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Development of Superparamagnetic Microparticles for Biotechnological Purposes

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Aqueous suspensions containing magnetic microparticles have been increasingly used in biosciences and biotechnology. This work describes an experimental procedure to produce superparamagnetic microparticles. The particles were prepared based on the coprecipitation of iron salts in alkaline medium. Afterwards, characterization was performed. Characterization data demonstrated that magnetite was the dominant phase in the analyzed sample, and 50% of them were in the size range of 0.5–5μm. The results suggest that the experimental protocol provided a simple synthesis route to produce superparamagnetic microparticles. Such properties may be very useful for biotechnological purposes.

Keywords magnetic particles; coprecipitation; superparamagnetism; magnetic drug targeting; magnetic separation

INTRODUCTION

Aqueous suspensions containing small magnetic particles have been increasingly used in biosciences and biotechnology. When a magnetic field is applied, such systems develop magnetic polarization and magnetophoretic mobility, and because of such unique features, these particles are promising candidates for delivering drugs to specific locations within the body (Alexiou et al., 2000; Voltairas, Fotiadis, & Michalis, 2002). By means of a selective application of a magnetic field on a desired area, active ingredients bound to these particles can be successfully carried to their site of action with high accuracy, minimum or no surgical intervention, and maximum concentration (Voltairas et al., 2002). Therefore, regional therapy efficacy could be improved by increasing local drug concentration, whereas systemic drug biodistribution and toxic side effects would be limited (Alexiou et al., 2000, 2002). Magnetic particles are effectively internalized into phagocytic cells or into other cells when targeted to them. Once inside the cells, particles are clustered within lysosomes. Degradation of iron oxides into iron and oxygen is presumed to occur in intracellular lysosomes of macrophages under the influence of a variety of hydrolytic enzymes, low pH, and proteins participating in iron metabolism. Iron oxides have been shown to degrade in vivo by iron mobilization and utilization according to natural iron pathways (Weissleder, Bogdanov, Neuwelt, & Papisov, 1995).

In a different approach, magnetic particles can be used to purify or detect cells, cell organelles, and biologically active compounds such as nucleic acids, proteins, and xenobiotics (Safarik and Safarikova, 2002). Magnetic separation implies several advantages in comparison with other techniques used for the same purpose. Direct isolation from crude samples such

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as blood, bone marrow, or tissue homogenates can be pointed out. Moreover, compared with conventional methods of cell separation, magnetic separation is simple and fast, and it is regarded as a sample enrichment step for further chromatographic and electromigratory analysis (Safarik & Safarikova, 1999). Ligands can be attached to their surface to provide particles with target specificity and selectivity. The separation process for the purification of target cells usually consists of the following fundamental steps. Initially, the suspension containing the cells of interest is mixed with magnetic particles. During incubation, such particles are bound to desired cells or molecules, and thus stable magnetic complexes are formed. Owing to their capacity of being attracted by a magnetic field, complexes can be removed from the sample by using an appropriate magnetic separator and the supernatant can be discarded or used for another application. Isolated cells remain phenotypically unaltered, and as a result, very pure cell populations with excellent viability can be isolated (Bergemann, Muller-Schulte, Oster, Brassard, & Lubbe, 1999; Safarik & Safarikova, 2002).

Superparamagnetism is an eligible property for magnetic separation and drug targeting as well. Concerning magnetic separation technique, superparamagnetic particles are specially desired because they can be easily resuspended and remain in suspension for a long time (Safarik & Safarikova, 2004). Concerning drug targeting, superparamagnetic particles may be regarded as safer carriers. Magnetic fields are known to interact with human tissues (Schenck, 2005). Therefore, as particles do not remain magnetized in the absence of a magnetic field, human tissue exposition to the magnetic field would occur only during its application. Such exposition would last about 1h (Alexiou et al., 2000; Goodwin, Peterson, Hoh, & Bittner, 1999).

Micrometric size range is also advantageous not only for magnetic separation but also for deep organ drug targeting. The diameter of magnetic particles used for cell separation is typically 1–5 μm, i.e., they are on the order of a cell diameter. Magnetic particles having the diameter larger than 1μm can be easily separated using simple magnetic separators, whereas separation of smaller particles (magnetic colloids with the particle size ranging between tens and hundreds of nanometers) may require the usage of high-gradient magnetic separators. Besides, when functionalized by antibodies, such particles are able to have many receptor contacts, which enhance their dislodging potential (Safarik & Safarikova, 1999, 2004). Considering drug targeting, some organs such as the liver and the lungs lie deeply in the body cavity (8–12 cm from the body surface). Therefore, focusing the magnetic flux density in such areas is hardly achieved (Alexiou et al., 2000). Small particles (< 0.1μm) have a small magnetic moment. Therefore, it is difficult to counteract the hydrodynamic forces in the blood stream by using currently feasible magnetic forces. The use of magnetic microparticles (0.5–5μm) was found to overcome such difficulty (Goodwin et al., 1999; Kuznetsov et al., 1999).

The synthesis of superparamagnetic nanoparticles has been extensively described in the literature. For instance, the interested reader may refer to a review paper (Tartaj, Morales, Gonzalez-Carreno, Veintemillas-Verdaguer, & Serna, 2005) for details. Although considerable research has been devoted to production of such nanometric superparamagnetic particles, rather less attention has been paid to micrometric superparamagnetic ones. In this article, a method to produce superparamagnetic microparticles is reported. Such experimental setup is based on Massart's coprecipitation of iron salts in an alkaline medium (Massart, 1981).

MATERIALS AND METHODS

Materials

All the chemicals were of reagent grade used without further purifications. Ferric chloride hexahydrate ($FeCl_3.6H_2O$ > 97%) and ferrous sulfate heptahydrate ($FeSO_4$. $7H_2O$ >97%) were from Synth (Diadema, Brazil). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from J. T. Baker (New Jersey, USA) and Vetec (Rio de Janeiro, Brazil), respectively.

Synthesis

The synthesis of magnetite particles ($FeO.Fe_2O_3$) has been carried out via chemical coprecipitation of di- and tri-valent iron salts in an alkaline medium (Gao et al., 1998; Kim, Zhang, Voit, Rao, & Muhammed, 2001b; Koneracka, Kopcansky, Timko, & Ramchand, 2002), and the precipitation procedure was as follows. Solutions of ferric chloride and ferrous sulfate were prepared as a source of iron by dissolving the respective chemicals in HCl 0.4 N solution under vigorous stirring using a mechanical stirrer (Nova Ética, São Paulo, Brazil) at 100 mA. As a second step, solutions were combined and a homogenous mixture of $FeCl_3$ (0.1 mol/L) and $FeSO_4$ (0.05 mol/L) was formed. An aqueous dispersion of particles was obtained just after adding 10 mL of the mixture of ferrous and ferric salts drop-wise into 250 mL of NaOH 1N under vigorous mechanical stirring, as in a previous step, at room temperature (25°C).

The product was believed to consist of magnetite particles. The black color and a strong spontaneous magnetization when subjected to an applied magnetic field supported this hypothesis (Gao et al., 1998). Characterization data confirmed it. For such synthesis, the chemical reaction is expected as follows (Kim et al., 2001b):

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \qquad (1)$$

The black powder settled to the bottom of the beaker after removing mechanical stirring. The supernatant was discarded and fresh ultra-pure water was added to the beaker. This procedure was repeated copiously until most of the ions in the suspension were removed. Dry powders were obtained at room temperature under reduced pressure.

Characterization

The structural properties of the particles were characterized by X-ray powder diffraction (XRPD), which was carried out in an X-ray diffractometer (XRD-6000, Shimadzu, Kyoto, Japan) using the Cu Kα line as a radiation source. The mean diameter of the particles was examined using a laser scattering particle size analyzer (1064L, Orleans, Cilas, France). Particle morphology analysis was conducted by scanning electron microscopy (SEM) on a scanning electron microscope (XL30, Philips, Eindhoven, The Netherlands). Magnetization measurements were performed using a homemade vibrating sample magnetometer (VSM). Particle characterization was performed in dry samples (XRPD, VSM) and liquid samples (SEM and laser scattering).

RESULTS

Structural Properties

According to XRPD patterns, as shown in Figure 1, magnetite was the dominant phase in the analyzed sample, with a primary scattering peak at around 2θ = 35° (Chen & Hu, 2003). It is known that iron oxide particles may undergo undesired critical oxidation during the synthesis process (Equation 2). Once oxidation takes place, ferric hydroxide may be produced and the physical and chemical properties of the particles may be critically affected (Kim et al., 2001a, 2001b). The XRPD pattern analysis suggested the absence of ferric hydroxide, which would produce a primary peak at 2θ = 26.38°. Additionally, no peaks of other compounds, such as goethite (2θ = 21.22°) and hematite (2θ = 31.15°), were observed. XRPD data also revealed that the coherence length in the crystal lattice was of the order of 5.8 nm.

$$Fe_3O_4 + 0.25O_2 + 4.5H_2O \rightarrow 3Fe(OH)_3 \qquad (2)$$

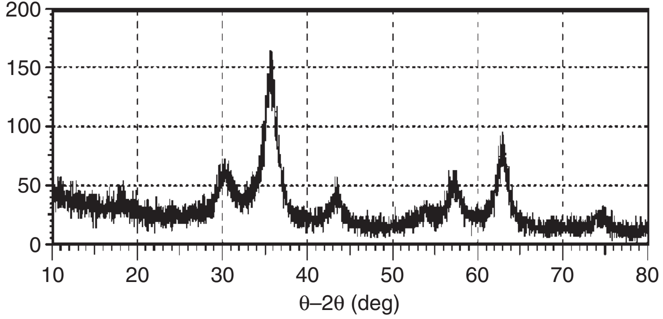

FIGURE 1. X-ray diffraction pattern of magnetite (intensity versus 2θ).

Size and Morphology

Particle size distribution was investigated by laser scattering particle size analysis. As it can be seen in Figure 2, more than 50% of the particles were found to be in the size range of 0.5–5 µm. About 90, 50, and 10% of the sample was smaller than 6.85, 2.48, and 0.73 µm, respectively. Scanning electron micrograph in Figure 3 shows that particles are roughly spherical to irregular in shape and they showed a size distribution in agreement with the laser scattering particle size data.

Magnetic Properties

Magnetization measurements on powder samples were made at room temperature using a VSM. The results were typically as shown in Figure 4, indicating that the particles readily displayed magnetization when subjected to a magnetic field. Two features of the magnetization curve deserve attention.

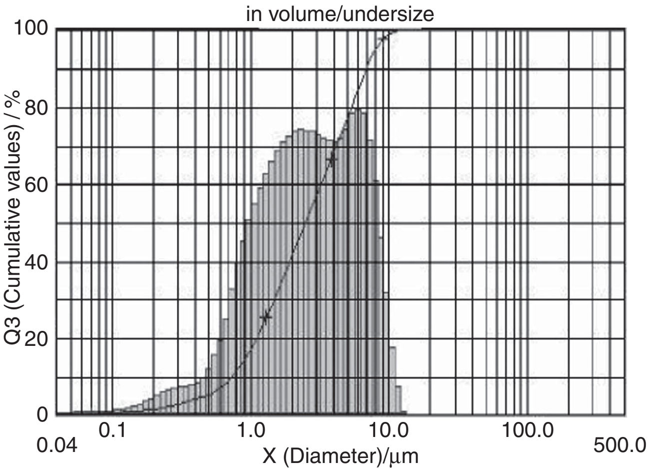

FIGURE 2. Particle size distribution for synthesized magnetite.

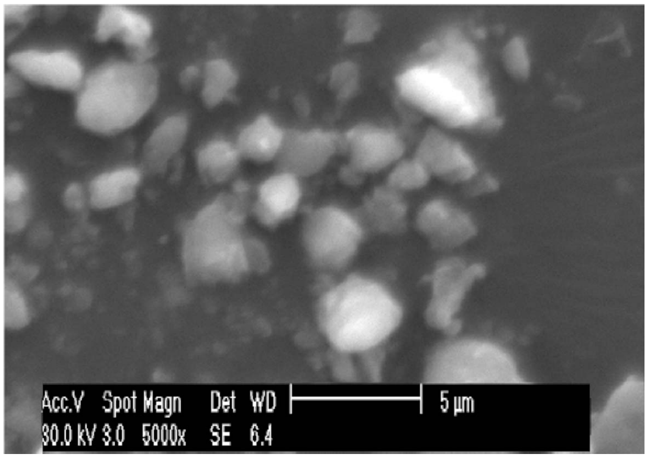

FIGURE 3. Scanning electron micrograph of magnetite particles.

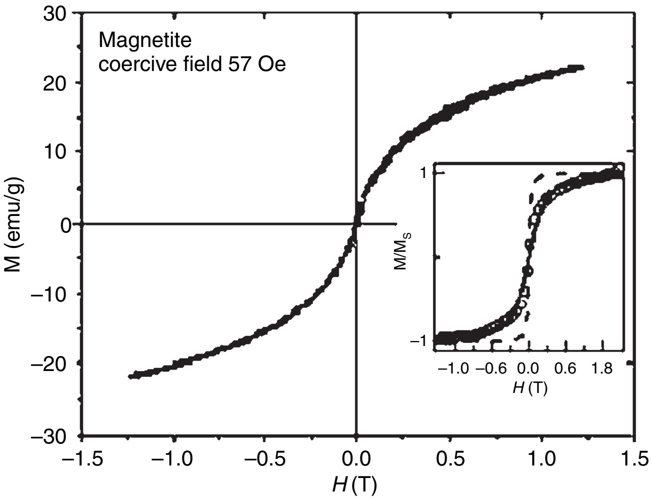

FIGURE 4. Magnetization versus applied magnetic field for Fe_3O_4 particles (see the text for the details of the curve fitting shown in the inset).

First, the magnetization is not saturated in fields of 12 kG. This field strength is two orders of magnitude larger than the coercive field of bulk magnetite, H_c = 310G (Cullity, 1972). Second, there is no remanent magnetization ($M = 0$ for $H = 0$), suggesting that the magnetite particles are superparamagnetic. In the inset of Figure 4, it can be observed that the increasing field branch of the magnetization curve (open symbol curve) was well reproduced by the magnetization $m(H,T)$ of a 4.8nm side cubic crystallite (continuous line curve). In the inset, the dashed line curve indicates the magnetization of a larger crystallite of 10 nm side. For the fitting, $m(H,T) = L(\mu H/k_BT)$ was used, where μ is the magnetic moment of the crystallite, H is the strength of the external field, T is the temperature, k_B is the Boltzmann constant, and $L(x) = \tanh(x) - 1/x$ is a Langevin function, as appropriate for superparamagnetic particles (Cullity, 1972). Note that the curve for the 10nm crystallite saturates at a rather small value of the external field strength, suggesting the particles contain a narrow distribution of crystallite sizes, centered at 4.8 nm.

DISCUSSION

Magnetic particles have been largely produced by means of coprecipitation technique. Although the reported method is based on coprecipitation protocol, it differs from the traditional Massart's procedure (Massart, 1981) in iron salt concentration, type of iron salts, nature, and concentration of the agent inducing precipitation. In the presented protocol, precipitation was carried out using solutions of $FeCl_3$ (0.1 mol/L), $FeSO_4$ (0.05 mol/L), and NaOH (1mol/L). In Massart's procedure, magnetic particles were obtained using $FeCl_3$ (1 mol/L), $FeCl_2$ (2 mol/L), and NH_4OH (0.7 mol/L). Besides, they were peptized after synthesis. Such facts are relevant because the structure, dispersity, and morphology of the particles depend on the temperature of the process, concentration of the original solutions, pH value of the medium, nature of the agent inducing precipitation, and the presence of surfactant (Gribanov, Bibik, Buzunov, & Naumov, 1990).

As presented above, samples consisted of nearly spherical particles with diameters of the order of 10^3 nm. Therefore, there should be a liquid magnetization in the absence of external field. One possible mechanism for this unique form of superparamagnetism is the independent thermal fluctuation of small ferrimagnetic domains inside the particles. The XRPD data supported this picture. The width of the peaks in the XRPD data revealed that the coherence length in the crystal lattice was of the order of 5.8 nm. The boundaries of these small crystallites, with linear dimensions of the order of a few nanometers, may contain lattice defects that impede the propagation of the magnetic order. Similar phenomena has been recently observed in Fe_3O_4 thin films (Margulies et al., 1997; Voogt et al., 1998), where stacking defects in the cation sublattices induced superparamagnetism by breaking up the thin films in a large number of uncorrelated small ferrimagnetic domains with dimensions of the order of a few nanometers.

Three factors may contribute to the formation of micrometric particles consisting of the assembly of superparamagnetic nanometric crystallites. First, sulfate ions are strong flocculating agents (Massart, 1981), and thus induce agglomeration of crystallites. Second, no surfactant was used in the coprecipitation process, favoring the formation of large particles. Third, no peptization was carried out. The boundaries of the nanometric crystallites composing the microparticle are very likely to contain lattice defects that impose a constraint on the propagation of the magnetic order, leading to uncorrelated superparamagnetic crystallites. Actually, the production of magnetite aggregates by using coprecipitation technique has already been reported. However, they were not found to be superparamagnetic (Lee, Lee, & Furubayashi, 1999).

The sizes of aggregates depend on synthesis parameters such as stirring condition (Mersch-Sundermann, Kevekordes, & Jenter, 1998). Depending on the size and the magnetic properties of the core material, particles can be used for different purposes. Particles in the size range of 1–5 and 0.5–5 μm are particularly desired for magnetic separation (Safarik & Safarikova, 1999) and deep organ target (Alexiou et al., 2000; Goodwin et al., 1999). Also, as discussed before, superparamagnetism is desirable for both techniques.

Iron–carbon particles and magnetite presenting such size range could be successfully produced. However, they were not superparamagnetic (Adam, Hartung, Okamoto, Saha-Moller, & Spehar, 2000; Kuznetsov et al., 1999; Mersch-Sundermann et al., 1998). In fact, some microparticles are superparamagnetic, but, in such a case, they are made of iron oxide dispersed in a cross-linked polymer matrix (Ngomsik, Bee, Draye, Cote, & Cabuil, 2005; Safarik & Safarikova, 2004). Owing to the presence of this non-magnetic material, magnetic properties are considerably reduced. Therefore, polymer content should be minimum (Ramanujan & Yeow, 2005).

In this article, it was presented an experimental setup to produce superparamagnetic microparticles whose content is pure magnetic material. As it can be expected, their magnetic properties is superior to the ones of many polymeric particles found in literature (Li et al., 2001; Liu et al., 2005; Pich, Bhattacharya, & Adler, 2005).

Different molecules, as drugs and antibodies, can be directly immobilized on the surface of magnetite particles by using carbodiimide as a coupling agent (Koneracka et al., 2002). Direct binding takes place because hydroxyl group is present on the surface of magnetic particles prepared by coprecipitation (Bacri, Perzynski, Salin, Cabuil, & Massart, 1990). By immobilizing drugs and antibodies on the surface of the microparticles produced by the present method, one can develop carriers for magnetic drug targeting and cell separation, respectively.

In conclusion, structure, dispersity, and morphology of magnetite particles depend on the synthesis procedure conditions, such as the order of reagent mixing, temperature of the process, concentration of the original solutions, pH value of the medium, and nature of the agent inducing precipitation (Gribanov et al., 1990). The precipitation procedure described above was capable of producing superparamagnetic particles whose properties are rather suitable for magnetic separation and deep organ targeting. Currently, we are investigating ways of modifying the reaction medium in order to control the particle size.

The presented experimental setup markedly differs from the prior art. For the first time, a synthesis method is developed which used to produce particles that are at the same time micrometric and superparamagnetic because of the lattice defects inside the cluster that impede the propagation of the magnetic order. Although the occurrence of clusters has already been reported (Gupta & Gupta, 2005), they exhibited strong magnetic dipole–dipole attractions between them and showed ferromagnetic behavior. Therefore, a synthesis approach to produce particles with superparamagnetic profile and micrometric size range had not been presented so far.

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