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Synthesis and Characterization of Magnetic Methyl Methacrylate Microspheres Loaded with Indomethacin by Emulsion Solvent Evaporation Technique

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Abstract

Magnetic nanoparticles encapsulated in Methyl methacrylate (Eudragit L-100) microspheres containing Indomethacin drug were prepared and their detailed structural and magnetic characteristics were studied. Iron oxide nanoparticles were obtained by chemical coprecipitation of Fe(II) and Fe(III) salts and stabilized with tetra-methyl ammonium hydroxide. Microspheres were prepared by solvent evaporation technique. We characterized the magnetic microspheres in terms of morphology, composite microstructure, size and size distribution, magnetic properties and *in-vitro* release patterns. The microspheres were uniform both in shape and usually also in size; their size distribution was narrow. All the magnetic parameters confirm superparamagnetic nature of the microspheres with magnetizations up to 20–30 emu/g of microspheres. The *in-vitro* release profile was studied in pH 7.4 phosphate buffer medium up to 8 hours using USP XXII dissolution apparatus. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 60-85% of the total drug content from the microspheres within 8 hours. From this study, it could be suggested that magnetic Methyl methacrylate microspheres could be retained at their target site in-vivo and such microspheres can be used in biomedical applications and research areas such as target drug delivery, selective blood detoxification, tissue engineering and replacement, and magnetic resonance imaging (MRI) contrast agents.

Keywords: Methyl methacrylate, Magnetite, Indomethacin, single emulsion solvent evaporation Technique, Chemical co-precipitation technique.

Introduction

In recent years, polymeric controlled drug delivery systems have evolved as one of the most attractive areas in drug delivery and drug targeting. 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 [1-2].

Despite several advantages offered by controlled drug release, a major problem associated with all

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these systems so far developed give release rates that are either constant or decrease with time, but not augmented delivery on demand[3]. It can be achieved with the systems, which are associated with external or feed back control such as magnetic control[4]. 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[5-7]. 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[8-10]. 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[11].

Recently, biodegradable magnetic carriers have attracted increasing interest in biomedical and clinical research[12-14]. For example, magnetic resonance imaging (MRI) contrasts agents[15]. The success of these technologies depends largely on the synthesis of magnetic spheres from biocompatible, non-toxic polymers such as Methyl methacrylate (Eudragit L-100) and ethyl cellulose[16-19]. For applications requiring *invivo* magnetic targeting, for example, magnetic drug delivery, the magnetic carriers must have a proper size range (i.e. between 200 nm and 3 mm) and high magnetizations to enable technically feasible external magnetic guidance within the vasculature. In these applications the microspheres (i.e. 1–2 mm) would be more advantageous than nanospheres in terms of better targeting and easier capture [20-24]. To do this, one of the main challenges has been the fabrication of magnetic spheres with high magnetizations, good drug encapsulation efficiency and high drug payload [25-26]. This generally requires that the magnetic carriers are biodegradable, smaller than red blood cells (i.e. 7–8 mm), have a proper size range, and contain high concentrations of the magnetic material. However, the hydrophilic surface properties of magnetite compounds make it challenging to attain high magnetite content in hydrophobic biodegradable polymers such as Eudragit (L-100) [27]. The usual method of encapsulating hydrophilic magnetite can be done by single emulsion solvent evaporation protocol to prepare biodegradable/biocompatible magnetic microspheres that are 1–2 mm in mean diameter and contain a large concentration of magnetic material by encapsulating a hydrophobic magnetite material [28]. Magnetic carrier technology would benefit from this simplified procedure and can be utilized in biomedical applications and research areas such as target drug delivery, selective blood detoxification, tissue engineering and replacement, and magnetic resonance imaging (MRI) contrast agents [29-30].

Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID) that reduces fever, pain, and inflammation [12]. IND was selected mainly because of its side effects such as stomach irritation or intestinal bleeding or ulcers and can increase blood pressure and decrease kidney function, it is important to decrease used dosages and side effects during the treatment. One of the possibilities to do it is direct delivery of the drug to the target area of the body by external magnetic field.

The aim of the present study was to prepare and characterize Eudragit magnetic microspheres loaded with Indomethacin. This study illustrates preparation of size controlled magnetic microspheres with high magnetization of the prepared microspheres by emulsion solvent evaporation technique. The prepared microspheres were then studied for their morphology, magnetic property, and drug release characteristics.

Materials and Method

Materials

Methyl methacrylate (Eudragit L-100) (Sigma-aldrich, USA), Ferrous sulphate and Ferric

chloride (Hi-Pure, Fine chem. Industries, Chennai), Indomethacin (Nu Therapeutics Pvt Ltd, Hyderabad), Tetra-methyl Ammonium hydroxide (SD fine chemicals), all other chemicals and solvents used are of analytical grade.

Methods

Preparation of magnetite nano particles

Magnetite nano particles were prepared by chemical co-precipitation method which in involves co-precipitation of ferrous and ferric forms in 1:2 ratio in alkaline medium. In brief, 6N NaOH was added to 100ml of a solution containing iron (Fe) in the ratio of 1:2 (Fe2+: Fe3+). The resulting mixture was allowed to digest for 30min at 60^{o}C to give colloidal magnetite suspension. 6g of oleic acid was then mixed with 9.6ml of 3N NaOH solution and 50ml of distilled water, the mixture was heated to 60^{o}C. Following the dissolution of sodium oleate, the solution was added to the magnetite suspension and stirred for 30min at 90^{o}C. After cooling, the pH was slightly acidified with 1N HCl solution, resulting in the flocculated suspension which was filtered and stored until use [3].

Preparation of eudragit magnetic microspheres

Microspheres were prepared by solvent evaporation technique with necessary modifications. Accurately weighed but varying amounts of Eudragit E-100 was dissolved in 10 ml of acetone over a cyclo-mixer, and accurately weighed drug was added in the polymer solution. 50 mg of magnesium stearate was then added to the solution of polymer and drug in acetone. Finally specified amount of magnetite was added to the drug-polymer solution. The organic phase was poured drop-wise to 25 ml of 1:1 mixture of light and heavy liquid paraffin with vigorous stirring over a mechanical stirrer. High stirring rates of approximately 4,000 rpm were employed to obtain microspheres of smaller size. Stirring was continued for eight hours. 20 ml of hexane was added to the stirred contents. The batch was filtered and washed thrice with hexane, 10 ml each, to remove any adhering liquid paraffin from the surface of microspheres. Then, several washings with distilled water were given to remove any un-entrapped drug on the surface of the microspheres. Several batches of microspheres were prepared by varying drug-polymer ratio, keeping all other formulation factors constant [11].

Characterization

Scanning Electron Microscopy

The magnetic microspheres characterized in terms of morphology, composite microstructure, size and size distribution 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 and were scanned at an accelerating voltage of 15kV before observation.

Magnetic properties of the microspheres

A vibrating sample magnetometer (VSM, Lake Shore Cryotronics Inc., OH) was employed to measure the magnetizations of starting magnetite and microspheres at room temperature. The samples were dried in a vacuum overnight by lypholizer. Several parameters like, saturated induced magnetization, magnetic susceptibility and hysteresis loops were measured. All the quantities were measured in the same sample.

X-ray diffraction studies

The physical nature of the drug entrapped in the magnetic microspheres was further confirmed by diffraction patterns obtained from X-ray diffraction studies using X-ray diffractometer (XD-D1, Shimadzu, Japan), in the range 5–70◦ of 2θ. The working conditions were CuK radiation, 30 kV, 20mA and with a slit of 1.1–0.3 mm.

In vitro release studies

Drug release tests were performed according to USP XXIV paddle method for each size fraction separately. Accurately weighed amounts (100mg) of microspheres were introduced into 900ml of PBS (phosphate buffer saline, pH 7.4) and stirred

with 100 rpm at 37 ± 0.5°C. Five milliliters samples were withdrawn and filtered at selected time intervals. The concentration of Indomethacin was determined spectrophotometrically at 318 nm [2].

Result and Discussion

The magnetic microspheres were spherical in geometry [Figure 1]. The surface was primarily smooth, although some roughness could be identified in certain areas of some spheres. Magnetite appears to be encapsulated heterogeneously within the microspheres. All samples are in the size range between 300 and 2.5 mm in diameter with a broad size distribution. The poly-dispersity index (PI) of 0.2, indicating a particle size distribution (PSD) characteristic of a sample with broad size range, is in agreement with microscopy.

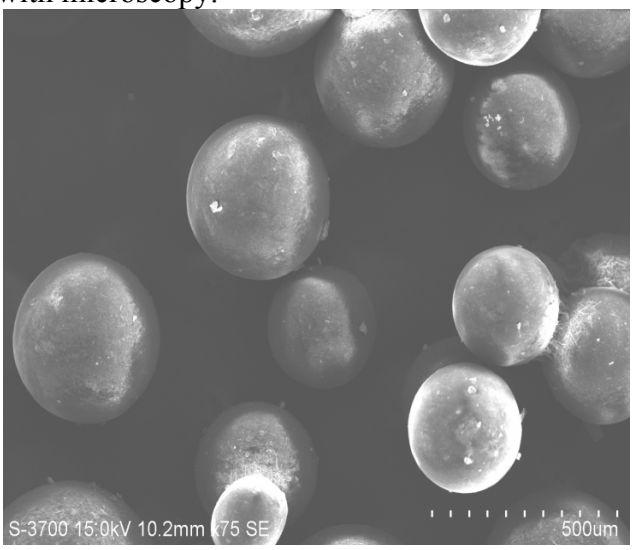

Figure 1: SEM of Eudragit (L-100) magnetic microspheres loaded with Indomethacin

Saturation magnetization (Ms) of Eudragit microspheres was determined from the hysteresis loops after subtraction of paramagnetic component using linear interpolation in the upper 30% of the field range [Figure 2]. This value (determined from the loops measured at room temperature) can be used to approximate the content of iron oxides in the microspheres (as of Fe3O4). The values of the induced magnetization measured at 7 T and 5K and their ratios to those obtained at 300K were in the range of 23.8 for smaller microspheres and 13.5 for larger microspheres. This ratio can be interpreted in terms of (super) paramagnetic contribution due to the energy of thermal vibrations. It is clear that while smaller microspheres show high (super) paramagnetic contributions, the largest microspheres exhibit the lowest magnetization ratios.

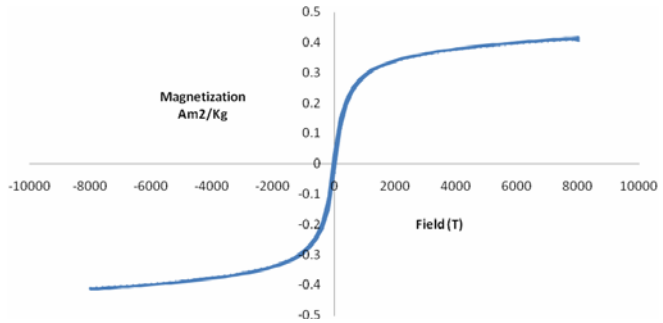

Figure 2: Vibrating sample magnetometer (VSM) of synthesized magnetic composite microspheres measured at 300 K.

Magnetic susceptibility (both in-phase and out-of-phase AC components) were measured by applying the AC field 4Oe (320 A/m) at frequencies 0.1, 1, 10, 100, 500 and 1000 Hz. Typical superparamagnetic behavior is evident from the hyperbolic decay of susceptibility from the curve, following the Curie–Weiss paramagnetic law. Microsphere show typical superparamagnetic pattern of the in-phase FDS, with maximum around 75–80 K, followed by hyperbolic decay. Therefore, despite the absence of blocking temperature in the thermal demagnetization of FC and ZFC remanences, this observation can be considered as an indicator of the transition from true superparamagnetic behavior to ferrimagnetic one at lower temperatures. However, interpretation of these features requires further study.

The X-ray diffraction patterns of the magnetic nanoparticles and magnetic microspheres are shown in [Figure 3]. All XRD peaks could be attributed to the characteristic peaks of iron oxide spinel structure (Fe3O4). The peak broadening (311) indicated that the average crystallite size of the fabricated particles was only a few nanometers. Characteristic peaks of magnetite in magnetic microspheres confirmed the presence of magnetite particles in the prepared microspheres. It can be observed that the X-ray diffraction patterns of drug containing microspheres showed sharp peaks due to crystalline nature of the drug.

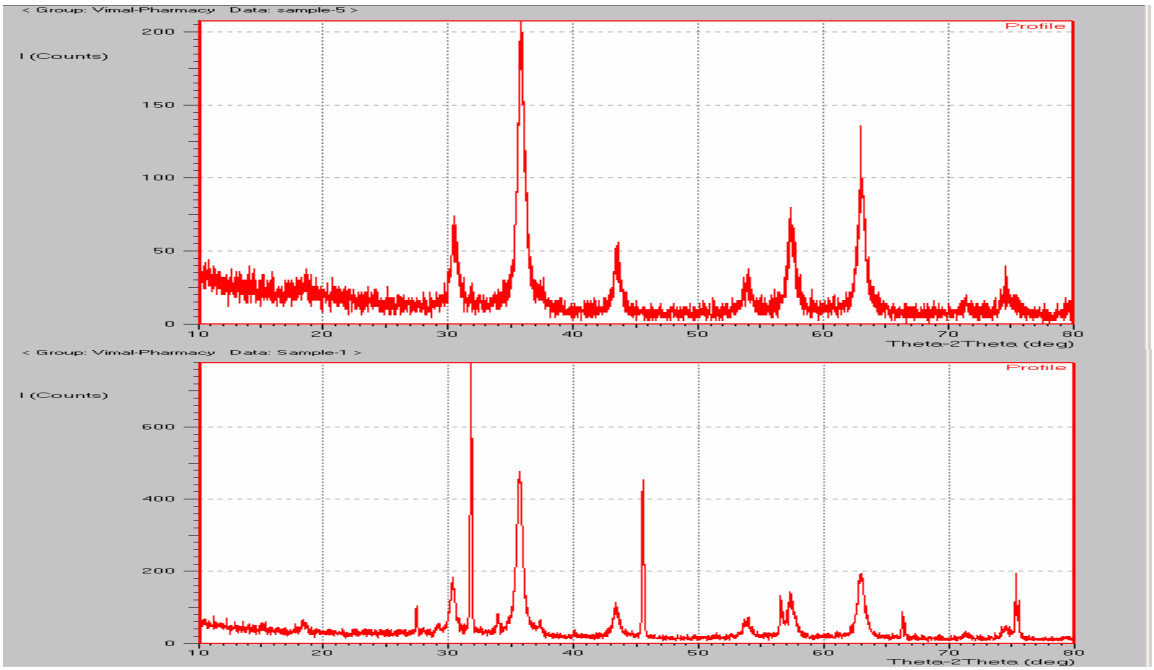

Figure 3: X-ay diffraction patterns of magnetite nano-particle and formulation of Eudragit magnetic microspheres containing magnetite.

[Figure 4] shows the release profile of Indomethacin from magnetic Eudragit microspheres (1:1, 1:2, 1:3) until 8 h after dispersion in pH 7.4 buffer solution. As evident from the graph, the curve of dissolution release profile indicates that, with the increase in the polymer ratio release of Indomethacin from the microspheres decreases. The concentration of the drug released from the microspheres increased and reached a maximum as the time proceeds until 6hrs and attains a steady state for the next two hours. The initial burst release could be

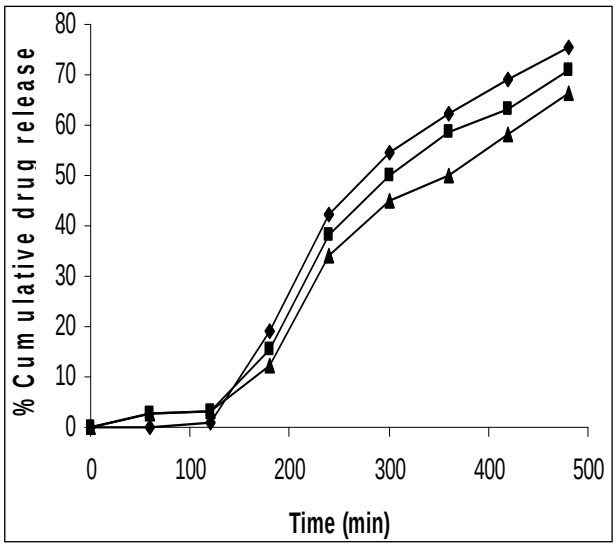

Figure 4: Release of Indomethacin from magnetic Eudragit microspheres up to 8h after dispersion (■) 1:1, (♦) 1:2, (▲) 1:3 of drug to polymer ratio

related to the surfacial drug as well as small size of the microspheres with increased surface area. The maximum concentration of Indomethacin released was 23.6µg/ml there by the maximum quantity of drug released after 8 h will be 82.4%.

Conclusion

Magnetic nanoparticles encapsulated in Methyl methacrylate (Eudragit L-100) microspheres containing Indomethacin drug were prepared and their detailed structural and magnetic characteristics were studied. Iron oxide nanoparticles were obtained by chemical coprecipitation method. Microspheres were prepared by solvent evaporation technique and were characterized in terms of morphology, composite microstructure, size and size distribution using SEM, magnetic properties by VSM and in-vitro release patterns. All the magnetic parameters confirm superparamagnetic nature of the microspheres with magnetizations up to 20–30 emu/g of microspheres. Characteristic sharp peaks of magnetite in magnetic microspheres confirmed the presence of magnetite particles in the prepared microspheres and the drug was in crystalline form. The in-vitro release profile was studied in pH 7.4 phosphate buffer medium up to 8 hours using USP XXII dissolution apparatus. Drug release in the first hour was found to increase and reached a maximum, releasing approximately 60-85% of the total drug content from the microspheres within 8 hours. From this study, it could be suggested that magnetic Eudragit microspheres could be prepared using simple technique and such microspheres can be used in biomedical applications and research areas such as target drug delivery, selective blood detoxification, tissue engineering and replacement, and magnetic resonance imaging contrast agents.

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