

Dispersion of super paramagnetic iron oxide nanoparticles in poly(D,L-lactide-co-glycolide) microparticles

Ronald A. Wassel^a, Brian Grady^{b,*}, Richard D. Kopke^{a,c},
Kenneth J. Dormer^{a,c}

^a Hough Ear Institute, Oklahoma City, OK, United States

^b University of Oklahoma School of Chemical, Biological and Materials Engineering, Norman, OK, United States

^c Departments of Physiology and Otolaryngology, University of Oklahoma Health Science Center, Oklahoma City, OK, United States

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Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs) coated with oleic acid were encapsulated into poly(D,L-lactide-co-glycolide) (PLGA) particles using an oil-in-water-in-oil emulsion technique. The use of the oleic acid-coated SPIONs, and their suspension in the first oil phase led to well-dispersed nanoparticles in the PLGA matrix. Relative amounts of SPIONs encapsulated in PLGA could be varied by increasing the concentration of SPIONs in the first oil phase: doubling the amount in that phase doubled the amount of SPIONs in the PLGA. The saturation magnetization scaled proportionally with the amount of SPIONs in the PLGA and was significantly larger than other efforts to encapsulate magnetic nanoparticles in PLGA. Size of the composite particles, as determined by dynamic light scattering (DLS), could be varied from 280 to 160 nm by varying either power or time of sonication while the zeta potential remained near -20 mV for the composite, independent of SPION content. Transmission electron microscopy images showed SPIONs ranging in diameter from 5 to 15 nm embedded inside the polymer and indicated that they were uniformly dispersed within the PLGA particles. Small angle X-ray scattering (SAXS) showed that only the particles containing the largest amount of encapsulated SPIONs displayed a peak indicative of aggregation.

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1. Introduction

Encapsulation of bioactive agents into submicron size particles of biocompatible and degradable polymers have been tested as prospective therapeutic delivery vehicles for numerous applications in nanomedicine [1–9]. Bioactive agents may be proteins or DNA; however, the therapeutic dose for these agents is often narrow. Conventional oral doses of these agents, such as proteins, are frequently useless, because of denaturation during gastrointestinal transit or poor absorption [10]. Further, biological agents required at one target organ could lead to toxic effects on neighboring organs and tissues. It would be of immense value to have external control of submicron size particles for targeting to specific tissues. Delivery of bioactive

agents into the blood stream and then directing them to a diseased organ or tissue is a major effort in nanomedicine today that would reduce both the cost and risk of pharmacological therapy.

Through the use of an external magnetic field, SPIONs can be directed to specific tissues in the body [11]. One method to accomplish controlled release with respect to both time and tissue location is to encapsulate SPIONs in biodegradable polymer particulates. This method may be a very efficient method of delivering a drug to a localized disease site. For the purposes of our research, however, magnetic nanoparticles were designed with the sole purpose of being pulled by magnetic forces into the inner ear by applying them directly to the round window membrane [12]. Thus, the ability to direct magnetic nanoparticles in the vascular system is not necessary.

One commonly used biocompatible polymer is poly(D,L-lactide-co-glycolide) (PLGA). Sub-micron PLGA particles have been shown to have the potential to act as non-viral vectors

* Corresponding author. Tel.: +1 405 325 4369; fax: +1 405 325 5813.
E-mail address: bpgrady@ou.edu (B. Grady).

for DNA and other biologically active compounds [8,13–20]. SPIONs can be incorporated into biodegradable polymers such as PLGA and could be used to serve the dual purpose of MRI contrast enhancement as well as direction of polymer particles to specific locations [21–26]. Previous methods for incorporation of SPIONs in PLGA gave extremely low saturation magnetizations of the composite submicron particle. The saturation magnetization of well-dispersed single SPIONs can theoretically be as high as 92 emu/g [27]; the saturation magnetizations of previously described PLGA particles containing SPIONs are typically two to three orders of magnitude lower. Although difficult to ascertain, SPIONs appear to be aggregated, which could be the basis of the low saturation magnetizations reported. Other possible causes of the low magnetic susceptibilities are reduced efficiencies of SPION encapsulation, and SPION oxidation during the sonication process in water [21,28,29].

High saturation magnetization is required so as to maximize the ability to target the particles using an external magnetic field. We have shown previously an 8% (w/w) magnetite in PLGA is sufficient to move the particles to a specific location in a guinea pig [12]. Low concentrations of SPIONs are strongly preferred because of the effect that high concentrations of SPIONs might have on the loading efficiency and/or activity of the bioactive agent. Hence, it is essential to design a process limiting oxidation so as to maximize the saturation magnetization for a given overall SPION concentration. Also of importance is limitation of SPION aggregation, which can lead to a reduction in saturation magnetization. Aggregation could also lead to an uneven distribution of the SPIONs within different polymeric particles; that is many SPIONs in a few PLGA particles and no SPIONs in most PLGA particles. If an even distribution of the therapeutic agent is assumed, polydispersity would cause a significant loss of drug use in PLGA particles that were not receptive to external magnetic fields.

In this study we showed that SPIONs with a hydrophobic coating can be incorporated into PLGA particles by using an emulsification-diffusion technique [9]. This process involves first forming a water-in-oil emulsion in which the polymer and the SPIONs are in the oil phase and any hydrophilic bioactive agent can be dissolved in the water phase. Our procedure differs from nearly all previously published attempts using a similar technique since SPIONs normally have a hydrophilic surface and hence are suspended in the water phase [4,24,29–33]. As stated previously, suspending SPIONs in the water phase generally leads to low saturation magnetizations. Marchais and co-workers [25] did incorporate SPIONs into PLGA using an emulsification-diffusion technique where SPIONs were in the oil phase via the use of fatty acid-coated SPIONs; however, their dispersions were poor and their PLGA particles showed an inconsistent distribution of SPIONs. The water-in-oil emulsion is added to an aqueous phase containing a stabilizer and then emulsified to form a water-in-oil-in-water emulsion. Over time the solvent diffuses out creating nanoparticles with the SPIONs and bioactive agent incorporated inside the polymer. This study describes the characterization of the resulting composite SPION-PLGA nanoparticle.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (50:50: lactide/glycolide, 1.13 dL/g viscosity) was purchased from Absorbable Polymers International (Pelham, AL, USA). Polyvinyl alcohol (PVA) (Mw 30–70 kDa) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Iron oxide (magnetite) nanoparticles with an oleic acid coating were purchased from Liquid Research Ltd. (Bangor, Gwynedd, LL527 2UP, UK) Chloroform (HPLC grade) was purchased from VWR (West Chester, PA, USA).

2.2. Preparation of PLGA particles

The desired concentration of SPIONs (0.1–10 mg/ml) was dispersed into 1 ml of chloroform, then 30 mg of PLGA was dissolved in the solution. Two hundred microliters of nanopure water was emulsified into the PLGA/SPION/chloroform solution for 1 min in an ice bath using a probe sonicator (Sonicator 2000, Misonix, NY, USA) to form a water-in-oil emulsion. This primary emulsion was emulsified again by adding the water-in-oil emulsion 6 ml of nanopure water containing 2% polyvinyl alcohol. The system was sonicated for 5 min and stirred for ~24 h to allow the chloroform to evaporate. The PLGA particles containing SPIONs were then recovered via centrifugation at 20,000 rpm for 25 min at 4 °C (Beckman Optima LE-90 K, Beckman Instruments Inc., Palo Alto, CA, USA). The particles were washed with nanopure water four times to remove any excess PVA and SPIONs and then dispersed in 1 ml of nanopure water in 2 ml cryotubes. The PLGA particles were then lyophilized for 2 days and stored until use.

2.3. Characterization of PLGA/SPION particles

Dynamic light scattering (DLS) and zeta potential measurements in water were performed to determine PLGA particle size and zeta potential, respectively (HPP5001 High Performance Particle Analyzer, Malvern Instruments, Worcestershire, United Kingdom and Brookhaven Zeta PALS, Long Island, NY, USA). Transmission electron micrographs (TEMs) were taken also to determine particle size and the qualitative state of aggregation of the SPIONs inside the PLGA particles (H7600 Electron Microscope, Hitachi, Pleasanton, CA, USA). For TEM images, a drop of the composite particles was placed on a formvar-coated copper grid. All samples for small angle X-ray scattering (SAXS) were made by encasing dry powder within tape; the displayed data is after the pattern of the tape was subtracted using the measured transmittances (Micro Max[®] System for Small Angle X-ray Scattering, Osmic Auburn Hills, MI, USA). A 10 cm × 10 cm wire detector was placed approximately 50 cm from the sample position and silver behenate was used to determine the exact pixel to q ($q = 4\pi \sin \theta / \lambda$; $\theta = 0.5 \times$ scattering angle, $\lambda = 1.54 \text{ \AA}$) conversion. Magnetization measurements were performed at room temperature using a vibrating sample magnetometer (Lakeshore Cryotronics Inc., Westerville, OH, USA).

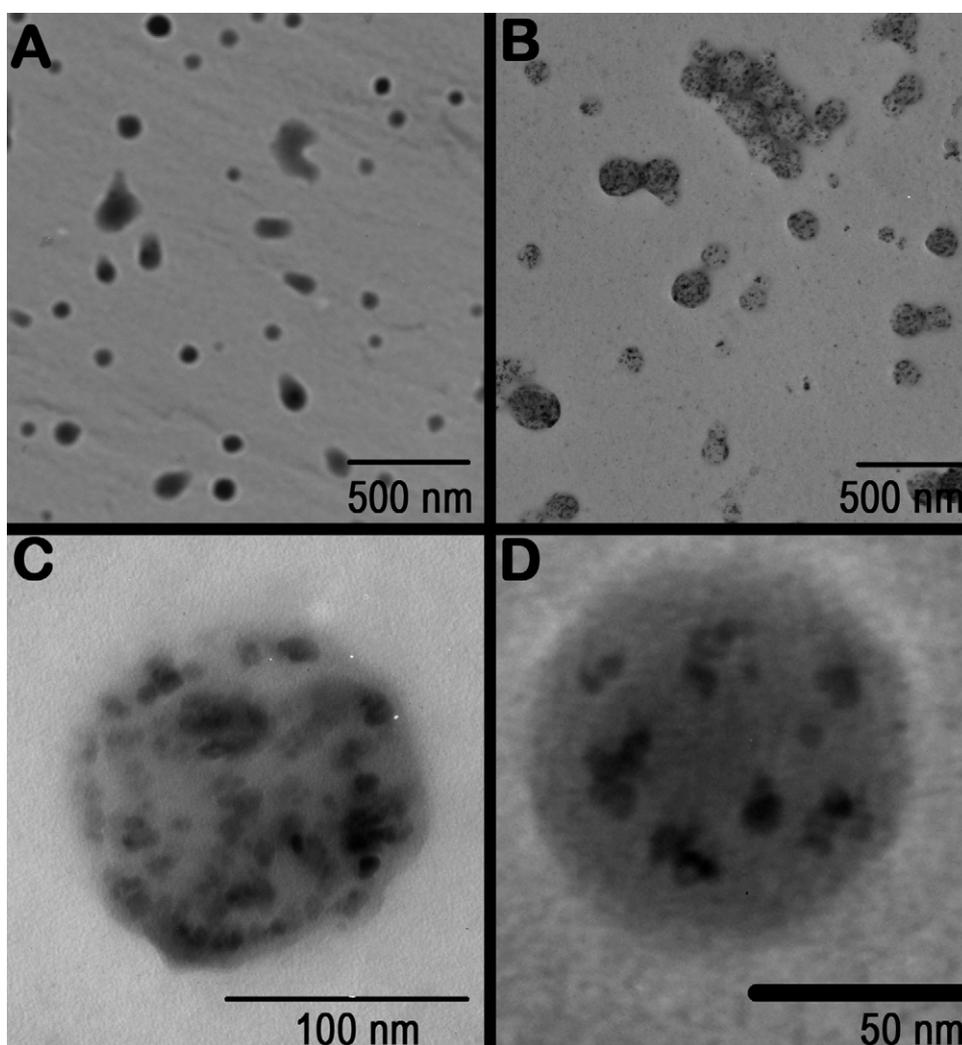


Fig. 1. Transmission electron micrographs. (A) PLGA particles. Magnification 7000 \times . (B) PLGA particles with magnetite incorporated inside. Magnification 7000 \times . (C) PLGA particles with magnetite made from a 5 mg/m solution. Magnification 70,000 \times . (D) PLGA particles with magnetite made from a 1 mg/m solution. Magnification 40,000 \times .

3. Results and discussion

Many factors with respect to emulsification procedure influence the size of the PLGA particles: the size of the vials that are used for the primary and secondary emulsions, the power of the ultrasound, the position of the sonicator head in the solution, and the duration of sonication. All of these factors affect how the energy from the sonicator is dispersed through the solution. Hence, it is impossible to exactly describe the conditions that led to a particular PLGA particle size. In general, longer sonication times and higher sonication powers led to smaller particles. By changing these parameters from a few seconds to a few minutes, and from 3 to 15 W, we were able to vary the PLGA particle size from approximately 300 to 150 nm. For these studies, the primary emulsion was formed by using a sonication time of 1 min and a power setting of 7 W. The secondary emulsion was formed by using a sonication time of 5 min with a power setting of 12 W.

Fig. 1A shows a TEM of the synthesized PLGA particles. The particles had an average size of 99 ± 44 nm versus a size of 175 nm and a polydispersity index of 0.045 from dynamic light

scattering. Fig. 1B shows a TEM of oleic acid-coated SPIONs incorporated into the PLGA microparticles. These composite particles had an average size of 85 ± 32 nm. The average size of the particles as measured via DLS was ~ 180 nm with a polydispersity index of ~ 0.1 . The oleic acid-coated SPIONs appeared to range in size from 5 to 15 nm yet incorporation of these SPIONs did not influence the size of the PLGA particles. It has been reported that PVA forms layers of aggregates around the surface of the PLGA [34]. DLS measurements are expected to give the hydrodynamic radius rather than the actual size of the nanoparticles. A measurement of the hydrodynamic radius of the nanoparticles would account for the larger DLS measurement than the TEM images because the PVA is expected to have a much smaller configuration dried on the TEM grid versus in water. This size discrepancy has been observed by others [14,35].

Fig. 1C suggests that the SPIONs have slightly aggregated inside the polymer, although this is difficult to determine since particles separated in the z -direction will appear aggregated in this 2D projection. This slight aggregation is probably not an

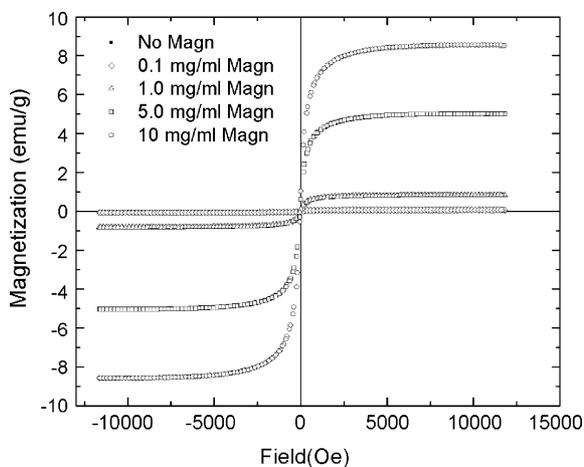
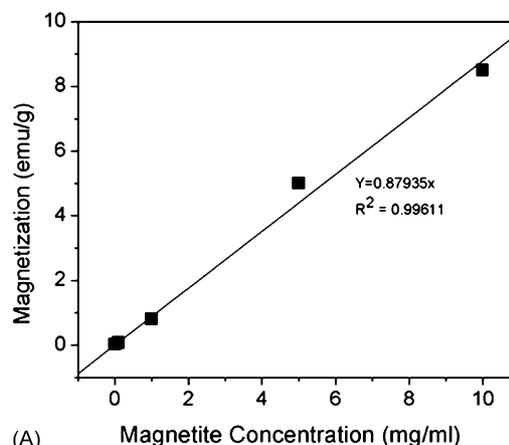


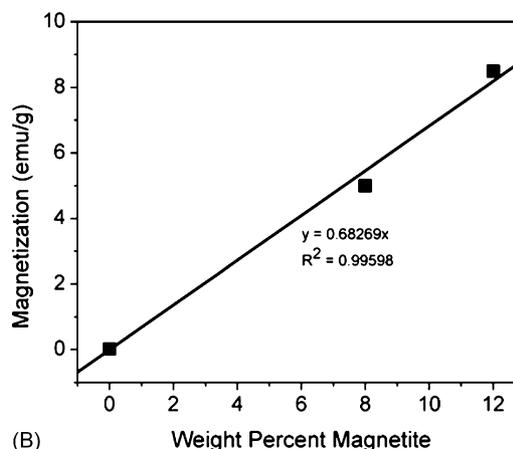
Fig. 2. Plot of magnetization of PLGA microparticles with magnetite incorporated inside. The different concentrations represent various initial concentrations of magnetite in the organic phase prior to emulsification.

artifact of evaporating the particles on the TEM plate since the oleic acid-coated SPIONs encapsulated in the PLGA were not freely mobile. When a lower concentration of magnetite was used (1 mg/ml versus 5 mg/ml) in the formation of the PLGA microparticles, fewer magnetite particles were incorporated into the polymer and their appeared to be no aggregation (Fig. 1D). The dispersion of SPIONs inside the polymer appears much less aggregated than other published examples [23,25,36,37]. There also does not appear to be partitioning of SPIONs from one PLGA particle versus another, except for expected partitioning due to randomization processes. This result is also very different than what appears in other laboratories [23,25,36,37]. Oleic acid-coated SPIONs can be incorporated into the PLGA at essentially any concentration of magnetite in the organic phase prior to emulsification. Fig. 2 shows magnetization curves for PLGA particles made with magnetite concentrations of 0, 0.1, 1.0, 5, and 10 mg/ml in the organic phase. The saturation magnetization is higher than previously reported by other groups [24,26]. If the maximum magnetization for each concentration is plotted versus the initial concentration of magnetite in the organic phase it can then be seen that the magnetization scales linearly with concentration (Fig. 3A). Even if aggregation were occurring, it is not clear that saturation magnetization would decrease because the oleic acid coating would decrease interactions (such as dipolar magnetic interactions) between the SPIONs [38]. In any case, encapsulation of the nanoparticles does not seem to affect the super paramagnetic nature of the SPIONs, as evidenced by the steep slope in the VSM data in Fig. 2.

The weight percent of the magnetite was determined for the 5 and 10 mg/ml samples by burning the samples and measuring the mass remaining; lower concentrations could not be measured reliably with this method because of the small amount of magnetite in the PLGA particles. Approximately 50% of the magnetite suspended in chloroform is incorporated into the PLGA, as compared to Landfester and co-workers [29] 40% incorporation using miniemulsion polymerization. The magnetization of the PLGA particles with 5 and 10 mg/ml scales linearly with the measured weight fraction, and the measured values for



(A)



(B)

Fig. 3. (A) Magnetization of PLGA particles plotted vs. magnetite concentration in the organic phase prior to emulsification. (B) Magnetization of PLGA particles plotted vs. weight percent of magnetite in PLGA particles.

the particles containing less magnetite fit on this line assuming an extrapolated weight value (i.e. the weight of 1 mg/ml was 10 times less than 10 mg/ml). The extrapolated value at 100% (w/w) magnetite, 68.3 emu/g, is within normally reported values for magnetite nanoparticles [27]. This value was in fact larger than the measured value for the pure magnetite ~ 57 emu/g. (The pure value was adjusted for the oleic acid coating weight; the actual measured value of magnetite + oleic acid coating was 45 emu/g.) The fact that the extrapolated value at 100% magnetite in Fig. 3B is higher than what we measured for the pure material is probably due to inaccuracies in the measurement of the pure magnetite sample. Further, the fact that the saturation magnetization did not go down indicates that the SPIONs did not oxidize with encapsulation; oxidation was thought to have occurred in the miniemulsion procedure described by Landfester and Ramirez [4].

SAXS was performed to qualitatively analyze aggregation of the SPIONs in the PLGA. Fig. 4 shows the comparison of the magnetite standard and the pure PLGA with PLGA filled with magnetite at various loadings. The scattering pattern of the PLGA particles is quite large, which is presumably due to surface scattering at the PLGA particle–air interface. The scattering from the pure magnetite shows a peak at approximately $q = 0.06 \text{ \AA}^{-1}$, which corresponds to a d -spacing of slightly larger

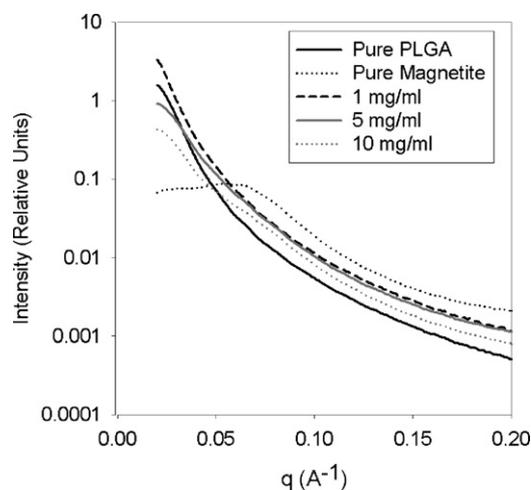


Fig. 4. Small-angle X-ray scattering data.

than 10 nm. This d -spacing is considerably smaller than twice the average radius of the SPIONs. However, form-factor scattering could be influencing the peak location; the form-factor of 8 nm spheres has a peak near this q -value. No evidence of any secondary peaks was found, indicating that the magnetite particles were of substantial polydispersity. The 10 mg/ml magnetite SPION-PLGA composite particles show a clear bump at about $q = 0.075 \text{ \AA}^{-1}$. Clearly the peak due to magnetite has shifted to lower distances after incorporation into the PLGA, for reasons that are not entirely clear. The extremely large electron density of the magnetite compared to the polymer, coupled with the absence of peaks for other concentrations, strongly suggests that this peak is due to interparticle interference. The movement of the peak to higher q with incorporation indicates either that smaller magnetite particles tend to aggregate together, or that smaller magnetite particles are preferentially incorporated in the PLGA. No evidence of the latter was found in TEM measurements (although this would be very difficult to determine given the 2D nature of the images).

4. Conclusion

A double-emulsion method to produce sub-micron PLGA particles with encapsulated iron oxide nanoparticles that uses oleic acid-coated magnetite nanoparticles dispersed in the oil phase has been developed. SPIONs were evenly distributed throughout the PLGA particles, both with respect to individual particles as well as with respect to many particles. Saturation magnetizations (magnetic susceptibilities) were only one order of magnitude less than the saturation magnetization of the pure iron oxide nanoparticles, as opposed to the two to three orders of magnitude reported elsewhere for PLGA/iron oxide nanoparticle composites. Within experimental error, saturation magnetizations were exactly proportional to the amount of magnetite incorporated. The weight fraction of magnetite incorporated inside the PLGA was readily controlled by adjusting the amount of magnetite in the feed, and the amount incorporated is also directly proportional to the amount in the feed.

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References

- [1] B.C. Simionescu, G. David, Functional Micro- and Nanoparticles Based on Poly(*N*-acylimino)Ethylene, Biomaterials: From Molecules to Engineered Tissues, Kluwer academic/plenum publication, New York, 2004, pp. 1–18.
- [2] B.A. Pfeifer, J.A. Burdick, R. Langer, Formulation and surface modification of poly(ester-anhydride) micro- and nanospheres, Biomaterials 26 (2005) 117–124.
- [3] S. Prabha, V. Labhasetwar, Nanoparticle-mediated wild-type p53 gene delivery results in sustained antiproliferative activity in breast cancer cells, Mol. Pharm. 1 (2004) 211–219.
- [4] K. Landfester, L.P. Ramirez, Encapsulated magnetite particles for biomedical application, J. Phys. Condens. Matter 15 (2003) S1345–S1361.
- [5] T. Cai, Z.B. Hu, M. Marquez, Synthesis and self-assembly of nearly monodisperse nanoparticles of a naturally occurring polymer, Langmuir 20 (2004) 7355–7359.
- [6] K.Y. Win, S.S. Feng, Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs, Biomaterials 26 (2005) 2713–2722.
- [7] L. Mu, P.H. Seow, S.N. Ang, S.S. Feng, Study on surfactant coating of polymeric nanoparticles for controlled delivery of anticancer drug, Colloid Polym. Sci. 283 (2004) 58–65.
- [8] M. Kumar, G. Hellermann, R.F. Lockey, S.S. Mohapatra, Nanoparticle-mediated gene delivery: state of the art, Expert Opin. Biol. Ther. 4 (2004) 1213–1224.
- [9] I. Bala, S. Hariharan, M. Kumar, PLGA nanoparticles in drug delivery: the state of the art, Crit. Rev. Ther. Drug Carrier Syst. 21 (2004) 387–422.
- [10] E. Kai, T. Ochiya, A method for oral DNA delivery with *N*-acetylated chitosan, Pharm. Res. 21 (2004) 838–843.
- [11] L. LaConte, N. Nitin, G. Bao, Magnetic nanoparticle probes, Mater. Today 8 (2005) 32–38.
- [12] R.D. Kopke, R.A. Wassel, F. Mondelek, B. Grady, K. Chen, J. Liu, D. Gibson, K.J. Dormer, Magnetic nanoparticles: inner ear targeted molecule delivery and middle ear implant, Audiol. Neurotol. 11 (2006) 123–133.
- [13] G. Heng, L. Yongjun, Z. Yuehong, L. Changwei, Y. Jing, S. Cunxian, W. Pengyan, Z. Sanmei, W. Zongli, S. Mingpeng, Nanoparticle as a new gene transferring vector in specific expression gene, Chin. Med. Sci. J. 17 (2002) 220–224.
- [14] S. Prabha, W.Z. Zhou, J. Panyam, V. Labhasetwar, Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles, Int. J. Pharm. 244 (2002) 105–115.
- [15] M.G. Qaddoumi, H.J. Gukasyan, J. Davda, V. Labhasetwar, K.J. Kim, V.H.L. Lee, Clathrin and caveolin-1 expression in primary pigmented rabbit conjunctival epithelial cells: role in PLGA nanoparticle endocytosis, Mol. Vis. 9 (2003) 559–568.
- [16] C.G. Oster, N. Kim, L. Grode, L. Barbu-Tudoran, A.K. Schaper, S.H.E. Kaufmann, T. Kissel, Cationic microparticles consisting of poly(lactide-co-glycolide) and polyethylenimine as carriers systems for parental DNA vaccination, J. Controlled Release 104 (2005) 359–377.
- [17] S.P. Kasturi, K. Sachaphibulkij, K. Roy, Covalent conjugation of polyethyleneimine on biodegradable microparticles for delivery of plasmid DNA vaccines, Biomaterials 26 (2005) 6375–6385.

- [18] S. Jilek, H. Zurkaulen, J. Pavlovic, H.P. Merkle, E. Walter, Transfection of a mouse dendritic cell line by plasmid DNA-loaded PLGA microparticles in vitro, *Eur. J. Pharm. Biopharm.* 58 (2004) 491–499.
- [19] E. Walter, H.P. Merkle, Microparticle-mediated transfection of non-phagocytic cells in vitro, *J. Drug Target.* 10 (2002) 11–21.
- [20] S.R. Little, D.M. Lynn, S.V. Puram, R. Langer, Formulation and characterization of poly(beta amino ester) microparticles for genetic vaccine delivery, *J. Controlled Release* 107 (2005) 449–462.
- [21] J.R. Jeong, S.J. Lee, J.D. Kim, S.C. Shin, Magnetic properties of Fe₃O₄ nanoparticles encapsulated with poly(D,L lactide-co-glycolide), *IEEE T Magn.* 40 (2004) 3015–3017.
- [22] S.J. Lee, J.R. Jeong, S.C. Shin, Y.M. Huh, H.T. Song, J.S. Suh, Y.H. Chang, B.S. Jeon, J.D. Kim, Intracellular translocation of superparamagnetic iron oxide nanoparticles encapsulated with peptide-conjugated poly(D,L lactide-co-glycolide), *J. Appl. Phys.* 97 (2005).
- [23] S.J. Lee, J.R. Jeong, S.C. Shin, J.C. Kim, Y.H. Chang, Y.M. Chang, J.D. Kim, Nanoparticles of magnetic ferric oxides encapsulated with poly(D,L lactide-co-glycolide) and their applications to magnetic resonance imaging contrast agent, *JMMM* 272–76 (2004) 2432–2433.
- [24] 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 with poly(D,L-lactide-co-glycolide), *Colloid Surf. A: Physicochem. Eng. Aspects* 255 (2005) 19–25.
- [25] L.N. 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.
- [26] P.C. Wang, C.F. Lee, T.H. Young, D.T. Lin, W.Y. Chiu, Preparation and clinical application of immunomagnetic latex, *J. Polym. Sci. Part A: Polym. Chem.* 43 (2005) 1342–1356.
- [27] L.A. Harris, J.D. Goff, A.Y. Carmichael, J.S. Riffle, J.J. Harburn, T.G. St Pierre, M. Saunders, Magnetite nanoparticle dispersions stabilized with triblock copolymers, *Chem. Mater.* 15 (2003) 1367–1377.
- [28] R.A. Ali-Zade, Structure and magnetic properties of polymer microspheres filled with magnetite nanoparticles, *Inorg. Mater.* 40 (2004) 509–515.
- [29] L.P. Ramirez, K. Landfester, Magnetic polystyrene nanoparticles with a high magnetite content obtained by miniemulsion processes, *Macromol. Chem. Phys.* 204 (2003) 22–31.
- [30] R.H. Muller, S. Maassen, H. Weyhers, F. Specht, J.S. Lucks, Cytotoxicity of magnetite-loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles, *Int. J. Pharm.* 138 (1996) 85–94.
- [31] W.M. Zheng, F. Gao, H.C. Gu, Magnetic polymer nanospheres with high and uniform magnetite content, *JMMM* 288 (2005) 403–410.
- [32] A. Chastellain, A. Petri, H. Hofmann, Particle size investigations of a multistep synthesis of PVA coated superparamagnetic nanoparticles, *J. Colloid Interface Sci.* 278 (2004) 353–360.
- [33] J. Lee, M. Senna, Preparation of monodispersed polystyrene microspheres uniformly coated by magnetite via heterogeneous polymerization, *Colloid Polym. Sci.* 273 (1995) 76–82.
- [34] M.F. Zambaux, F. Bonneaux, R. Gref, P. Maincent, E. Dellacherie, M.J. Alonso, P. Labrude, C. Vigneron, Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method, *J. Controlled Release* 50 (1998) 31–40.
- [35] M. Kumar, U. Bakowsky, C.M. Lehr, Preparation and characterization of cationic PLGA nanospheres as DNA carriers, *Biomaterials* 25 (2004) 1771–1777.
- [36] P. Chattopadhyay, R.B. Gupta, Supercritical CO₂ based production of magnetically responsive micro- and nanoparticles for drug targeting, *Ind. Eng. Chem. Res.* 41 (2002) 6049–6058.
- [37] X.Q. Liu, Y.P. Guan, Y. Yang, Z.Y. Ma, X.B. Wu, H.Z. Liu, Preparation of superparamagnetic immunomicrospheres and application for antibody purification, *J. Appl. Polym. Sci.* 94 (2004) 2205–2211.
- [38] M.A. Morales, T.K. Jain, V. Labhasetwar, D.L. Leslie-Pelecky, Magnetic studies of iron oxide nanoparticles coated with oleic acid and pluronic (R) block copolymer, *J. Appl. Phys.* 97 (2005).