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Preparation and *in vitro* evaluation of Diclofenac Sodium loaded Ethyl cellulose composite magnetic microspheres

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Abstract- In this study Diclofenac sodium-containing ethyl cellulose micro particles were prepared by the Emulsion-solvent evaporation method with a view for use in the application of magnetic carrier technology. The properties of these magnetic microspheres, such as morphological, magnetic susceptibility and polymer-drug interactions were characterized by different techniques (i.e. SEM, magnetometry and FT-IR). The loading efficiency and swelling kinetics magnetic microspheres were also studied. The formulated microspheres were below 5µm and spherical in nature as evidenced from SEM. FT-IR 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 57.46% to 81.44% 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 ethyl cellulose 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- Emulsion-Solvent evaporation technique, Ethyl cellulose, Diclofenac sodium, Magnetite, Electromagnet, Magnetic microspheres, Magnetometry, Magnetically modulated drug delivery systems.

Introduction

In recent years, polymeric controlled drug delivery systems have evolved as one of the most attractive areas in drug delivery and drug targeting [1-3]. The drug release is controlled by the properties of the polymer-drug systems and to some extent environmental factors such as pH, enzymes and inter-patient variance. Despite several advantages offered by controlled drug release, a major problem associated with all these systems so far developed give release rates that are either constant or decrease with time, but not augmented delivery on demand [4]. It can be achieved with the systems, which are associated with external or feed-back control such as magnetic control. Magnetically targeted drug delivery system (MT-DDS) will be a promising way, which involves binding a drug to a small biocompatible magnetically active component, entrapped in the biodegradable polymeric matrix and formulating in to a pharmacologically active stable formulation, which is injected into the blood stream and using a high-gradient magnetic field to pull them out of suspension in the target region [5]. Magnetic microspheres will be formulated with an intension to produce a depot near the target organ, by placing a suitable magnet near it. From the depot, drug will be released slowly & carried to the target organ through blood. By localizing the drug carrier near the target organ, unwanted distribution of drug to non target organ can be avoided. This approach will localize the drug only at target site & minimize the drug-induced toxicity [6-9]. Magnetism play an important role in different applications of health care, magnetically active component is composed of magnetite, which are well tolerated by the body. Magnetite (Fe_3O_4) is a common magnetic iron oxide, and it has a cubic inverse spinel structure with oxygen forming a FCC closed packing and Fe cations occupying the interstitial tetrahedral sites and octahedral sites [10-11]. The electrons can hop between Fe^{2+} and Fe^{3+} ions in the octahedral sites at room temperature, rendering magnetite an important part of half-metallic materials. Magnetite nanoparticles have been widely studied because of their applications in ultrahigh density magnetic storage media, biological labeling, tracking, imaging, detection, and separations, and ferrofluid [12-16]. Various chemistry-based processing routes have been developed to synthesize nanosized magnetite particles, including coprecipitation or precipitation, sol–gel method, emulsions technique, mechanochemical processing, hydrothermal preparation and DC thermal arc-plasma method [17-19]. 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 [2]. To overcome the toxicity produced by the Diclofenac sodium and to get prolonged therapeutic effect, in the present study, ethyl cellulose magnetic microspheres are formulated to target the drug at its site of action.

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The ethyl cellulose magnetic microspheres were prepared by solvent evaporation technique. The formulated microspheres were characterized by particle size distribution, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and evaluated for their in-vitro magnetic responsiveness and in-vitro drug release characteristics.

Materials and method

Ethyl cellulose (SD Fine chemicals, Mumbai), Diclofenac sodium (KAPL, Banglore), Neodymium magnet, 8000 G field strength (ABY systems, Chennai), Acetone (SD Fine chemicals, Mumbai), all other chemicals used was of analytical grade.

Experiment

3.1. Preparation of composite magnetic microspheres

It involves two steps; firstly preparation of magnetic fluid from ferric and ferrous salts by co-precipitation method. Secondly preparation of micro particles using biodegradable polymer and magnetite from first step and drug. Polymer is a means of encapsulating the drug in matrix and helps in controlled release while magnetite imparts magnetic properties to the formulated micro particles. The two steps in brief is given as follows:

3.1.1. Preparation of magnetic fluid

Magnetic fluid was synthesized as follows: a 35% (w/v) ferrous sulfate solution, 54% (w/v) ferric chloride solution and 36% (w/v) sodium hydroxide solution were prepared using distilled water. Then the ferric salt and ferrous salt were mixed, stirred and heated. When the temperature reached 55 1C, the alkaline solution was added. The mixture was stirred for 30 min, and then 5 g of polyethylene glycol-10000 (PEG-10000) was added. The temperature was raised to 80 1C and maintained for 30 min. The mixture was then neutralized while cooling, and the magnetic fluid was prepared.

3.1.2. Synthesis of magnetic composite microspheres

In a typical procedure, three batches of ethyl cellulose magnetic microspheres were prepared with 1:1, 1:2 and 1:3 drug to polymer ratios. The procedure for preparation of different batches was same, in brief, specified quantities of ethyl cellulose was dissolved in acetone and methanol solution mixture followed by addition of specified quantity of drug (100mg) in the polymer solution and shaken vigorously to form uniform drug polymer dispersion. To the above dispersion 50mg of magnetic fluid was added and dispersed which imparts magnetic properties to the prepared microspheres. The dispersion was poured in to 100ml of light liquid paraffin containing 0.5ml of Tween 80 and stirred for 4hours at 400 RPM at room temperature. After 4 hours, acetone was completely removed by evaporation. The light liquid paraffin was decanted at the end of this period and the magnetic microspheres were collected using a magnet and washed consecutively with petroleum ether and n-Hexane and stored in a cool place till further use.

3.2. Characterization:

The particle size of the microspheres prepared was determined by using a particle size analyzer (Hiac/Royco, 4100-Pacific Scientist) to determine the average particle size suitable for administration through i.v. The SEM analysis of the microspheres was carried out by using Jeol JSM 5300, Japan, to determine size, shape and surface morphology of the prepared magnetic microspheres. FT-IR of Diclofenac sodium, magnetite and microspheres were performed using KBr pellet method using Fourier-transform infrared (FT-IR) spectrometer to determine the possible drug-polymer interaction and the physical state of the drug in the microspheres.

3.3. Evaluation

3.3.1. Magnetic responsively of the drug-loaded magnetic microspheres [1]

The apparatus shown in Fig. 1 was designed for this study.

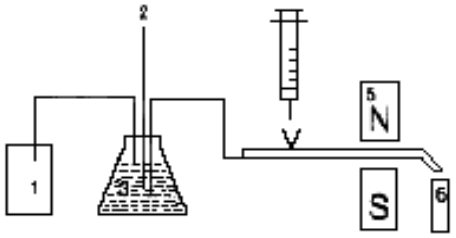

Fig. 1 - Pump, 2 – Thermometer, 3 – Normal saline, 4 – Injection site, 5 – Magnet, 6 – collecting sample

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℃). 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 were 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.

3.4. In-vitro drug release from magnetic ethyl cellulose microspheres

Drug loaded microspheres containing 30mg equivalent weight of the drug was taken and dispersed in 900 mL of pH1.2 phosphate buffer solution for the first two hours followed by transferring them in to pH 7.4 buffer solution for the next 6 hours in beaker of the USP dissolution apparatus 2. The temperature was kept at 37 + 0.5°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 8 h and 5 mL samples were removed from the test beakers at regular intervals up to 8 h. 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 its blank. Similar procedure was followed for all the formulations in which the drug to polymer ratio (1:1, 1:2, 1:3) was varied keeping the amount of drug, magnetite and other additives constant in all the cases and increasing the amount of polymer used

Results and discussion

The prepared ethyl cellulose magnetic microspheres loaded with Diclofenac sodium were characterized for particle size, SEM, FT-IR 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 8 h using the USP dissolution apparatus. The size distribution of ethyl cellulose microspheres {Fig. (1)} was between 0.4 and 5 µm. The average particle size of these microspheres was found to be 2.4 µm. The size and shape of ethyl cellulose magnetic microspheres were further studied by SEM. As shown in Fig. (2), the formulated microspheres were spherical and compact in nature. The particle size of the formulated ethyl cellulose microspheres was less than 5µm as evidenced by the SEM photograph. FT-IR of pure drug and formulation were carried out, the prominent peaks present in the pure drug and the formulation were similar indicating there was no significant interaction between polymer and the drug as evident from the peaks of specific functional groups present in both the pure form of the drug and the formulation respectively.

4.1. Magnetic responsively

In fig. (3), 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 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. 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

4.2. In-vitro release

Fig. (4) shows the release profile of Diclofenac sodium from microspheres (1:1, 1:2, 1:3) until 8 h after dispersion. As evident from the graph, the curve of dissolution release profile indicates that, with the increase in the polymer ratio release of Diclofenac sodium from the microspheres decreases. In the third hour i.e. in the alkaline medium, the concentration of the drug released from the microspheres increased and reached a maximum. The initial burst release could be related to the surface drug as well as small size of the microspheres with increased surface area. The maximum concentration of Diclofenac sodium released was 17.5µg/mL there by the maximum quantity of drug released after 7 h will be 72.4%.

Conclusion

In conclusion, Diclofenac sodium-loaded ethyl cellulose magnetic microspheres were prepared by solvent evaporation technique and the microspheres showed good spherical geometry as confirmed through SEM. FT-IR 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 ethyl cellulose 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.

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However, further studies need to be carried out to determine the 13effects 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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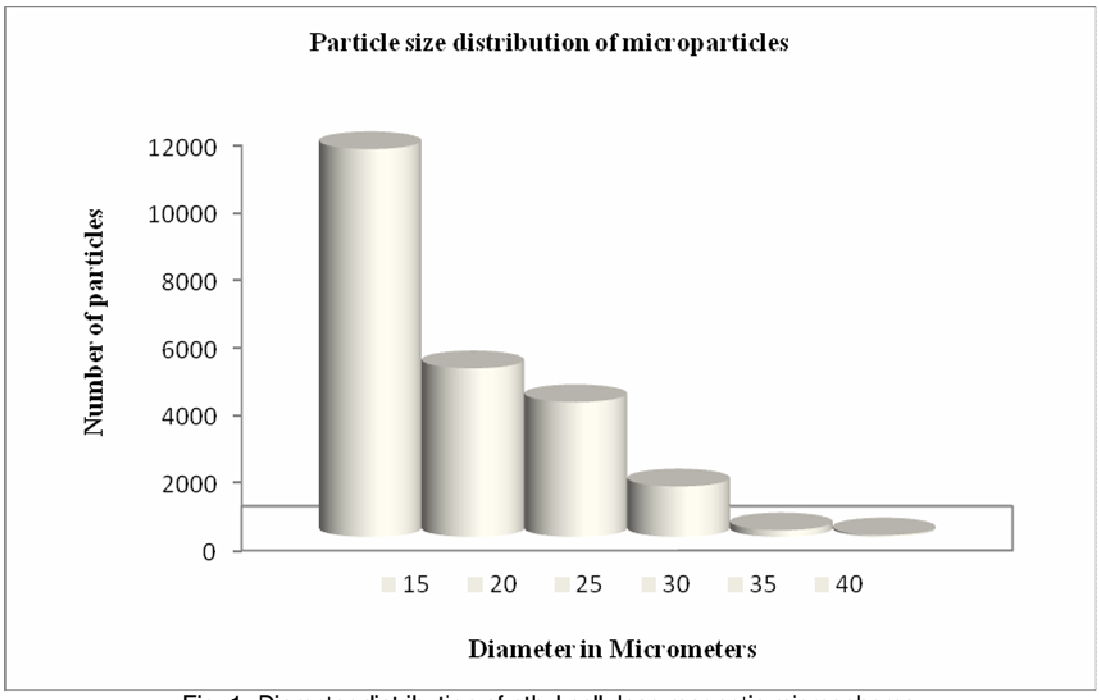

Fig. 1- Diameter distribution of ethyl cellulose magnetic microspheres

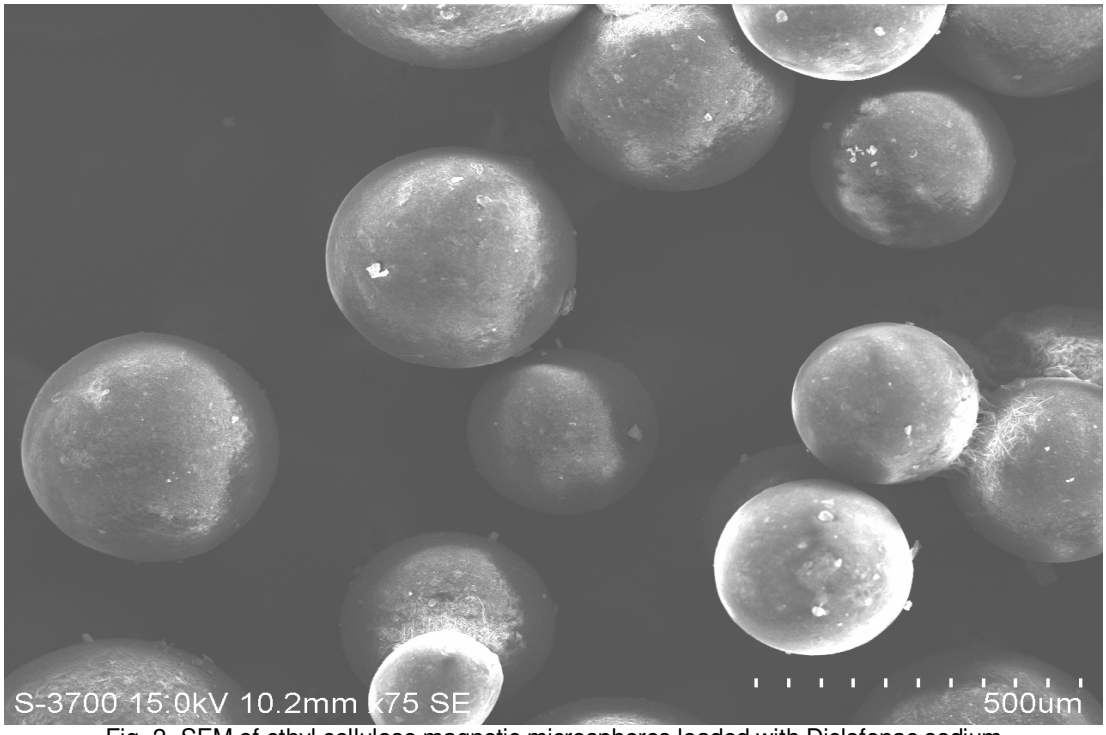

Fig. 2- SEM of ethyl cellulose magnetic microspheres loaded with Diclofenac sodium

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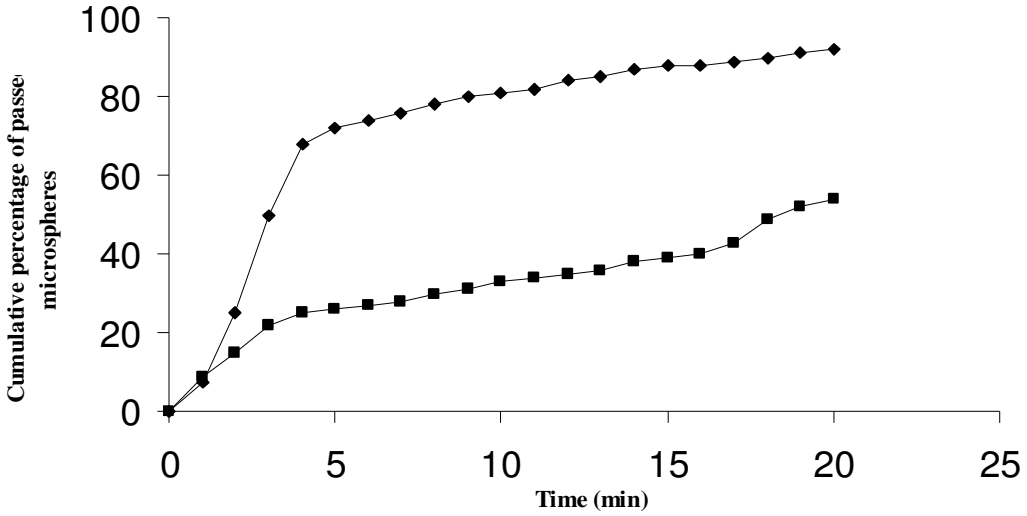

Fig. 3- Transport of microspheres through the tube in the presence and absence of the magnetic field; Symbols (□) without magnetic field, (■) with magnetic field

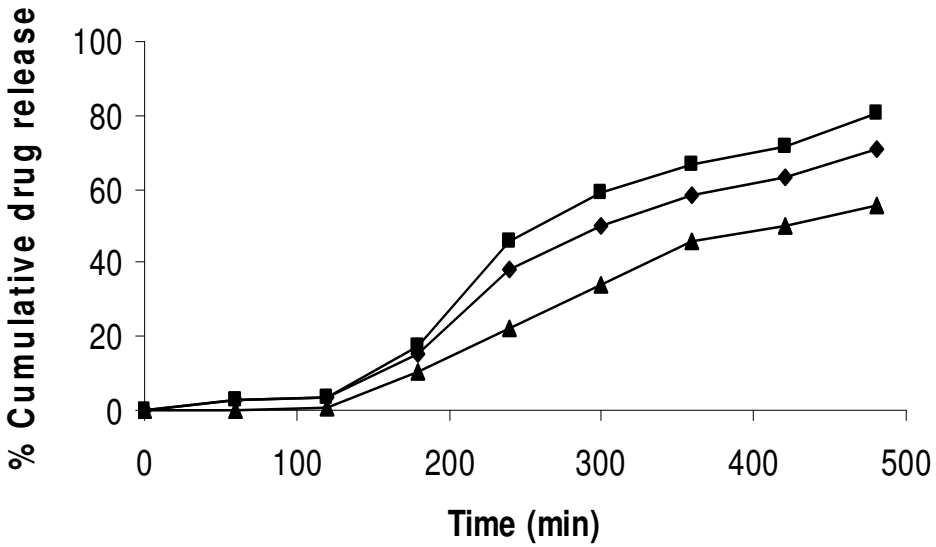

Fig. 4- Release of Diclofenac sodium from magnetic ethyl cellulose microspheres up to 7h after dispersion (■) 1:1, (♦) 1:2, (▲) of drug to polymer ratio.