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Investigation of adsorption kinetic of Doxorubicin onto iron oxide magnetic nanoparticles functionalized with poly(acrylic acid)/Allyl Alchohol

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ABSTRACT

The study aimed to prepare iron oxide magnetic nanoparticles(MNPs) loaded with anticancer drug Doxorubicin (DOX) in order to evaluate the adsorption isotherms and adsorption kinetic of particles. Nanoparticles were prepared by a reduction–precipitation method and coated with poly(acrylic acid)/Allyl Alchohol as a pH-sensitive co-polymer followed by loading with DOX. They were characterized by FTIR, XRD and TEM techniques. Shapely nanoparticles with the size of 27 nm and loading efficiency of 90% were produced. Also it was cleared that DOX adsorption onto MMNP follows pseudo first-order kinetics. Finally, the proper results of study suggested evaluating the efficacy of nanoparticles against a cancer cell line in vitro environment.

Key words: Drug delivery, Functionalized magnetic nanoparticles, DOX, kinetic study.

INTRODUCTION

Cancer is a serious health threat worldwide with millions death every year[1]. Radiotherapy and chemotherapy are two main options of cancer therapy [2]. The efficacy of chemotherapy however, is restricted by nonspecific cytotoxicity, poor aqueous solubility and bioavailability [3]. Targeted drug delivery to cancerous cells without affecting the normal cells is forcefully recommended in cancer treatment [4]. Nanotechnology materials such as magnetic iron oxide nanoparticles (MNPs) have shown the promising results as chemotherapeutics carrier [5]. Nontheless MNPs could not used directly for drug delivery since because of their aggregation and consequently thromboses. In addition, bare iron oxide NPs are easily oxidized and induce harmful free radicals [6]. To solve the problems various polymeric agents have been recruited as NPs coating agents[7].Poly acrylic acid (PAA) is one of the widely used polymer for coating the MNPs. The polymer is a biocompatible water soluble material, having many reactive functional groups. As coating agent, PAA reduces the electrostatic interactions between particles [7].

One of the basic concept in adsorption science is adsorption isotherm. At constant temperature, adsorption isotherm is the equilibrium relation between the quantity of the adsorbed substance and the concentration in the bulk fluid phase[8]. The adsorption isotherm make it possible to describe the adsorption phenomena that occur at different types of interfaces. Langmuir, Freundlich and Temkin were three models of adsorption isotherm that were investigated in this study[9]. Langmuir model has been broadly employed for many process of metal ions sorption[10].The model quantitatively explains the development of a monolayer of adsorbate on the outer surface of the adsorbent which followed by no further adsorption. As a consequence, the Langmuir displays the equilibrium distribution of metal ions between the liquid phases and solid. The model presumes integrated energies of adsorption onto the surface and no displacement of adsorbate in the sheet of the surface[10]. Freundlich model was used for study the adsorption isotherm as well. This model is used to describes the adsorption properties of heterogeneous surface of adsorbents. Freundlich model assumes that the ratio of the amount of dissolved material that adsorbed onto a distinct mass of sorbent to the concentration of the dissolved material is not constant at various concentrations in the solution [11]. The heat of adsorption decreases logarithmically with increasing the quantity of adsorption [12].The model of Temkin for adsorption isotherm has a factor that clearly considers the adsorbent–adsorbate interactions. The model has two rules as follows: 1) The adsorption heat of entire molecules of the layer decreases linearly with coverage owing to adsorbent–adsorbate interactions and 2) the adsorption is described by an integrate distribution of binding energies, up to some maximum binding energy [13]. kinetic adsorption is also important for adsorption studies because it describes the relationship between drug adsorption rate and contact time[14]. There are three famous kinetic models to investigate the adsorption mechanism including Pseudo-First-Order Kinetic, Pseudo-Second-Order Kinetic and Intra-Particle Diffusion Kinetic[9].

In the present study, MNPs were prepared and functionalized with PAA/Allyl Alchohol. After that DOX was loaded onto the particles. Kinetic adsorption and adsorption isotherm of the formulation were investigated and Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD), spectrophotometry and Transmission Electron Microscopy (TEM) techniques were used for nanoparticle characterization.

MATERIALS AND METHODS

Instruments

FTIR technique (FT-IR Nexus 870; Nicolet, USA) was used to evaluate the chemical bonds and their modification in each step of NPs preparation. For pH measurements Metrohm pH meter model 744 (Metrohm AG,. Herisau, Switzerland) was employed. The UV-Visible spectra were prepared by a UV/Vis spectrophotometer (JASCO V-530 –Japan). Morphology of NPs was evaluated by a CM 30 model TEM (Philips, Amsterdam, Netherlands).The sample were analyzed by XRD to identify Crystal phase of MMNPs in the 2θ in region of 10-70o at 25°C using a D8 advance model (Bruker, Germany).

Chemicals

Allyl Alchohol(AA) was prepared from Fluka Company (Switzerland). Acrylic acid (AA), C2H5OH, FeCl2.4H2O, NH4OH, FeCl3.6H2O and Dryed toluene were purchased from Merck (Darmstadt, Germany). 2, 2-azoisobutyronitrile (AIBN) and 3-mercaptopropyltrimethoxysilane (MTPMS) were obtained from Aldrich chemical company (Germany). DOX hydrochloride was supplied from Sobhan daru company (Iran). All chemicals used were of analytical grade. Initial stock solution of DOX (500 ppm) was prepared by dissolving 50 mg of drug in 100 ml deionized water. phosphate buffer solution (pH=6.5-7.5 containing 0.1 M Na2HPO4, NaH2PO4) and 0.01 M acetate buffer (pH=3-6.5 containing 0.1 M CH3COOH, CH3COONa) was used to determining of solutions pH.

Synthesis of co-polymer grafted modified MNPs

Synthesis of iron oxide MNPs

Iron oxide magnetic nanoparticles were prepared by the co-precipitation method [9]. Briefly, ferric chloride and ferrous chloride (molar ratio of 2:1) were dissolved in 200 ml deionized water and stirred (300 rpm, 70 °C) under nitrogen atmosphere. subsequently aqueous solution of 2.5 M NH4OH (10 ml) was added into the vessel and the process was kept for one hour at 70 °C under stirring. Obtained precipitation was separated by centrifugation and washed several times with water and ethanol and dried in oven at room temperature.

Modification of MNPs with 3-mercaptopropyltrimethoxysilane

In second stage, TO Stabilizing of synthesized magnetic Fe3O4 nanoparticles [15, 16], obtained NPs (3g) was dispersed into 50 ml of toluene solution containing 3-mercaptopropyltrimethoxysilane and refluxed at 70 °C for 24

h. The reaction products were precipitated by an external magnet, washed with 20 ml toluene and dried at room temperature (RT).

Graft polymerization of modified MNPs

The third step was polymerization and grafting of AGE/AA onto above NPs. To this, the modified MNPs (MMNPs) were transferred into a vessel containing 40 ml ethanol, 15 ml AGE, 5 ml AA and 0.1 mg AIBN and stirred (300 rpm, 70 °C) under a nitrogen atmosphere for 8 h. After that the grafted MMNPs (GMMNPs) were separated by an external magnet and washed with 40 ml ethanol and dried at RT. The steps of GMMNPs synthesis are shown in Figure1. The Final products were characterized by FT-IR, transmission electron microscopy (TEM), scanning electron microscopy (SEM).

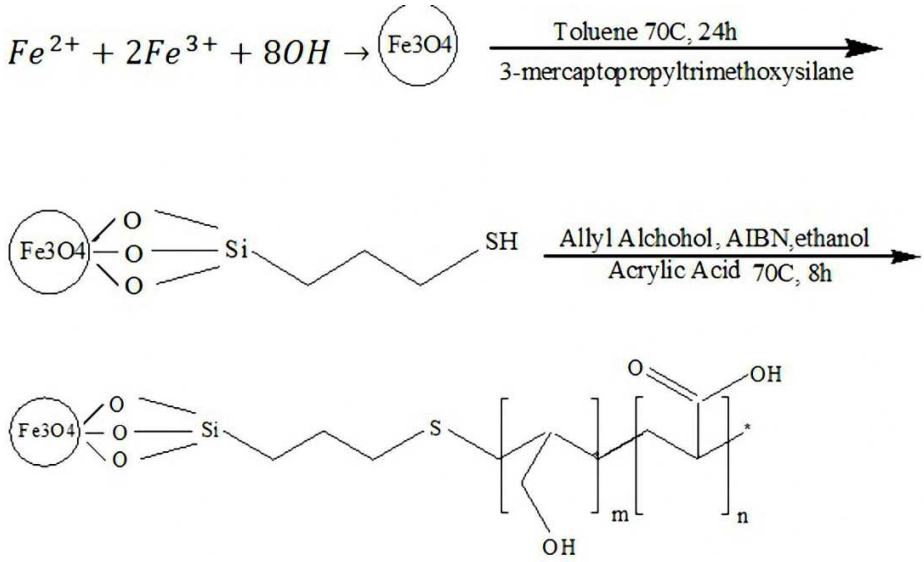

Figure 1: Schematic of synthesis process of Functionalized MNPs

Drug loading process

DOX dissolved in 100 mL deionized water containing 5 mL buffer PBS (pH= 7) to prepare a solution with the concentration of 0.2 mg/ml. Sorbent (0.4 g) with 50 mL drug solution shacked at room temperature. After 30 min, solid/liquid phases were separated by centrifuging (15 min ,13000 rpm). The content of unloaded free DOX in the supernatant was determined by UV–Vis spectrophotometer at wavelength of 480 nm and with following equation. Drug loading efficiency was calculated using following equation:

$$Loading\ efficiency(\%) = \left(\frac{total\ amount\ of\ drug\ added - \text{amount of drug in supernatant}}{total\ amount\ of\ drug\ added}\right) * 100 \quad (1)$$

Batch adsorption and isotherm studies

Batch technique used to study of DOX Adsorption rate onto adsorbent (GMMNPs) [17]. In this system, Briefly, 0.01 g of adsorbent added into 10 mL of buffer (1 mM, pH=5) containing various concentrations of drug (2-100 mg l^{-1}). The solutions agitated for 15 min (200 rpm). Then, the solutions centrifugated at for 15 min(10,000 rpm) and solid/liquid phases were separated. The concentrations of DOX in supernatants, C_e, were measured by a Cary 1E UV-Vis Spectrophotometer. The amount of adsorbed DOX on adsorbent (mg DOX/g adsorbent) was determined from the following equation:

$$q_e = \frac{(C_i - C_e)V}{m} \quad (2)$$

WhereC_i and C_e (mg l-1) are the initial and the final concentration of DOX in the solution phase, respectively. V is the volume of DOX solution (l) and m is the mass of the adsorbent used (g).

The amount of adsorption at any time t, q_t (mg.g-1), was calculated by [20]:

$$q_t = \frac{(C_i - C_t)V}{m} \quad (3)$$

Where C_t (mg L-1) is concentration of DOX in the liquid phase at any time t.

Statistical analysis
Data of study was analysed by SPSS software version 18.0 and P<0.05 was considered significant.

RESULTS AND DISCUSSION

Characterization of adsorbent
FTIR spectra, were employed to investigate the chemical composition of the bare, modified and grafted and MNPs. FTIR spectrum of bare Fe3O4 is represents two bonds with the wavelength of 564.34cm-1 belongs to vibrations of the Fe-O bond and the wavelength 3376.3 cm-1 belongs to O-H bond. Figure 2(b) shows Additional peaks of CH_2 (1367.44cm^{-1}) and Si–O (1042.02cm^{-1}).Figure 2(c) confirms Polymer grafting onto MNPs according to the peaks of bendingCH_2 (2355.02cm^{-1}), stretching CH_2 (1396.75cm^{-1}), OH (3401.38cm^{-1}), Si–O (1038.054cm^{-1}) and C=O (1624.28cm^{-1}) bonds. Furthermore, the results of drug loading efficiency indicated the high competence of NPs in drug adsorption in that 90% of primary drug used become adsorbed on the nanoparticles. In other word drug loading efficiency was calculated 90%.

The TEM image presented inFigure2 shows that functionalized MNPs have a spherical shape.

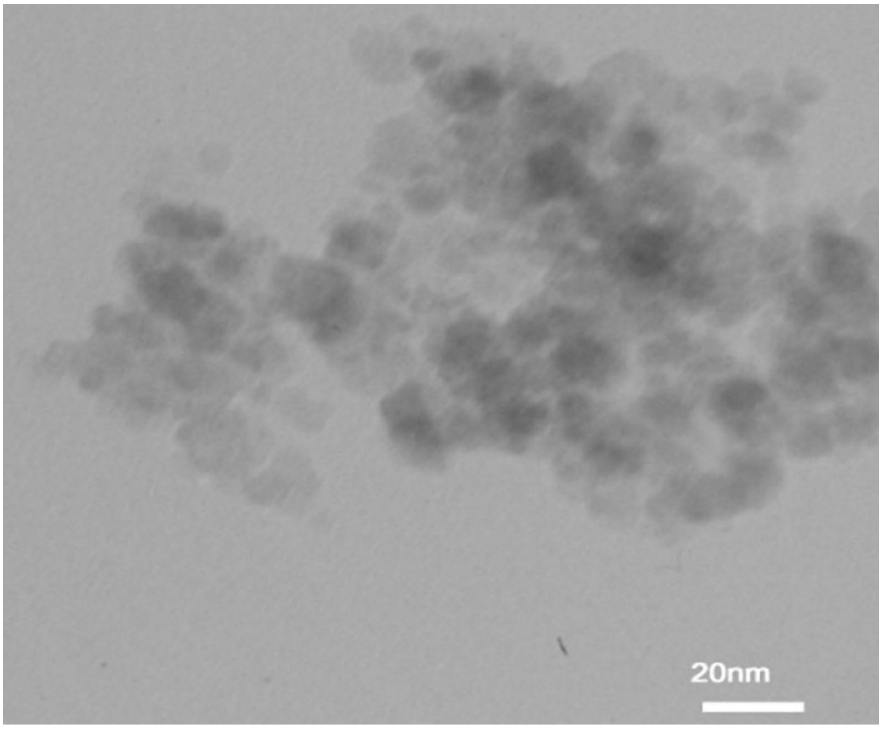

Figure2: TEM photograph of functionalized NPs

XRD pattern of the functionalized MNPs are shown in Figure 4. The average size of MNPs (D) can be estimated by Scherrer's equation[1]:

$$D = \frac{K\lambda}{B\cos\theta} \quad (4)$$

k = 0.94 is the constant crystal lattice, λ = 0.154 nm is the X-ray wavelength, β is the full width of the peak measured at half maximum intensity and θ is the Bragg's angle of the peak.

Where λ (0.15406nm) is the X-ray wavelength, B is the full width of the peak at half maximum intensity; θ is the diffraction angle, and K is the shape parameter, which is 0.89 for magnetite.

According to the Scherrer's equation, the average size of nanoparticles was calculated to be about 26.73 nm(2θ = 32.36°).

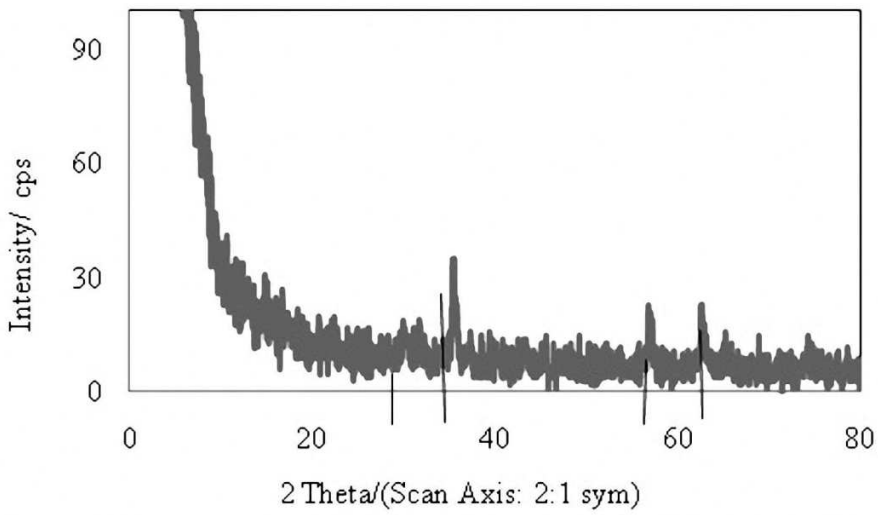

Figure 3: Spectra of Functionalized Fe3O4 magnetic nanoparticles

Adsorption kinetic models

Contact time is an important parameter which reflects the adsorption kinetics of an adsorbent at given initial adsorbate concentration. In this work, three common kinetic models were used to investigate the adsorption kinetics of DOX onto sorbent. Experiments were carried out for different contact times (0.5-90 min) with initial concentration (20 mg L-1) and pH= 7. The linear forms of the pseudo-first order, pseudo-second order models and Elovich kinetic model can be expressed by Equations 9, 10 and 11, respectively.

$$\mathrm{Ln}(q_e - q_t) = \mathrm{Ln}q_e - \frac{k_1}{2.303}t \qquad (5)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (6)$$

$$q_t = \frac{1}{\beta}Ln\alpha\beta + \frac{1}{\beta}Lnt \qquad (7)$$

Where k_1(min^{-1}) and k2 (g.mg^{-1}.min^{-1}) are the equilibrium rate constant of models.q_e and q_t refer to the adsorption capacity of DOX (mg g−1) at equilibrium and at any time, respectively.

α is the initial adsorption rate (mg/gmin) andβ(g/mg)is a desorption constant.

As shown in Table 1 correlation coefficient value for this model is close to 1.0(R^2=0.976)and the calculated q_e value (0.0104) is similar to the experimental $q_e$value (0.01).

Table 1: Parameters of kinetic models for adsorption of DOX onto MMNPs

Model	Parameter		
Pseudo-first order	k_1(min^{-1})	q_e (mg.g^{-1})	R^2
	0.0766	0.0104	0.9766
Pseudo-second order	k_2 (g.mg^{-1}min^{-1})	q_e (mg.g^{-1})	R^2
	29.544	0.1359	0.999
Elovich	α*10^{-8}	B	R^2
	2.68	384.61	0.9116

The correlation coefficient of pseudo-second-order model is the maximum value and calculated q_e value (0.1359) is equal to the experimental $q_e$value, so has the best conformity with it. This suggests that DOX adsorption on MMNP follows pseudo first-order kinetics.

CONCLUSION

We have constructed DOX loaded Fe3O4 NPs and functionalized them with Poly acrylic acid/Allyl Alchohol copolymer. The NPs were characterized in terms of morphology, drug loading efficiency, adsorption drug kinetics and isotherm study. Shapely NPs with nanoscale size and high drug loading efficiency were prepared. Furthermore, NPs showed isotherm and follow of pseudo first-order kinetics. The evaluation of the efficacy of formulation in vitro environment against a cancer line can be considered in the future study.

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