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A Novel Vehicle for Entrapment of Drugs: Doxorubicin-loaded bilayer-surface magnetite nanoparticle

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Abstract. In the past few decades, Magnetite (Fe_3O_4) nanoparticles have attracted growing research interest that this material has many applications in medicine and drug delivery, Coated magnetic particles, called carriers are very useful for delivering chemotherapeutic drugs. We are herein reporting a synthesis of doxorubicin-loaded bilayer-surface magnetite nanoparticles. The particles were first stabilized with Stearic acid as a primary surfactant, followed by Maleic anhydride-methyl acrylate (MAN-MA) copolymer as a secondary surfactant to form nanoparticles with hydrophobic inner shell and hydrophilic corona. Then anticancer drug doxorubicin (DOX) was selected as a model Drug, That loaded at modified magnetic nanoparticles. The structural, morphological and magnetic properties of as-prepared sample were characterization by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectra, scanning electron microscopy/energy dispersive x-ray analysis (SEM-EDAX) and magnetic measurements were investigated using vibrating sample magnetometer (VSM). The particles were exhibited super paramagnetic behavior at room temperature with saturation magnetization (Ms) about 50 emu/g magnetite. We demonstrate that the drug DOX is attached to the nanoparticles surface, that the binding of DOX to the nanoparticles was confirmed by FT-IR analysis. The present finding show that DOX loaded nanoparticles coated by copolymer are promising for magnetically targeted drug delivery.

Key words: Doxorubicin, loaded bilayer, magnetite nanoparticle, novel vehicle, entrapment of drugs

1. INTRODUCTION

Magnetic nanoparticles are a class of nanoparticle which can be manipulated using magnetic field. Such particles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. Water dispersible magnetic nanoparticles coated with water soluble polymeric surfactants offer intriguing new opportunities for various biomedical applications such as magnetic resonance imaging (MRI) contrast enhancing agents [1-5], magnetic field guided drug delivery [6, 7], hyperthermia treatment of tumors [8] and bimolecular magnetic separation and diagnosis [9]. Especially in drug delivery applications, drug-loaded magnetic nanoparticles have been widely studied in an attempt to obtain the particles with high drug loading capacity, good stability in aqueous solutions, good biocompatibility with cells and tissue, desired releasing profile and retention of magnetic properties after modification with polymeric stabilizers [10-14]. Amongst these studies, therapeutic drugs were either physically adsorbed, chemically conjugated or ionically bound to the polymeric stabilizers [15]. Too low drug loading capacity is the major problem for these approaches. Formation of degradable microspheres loaded magnetic nanoparticles and therapeutic drugs has also been widely studied for uses in these applications [16-18]. Nonetheless, the magnetic response of the carriers is so low that it could not be effectively localized to a target area due to only a few percent of magnetic nanoparticles entrapped in the microspheres. Many attempts have recently been made on preparing core/shell magnetite nanoparticles possessing polymer-coated surfaces [19–23]. Riffle et al. have recently reported the synthesis of poly- (lactide-b-siloxane-b-lactide) triblock copolymers as magnetite nanoparticle stabilizers [24].

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While nanoparticles are smaller than 1 micrometer in diameter (typically 5-500 nanometers), the larger micro beads are 0.5-500 micrometer in diameter. In the current work, We are herein reporting a synthesis of doxorubicin-loaded bilayer-surface magnetite nanoparticles.

2. MATERIALS AND METHODS

All the reagents were of analytical grade and used as received without further purification, including $FeCl_2.4H_2O$, $FeCl_3.6H_2O$, ethanol, NaOH, ammonia solution (25%), stearic acid, chloroform. and Doxorubicin hydrochloride (DOX). Deionized water was used as a solvent. Methyl acrylate (MA) was obtained from Merck and distilled under reduced pressure to remove inhibitors before use. Maleic anhydride (MAN; Merck) was recrystallized from chloroform. The radical initiator of α,α-azobis (isobutyronitryle) (AIBN; Fluka) and recrystallized from methanol.

2.1. Synthesis of the poly(MAN-co-MA)

Poly(MAN-co-MA) (III) has been synthesized as general method using 1.00 g (10 mmol) of MMA and MA or 1.00 g (10 mmol) and 0.325 g (0.2 mmol) of AIBN dissolved in 15 ml of ethyl acetate. The reaction mixture was heated to 70±1 °C, with constant stirring and under a nitrogen atmosphere. The reaction conditions were maintained for 48 h. copolymer synthesized was purified by precipitation and subsequent re-precipitation in cooled methanol; after wards it was dried till constant weight at room temperature under reduced pressure.

2.2. Preparation of the copolymer-stabilized aqueous-based magnetite nanoparticles

The stable Fe_3O_4 ferro fluid was synthesized using the method of co-precipitation. $FeCl_3$ solution (1.66 g in 20 ml deionized water) and $FeCl_2.4H2O$ solution (1.00 g in 20 ml deionized water) were mixed together with stirring, followed by addition of 25% NH_4OH (20 ml). stearic acid solution in chloroform ($CHCl_3$) (2 g in 10 ml chloroform) was then introduced into the magnetite dispersion. Dispersion was continuously stirred for another 30 min to complete the reaction. It was then centrifuged at 5000 rpm for 20 min to precipitate large aggregate and the aqueous supernatant was discarded.

$$2FeCl_3.6\,H_2O + FeCl_2.4H_2O + 8NH_3 \rightarrow Fe_3O_4 + 8NH_4Cl + 6H_2O$$

To prepare the copolymer-stabilized nanoparticles, 20 ml of the dispersion was introduced into the copolymer solutions in deionized water (20 ml). The mixture was then sonicated for 4 h, followed by centrifugation at 3000 rpm for 20 min to remove large aggregate. The aqueous dispersion was dialyzed against deionized water for 24 h and refreshed twice to remove the excess copolymer in the dispersion.

2.3. Doxorubicin (DOX) drug loading

The water-soluble anti-cancer drug DOX was chosen as a model drug. The DOX loading was carried out by dispersing 5 mg of core/shell Magnetite nanoparticles in 5 mL aqueous DOX solution (drug concentration=0.1 mg/mL) following the experimental procedure described by Kuznetsov et al [10]. The mixture of silica coated Magnetite nanoparticles in DOX was shaken in a rotary shaker (200 rpm) at 37 °C for 26 hours to facilitate DOX uptake (Figure 1).

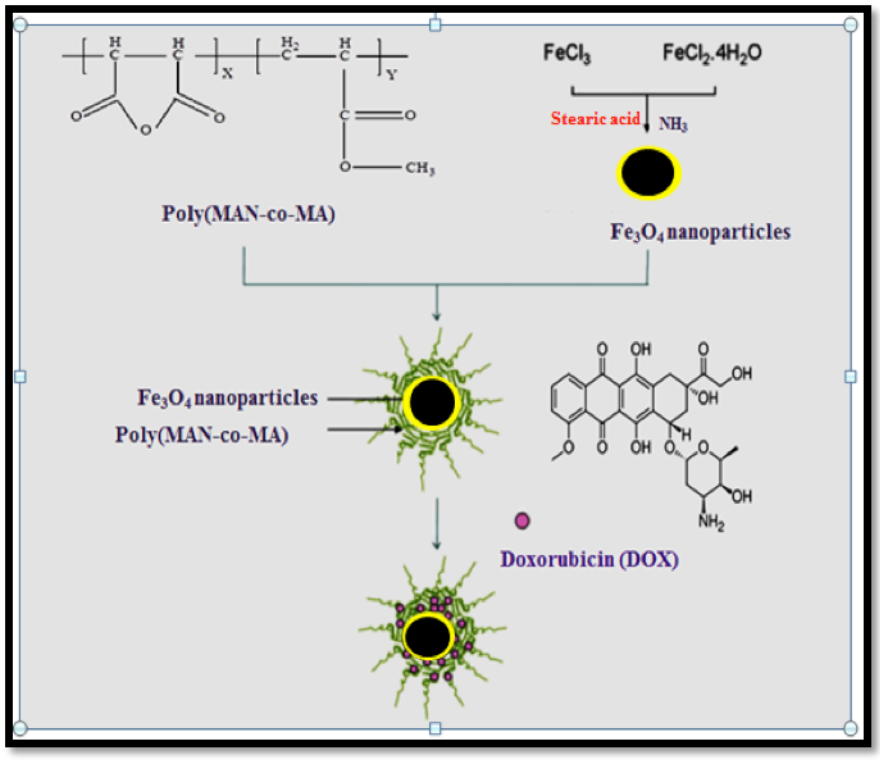


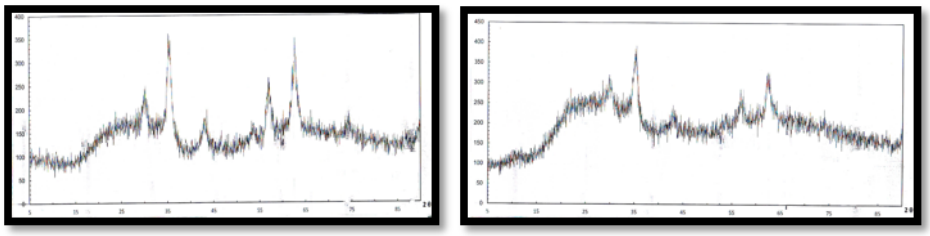
Figure 1. Proposed entrapment mechanism of indomethacin in the hydrophobic inner-layer surfaces of the copolymer-stabilized magnetite nanoparticles.

3. Results and Discussion)

The powder XRD was performed at room temperature with a PW 1800 (PHILIPS) X-ray diffractometer equipped with a Cu Ka radiation source (k = 0.154056 nm). The lattice constant and the average crystallite size were determined by the positions and full width at half maximum (FWHM) of the (311) peaks by using the Scherrer formula. The element analysis was carried out by an energy dispersive X-ray analysis (EDAX) on a Field emission scanning electron microscopy (FESEM, Hitachi F4160 oxford instrument). The Fourier transform infrared spectra (FT-IR) (NICOLET 5700) were also measured. The magnetic measurements were performed by using a vibrating sample magnetometer (VSM) on a physical property measure system (VSM Lecshore). The DOX concentration was measured by a UV–VIS (Shimadzu UV 1700) spectrophotometer equipped with quartz 1 cm optical length cuvettes (Hellma).

3.1 X-Ray Diffraction (XRD)

The structure of the Fe_3O_4 nanoparticles was investigated by X-ray diffraction. Figure 2 shows the x-ray diffraction patterns of the as-prepared Fe_3O_4 and Fe_3O_4/co-polymer nanoparticles. All peaks can be seen with the standard pattern XRD (JCPDS card No. 86-2267) and crystal nanoparticles corresponded to confirm. X-ray diffraction pattern of the nanoparticles coated with co-polymer, a broad peak at 18-22° can be observed. Particles size using Debye Scherrer formula was calculated as follows.

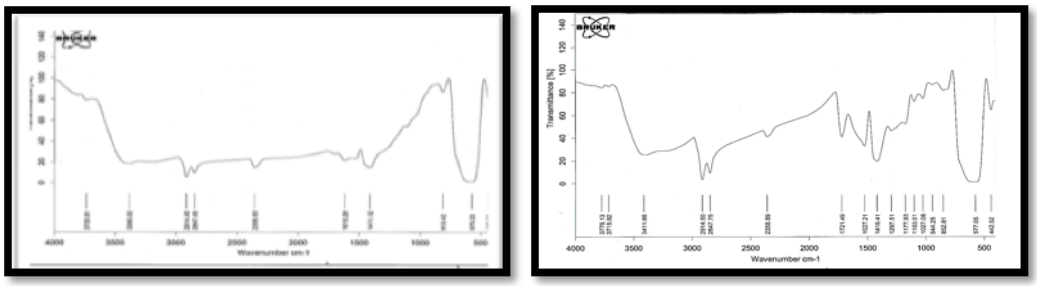


a **b**

Figure 2. X-ray diffraction patterns for (a) Fe_3O_4 and (b) Fe_3O_4/co-polymer nanoparticles.

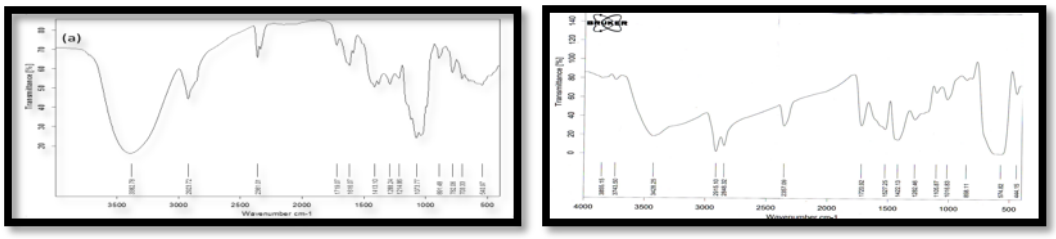
3.2. FT-IR Spectra

Figure 3 shows the FT-IR spectra of Fe_3O_4 (a) and Fe_3O_4/co-polymer (b) nanoparticles. In the spectra, the strong peaks near 582 cm^{-1} and 465 cm^{-1} are associated with the stretching vibrations of the Fe-O bonds; which represent the presence of magnetic NPs. The peaks around 3000-3500 cm^{-1} and 1624 cm^{-1} are related to the stretching and bending vibrations of the H–O–H bond, respectively, showing the physical absorption of H_2O molecules on the surfaces. As shown in Fig. 3(b), the characteristic peaks of spinel Fe_3O_4 appear in the infrared spectrum of Fe_3O_4/co-polymer nanoparticles. The band at 1712 cm^{-1} can be attributed to the C=O anhydride vibration and the band at 1097 and 1022 cm^{-1} can be attributed to the C-O-C asymmetric stretching vibrations; these vibrations are expected to have strong IR absorption consistent with how they appear in the spectrum. The weak band at 680 and 740 cm^{-1} is due to C–H. Figure 4 shows the FTIR spectra of pure DOX and doxorubicin-loaded bilayer-surface magnetite nanoparticles. This FTIR result can be interpreted that the DOX entrapment of the copolymer micelles.



a **b**

Figure 3. FT-IR spectra of (a) Fe_3O_4 and (b) Fe_3O_4/co-polymer nanoparticles



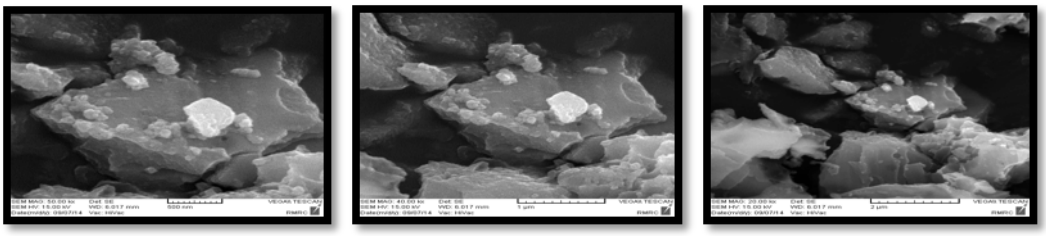
a **b**

Figure 4. FTIR spectra of pure (a) DOX and (b) doxorubicin-loaded bilayer-surface magnetite nanoparticles.

3.3. SEM and EDAX

The surface morphology of the magnetic nanoparticles have been studied by Scanning Electron Microscopy (SEM) method. Figure 5 (a,b) represents SEM images of Fe_3O_4 and Fe_3O_4/co-polymer core-shell nanoparticles. The x-ray energy dispersive data (EDAX) of these two materials are given in Figure 6 (a,b). the elemental analysis shows the presence of Fe, O (see Figure 6.a) indicates the formation of Fe_3O_4. Also elemental analysis shows the presence of Fe, O, C (see Figure 6.b) indicates the formation of Fe_3O_4/co-polymer nanoparticles.

(a)



(b)

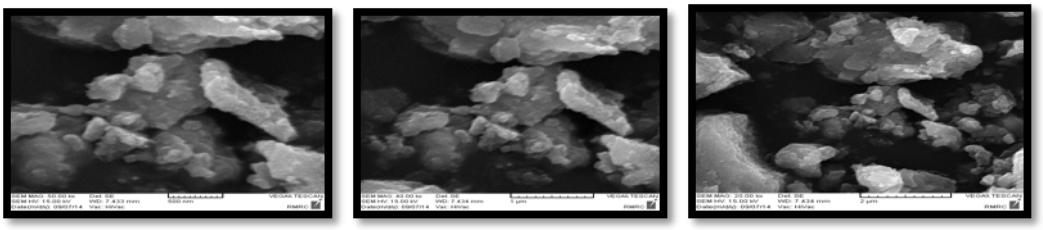
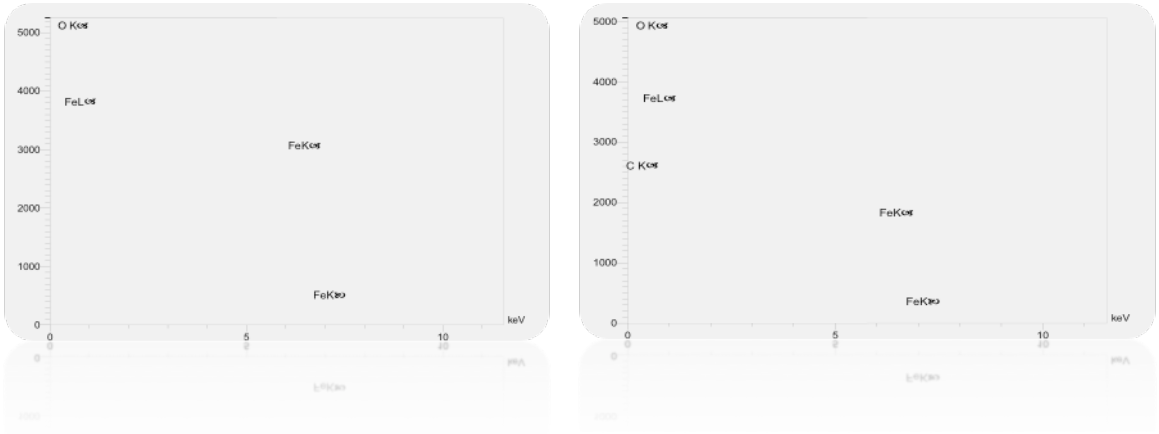


Figure 5. SEM images (a) Fe_3O_4 and (b) Fe_3O_4/co-polymer nanoparticles.



a **b**

Figure 6. EDAX analysis shows presence of Fe, O, C (a) Fe_3O_4 and (b) Fe_3O_4/co-polymer nanoparticles.

3.4. Magnetic properties of nanoparticles

Vibrating Sample Magnetometer (VSM) curve in terms of the (Figure 7) is shown. The absence of remanence in the hysteresis curves indicates that magnetic particles are super paramagnetic. The magnetic parameters such as saturation magnetization (Ms) and coercivity (Hc) of Fe_3O_4 determined by hysteresis loops, clearly decrease upon coating. Core/shell ferrite nanoparticles show lower magnetization saturation than the uncoated ferrite nanoparticles; this is due to the effect of co-polymer shell coating where each particle was separated from its neighbors by the shell layer leading to decreased magnetostatic coupling between

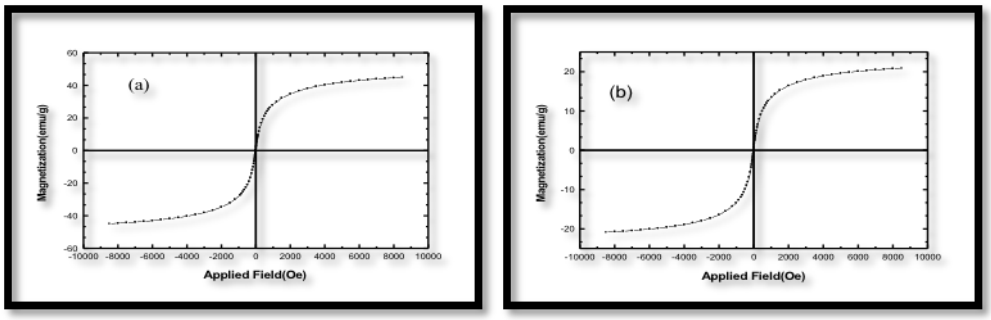


Figure 7. Hysteresis loops of the as-prepared (a) Fe_3O_4 and (b) Fe_3O_4/co-polymer nanoparticles.

4. CONCLUSIONS

- Magnetite nanoparticles were synthesized by co-precipitation method, then coated with co-polymer.
- The structure, morphological and magnetic properties of as-prepared sample was characterization by X-Ray Diffraction (XRD), Scanning Electron Microscopy / Energy Dispersive X-ray analysis (SEM- EDAX) and magnetic measurements were investigated using Vibrating Sample Magnetometer (VSM).
- The magnetic properties of the nanoparticles were determined using VSM results indicate that the properties of super paramagnetic nanoparticles was produced.
- The present data show that DOX loaded on co-polymer coated Fe_3O_4 nanoparticles is promising magnetically targeted drug delivery.

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