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Encapsulation of anticancer drug and magnetic particles in biodegradable polymer nanospheres

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Abstract

In this study, we have prepared PLGA (poly-D,L-lactide-co-glycolide) nanospheres loaded with biocompatible magnetic fluid and anticancer drug taxol by a modified nanoprecipitation technique and investigated their magnetic properties. A magnetic fluid, MF-PEG, with a biocompatible layer of polyethylene glycol (PEG), was chosen as a magnetic carrier. The PLGA, whose copolymer ratio of D,L-lactide to glycolide is 85:15, was utilized as a capsulation material. Taxol, as an important anticancer drug, was chosen for its significant role against a wide range of tumours. The morphology and particle size distributions of the prepared nanospheres were investigated by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and showed a spherical shape of prepared nanospheres with size 250 nm. Infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and thermogravimetry (TGA) analysis confirmed incorporation of magnetic particles and taxol into the PLGA polymer. The results showed good encapsulation with magnetite content 21.5 wt% and taxol 0.5 wt%. Magnetic properties of magnetic fluids and taxol within the PLGA polymer matrix were investigated by SQUID magnetometry from 4.2 to 300 K. The SQUID measurements showed superparamagnetism of prepared nanospheres with a blocking temperature of 160 K and saturation magnetization 1.4 mT.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Polymeric magnetic nanospheres (NPs) have been considered as promising carriers for anticancer agents. This means of delivering and concentration of drug at the desired place by the influence of external magnetic field could reduce side-effects and increase therapeutic efficacy. Taxol, as an portant anticancer drug, has been chosen for encapsulation in the polymer for its significant role against a wide range of tumours (breast cancer, ovarian carcinoma, lung cancer, head and neck carcinomas and acute leukaemias) [1]. The structure of taxol is shown in figure 1. Taxol is one of the natural diterpenoid alkaloids first isolated from the bark of the yew (*Taxus brevifolia*) [2]. It takes all of the bark of six large trees, each 100 years old, to produce enough taxol to treat just one patient. This is not affordable from nature. The total chemical synthesis of taxol has been achieved, but it may not be commercially practical because of the complex and unusual chemistry. The possible solution may be semisynthesis of the drug from a more abundant source such as English yew trees and Chinese red bean yew trees. The other limitation of taxol is its high insolubility in water and most pharmaceutical solvents [3]. Adjuvants such as Cremophor EL have to be used in its current clinical administration, which, by itself, causes serious side-effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity, cardiotoxicity and

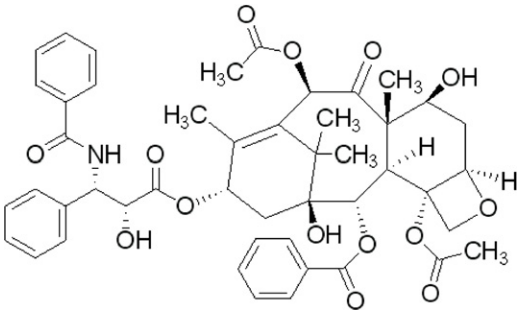

Figure 1. Chemical structure of taxol.

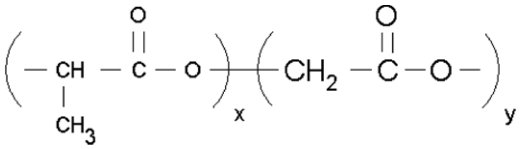

Figure 2. Chemical structure of PLGA polymer.

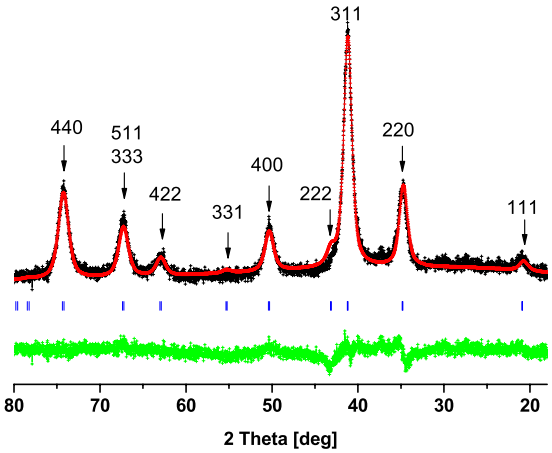

Figure 3. XRD spectrum of prepared magnetite particles.

other inconvenience in application. In order to eliminate the side-effects of the adjuvant and improve the therapeutic efficacy of the drug, alternative dosage forms have been suggested, including liposomes, microspheres and polymeric nanoparticles. Other important advantages associated with the use of nanoparticles include their ease of preparation with well defined biodegradable polymers and their high stability in biological fluids and during storage.

Poly(lactic-co-glycolic acid) (PLGA) (figure 2), from the ester family, has been widely used in the biomedical industry as a major components in biodegradable sutures, bone fixation nails and screws. It is a well characterized polymer; its subproducts are nontoxic; monomeric units lactic and glycolic acid occur naturally in the human body and are easily eliminated through the glycolytic pathway as carbon dioxide and water. There are numerous publications [4] about factors that influence controlled drug release profiles by changing the PLGA copolymer ratio, which affects the crystallinity (low crystallinity, more amorphous polymer means faster degradation) of PLGA. This fact is characterized by glass transition T_g. The next factors that influence the degradation process and release profile of the entrapped drug are polymer molecular weight, polymer–drug ratio, environmental temperature, pH and finally geometry of the delivery system. In general, low molecular weight PLGA with higher amounts of glycolic acid offers faster degradation rates [5].

The aim of our paper is to prepare PLGA (poly-D,L-lactide-co-glycolide) nanospheres loaded with biocompatible magnetic fluid and anticancer drug taxol by a modified nanoprecipitation technique and to investigate their magnetic properties. The infrared spectroscopy FTIR, DSC and TGA measurements were used to confirm incorporation of magnetic particles and drug in the PLGA polymer. The morphology and the particle size was observed by TEM and SEM. To investigate the magnetic properties of the magnetic fluids and drug loaded magnetic PLGA nanospheres, magnetization measurements have been made using a SQUID.

2. Experimental methods and results

2.1. Materials

Taxol was obtained from Indena. Poly(D,L-lactide-co-glycolide) (PLGA) with D,L-lactide to glycolide ratio of 85:15, average molecular weight of 50 000–75 000 and glass transition temperature T_g = 50 °C, Pluronic F 68, was purchased from Sigma and poly(ethylene glycol) with M_W = 100 000 was supplied by Merck.

2.2. Preparation and characterization of biocompatible magnetic fluids

The coprecipitation method of ferric and ferrous salts in an alkali aqueous medium was used to prepare magnetite particles [6]. X-ray diffraction measurement was performed to identify the crystallographic structure of prepared iron oxide particles. The XRD spectrum of the prepared magnetite is shown in figure 3. The six peaks in the figure corresponds to Miller index values {*hkl*} of {220}, {311},{400}, {422}, {511, 333}, and {440}, respectively. Evidently, the sample is indeed magnetite.

The magnetic properties of magnetic particles were characterized by SQUID magnetometer at room temperature and confirmed a superparamagnetic behaviour (data not shown). To prepare a stable colloidal suspension of magnetic particles, sodium oleate ($C_{17}H_{33}COONa$) as a first surfactant was used for the modification of prepared magnetic particles to prevent their agglomeration. To improve stability and increase the circulation half-life of the particles, poly(ethylene glycol) (PEG) as a second surfactant was added to the magnetite—oleate system and stirred for 3 h. Preliminary *in vitro* and *in vivo* experiments have already shown the effectiveness of such a coating [7]. Surfaces covered with PEG are biocompatible, i.e. nonimmunogenic, nonantigenic and protein resistant. This magnetic fluid (MF-PEG) had a

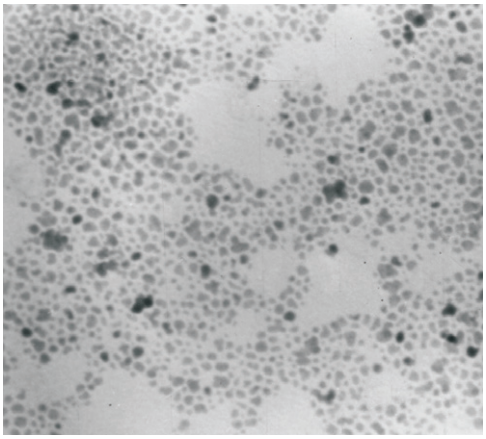

Figure 4. TEM image of magnetic fluid (1 mm = 6 nm).

solid loading of 110.8 mg ml^{-1}. The solid loading in this case includes the weights of the iron oxide, sodium oleate and PEG. The composition 37.3 wt% magnetite, 50.6 wt% sodium oleate and 12.1 wt% PEG was determined by TGA and also confirmed by SQUID measurements. Examination of the prepared biocompatible magnetic fluid MF-PEG by TEM was done using a Tesla BS 500 microscope normally operated at 90 kV and 80 000 × magnification by the replication technique (figure 4). A drop of magnetic fluid sample containing 5×10^{14} particles cm^{-3} was deposited on the 400 mesh copper grid and air dried before the picture was taken. About 1420 particles were analysed in the TEM image data. Figure 4 clearly shows that the basic particle size is about 12 ± 0.2 nm. The saturation magnetization of the studied MF-PEG (with a concentration of $Fe_3O_4 c = 40$ mg ml^{-1}) and average diameter of magnetite particles were estimated by SQUID to be 3.4 mT and 10 nm, respectively.

2.3. Encapsulation of taxol and MF-PEG into PLGA polymer and their characterization

The modified nanoprecipitation method was used to entrap magnetic fluids and anticancer drug taxol into polymer nanospheres [8]. Briefly, 100 mg of PLGA and 1 mg of taxol were dissolved in 10 ml acetone to prepare a organic phase. Next, the aqueous solution was prepared by mixing Pluronic F66 as a stabilizing agent, and 2 ml MF-PEG (40 mg ml^{-1} Fe_3O_4). Then, the organic phase was added dropwise into the aqueous phase and stirred vigorously for several hours to allow complete evaporation of the organic solvent at room temperature. A turbid nanosphere suspension was formed. The prepared samples were observed by TEM and SEM microscopy (figures 5, 6, 7) to obtain information about the morphology, surface characterization (shape, distribution, aggregation) and particle size of the magnetite and drug loaded polymeric nanospheres. Figure 5 shows a TEM image of magnetic nanoparticles embedded in the PLGA polymeric matrix, where dark-contrast images correspond to the magnetic particles implanted in PLGA. The SEM images (figures 6, 7) clearly show that nanospheres have nearly spherical shape with size ∼250 nm.

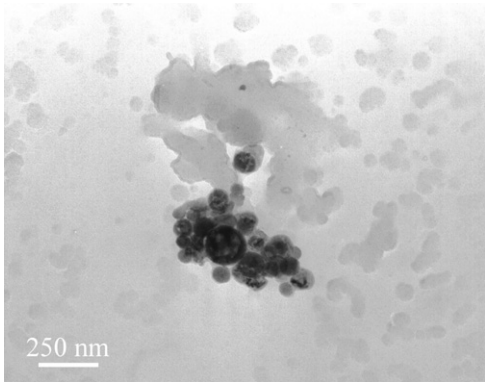

Figure 5. TEM image of magnetite loaded PLGA nanospheres.

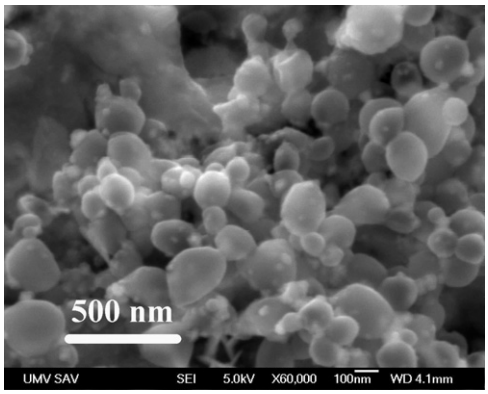

Figure 6. SEM image of magnetite–PLGA nanospheres.

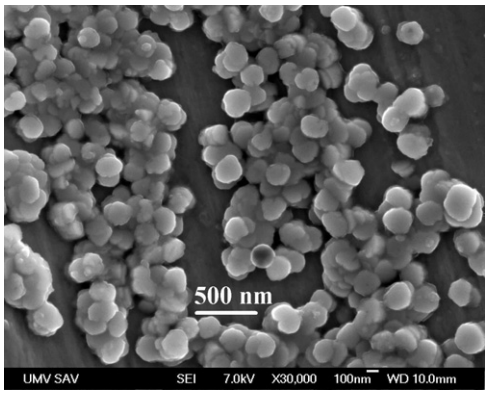

Figure 7. SEM image of taxol–magnetite–PLGA nanospheres.

With the aim to confirm adsorption of PEG on the surface on magnetic particles as well as encapsulation in PLGA matrix, FTIR spectroscopy was used. The IR spectra were measured using an AVATAR 330 FT-IR Nicolet spectrometer. The infrared spectra of magnetite, pure PEG, pure PLGA, and magnetite–PEG/PLGA were obtained by the KBr pellet method. In this method, the solid sample was finely pulverized with pure, dry KBr, the mixture was pressed in a hydraulic press to confirm a transparent pellet, and the spectrum of the pellet was measured. The MF-PEG/PLGA IR spectra (figure 8) show the presence of all bands of the PEG (C–O–C and

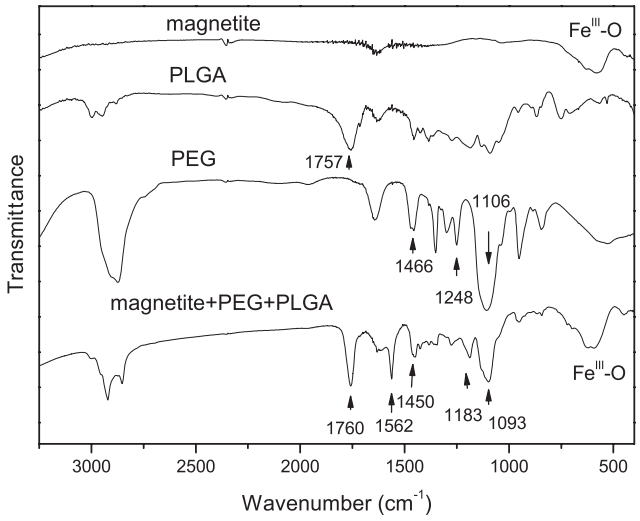

Figure 8. FTIR spectra of magnetite, pure PLGA, pure PEG, and MF-PEG/PLGA. (The spectra are shifted vertically for clarity.)

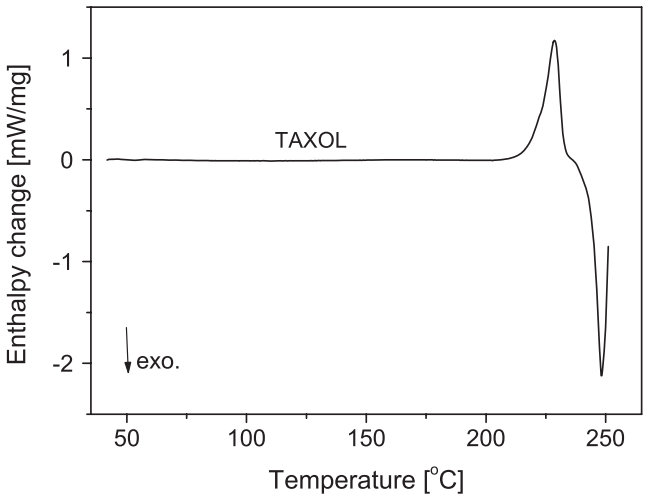

Figure 9. DSC thermogram of pure taxol.

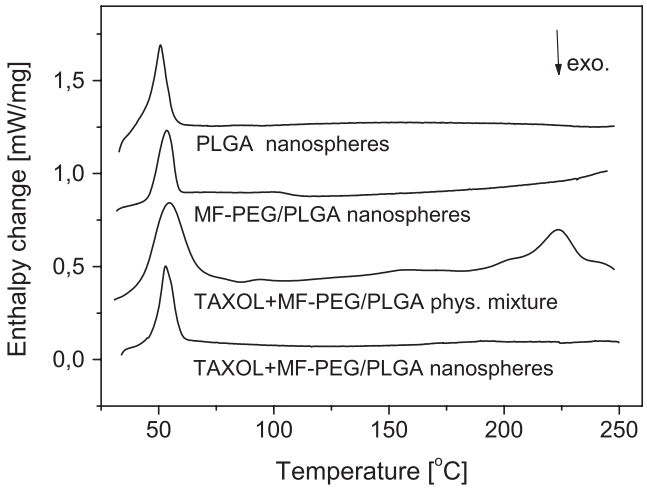

Figure 10. DSC traces of prepared nanospheres.

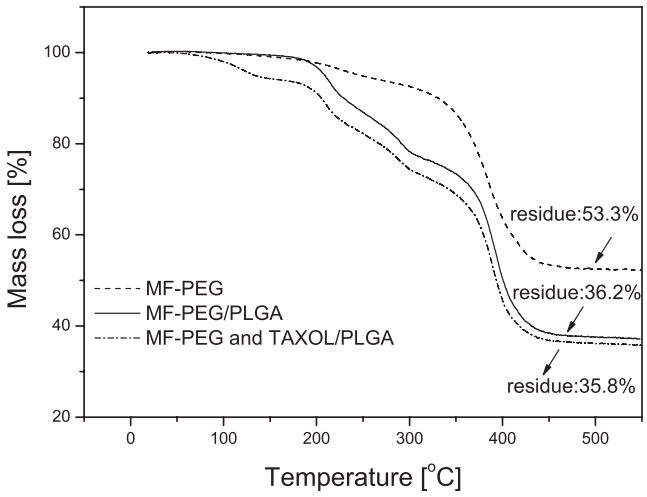

Figure 11. TGA traces of MF-PEG, MF-PEG and MF-PEG/taxol encapsulated in PLGA.

C–O–H in the region from 1093 to 1348 cm^{-1}) and PLGA (C=O stretch band at 1760 cm^{-1}), and a weak peak at 582 cm^{-1} that is attributed to the magnetite particles.

DSC and TGA methods are a very useful tool in the investigation of thermal properties of nanospheres. DSC measurements were carried out using a Perkin Elmer DSC 7 calorimeter under the heating rate of 10 °C min^{-1}. DSC studies were performed to investigate the physical state of drug in the nanoparticles, because this aspect could influence the *in vitro* and *in vivo* release of drug from the systems. There are several different combinations of the coexistence of drug/polymer in the polymer carrier [9]. Moreover, a drug may be present either as a solid solution or a solid dispersion in an amorphous or crystalline polymer. Figure 9 shows a representative thermogram of pure taxol. As can be seen, a single melting endotherm at 228.6 °C, just prior to degradation, is observed. In figure 10 there are DSC curves of unloaded PLGA nanospheres, magnetite loaded PLGA nanospheres, taxol unloaded magnetic PLGA nanoparticle physical mixture and taxol loaded magnetic PLGA nanospheres. The endothermic melting peak of pure taxol (228.6 °C) (figure 9) is slightly shifted to a lower temperature (224.8 °C) in the thermogram of the physical mixture. However, the taxol melting peak totally disappeared in the DSC curve of loaded nanospheres, evidencing the absence of crystalline drug in the nanospheres samples, at least at the nanosphere surface level. Therefore, it could be concluded that taxol in nanospheres was in an amorphous or disordered crystalline phase of molecular dispersion or a solid solution state in the polymer matrix after the production. The polymer glass transition temperature was not influenced by the preparation procedure.

TGA is a useful method for the determination of the presence or absence of residual components in nanospheres. It is based on the observation of the mass loss of individual components. Figure 11 shows the TGA thermograms of the dried PEG coated magnetite particles, magnetite loaded PLGA nanospheres and taxol loaded magnetic PLGA nanospheres. The thermogravimetric investigations were carried out on a SETARAM model TGDTA92 thermobalance at the heating

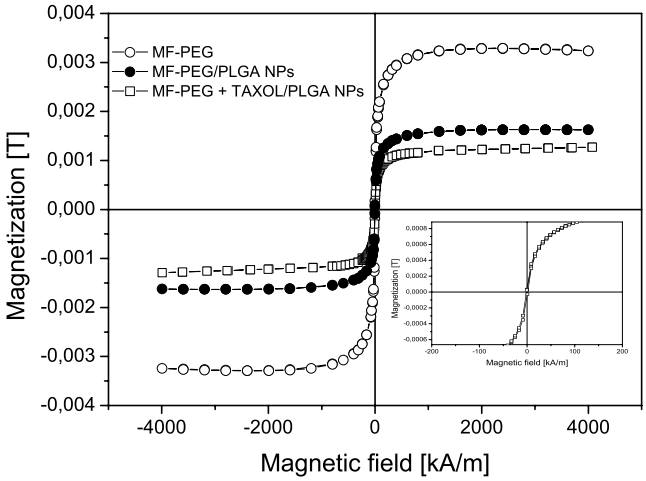

Figure 12. Hysteresis cycles of MF-PEG/PLGA and MF-PEG and taxol/PLGA at 300 K.

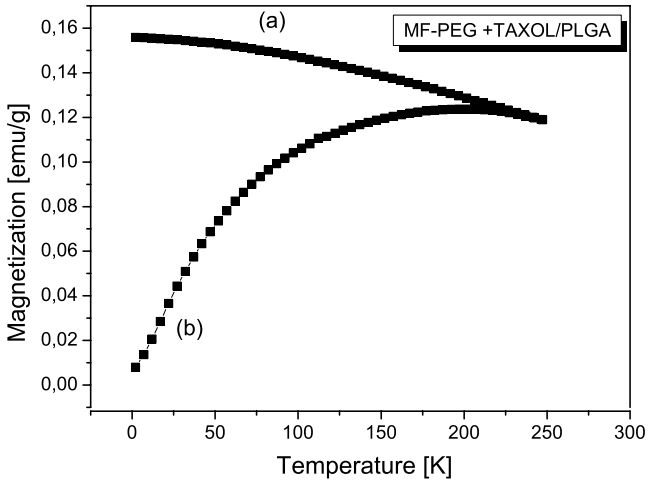

Figure 13. Temperature dependence of the magnetization curves of taxol + MF-PEG/PLGA under (a) FC and (b) ZFC at 10 mT.

Table 1. TGA data combined with data collected from the SQUID measurement.

Sample	I_S (mT)	Fe_3O_4 (mg ml^{-1})	% shell TGA	% shell SQUID
MF-PEG	3.4	40.7	—	—
MF-PEG/PLGA	1.6	18	17.1	15.5
Taxol + MF-PEG/PLGA	1.4	15.1	17.5	15.9

rate of 10 °C min^{-1} in the temperature interval from 30 to 600 °C in dynamic conditions in argon atmosphere. The TGA residue for MF-PEG at 600 °C was 53.31 wt%, that for MF-PEG/PLGA was 36.2 wt% and that for taxol loaded magnetic PLGA was 35.8 wt%. The 17.1 wt% difference between up and down thermograms was associated with the presence of PLGA and taxol. Compared with the amount of taxol added (theoretical taxol loading: 0.6 wt%/wt), it was shown that most of the taxol was encapsulated into the PLGA polymer matrix.

To investigate the magnetic properties of encapsulated MF-PEG and taxol in the PLGA matrix, FC/ZFC measurements have been made using SQUID. From figure 12 it is seen that all types of samples exhibit similar overall superparamagnetic behaviour at room temperature. The presence of the nonmagnetic shell is evident from the fact that I_S of MF-PEG + taxol/PLGA and MF-PEG/PLGA nanospheres are about one half of the pure MF-PEG. The saturation magnetization values are summarized in table 1.

The calculated values of coating for PLGA 15.5 wt% and for taxol 0.5 wt% are in good agreement with PLGA and taxol concentrations obtained from TGA. Figure 13 shows temperature-dependent magnetization plots at 10 mT for the zero-field-cooled (ZFC) and the field- (10 mT) cooled (FC) cases. The ZFC curve (figure 13) exhibits a broad maximum at 200 K, which is associated with the average blocking temperature of the nanocomposites. Below T_B, the magnetization curves exhibit hysteresis as expected. Both hysteresis loops for the ZFC and the FC cases were symmetric about the origin. By measuring the temperature dependence of coercivity below the temperature $T = 200$ K and using the procedure described in [10], we have determined the average blocking temperatures as $T_B = 160$ K. The next result obtained from SQUID measurements is the fact that each prepared individual PLGA nanosphere contains approximately 10 magnetite particles inside the polymer matrix.

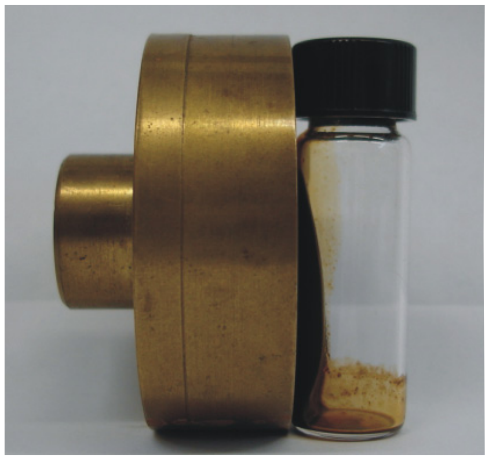

Figure 14. Encapsulated taxol and MF-PEG in PLGA attached to a magnet.

2.4. Conclusion

The surface modification of magnetite with PEG was a useful approach to prepare biocompatible magnetic fluid suitable for entrapment into a hydrophobic polymer PLGA together with the anticancer drug taxol by the nanoprecipitation method. The nanospheres were nearly spherical with mean diameter 250 nm and with entrapment efficiency of magnetite 21.5 wt% and taxol 0.5 wt%. They were superparamagnetic, with saturation magnetization 1.4 mT. Nevertheless, the prepared taxol loaded magnetic polymeric nanospheres still show sufficient magnetization for their magnetic properties to be useful from the point of view of magnetic carrier technology (figure 14).

Acknowledgments

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