Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 31–38
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Optimization of iron oxide nanoparticles encapsulation within poly(D,L-lactide-co-glycolide) sub-micron particles

Lazare Ngaboni Okassa, Hervé Marchais *, Laurence Douziech-Eyrolles, Katel Hervé, Simone Cohen-Jonathan, Emilie Munnier, Martin Soucé, Claude Linassier, Pierre Dubois, Igor Chourpa

Magnetically Targeting Anticancer Drugs Research Group, School of Pharmaceutical Sciences, University François Rabelais of Tours, France

Received 28 July 2006; accepted in revised form 19 December 2006
Available online 12 January 2007

Abstract

This work describes a method for preparation of sub-micron poly(D,L-lactide-co-glycolide) (PLGA) particles loaded with magnetite/maghemite nanoparticles to be used as magnetically-controlled drug delivery systems. The methodology of simple emulsion/evaporation technique has been optimized to provide greater iron oxide loading rates. The surface of iron oxide nanoparticles was coated with oleic acid (OA) for better compatibility with organic phase containing the polymer. To increase their loading into polymeric sub-micron particles, we added dried iron oxide nanoparticles in variable ferrite/polymer ratio of 1:1; 1:1.5 and 1:2 w/w. Composition and surface properties of obtained composite sub-micron particles have been studied in comparison with those of ferrite-free PLGA sub-micron particles. Presence of magnetite/maghemite was qualitatively confirmed by characteristic bands in the FT-IR spectra of composite sub-micron particles. Quantification of the incorporated iron was achieved by AAS. The highest incorporation rates of ferrite (up to 13.5% w/w) were observed with initial ferrite/polymer ratio of 1:1 w/w. TEM images indicate that the composite sub-micron particles are nearly spherical. According to laser granulometry data, average hydrodynamic diameter of the composite sub-micron particles is close to 280 nm, independently of ferrite presence. Electrophoretic properties (zeta potential) were very similar for both composite and ferrite-free PLGA sub-micron particles, thus indicating that the polymeric coating should mask the surface of ferrite nanoparticles buried inside. Finally, composite sub-micron particles exhibit superparamagnetic property.

Keywords: Oleic acid-coated magnetite nanoparticles; PLGA; Composite sub-micron particles; Emulsion–evaporation technique; Magnetic carrier technology

1. Introduction

Magnetic polymeric sub-micron particles offer promising drug delivery applications. An external localized magnetic field gradient may be applied to a chosen site to retain drug-loaded magnetic polymeric sub-micron particles from blood circulation [1]. If the magnetic polymeric sub-micron particles are designed appropriately, they could be used to increase antitumor efficacy and to reduce systemic side-effects of anticancer drugs.

To make such magnetic polymeric drug delivery systems, both drug and magnetic nanoparticles should be incorporated in sub-micron particles of biodegradable/biocompatible polymers. Magnetic nanoparticles widely described in the literature to be useful for this purpose are nanocrystals of magnetite [2,3]. Recently we have shown that magnetite nucleus is covered with a thin layer of maghemite [4]. Suitable biodegradable polymers, such as poly(ε-caprolactone), polylactide or poly(lactide-co-glycolide), are usually chosen to prepare non-magnetic

---

* Corresponding author. Department of Pharmaceutical Technology, School of Pharmaceutical Sciences, University François Rabelais of Tours, 31 Avenue Monge, 37200 Tours, France. Tel.: +33 2 4736 7197; fax: +33 2 4736 7198.

E-mail address: marchais@univ-tours.fr (H. Marchais).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.12.020

drug-loaded sub-micron particles. These polyesters have biocompatible and biodegradable properties and are approved by FDA for human therapeutic use. In such drug delivery system, the release mechanism of drug molecules includes diffusion plus biodegradation. The degradation rate of the polymer could be modulated by different factors such as the chemical composition, the molecular weight or the water permeability [5].

Several processes have been developed to prepare magnetic polymeric sub-micron particles including emulsion polymerization [6], double emulsion (w/o/w) evaporation [7], inverse microemulsion polymerization [8], miniemulsion polymerization [9] and emulsification–diffusion method [10]. The main challenge is that the conditions must be such that all the magnetite nanoparticles are transferred uniformly into the resulting composite sub-micron particles. It often carries the risk of incomplete and non-uniform encapsulation in which the magnetite content should not be large enough for quick magnetic separation.

The main goal of this study was to incorporate a significant amount of magnetite nanoparticles into PLGA sub-micron particulate matrix in order to obtain sufficient magnetic property to the carrier system for effective targeting. The above approach is further limited by the amount of pure magnetite nanoparticles that can be incorporated into other sustained-release polymeric drug carrier systems (such as micro- or nanoparticles) formulated from polylactides (PLA) or poly(lactide-co-glycolide) (PLGA) [11]. This was mainly due to the hydrophilic nature of the magnetite nanoparticles which causes low incorporation in the hydrophobic polymer matrix. To avoid this problem, we have firstly synthesized oleic acid-coated magnetite (OA-coated magnetite) nanoparticles with increased lipophilicity and stability [12]. Then, these OA-coated magnetite nanoparticles were dispersed into an organic phase containing the polymer (PLGA) and the resulting dispersion was emulsified into an aqueous phase containing polyvinyl alcohol (PVA) as emulsifier, according to the emulsion/evaporation technique. In the present study, we investigate the effect of weight ratio of OA-coated magnetite nanoparticles to PLGA on the magnetite entrapment efficiency and other important characteristics of the composite sub-micron particles, including overall composition, morphology, size distribution, zeta potential and magnetic character.

2. Materials and methods

2.1. Materials

Biodegradable PLGA (50:50 lactide:glycolide, Mw 40,000–75,000) was provided by Sigma–Aldrich (St-Quentin Fallavier, France).

Iron (II) chloride anhydrous ($FeCl_2$), iron (III) chloride anhydrous ($FeCl_3$), oleic acid (density: 0.89, M_w: 282), methylene chloride and polyvinyl alcohol 4–88 (M_w: 31,000) were purchased from Sigma–Aldrich (St-Quentin Fallavier, France) and used as received. All aqueous solutions were prepared with deionized water filtered through a 0.45 μm pore size filter (Interchim, France).

2.2. Synthesis of OA-coated magnetite nanoparticles

The synthesis of surfactant-stabilized magnetite nanoparticles was previously described with some modifications [2]. We have checked the particular conditions (salt concentrations, pH) needed to obtain magnetite coated by oleic acid. The procedure consists of the following steps:

(a) Mixing Fe^{+2} and Fe^{+3} solutions: 0.42 g $FeCl_2$ and 1.09 g $FeCl_3$ ($Fe^{3+}/Fe^{2+} = 2.59$) were dissolved in 10 ml of 1N HCl by mechanical stirring.
(b) Co-precipitation step: this acid solution was added dropwise to an aqueous solution (90 ml) of 1N KOH under N_2 atmosphere leading to a black precipitate.
(c) Washing with deionized water: the precipitate was isolated by decantation and washed several times with deionized water until the pH of the medium was around 9.
(d) Oleic acid adsorption: after the washing step, 20 ml of oleic acid was added to the alkaline medium containing magnetite nanoparticles under vigorous stirring for 1 h at room temperature.
(e) Washing with organic solvent: finally, the oleic acid-treated magnetite nanoparticles were washed 3 times with 30 ml acetone and redispersed in methylene chloride.
(f) Drying step: the wet precipitate was dried in an oven at 40 °C during 48–72 h before used.

2.3. Preparation of composite sub-micron particles

The procedure followed to prepare composite sub-micron particles from OA-coated magnetite nanoparticles and PLGA was the conventional simple emulsion/evaporation technique. Briefly, 100 mg of dried OA-coated magnetite nanoparticles was mixed with an organic solution of the polymer (100–200 mg of PLGA in 2 ml methylene chloride) by vortexing. This organic solution was then emulsified by sonication in 4 ml of an aqueous PVA solution (3% w/v) for 30 s (15 W). The resulting simple emulsion was diluted in 50 ml aqueous PVA solution (0.3% w/v) under mechanical stirring and the solvent was rapidly eliminated by evaporation under reduced pressure.

2.4. Purification of composite sub-micron particles

After solvent removal, the composite sub-micron particles were collected by centrifugation at 15,000*g* for 30 min at 4 °C. The particles were washed with deionized water and redispersed in 4 ml of aqueous solution of 0.05 M HNO_3 . To remove unloaded magnetite nanoparticles, the suspension was neutralized in Tris buffer pH 7.4

and the resulting aggregates were eliminated by mild centrifugation (1000*g* for 15 min at 4 °C). Then, the composite sub-micron particles remaining in the supernatant were collected by centrifugation at 20,000*g* for 1 h at 4 °C, washed with deionized water and resuspended in 2 ml of aqueous solution of trehalose as cryoprotectant (5% w/v). The washed composite sub-micron particles were then stored under frozen conditions (−20 °C) until they were freeze-dried for 24 h (Lebold-Heraeus freeze-dryer, Heraeus, France).

2.5. Physicochemical characterization of magnetite nanoparticles and composite sub-micron particles

2.5.1. Transmission electron microscopy (TEM)

The morphology examination of various particle samples was performed using an electronic transmission microscope at 88 kV (Jeol 1010, Jeol, Japan). The samples were placed on a carbon-coated copper TEM grid, stained with 3% (w/v) uranyl acetate for 1 min (for composite sub-micron particles only) and then allowed to air-dry before TEM viewing.

2.5.2. FT-IR spectrometry

Measurements were carried out on a Bruker Vector® 22 FT-IR spectrometer (Bruker, Germany). FT-IR spectra were recorded before and after processing the coating of magnetite nanoparticles by oleic acid and their incorporation in the PLGA polymer matrix. Dried samples were mixed and pressed with KBr to obtain pellets for FT-IR analysis.

2.5.3. Composite sub-micron particles size determination

The mean diameter and polydispersity index of the composite sub-micron particles were determined by photon correlation spectroscopy (PCS) using a Malvern Autosizer® 4700 (Malvern Instruments, Malvern, UK) with photodetector perpendicular to the laser beam. The nanoparticle samples were resuspended in distilled water by sonication before measurement. Each measurement was done in triplicate.

2.5.4. Surface charge

Magnetite-free PLGA and composite sub-micron particles were characterized with respect to zeta potential by using a Malvern Zetasizer® 3000 (Malvern Instruments, Malvern, UK). The freeze-dried composite nanoparticle samples were suspended in an aqueous solution of NaCl 10^{-3} M (pH 6.5–6.8) and sonicated before measurement. The obtained homogeneous suspensions were examined to determine the zeta potential. Each measurement was done in triplicate.

In addition, the surface charge properties of different types of composite nanoparticles were analysed by electrophoresis measurement (zeta potential) as a function of pH (ranging from 2 to 9) in the presence of 10^{-3} M NaCl using the same device. Each measurement was done in triplicate.

2.5.5. Magnetite entrapment efficiency and magnetite content determination

To determine the amount of magnetite incorporated in the composite sub-micron particles, a weighed sample of freeze-dried composite sub-micron particles was digested by 5 ml of DMSO during 30 min. Then, 10 ml of HCl 6N was added and the digestion was maintained up to 2 h and the resultant solution was diluted with HCl 1%. The iron concentration in this solution was determined by atomic absorption spectrophotometry (AAS) measurements at 248 nm (SpectrAA-10 plus, Varian, France). A calibration curve was obtained by treating an acidic solution of $FeCl_3$ (1 g/l) in the same conditions. The amount of the loaded magnetite was calculated from the amount of iron measured.

Magnetite entrapment efficiency (%) and magnetite content (% w/w) were calculated by Eqs. (1) and (2), respectively:

Magnetite entrapment efficiency (%):

$$= \frac{\text{Mass of magnetite in composite sub-micron particles}}{\text{Mass of magnetite used in formulation}} \times 100 \tag{1}$$

Magnetite content (% w/w):

$$= \frac{\text{Mass of magnetite in composite sub-micron particles}}{\text{Mass of composite sub-micron particles}} \times 100 \tag{2}$$

2.5.6. Magnetization measurements

A Quantum Design MPMS SQUID magnetometer was used to investigate the magnetic properties of the OA-coated magnetite nanoparticles and PLGA composite sub-micron particles. The magnetization (M, emu/g) of these different particles, pressed in the form of pellets, was measured as a function of the magnetic field (H, Oe) at 5 and 300 K.

3. Results and discussion

3.1. Characterization of OA-coated magnetite nanoparticles

The OA-coated magnetite nanoparticle synthesis procedure described in the experimental section led to a stable colloidal dispersion of iron oxide nanoparticles in methylene chloride. The magnetite concentration in the iron oxide nanoparticles synthesized is $62.1 \pm 4.8\%$ m/m (according to iron dosing by AAS and molecular fraction determination by Raman spectrometry; see Ref. [4]). Transmission electron microscopy was used to gain information on the size and shape of the OA-coated magnetite nanoparticles with the aim of determining the optimal synthesis conditions that provide the lowest size and the best monodispersity. According to different TEM photomicrographs like Fig. 1, the estimated particle diameter was below 10 nm with a relatively good monodispersity. The presence of some clusters could be due to the aggregation of individual particles with an incomplete coating by oleic acid molecules. Nevertheless, no difference in size was observed with uncoated magnetite nanoparticles [12].

The FT-IR spectra of uncoated and OA-coated magnetite nanoparticles exhibit the characteristic band of magnetite at 587 cm^{-1} and 593 cm^{-1}, respectively, (Figs. 2a and b). FT-IR spectrometry is also used to evidence the adsorption of oleic acid on iron oxide surface. The spectrum of OA-coated magnetite nanoparticles shows that both stretching modes appear: the peaks at 2855 and 2926 cm^{-1} correspond to the symmetric and asymmetric CH_2 stretching bands, respectively, (Fig. 2b). In addition, it could be noted the presence of two bands in the 1500–1400 cm^{-1} region which were attributed to the oleate ion immobilized on the surface of the magnetite nanoparticles. Montagne et al. [2] demonstrated that this complexation was characterized by two bands at 1425 cm^{-1} (V_s: COO^-) and 1520 cm^{-1} (V_{as}: COO^-). The oleate form is due to the alkaline (pH 9) reaction conditions for the preparation of the OA-coated magnetite nanoparticles. According to Deacon and Phillips [13], the carboxylate ion may be coordinated to a metal atom in one of the following structures (Fig. 3):

- structure I: unidendate complex where one metal ion is binding with one carboxylic oxygen atom
- structure II: bidendate complex where one metal ion is binding with two carboxylate oxygens
- structure III: bridging complex where two metal ions are binding with two carboxylate oxygens

OA-coated magnetite nanoparticle infrared spectra indicated the presence of two bands, 1438 cm^{-1} (V_s: COO^-) and 1538 cm^{-1} (V_{as}: COO^-), attributed to the oleate ion immobilized on the magnetite surface. The maxima asymmetric and symmetric frequency values and the Δ value equal to 100 cm^{-1} indicate the existence of a bidentate structure II or a bridging structure III (Fig. 3) where the carboxylate oxygen atoms are binding with an iron atom or hydroxyl hydrogen due to potential presence of amorphous non-stoichiometric oxyhydroxide [14]. However, the bridging mode (structure III) was less likely than structure II because the distance between two next iron atoms or hydroxyl hydrogen atoms in spinet structure is more important than the distance of the two carboxylate oxygens.

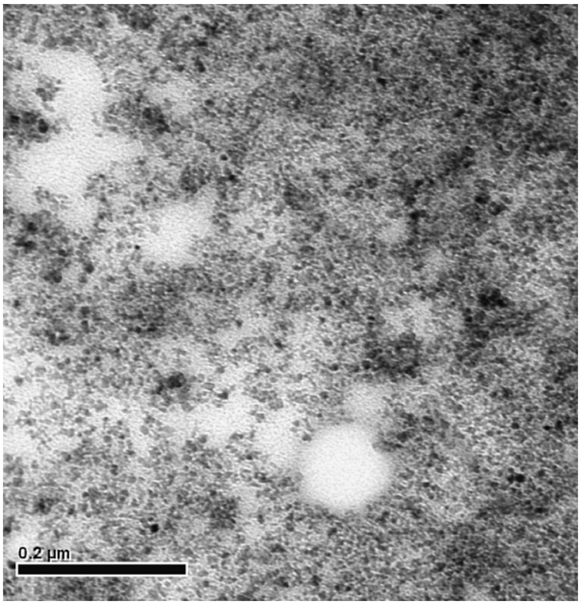

Fig. 1. TEM photomicrograph of OA-coated magnetite nanoparticles.

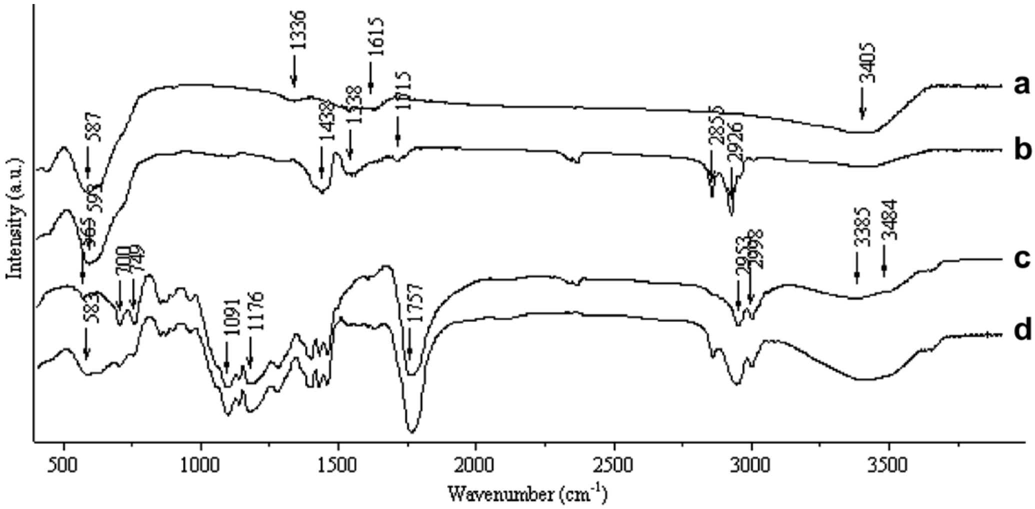

Fig. 2. FT-IR spectra of uncoated magnetite nanoparticles (a), OA-coated magnetite nanoparticles (b), magnetite-free PLGA sub-micron particles (c) and PLGA composite sub-micron particles (d).

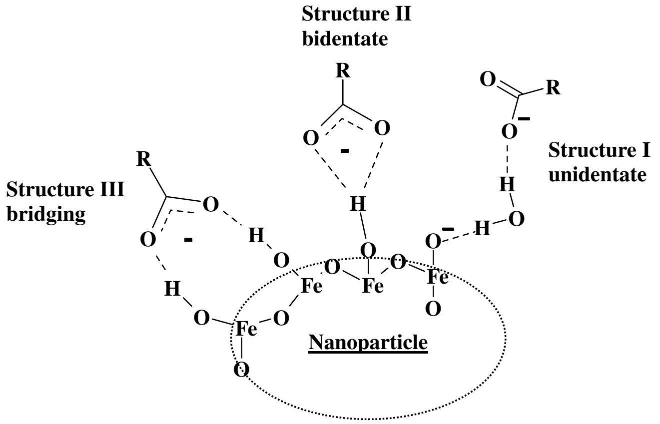

Fig. 3. Three possible interactions between oleic acid and iron oxide nanoparticle (adapted from Deacon and Phillips [13]) (see text).

Dried OA-coated magnetite nanoparticles could be easily redispersed in methylene chloride allowing the production of a stable magnetite nanoparticles suspension. As reported by several authors [15,16], the double bond in the hydrocarbon chain of oleic acid seems to play an important role for an effective stabilization of iron oxide in alkane medium.

Magnetization curve obtained at 300 K from dried OA-coated magnetite nanoparticles shows that the magnetization decreases with the applied magnetic field (data not shown). The absence of coercivity and negligible remanent magnetization indicate that the OA-coated magnetite nanoparticles exhibit a superparamagnetic behavior. Specific saturation magnetization is around 73 emu/g. This value is below the specific saturation magnetization known for bulk magnetite ($M_s = 92$ emu/g [17]) and the decrease of the saturation magnetization is most likely attributed to the existence of surfactants on the surface of magnetite nanoparticles [18].

3.2. Characterization of PLGA composite sub-micron particles

The presence of oleates at the surface of iron oxide nanoparticles is expected to facilitate their incorporation in the matrix of hydrophobic polymers that is of a great interest in the design of composite sub-micron particles because these colloidal magnetic particles could be used as drug carriers for site-specific magnetic targeting [19]. For this application, the magnetic properties, size, charge and surface chemistry of these particles are important and strongly affect both the blood circulation time as well as biovailability of the nanoparticles within the body [20].

The size and morphology of the PLGA composite sub-micron particles were determined by TEM. Fig. 4 shows the spherical shape of the particles with an approximate diameter around 150 nm and a narrow size distribution. The picture also reveals aggregates of OA-coated magnetite particles associated to numerous composite nanoparticles. This could be due to the aggregation of magnetite particles with an incomplete coating by oleic acid molecules [21].

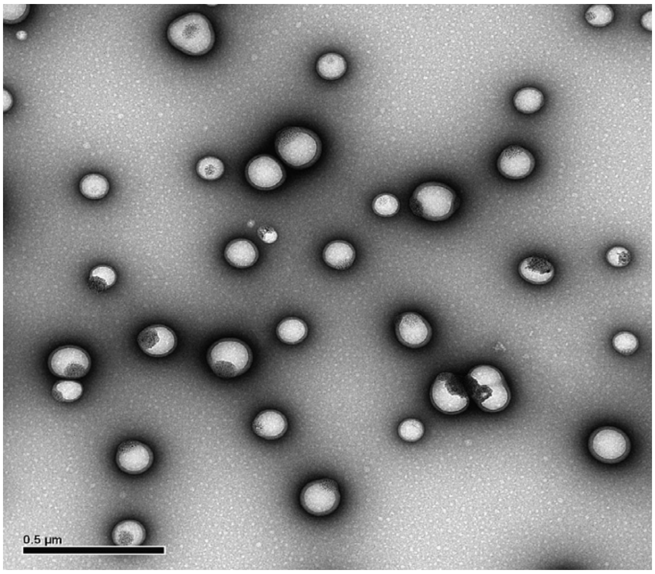

Fig. 4. TEM photomicrograph of PLGA composite sub-micron particles.

The characteristics (mean diameter, polydispersity index, zeta potential, magnetite entrapment efficiency and magnetite content) of composite nanoparticles as a function of weight ratio of magnetite to polymer are shown in Table 1.

The hydrodynamic diameters were below 300 nm and in the range of 272–281 nm with polydispersity index in the range of 0.1–0.2 indicating a narrow size distribution. In comparison with the size obtained with TEM viewing, the hydrodynamic diameter is probably larger because of the oleic acid layer on the magnetite nanoparticle surface. The hydrodynamic average diameters of the three types of PLGA composite sub-micron particles were very close whatever the weight ratio of OA-coated magnetite nanoparticles to PLGA used in the formulation.

The FT-IR spectrum of the PLGA composite sub-micron particles is shown in Fig. 2d. The characteristic band of magnetite is present at 583 cm^{-1} but not in the spectrum of magnetite-free PLGA sub-micron particles (Fig. 2c). In addition, the peaks around 3000 cm^{-1} are probably due to the contribution of the CH_2 stretch mode both from oleate and PLGA molecules suggesting the presence of OA-coated magnetite nanoparticles in the PLGA matrix. The main goal is to have enough amount of magnetite into polymeric sub-micron particles necessary to obtain significant magnetic properties. Such polymeric magnetic colloidal particles can be driven and held in the specific area for a desired period of time by applying external magnetic field [1]. In order to investigate to what extent this target is achieved, we have compared the surface properties of magnetite-free PLGA sub-micron particles with those of the PLGA composite sub-micron particles.

Previously, Arias et al. [6] have proposed the electrophoresis measurement of poly(ethyl-2-cyanoacrylate)

Table 1
Characteristics of PLGA composite sub-micron particles prepared with different OA-coated magnetite nanoparticles/PLGA weight ratios

OA-coated magnetite/PLGA weight ratio	Mean diameter ± SD (PI)[a] (nm)[b]	Zeta potential ± SD (mV)[b]	Magnetite entrapment efficiency (%)[b]	Magnetite content (% w/w)[b]
1:1	281 ± 3 (0.2)	−21.5 ± 1.9	15.6 ± 0.7	13.5 ± 0.8
1:1.5	282 ± 3 (0.1)	−15.5 ± 0.9	11.9 ± 0.4	8.5 ± 0.3
1:2	272 ± 5 (0.1)	−16.3 ± 1.5	10.3 ± 0.5	7.3 ± 0.4

[a] PI = polydispersity index.

[b] $n = 3$.

nanoparticles with a magnetic core as a useful tool for qualitatively checking the efficiency of the magnetite coating by the polymer. Thus, the zeta potential of magnetite-free PLGA sub-micron particles and PLGA composite sub-micron particles prepared with different weight ratio of OA-coated magnetite to PLGA was assessed as a function of pH in the presence of 10^{-3} M NaCl. As observed in Fig. 5, when the pH of the medium increases up to 9, the zeta potential decreases due to the dissociation of negative charge generating groups like carboxylic groups of PLGA polymer. For values of pH up to 7, the zeta potential of the PLGA composite sub-micron particles was in the same order of magnitude to that of magnetite-free PLGA sub-micron particles. When the pH is above 7, the phenomenon is more important with the PLGA composite sub-micron particles than with the magnetite-free PLGA sub-micron particles. In this case, the contribution of the carboxylic groups from the oleic acid located at the surface of the magnetite nanoparticles incorporated in the matrix of PLGA composite sub-micron particles is probably added to the important dissociation of carboxylic groups of PLGA polymer. However, for the PLGA composite sub-micron particles, the weight ratio of OA-coated magnetite to PLGA used in the formulation seems to have a moderate influence on the zeta potential values. In addition, PLGA composite sub-micron particles show no isoelectric point in the vicinity of pH 7 but rather around pH 3 in the opposite that it could be observed for magnetite nanoparticles. This result is in agreement with previous determination obtained with magnetite/biodegradable PLA composite particles [7]. If an entrapment of OA-coated magnetite nanoparticles in PLGA matrix is achieved, it could be supposed that PLGA composite sub-micron particles will have an isoelectric point lower than pH 7. These results can help in giving clues on the efficiency of the incorporation of OA-coated magnetite nanoparticles in PLGA matrix.

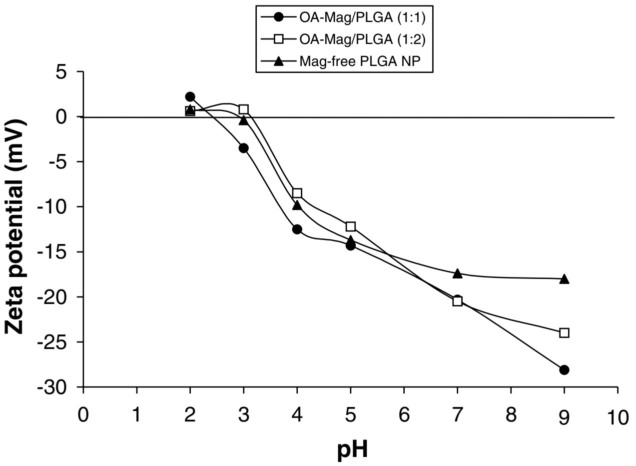

Fig. 5. Zeta potential of magnetite-free PLGA sub-micron particles and PLGA composite sub-micron particles (prepared with a magnetite/PLGA weight ratio of 1:1 and 1:2) as function of pH in the presence of 10^{-3} M NaCl.

On the other hand, it is also important to quantify exactly the magnetite content in the PLGA composite sub-micron particles. This was achieved by dosing iron by AAS after dissolution in DMSO of a weighted known sample of composite sub-micron particles. The magnetite content is higher compared with that obtained with uncoated magnetite nanoparticles (13.5% vs 1%) [12]. Moreover, ferrite/polymer weight ratio is an important factor in controlling the magnetite content of the PLGA composite sub-micron particles. It could be also observed that the magnetite content increased with increasing magnetite/polymer weight ratio. The highest incorporation of OA-coated magnetite was obtained when the weight ratio of OA-coated magnetite to PLGA was 1:1. In our previous study [12], this ratio was only 1.3:100 and this could explain why the magnetite content of the composite sub-micron particles was slightly above 1% w/w even when OA-coated magnetite was used in the formulation. Similar results have been reported by Zheng et al. [9] with SDS/oleic acid bilayer coated magnetite particles incorporated into polystyrene nanospheres by a new process based on miniemulsion polymerization. The encapsulation of a large amount of magnetite into the composite sub-micron particles is interesting in order to have significant magnetic sensitivity under an outer magnetic field. The magnetization curve of PLGA composite sub-micron particles loaded with 13.5% w/w of magnetite is shown in Fig. 6. These nanoparticles exhibit superparamagnetic properties at 300 K. The hysteresis loops have negligible coercivity and consequently no remanence was observed when the magnetic field was removed (see inset of Fig. 6), indicating that the single domain magnetic nanoparticles remained in these composite sub-micron particles. On the contrary, the composite sub-micron particles were not superparamagnetic at 5 K. The specific saturation magnetization (M_s) at 300 K of OA-coated magnetite sub-micron particles is 42 emu/g,

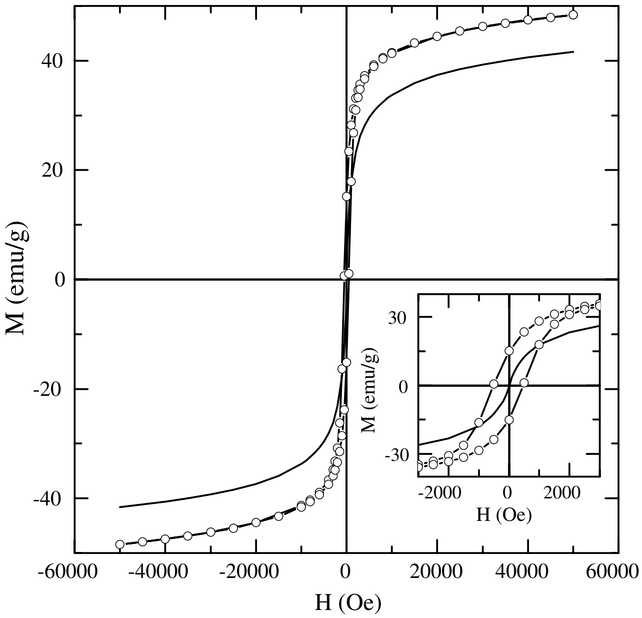

Fig. 6. Magnetization curve of PLGA composite sub-micron particles at 5 K (circles) and 300 K (solid line). The inset shows that no remanence was observed (at 300 K) when magnetic field was removed.

about half of the M_s of bulk magnetite. This significant loss in magnetization of the magnetic material is attributed to the presence of polymer matrix [22,23]. Despite this fact, PLGA composite sub-micron particles have enough magnetic sensitivity to move toward an external magnetic field (magnet) leading to complete seperation from water (data not shown). Magnetic properties (saturation magnetization and super paramagnetic behaviour) of the obtained PLGA composite sub-micron particles are potentially of interest for drug targeting purposes but further experiments are needed to check this latter point.

4. Conclusion

We have reported in this work a process to obtain magnetite nanoparticles coated with oleic acid and their incorporation into PLGA matrix. TEM examination, FT-IR analysis, magnetite entrapment efficiency and magnetite content of composite sub-micron particles give strong evidence of the presence of nanoscale magnetic material within the biodegradable polymer matrix. These composite sub-micron particles were nearly spherical in shape with a mean size near 280 nm. The weight ratio of OA-coated magnetite nanoparticles to polymer plays an important role in controlling the magnetite content of the composite sub-micron particles. 13.5% (w/w) magnetite content was achieved when a OA-coated magnetite nanoparticles to polymer ratio of 1:1 was used. Zeta potential of composite and magnetite-free polymer sub-micron particles varies in the same way with the pH of the medium, indicating that the electrical surface properties of magnetite nanoparticles are probably masked by the polymer coating. In addition, the presence of magnetite nanoparticles embedded in the polymer matrix is also demonstrated by the FT-IR spectra of composite sub-micron particles showing the characteristic band of magnetite. Finally, PLGA composite sub-micron particles exhibit superparamagnetic behavior indicating that this drug carrier system will be promising for magnetically targeting anticancer drugs.

Acknowledgements

The authors are grateful to Pr F. Boury (INSERM U646, University of Angers, France) for his assistance with Zeta potential analysis and Drs B. Arbeille and P.-Y. Sizaret (PPF Analyse des Systèmes Biologiques, University of Tours, France) for their assistance in Transmission Electronic Microscopy. Thanks are also due to Dr O. Cador and T. Guizouarn (UMR CNRS 6226, University of Rennes, France) for magnetic measurements.

References

[1] R. Asmatulu, M.A. Zalich, R.O. Claus, J.S. Riffle, Synthesis, characterization and targeting of biodegradable magnetic nanocomposites particles by external magnetic fields, J. Magn. Magn. Mater. 292 (2005) 108–119.

[2] F. Montagne, O. Mondain-Monval, C. Pichot, H. Mozzanega, A. Elaïssari, Preparation and characterization of narrow sized (O/W) magnetic emulsion, J. Magn. Magn. Mater. 250 (2002) 302–312.

[3] M. Igartua, P. Saulnier, B. Heurtault, B. Pech, J.E. Proust, J.L. Pedraz, J.P. Benoit, Development and characterization of solid lipid nanoparticles loaded with magnetite, Int. J. Pharm. 233 (2002) 149–157.

[4] I. Chourpa, L. Douziech-Eyrolles, L. Ngaboni Okassa, J.-F. Fouquenet, S. Cohen-Jonathan, M. Souce, H. Marchais, P. Dubois, The Analyst 130 (2005) 1395–1403.

[5] J.M. Anderson, M.S. Shive, Biodegradation and biocompatibility of PLA and PLGA microspheres, Adv. Drug Del. Rev. 28 (1997) 5–24.

[6] J.L. Arias, V. Gallardo, S.A. Gomez-Lopera, R.C. Plaza, A.V. Delgado, Synthesis and characterization of poly(ethyl-2-cyanoacrylate) nanoparticles with a magnetic core, J. Cont. Release 77 (2001) 309–321.

[7] S.A. Gomez-Lopera, R.C. Plaza, A.V. Delgado, Synthesis and characterization of spherical magnetite/biodegradable polymer composite particles, J. Colloid Interface Sci. 240 (2001) 40–47.

[8] Y. Deng, L. Wang, W. Yang, S. Fu, A. Elaïssari, Preparation of magnetic polymeric particles via inverse microemulsion polymerization process, J. Magn. Magn. Mater. 257 (2003) 69–78.

[9] W. Zheng, F. Gao, H. Gu, Magnetic polymer nanospheres with high and uniform magnetite content, J. Magn. Magn. Mater. 288 (2005) 403–410.

[10] S.J. Lee, J.R. Jeong, S.C. Shin, J.C. Kim, Y.H. Chang, K.H. Lee, J.D. Kim, Magnetic enhancement of iron oxide nanoparticles encapsulated withpoly(D,L-lactide-co-glycolide), Colloids and Surfaces A: Physicochem. Eng. Aspects 255 (2005) 19–25.

[11] T.K. Jain, M.A. Morales, S.K. Sahoo, D.L. Leslie-Pelecky, V. Labhasetwar, Iron oxide nanoparticles for sustained delivery of anticancer agents, Mol. Pharm. 2 (2005) 194–205.

[12] L. Ngaboni Okassa, H. Marchais, L. Douziech-Eyrolles, S. Cohen-Jonathan, M. Souce, P. Dubois, I. Chourpa, Development and characterization of sub-micron poly(D,L-lactide-co-glycolide) particles loaded with magnetite/maghemite nanoparticles, Int. J. Pharm. 302 (2005) 187–196.

[13] G.B. Deacon, R.J. Phillips, Relationship between the carbon–oxygen stretching frequencies of carboxylato complexes and the type of carboxylate coordination, Coord. Chem. Rev. 33 (1980) 227–250.

[14] M.H. Sousa, F.A. Yourhino, J.C. Rubin, Use of Raman micro-spectroscopy in the characterization of $M^{II}Fe_2O_4$ (M = Fe, Zn) electric double layer ferrofluids, J. Raman Spectrosc. 31 (2000) 185–191.

[15] A. Wooding, M. Kilner, B. Lambrick, Studies of the double surfactant layer stabilization of water-based magnetic fluids, J. Coll. Interf. Sci. 144 (1991) 236.

[16] R. Tadmor, R.E. Rosensweig, J. Frey, J. Klein, Resolving the puzzle of ferrofluid dispersants, Langmuir 16 (2000) 9117–9120.

[17] V.S. Zaitsev, D.S. Filimonov, I.A. Presnyakov, R.J. Gambino, B. Chu, Physical and chemical properties of magnetite and magnetite-polymer nanoparticles and their colloidal dispersions, J. Coll. Interf. Sci. 212 (1999) 49–57.

[18] S. Si, C. Li, X. Wang, D. Yu, Q. Peng, Y. Li, Magnetic monodisperse Fe_3O_4 nanoparticles, Cryst. Growth and Design 5 (2005) 391–393.

[19] U.O. Häfeli, Magnetically modulated therapeutic systems, Int. J. Pharm. 277 (2004) 19–24.

[20] A.S. Lübbe, C. Alexiou, C. Bergemann, Clinical applications of magnetic drug targeting, J. Surg. Res. 95 (2001) 200–206.

[21] M.T. Lopez-Lopez, J.D.G. Duran, A.V. Delgado, F. Gonzalez-Caballero, Stability and magnetic characterization of oleate-covered magnetite ferrofluides in different nonpolar carriers, J. Coll. Interf. Sci. 291 (2005) 144–251.

[22] L.P. Ramirez, K. Landfester, Magnetic polystyrene nanoparticles with a high magnetite content obtained by miniemulsion processes, Chem. Mater. 204 (2003) 22–31.

[23] Y. Wu, J. Guo, W. Yang, C. Wang, S. Fu, Preparation and characterization of chitosan-poly(acrylic acid) polymer magnetic microspheres, Polymer 47 (2006) 5287–5294.