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Green synthesis of Iron oxide nanoparticles using carum carvi L. and modified with chitosan in order to optimize the anti-cancer drug adsorption

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ABSTRACT: Magnetic iron oxide nanoparticles have gained a lot of attention in drug delivery systems because they can control a drug pathway to deliver it to the specific site under a magnetic field which is related to their magnetic core and surface coating. Chitosan-coated FeNPs, have prominent antimicrobial and biological properties that make chitosan a promising biopolymer for drug delivery application, especially in cancer treatment. In this research, FeNPs were green synthesized using the aqueous extract of Carum carvi L. and under optimum conditions. Formation of FeNPs was confirmed by UV-Vis spectroscopy, XRD analysis, and SEM. Also, chitosan-coated FeNPs were synthesized to increased biocompatibility and the absorption capacity of nanoparticles. Chitosan coating on FeNPs was detected by FTIR. After the production of nano-absorbent, the maximum absorbance of different concentrations of doxorubicin was determined. The effect of pH was investigated on the absorption of doxorubicin in maximum absorbance at pH 3-10 by UV-Vis spectroscopy. The results obtained from the characterization of FeNPs showed they are spherical particles with less than 300 nm in size. The maximum absorbance of different concentrations of doxorubicin was in 280 nm. Doxorubicin showed maximum absorption at pH 7. This green biosynthesis method has been found to be eco-friendly, cost-effective and promising for different applications. The seeds extract of Carum carvi L. have a great ability to reduce Fe ions to FeNPs. Also, doxorubicin loaded chitosan-coated FeNPs can successfully use in drug delivery systems.

Keywords: *Doxorubicin; Drug delivery; Green synthesis; Iron nanoparticles; Surface modification*

INTRODUCTION

Nanotechnology is concerned with synthesis and manipulation of particles on an atomic or molecular scale ranging from one to one hundred nm. Nanoparticles have a large surface area to volume ratio, which is responsible for the widespread use of them in biotech-

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nology, microbiology, medicine and many other areas (Saif, *et al.*, 2016). Recently, magnetic nanoparticles, have gained much attention due to their ability in cancer therapy. Iron nanoparticles can be targeted to the tumor site in a magnetic field (Pilar Vinardell, *et al.*, 2015). Targeted delivery of the drug via nanoparticles can solve the problems related to conventional chemotherapeutic agents such as insolubility under aqueous situations and lack of selectivity.

Also, the problem of drug resistance can be solved (Singh and Lillard, 2009, Xin, 2017). Iron nanoparticles have been reported to have anti-inflammatory and antimicrobial properties, which have a great potential in human medicine. Synthesis of iron nanoparticles is one of the areas of interest to the scientific community due to their wide range of applications (Saranya, *et al.*, 2017). A number of methods are available for the synthesis of Iron nanoparticles. Among them, use of microorganisms and plant materials provides advancement over physical and chemical methods as it is eco-friendly, cost-effective and there is no need to use high pressure, temperature and toxic chemicals (Gottimukkala, *et al.*, 2017). The use of plant extracts for the synthesis of iron nanoparticles could be more advantageous because it does not need some processes like intracellular synthesis and multiple purifications or the preservation of microbial cell cultures (Shanmugavadivu, *et al.*, 2014). The plant extracts contain polyol components are responsible for the bioreduction of iron ions in the green synthesis of iron nanoparticles (Rosi, *et al.*, 2006, Shchukin, *et al.*, 2003).

Carum carvi L. the plant used in this study belongs to the Apiaceae family. Traditionally, this plant has been used for stomach disorders, diarrhea and in veterinary medicine. The seeds of this plant contain 1–9% essential oils including more than 30 compounds. Monoterpenes, sesquiterpenes, saturated and unsaturated fatty acids, aldehydes, ketones and esters are some chemical groups which were isolated from the seeds (Abdalaziz, *et al.*, 2017, Miraj and Kiani, 2016). There are no reports on the use of *Carum carvi L.* in the biosynthesis of iron nanoparticles.

Chitosan, the second most plentiful polysaccharide in exoskeletons of crab and shrimp has great biological features (Shi, *et al.*, 2006). Chitosan-coated FeNPs are a noticeable pharmaceutical delivery carrier, due to their ability to protect sensitive macromolecules from enzymatic and chemical degradation in vivo situation (Mao, *et al.*, 2001). Their chitosan covering helps them to bind to different chemical groups and ions with free amino and hydroxyl groups leading to numerous applications such as metal adsorption, drug and gene delivery and tissue engineering (López, *et al.*, 2013).

Doxorubicin is one of the most commonly used drugs against different types of cancer. But, side effects and drug resistance limited its application (Liang, *et al.*, 2016). To reduce the side effects of Doxorubicin and improve its half-life in blood circulation, targeted delivery of the drug through nanoparticles is an alternative method for cancer therapy (Unsoy, *et al.*, 2014, Parvanian, *et al.*, 2017).

In the present study, iron nanoparticles were synthesized using a green biosynthetic method employing aqueous extract of *Carum carvi L.* as the reducing agent and modified with chitosan as nano-absorbent. Further, optimal pH in maximum absorbance was determined for Doxorubicin absorption.

MATERIAL AND METHODS

Materials

Doxorubicin was obtained from (Sigma-Aldrich, USA), $FeCl_3$ and $FeSO_4$ was obtained from (Merck, Germany). The plant was purchased from the local grocery store and authenticated as *Carum carvi L.* by a botanist.

Preparation of the extract

Seeds were washed and then were air-dried, under the shade for a week. Then the plant was easily powdered. The aqueous extract solutions of *Carum carvi L.* were collected by weighing 20 gr of the powdered plant seeds in an Erlenmeyer containing 100 mL distilled water and boiling the mixture at 100°C for 5 min. The aqueous extracts were filtered using filter paper and vacuum pump to remove insoluble materials. The filtered extracts were collected in a dark container and stored in the refrigerator (4°C) for further experiments.

Synthesis of FeNPs

The synthesis of FeNPs was done by dissolving 3.04 g of $FeSO_4$ and 1.62 g of $FeCl_3$ in a balloon and added distilled water to100 ml. The amount of 70 ml of the final solution was sonicated for 10 min to remove oxygen. Then the aqueous extract of plant seeds was dropwise added to this solution in anaerobic conditions and was mixed for 2 hours. The reaction mixture was centrifuged at 10000 rpm for 10 min and the supernatant was discarded at each stage. Finally, the pellet was rinsed with distilled water and was centrifuged to remove any impurities.

Synthesis of nano-adsorbent (Chitosan-coated FeNPs)

Chitosan (0.5 g) was dissolved in 50 ml of 1% acetic acid and the pH was adjusted to 4.8 by 1 M NaOH. The solution was then mixed with synthesized FeNPs for 2 hours at room temperature. The colloidal chitosan coated FeNPs were then centrifuged at 10000 rpm for 15 min and the supernatant was discarded. The precipitate was extensively washed (3 times) with distilled water and after centrifugation, dried in an oven at 50°C for 24 hours. The dried sample was stored at room temperature for further use.

Characterizations of FeNPs and Chitosan-coated FeNPs

The absorption spectra of the extracts were measured in two steps before and after the addition of $FeSO_4$ and $FeCl_3$ aqueous solution using a Cary 300 UV-Vis spectrophotometer in a wavelength of 300-700 nm at room temperature. The size and shape of synthesized FeNPs were characterized employing a Philips, EM-400 TEM. Ethanol was used as the solvent to prepare the colloidal solution of FeNPs.

A Thermo Nicolet NEXUS 870 FTIR was used to confirm the binding of chitosan polymer on the surface of nanoparticles. Solid powders of FeNPs and KBr powders were mixed and compressed to a transparent disc, and the final spectrum was obtained at a range of 400-4000 cm^{-1}. To investigate the general structure of nanoparticles, the KYKY-EM3200 SEM was used. The X-ray diffraction patterns were collected with a Siemens D500 X-ray diffractometer (XRD) to investigate the mineral phase of chitosan-coated FeNPs. XRD measurements were carried out with the operation voltage and current at 40 kV and 30 mA, respectively.

Determination of optimum pH

The optimum pH value was determined by preparing 25 mL of doxorubicin solutions (25 ppm) containing 2.5 mL of buffers with pH 3, 4, 5, 6, 7, 8, 9, 10 and mixing 10 mL of each solution with 0.005 g of nano-adsorbent. Samples were shaken, centrifuged and the supernatant solution was evaluated by a standard solution of maximum absorbance at λmax by a spectrophotometer. In order to determine the optimal pH, the standard and sample adsorption at each pH were separately evaluated and the optimum pH was determined using the eq. (1).

$$Q = \frac{C_0 - C_e \times V}{W} \quad (1)$$

Where C_0 and C_e (mg L^{-1}) stand for primary and equilibrium concentrations of the doxorubicin respectively, V (L) represents the volume of the solution, and W (g) is the mass of the chitosan-coated FeNPs.

Calibration curve of maximum absorbance of Doxorubicin

UV spectrum of all concentrations of doxorubicin in the wavelength of 200-800 nm was obtained by ultraviolet (UV-Vis) spectroscopy to determine the maximum absorbance. Then, the spectrum of all concentrations of doxorubicin in maximum wavelength was investigated and its calibration curve was drawn.

Determination of sorption capacity

A set of solutions (the volume of each 10 mL) containing 2, 5, 10, 20, 30, 40, 60, 80, and 100 ppm of doxorubicin were performed.

Their pH was adjusted to the optimum value. Chitosan-coated FeNPs (0.005 g) was added to each solution, which was called the sample. The samples were vortexed and then centrifuged. The supernatant phase was separated and the absorbance was compared with the standard solution in λ max by UV-Vis spectrophotometry. For other concentrations, the same procedure was performed and the optimum concentration was determined.

Kinetic studies

To determine the adsorption kinetics, various samples were vortexed at optimal pH and 20 ppm concentration at different times (2, 5, 10, 15, 30, 45, 60, 90 and 120 min). The samples were then centrifuged and compared with each other and with standard absorbance by UV-Vis spectrophotometry.

RESULTS AND DISCUSSION

Characterizations of FeNPs and Chitosan-coated FeNPs

After mixing the plant seed extracts with the aqueous solution of $FeCl_3$ and $FeSO_4$, the color of the resulting solution started to change after 2 hours. More darkening of the solution indicates the synthesis of more FeNPs, which depends on the time and initial concentration of the extract and the aqueous solution of $FeCl_3$ and $FeSO_4$ (Fig. 1). Tessier *et al.*, 2000 reported that the brown color of the solutions is due to the particles much smaller than the wavelength of visible light (Tessier, *et al.*, 2000).

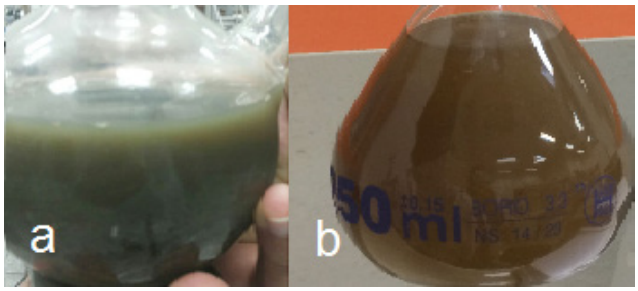

Fig. 1. a) Aqueous solution of plant seeds extract. b) Aqueous solution of FeNPs in plant seeds extract.

Fig. 2. TEM image of FeNPs synthesized by aqueous extract of *Carum carvi L.*

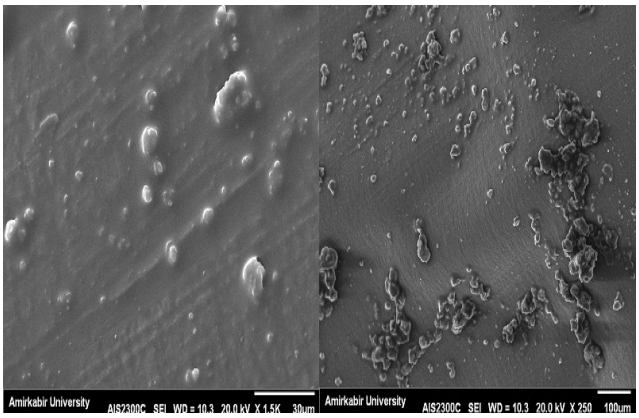

Fig. 3. SEM images of FeNPs synthesized by aqueous extract of *Carum carvi L.*

TEM images showed the spherical nanoparticles with an average size of about 300 nm (Fig. 2). Awwad, *et al.*, 2013 by the extract of Carob, Sathyavathi, *et al.*, 2011 by Coriandrum sativum extract and Ponarulselvam *et al.*, 2011 by the extract of *Catharanthus roseous* produced nanoparticles with the sizes of 5-40, 8-75 and 35-55 nm, respectively (Awwad, *et al.*, 2013, Sathyavathi, *et al.*, 2010, Ponarulselvam, *et al.*, 2012).

The images of Scanning Electron Microscopy showed spherical and porous nanoparticles of less than 300 nm in size (Fig. 3). In some parts, the nanoparticles are agglomerated.

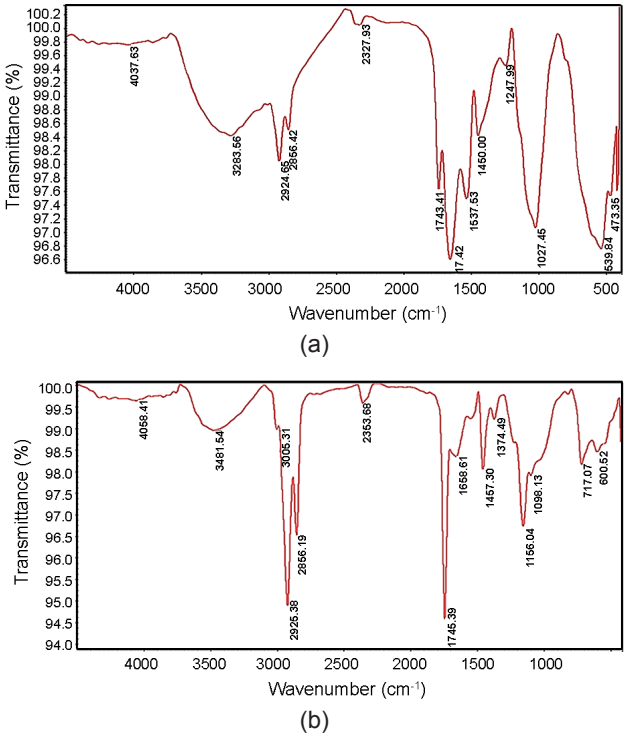

Fig. 4 a) the FTIR spectrum of synthesized FeNPs b) FTIR spectrum of the chitosan-coated FeNPs.

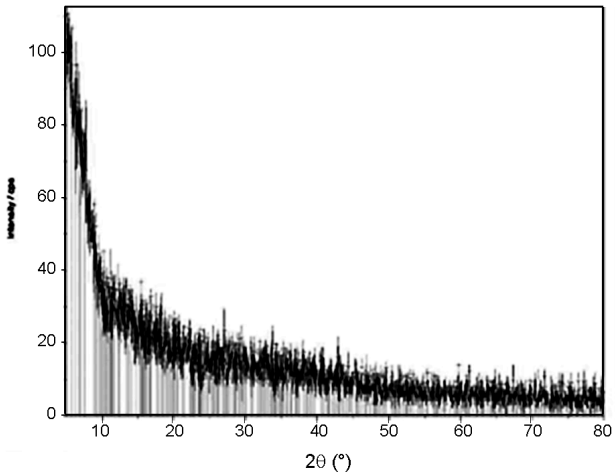

Fig. 5. XRD spectra of chitosan-coated FeNPs

Fig. 4 shows the FTIR spectra for FeNPs and chitosan-coated FeNPs. The peak at 539 cm^{-1} related to the Fe-O band, confirmed the presence of FeNPs in the synthesized samples. For chitosan-coated FeNPs, the peak at 3481 cm^{-1} assigned to OH and N-H groups. The bands at 2925 and 1745 cm^{-1} were due to C-H and C=O, respectively. So, FTIR study confirmed the coating of chitosan on the surface of FeNPs.

Fig. 5 shows the XRD pattern of chitosan-coated FeNPs. Crystalline nanoparticles at (111), (220), (311), (400), (422), and (511) showed peaks at values of 18/29°, 30/03°, 35/68°, 43/2°, 57/18° and 62/79°, respectively which are the characteristic peaks of the magnetite (Fe_3O_4).

Adsorption studies

Different concentrations of doxorubicin showed maximum absorbance at 280 nm. Fig. 6 shows the calibration curve of all concentrations of doxorubicin in maximum wavelength.

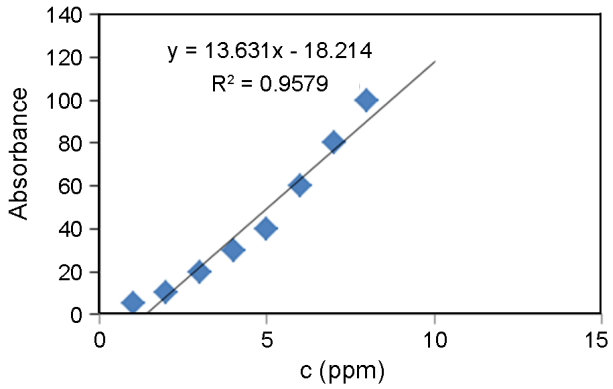

Fig.6. Calibration curve of maximum absorbance of Doxorubicin.

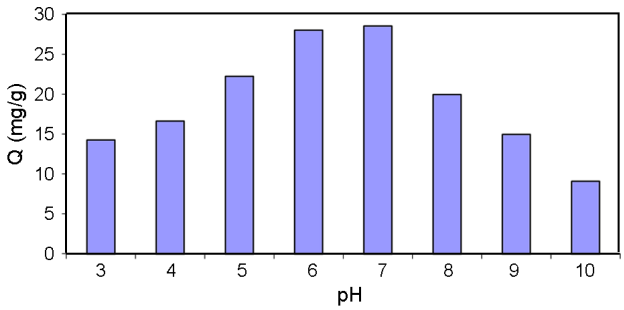

Fig. 7. The curve of optimum pH

The optimum pH of samples at 280 nm was 7 (Fig. 7).

Kinetic studies

A kinetic study of surface absorption provides a deeper understanding of the rate of drug loading on the adsorbent and the mechanism of adsorption process. The drug absorption rate determines the residence time required to complete the absorption process. Therefore, the pseudo-first-order (eq. 2), pseudo-second-order (eq. 3), and intra-particle diffusion (eq. 4) models were used to study the adsorption of Doxorubicin on modified FeNPs:

$$= q_e(1-e^{-k_1 t}) \quad (2)$$

$$q_t = \frac{k_2 q_e^2 t}{1+k_2 q_e t} \quad (3)$$

$$q_t = k_i t^{0.5} + C \quad (4)$$

Where q_e(mg g^{-1}) is the absorption capacity at equilibrium time. k_1(min^{-1}), k_2(g $mg^{-1}min^{-1}$), k_i(mg $g^{-1}min^{-1/2}$) are the pseudo-first-order, pseudo-second-order and intra-particle diffusion rate constants respectively. Kinetic parameters and determination coefficients are shown in Table 1. The values of the determination coefficient of pseudo-second-order model is higher than other two models. It is indicating that the experimental adsorption capacities ($q_{e,exp}$) are well described by pseudo-second order model. The intra-particle diffusion model can utilize to elaborate the adsorption mechanism. If the parameter C is equal to zero in the (eq. 4), the absorption process is controlled only by the intra-particle diffusion mechanism. The calculated values for parameter C at different temperatures are opposite to zero. Therefore, intraocular infiltration is

Table 1. Parameters of three kinds of kinetic models.

Kinetics models	Parameters	Content
Pseudo-first order	k_1 (min^{-1})	0.203125
	$q_{e,cal}$ ($mg\ g^{-1}$)	1.596337
	R^2	0.9959
	$q_{e,exp}$ ($mg\ g^{-1}$)	39.48387
Pseudo- second order	k_2 ($g\ mg^{-1}\ min^{-1}$)	0.426727
	$q_{e,cal}$ ($mg\ g^{-1}$)	39.52569
	R^2	1
	$q_{e,exp}$ ($mg\ g^{-1}$)	39.48387
Intra-particle diffusion	k_i ($mg\ g^{-1}\ min^{-1/2}$)	0.065
	C ($mg\ g^{-1}$)	38.577
	R^2	0.9471

not the only determining factor in the rate of adsorption of doxorubicin on Chitosan-coated FeNPs, but other processes such as exchange, sharing, and electron transfer can affect surface adsorption kinetics (Figs. 8 (a, b, c)).

The effect of time on the adsorption of doxorubicin by nano-adsorbent

At this stage, the goal is to investigate the effect of nano-absorbent contact time with doxorubicin on the adsorption of drug by nano-adsorbent. The results are shown

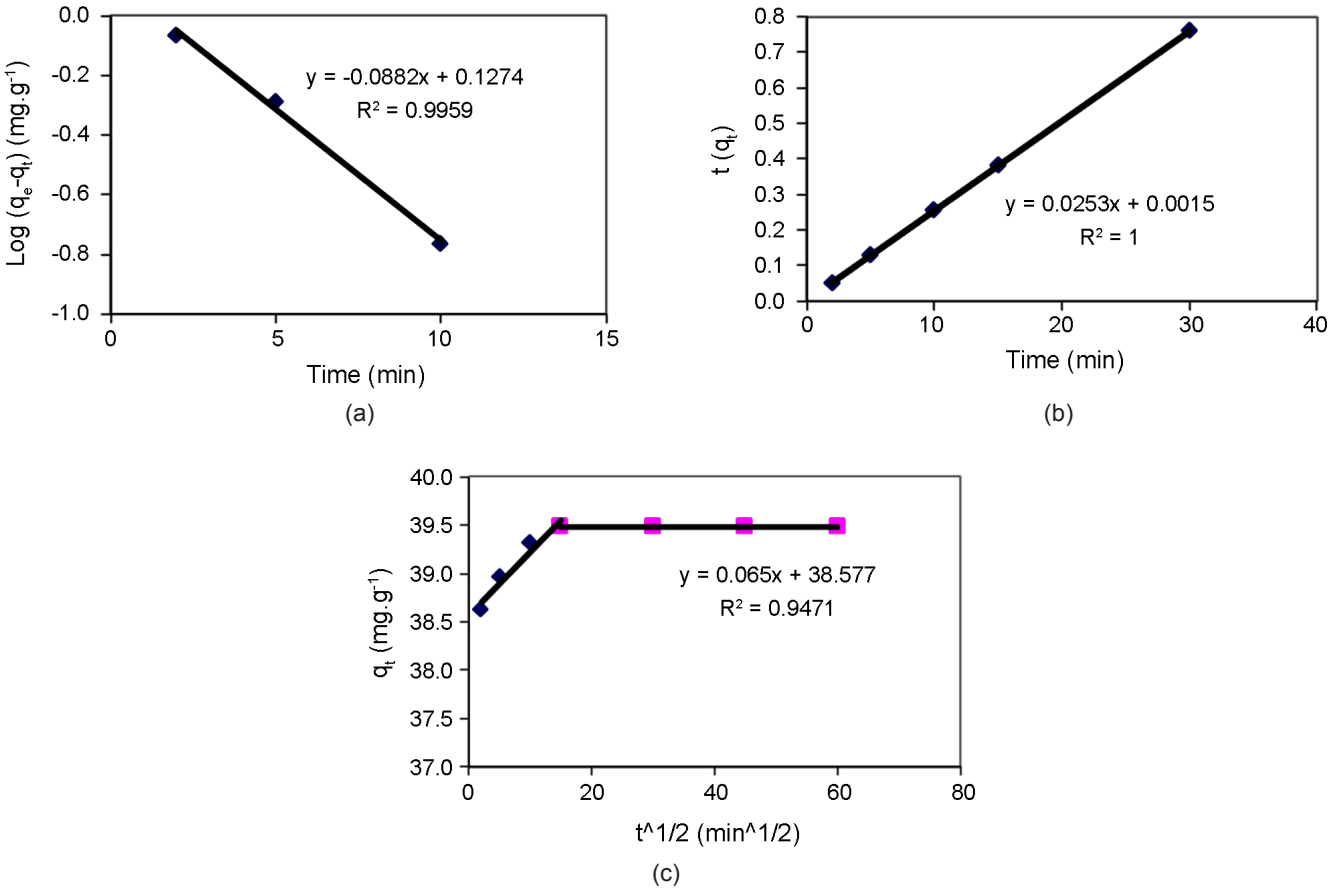

Fig. 8. Adsorption kinetics of doxorubicin on 0.005 g FeNPs at temperature of 298 K: (a) pseudo-first-order; (b) pseudo-second-order; (c) and intra-particle diffusion models.

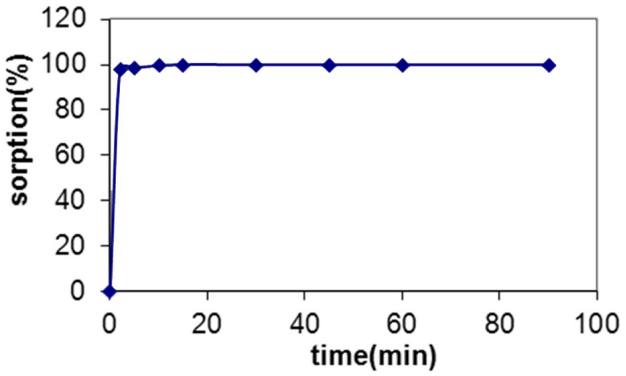

Fig. 9. Percentage of absorption at different times.

in Fig. 9. As these results indicate, the nano-adsorbent in the first two minutes exhibited the highest adsorption for doxorubicin, and then, with increasing time, the adsorption rate was a constant trend. Therefore, there is no need for long-term contact for the interaction of nano-absorbent and doxorubicin. It can also be concluded that the sites that are on the nano-absorbent drug are rapidly absorbed and available for the drug.

CONCLUSIONS

FeNPs were synthesized successfully by biological synthesis in an easy and less time-consuming manner using aqueous the extract of *Carum carvi L.* and under optimum conditions. Formation of FeNPs was confirmed by UV-Vis spectroscopy. FeNPs solutions showed maximum absorbance at 280 nm. The results obtained from the characterization of FeNPs showed they are spherical particles with less than 300 nm in size. Also, chitosan-coated FeNPs were synthesized to increased biocompatibility and the absorption capacity of nanoparticles. After the production of nano-absorbent, the maximum absorbance of different concentrations of Doxorubicin was determined which was 280 nm. The effect of pH was investigated on the absorption of Doxorubicin in maximum absorbance at pH 3-10. Doxorubicin showed maximum absorption at pH 7.

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