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RESEARCH PAPER

$FeMn_2O_4$ nanoparticles coated dual responsive temperature and pH-responsive polymer as a magnetic nano-carrier for controlled delivery of letrozole anti-cancer

Mitra Amoli-Diva [1], Maryam Daghighi Asli [2], Shahla Karimi [2]*

[1]*Department of Chemistry, Payame Noor University (PNU), Tehran, Iran*

[2]*Department of Chemistry, Islamic Azad University Central Tehran Branch, Tehran, Iran*

ABSTRACT

Objective(s): For cancer cells, an efficient and selective drug delivery vehicle can remarkably improve therapeutic approaches. This paper focuses on the synthesis and characterization of magnetic $MnFe_2O_4$ NPs and their incorporation in a dual temperature and pH-responsive polymer, which can serve as an efficient drug carrier.

Materials and Methods: $MnFe_2O_4$ NPs were synthesized by chemical co-precipitation technique and coated with tetraethyl orthosilicate (TEOS) and modified with 3-mercaptopropionic acid (MPA). Then, it was used in the reaction medium during the synthesis of a temperature and pH-responsive poly (N-isopropylacrylamide-co-vinyl acetate-co-methacrylic acid). The prepared vehicle was characterized by FESEM, XRD, VSM, and FT-IR. Letrozole was used as a model drug and its loading and release and LCST of the vehicles were evaluated.

Results: The results for LCST measurements reveal that the phase transition of polymer occurs at temperatures in the range of 37-40 °C which is in the range of body conditions. Results for loading efficiency shows that maximum loading occur in about 10 h. The loading % for nano-carrier was lower than plain polymer which was due to lower polymer content in the nano-carrier with the same weight compare to the plain polymer. The results for drug release showed that the release of letrozole in pH 1.2, 5.5 and 7.2 was about 80, 45 and 35% for plain polymer and 81, 56 and 50% for the nano-carrier respectively.

Conclusion: The results indicate that the prepared magnetic nano-carrier can be a suitable candidate for site-specific and controlled anti-cancer delivery through oral administration.

Keywords: *Drug delivery, Dual responsive polymers, Letrozole, $MnFe_2O_4$ nanoparticles*

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INTRODUCTION

Recently, magnetic nanoparticles (MNPs) have attracted much attention for their potential use in biomedical applications like controlled drug delivery, cell separation, magnetic resonance imaging and localized hyperthermia and etc. [1-4]. Among them, iron oxide-based NPs have particular significance because of their remarkable magnetization property, appropriate biocompatibility and low toxicity. Such NPs can be directly injected to cancer cells, delivered by magnetic field gradient or delivered by other efficient drug delivery system to release their drug(s) [5]. Furthermore, these NPs can generate heat by hysteresis loss, and Néel and Brownian relaxations depending on the size of the particles by applying high frequency magnetic fields [6]. To more improve the efficacy of MNPs therapeutic property, researchers begin to coat them with stimuli-responsive polymers which can respond when exposed to external stimuli and can produce physicochemical changes in the structure which is in favor of drug release in controlled manner or at the specific site. These external stimuli include physical signals such as temperature, electric field, magnetic field, and ultrasound; or chemical signals such as pH, ionic strength and etc. [7-9]. Among

* *Corresponding Author Email: amoli@physics.sharif.edu*

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these stimuli, temperature and pH have been widely used to design delivery vehicles.

Temperature sensitivity is one of the most interesting characteristics in stimulus-responsive polymeric nano-carriers and has been extensively applied for drug and gene delivery systems [10]. On the other hand, using a pH-sensitive polymer as drug carrier has benefit of responding to acidic environment of cancer cells [11, 12]. So, it is anticipated that the pH-responsive drug-delivery systems will show a main role in applications in new horizons of cancer therapy [13, 14]. It was also proved that cancer tissues show lower pH and higher temperature than that of normal tissues. These features can be applied for formulating the dual-sensitive carriers that smartly differentiate between normal and cancer tissues [7].

To prepare such a dual responsive carrier, copolymer of a temperature-responsive monomer N-isopropylacrylamide (NIPAAm) and a pH-responsive monomer such as methacrylic acid (MAA) is needed [15]. NIPAAm has a lower critical solution temperature (LCST) of 32 °C which is near the physiological condition of body and can react with MAA or other polymers to results new multifunctional materials with optimum LCST. The prepared vehicle can act much faster than rigid particles and can be controlled by changing of the outer environment.

In this study, synthesis and application of a smart core/shell $MnFe_2O_4$ NPs was reported which its surface was decorated by a dual responsive copolymer of poly (N-isopropyl acrylamide-co-vinyl acetate-co-methacrylic acid) and used as a carrier of an anti-cancer drug. The carrier was characterized by XRD, SEM, VSM and FT-IR techniques and then, its LCST was investigated. Letrozole was used as a model drug and its loading and release efficiency was evaluated. The results revealed its potential for controlled and targeted delivery.

Experimental

Materials

All the solvents and chemicals were listed below were purchased from Merck Company and used without further purification except for 2,2'-azobisisobutyronitrile (AIBN, 98%) which was purchased from ACROSS. Ferrous chloride hexahydrate ($FeCl_3.6H_2O$), manganese nitrate ($Mn(NO_3)_2$), 3-mercaptopropionic acid (MPA), aqueous ammonia (25% w/w), tetraethyl ortho silicate (TEOS), methanol, acetic acid, sodium acetate trihydrate ($CH_3COONa.\ 3H_2O$), sodium hydroxide, potassium hydrogen phosphate, glycerol (about 87%), hydrochloric acid, potassium chloride, N-isopropyl acrylamide (NIPAM), vinyl acetate (VA), methacrylic acid (MAA), and ethylene glycol dimethacrylate (EGDMA, 98%). Letrozole standard was purchased from Sobhan Daru pharmaceutical company and deionized water was used throughout the experiments.

Instrumentation

A Hitachi S-4160 scanning electron microscope (SEM) was used for characterization of size and morphology of modified $MnFe_2O_4$ NPs and the nano-carrier. Phase characterization of the modified NPs was performed using Phillips PW-1800 X-Ray diffraction (XRD). UV-V is spectra of loading and release solutions of the drug were carried out by UV240 Shimadzu UV-Vis spectrophotometer. A Metrohm 827 pH meter was used for measuring and adjusting the pH of the solutions. A Memmert WB10 water bath was used to precise control of the temperature (37 ± 0.1 °C). The magnetization measurement of $MnFe_2O_4$ NPs was performed using an AGFM/VSM 3886 vibrating sample magnetometer at room temperature.

Synthesis of the modified $MnFe_2O_4$ NPs

The $MnFe_2O_4$ NPs were prepared via a chemical co-precipitation method based on the previously reported method with slight modification [16]. Briefly, 0.1 mole $Mn(NO_3)_2$ and 0.2 mole g $FeCl_3.6H_2O$ were dissolved in deionized water under N_2 atmosphere with vigorous stirring. Then, 0.2 mole NaOH was rapidly added into the solution with the final volume of 50 mL followed by vigorous stirring at 85 °C. The mixture was stirred for 15 min under the same condition and then, was washed with deionized water (200 mL, three times) and methanol (100 mL, once). The precipitate was dispersed in an aqueous solution of TEOS (10% v/v, 80 mL followed by 60 mL glycerol. The suspension was stirred at 90 °C for 2 h under nitrogen atmosphere. After cooling down to room temperature, the suspension was washed sequentially with deionized water (3 × 200 mL), methanol (2 × 100 mL) and deionized water (3 × 200 mL). After that, TEOS coated $MnFe_2O_4$ NPs were homogeneously dispersed in 150 mL solution of 1.0% (w/v) of MPA and sonicated for 2 h. The

resulting NPs were washed five times with 250 mL of deionized water and dried as black powder at 45 ºC in an oven. Then, the obtained powder was calcined in a horizontal electrical furnace at 900 °C.

Synthesis of plain polymer and the nano-carrier

Plain polymer was synthesized as follows: an amount of 0.4 g of NIPAM, 1.5 mL of MAA and 1.5 mL of VA were added to 25 mL acetonitrile in a 50-mL conical flask. Then, an amount of 0.7 g EGDMA as the cross-linker and 0.2 g of AIBN as the initiator were added to the mixture. The solutions were bubbled with N_2 gas for 20 min and placed in a water bath at 60 °C for 6 h with vigorous stirring. After that, the reaction solution allowed to cooling down to ambient temperature and the prepared polymer was sequentially washed with 200 mL deionized water (twice), 200 mL of ethanol (twice), and 200 mL deionized water (three times) to remove un-reacted monomers and reagents. Finally, the plain polymer was dried in vacuum oven at 50 °C for 24 h. The nano-carrier was prepared in the same manner by adding 50 mg of modified $MnFe_2O_4$ NPs in the polymerization reaction medium.

LCST measurements of the plain polymer

Volume-phase transition temperature or LCST of the plain polymer was determined from its transmittance measurements in deionized water as a function of temperature. An amount of 0.5 g of plain polymer was transferred to 3 mL of to a quartz cell which placed in a water bath with desired temperature and after 5 min, its transmittance was measured. Three replicate measurements were performed for each temperature point.

Drug loading and release experiments

Loading kinetics measurements of letrozole for the plain polymer and the nano-carrier were carried out based on the previously reported method [17] with slight modification. Briefly, an amount of 0.5 g of each carrier was transferred to a 100-mL beaker containing 25 mL of 40 ppm letrozole and variation in drug concentration for each time was monitored for about 12 h. For each time, drug concentration in supernatant was measured spectrophotometrically at λ_{max} of letrozole and appropriate dilution performed to ensure the absorbance is in linearity range of Beer's law.

Then, in-vitro release experiments were performed in buffered solutions with pH 1.2, 5.5 and 7.4 which were selected for release medium to simulate stomach, cancer cells and intestine conditions. For these experiments, letrozole loaded plain polymer/nano-carrier was washed twice with 100 mL of ethanol and then, immersed into a beaker containing 25 mL of each buffered solution at 37 °C. At predetermined time interval, upper solution was analyzed spectrophotometrically to determine the cumulative drug released from the carrier.

RESULTS AND DISCUSSION

Characterization

The results from XRD analysis of the modified $MnFe_2O_4$ NPs was presented in Fig. 1. The data clearly confirm the crystalline phase of ferrite part in $MnFe_2O_4$ to be very close to the JCPDS No. 74-2403. For the modified $MnFe_2O_4$ NPs, the most intensive lines (311) and (440) diffraction peaks were observed at peak position of 36.47 and 61.44. The XRD pattern shows the prepared NPs have cubic structure. Moreover, the sharp peaks represent the crystalline order and high crystalline structure of $MnFe_2O_4$ NPs. There were no additional peaks, due to the existing impurity in the prepared powder.

The saturation magnetization curve of the modified $MnFe_2O_4$ NPs was presented in Fig. 2. As can be seen, the modified NPs have a saturation magnetization of about 22 emu/g indicating enough magnetic responsiveness in the applications with magnetic manipulation. In this study, it is expected to be held in place by an external magnetic field and the carrier releases the drug in a controlled manner.

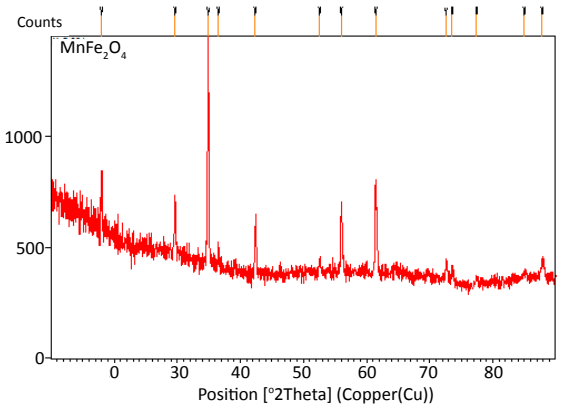

Fig 1. The XRD pattern of the prepared $MnFe_2O_4$ NPs

Fig. 3 shows the SEM images of the modified $MnFe_2O_4$ NPs and the nano-carrier. As can be seen, the particles have relatively uniform structure and quasi-spherical in shape and the nano-carrier has mean diameter of 40 nm.

Fig. 4 represents FT-IR spectra of the modified $MnFe_2O_4$ NPs and the nano-carriers. Two strong absorption bands at about 547 and 451 cm^{-1} are due to the stretching vibration of Fe-O bond [6, 18]. This band is also present in the nano-carrier spectrum confirming the presence of $MnFe_2O_4$ NPs in the structure of the carrier. The absorption band at 1096 cm^{-1} corresponds to the stretching vibration of Si–O band and absorption band at 3437 cm^{-1} is due to the N-H stretching vibration of NH group. Although, this band can be attributed to the OH groups of MAA units or existence of surface water in sample powder. The absorption peaks at 1626 cm^{-1} and 1747 cm^{-1} belong to the vibrational bands of C=O. Furthermore, two vibrational bands at about 2922 and 2848 cm^{-1} correspond to the asymmetric stretching of -CH in the structure of the polymer and the intense characteristic band at 1379 cm^{-1} is assigned to bending vibration of -CH groups. The results confirm successful synthesis of both modified NPs and the nano-carrier.

LCST measurements of the plain polymer

The LCST or volume-phase transition temperature of the plain polymer was studied at different temperature in deionized water. Fig. 5 shows the results for LCST determination and as can be seen; the polymer network undergoes a volume phase transition from shrunken to swollen state by increasing temperature. As can be seen, the LCST of both plain polymer and the nano-carrier was about 37-40 °C which is in the range of body conditions and phase transitions needed for drug release from the polymer can be occur in this situation. The balance between hydrophobic/ hydrophilic interactions between network chains and water molecules is the driving force for such phase transition. In fact, there is a greater degree of hydrogen bonding between monomer units of polymer chains at low temperature. Upon increasing the temperature, these attraction forces become weak and hydration occurred in the polymer network leading to the swelling.

Loading efficiency

To obtain the loading kinetics of letrozole on the plain polymer and the nano-carrier, an amount

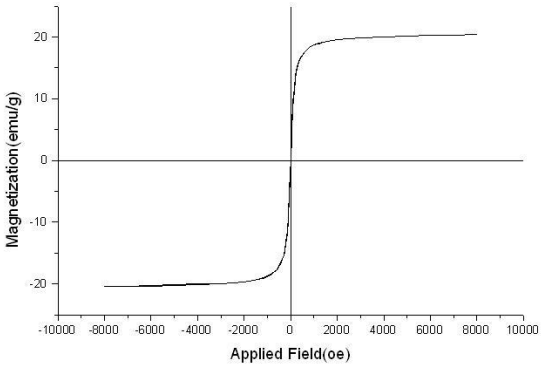

Fig 2. The VSM analysis of modified $MnFe_2O_4$ NPs

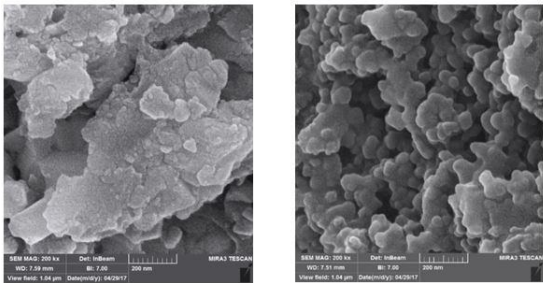

Fig 3. SEM images of (a) modified $MnFe_2O_4$ NPs and (b) the nano-carrier

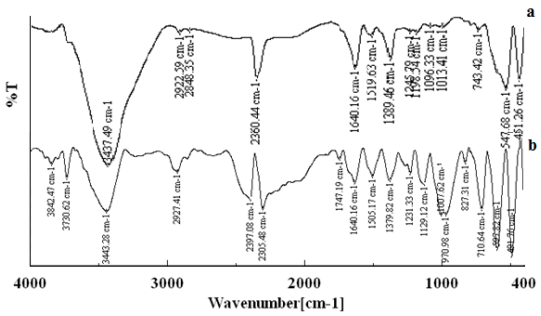

Fig 4. FT-IR spectra of (a) modified $MnFe_2O_4$ NPs and (b) the nano-carrier

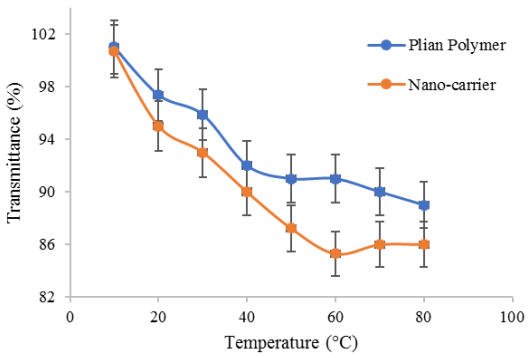

Fig 5. LCST measurements for the plain polymer

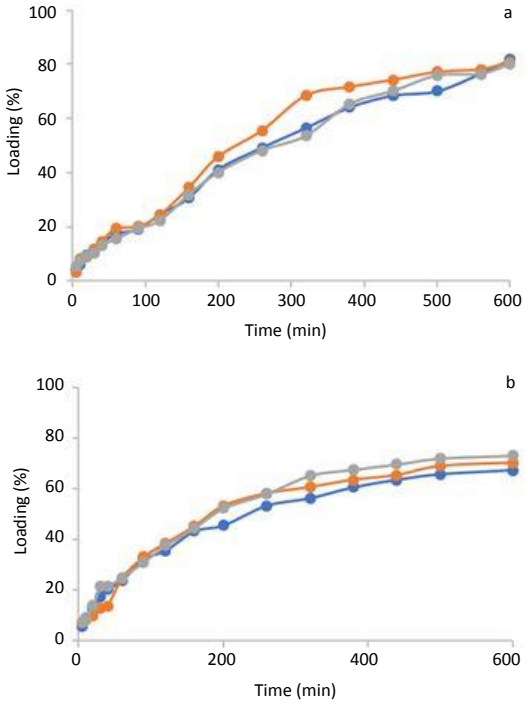

Fig 6. Results for loading efficiency/kinetics of (a) the plain polymer and (b) the nano-carrier

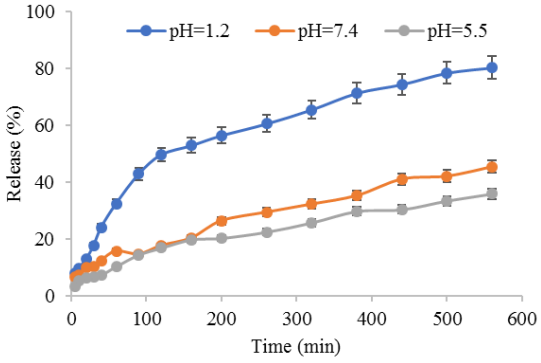

Fig 7. Results for letrozole release from the plain polymer

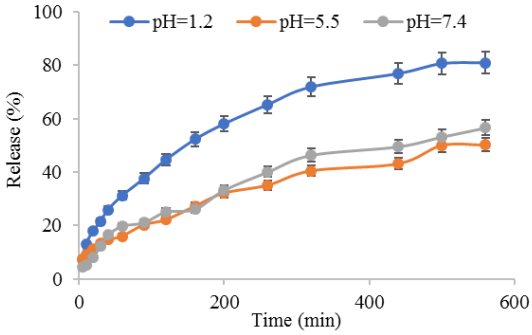

Fig 8. Results for letrozole release from the nano-carrier

of 0.5 g of each carrier was transferred to a 100-mL beaker containing 25 mL of 40 ppm letrozole solution and variation in drug concentration in different times was monitored for about 10 h. The results were shown in Fig. 6 and shows that maximum loading can be occurred in about 8 h. Although, more than 85% of these loadings were occurred in 5 h. As can be seen, the amount of letrozole loading (%) decreases in the nano-carrier with respect to the plain polymer. This is due to the decreasing hydrodynamic volume sizes and the polymer content in the prepared magnetic nano-carrier. Similar results were reported previously [17, 19, 20]. Loading to the nano-carrier has smoother behavior and reach almost constant at above 6 h.

Drug release studies

The results for release of letrozole from plain polymer and the nano-carrier in different pH were shown in Fig. 7 and 8 respectively. As can be seen, drug release at pH 1.2 is much faster than the release at pH 5.5 and 7.2. As reported in the literature, the pK_a value of poly MAA is about 5-6 depending on the block composition. In the lower pH values than the pK_a, most carboxylic acid groups in the polymer structure are in the form of COOH and when the pH increases from 1.2 to 7.2 (above the pK_a of PMAA), an increase in dissociation of polymer carboxylic groups occurs which facilitate the interaction of functional groups of the polymer with targeted drug. Furthermore, when the interaction between the polymer and the drug occurs, an insoluble complex produces, which further retards the release. In addition, at pH values above the pK_a's, the carboxylic acid groups of the polymer ionized and repulsion of the chains cause expansion of the polymer and form a gel that slows drug release rate.

CONCLUSIONS

A novel modified magnetic $MnFe_2O_4$ NPs coated by a dual temperature and pH-responsive poly (N-isopropylacrylamide-co-vinyl acetate-co-methacrylic acid) was successfully designed and synthesized as an efficient carrier of letrozole anti-cancer. The loading kinetic/efficiency and release characteristics of both plain polymer and the nano-carrier were investigated in details. The pH-sensitive property of the polymeric shell enables the carrier to control letrozole release in different pH solutions and LCST of about 40 ºC

makes the polymer suitable for release the drug in cancer tissues which has higher temperature with respect to normal tissues. The results indicate that the prepared magnetic nano-carrier has good potential as a candidate for targeted and controlled release of letrozole.

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CONFLICT OF INTEREST

Author has no received research grants. The author declares that he has no conflict of interest.

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