



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Journal of Magnetism and Magnetic Materials 293 (2005) 62–68

Journal of
magnetism
and
magnetic
materials

www.elsevier.com/locate/jmmm

Preparation and characterization of phospholipid stabilized uniform sized magnetite nanoparticles

Jyotsnendu Giri^a, Sanjukta Guha Thakurta^a, Jayesh Bellare^b,
Arun Kumar Nigam^c, Dharendra Bahadur^{a,*}

^aDepartment of Metallurgical Engineering and Materials Science, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

^bSchool of Bio-science and Bio-engineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

^cTata Institute of Fundamental Research, Colaba, Mumbai 400005, India

Available online 3 March 2005

Abstract

The effects of phosphatidylcholine (PC) on the growth of magnetite superparamagnetic particles have been investigated. These coated particles give stable suspension in solvents like chloroform and were used for magnetoliposome preparation. The superparamagnetic particles and magnetoliposomes were characterized by X-ray diffraction (XRD), FTIR, Transmission electron microscopy (TEM) and SQUID measurements. The result shows that PC acts as capping agent to restrict the growth of the particles.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Magnetic liposome; Nanoparticles; Phosphatidyl choline stabilized magnetite; Magnetic hyperthermia; Size

1. Introduction

In the field of biotechnology, the use of magnetic nanoparticles is gradually gaining importance [1]. The application of magnetoliposome (lipid vesicles containing magnetic particles) as carrier or vehicles for targeted delivery of drugs and magnetic field hyperthermia, appears to be a promising technique. Among the magnetic oxides,

Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ are most suitable due to its relatively low toxicity and suitable magnetic properties [2]. For any biomedical application, a strict control of size is necessary. In magnetic-field-induced hyperthermia, particles transform the energy of AC magnetic field into heat, which strongly depends on the particle size [3]. However, synthesis of magnetic nanoparticles with controlled morphology and size has been a challenging area of study.

Among the different soft chemical methods of synthesis of magnetic nanoparticles, the easiest and most common method is co-precipitation. But

*Corresponding author. Tel.: +91 22 5767632/5723480 (O); fax: +91 22 25723480.

E-mail address: dhiren@met.iitb.ac.in (D. Bahadur).

the rapid kinetics of this process leads to broad distribution of particle size and uncontrolled morphology. Due to a large ratio of surface area to volume, there is a tendency to agglomerate so as to reduce their surface energy by strong magnetic dipole–dipole attraction between particles. This can be controlled by precipitation of metal ions in the presence of long-chain capping molecules. The effect of several organic anions such as carboxylate and hydroxyl carboxylate ions on the formation of magnetic oxides has been studied extensively in the context of magnetic oxides [4]. Kandori et al. [5] reported the effect of phosphate ions on the formation of ferric oxide particles. The effect also depends on the structure of capping molecules. It is expected that phospholipid molecules (egg phosphatidylcholine), which are essential precursors for liposome formation may also influence the nucleation and growth characteristics of magnetite. In this paper, we describe the effects of phosphatidylcholine (PC) on the synthesis of magnetite nanoparticles and magnetoliposomes and their properties.

2. Experimental

2.1. Preparation of phospholipid stabilized magnetite

PC coated magnetite was prepared by co-precipitation method which was carried out in N₂ atmosphere. A 0.2 M aqueous solution of FeCl₂ and FeCl₃ salt (molar ratio 2:1) was prepared. Different concentrations of phospholipid (PC) solutions were prepared in methanol such that the mol% of PC to Iron ions (Fe²⁺ and Fe³⁺) was varied from 0 to 6.12. The metal ion solution was mixed with PC solution by stirring. Precipitation of metal ions was carried out by addition of 0.93 M NH₃ (25% solution) to the 0.2 M metal ion solution with vigorous stirring (2000 rpm). Then the reaction mixture was heated at 60–85 °C for 15 min with continuous stirring. The PC coated magnetite was immobilized with a permanent magnet and the aqueous supernatant decanted. The precipitate was then washed 5–6 times with cold water to remove Cl⁻ ions and with acetone

followed by methanol. The resulting precipitate is PC stabilized magnetite, a part of which was then air dried for further characterization. Rest of PC coated magnetite was then dispersed in CHCl₃ with the variable concentration to prepare CHCl₃-based suspension.

2.2. Preparation of magnetoliposome

Mixtures of phospholipid and cholesterol were employed for the liposome preparation. Here, to optimize the preparation of magnetoliposome, required amounts of PC and cholesterol (in a 2:1 ratio) were added into CHCl₃ dispersion of PC-coated magnetite. The magnetoliposomes were prepared by the thin film hydration method. The phospholipids and CHCl₃-based stable suspension was further added to a suitable volume of chloroform/methanol solution (10/1 v/v) and evaporated to make the thin film in a rotary evaporator. The resulting film was then dried under vacuum overnight. The lipids were hydrated in a rotary shaker to prepare multilamellar magnetoliposomes which were then subjected to sonication for 15–30 min to prepare unilamellar magnetoliposomes.

Thermo gravimetric analysis (TGA) was done on the air-dried precursors using Thermowage L 81 (Germany) TGA units. Determination of phase purity and their identification were done by X-ray diffraction (XRD) studies using Philips powder diffractometer PW1710 with CuK_α radiation. The crystallite size is determined from the X-ray line broadening using Scherrer formula

$$D = 0.9\lambda / \beta \cos \theta,$$

where D is the average crystalline size, λ the X-ray wavelength used, β the angular line width of half maximum intensity and θ the Bragg's angle in degree. The infrared spectra of the synthesized materials were obtained using a Nicolet spectrometer (Magna IR, 550). The micrograph was taken in Cryo-transmission electron microscopy (Cryo-TEM) and room temperature TEM [CM 200 (Philips)] for magnetoliposome and powder samples, respectively. For the TEM observation, the powder was first dispersed in chloroform by an ultra sonication bath (20 kHz, 500 W) and then the

suspension was deposited drop-wise on to a copper grid that was coated on carbon film and dried. For magnetoliposomes, the sample was prepared using the cryo-plunger. It is a machine where the liposome containing grid could be quickly freezing to a liquid nitrogen temperature (-195°C) to avoid the cubic ice crystal. The low temperatures as well as room temperature magnetization were measured by SQUID Magnetometer (Quantum Design) MPMS-XL7. The blocking temperature (T_B) was measured based on zero-field-cooled (ZFC) and field-cooled (FC) measurement with M vs. T curve at the applied external magnetic field of 200 Oe. The ZFC curve was obtained by cooling the sample to 4.2 K in the absence of a magnetic field followed by applying the field and performing the measurement. Then without turning off the magnetic field the sample was cooled to 4.2 K with a measurement of magnetization at each intermediate temperature (FC).

3. Result and discussion

Fig. 1 shows the X-ray diffraction (XRD) pattern of the uncoated (unmodified) as well as modified (with 0.5 mol% of PC) samples. It shows single cubic magnetite phase formation. From XRD, the crystallite size has been estimated

between 6 and 20 nm. The effect of different groups such as phosphates, carboxyl, etc. on crystallinity and morphology are also reported [4–6]. Two processes are possible: (1) These molecules or ions chelate with metal ions to inhibit nuclei formation. (2) The absorption of these ions on these nuclei may restrict the growth of nuclei [7]. These two processes, which depend on concentration of molecules and pH of solution, control the morphology and crystallite size.

The FTIR spectra of the pure magnetite, PC coated magnetite and pure PC samples were recorded between $4000\text{--}400\text{ cm}^{-1}$ (Fig. 2(a)–(c), respectively). Three strong (3420 , 1630 , 631 cm^{-1}) and two weak (2361 , 442 cm^{-1}) absorption bands were observed for pure magnetite. The adsorbed water is featured by the bands at 3420 and $1620\text{--}1630\text{ cm}^{-1}$ which are assigned to O–H stretching and H–O–H bending modes of vibration [8,9]. The bands around 2361 cm^{-1} may be due to the environmental CO_2 molecules. These bands are observed in all the coated and uncoated samples. But the intensity of these bands is different for different samples. For pure magnetite bands around 600 and 400 cm^{-1} are observed, which are the stretching vibration due to the interactions produced between the oxygen and the cations occupying the octahedral and tetrahedral sites [10]. Spectra of the PC-coated magnetite as

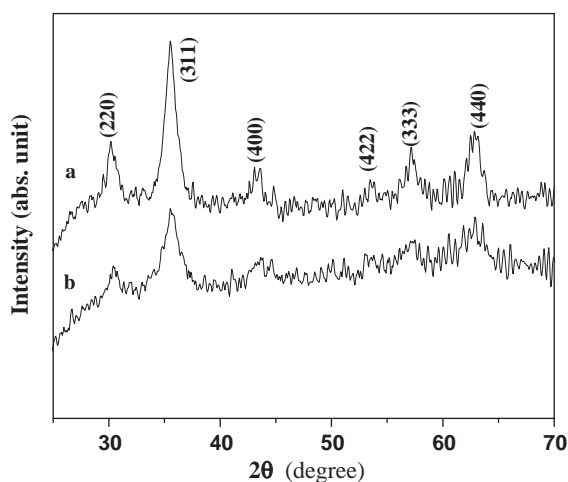


Fig. 1. XRD diffraction patterns of magnetite nanoparticles prepared (a) in absence of PC (b) in the presence of 0.5 mol% PC.

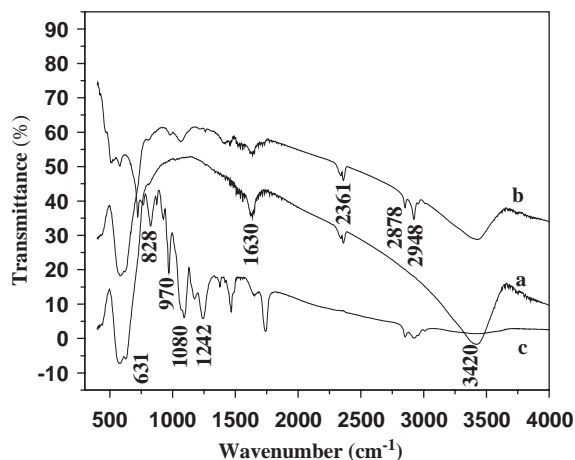


Fig. 2. Room temperature FTIR spectra of (a) uncoated magnetite nanoparticles (b) PC coated magnetite nanoparticles (c) phosphatidylcholine (PC) sample.

well as in the PC sample consist of the bands at 2878 and 2948 cm^{-1} and are attributed to symmetric and asymmetric methylene ($-\text{CH}_2-$) and methyl ($-\text{CH}_3$) vibrations. The bands observed between 1405 and 1460 cm^{-1} are due to bending vibration of $-\text{CH}_2-$ group [11]. But for pure PC and coated magnetite clear bands are observed at around 1242 , 1080 , 970 and 828 cm^{-1} which are attributed to the presence of $-\text{PO}_4^{3-}$ group vibration mode. The PC stabilized magnetite has bands of pure PC as well as pure magnetite. This supports the presence of absorbed PC over the magnetite.

Fig. 3 shows the % of weight losses with temperature of magnetite nanoparticles prepared in the presence of different % of PC. The uncoated magnetite shows the total weight loss of about 4%. The maximum loss at around 100°C and negligible loss beyond this temperature may be due to the associated water and surface $-\text{OH}$ groups. The weight loss at 100°C is common for all samples. Around 300°C there is a sharp weight loss that could be attributed to the decomposition of capped organic molecules (PC or glycerophosphoric acid) from the magnetic particles surface. From the curve, it is observed that with increasing mol% of PC, the % weight loss increases, indicating more adsorption of these molecules with increasing mol% of PC.

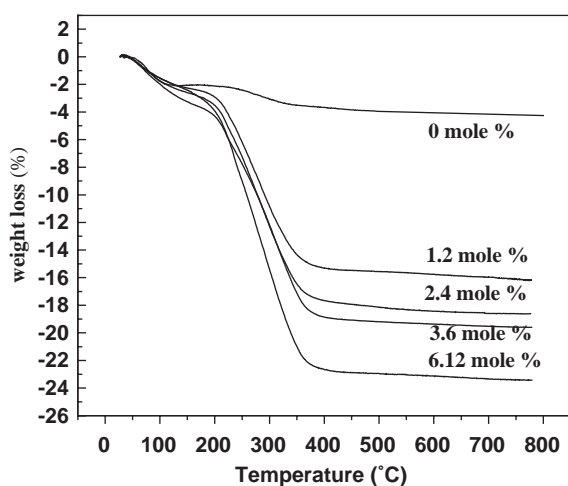


Fig. 3. Weight loss (%) with temperature of magnetite nanoparticles prepared in presence of 0 to 6.12 mol% of PC.

The magnetization loops of magnetite nanoparticles prepared in the absence of PC and in the presence of 3.06 mol% of PC are depicted in Fig. 4 as a typical example. The result shows zero coercivity and remanence and magnetization does not saturate at 20 kOe, indicating superparamagnetism. But magnetization measured at 4 K exhibits a hysteresis (inset of Fig. 4) which indicates that this temperature is lower than the blocking temperature (T_B). It can be seen from Fig. 4 that the magnetization decreases with increasing mol% of PC. This is due to the presence of increasing amounts of nonmagnetic materials with increasing % of PC. The change in mass profile data with temperature (TGA) is indicative of the amount of organic molecules associated with magnetite nanoparticles.

The blocking temperature (T_B) is determined from the branching of the ZFC and FC curves or from the maxima in the ZFC data. As is well known, above T_B , superparamagnetic particles become thermally unstable and magnetization decreases exponentially as KV/kT is less than 1 where the symbols K , V , k and T have their usual significance. In Fig. 5, the magnetite sample (with 0.6 mol% PC) shows the splitting of ZFC and FC curve at 280 K; whereas for the sample (with 3.06 mol% PC), the splitting is at around 175 K. Considering that T_B is the temperature where the

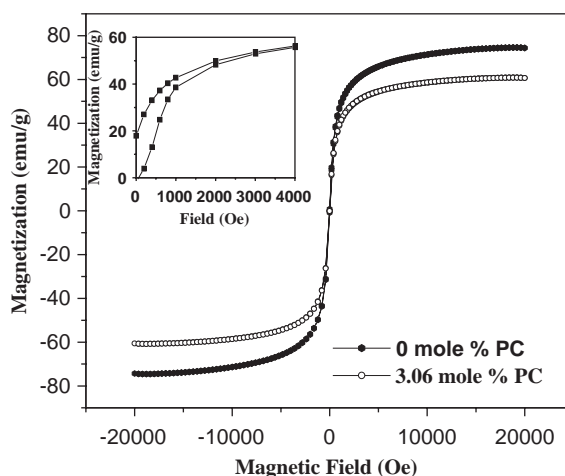


Fig. 4. Magnetization vs. Field curve of magnetite nanoparticles with 3.06 and 0.6 mol% PC at 300 K and inset at 4 K showing the presence of remanence.

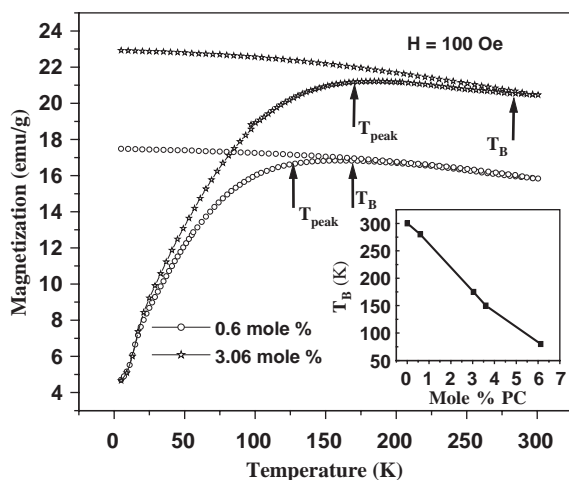


Fig. 5. The ZFC and FC curve for magnetite nanoparticles prepared in presence of 0.6 and 3.06 mol% PC. Inset showing the variation of T_B of magnetite prepared in presence of different mol% of PC.

branching takes place, 3.06 mol% of the PC sample has lower blocking temperature than 0.6 mol% PC sample. The peak magnetization of ZFC curve is also different for different samples. For sample with 0.6 mol% of PC it is 170 K, whereas for the sample with 3.06 mol% PC it is 135 K. T_B depends on the interaction between particles as well as particle size. It is noteworthy that the difference between T_B and T_{peak} is much higher for the sample with 0.6 mol% PC (280 vs. 170 K) compared to sample with 3.06 mol% PC which is relatively smaller (175 vs. 135 K). This has been discussed in terms of particle size distribution and inter-particle dipole–dipole interaction [12]. From the ZFC and FC data it is clear that the T_B shifts to lower temperatures (inset Fig. 5) and the difference between T_B and T_{peak} also narrows down with increasing mol% of PC. This shows that the PC controls the particle size and distribution. If the distance between the particles is more, then the inter-particle dipole–dipole interaction is less, thereby shifting T_B to a lower temperature. A similar trend is observed for small particle sizes. As the particles are smaller, T_B shifts to lower temperature.

Fig. 6 shows the typical TEM photograph of PC stabilized magnetite nanoparticles, the majority of

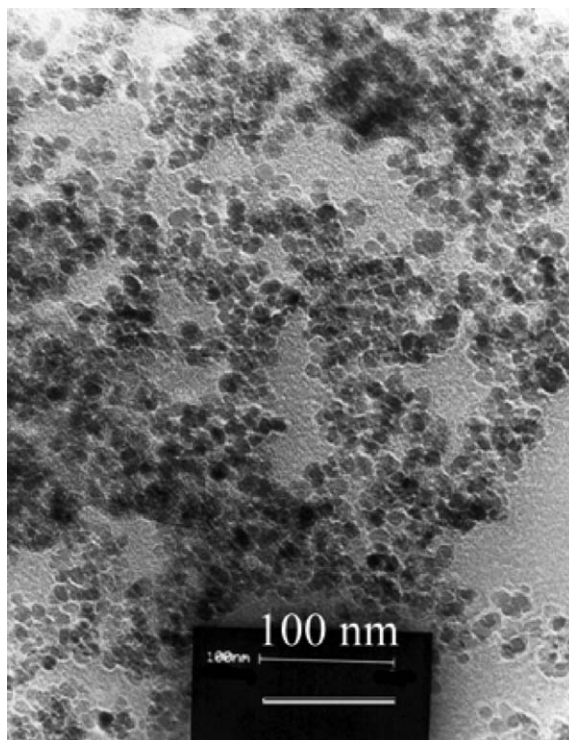


Fig. 6. TEM photograph of PC coated magnetite nanoparticles.

which are in the range of 10–15 nm. To get uniform spherical size particles, it is essential to have a controlled precipitation of metal ions. It can be done in the presence of surfactant or long-chain molecules. For this purpose, we have used PC (phosphatidylcholine), to control the precipitation. To increase the solubility of phospholipid in water, methanol was used. It helps to increase the solubility of PC, i.e. the possibility of direct interaction between PC and magnetic particles, which is important for preventing the agglomeration during the early stages of crystal growth. When the reaction mixture was heated at 80 °C, methanol rapidly evaporated, ensuring the large contact area between freshly formed particles and surfactant. The strong affinity between magnetite and phosphate groups helps in the process of chemisorptions as well as orientation between particles and surfactant. Once the particles are capped by PC, the number of accessible sites for

further crystal growth during heating is reduced, leading to termination of particle growth. Thus the initial presence of PC during the rapid precipitation process controls the particle growth and prevents agglomeration. So it is expected that the presence of increasing concentration of PC (mol%) during precipitation will control particle growth more, as the number of capping molecules increases and results in smaller particles with narrow size distribution.

The Cryo-TEM photos of magnetoliposome are shown in Fig. 7(a) and (b). The spherical magnetoliposomes are seen in these figures. The

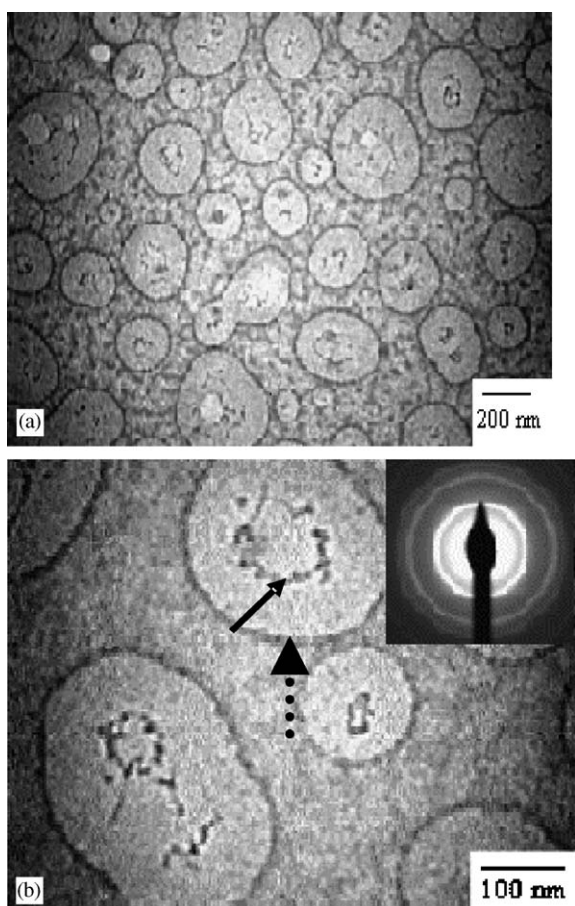


Fig. 7. Cryo-TEM micrograph of PC based spherical magnetoliposome (a) at lower magnification (b) at higher magnification showing the self assembled magnetic nanoparticles shown by arrow (the dotted arrow points towards the liposome surface). Inset shows electron diffraction pattern of this area.

magnetic particles are assembled within the liposome. This is expected from the fact that the PC/glycerophosphoric acid is chemically bonded through the polar end with magnetic particles. During the liposome synthesis, the lipid molecules are expected to arrange in bilayer such that the polar head is exposed to polar media (water). The inset figure has shown the diffraction pattern of magnetoliposome, indicating the presence of iron oxide particles within the liposome.

4. Conclusion

The presence of PC molecules controls the particle morphology and size of magnetite particles and their distribution by inhibiting growth. The presence of increasing concentration of PC during precipitation decreases the particle size as well as narrows down the particle size distribution. Higher mol% of PC helps in obtaining smaller and narrow size distribution of particles. Magnetic behavior is characteristic of superparamagnetic nature. These PC-stabilized magnetite particles are easily dispersed in chloroform and can be used to make encapsulated liposome. All these properties suggest that these surface-modified-magnetite nanoparticles within the liposomes may be suitable for hyperthermia applications.

Acknowledgments

Thanks are due to the Department of Science and Technology (DST) and the Department of Biotechnology (DBT), Govt. of India for financial support.

References

- [1] I. Safarik, M. Safarikova, *Monatsh. Chem.* 133 (2002) 737.
- [2] M. Shinkai, *J. Biosci. Bioeng.* 94 (2002) 606.
- [3] F. Janet, *Drug Discovery Today* 6 (2001) 387.
- [4] T. Ishikawa, S. Kataoka, K. Kandori, *J. Mater. Sci.* 28 (1993) 2693.

- [5] K. Kandori, S. Uchida, S. Kataoka, et al., *J. Mater. Sci.* 27 (1992) 719.
- [6] U. Schwertmann, *Nature* 212 (1966) 645.
- [7] R.M. Cornell, P.W. Schindler, *Colloid Polym. Sci.* 258 (1990) 1171.
- [8] A.A. Kamnev, M. Risti, M. Angelov, *J. Mol. Struct.* 349 (1995) 77.
- [9] Q. Fu, C. Cao, H. Shu, *Thin Solid Films* 348 (1991) 99.
- [10] Li. Xinyong, Lu. Gongxuan, Li. Shuben, *J. Alloys Compounds* 235 (1996) 150.
- [11] J. Giri, T. Sriharsha, D. Bahadur, *J. Mater. Chem.* 14 (2004) 875.
- [12] M.F. Hansen, S. Mørup, *J. Magn. Magn. Mater.* 203 (1999) 214.