Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 324 (2006) 185–190
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Preparation of poly ε-caprolactone nanoparticles containing magnetite for magnetic drug carrier

J. Yang [a,1], S.-B. Park [b,2], Ho-Geun Yoon [c,3], Y.-M. Huh [d,*], S. Haam [a,**]

[a] Department of Chemical Engineering, College of Engineering, Yonsei University, Seoul 120-749, South Korea
[b] Pharmaceutical Development Laboratory, Yuhan Research Institute, 27-3 Dangjeong-dong, Gunpo-si Gyeonggi-do 120-749, South Korea
[c] Department of Biochemistry and Molecular Biology, Center for Chronic Metabolic Disease Research, College of Medicine, Yonsei University, Seoul 120-752, South Korea
[d] Department of Radiology, College of Medicine, Yonsei University, Seoul 120-749, South Korea

Received 5 October 2005; received in revised form 25 April 2006; accepted 6 June 2006
Available online 23 June 2006

Abstract

Magnetic poly ε-caprolactone (PCL) nanoparticles were prepared in a well shaped spherical form by the o/w emulsion method. The influence of some preparative variables on the size and surface property was investigated. Nanoparticles were smooth, well individualized and homogeneous in size. The presence of magnetite and its superparamagnetic characteristic were confirmed by transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FT-IR) and vibrating sample magnetometer (VSM), respectively. The anti-cancer drug was encapsulated in the magnetic nanoparticle during preparation. A typical release behavior was observed for 30 days. In vitro experiment of magnetic susceptibility under external magnetic field demonstrated that the magnetic PCL nanoparticles have sufficient magnetic susceptibility for a potential magnetic drug carrier for targeted delivery.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Poly ε-caprolactone; Magnetite; Emulsion; Nanoparticle; Magnetic drug targeting

1. Introduction

Therapeutic treatments using drug carrier has been researched for the past decade. These drug carriers can be made of various biocompatible (or biodegradable) materials such as polymer, liposome, dendrimer (Soppimath et al., 2001; Løklinga et al., 2004; Boas and Heegaard, 2004). In case of polymer, morphology of drug carrier and drug release pattern can easily be modified by control of preparation method and processing variables (Murakami et al., 2000).

The methods to deliver drug to specific site using nano-sized drug carrier (or particle) have been extensively studied for the purpose of reducing side effect and increasing efficacy of drug. As targeting moieties of drug carrier, antigen–antibody reaction, pH, temperature and magnetic field have been widely used (Torchilin, 2000).

In case of magnetic drug targeting, magnetic materials for delivery the carrier to specific site and polymer matrix for drug loading are the core of targeted delivery using magnetic field. Magnetic particles are usually made of magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), cobalt ferrite (Fe_2CoO_4), chromium dioxide (CrO_2), etc (Cabuil, 2002). These magnetic particles have been widely used for MRI (Tiefenauer et al., 1996), hyperthermia (Jordan et al., 1999), cell separation (Zborowski et al., 1999), etc. Among them, magnetite is a well-known magnetic material, properly characterized in many aspects, whose toxicity has been demonstrated to be low, and well tolerated in the human body (Iannone et al., 1991). For nano-sized magnetite particles ($d < 10$ nm, superparamagnetic), biodegradation can occur in the lysosomes of monocyte phagocytes system cells (Okon et al., 1994).

Magnetite conjugated with specific drug has some limitations; difficult drug release control and low drug loading

* Corresponding author. Tel.: +82 2 2228 2375; fax: +82 2 362 8647.
** Corresponding author. Tel.: +82 2 2123 2751; fax: +82 2 312 6401.
E-mail addresses: 177hum@yonsei.ac.kr (J. Yang), onlyou@yuhan.co.kr (S.-B. Park), yhgeun@yumc.yonsei.ac.kr (H.-G. Yoon), ymhuh@yumc.yonsei.ac.kr (Y.-M. Huh), haam@yonsei.ac.kr (S. Haam).
[1] Tel.: +82 2 2123 3554; fax: +82 2 312 6401.
[2] Tel.: +82 31 452 4111; fax: +82 31 456 4418.
[3] Tel.: +82 2 2228 1683; fax: +82 2 362 8647.

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.06.029

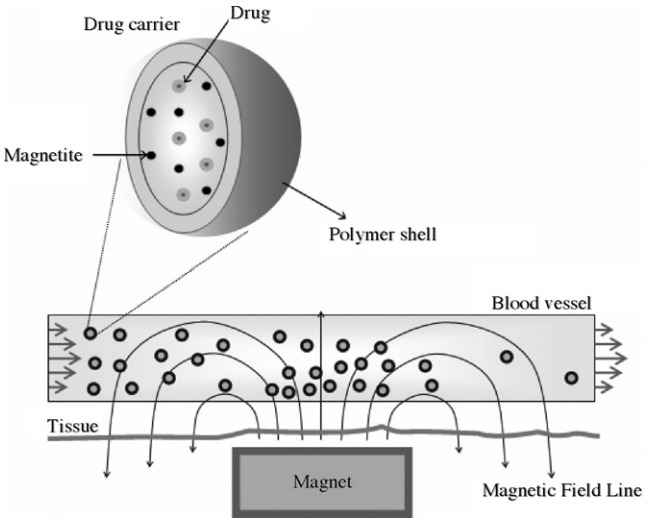

Fig. 1. The schematic representation of the magnetic drug targeting; magnetic drug carriers disintegrate in the target zone and release the drug.

capacity. To resolve these problems, many researchers have used specific organic polymers such as poly(D,L lactide-*co*-glycolide) (Jeong et al., 2003), poly(ethyl-2-cyanoacrylate) (Vauthier et al., 2003), and poly(ε-caprolactone) (Luo et al., 2002), because of their biocompatibility and biodegradable properties as well as low toxicity.

In this study, we synthesized and characterized the magnetic PCL nanoparticles containing anticancer drug (Gemcitabine and Cisplatin) as a potential drug carrier for targeted delivery (Fig. 1). To determine the physical characteristics of magnetic PCL nanoparticles, particle size and size distribution were analyzed TEM and dynamic light scattering (DLS). The presence of magnetite was confirmed by FT-IR spectra. Loading amount of magnetite and drug were analyzed using thermogravity analysis (TGA) and UV spectrometer, respectively. Saturation of magnetization and superparamagnetic characteristic of magnetite were measured by VSM. In vitro drug release experiments and magnetic mobility measurement under external magnetic field demonstrated that magnetic PCL nanoparticles can be a highly versatile magnetic drug carrier with sustained release behavior and sufficient magnetic susceptibility.

2. Materials and method

2.1. Materials

For synthesis of magnetite, ferric chloride anhydrous ($FeCl_3$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were purchased from Aldrich Chemical Co., (USA). Poly ε-caprolactone (M.W. 42,500) and poly vinyl alcohol (M.W. 15,000–20,000) was purchased from the Aldrich Chemical Co., (USA). Chloroform, ammonium hydroxide (28–30%) were purchased also from Duksan Pure Chemicals Co. (Korea). Cisplatin (Diamminedichlroplatium) and Gemcitabine (GEMZAR^{TM}) were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Lilly Co. (France S.A.), respectively.

2.2. Synthesis of hydrophobic magnetite

The hydrophobic magnetite was synthesized by co-precipitation from an aqueous Fe^{3+}/Fe^{2+} solution using concentrated ammonium hydroxide in excess (Reimers and Khalafalla, 1974). Twenty-one grams of $FeCl_3$ and 17.3 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 50 ml distilled water. Forty milliliters of ammonium hydroxide were added rapidly. After the co-precipitation of magnetite particles, oleic acid was added, and the suspension was heated to 80 °C for 30 min. The black-rump was washed with distilled water (Ramirez and Landfester, 2003).

2.3. Magnetic PCL nanoparticles by o/w emulsion method

PCL (0.5 g), magnetite (20 mg) and drugs (15 mg) were dissolved in 10 ml of dichloromethane. The organic phase was added into 20 ml of aqueous phase containing stabilizer. After mutual saturation of organic and continuous phase, the mixture was emulsified for 10 min with ultrasonicator (ULH700S, Ulssohitech, Korea) at 350 W. After solvent evaporation, sample was obtained through cleaning procedure that included serum replacement in a stirred filtration cell and three cycles of centrifugation at 10,000 rpm for 30 min. The magnetic PCL nanoparticles were dried in a vacuum oven at 35 °C and stored in ice cool bath (Fig. 2). To perform in vitro microscopic visualization, magnetic PCL nanoparticles stained with fluorescence dye (rhodamine B) were made by adding dye in solvent.

2.4. Magnetic mobility test

We made up a similar vascular system using borosilicate micro-channel and investigated for the behavior of dyed magnetic PCL nanoparticle under magnetic field using epi-fluorescence microscope (Olympus, BX51). Dyed particles were injected in sq i.d. 500 μm micro-channel (VitroCom Inc.). Magnetic field applied with permanent magnet (Nd–B–Fe).

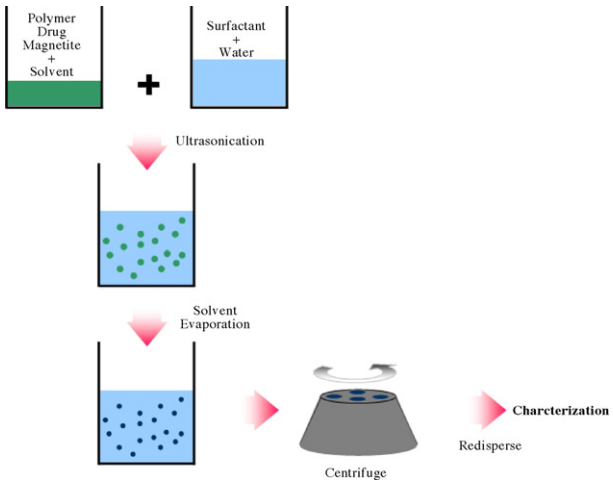

Fig. 2. Schematic representation for the process of o/w emulsion method.

2.5. Drug release studies

Drug release experiments were carried out in an aqueous release medium with the phosphate buffer solution (pH 7.4) at 37.5 ± 0.5 °C. Dried magnetic PCL nanoparticles in buffer solution put into dialysis tube of a flask that was immersed into 30 ml buffer solution. The flask was placed in a shaking incubator (SI-900, J.O Tech., Korea). At regular time intervals, 3 ml of the aqueous solution were withdrawn and replenished with 3 ml of buffer solution. The amount of released drug was monitored by measuring the absorbance using UV spectrophotometer (Optizen 2120UV, MECASYS Co., Korea).

2.6. Characterization

The magnetite concentration of the magnetic PCL nanoparticles was determined by TGA. Direct light scattering (DLS, Zetaplus, Brookhaven 9000 instruments) analysis of the magnetic PCL nanoparticles was used to determine the size and the size distribution. TEM (JEOL JEM2000, Nikon, Japan) analysis was performed to observe morphology and size of the nanoparticles. FT-IR spectra (ATI Mattson, Genesis series FT-IR) analysis was used to verify the presence of the magnetic PCL nanoparticles. The crystallinity of magnetite was confirmed by X-ray diffraction. The saturation of magnetization was evaluated using vibrating-sample magnetometer (Lakeshore, model 7300).

3. Results and discussion

A mean diameter of 9 nm of magnetite (Fig. 3) was obtained by co-precipitation process. The molar ratio of Fe^{2+}/Fe^{3+} was 3:2, which allowed the compensation of the oxidation of some iron II to iron III during the co-precipitation in an open reactor (Reimers and Khalafalla, 1974). By adding oleic acid at melting point, the magnetite was hydrophobized as illustrated in Figs. 4–5 shows X-ray diffraction patterns of the prepared magnetite nanocrystals.

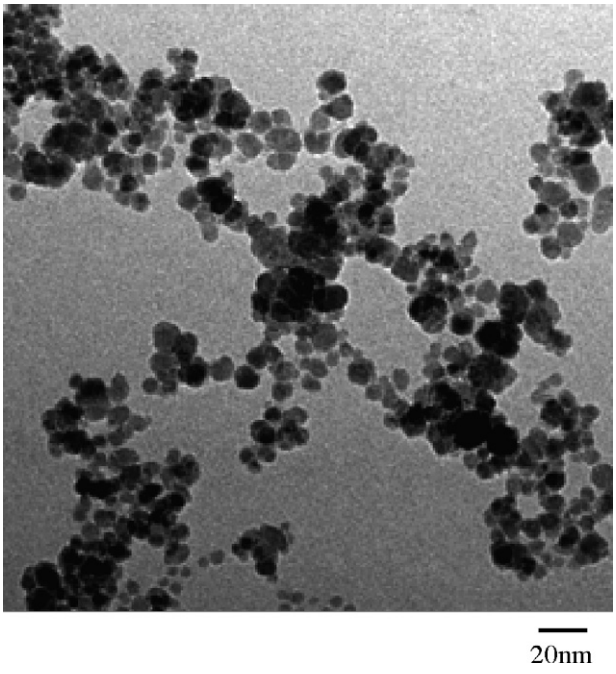

Fig. 3. TEM image of magnetite by co-precipitation method.

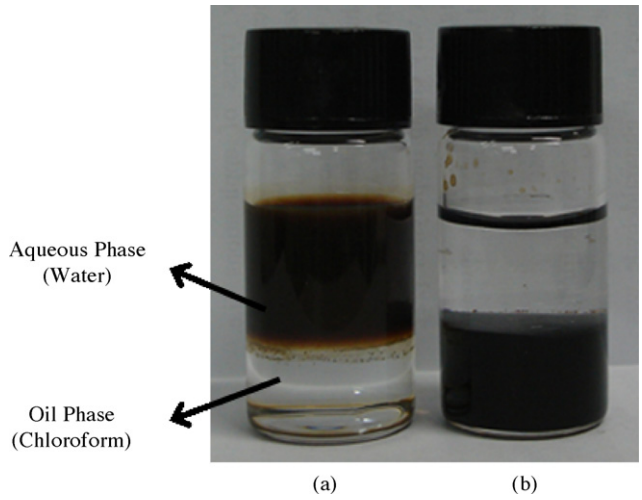

Fig. 4. The magnetite was hydrophobized by coating of oleic acid: (a) un-coated magnetite; (b) oleic acid coated magnetite.

The spherical nanoparticles with smooth surfaces and moderately uniform size distributions were obtained by the o/w emulsion method. The mean size and the size distribution of prepared particles measured by laser scattering showed 160 ± 5 nm narrow size distributions. TEM photography of magnetic PCL nanoparticles and PCL nanoparticle are shown in Fig. 6. These images clearly demonstrate that the magnetic particles are well incorporated in the core of PCL nanoparticles.

The amount of magnetite loaded in the magnetic PCL nanoparticle was measured by thermo-gravimetric analyzer (TGA) and the results were shown in Fig. 7. In Fig. 7, the maximum amount of magnetite was determined as 25 wt.%.

Fig. 8 shows the FT-IR spectra of magnetic PCL nanoparticles prepared by o/w emulsion method. The FT-IR spectra of the pure PCL nanoparticles (Fig. 8(b)) showed the characteristic bands at 1726 cm^{-1} of carbonyl band. The FT-IR spectra of magnetite exhibited in the low-frequency region (600–400 cm^{-1}) due to the iron oxide structure. Depending on Fe (II) content, the pattern of magnetite (Fe_3O_4) spectrum shows at 570 cm^{-1} (Zaitsev et al.,

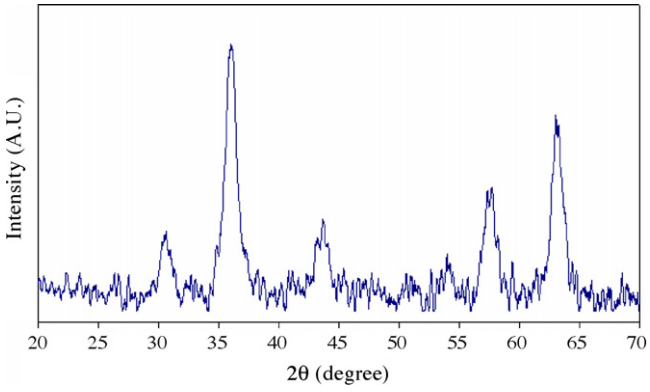

Fig. 5. X-ray diffraction patterns of prepared magnetite nanocrystals.

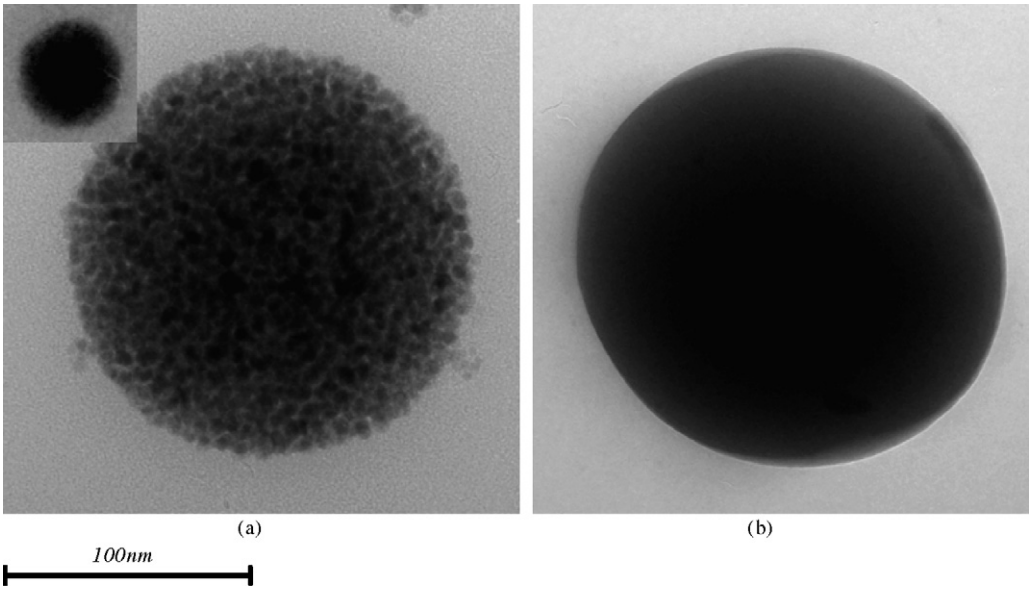

Fig. 6. TEM images of magnetic PCL nanoparticle (a); PCL nanoparticle (b). Magnetic particles are well encapsulated in PCL nanoparticle.

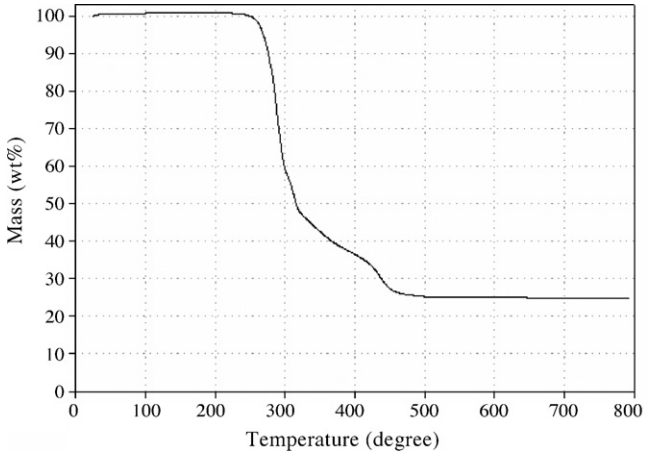

Fig. 7. Thermo-gravimetric curves of magnetic PCL particles. Encapsulated magnetite was coated with hydrophobic agent, oleic acid.

1999). As seen in Fig. 8(b) and (c), the most significant bands of magnetic PCL particles were identified in comparison with magnetite (570 cm^{-1}). In addition, the characteristic bands of PCL nanoparticles (Fig. 8(b)) and magnetite (Fig. 8(c)) were observed in the spectrum of the magnetic PCL nanoparticles (Fig. 8(a)).

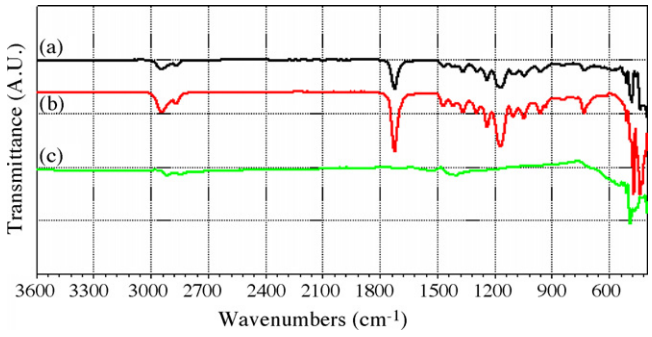

Fig. 8. FT-IR analysis of magnetic PCL particles: (a) magnetic PCL nanoparticles; (b) PCL nanoparticles; (c) magnetite coated with oleic acid.

The hysteresis loops of magnetite and the magnetic PCL nanoparticles were observed using a vibrating sample magnetometer (VSM) at 25 °C (Fig. 9). The prepared particles exhibited the superparamagnetic behavior without magnetic hysteresis. The saturation of magnetization of magnetic PCL nanoparticles was 17.6 emu/g at 9000 Gauss. The saturation of magnetization of magnetic PCL nanoparticles was smaller than that of bulk magnetite, but both particles had similar properties that were close to the paramagnetic behavior.

In vitro microscopic visualization represents that the prepared particles have the magnetic mobility under external magnetic field (Fig. 10). The magnetic PCL nanoparticles stained with fluorescence dye (rhodamine B) were suspended in the rectangular channel of sq. i.d. 1.0 mm (Fig. 10(a)). When the commercial Nd–Fe–B magnet (3500 Gauss) was placed on the wall of the channel, the particles gathered on the wall within a minute

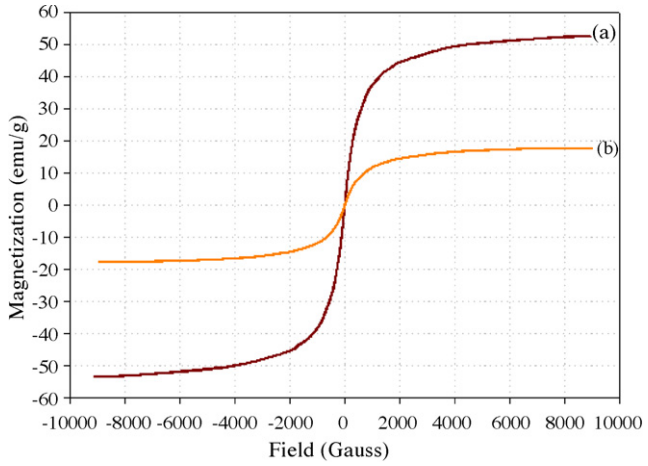

Fig. 9. Hysteresis loop by VSM of: (a) magnetite; (b) magnetic PCL nanoparticles.

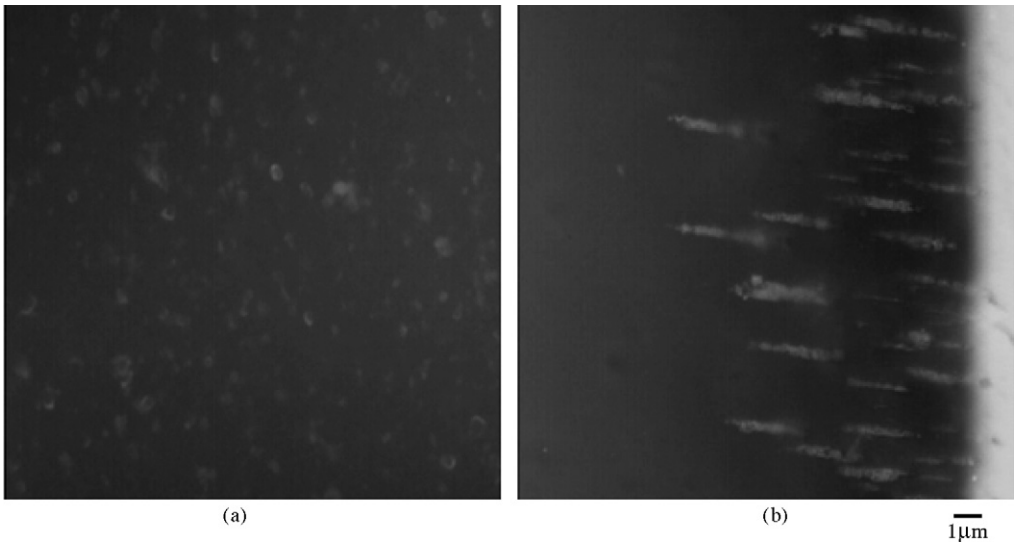

Fig. 10. Images of magnetic PCL nanoparticles by epi-fluorescence microscopy (1000×): (a) without magnetic field; (b) with magnetic field. Nd–Fe–B magnet is located at right-side of the channel.

(Fig. 10(b)). It demonstrated that the magnetic PCL nanoparticles were sensitive to external magnetic fields and seemed to have sufficient magnetization as a magnetic drug carrier for targeted delivery.

The amount of encapsulated drug in the nanoparticles and the drug release profile (Fig. 11) were obtained using UV spectrophotometer. The release test was performed with three times and obtained values used for calculation of mean value and standard deviation. The drug loading amount of Cisplatin and Gemcitabine were 24.6 and 7.6 wt.%, respectively.

This difference in the drug loading amount was caused by the solubility of drug in oil phase. The drug release of Cisplatin (hydrophobic) resulted in more sustained release behavior than that of Gemcitabine (hydrophilic) since the affinity of Cisplatin to oil phase was higher than that of Gemcitabine. In early stage of release, an initial burst effect was observed in both drugs. This behavior was probably due to small amount of poorly encapsulated drug bound to the nanoparticle surface and/or to residual drug from manufacturing and handling (Ammoury et al., 1993). Thus, the mechanism of the drug release from magnetic PCL nanoparticles is responsible for mainly a diffusion process from the oil core through the polymeric network constituting the nanoparticle.

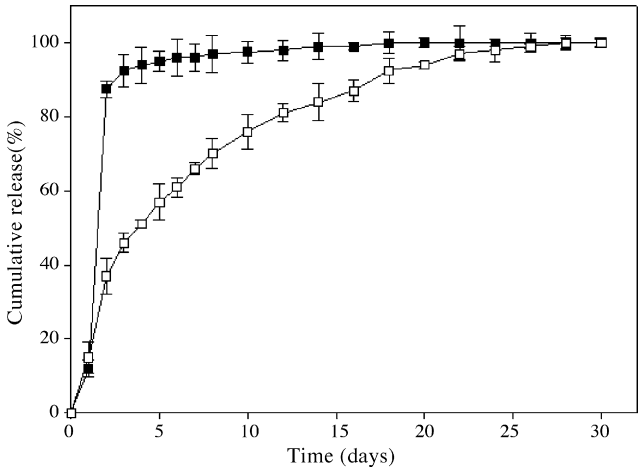

Fig. 11. Release profiles of drug from magnetic PCL nanoparticles. Cisplatin (□) and Gemcitabine (■).

4. Conclusion

Biodegradable poly ε-caprolactone (PCL) nanoparticles containing magnetite and anticancer drug were successfully synthesized by emulsion techniques for magnetic drug targeting. FTIR spectra and TEM showed the presence of the magnetite, which were well incorporated in the PCL nanoparticles. In addition, TEM images and dynamic laser scattering analysis demonstrated that the magnetic PCL nanoparticles have smooth surfaces and moderately uniform size distributions (160 ± 5 nm). The possession of sufficient paramagnetic property was confirmed by in vitro microscopic visualization and VSM. Even though the drug loading capacity differs from the hydrophobicity of drugs, the release kinetics of o/w emulsion method showed that the entrapment of drugs in the nanoparticles could highly retard its in vitro release test. These results suggest that the magnetic PCL magnetic nanoparticles may have a potential as a highly versatile carrier for targeted delivery approach.

Acknowledgement

This work was supported by KOSEF through National Core Research Center for Nanomedical Technology (R15-2004-024-00000-0) and (R01-2006-000-10023-0), Republic of Korea.

References

Ammoury, N., Dubrasquet, M., Fessi, H., Devissaguet, J.P., Puisieux, F., Benita, S., 1993. Indomethacin-loaded poly(D,L-lactide) nanocapsules,protection from gastrointestinal ulcerations and antiinflammatory-activity, evaluation in rats. Clin. Mater. 13, 121–130.

Boas, U., Heegaard, P.M.H., 2004. Dendrimers in drug research. Chem. Soc. Rev. 33, 43–63.

Cabuil, V., 2002. Preparation and properties of magnetic nanoparticles. In: Hubbard, A.T. (Ed.), Encyclopedia of Surface and Colloid Science. Marcel Dekker Inc., New York, pp. 4306–4321.

Iannone, A., Magin, R.L., Walczack, T., Federico, M., Swartz, H.M., Tomasi, A., Vannini, V., 1991. Blood clearance of dextran magnetite particles determined by a non-invasive in vivo ESR method. Magn. Reson. Med. 22, 435–442.

Jeong, J.H., Byun, Y., Park, T.G., 2003. Synthesis and characterization of poly(L-lysine)-*g*-poly(D,L-lactic-*co*-glycolic acid) biodegradable micelles. J. Biomater. Sci. Polym. Ed. 14, 1–11.

Jordan, A., Scholz, R., Wust, P., FaK hling, H., Felix, R., 1999. Magnetic fluid hyperthermia (MFH): cancer treatment with AC magnetic field induced excitation of biocompatible superparamagnetic nanoparticles. J. Magn. Magn. Mater. 201, 413–419.

Løklinga, K.-E., Fossheimb, S.L., Klavenessa, J., Skurtveit, R., 2004. Biodistribution of pH-responsive liposomes for MRI and a novel approach to improve the pH-responsiveness. J. Controlled Release 98, 87–95.

Luo, L., Tam, J., Maysinger, D., Eisenberg, A., 2002. Cellular internalization of poly(ethylene oxide)-b-poly(E-caprolactone) diblock copolymer micelles. Bioconjug. Chem. 13, 1259–1265.

Murakami, H., Kobayashi, M., Takeuchi, H., Kawashima, Y., 2000. Further application of a modified spontaneous emulsification solvent diffusion method to various types of PLGA and PLA polymers for preparation of nanoparticles. Powder Technol. 107, 137–143.

Okon, E., Pouliquen, D., Okon, P., Kovaleva, Z.V., Stepanova, T.P., Lavit, S.G., Kudryavtsev, B.N., Jallet, P., 1994. Biodegradation of magnetite dextran nanoparticles in the rat: a histologic and biophysical study. Lab. Invest. 91, 895–903.

Ramirez, L.P., Landfester, K., 2003. Magnetic polystyrene nanoparticles with a high magnetite content obtained by miniemulsion processes. Macromol. Chem. Phys. 204, 22–31.

Reimers G.W., Khalafalla S.E., Production of magnetic fluids by peptization techniques, 1974. US Patent No. 3,843,540.

Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Controlled Release 70, 1–20.

Tiefenauer, L.X., Tschirky, A., Kuhne, G., Andres, R.Y., 1996. In vivo evaluation of magnetite nanoparticles for use as a tumor contrast agent in MRI. Magn. Reson. Imaging 14, 391–402.

Torchilin, V.P., 2000. Drug targeting. Eur. J. Pharm. Sci. 11, S81–S91.

Vauthier, C., Dubernet, C., Fattal, E., Pinto-Alphandary, H., Couvreur, P., 2003. Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications. Adv. Drug Deliv. Rev. 55, 519–548.

Zaitsev, V.S., Filimonov, D.S., Presnyakov, I.A., Gambino, R.J., Chu, B., 1999. Physical and chemical properties of magnetite and magnetite-polymer nanoparticles and their colloidal dispersions. J. Colloid Interface Sci. 212, 49–57.

Zborowski, M., Sun, L., Moore, L.R., Stephen Williams, P., Chalmers, J.J., 1999. Continuous cell separation using novel magnetic quadrupole flow sorter. J. Magn. Magn. Mater. 194, 224–230.