apparatus consists of a Millipore pump, which pumped air into the flask containing normal saline. This resulted in the flow of normal saline through the glass tube, which was exposed to an electromagnet. By regulating the pump pressure, the flow rate within the glass tube was controlled at 3 mL/min (0.5 cm/s). The experiment was carried out at room temperature (21°C). Prior to injection, microspheres (25 mg/mL) were dispersed in normal saline containing 0.1% w/v Tween 80 and a stock solution was prepared. A flow of 0.5 cm/s of normal saline, resembling the blood flow rate passing through the capillaries was established. A 1 mL aliquot of the microspheres suspension in the test vehicle was then injected into the injection site. The 8000 G magnetic field was established for 15 min and one sample was collected every minute. The magnetic field was then removed and samples collected for a further 5 min. Microspheres content of the collected samples were then evaluated using UV-Vis. Spectrophotometer, (Shimadzu Ltd. Japan) at 277nm wavelength.

In Vitro Drug Release From Magnetic Albumin Microspheres

Drug loaded microspheres containing 30mg equivalent weight of the drug was taken and dispersed in 400 mL of normal saline in beaker of the USP dissolution apparatus 2. The temperature was kept at $37 \pm 0.5^\circ\text{C}$ throughout the experiment and the stirring rate of the paddles was set at 90 ± 5 rpm. The experiment was run for a total period of 7 h and 5 mL samples were removed from the test beakers at regular intervals up to 7h. The drug concentration in the samples was then determined spectrophotometrically at 277 nm, using a Shimadzu 160A UV-Vis. spectrophotometer. The absorption of each sample was read against it's blank.

Results and Discussion

The prepared albumin magnetic microspheres loaded with Diclofenac sodium were characterized for particle size, SEM, DSC and evaluated for their in-vitro accumulation in the presence and absence of a magnetic field and their in-vitro drug release profile up to 7 h using the USP dissolution apparatus. The size distribution of albumin microspheres (Fig. 2) was between 0.4 and 5 μm . The average particle size of these microspheres was found to be 2.4 μm .

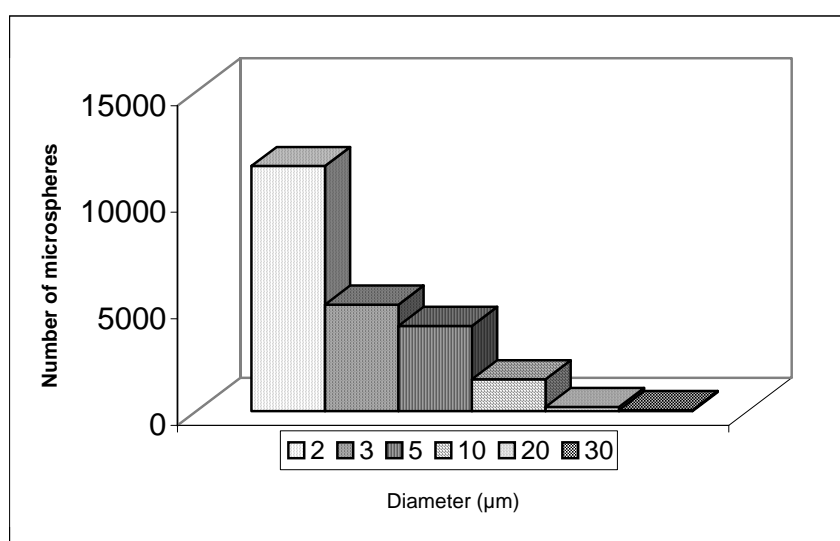


Fig 2. Diameter distribution of albumin magnetic microspheres.

The size and shape of albumin magnetic microspheres were further studied by SEM. As shown in Fig. 3, the formulated microspheres were spherical and compact in nature as evidenced by the SEM photograph.

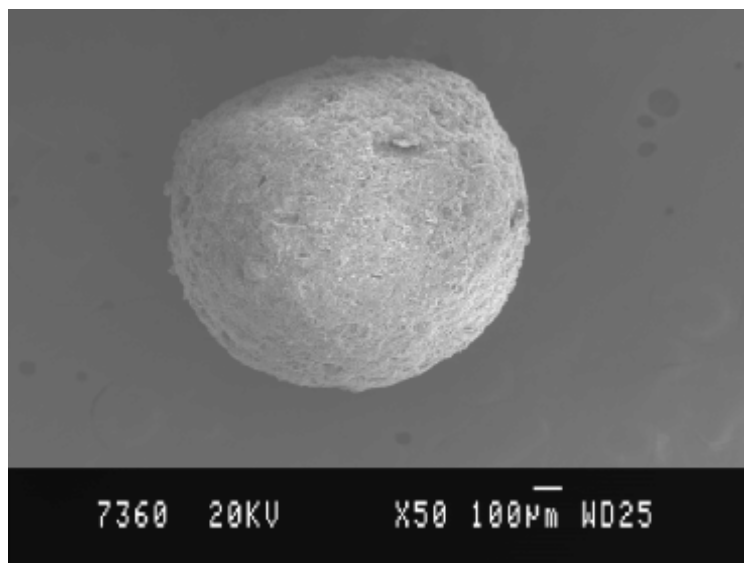


Fig 3. SEM of albumin magnetic microspheres loaded with Diclofenac sodium

The thermogram of Diclofenac sodium showed a peak at about its melting point (297°C). Diclofenac sodium peak was absent in the thermogram of drug-loaded albumin magnetic microspheres, which revealed the amorphous nature of entrapped drug in the formulated microspheres.

Magnetic Responsivity

In Fig. 4, the percentage of the microspheres failed to remain in the glass tube in the absence and presence of the magnetic field (8000 G) has been compared. It was observed that the majority of the microspheres with approximately 17% w/w magnetite in a 8000 G magnetic field and in a flow rate equal to 0.5 cm/s were retained and did not exit the glass tube. Therefore, it is predicted that the microspheres prepared by this method can accumulate in the capillaries following *in vivo* administration. Nevertheless, it is important to note that the experimental conditions encountered in this study, e.g. temperature was lower than the body temperature, were not exactly the same as those seen *in vivo*. Hence, extensive *in vivo* studies are needed to be carried out in order to determine the actual percentage of the micro- spheres, which will be retained in the capillaries.

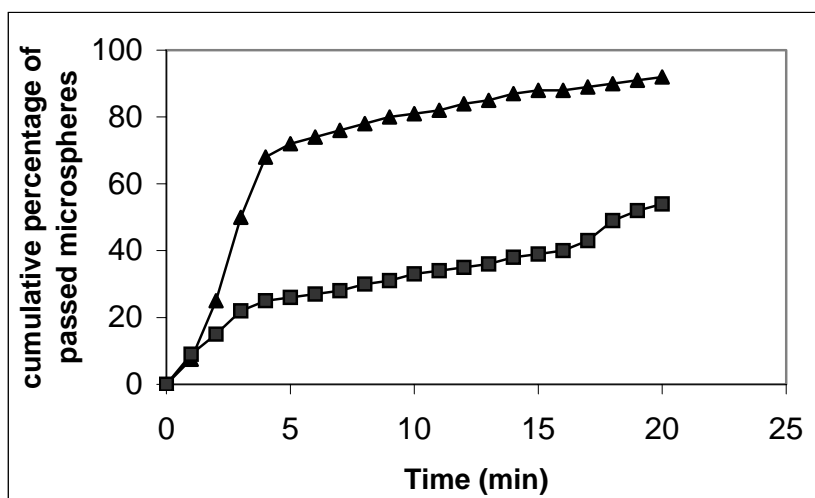


Fig 4. Transport of microspheres through the tube in the presence and absence of the magnetic field; Symbols (▲) without magnetic field, (■) with magnetic field

Magnetic targeting of the microspheres was developed to overcome the two major problems encountered in drug targeting, namely the reticuloendothelial and target site specificity. 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. Restriction of the microspheres at the micro vascular level can be achieved by taking advantage of the physiological differences between the linear flow velocity of blood in large arteries (approximately 30 cm/s) against that of the capillaries (0.5 cm/s). A greater field is necessary to retard the microspheres in faster moving arterial system as opposed to intra-capillary retention. [4]

In-vitro release

Fig. 5 shows the release profile of Diclofenac sodium from microspheres until 7 h after dispersion. In the first hour, the concentration of the drug released from the microspheres increased and reached a maximum. The initial burst release could be related to the surficial drug as well as small size of the microspheres with increased surface area. The maximum concentration of Diclofenac sodium released was 17.5 μ g/mL there by the maximum quantity of drug released after 7 h will be 30%.

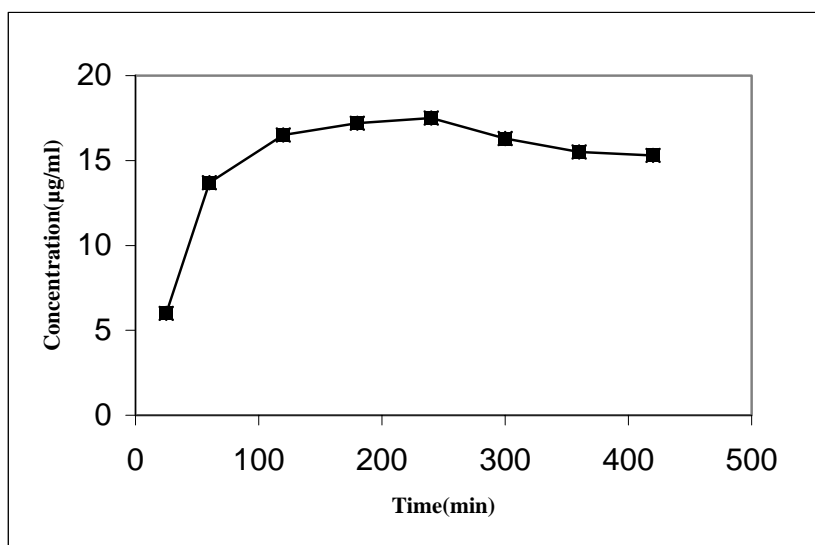


Fig 5. Release of Diclofenac sodium from magnetic albumin microspheres up to 7h after dispersion

The release of water-soluble drugs from albumin microspheres is usually characterized by an initial rapid release (burst effect), followed by a slower release of the remaining drug [14]. Diclofenac release from magnetic albumin microspheres also obeys this pattern.

Natsume et al suggested that the mitomycin C release from albumin microspheres with various diameters can be evaluated by a set of first order release kinetics. Drug diffusion rate from the spheres to the sink solution is very low in the albumin microspheres and thus the drug in the core and peripheral regions of the spheres release at the same rate. The results obtained also suggest that the initially rapid drug release could mainly be due to the smaller microspheres.

It was suggested by Okada et al [15] that the release kinetics is first-order, and the derived first-order release rate constant is a function of the following parameters: radius of core particle, radius of the microspheres, solubility of the core substance in the dissolution media, density of the core particle, and the permeability constant of the core substance in the swollen matrix.

The permeability constant varies depending on the preparation conditions, and the reason for variation is shown clearly to be the difference in the degree of swelling of the microsphere [16].

The volume within the beakers of the dissolution apparatus is constant and it is therefore predicted that the concentration of the released drug following the initial burst effect should be increasing at a constant rate. In contrary, the results obtained showed no increase in the Diclofenac sodium concentration within the beakers following the initial rapid release. This may be due to the slow release of the remaining drug in the microspheres, as well as numerous sampling through the experiment

Another factor, which affects the drug content within the microspheres, is the partition co-efficient of the drug. An increase in the partition coefficient causes a decrease in the

drug content within the microspheres prepared, due to the drug migration towards the outer organic phase, during preparation. The drug partition coefficient affects the release in such a way that drugs with higher partition coefficients are released faster (and to a greater extent) than those with lower partition coefficients [17]. The partition co-efficient of Diclofenac is very low and the slow release of it from the microspheres could be due to the high solubility of it in water.

Conclusion

In conclusion, Diclofenac sodium-loaded albumin magnetic microspheres were prepared by emulsification heat stabilization technique and the microspheres showed good spherical geometry as confirmed through SEM. DSC studies confirmed the absence of drug-polymer interaction and amorphous nature of entrapped drug in the microspheres. The results obtained from this study clearly suggest that magnetic albumin microspheres containing Diclofenac sodium are retained at the target site, in the presence of a 8000 G magnetic field, and are capable of releasing their drug for an extended period of time. Hence, it is predicted that these microspheres could be retained on the target tissue *in vivo* and release their drug for prolonged periods of time thus offering an alternative approach in achieving drug targeting. However, further studies need to be carried out to determine the effects of various factors such as the strength of the magnetic field, concentration and size of the magnetite in the microspheres, size and density of microspheres and experimental conditions.

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