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RESEARCH PAPER

# Preparation of Degeredable Polyprolactone Polymer (PCL)/ Magnetic nanocomposite for Drug Delivery Systems Against Anticancer Compounds

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**ABSTRACT**

Magnetic nanoparticles have been used primarily for medical advances, chemotherapy, and specialized tissue repair for targeted drug delivery. In this research, magnetic iron nanoparticles were first prepared and identified. Then, biodegradable copolymer of polypro-pylene capro-lactone-polyethylene glycol PCL-PEG1000-PCL was synthesized. Doxorubucin nanoparticles were prepared by using copolymer containing magnetic nanoparticles by solvent-evaporation method. VSM, FT-IR, UV-vis, $^{1}$H-NMR and SEM were used to determine the structural properties of copolymer nanoparticles. The synthesis of PCL-PEG1000-PCL triple-block copolymer and doxorubicin and iron nanoparticles encapsulation were confirmed by the mentioned characterization methods. The resulting nanoparticles have superparamagnetic properties and the drug encapsulation yield was about 95%. The effect of pH and heat on drug release curve was investigated. The results showed that the copolymer synthesized is suitable for the encapsulation of doxorubicin and iron nanoparticles and can be effective as a carrier of novel nanostructures in the delivery of anticancer drugs. The results showed that due to the properties of magnetic nanoparticles and copolymers they can be used for targeted drug delivery for targeted drug delivery.

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## INTRODUCTION

Cancer is a major threat to human life, and chemotherapy is still a common method used to treat cancers [1]. Cancer has become a big challenge in medical science in recent years, especially due to the increase in the use of chemicals and other mutagenic agents, and on the other hand, the increase in life expectancy due to medical advances and its increasing prevalence

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in different societies [2]. The highest percent of cancers include lung cancer, colon cancer, liver cancer, breast cancer in women, and prostate cancer in men respectively. Many drugs have been used to treat different cancers depending on the type and stage of the disease. In many cases, the use of a very effective drug due to properties such as hydrophobicity or instability in the body environment and these disadvantages make the drug effectiveness impossible or very difficult in treating the disease. Many drug delivery systems have been developed by various research groups to solve these problems, and some of them have reached the commercial stage or are at the clinical trial stages.

The use of nanoparticles to transfer drugs to cancer cells is a promising way to overcome this problem [3]. Nanoparticles are defined as very fine particles with a size between 1-100 nm. The nanoparticle drug delivery systems lead to drug stability, increase in blood shelf life, the provision of lower doses of effective chemotherapy agents, nanoparticle control, and drug distribution inside the body [4].

The main purpose of designing any effective drug delivery system is to target drugs to specific cells or tissues in the body that increase the therapeutic effects of drugs, reduce drug side effects, and reduce the therapeutic doses of drugs, and ultimately modify the physicochemical properties of drugs [5]. Nanostructured compounds and synthetic copolymers have significantly achieved these goals and overcome limitations such as low adsorption, low solubility, low stability, explosive release, or incomplete encapsulation of drugs [6]. Furthermore, the modification and development of structures of amphiphilic copolymers, which are composed of hydrophilic and hydrophobic monomers, have largely solved the problems with solubility or insufficient absorption of drug-polymer systems.

Conventional chemotherapy drugs are inactively distributed throughout the body, affecting cancer and healthy cells together. Therefore, they limit the dose that can be absorbed inside the tumor. Targeted cell therapy has been developed as a method to overcome the limitations of drugs in chemotherapy. The higher resistance in cell cancer can be poisoning. Designing the dynamic biological systems on a nano-micro scale and creating pharmaceutical carriers, the researchers have created promising hopes in this regard. The nanoparticle structure, with its small size, can penetrate the blood-brain barrier that is impermeable to most therapeutic matters and imaging agents. The use of magnetic nanoparticles in medicine, especially in cancer diagnosis and treatment, has attracted the attention of many researchers in the last two decades. A review of authoritative references and journals about the research subject indicated that the targeted transfer of magnetic nanoparticles to cancer cells was through active and inactive methods. In the active method, the targeted transfer of nanoparticles to the tumor takes place using specific molecular ligands of tumor cells and external magnetic field radiation to the tumor area, but in the inactive method,

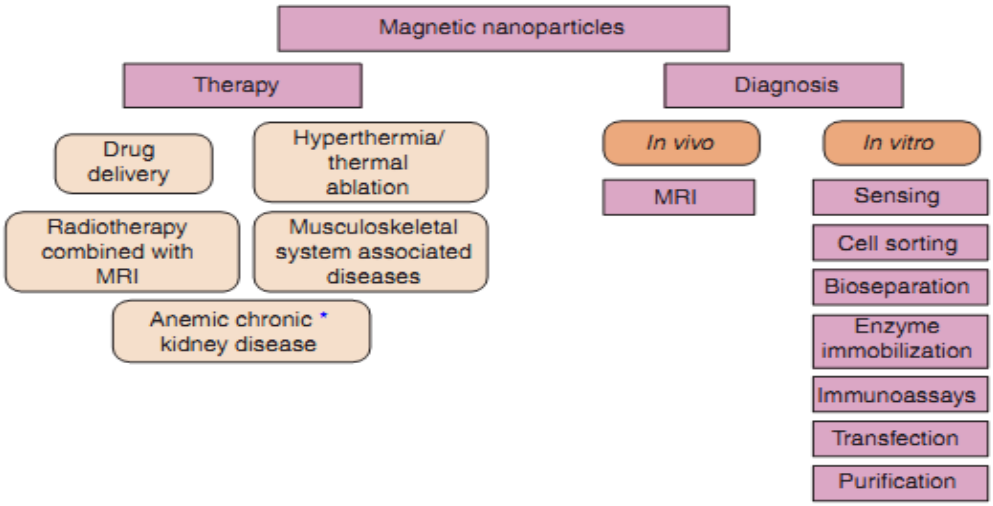


Fig. 1. Applications of iron oxide superparamagnetic nanoparticles [9]

they penetrate the tumor due to the enhanced permeability and retention of nanoparticles. Considering the magnetic behavior of magnetic nanoparticles as well as their ability to carry drugs, these particles have multiple applications in biomedicine, including targeted drug delivery to tumors, magnetic resonance imaging, and cancer treatment by hyperthermia. According to a study by Mohammad Ghanbari and Ali Ghanbari, the use of magnetic nanoparticles in medicine leads to the development of targeted and effective therapies in the treatment of cancer, and thus reduces the side effects and biological damage caused by chemotherapy in patients [7].

Magnetic nanoparticles are widely used in medical advances and therapeutic and diagnostic applications such as high-resolution magnetic resonance imaging (MRI), cell isolation, biosensors, hyperthermia, chemotherapy of special sites, repair of special tissues, and targeted drug delivery. In this regard, much effort has been made into the preparation of monodispersed magnetic nanoparticles and functionalization of the surface of these nanoparticles with biocompatible and water-soluble groups and target ligands (e.g. folic acid) [8].

In recent years, magnetic nanoparticles have been increasingly used as a solid basis for fixing various types of biomolecules. The advantage of such magnetic nanoparticles is the ease and acceleration of the release of special biomolecules from their complex mixture. This is possible because the biomolecules stabilized on magnetic nanoparticles are easily controlled by an external magnetic field [3].

Polymer coatings or coatings such as dextran and agarose can be used to fix biomolecules and drugs on magnetic nanoparticles. However, direct binding of proteins to the surface of magnetic nanoparticles has also been reported due to the presence of free hydroxyl groups (OH) on magnetic nanoparticles of $Fe_3O_4$. In recent studies, proteins such as bovine serum albumin (BSA), Glucose oxidase, streptokinase, chymotrypsin, and dispase successfully bind magnetic nanoparticles without polymer coating mediated by carbodiimide (CDI) as a bridge and cross-linker [10].

## MATERIALS AND METHODS

Table 1 presents the list of materials used in the present research.

The list of equipment used in the project is as follows:

Chemical structures of copolymers were used according to FT-IR (Shimadzu 8400, Japan) and device $^1$H-NMR Bruker Avance 400 apparatus. The sample was dissolved in deuterated chloroform ($CDCl_3$).

Table 1 presents the list of materials used in the present research

| materials | Fabricant |
|---|---|
| (PVA) | Sigma-Aldrich |
| (PEG) | Sigma-Aldrich |
| (ε-Cl) | Sigma-Aldrich |
| (DEE) | Sigma-Aldrich |
| ($SnOct_2$) | Sigma-Aldrich |
| (DCM) | Sigma-Aldrich |
| (DOX) | Pharmacy grade |
| (PBS) | Merck |
| ($NaH_2Po_4$) | Merck |
| ($Na_2HPo_4$) | Merck |
| ($FeCl_3$) | Sigma-Aldrich |
| ($FeCl_2$) | Sigma-Aldrich |
| ($NH_3$) | Sigma-Aldrich |

The polymerization reactions were performed using a vacuum pump (Heidolph, Germany) at low pressure. The products were isolated by using an ultracentrifuge model Beckman, Optima TLX, USA. To investigate the size and surface morphology of nanoparticles, scanning electron microscope (SEM, VEGA/TESCAN model 440i) was applied. Also a freeze dryer model Christ Alpha 1-4, the USA was used to dry nanoparticles after ultracentrifugation.

To measure the amount of drug, a spectrophotometer UV Shimadzu 160 is utilized. and used to filter particles. Homogenizer (Edmund Buhler HO 4AP, 10000 rpm, 3×10 s) and 1.2-mm filters (Millex AP, Millipore) were used during preparing of nano products.

*Synthesis and characterization methods*

*Preparation of magnetic nanoparticles ($Fe_3O_4$) using the co-precipitation method*

The conventional co-precipitation method is applied to prepare magnetic iron nanoparticles, an exact amount of 7.806 g (28 mmol) of $FeCl_3.6H_2O$ and 2.967 g (16 mmol) of $FeCl_2.4H_2O$, was dissolved into 50 ml of oxygen-free distilled water (deoxygenation for half an hour) (the interval between dissolving iron chlorides and starting the reaction should not be too long as it causes oxidation of $Fe^{3+}$ and $Fe^{2+}$). Molar ratio of $Fe^{3+}$ to $Fe^{2+}$ was 1:1.75. The solution was poured into a three-opening balloon. After dissolving iron chlorides in distilled water, the solution was deoxygenated by passing a stream of nitrogen gas and the balloon was transferred to a silicone bath at 85°C. After a few minutes, a bromine ampoule with 25% ammonia was applied to add 90 ml of ammonia into the reaction mixture within a few seconds (it should be noted that the speed of the mechanical stirrer is about 400 rpm at the beginning of the reaction but it will reach 1300 rpm as soon as the ammonia solution is poured). The increase in ammonia continues until the pH reached to 9.5-11 (approximately 40 ml). As soon as ammonia is added, the yellow solution suddenly turns black, indicating the formation of magnetic nanoparticles. The amount of $Fe_3O_4$ product was about 2.760 g and the reaction efficiency was 85%. Magnetic nanoparticles can be separated from water by a magnet and decanting the supernatant.

$$2FeCl_3 + FeCl_2 + 4H_2O + 8NH_3 \rightarrow Fe_3O_4 + 8NH_4Cl$$

*Synthesis of PCL-PEG-PCL Triblock Copolymer*

For polymer synthesis, polyethylene glycol and caprolactone monomer were used in a ring-opening polymerization by mass polymerization. Certain amounts of polycaprolactone (PCL) and Polyethylene glycol (PEG) with a molecular mass of 1000 ($PEG_{1000}$) were carefully weighed and placed in a three-opening balloon equipped with the nitrogen gas inlet and outlet. The balloon was heated on a magnetic stirrer equipped with a heater. To accurately control the temperature at all stages, a thermometer was placed in the bath at a constant height equal to the bottom of the balloon. After PEG melting, the temperature increased up to 130 °C. Tin octoate catalyst ($Sn(oct)_2$) (0.05 wt% of raw material) was then added to the melt as a catalyst to initiate the polymerization reaction. Polymerization continued at this temperature with gentle stirring and nitrogen gas flow (At the first 1 to 2 hours of polymerization, it is necessary to completely prevent the entry of oxygen gas into the balloon. This is done by oxygen degassation through blowing $N_2$ gas during the polymerization). After polymerization, the solution was cooled to room temperature. To purify the impure polymer and separate it from the remaining monomers, the solid polymer was dissolved in dichloromethane and poured into a large volume of dry diethyl ether. Diethyl ether acted as a solvent and led to the precipitation of the polymer. The process was repeated twice. The polymer was isolated from the solvent by filtering and dried in a vacuum attached to the desiccator.

Various PCLx -PEGy-PCLx triblock copolymers can be synthesized depending on the choice of the molecular mass of the raw materials. In the present study, polycaprolactone (1000g/mol) and the molecular mass of polyethylene glycol (1000 g/mol) were chosen.

*Preparation of magnetic nanoparticles of PCL-PEG-PCL loaded with doxorubicin*

Drug-containing magnetic nanoparticles were prepared by the emulsion solvent evaporation method. First, 200 mg of polymer and 20 mg of doxorubicin, and 5 mg of Iron oxide nanoparticles were mixed in 20 ml of dichloromethane and stirred by a vortex mixer. The organic solution was poured into 100 ml of the aqueous solution containing 1% polyvinyl alcohol as an emulsion stabilizer and stirred with a homogenizer. Then this organic solvent was then removed by a rotary

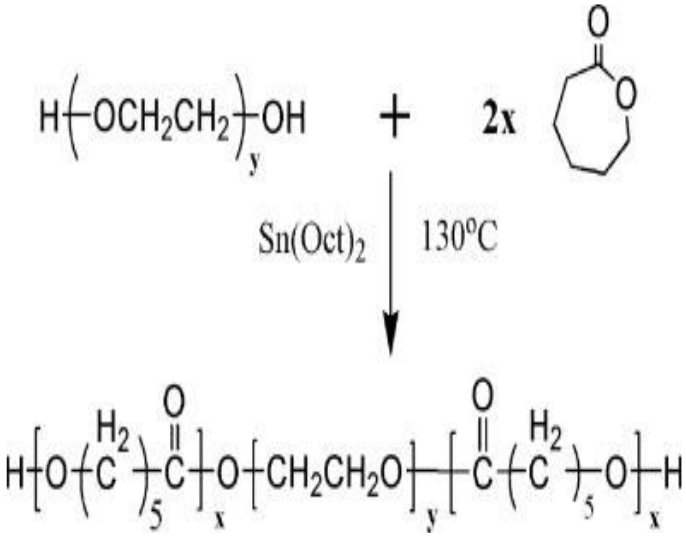


Fig. 2. Schematic image of the synthesis of PCL-PEG-PCL triblock copolymer (polycaprolactone- Polyethylene glycol-polycaprolactone)

evaporator.

Centrifuge separation was repeated 3 times (each time at 12000 rpm for 15 minutes) after complete evaporation of organic solvent for half an hour by rotation on the sample. Then the nanoparticle sediment solution was separated and nanoparticles was dried in a freezer dryer. The supernatant was stored to measure the concentration of the unencapsulated drug.

*Determination of physicochemical properties of polymer and drug loading in polymer*

Infrared spectroscopy studies of the synthesized drug-loaded nanocomposite indicated for presentation of different functional groups in the structures of the resulting branched polymers, such as carbonyl, ether, methylene, methyl, and other groups. First, a certain concentration of each sample is prepared in dichloromethane solvent, and then a thin film on KBr plates is formed. Then, FT-IR spectra was taken.

*Scanning electron microscope (SEM) images*

Scanning electron microscope (SEM) images are applicable to examine the surface structure, cross-sections, sizes, and morphology of drug-containing nanoparticles. SEM was applied to scanning point-by-point of nanoparticles containing DOX at a voltage below 30 kV in different magnifications and under vacuum pressure of 5-10. Drug-containing copolymers was dispersed in a small amount of water and SEM imaging was performed after spraying the suspension on the gold grade. The gold deposition apparatus in the electron microscope laboratory was a Suptter coater made by the KYKY Company and had a model SBCI2. The lyophilized samples of DOX-containing nanoparticles coated with magnetic iron particles in the form of a powder containing nanoparticles were put on a special double-sided adhesive stick on an aluminum piece. The surface of the samples was then covered with a thin layer of gold by a particle coating apparatus in the presence of argon plasma. The cross-section of the nanoparticles was created by mechanical stress. Finally, the SEM imaging of nanoparticles containing two drugs was performed.

*Investigation of vibration of nanoparticles using the vibrating-sample magnetometer (VSM)*

Magnetic property of the drug loaded sample was analysed by using the vibrating-sample magnetometer (VSM) and compared it with samples of free iron nanoparticles.

*Calculation of the amount of drug loading*

After drying by solvent evaporation, the drug loading by the free drug concentration method using UV-Vis spectrometry was calculated.

*Determining the loading capacity of doxorubicin in drug-containing polymer nanoparticles using the ultraviolet spectrophotometer*

50 mg of the drug-containing polymer

nanoparticles was dispersed in 25 ml of decinormal hydrochloric acid and ethanol with a volume ratio of 1:1. The sespension was mixed by a magnetic stirrer at a low speed for 24 hours and then centrifuged at 10,000 rpm. The supernatant was separated and passed through Whatman filter paper and the drug content was examined using an ultraviolet spectrophotometer at a maximum wavelength of 484 nm.

To have an accurate comparison, another sample containing 50 mg of the drug loaded nanoparticles was dispersed in 25 ml of decinormal hydrochloric acid and ethanol with a volume ratio of 1: 1. All praparing condition was same to the previous sample (drug-containing polymer nanoparticles). Using equation 1, the drug loading capacity in nanoparticles as the drug carrier was calculated by using ultraviolet spectrophotometer [11].

Drug loading percentage=(actual drug content/ theoretical drug content)×100 Eq (1)

Using standard solutions, the grading curve was plotted and the actual drug concentration was calculated according to the grading equation.

*Investigation of drug encapsulation efficiency in nanoparticles*

The amount of drug loading on the nanoparticles was measured indirectly by measuring the drug in the remaining solution after the formation of nanoparticles through UV spectroscopy at 303 nm, and determined the drug encapsulation efficiency in nanoparticles indirectly by measuring the drug concentration in the remaining solution of emulsification using the following equation.

EE%= (mtotal - msupernatant)/mtotal × 100 Eq (2)

EE%=[(5-0.25)/5]*100=95%

Where, EE% indicates the drug encapsulation efficiency; mtotal indicates the concentration of drug used in the encapsulation phase; and msupernatant indicates the concentration of drug in the solution remaining after centrifugation and separation of nanoparticles.

*The general method for releasing drugs from drug-loaded carriers*

In order to investigate the drug release in the external environment, at first the drug-containing carriers was dried at room temperature. Then, the drug release from the complexes at room temperature and at specific pH was examined using the dialysis bag of spectroscopic procedures. To this end, 1 mg of the complex was dispersed in a buffer solution (at pH of 7.4 and 5.8), then the mixture was transferred to a dialysis bag, and put in a beaker with 25 ml of the same buffer at 37 °C, and was stirred using a shaker. The amount of drug absorbed was determined by the UV spectrophotometer and the drug release concentration was measured using a calibration curve.

*Preparation of necessary buffer solutions*

The needed buffers were prepared according to the following instruction:

Phosphate buffer, pH= 7.4

A: 8.01 g of NaCl

B: 0.2 g of KCl

C: 1.78 g of $Na_2HPO_4$

D: 0.27 g of $KH_2PO_4$ was dissolved in some distilled water and then its valume was diluted it to 1 liter.

Acetate buffer, pH= 5.8

A: 5.98 g of sodium acetate

B: 3 ml of acetic acid 2 Normal was dissolved in some water and then diluted it to 1 liter.

*Drawing the necessary calibration curves*

*Doxorubicin calibration curve*

The standard solution of 100 ppm Doxorubicin was used to prepare a control solution at pH of 7.4 and 5.8 (since the pure drug was 50 mg in 25 ml, we dissolved 0.5 ml of the drug in 9.5 ml of buffer). Control solutions were prepared at a concentration range of 5-100 ppm (5, 10, 15, 20, 25, and 100) from the standard solution, and standard curves were drew for different solvents.

*In-vitro experiments in buffer environment (Drug*

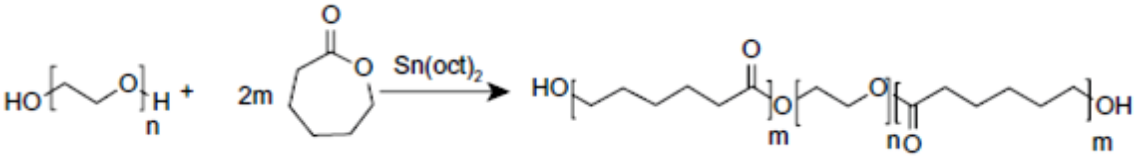


Fig. 3. The chemical structure and PCL-PEG-PCL triblock copolymer preparation method

*release experiment)*

A specific weight of lyophilized nanoparticles containing doxorubicin was suspended in 30 ml of 5.0 M phosphate buffer solution (0.35 mol of disodium hydrogen phosphate at pH of 4.7).

This solution was put in a shaking incubator at 37 °C and a 3-ml sample was took from the transparent part of the solution at regular intervals for 14 days, and at the same time it was replaced with 3 ml of phosphate buffer solution. At the end of 14 days, about 40 samples were prepared and analysed for doxorubicin contents using the UV spectrophotometer.

To examine the impact of pH in the drug release, the drug release test was repeated in acetic acid buffer solution at pH= 5.8 at a temperature of 40 °C.

## RESULTS AND DISCUSSION

*Results of synthesis and spectral study of copolymer structure of PCL-PEG-PCL*

The prepared PCL-PEG copolymer has the following chemical structure. This copolymer is obtained during the ring-opening polymerization with $Sn(oct)_2$ catalyst.

The following table presents the synthesis conditions and specifications of the synthesized copolymer.

Figs. 4 and 5 show the infrared spectrum (FT-IR) and $^1$H-NMR of the prepared copolymer respectively.

An absorption band in 1731 $cm^{-1}$ represents the stretching vibration of the ester band of the main polymer chain. Absorption bands at 1116 $cm^{-1}$ and 1246 $cm^{-1}$ are the stretching vibration of the C-O-C band of $-OCH_2CH_2$ units of Polyethylene glycol and -COO- band of polymer chains. Also absorption bands at 2944 $cm^{-1}$ and 2866 $cm^{-1}$ are special bands for stretching vibration of C-H bands of $-CH_2CH_2$ of caprolactone. An absorption band in 3742 $cm^{-1}$ indicates the end hydroxyl group of PCL-PEG-PCL.

As shown in the $^1$H-NMR spectrum for the synthesized copolymer, the chemical proton displacement of 1.24, 1.3, 2.2, and 4.06 belongs to $-(CH_2)_3$, $-OCCH_2-$ and $-CH_2OOC$ in polycaprolactone. A sharp peak in 3.37 ppm belongs to the chemical displacement of polyethylene glycol protons. Chemical displacements in 3.9 ppm to 4.2 ppm are due to $-O-CH_2-CH_2$ protons of Polyethylene glycol attached to polycaprolactone [11-12].

*Characterization of the magnetic nanoparticles*

Magnetic nanoparticles was preoared by the coprecipitation method using divalent and trivalent iron salts in the presence of ammonia to alkalize the medium and their structural and magnetic properties was determined using spectroscopy and magnetometry.

*FT-IR Spectrum of magnetic nanoparticles*

Fig. 6 shows the FT-IR spectrum of magnetic nanoparticles of $Fe_3O_4$. The FT-IR spectrum of magnetic nanoparticles of $Fe_3O_4$ shows an intense absorption band at a frequency of about 571 $cm^{-1}$ for FeO and a frequency of 3382 $cm^{-1}$ for OH functional group.

*SEM images of magnetic nanoparticles*

Scanning electron microscopy is applicable to determine the surface topology, cross-sections, and morphology of microparticles and nanoparticles [13]. Results for SEM analysis in different magnifications are shown in Fig. 7a to 7c. After a careful and point-to-point study of different regions of the sample, the sizes of magnetic nanoparticles was obtained to be 20-50 nm (Fig. 7a to 7c). Also, according to the SEM result, the product has spherical morphology.

*Investigation of magnetic properties of $Fe_3O_4$ nanoparticles*

Fig. 8 shows the hysteresis curve (delay of change in magnetic flux density vs. magnetic field strength) of as prepared $Fe_3O_4$ nanoparticles.

The hysteresis curve of magnetite was obtained using a magnetometer at a temperature of 25 ºC according. The nanoparticles show the super-paramagnetic behavior without magnetic

Table 2 - Synthesis conditions and specifications of PCL-PEG-PCL copolymer

| Polymer sign | PEG to CL ratio | Molecular mass of PEG | Polymerization efficiency | Molecular mass of the polymer | Dispersion Index |
|---|---|---|---|---|---|
| $PCL_{1000}$-$PEG_{1000}$-$PCL_{1000}$ | 2:1 | 1000 | 87% | 8000 | 1.9 |

hysteresis, in other words, they show magnetic properties of 80 emu/g in the presence of a magnetic field with increasing field strength up to about 8000 Gauss. But as soon as the magnetic field is removed, these particles completely lose their magnetic properties (0 emu/g). Therefore, magnetic orientations is observed in the presence of the externan magnetic field, and it disappear by removing the ecternal field. So, we can conclude that $Fe_3O_4$ is a super-paramagnetic particle that shows a strong magnetic property of 80 emu/g by applying a magnetic field, and the magnetic nanoparticles spread with a gentle shake as soon as this field is removed. This property of magnetic nanoparticles is important in their wide applications in biomedical and bioengineering disciplines.

*Results for characterization of drugs loaded magnetic nanoparticles*

FT-IR spectroscopy and scanning electron microscopy was applied to study the chemical structures and surface morphology of PCL/ $Fe_3O_4$ nanocomposite loaded with doxorubicin.

*FT-IR spectrum of PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin*

To investigate the vibrations of functional groups in the structure of drug loaded nanocomposites, the FT-IR technique was applied.

A certain concentration of doxorubicin encapsulated nanocomposite was prepared and analyzed. Fig. 9 shows the FT-IR spectrum of nanocomposite loaded with doxorubicin. Absorption bands of 416 $cm^{-1}$, 583 $cm^{-1}$, and 516 $cm^{-1}$ belongs to band vibration of Fe-O, and absorption bands of 3560 $cm^{-1}$ belong to free hydroxyl groups (OH) no the magnetite surface. The most important characteristic of the magnetite IR spectrum is the presence of a 583 $cm^{-1}$ band belonging to the stretching vibration of Fe-O. Absorption bands of 2869-2938 $cm^{-1}$ is belonged to stretching vibration of C-H, a band at 1731 $cm^{-1}$ is belonged to carbonyl ester band, absorption band 1162 $cm^{-1}$ belonged to C-C and C=O bands, and 1239 $cm^{-1}$ belonged to ether band of polyethylene glycol. There were also absorption bands of 1357, 953, 1010, 1587, 1648, and 1460 $cm^{-1}$ that corresponded with the IR spectrum of pure doxorubicin indicating the drug encapsulation inside the relevant nanoparticle.

*SEM images of PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin*

Fig. 10 shows results for SEM imaging of nanoparticles in three different magnifications. Scanning electron microscopy is applicable to determine the surface topology, cross-section examination, and morphology of nanoparticles.

As shown in Fig. 10, the sizes of nanoparticles

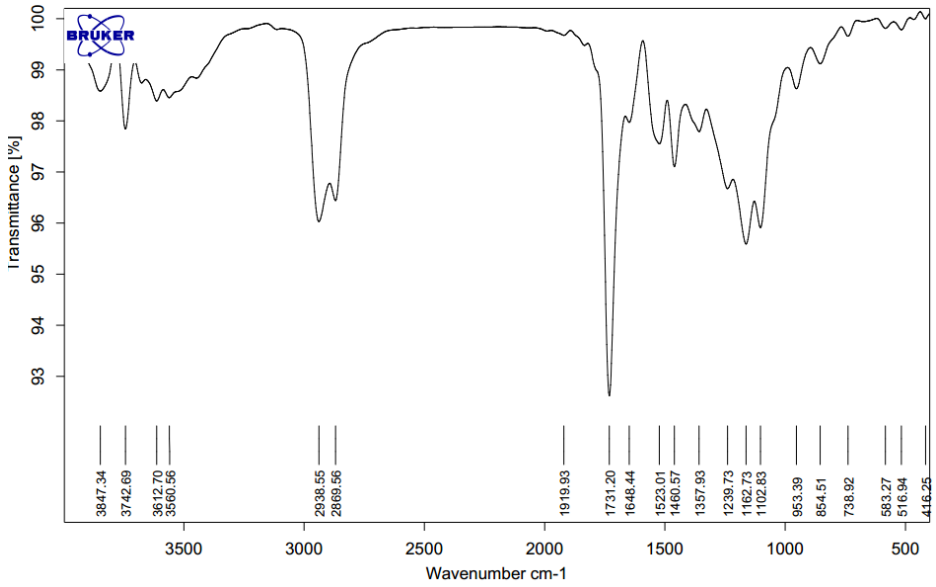


Fig. 4. The FT-IR spectrum of PCL-PEG-PCL copolymer

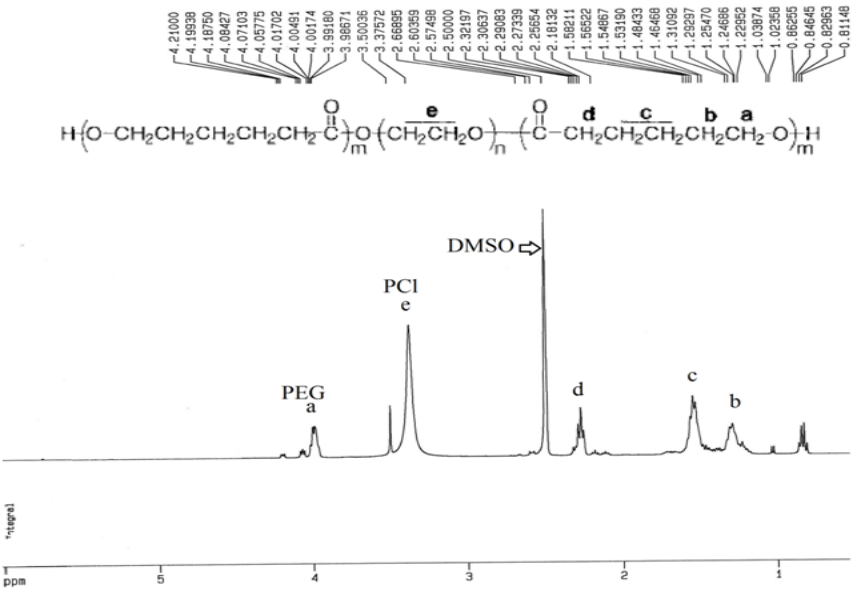


Fig. 5. $^{1}$H-NMR spectrum of PCL-PEG1000-PCL copolymer

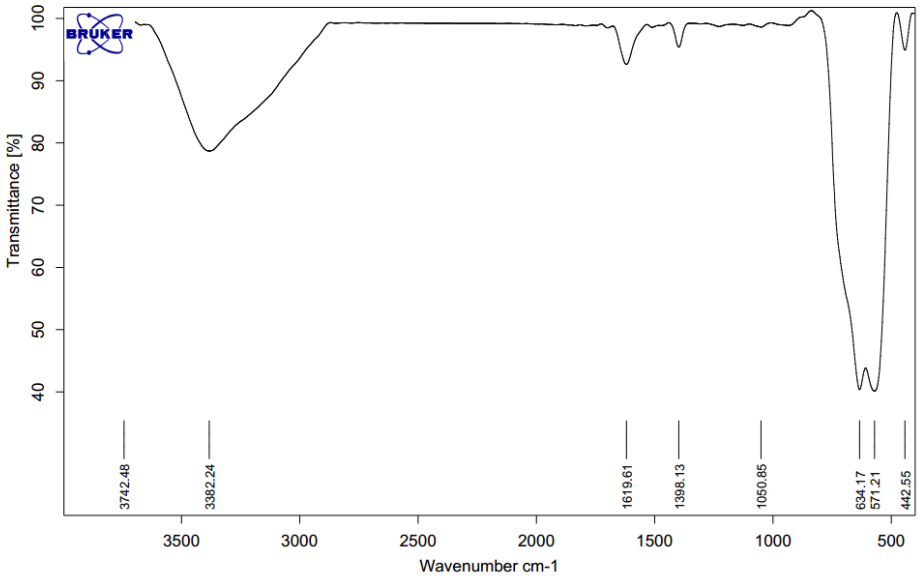


Fig. 6. FT-IR Spectrum of magnetic nanoparticles of $Fe_3O_4$

varied between 50 and 200 nm. However, the aggregation and agglomeration has been occured in some regions, which can be redispersed by sonication in Phosphate-buffered saline (PBS), before examination by the SEM.

*Results of measuring the efficiency of PCL/ $Fe_3O_4$ nanocomposites in the encapsulation of doxorubicin drug*

The drug encapsulation efficiency in nanoparticles was determined indirectly by measuring the drug concentration in the residual solution of emulsification and according to the following equation.

EE%= (mtotal - msupernatant)/mtotal × 100

EE%=[(5-0.25)/5]*100=95%

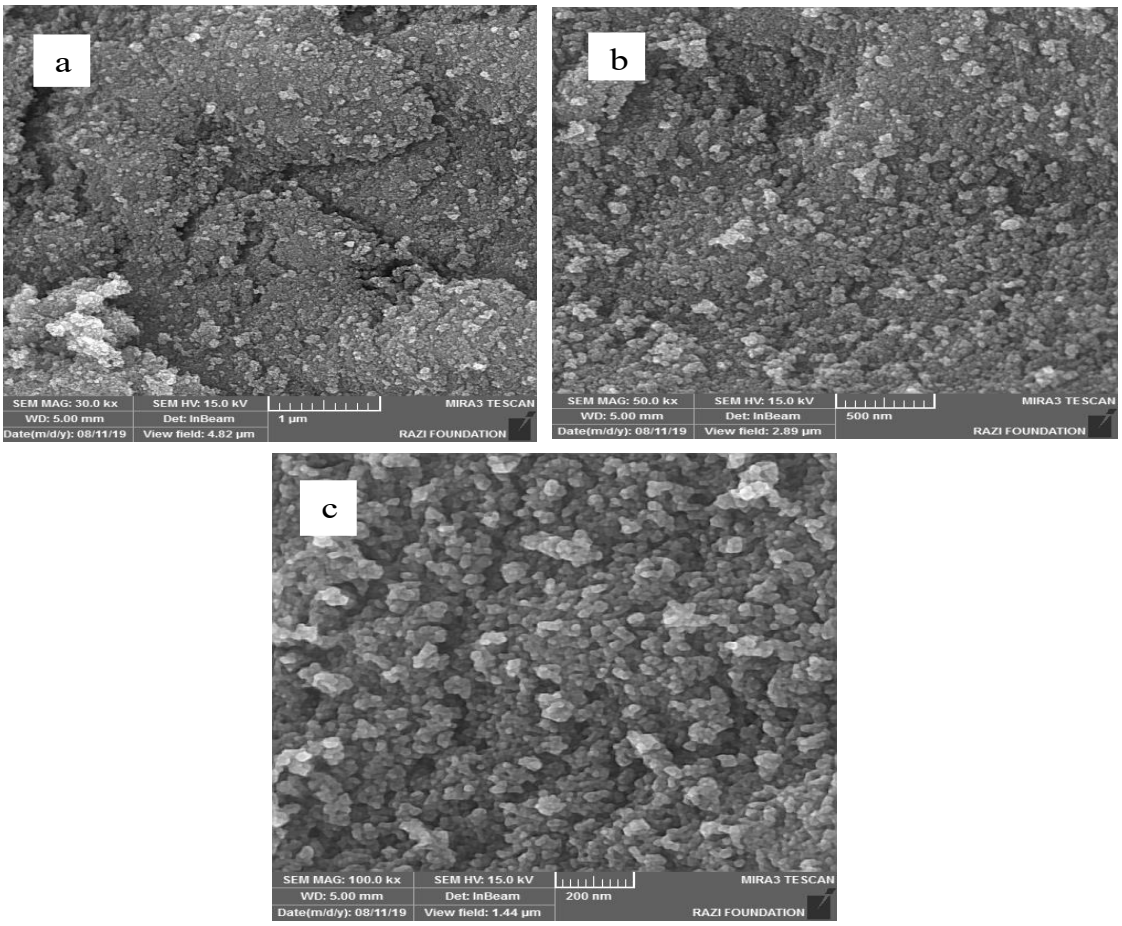


Fig. 7. a) SEM Image of $Fe_3O_4$ magnetic nanoparticles (30 kx) b) SEM Image of $Fe_3O_4$ magnetic nanoparticles (50 kx) c) SEM Image of $Fe_3O_4$ magnetic nanoparticles (100.0kx)

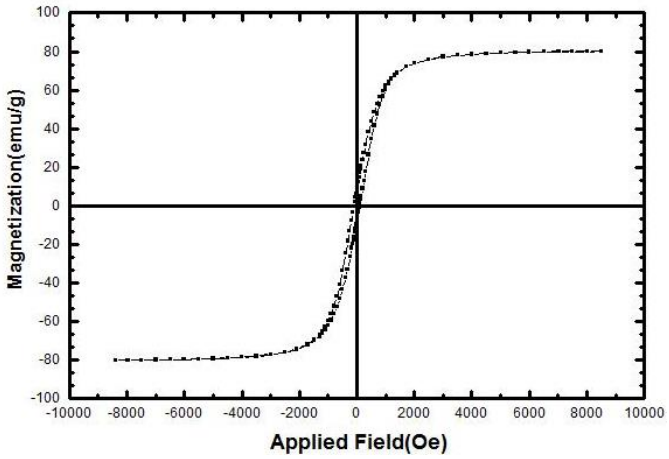


Fig. 8. VSM curve of magnetic iron nanoparticles

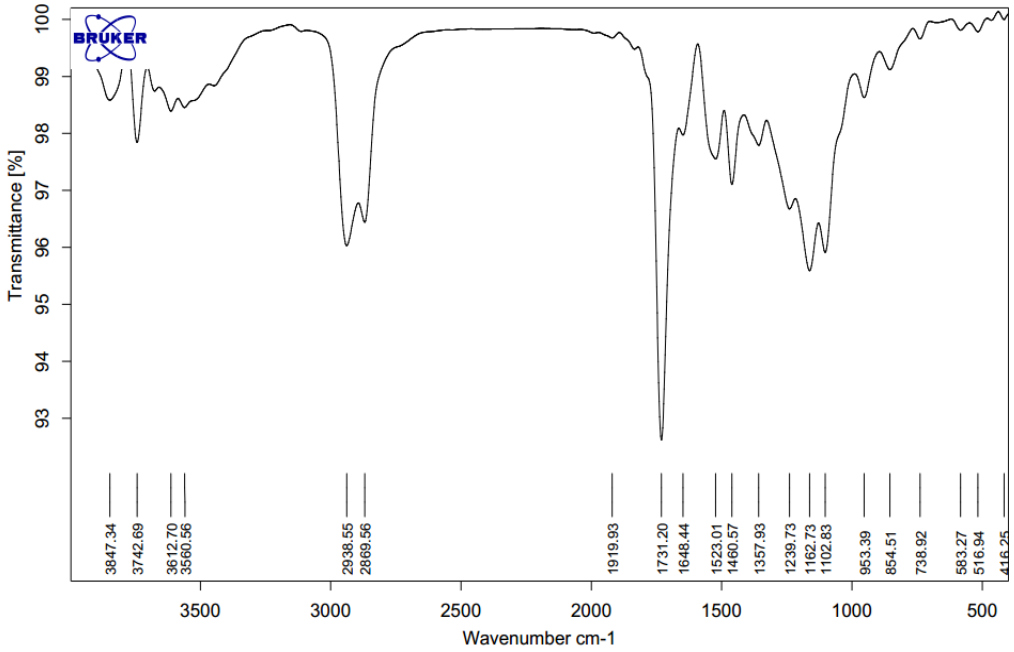


Fig. 9. FTIR Spectrum of the PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin

Table 3. Physicochemical characteristics of doxorubicin loaded PCL/ $Fe_3O_4$ nanocomposites

| Polymer sign | PEG to CL ratio | Drug-polymer ratio | Encapsulation efficiency | Particle size (nm) |
|---|---|---|---|---|
| PCL-PEG-PCL | 2:1 | 10:1 | 95 | 70-150 |

Where, EE% represents the drug encapsulation efficiency, mtotal indicates the doxorubicin concentration used in the encapsulation phase, and msupernatant indicates the doxorubicin concentration in the solution remaining after centrifugation and nanoparticle separation.

The following table summarizes the physicochemical properties of doxorubicin-containing PCL-PEG-PCL nanoparticles.

*Investigation of magnetic properties of doxorubicin-loaded PCL/ $Fe_3O_4$ nanocomposites*

Diagram (2) shows the magnetite hysteresis curve and magnetic nanoparticles with a polymer coating containing doxorubicin at room temperature. As shown in Diagram (4-1), the magnetic hysteresis of about 80 emu/g was seen for solid magnetic nanoparticles in the presence of a magnetic field of about 8000 G, but the magnetic hysteresis decreased by 30 emu/g due to encapsulation of these magnetic nanoparticles with nonmagnetic doxorubicin drug. The reduction of about 50 emu/g in the magnetic hysteresis indicated the physical coating of magnetic nanoparticles by copolymers. As soon as the magnetic field is removed, magnetization becames zero, indicating the super-magnetic properties of the nanocomposites. This property is very effective in the application of nanoparticles in targeted drug delivery.

*In-vitro studies*

*Evaluation of drug release rate*

*Doxorubicin release mechanism and comparison of drug release rates in buffer media with various pH (pH= 7.4 and pH= 5.8)*

The measurement of pH of extracellular fluids of solid tumors indicated that the acidic pH is suitable for the growth of solid tumors. Therefore, nanoparticles sensitive to pH were included in anti-cancer drugs.

Given that the release of doxorubicin from DOX-PCL-PEG1000 $Fe_3O_4$ at a pH of 5.8 is more than a pH of 7.4, we can expect that the drug release rate

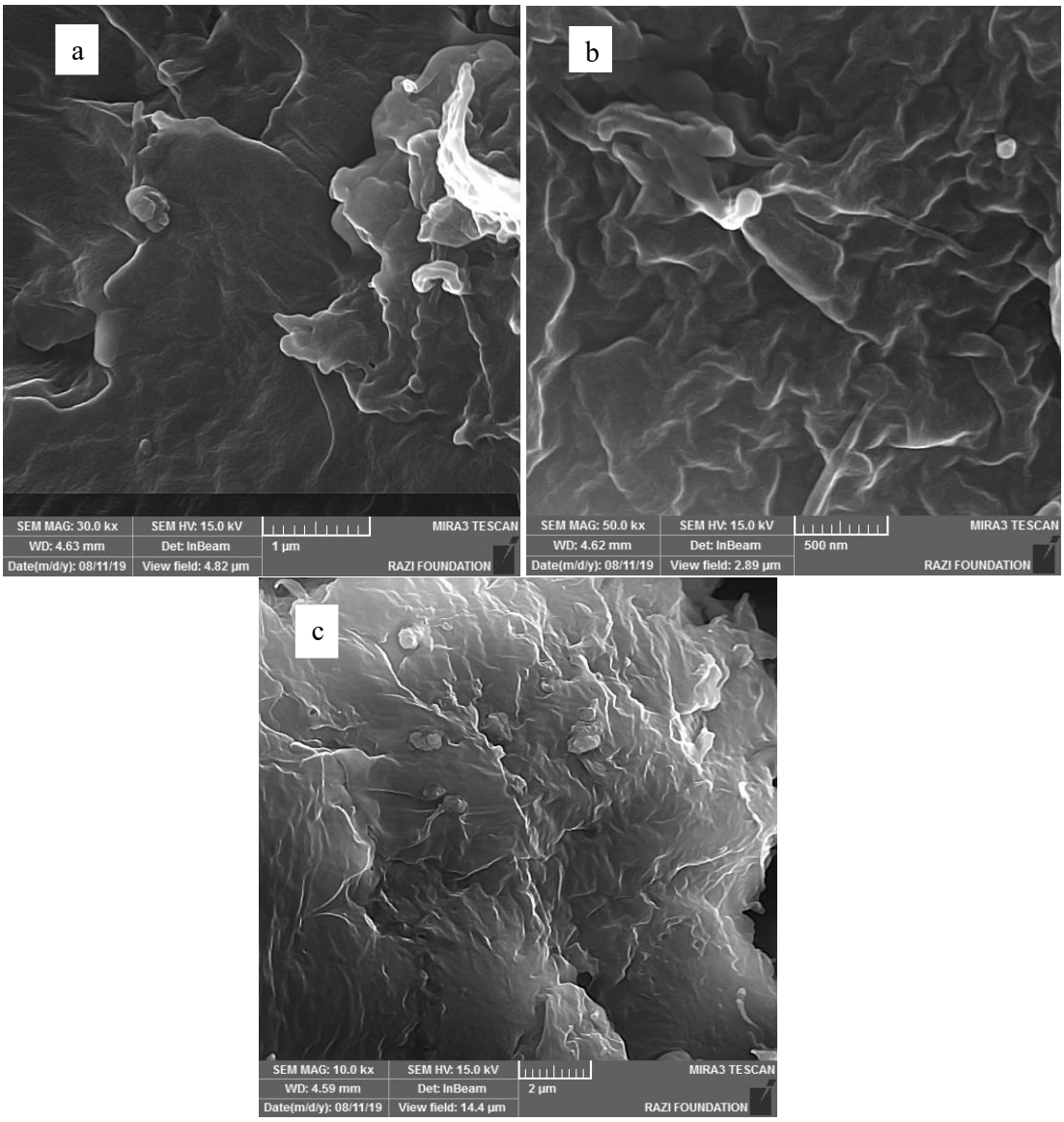


Fig. 10. a) SEM image of PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin (30.0 kx) b) SEM image of PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin (50 kx) c) SEM image of PCL/ $Fe_3O_4$ nanocomposites loaded with doxorubicin (10.0 kx)

in the acidic medium of the extracellular fluid of the tumor will be better than other cells.

The relatively high drug release rate in acidic pH can be possibly attributed to ester hydrolysis of polymers and faster dissolution of nanoparticles at low pH.

*Doxorubicin release mechanism and comparison of drug release rates at different temperatures (37 ºC and 40 ºC)*

The nanoparticles with a certain weight was incubated into a buffer tube with shaking for 6 days and samples were took from them at regular intervals.

The drug release increased at a temperature of 40 ºC when the temperature was similar to the environment of cancer cells.

## CONCLUSION

In the modified method of the present study, we first used Polyethylene glycol at a relatively low molecular weight ($PEG_{1000}$) to prepare PCL-PEG-

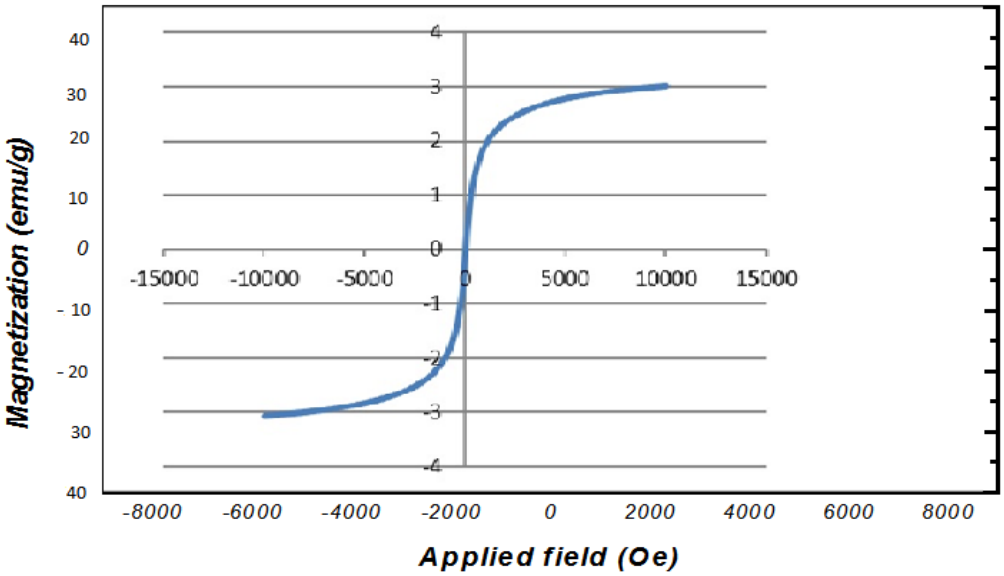


Fig. 11. VSM result for doxorubicin-loaded PCL/ $Fe_3O_4$ nanocomposites

PCL triblock copolymer. The molecular mass of the polymer was about 8000 and the dispersion index of the molecular mass was about 1.9. The polymerization efficiency was 87%. Using the synthesis and purification, pure polymer was obtained in which chemical structures were confirmed by spectroscopic methods. At the next step, coprecipitation method was used to prepare magnetic nanoparticles. The results of VSM, FT-IR, and SEM images indicate successfully synthesis of magnetic nanoparticles.

According to the review of several sources and studies in this field, we can conclude that the loading of anticancer drugs on magnetic nanoparticles has an effective role in enhancing drug function and killing cancer cells. Research results indicate that magnetic nanoparticles enhance the functions of anticancer drugs (e.g. doxorubicin and Cisplatin) in killing cancer cells by boosting the production of reactive oxygen species or other unknown mechanisms. In other words, magnetic nanoparticles increase the cytotoxicity of anti-cancer drugs and also play important roles in drug delivery to tumor cells [13-14].

## CONFLICT OF INTEREST

The authors declare there is no competing interest.

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