Volume 7, Issue 6, 2017, 2249 - 2252 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 25.11.2017 / Revised: 12.12.2017 / Accepted: 14.12.2017 / Published on-line: 15.12.2017

Optimal synthesis and characterization of magnetic $CuMnFe_2O_4$ nanoparticles coated by PEG for drug delivery

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ABSTRACT

In this study, we describe the synthesis of a novel PEG-functionalized magnetic nanoparticle (PEG-MNPs) activated with a stable ligand, folic acid and conjugated with an anti-cancer drug called imatinib. The $CuMnFe_2O_4$ magnetic nanoparticles were prepared using a combination of co-precipitation and the hydrothermal approach. Response surface methodology (RSM) was used to assessment the particle size (PS) of MNPs. The influence of temperature, in the range 60-85°C, and stirring rate, in the range 500-1500 rpm, was investigated. The optimum conditions for the synthesis of MNPs were found to be: 65°C and 1500 rpm. In this condition, a nanoparticle with the dimensions of about 43 nm was obtained and it was found to be appropriate for biomedical applications. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and vibrating sample magnetometry (VSM) were used to characterize these nanoparticles. Based on these results, the composite nanoparticles were observed to have a spherical shape and a uniform size distribution; therefore, they could be considered for targeted drug delivery.

Keywords: *PEG-MNPs, folic acid, imatinib, RSM, targeted drug delivery.*

1. INTRODUCTION

Magnetic nanoparticles (MNPs) have wide applications in industry and medicine due to their unique chemical, thermal and magnetic properties [1]. They can be potentially used in a variety of fields including catalyst, sensors, magnetic separation and drug delivery [2]. In the last decade, application of MNPs in biomedicine has received considerable attention [3]. Due to the controllable size range of these particles, which are smaller or comparable to a cell (1-100 μm), a virus (20-450 nm), protein (5-50 nm) or gen (2-100 nm), they can be of biological interest [4]. These nanoparticles have magnetic properties, so they can be manipulated by an external magnetic field [5]. This application makes them suitable as a carrier in drug delivery; for instance, they can serve as anticancer drugs to a targeted tumor [6]. Further, these nanoparticles can be used as hyperthermia agents. In this method, nanoparticles are injected to the tumor and by applying a constant magnetic field; the malignant cell is warmed up to 41°C and consequently, destroyed [7].

Iron oxide based magnetic nanoparticles are very appropriate for biomedical research purposes [8]. They are stable, non-toxic and bio-compatible, along with high magnetic properties [9]. They can be employed in the delivery of drugs to magnetically induce hyperthermia in the treatment of cancer [10]. Ferrites with the general formula MFe_2O_4 (M= Mn, Co, Ni, Cu, Ca, Zn, Pd, Ga and other divalent metal) are a group of material with an inverse spinel structure; they are chemically and thermally stable materials [11].

The surface of synthesized MNPs can be modified by different types of material including polymers, surfactants and inorganic materials to develop a more suitable drug delivery system [12]. Among them, many synthetic and natural polymers such as dextran, polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) have been used to modify the surface of the MNPs [13]. Recently, several articles have been published, reporting the progress in using MNPs for biomedical and therapy applications [14]. One of the major challenges in this method is to present a surface coating material that can provide active functional groups and also prevent the biofouling of MNPs in the blood plasma [15]. PEG is bio-compatible, non-toxic and non-antigenic; also, since PEG is potentially solved in polar and non-polar solvents, PEG functionalized surfaces can establish appropriate cellular uptake [16].

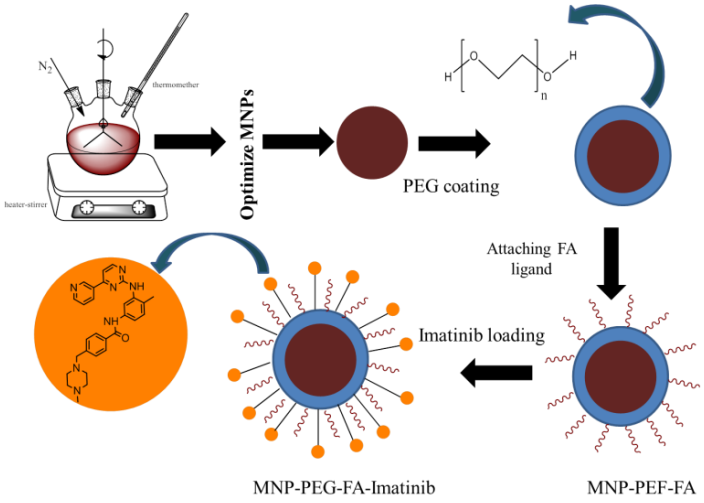

Figure 1. Overview process for synthesis of MNP-PEG-FA-Imatinib.

Different methods have been reported for the synthesis of nanoparticles; these include hydrothermal treatment, sol-gel method, co-precipitation, microwave, etc. [17-20]. In the present work, we used a combination of hydrothermal treatment and co-precipitation strategy for the preparation of $Cu_{0.5}Mn_{0.5}Fe_2O_4$

nanoparticles. An experimental design based on response surface methodology (RSM) technique was used to reduce the number of experiments and determine the interactions of the experimental variables [21]. The aim of this study was, therefore, to synthesize and characterize a novel carrier system based on PEG-MNPs loaded with imatinib; this could be appealing for anti-cancer drug delivery. Figure 1 shows the overview process involved in this study.

2. EXPERIMENTAL SECTION

2.1. General. All chemical and solvent were purchased form Merck and Sigma-Aldrich Company and used without further purification otherwise mentioned. Deionized water was used for all the experiments. The crystal structure of magnetic nanoparticles was characterized by X-ray diffractometer (STOE, STADIP), with Cukα irradiation (λ=1.54) in the 2θ scanning range 10 to 80 and particle size (PS) was calculated via Scherrer's formula. The shape and surface morphology of the MNPs and PEG-MNPs were investigated by scanning electron microscope (SEM, KYKY-EM3200). The functional groups were identified by FTIR spectrophotometer (Shimidzo 300 spectrometer) from 4000-400 cm-1 using KBr discs. Magnetic behavior was characterized by vibration sample magnetometer (VSM, BHV-S5) with the external magnetic field between 10 kOe

2.2. Synthesis of $CuMnFe_2O_4$ MNPs. $CuMnFe_2O_4$ nanoparticles were synthesis with combined co-precipitation and hydrothermal approach. Central composite design (CCD) of response surface methodology (RSM) for experiment design and Minitab 17.1.0 software were used to optimize the PS of MNPs. Numerous parameters may affect the response of the experiment. In this study two important variations were evaluated: temperature and stirring rate. The design contains low and high levels (–1, +1), and a total of 13 runs were carried out using reaction temperature (X1) and rate (X2) as the independent variables and particle size (Y) as the response. The independent variables in RSM are performed in Table 1. It should be mentioned the reaction time and pH were steady in all the experiments.

Table 1. Independent variables in RSM.

Factor/Level	Temperature (°C)	Rate (rpm)
-1	60	500
+1	85	1500

$FeCl_3.6H_2O$ (10mmol), $MnCl_2.4H_2O$ (5mmol) and $CuCl_2.2H_2O$ (5mmol) were dissolved in 100 mL deionized water in room temperature and stirred for 30 min at 1000 rpm. 6g NaOH was added to the mixture with vigorous stirring and bring the pH about to12. In the next step, the reaction mixture placed in an oil bath and heated according to the conditions which mentioned in Table 2 under nitrogen-gas atmosphere for 30 min. The mixture transferred to a Teflon autoclave and heated for 12 h at 180 ºC. The product cooled down to room temperature, washed with deionized water several times and separated with magnetic decantation by placing a magnet below the beaker and allowing the solution to clear. The precipitate dry in vacuum oven for 10 h at 100 ºC. Finally, nanoparticles heated in oven at 800 ºC for 120 min.

2.3. Synthesis of PEG functionalized MNPs. To synthesize PEG-functionalized nanoparticle, the $CuMnFe_2O_4$ nanoparticle (MNP) were mixed with the PEG solution in the ratio 1:2 ($CuMnFe_2O_4$: PEG). The resulting mixture was dispersed in 100mL toluene and sonicated for 4h at 60°C under nitrogen-gas atmosphere. MMNP-PEG was separated by magnetic decantation and washed with ethanol several times.

2.4. Folic acid modified PEG-MNPs. Conjugation of folic acid (FA) to the surface of the PEG is difficult due to the weak reactivity of the carboxylic acid group linked to the PEG [22]. To overcome this issue, dicyclohexyl carbodiimide (DCC) was first used to activate the carboxyl group of FA. Folic acid activated using the following procedure: 10 mg folic acid was dissolved in 10 mL DMSO (dimethyl sulfoxide). In the next step, 5 mg DCC was added to previous solution and stirred at 500 rpm for 2h under a nitrogen-gas atmosphere. Finally, PEG-MNPs (20 mg) was added to the solution and stirred vigorously for 2h under a nitrogen-gas atmosphere. PEG-FA-MNPs were separated with magnetic decantation and washed with water repeatedly.

2.5. Conjugation of drug to PEG-FA-MNPs. The anticancer drug imatinib (5 mg) was dissolved in 5 mL deionized water. 50 mg PEG-FA-MNPs was added to the solution and stirred at room temperature then, placed in a shaker (rpm=300) for 12h. Drug loaded composite (PEG-FA-MNPs-imatinib) was separated by magnetic decantation and dried in a vacuum desiccator.

3. RESULTS SECTION

3.1. Preparation of MNPs and surface modification. $CuMnFe_2O_4$ magnetic nanoparticles were synthesis by combined co-precipitation and hydrothermal method and surface of MNPs modified with PEG and FA to develop a suitable drug delivery system. PEG has high solubility in both polar and nonpolar solvents and this increased the solubility of nanoparticles which coated with PEG in the cellular membrane. To determine the optimal condition for synthesis of MNPs with suitable particle size, CCD was chosen as a reliable methodology for evaluating the interaction between independent variations. In this study, the influences of temperature in the range 60-85°C and stirring rate in the range 500-1500 rpm were investigated. In Table 2 the PS of MNPs which obtained in each condition were listed.

Three-dimensional response surface and contour plots have been used to illustrate the effect of interactions between two variables on the size of nanoparticles (Figure 2). As shown in Fig. 2, the 60°C and 1500 rpm was chosen as the best condition for preparation the optimal MNPs. In this condition nanoparticle with dimensions of about 43 nm was produced. The appropriate size range of magnetic nanoparticles for drug loaded in hyperthermia is about 10-50 nm. Therefore, size of nanoparticles is suitable for biomedical applications.

Table 2. Particle size of MNPs obtained with RSM.

Std order	Run order	Pt type	Blocks	X1= Temperature (°C)	X2= Rate (rpm)	Y= particle size (nm)
9	1	0	1	72.5	1000	62.381
1	2	1	1	60	500	52.775
3	3	1	1	60	1500	43.169
6	4	-1	1	90	1000	62.387
10	5	0	1	72.5	1000	62.381
5	6	-1	1	55	1000	62.388
11	7	0	1	72.5	1000	62.381
2	8	1	1	85	500	56.107
4	9	1	1	85	1500	59.244
13	10	0	1	72.5	1000	62.381
8	11	-1	1	72.5	1700	60.212
12	12	0	1	72.5	1000	62.381
7	13	-1	1	72.5	300	56.126

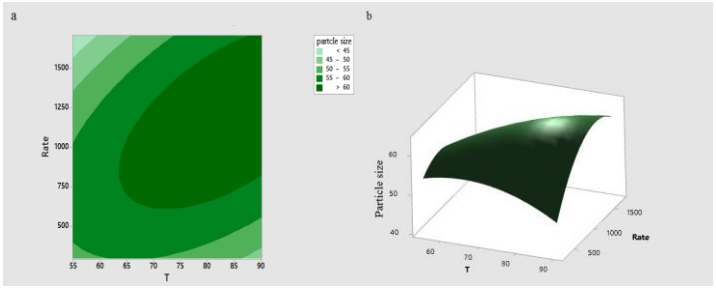

Figure 2. The contour (a) and 3D Response surface (b) plots from the RSM for particle size.

3.2. Characterization. The structure of magnetic nanoparticle ($Cu_{0.5}Mn_{0.5}Fe_2O_4$) was characterized by applying XRD, VSM, SEM and FTIR analyses. The peaks in XRD pattern of MNPs are in good agreement with the standard Fe_3O_4 (JCPDS file NO. 19-0629). The appearance of these peaks demonstrates that the crystalline spinel ferrite core structure during the preparation of nanoparticle was survival. The average particle size of samples has been calculated from the XRD pattern using Debye-Scherrer's formula ($D = \frac{B\lambda}{\beta Cos\theta}$) where, D is the crystallite size (nm), B is the Scherrer constant (0.9), λ is the wavelength of X-ray (λ=0.154 nm), β is the full-width at half maximum (FWHM) of plane (311) and θ is the Bragg's angle. Figure 3 shows the XRD spectra of $CuMnFe_2O_4$ (a), $CuMnFe_2O_4$-PEG (b) and $CuMnFe_2O$ -PEG-Imatinib (c) nanoparticles.

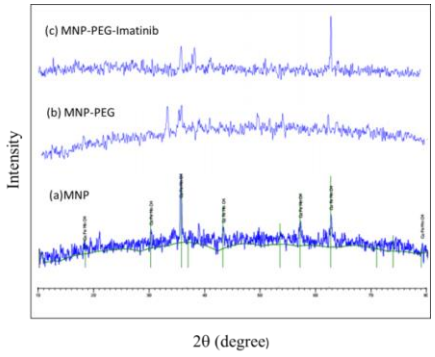

Figure 3. XRD diffraction Pattern of (a) MNP, (b) PEG-MNPs and (c) MNPs-PEG-Imatinib.

Figure 4 shows the VSM plot of the MNPs and MNP-PEG-FA-Imatinib at room temperature. On the basis of Figure 4 the saturation magnetization (Ms), remanent magnetization (Mr) and coercivity (Hc) can be measured. The saturation magnetization (Ms) quantity of MNPs and MNP-PEG-FA-Imatinib was measured 62 and 37emu/g, respectively. The loos of saturation magnetization of the drug loaded nanoparticle are due to the diamagnetic contribution of polymer and drug shells. The absence of Mr and Hc indicate that the magnetic particles are superparamagnetic.

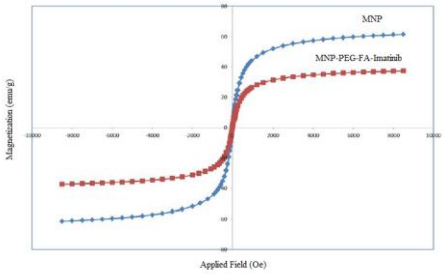

Figure 4. VSM plot of MNP and MNP-PEG-FA-Imatinib.

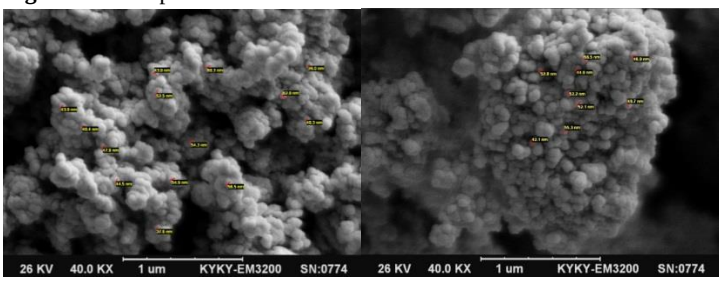

Figure 5. SEM images of (a) MNP and (b) MNP-PEG.

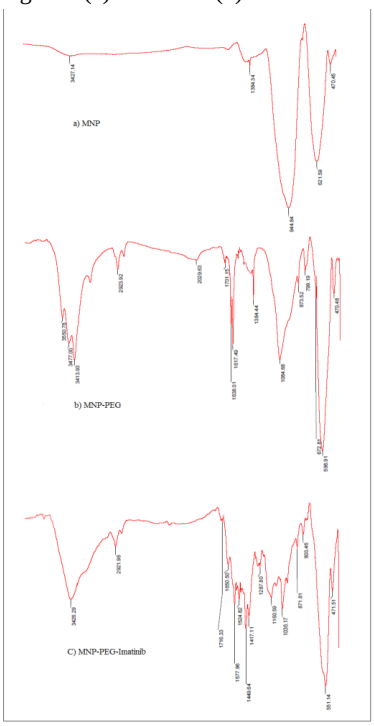

Figure 6. FTIR spectra of (a) MNP, (b) MNP-PEG and (c) MNP-PEG-Imatinib.

The SEM images of the pure MNPs and the PEG-MNPs are presented in Figure 5. The minimum PS observed in SEM image confirmed the XRD results. The agglomeration of the corresponding MNPs is strong due to the van der Waals forces between the particles. After coating this surface with PEG, the agglomeration of nanoparticle was reduced because of low molecular weight of PEG. The SEM images of magnetic nanoparticles reveals their spherically shape with uniform size distribution and this feature makes this nanoparticle appropriate for drug loading in next step.

In the FTIR spectra of MNPs (Figure 6a), absorption bands at 470, 620 and 944 cm^{-1} assigned to M-O (M=metal) stretch mode of tetrahedral and octahedral sites in $CuMnFe_2O_4$ particle.

Absorption bands of 3413 and 2923 cm^{-1} belong to OH and –CH groups of PEG respectively, and confirmed the linkage of this macromolecule to the MNPs. The C=O functional group of PEG was observed at 1701 cm^{-1} (Figure 6b). In the FTIR spectra of MNP-PEG-FA-Imatinib (Figure 6c), existence of the characteristic peak in the range 1524-1650 cm^{-1} may be assigned to phenyl groups of Imatinib drug, also a broad band in the range 3000-3426 cm^{-1} demonstrated the hydroxyl (OH) stretching and N-H group of FA. Furthermore, the C=O bond of folic acid appears at 1716 cm^{-1}, while the band at 1650 cm^{-1} belong to the C=O bond stretching of $–CONH_2$.

4. CONCLUSIONS

In this study, we have successfully synthesized the novel $Cu_{0.5}Mn_{0.5}Fe_2O_4$ magnetic nanoparticle by applying combined co-precipitated and hydrothermal method. To reduce number of experiments and determine the interaction of variables experimental design based of response surface methodology (RSM) was used. The influences of temperature in the range 60-85°C and stirring rate between 500-1500 rpm were investigated. The optimal MNPs were achieved in 65°C and 1500 rpm. In this condition nanoparticle with dimensions of about 43 nm was formed. The surface of nanoparticles was modified with PEG and FA to develop a suitable drug delivery system. SEM images revealed the spherical shaped morphology for nanoparticles. Finally, the MNPs which coated with PEG were loaded with Imatinib anti-cancer drug.

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6. ACKNOWLEDGEMENTS

The authors would like to thank Dr. Hosseini (Department of Chemistry, Islamic azad University, Tehran North Branch, Tehran, Iran) for assistance with the use of the laboratory