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DEVELOPMENT OF DOXORUBICIN – ADSORBED MAGNETIC NANOPARTICLES MODIFIED WITH BIOCOMPATIBLE COPOLYMERS FOR TARGETED DRUG DELIVERY IN LUNG CANCER

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Abstract. Iron oxide nanoparticles are smart materials that have been commonly used in medicine for diagnostic imaging, drug delivery, and therapeutic applications. In this study, Iron oxide nanoparticles and doxorubicin hydrochloride, were absorbed into poly (D, L-lactic-co-glycolic acid) poly (ethylene glycol) (PLGA-PEG) nanoparticles for narrow behavior. PLGA:PEG triblock copolymers were synthesized by ring-opening polymerization of D, L-lactide and glycolide with polyethylene glycol (PEG3000) as an initiator. The bulk properties of these copolymers were characterized using Fourier transform infrared spectroscopy. In adding together, the consequential particles were characterized by scanning electron microscopy, x-ray powder diffraction, and vibrating sample magnetometry. The drug adsorbed efficiency achieved for Fe_3O_4 magnetic nanoparticles modified with PLGA:PEG3000 copolymers was 70%, and the release profile were controlled. The in vitro cytotoxicity test showed that the Fe_3O_4-PLGA:PEG3000 magnetic nanoparticles had no cytotoxicity. There is potential for apply of Fe_3O_4-PLGA:PEG3000 magnetic nanoparticles for biomedical purpose.

Keywords: *PLGA-PEG, Iron oxide nanoparticles, Triblock copolymer, Doxorubicin adsorbed*

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1. Introduction

Humanity attempt to cope with diseases dates back to early civilization. Substances taken from nature were experienced and used to treat dysfunctions of physiological life processes, pain and restlessness with the development of

science, the vigorous ingredients of these materials, the drugs, were recognized, isolated and in a lot of cases their methods of action elucidated. Even today new drug Nominees are Examined, in an effort to add an increasingly effective instrument against the disease [58, 63, 42]. Recently, we have seen remarkable developments in the understanding of neoplastic diseases and how they have been translated into improvements of therapy.

A growing amount of more particular therapeutic options to manage different tumour types are now available, but typical chemotherapy remains, generally a backbone option for many tumours [22, 23]. The last decade has witnessed Extensive progress in the improvement and use of nanotechnology in cancer detection, diagnosis, and therapy culminating in the development of the nascent field of "cancer nanomedicine. Most deaths from cancer in the world are related lung cancer and most of them being active tobacco smokers. Non small cell lung cancer accounts for around 85% to 90% of deaths, while the rest is contributed by small cell lung cancer. The incidence of lung cancer can be generally classified into two main types on the basis of histologic appearance, one being small cell lung cancer (SCLC) and the another one being non-small cell lung cancer (NSCLC). existing therapeutic strategies like chemotherapy and radiation therapy is only effective in the primary stages of treatment of SCLC, while NSCLC are less sensitive to such treatment modalities, which leaves surgery (only in stages I, II, and some of IIIA) and gene therapy as further feasible substitute to tackle NSCLC [2] and lung cancer stem cells. The main problem of the most chemotherapeutic ways that most of them are non-specific. As a result, in this method in addition to the target cells and primary tumor, normal and healthy cells attacked by cytotoxic drug [10, 16] nanoparticles for cancer treatment can be used in three ways:

1- particular antibodies can be conjugated to the MNPs to selectively bind to associated receptors and inhibit tumor growth
2- targeted MNPs can be used for hyperthermia for tumor therapy
3- Drugs can be loaded onto the MNPs for targeted therapy [20, 48].

Magnetic drug targeting, using superparamagnetic iron oxide nanoparticles (SPION) as carriers, is getting a considerable attention in research. Vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates. Effect of preparative variables on the properties of poly(dl-lactide-co-glycolide)-methoxypoly(ethyleneglycol) copolymers associated to their uses in restricted drug delivery [30]. Drug activity is a result of molecular interaction(s) in particular cells; it is consequently simply deduced that it is necessary for the drug to reach somehow the site of action next administration (oral, intravenous, local, transdermal, etc.) at sufficient concentrations Drug delivery systems, ranging from implantable electronic devices to single polymer chains, are essential to be compliant with processes in the body as well as with the drug to be delivered [14, 24]. Doxorubicin or 14-hydroxydaunomycin is renowned drug used in cancer chemotherapy. Biochemical data confirms that this drug makes complexe with DNA thereby blocking the any replication or transcription [1, 37, 39, 45]. One way that doxorubicin mechanism is by blocking an enzyme called topo isomerase. That cancer cells need in order to divide and grow.

Nanoparticles occupy a more and more prominent place in the armory of injectable, colloidal drug delivery systems, [12, 33, 53]. Substitute carriers counting polymer-drug conjugates [36], micelles [46], dendrimers [31], lipid and polymeric vesicles [56], nanocapsules [3, 54], are also the topic of investigate and clinical evaluation. Ideally, nanoparticles would deteriorate in products which are naturally excreted, or absorbed by the body. Additionally to systemic targeted management, localized drug delivery may be done by introducing a drug depot directly at the target site [4, 28, 29].

Nano science is one of the most significant study and development frontiers in modern science. The employ of nanoparticles (NPs) materials offers numerous benefits because of their unique size and physical properties. Due to the extensive uses of magnetic nanoparticles (MNPs), in biotechnology, biomedical, material science and engineering, a lot notice has been paid to the preparation of diverse types of MNPs. These nanosized particles are of great consideration for researchers from a wide range of disciplines, including magnetic fluids, biotechnology/biomedicine, catalysis, data storage, magnetic resonance imaging and environmental remediation [11, 25, 26, 35, 40, 50, 51, 52].

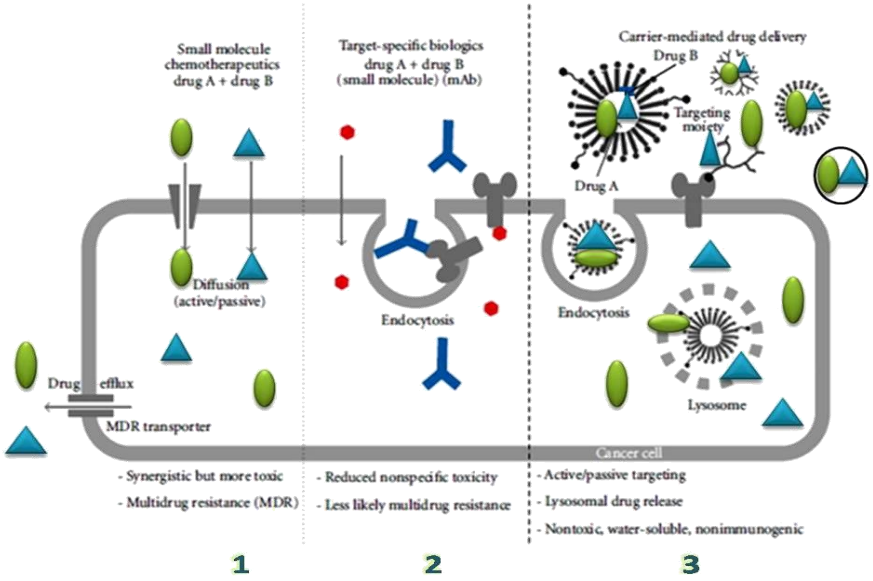

Fig. 1. Schematic image of diverse combination drug delivery approaches for treatment of cancer. (1) Combination of small molecule chemotherapeutic agents, (2) combination of target exact biologic agents including monoclonal antibodies, and small molecule chemotherapeutics (3) carrier-mediated combination drug delivery.

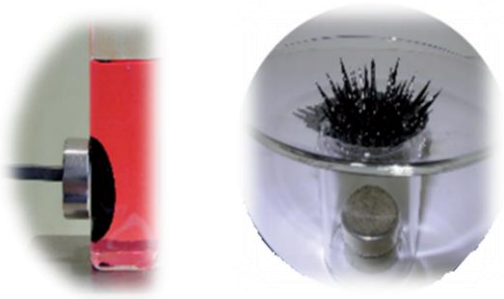

Fig. 2. Image of magnetic nanoparticles (MNPs)

In the 1970s Freeman et al were the first to establish the concept of use of magnetism in medicine [15] since then, much study has been done in this area, foremost to the design of different magnetic particles and vectors [21]. Nanoparticles that called Iron oxide nanoparticles are iron oxide particles with diameters in the range of 1 and 100 nanometers. The two main forms are magnetite (Fe_3O_4) and its oxidized form maghemite (γ-Fe_2O_3). Super paramagnetic iron oxide nanoparticles (SPIONs) consist an essential technology class within the emerging field of nanomedicine and have been mostly researched for cancer therapy and imaging [13]. One of the most pursued zones of research in the development of cancer treatment strategies is delivery of anticancer drugs by combination with functionalized SPIONs to their targeted site. SPION induced hyperthermia has also been used for localized killing of cancer cells. in spite of their possible biomedical use, alteration in gene expression profiles, interruption in iron homeostasis, oxidative stress, and changed cellular responses are some SPION-related toxicological aspects which necessitate due deliberation [43, 53].

A extensive diversity of agents from very hydrophobic to highly hydrophilic1can be encapsulated in PLGA nanoparticles, drug release rates can be modified to exacting applications, and size and loading are simply manipulated to provide additional control over drug delivery [18]. Similar to colloidal drug delivery systems, when PLGA nanoparticles are intravenously administered, they are rapidly removed from the systemic circulation by macrophages in the spleen and the mononuclear phagocyte system in Kupffer cells of the liver. Consequently, it is essential to alter the PLGA nanoparticle surface so that it can run away uptake by the mononuclear phagocyte system and have a prolonged circulation time in the blood [55]. This issue is easily accessible in PLGA nanoparticles when their surface is altered by hydrophilic poly(ethylene glycol) (PEG) PEG-coated nanoparticles keep away from detection by the mononuclear phagocyte system and removal because of the shielding effect of the polymer chains in PEG [14]. In the meanwhile poly ethylene glycol to nanoparticle surfaces such as PEGylation is known to improve circulation time by inhibition of nonspecific protein adsorption, opsonization, and following clearance. The difficulty associated with surface-modifying PLGA particles has been the lack of functional chemical groups on the aliphatic polyester backbone of the polymer. A diversity of methods have been urbanized for PEGylation of PLGA nanoparticles [64]. As shown in Figure3, PEG is a coiled polymer of repeating ethylene ether units with dynamic conformations [5, 44].

Fig. 3. PEGylated NP [48].

In drug delivery uses, the addition of PEG to NPs decreases RES uptake and increases circulation time opposed to without coverage counterparts [19]. Nanoparticles are passively targeting and amass in tumors by the improved penetration and retention effect after systemic organization [6, 27]. Drug concentration increases at the tumor locate to recover their remedial efficacy and decrease nonspecific side effects [60]. As can be seen in Figure 4, the drug loaded nanoparticles via angiogenesis is moreover not greatly effective because of the formation of neovessels which are often Confounded and irregular and so less efficient in oxygen, nutrient transport and drug delivery Magnetic drug delivery system acts on the delivery of magnetic nanoparticles loaded with drug to the tumor site Affected by the external magnetic field [7]. Though, advance of this delivery system mandates that the nanoparticles perform magnetic only Affected by an external magnetic field and after removal of the external magnetic field are inactive [8].

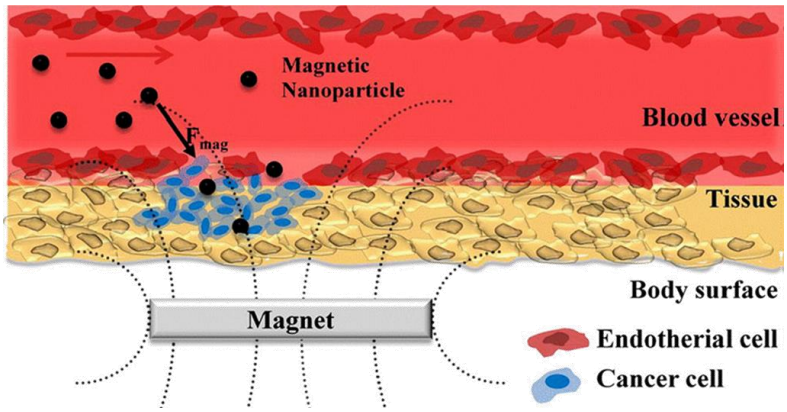

Fig. 4. Schematic image of Magnetic drug delivery system under the influence of external magnetic field [32].

2. Materials and methods

Materials. Ferric chloride hexahydrate ($FeCl_3$.$6H_2O$), ferrous chloride tetrahydrate ($FeCl_2$.$4H_2O$), and ammonium hydroxide (25 wt%) were purchased from Fluka (Buchs, Switzerland). D, L-lactide and glycolide were purchased from Sigma-Aldrich (St Louis, MO) and recrystallized with ethyl acetate. Stannous octoate (Sn (Oct)2: stannous 2-ethylhexanoate), PEG (molecular weight 2000, 3000, and 4000), and dimethyl sulfoxide were purchased from Sigma-Aldrich. PEGs were dehydrated under vacuum at 70°C for 12 hours and used without further purification. Doxorubicin hydrochloride was purchased from Sigma-Aldrich. X-ray diffraction, Rigaku D/MAX-2400 x-ray diffractometer with Ni-filtered Cu Kα radiation, and scanning electron microscopy (SEM) measurements were conducted using VEGA/TESCAN. DSC measurements were conducted by the Perkin Elmer 7 series. The drug-loading capacity and release behavior were

determined using an ultraviolet visible 2550 spectrometer (Shimadzu, Tokyo, Japan). Infrared spectra were recorded in real-time with a Perkin Elmer series FTIR. The magnetic property was measured on a vibrating sample magnetometer (Meghnatis Daghigh Kavir, Iran) at room temperature. 1H NMR spectra was recorded in real time with a Brucker DRX 300 spectrometer operating at 300.13 mHz. The average molecular weight was obtained by gel permeation chromatography performed in dichloromethane (CH2Cl2) with a Waters Associates Model ALC/gel permeation chromatography 244 apparatus. The samples were homogenated using a homogenizer (Silent Crusher M, Heidolph Instruments GmbH, Schwabach, Germany). The organic phase was evaporated by rotary (Rotary Evaporators, Heidolph Instruments, Hei-VAP series).

Synthesis of superparamagnetic magnetic nanoparticles. SPIONs were synthesized using an improved chemical coprecipitation way [38]. According to this process, 0.31736 g of $FeCl_2$.$4H_2O$ (1.6 mmol) and 0.075684 g of $FeCl_3$ $6H_2O$ (2.8 mmol) were dissolved in 32 mL of deionized water, such that Fe^{2+}/Fe^{3+}1/1.75. The mixed solution was stirred under nitrogen at 80˚C for 1 hour (Fig. 5). Then, NH_4OH 4 mL was injected into the mixture rapidly, stirred under nitrogen for one more hour, and then cooled to room temperature. The precipitated particles were washed with hot water and separated by magnetic decantation. Finally, the magnetic nanoparticles were dried under vacuum at 70˚C Reaction formation of magnetic nanoparticles can be seen in the Fig.5

$$\mathbf{2\ FeCl_3 + FeCl_2 + 4\ H_2O + 8\ NH_3 \longrightarrow Fe_3O_4 + 8\ NH_4Cl}$$

Fig.5. Reaction formation of magnetic nanoparticles

***Preparation of PLGA-PEG triblock copolymer*.** PLGA-PEG copolymers with PEG 3000 as an initiator were prepared by a melt polymerization process under vacuum using stannous octoate [Sn(Oct)2:stannous 2-thylhexanoate] as a catalyst [9, 57].

DL-lactide (1.44 g), glycolide (0.386 g), and PEG 3000 0.8 g (45% w/w) in a bottleneck flask were heated to 140°C under a nitrogen atmosphere for complete melting. The molar ratio of DL-lactide and glycolide was 3:1. Then 0.05% (w/w) stannous octoate was added and the temperature of the reaction mixture was raised to 180°C. The temperature was maintained for 4 hours. The polymerization

was carried out under vacuum. The copolymer was recovered by dissolution in methylene chloride followed by precipitation in ice-cold diethyl ether. The synthesis process of PLGA-PEG copolymer is shown in Fig. 6. A triblock copolymer of PLGA-PEG was prepared by ring opening polymerization of DL-lactide and glycolide in the presence of PEG 3000.

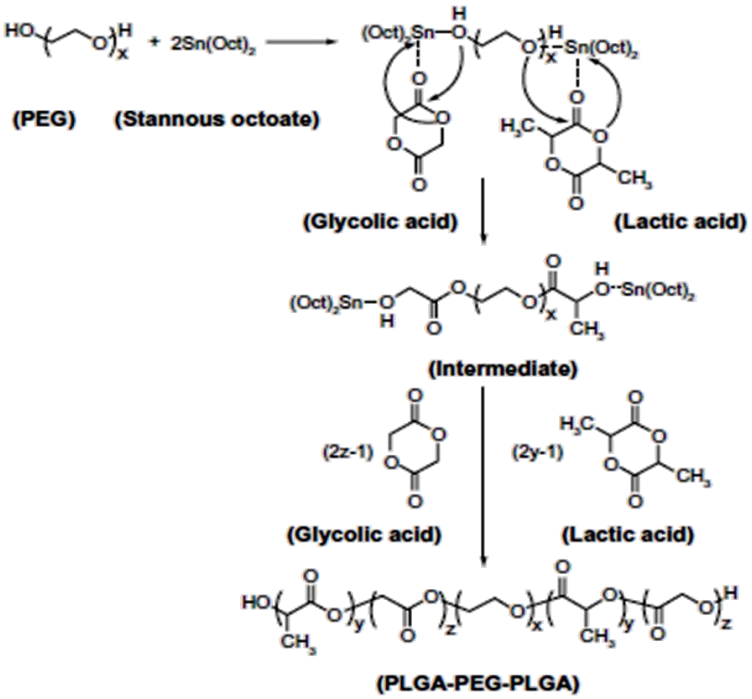

Fig.6. Mechanism of PLGA-PEG prepared by Sn (Oct)2 as catalyst [59].

Measurement of copolymer. The FTIR (Perkin Elmer series) spectrum was obtained from a neat film cast of the chloroform copolymer solution between KBr tablets. Gel permeation chromatography was performed in dichlorom using a Waters Associates (Milford, MA) Model ALC/gel permeation chromatography 244 apparatus. The molecular weight and molecular weight distribution of the copolymer were calculated using polystyrene as the standard.

Doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers. Doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers were ready using the double emulsion method (w/o/w) in use by Song et al [62]. with minor modifications. An aqueous solution of doxorubicin 10 mg/1 mL was emulsified in 5 mL dichloromethane, in which 200 mg of the copolymer and 5 mg magnetic nanoparticles had been dissolved, using a probe homogenizer or sonication at 20,000 rpm for 40 seconds. This w/o emulsion was transferred to a 20 mL aqueous solution of polyvinyl alcohol 0.5% and the mixture was probe-homogenized (or sonicated) at 72,000 rpm for one minute. The w/o/w emulsion shaped was gently stirred at room temperature until evaporation of the organic phase was completed or the organic

phase was evaporated (Heidolph Instruments). The nanoparticles were purified by applying two cycles of centrifugation (12,000 rpm for 30 min in a Biofuge 28 RS, Heraeus centrifuge) and reconstituted with deionized and distilled water.

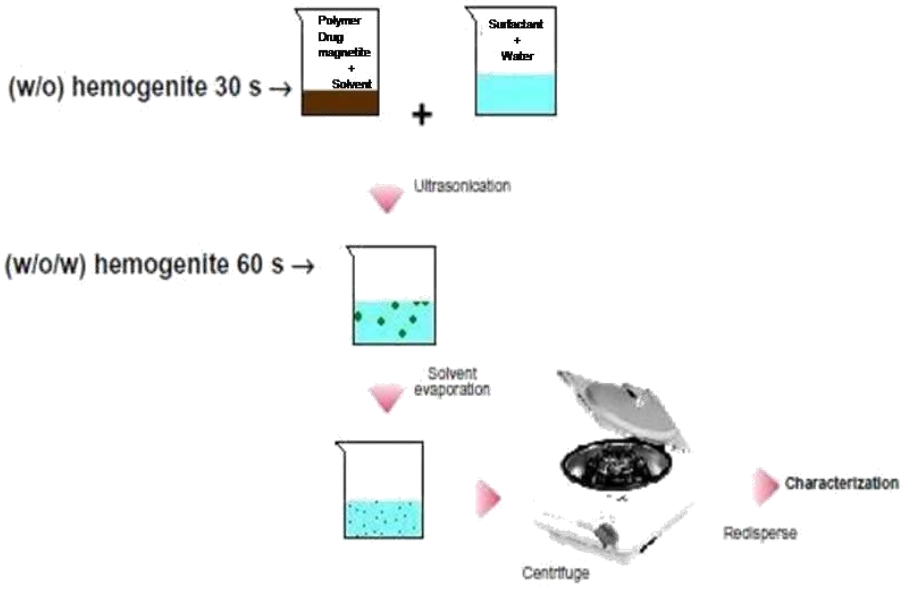

Fig. 7. Schematic image of Doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers.

The nanoparticles were finally filtered through a 1.2 mm filter (Millipore, Bedford, MA). In order to increase doxorubicin entrapment in the nanoparticles, the external aqueous phase used during the second emulsification step was saturated with doxorubicin.Blank nanoparticles were also prepared by the same method without adding doxorubicin at any stage of the preparation (Fig. 7) [58].

Drug loading and determination of doxorubicin entrapment efficiency. Doxorubicin as an anticancer drug, was used for drug loading and release studies. Briefly, 20 mg of lyophilized nanoparticles and 5 mg of doxorubicin were dispersed in phosphate-buffered solution. The solution was stirred at 4°C for 3 days to allow doxorubicin to entrap within the nanoparticle network. This value was then compared with the total amount of doxorubicin to determine the doxorubicin loading efficiency of the nanoparticles. The amount of nonentrapped doxorubicin in aqueous phase was determined using an ultraviolet 2550 (λ470 nm and λ585 nm) spectrophotometer (Shimadzu). This procedure permits analysis of a doxorubicin solution with elimination of most interfering substances.45 the amount of doxorubicin entrapped within the nanoparticles was calculated by the difference between the total amount used to prepare the nanoparticles and the amount of doxorubicin present in the aqueous phase, using the following formula [17]:

Loading efficiency % = [(amount of loaded drug in mg) /(amount of added drug in mg)] *100%

In vitro drug release profile. To study the drug release profile of the synthesized doxorubicin loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers, 3 mg of drug-loaded nanoparticles were dispersed in 30 mL of phosphate-buffered solution (PH 7.4) and acetate buffer (pH 8, the pH value for survey pH-dependent and pH sensitivity of drug release kinetics). Samples were incubated at a range of temperatures from 37°C to 40°C. At designated time intervals, a 3 mL sample was removed and same volume was reconstituted by adding 3 mL of fresh phosphate-buffered solution and acetate buffer to all samples. After the experiment, the samples were analyzed using ultraviolet spectrofluorometry to conclude the amount of doxorubicin released λ470 nm and λ585 nm for doxorubicin measurement) [47].

Nanoparticle characterization. Power x-ray diffraction (Rigaku D/MAX-2400 x-ray diffractometer with Ni-filtered Cu K β radiation) was used to study the crystal structure of the magnetic nanoparticles. The size and shape of the nanoparticles was determined by SEM. The sample was dispersed in ethanol and a small drop was spread onto a 400 mesh copper grid. The magnetization curves of the samples were measured using vibrating sample magnetometry at room temperature. The infrared spectra were recorded by a FTIR spectrophotometer (Perkin Elmer series), and the sample and KBr were pressed to form a tablet.

X-ray diffraction patterns. The x-ray diffraction patterns for pure Fe3O4 and doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers can be seen in Fig. 8. It is obvious that the diffraction pattern for our Fe3O4 nanoparticles is close to the standard pattern for crystalline magnetite. Six characteristic peaks for Fe3O4 corresponding to (220), (311), (400), (422), (511) and (440) were observed in all samples which could be well indexed to the inverse cubic spinel structure of Fe3O4 (JCPDS card 85–1436). Characteristic diffraction peaks were also observed for doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers. This demonstrates that modification of the Fe3O4 nanoparticles did not lead to any crystal phase change. The average crystallite size D was about 15 nm and obtained from the Sherrer equation $D = K\lambda/(\beta\cos\theta$, where K is the constant, λ is the x-ray wavelength, and β is the peak width of half-maximum [41].

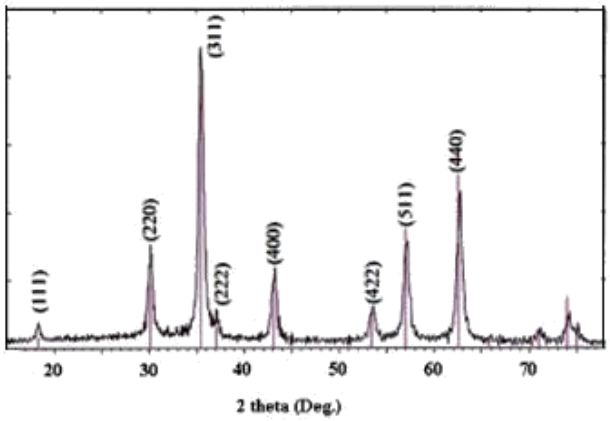

Fig. 8. XRD patterns of Fe_3O_4 nanoparticles

Size and size distribution. The surface morphology of the nanospheres during the incubation period were investigated using SEM techniques. Figure 9A, B and C show The nanographs of pure Fe3O4 nanoparticles, PLGA-PEG copolymers and doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers n. Observing the photograph, it can be seen that the nanoparticles were well aggregated, which was by reason of the nanosize of the Fe3O4 of about 20 nm. As seen in Figure 9B and C after encapsulation and modification of the doxorubicin-loaded Fe3O4 magnetic nanoparticles with PLGA-PEG copolymers, the size of the particles changed to 25–75 nm and dispersion of the particles was greatly enhanced, which can be explained by the electrostatic repulsion force and steric hindrance between the copolymer chains on the encapsulated Fe3O4 nanoparticles. The samples were coated with gold particle.

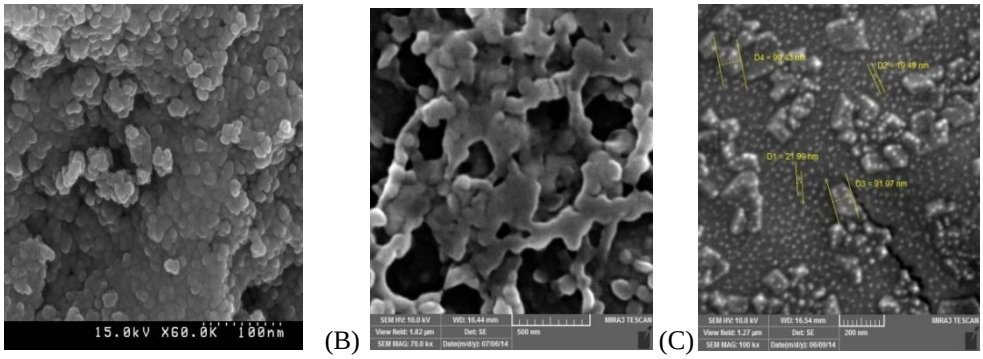

Fig. 9. Scanning electron microscopy of (A) Fe3O4 magnetic nanoparticles, (B) PLGA-PEG nanoparticles, and (C) doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers.

Magnetism test. We analyzed the magnetic properties of the nanoparticles by vibrating sample magnetometry at room temperature. Figure 10 shows the hysteresis loops of the samples. The saturation magnetization was found to be 18.5 emu/g for doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers, For example, less than for the pure Fe3O4 nanoparticles (70.9 emu/g).

This variation shows that a large amount of polymer encapsulated the Fe3O4 nanoparticles and doxorubicin. With the large saturation magnetization, the doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with PLGA-PEG copolymers could be separated from the reaction medium quickly and easily in a magnetic field. Additionally, there was no hysteresis in the magnetization, with both remanence and coercivity being zero, suggesting that these magnetic nanoparticles are superparamagnetic. When the external magnetic field was eliminated, the magnetic nanoparticles could be well dispersed by gentle shaking. These magnetic features are critical for use in the biomedical and bioengineering fields [59].

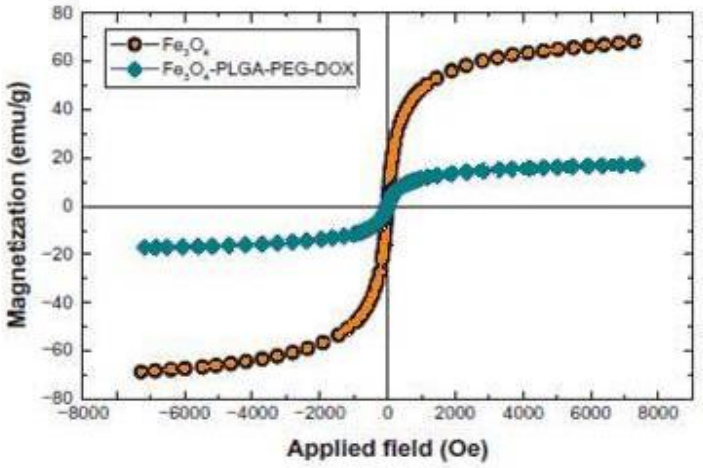

Fig.10. Magnetic behaviors of magnetic nanoparticles.

3. Results

The FT-IR spectrum is consistent with the structure of the expected copolymer. FTIR spectroscopy was used to show the structure of Fe3O4 and PLGA-PEG copolymer nanoparticles (Fig.11).

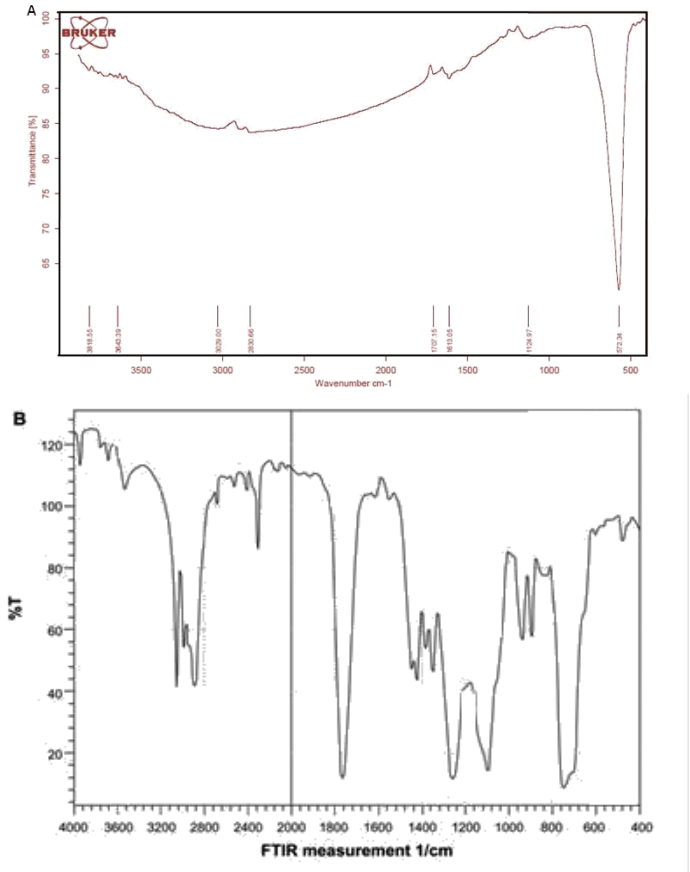

Fig.11. FTIR spectrum of pure Fe_3O_4 (A), PLGA-PEG copolymer nanoparticles (**B**).

Fourier transforms infrared spectra of Adriamaycin-Encapsulated magnetic nanoparticles modified with biocompatible copolymer can be seen in figure 13. From the infrared spectra shown in figure 12, the absorption peaks at 580 cm^{-1} belonged to the stretching vibration mode of Fe–O bonds in Fe_3O_4 (Table 1).

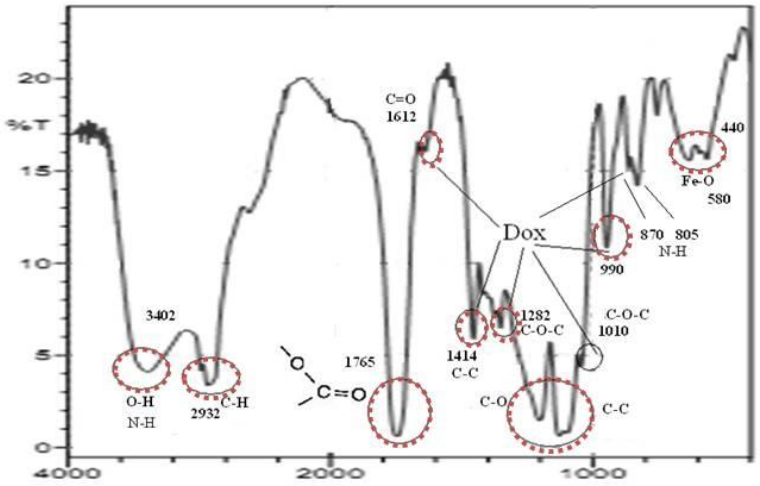

Fig.12. Fourier transforms infrared spectra of Adriamaycin-Encapsulated magnetic nanoparticles modified with biocompatible copolymer

Table 1: Fourier transform infrared spectrum for Fe3O4

System	Infrared bands (cm^{-1})	Description
Fe_3O_4	440	Absorption band of Fe–O
	580	Absorption band of Fe–O
	620	Absorption band of Fe–O
	3402	–OH vibrations

Absorption band at 3509.9 cm^{-1} is assigned to terminal hydroxyl groups in the copolymer from which PEG homopolymer has been removed. The bands at 3010 cm^{-1} and 2955 cm^{-1} are due to C–H stretch of CH, and 2885 cm^{-1} due to C–H stretch of CH. A strong band at 1762.6 cm^{-1} is assigned to C=O stretch. Absorption at 1186–1089.6 cm^{-1} is due to C–O stretch [59].

The in vitro doxorubicin release profiles were achieved by representing the percentage of doxorubicin release. Concerned with the amount of doxorubicin encapsulated. In experiment doxorubicin release occurred in two phases: A release, with a remarkable amount of drug released within 12 h, 30% for Fe_3O_4 magnetic nanoparticles customized with PLGA-PEG 3000 nanoparticles and after 12 h, the doxorubicin release profiles showed a sustained release pattern.

The doxorubicin release rate from the Fe_3O_4 -PLGA-PEG nanoparticles was also pH-dependent and enhanced at pH 5.8. It is usually assumed that a drug is released by several processes, including distribution through the polymer matrix, release by polymer degradation, and solubilization and diffusion through microchannels that exist in the polymer matrix or are formed by erosion. The magnetic-coated copolymer prepared in the present work that is not soluble in

water are AB triblock copolymer composed of hydrophobic A blocks (lactide-co-glycolide) and hydrophilic B blocks (central PEG). This copolymer exhibit reverses thermal and pH-dependent gelation properties. The gel becomes extremely pH-sensitive as hydrolysis proceeds, and carboxylic acid groups are generated in the structure. We can consider that drug is released from the Fe_3O_4 - PLGA-PEG nanoparticles by a diffusion mechanism in vitro within about 10 days, the swelling of the particles grows in acidic buffered solutions owing to protonation of central PEG groups and formation of positively charged chains in the polymer structure.

3. Conclusion

Superparamagnetic iron oxide nanoparticles were synthesized whit an enhanced chemical coprecipitation procedure, and then PLGA-PEG copolymer was used to encapsulate Fe_3O_4 nanoparticles by an emulsion method (w/o/w). The outcomes show that the copolymer chains effectively encapsulated the Fe_3O_4 nanoparticles. Saturation magnetization was found to be 19 emu/g. These particles were used in the encapsulation of doxorubicin under mild situations and could be used in drug delivery. Our results offer that supercritical liquid technology is a talented technique to produce drug polymer magnetic composite nanoparticles for the plan of controlled-release drug systems. As a result, these nanoparticles could become a powerful chemopreventive and chemotherapeutic system for lung cancer patients and constituents of these nanoparticles could be suitable candidates for drug improvement.

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