Iranian Journal of Chemical Engineering

Journal Homepage: www.ijche.com

pISSN: 1735-5397
eISSN: 2008-2355

Regular Article

Synthesis and Characterization of Doxorubicin Coated with Magnetic Copolymer Polycaprolactone-Polyethylene Glycol for Use in the Cancer Treatment

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ARTICLE INFO

Article history:
Received: 2021-12-20
Accepted: 2022-01-23
Available online: 2022-02-23

Keywords:
Magnetic Nanoparticles,
Doxorubicin,
Polycaprolactone-Polyethylene Glycol Copolymer,
Targeted Drug Delivery,
Kinetics of Drug Release

ABSTRACT

In recent years, the development of nanoparticles has received much attention in the controlled drug release and biomedicine fields. This research aims to develop new methods for the physical modification of Fe_3O_4 superparamagnetic nanoparticles with polymers through the physical retention. In this study, first, the degradable polycaprolactone-ethylene glycol copolymer and magnetic nanoparticles were synthesized. The anticancer drug doxorubicin was prepared using a dual-emulsion (w/o/w) copolymer containing magnetic iron nanoparticles. FT-IR, NMR, XRD, VSM, and, SEM analyzes were used to characterize copolymers and magnetic nanoparticles with drug-containing copolymer coatings. The results showed that nanoparticles had superparamagnetic properties and their particle size was between 70-150 nm. The drug encapsulation efficiency was about 96 %. The influence of pH and temperature on the drug release curve was investigated. The drug release was 31 % and 26 % after 144 hours in pH = 5.8 and 7.4 respectively. Since the extracellular fluid of the tumor is acidic, the rate of the drug release in these media will be better than the same in other cells. The kinetics of the drug release was also studied based on zero-order, first-order, Higuchi and Korsmeyer-Peppas models. Among the kinetic models, Higuchi was found to be the best model based on the correlation coefficient. The performance of the drug-loaded magnetic-copolymer nanoparticles with that of other similar studies was compared. The results revealed that the magnetic PCL-PEG copolymer with pH-sensitive properties can be used as an effective carrier for anticancer drugs delivery.

DOI: 10.22034/ijche.2022.320737.1421 URL: http://www.ijche.com/article_145331.html

1. Introduction

The development of nanotechnology in life

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sciences has led to the creation of a new branch called nanomedicine, which has been accompanied by a dramatic change in various fields of these sciences. The advent of nanoscale tools has opened a wide horizon in the treatment and diagnosis of diseases, especially the diagnosis and prevention of cancer, drug delivery, imaging, and biosensors. These systems can easily interact with biological molecules both on the cell surface and inside them and by accessing the areas of the body that were previously unthinkable, they can detect many of the biological processes that lead to certain diseases, including cancer, on time [1].

Cancer is still one of the most challenging diseases. With the spread of knowledge about this disease, many advancements have been made to treat it. Nevertheless, the toxic effects of chemotherapy drugs remain a treatment problem; because these drugs often work non-specifically. Over the past two decades, new drug delivery systems have been developed that have, to some extent, solved the problems associated with chemotherapy. These systems include nanoparticles containing organic and inorganic compounds. Many new nanoparticles have also solved the problem of cellular resistance to the drug and provided a new field in the treatment of cancer. The major advantages of nano delivery systems that make them superior to conventional delivery systems include the drug targeting, high cellular uptake, reduced side effects, controlled release, reduced therapeutic dose of the drug, improved physicochemical properties, and bioavailability of drugs [2-4].

The broad-spectrum drugs, such as hydrophobic or hydrophilic small drugs, biological macromolecules and vaccines, can be delivered using nanoparticles. Investigations in this field included the development of multifunctional pharmaceutical nanocarriers such as the inorganic nanoparticles, biodegradable polymers, micelles, … and surface engineering of carrier molecules [5].

Natural, synthetic, and semi-synthetic polymers are the most important materials in the preparation of environmentally friendly compounds and among them, synthetic copolymers with fully controlled structures and adjustable physical and chemical properties have been studied more than other polymers, especially in the field of pharmaceutical nanotechnology [6-8].

Among nanoscale materials, magnetic nanoparticles (MNPs) are known for the superior properties for medicine applications [3]. On the other hand, by placing specific ligands on the surface of nanoparticles, they can be used to bind to specific cellular receptors, resulting in targeted drug delivery to the cell. An example of targeted drug delivery is the placement of antibodies on the surface of nanoparticles to bind to a specific antigen on the tumor surface (Figure 1) [9].

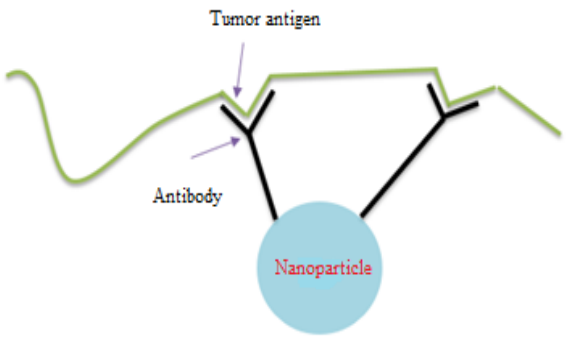


Figure 1. Binding of antibodies on the nanoparticle surface to a specific antigen on the tumor surface [9].

Poly(caprolactone) (PCL) is a biodegradable semi-crystalline poly α-hydroxy ester, generally considered as a

nontoxic, tissue-compatible polymer. However, the rate of the degradation of PCL is relatively low due to its high crystallinity and hydrophobic nature. Poly ethylene glycol (PEG) is known as a hydrophilic biocompatible polymer with significant biological or pharmaceutical applications. PEGylated therapeutics critically lead to the high aqueous solubility and in vivo circulation time with low enzymatic degradation. In addition, the PEGylation process leads to the passive tumor targeting with the enhanced permeability and retention (EPR) result [10-12].

One of the most potent and widely used anticancer drugs is doxorubicin, which works through preventing the formation of nucleic acids within cancer cells. The chemical structure of this drug is shown in Figure 2 [13, 14].

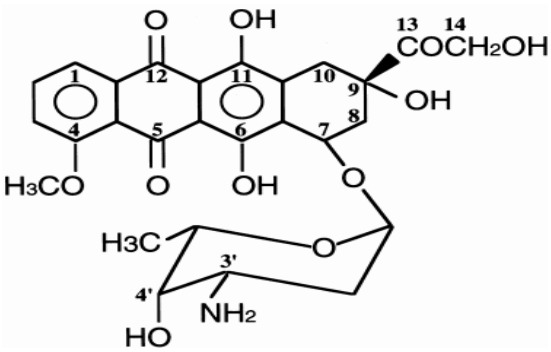


Figure 2. Chemical structure of doxorubicin [13].

In this research, the magnetite nanoparticles coated with PCL-PEG-PCL have been reported as a successful drug delivery system. For this purpose, the tri-block copolymer synthesized by ring-opening polymerization was modified by magnetic nanoparticles. Then, doxorubicin encapsulation was performed by the dual emulsion method (w/o/w) [11,12].

2. Materials and methods

2.1. Materials

The list of used materials and their manufacturer is given in Table 1.

2.2. Preparation of magnetic nanoparticles (Fe_3O_4) by the co-precipitation method

The conventional co-precipitation method was used to prepare magnetic iron nanoparticles [5]. 0.2242 g (7 mmol) of $FeCl_3.6H_2O$ and 0.4184 g (4 mmol) of $FeCl_2.4H_2O$ were mixed and dissolved in 10 ml of oxygen-free distilled water. The solution was placed inside a three-hole balloon (Figure 3). The balloon was transferred to a silicone bath at 85 °C. After a few minutes, an ampoule of bromine-containing ammonia was introduced into the reaction mixture. as soon as ammonia was added, the yellow solution suddenly turned black, indicating the formation of magnetic nanoparticles. Magnetic nanoparticles can be separated from water by a magnet.

$$2FeCl_3 + FeCl_2 + 4H_2O + 8NH_3 \rightarrow Fe_3O_4 + 8NH_4Cl \quad (1)$$

Table 1
List of materials used and their manufacturer.

Materials	Manufacturer
Polyvinyl alcohol	Sigma-Aldrich
Polyethylene glycol	Sigma-Aldrich
Capro lacton	Sigma-Aldrich
Diethyl ether	Sigma-Aldrich
$SnOct_2$	Sigma-Aldrich
Dichloromethane	Sigma-Aldrich

Doxorubicin	Merck
PBS	Merck
NaH_2PO_4	Merck
Na_2HPO_4	Merck
$FeCl_3.6H_2O$	Sigma-Aldrich
$FeCl_3.4H_2O$	Sigma-Aldrich
NH_3	Sigma-Aldrich

Figure 3. Preparation of magnetic nanoparticles and reaction formation.

2.3. Synthesis of polycaprolactone co-polymer-polyethylene glycol

For the synthesis of polymers, the ring-opening polymerization method was used. A certain amount of polycaprolactone (PCL) and polyethylene glycol with a molecular mass of 4000 (PEG_{4000}) were weighed and placed in a three-mouth balloon. The balloon was heated on a magnetic stirrer equipped with a heater. After melting the PEG, the temperature was raised to 120 °C. Then Tin octoate was added as a catalyst to initiate the polymerization reaction. After polymerization, the polymer was dried by filtering the solvent isolated in a vacuum attached to the desiccator. Figure 4 shows a schematic representation of the synthesis of copolymer poly caprolactone-ethylene glycol-caprolactone.

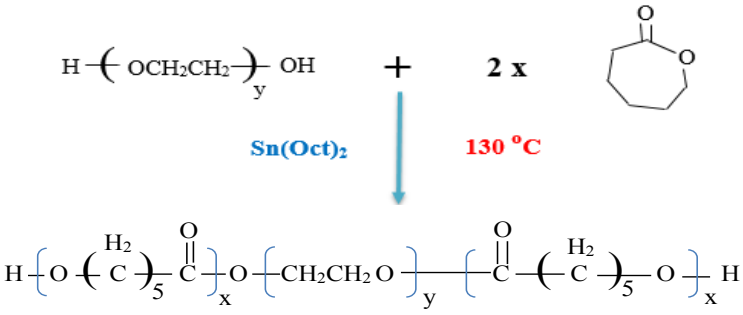


Figure 4. Schematic representation of the synthesis of copolymer poly caprolactone-ethylene glycol-caprolactone.

2.4. Preparation of PCL-PEG-PCL magnetic nanoparticles loaded with doxorubicin

Drug-containing nanoparticles and magnetic nanoparticles were prepared by the dual emulsion (W/O/W) method. Initially, 200 mg of polymer and 10 ml of doxorubicin, and 5 mg of iron oxide nanoparticles were dissolved in 15 ml of dichloromethane and stirred by Vertex. The organic solution was added to 50 ml of the aqueous solution containing polyvinyl alcohol as a stabilizer and stirred by a homogenizer. Then the organic solvent was removed by a rotary evaporator. After complete evaporation of the organic solvent, centrifugation was performed on the sample 3 times (15000 rpm each time for 15 minutes) for half an hour. The nanoparticle precipitate solution was isolated. The nanoparticles were dried in a freezer dryer. The supernatant was stored to measure the concentration of the unencapsulated drug [11, 12].

2.5. Investigation of the efficiency of the drug encapsulation in nanoparticles

The number of drugs loaded in the nanoparticles was measured indirectly by measuring the concentration of drugs in the residual solution after the formation of nanoparticles by UV spectroscopy for doxorubicin at 484 nm and the encapsulation efficiency was determined using the following equation:

Encapsulation efficiency (%) = (actual drug loading / theoretical drug loading) × 100

2.6. In vitro experiments in a buffer medium

A certain weight of lyophilized nanoparticles containing doxorubicin was suspended in 30 ml of a 0.5 M phosphate buffer solution (pH = 7.4). The solutions were incubated at 37 °C. For 7 days and at regular intervals, 3 ml of the transparent part of the solutions were sampled and at the same time, 3 ml of phosphate buffer solution was replaced by them. At the end of 7 days, about 20 samples were prepared, and using UV spectrophotometer and drawing calibration curves, the amounts of doxorubicin were measured in the collected samples. To investigate the effect of pH on the drug release, the drug release test was repeated in the acetic acid buffer solution at pH = 5.8 and the temperature of 40 °C [15-17].

2.7. Kinetic modeling

To study the kinetics of drug release from PCL-PEG-PCL magnetic nanoparticles loaded with doxorubicin, the release data were fitted to Zero order (Eq. (2)), First order (Eq. (3)), Higuchi (Eq. (4)) and Korsemeyer-Peppas (Eq. (5)). These kinetic models were analyzed by using Microsoft Office Excel (2016) to obtain the best fit model for the in-vitro release. In these equations, M_t/M_∞ represents the release fraction up to time t, K is the constant release rate and n is the delivery mode [18].

$$M_t/M_\infty=K_0t \quad (2)$$

$$M_t/M_\infty=1-\exp(-K_1t) \quad (3)$$

$$M_t/M_\infty=K_H\sqrt{t} \quad (4)$$

$$M_t/M_\infty=Kt^n \quad (5)$$

3. Results and discussion

3.1. Determination of the properties of the synthesized copolymers and nanoparticles

The structure and composition of the synthesized PCL-PEG-PCL tri-block copolymers were determined by Proton Nuclear Magnetic Resonance (1H NMR)

(Figure 5). The presence of ethylenes in PCL was observed around 1.24, 1.2, 1.3, and 4.06 ppm, the methylene groups of PEG were around 3.66 ppm.

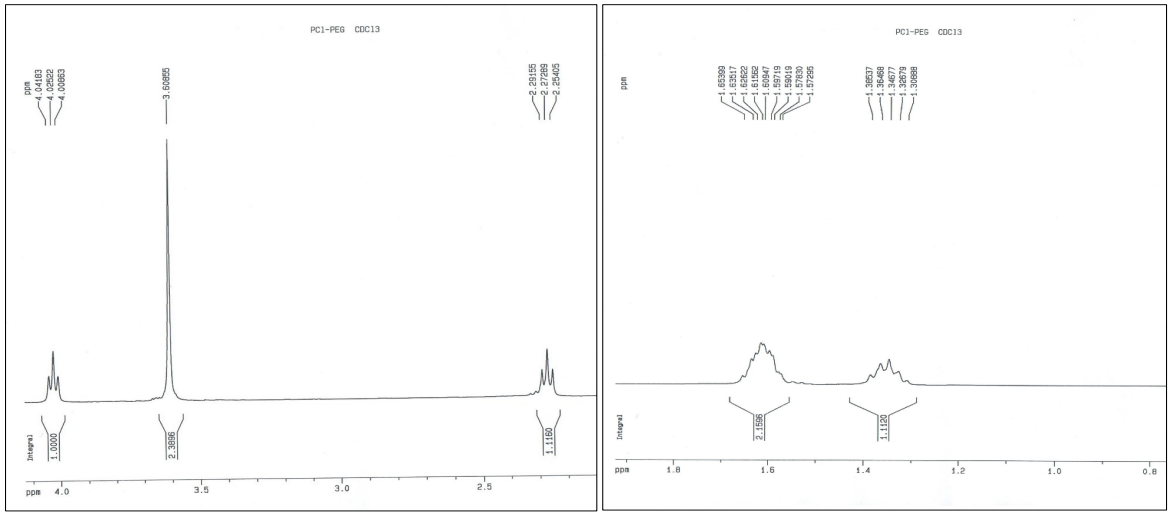


Figure 5. H-NMR of PCL-PEG-PCL triblock copolymers.

The XRD spectrum of PCL_{1000}-PEG_{4000}-PCL_{1000} is shown in Figure 6. As it can be seen from this figure, three index peaks appear at 2θ = 19.5, 22.05 and 23.08. Comparing this spectrum with the XRD spectrum of polyethylene glycol, it is concluded that the intensity of the peaks related to polyethylene glycol is reduced instead of that of the peaks related to caprolactone and this indicates an increase in the crystalline percentage of the resulting tri-block co-polymer compared to pure polyethylene glycol.

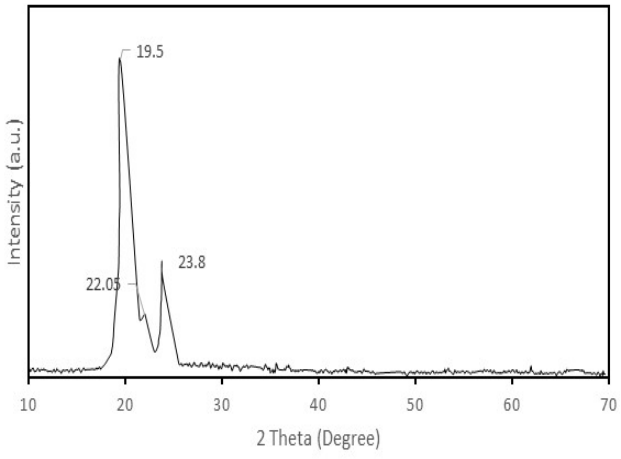


Figure 6. XRD spectra of PCL_{1000}-PEG_{2000}-PCL_{1000} copolymer.

FTIR spectra were used to show the structure of Fe_3O_4, the copolymer PCL_{1000}-PEG_{4000}-PCL_{1000}, and the structure of Fe_3O_4-PCL-PEG-Dox.

Figure 7 (a) shows the absorption bands of 446.75, 576.137 and 671 cm^{-1}, which are related to the vibration of the Fe-O bond. The absorption band of 3433.38 cm^{-1}, which belongs to the hydroxyl groups (OH) of Fe_3O_4 magnetite, was also observed.

For the copolymer, the tensile vibration of the hydroxyl group is visible in the region of 3457 cm^{-1}. In the region of 2856 cm^{-1} and 2922 cm^{-1}, it is related to the tensile vibration of the group of methylene in the tri-block copolymer. stretching vibrations in the 1739 region are related to the carbonyl group, vibration in the range of 977 cm^{-1} to 1133 cm^{-1} is related to the tension of carbon-oxygen bonds (Figure 7(b)).

For the drug loaded in magnetic-polymer nanoparticles, NH_2 stretching vibrations are observed in 3622, 3680 and 3740 cm^{-1}. The absorption bands of 2870 cm^{-1} and 2946 cm^{-1} are related to the tensile vibrations of C-H and 1650 cm^{-1}, 1732 cm^{-1} to carbonyl bonds C =

O, and 1177 cm^{-1}, 1242 cm^{-1} to C-C, C-O bonds; 671 cm^{-1} is related to an Fe-O bond (Figure 7(c)).

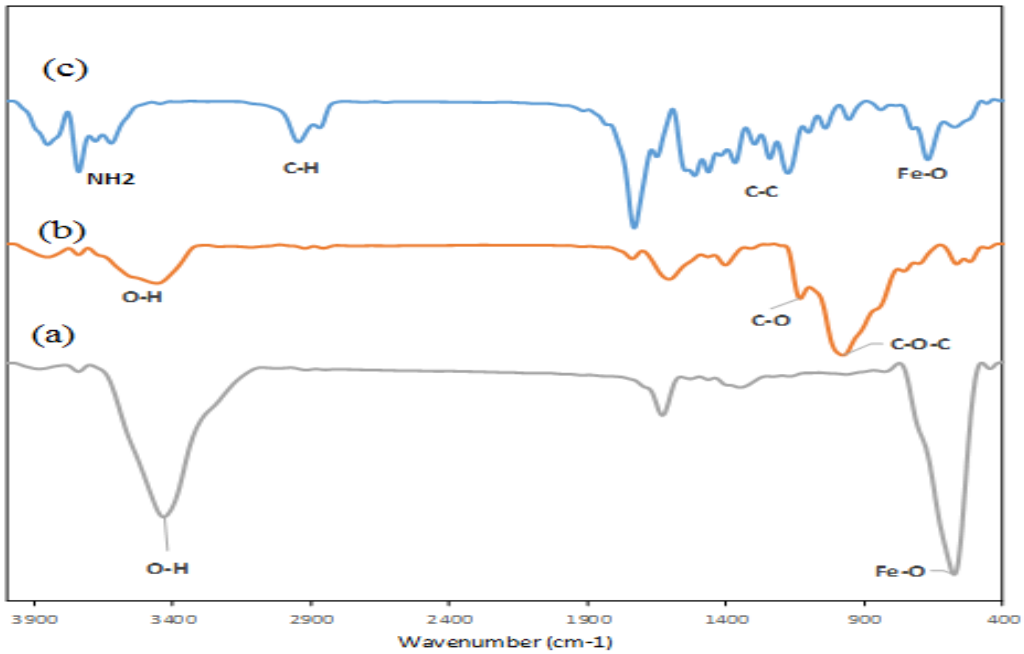
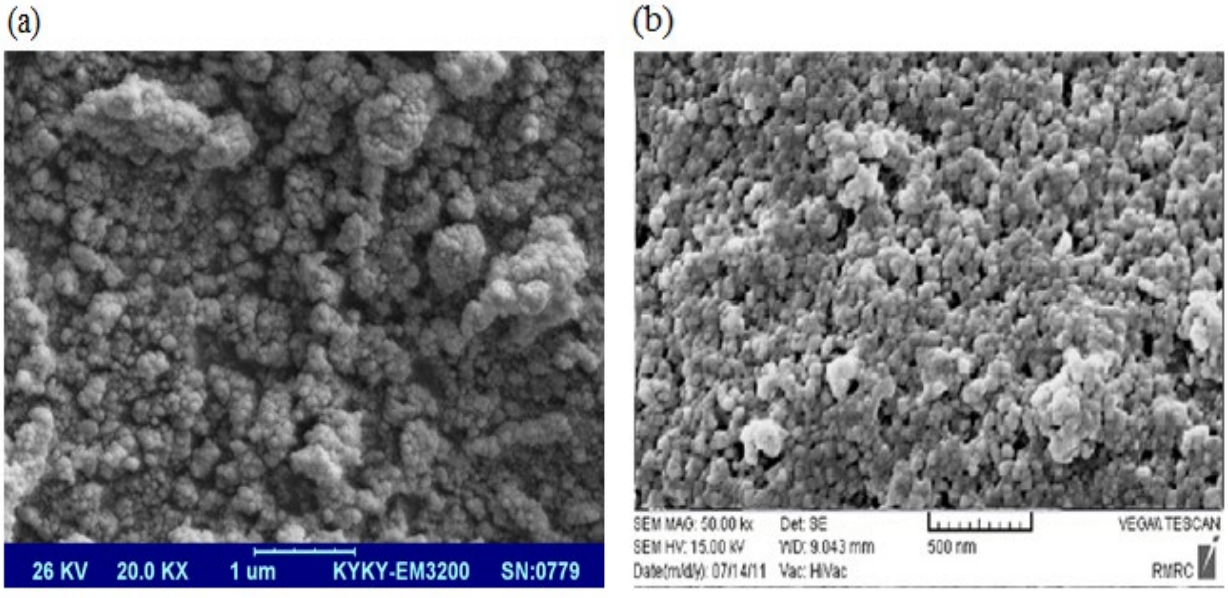


Figure 7. FTIR spectrum (a) Magnetite (Fe_3O_4), (b) Copolymer PCL1000-PEG4000-PCL1000, (c) Drugs loaded with magnetic nanoparticles – copolymer.

The morphology of magnetic nanoparticles, copolymers, and the drugs loaded with magnetic-copolymer nanoparticles was observed by SEM (Figure 8). Figure 8 (a) shows the SEM image of Fe_3O_4 magnetic nanoparticles. The size of magnetic nanoparticles was determined between 8 and 40 nm. Figure 8 (b) shows PCL-PEG copolymers of which the particles have a spherical morphology. SEM images of the drugs loaded with magnetic-copolymer nanoparticles are shown in Figures 8 (c) and (d). The size of nanoparticles varies between 15 and 100 nanometers.



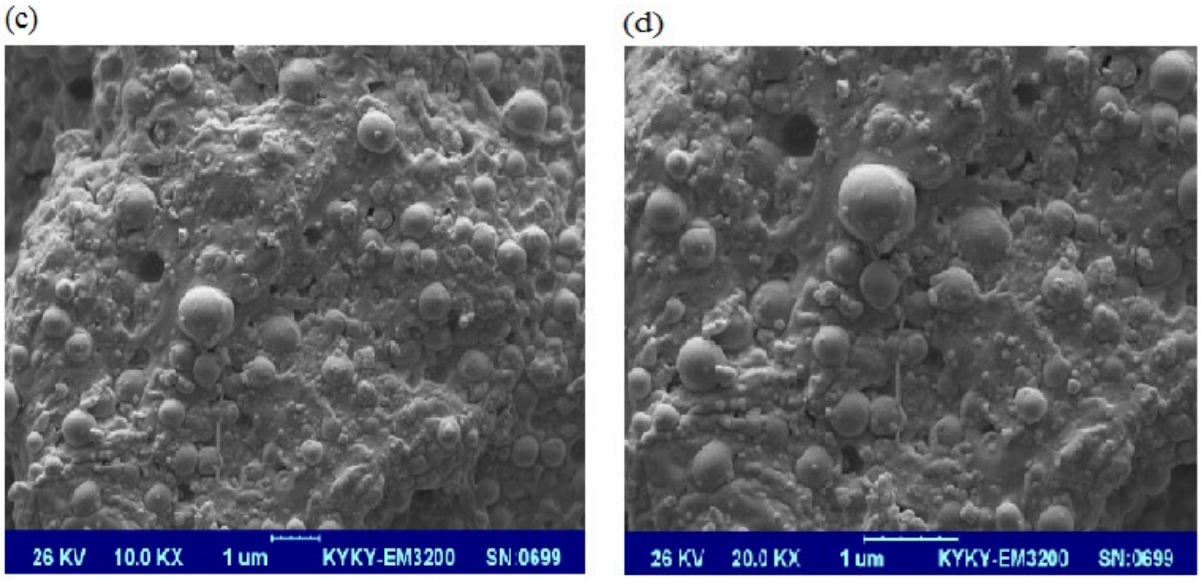


Figure 8. SEM images (a) Fe_3O_4 magnetic nanoparticles, (b) Copolymer sample (c) and (d) Drugs loaded with magnetic nanoparticles and copolymer.

3.2. Evaluation of the drug release rate

The measurement of the pH of extracellular fluids in solid tumors has shown that acidic pHs are suitable for the growth of solid tumors [19]. Due to this fact, pH-sensitive nanoparticles containing anti-cancer drugs have been prepared.

Figure 9 shows the pH-dependent release of doxorubicin from Fe_3O_4- PCL_{1000}-PEG_{4000}-PCL_{1000}-DOX.

The amount of Doxorubicin released from magnetic-copolymer nanoparticles at pH = 5.8 is more than the same at pH = 7.4. Therefore, it can be expected that the drug release rate in the acidic medium of the extracellular fluid of the tumor is higher than in other cells.

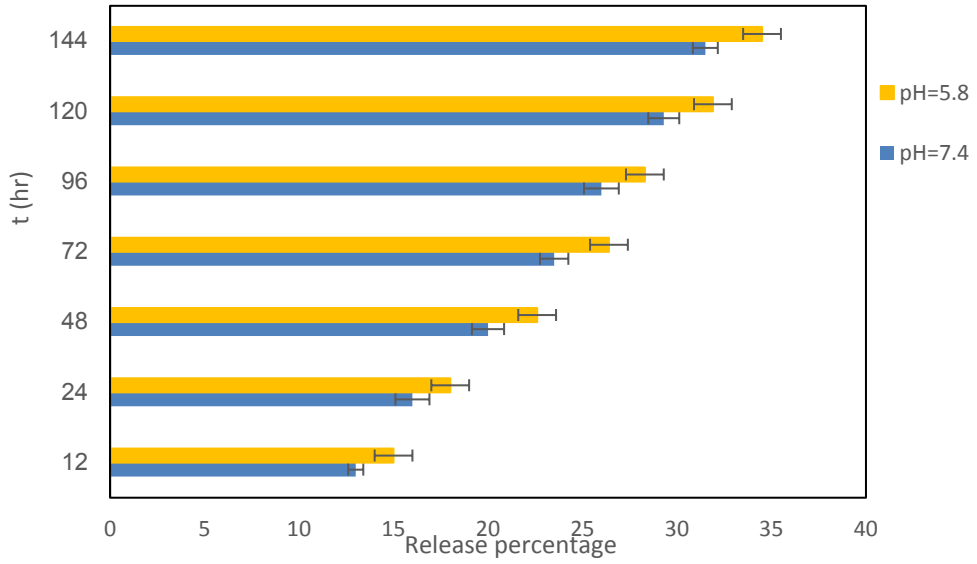


Figure 9. The rate of the release of doxorubicin from Fe_3O_4-PCL1000-PEG4000-PCL1000-DOX at different pHs.

3.3. Comparison of the performance of the drug with similar researches

Table 1 compares the performance of the drug-loaded magnetic-copolymer nanoparticles with other similar studies. As shown in the table, none of these studies used magnetic nanoparticles. The encapsulation efficiency obtained in the present study is higher than those of other researches and as for the drug release at lower pH values, similar results have been obtained in all studies.

Table 1
Comparison of the performance of loaded drug with the same in similar researches.

Use of magnetic nanoparticles	encapsulation efficiency	pHs studied in drug release	
-	86.7 %	pH = 5.5 pH = 7 More release at pH = 5.5	Zhang et al. [20]
-	70 %	pH = 5 pH = 7.4 More release at pH = 5	Liang et al. [21]
-	86.6 %	pH = 5.5 pH = 7.4 pH = 8.4 More release at pH = 5.5	Xu et al. [22]
Use of iron oxide nanoparticles and reduction of magnetic saturation from 60 to 5	96 %	pH = 5.8 pH = 7.4 More release at pH = 5.8	present study

3.4. Kinetic studies of the drug release

The release kinetics of the encapsulated drug was investigated based on four models of Zero order, First order, Higuchi, and Korsmeyer-Peppas. The data obtained from the mentioned models were fitted and evaluated based on the correlation coefficient (R^2) in Table 2.

Table 2
Kinetic parameters of the release of doxorubicin from nanoparticles.

Model type	Zero order model		First order model		Higuchi model		Korsmeyer-Peppas model		
	K	R^2	K	R^2	K_H	R^2	N	K	R^2
Doxorubicin	0.0015	0.7982	0.0008	0.8252	0.0204	0.9017	0.2794	0.0617	0.8353

The release kinetics of doxorubicin at pH = 5.8 are shown in Figures 10, 11, 12 and 13 for the Zero order, First order, Higuchi, and Korsmeyer-Peppas models respectively.

In this study, according to Figure 10 and the correlation coefficient of doxorubicin released from magnetic-polymer nanoparticles, the Higuchi model was followed.

In the Higuji model, R^2 = 0.9017 was calculated, which is higher in this model and is closer to one. The higher the R^2, the better the data fits the model and the most appropriate the model. Therefore, it can be concluded that the release of doxorubicin

follows this model which implies that the release of the drug from the matrix is a square root of the time-dependent process and the diffusion is controlled [23].

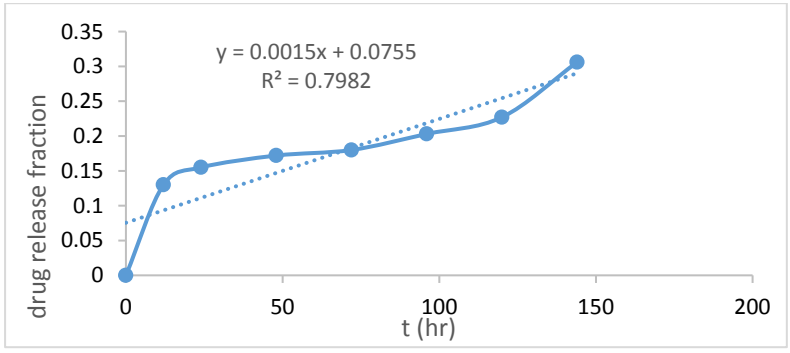


Figure 10. Study of the release kinetics of doxorubicin for zero-order model.

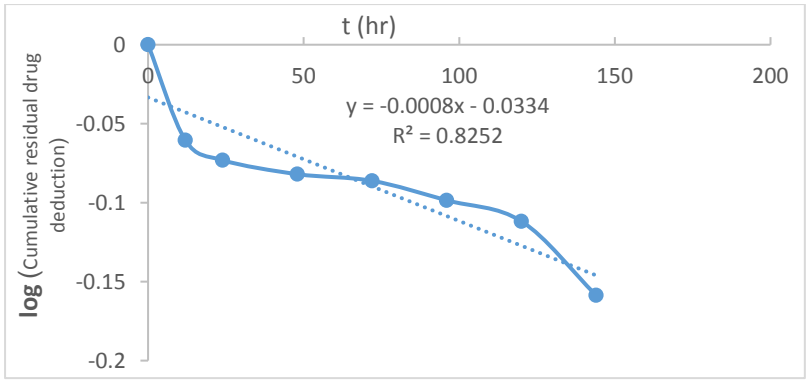


Figure 11. Study of the release kinetics of doxorubicin for the first-order model.

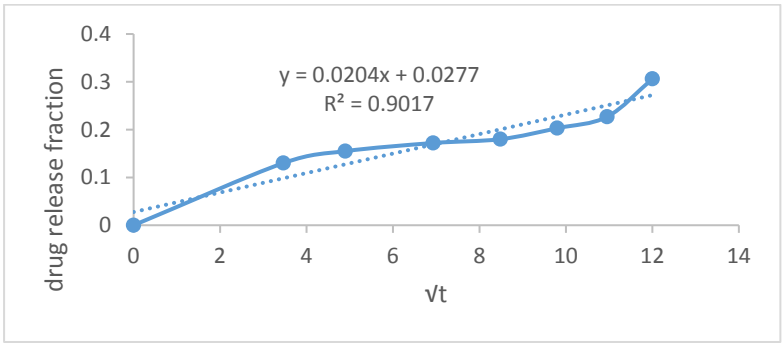


Figure 12. Study of the release kinetics of doxorubicin for Higuchi model.

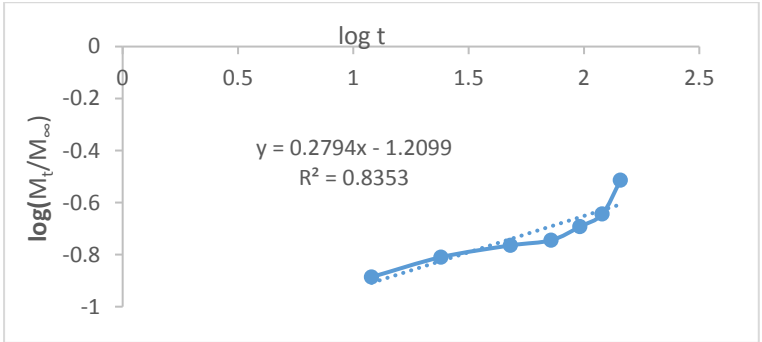


Figure 13. Study of the release kinetics of doxorubicin for Korsmeyer-Peppas model.

4. Conclusions

Nanoparticles with a size of less than 100 nm are highly desirable for pharmaceutical delivery applications. The first step to achieve this goal was to prepare the core of hybrid nanoparticles. In the present research, iron superparamagnetic nanoparticles (Fe_3O_4) with a size of less than 50 nm were prepared by the co-precipitation method. PCL-PCL-PEG nanoparticles (PEG_{4000}) were synthesized by the ring-opening polymerization method. The structure of the synthesized copolymer was confirmed by reviewing the results of various tests. Magnetic iron oxide nanoparticles with doxorubicin were encapsulated in copolymer nanoparticles, and a dual emulsion method was used. The percentage of the drug encapsulation for this copolymer was evaluated and was obtained as 96 %. By encapsulating these magnetic nanoparticles with the drug, the magnetic residue is reduced to 5 emu/g, indicating the physical coating of the magnetic nanoparticles by the copolymer. This property of the nanoparticle is very effective in the targeted drug delivery.

Samples were lyophilized for in-vitro testing and stored in a freezer. The release rate of the drug was investigated at different pH values. It was observed that at the acidic pH, the release rate of the drug was higher than in the neutral environment. Because the extracellular fluid of the tumor has an acidic environment, the release of the drug in this environment is faster. Also, kinetic studies were performed and according to their results and the correlation coefficient of doxorubicin released from magnetic-co-polymer nanoparticles, it followed the Higuchi model.

The attraction of magnetic nanoparticles towards magnetic fields is a property that leads to the targeting of drugs attached to these particles in the body. So magnetic nanoparticles are a solution to carry the drug to the desired areas in the body. The external magnetic field application guides magnetic nanoparticles to tumor tissue and helps to effectively treat cancerous tumors.

The use of nano drugs, especially magnetic nanoparticles coated with biocompatible polymers, instead of traditional chemotherapy methods, can be a safe, very convenient and inexpensive method.

Acknowledgement

The authors thank the Department of Chemical Engineerig, Ahar Branch, Islamic Azad University for all their supports.

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