Synthesis and Characterization of Spherical Magnetite/Biodegradable Polymer Composite Particles

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INTRODUCTION

A method for preparing colloidal particles formed by a magnetite nucleus and a biodegradable poly(DL-lactide) polymer coating is first described. The method is based on the so-called double-emulsion technique, employed to obtain polymeric spheres loaded with therapeutic drugs, to be used as drug delivery vectors. The aim of this work was to obtain, in a reproducible and rather simple way, colloidal particles that were both magnetic field responsive, and useful as drug delivery systems. In order to investigate to what extent is this target achieved, we compare the structure, chemical composition, and surface properties of the composite particles with those of the nucleus and the coating material. Although the surface properties of the magnetite core are not completely masked, this preliminary study shows that the synthetic new material displays a behavior intermediate between that of magnetite and poly (DL-lactide) spheres. Thus, electrophoresis measurements as a function of pH shows that the isoelectric point \( \text{pH}_{\text{iep}} \approx 5.2 \) of core/shell colloids is in between those of magnetite \( \text{pH}_{\text{iep}} \approx 7 \) and polymer \( \text{pH}_{\text{iep}} < 2 \). A similar conclusion is reached when a surface thermodynamic study is performed on the three types of particles: the electron-donor component of the surface free energy of the solids is the quantity that appears to be most sensitive to the surface composition. The fact that poly(DL-lactide) is close to being a nonpolar material gives rise to a measurable decrease in the electron-donor component of the surface free energy, although the effect of coating is also observable in the electron-acceptor and the apolar van der Waals component.

Key Words: magnetic colloids; magnetite; biodegradable polymers; poly(DL-lactide); core/shell colloidal particles; electrophoresis; surface thermodynamics; coating; drug delivery systems; magnetic carrier technology.

One of the various fields in which colloidal particles have found applications is the so-called magnetic carrier technology. Its aim is to control the physical, chemical and surface properties of magnetic colloidal particles in such a way that they can bind, selectively or not, target materials ranging from simple molecules to macromolecules, cells, or other colloids (1, 2). One of the possible and most promising applications is using such colloidal magnetic particles as drug carriers: ideally, they could bear on their surface or in their bulk a pharmaceutical drug that could be driven to the target organ and released there. Considerable work that demonstrates that biodegradable polymers are ideal as drug carriers because of their minimum toxicity and immunological response has been performed (3–6). Hence, the main aim of the present contribution is to explore the possibilities of preparing mixed particles, susceptible to external magnetic fields but covered with a shell of biodegradable polymer. Furthermore, since most theoretical treatments of the main properties of colloidal systems assume spherical geometry, it seemed useful to have particles with the above-mentioned properties as well as having a spherical shape and narrow size distribution.

The magnetic nuclei chosen were spherical magnetite particles, since Sugimoto and Matijević (7) have determined procedures to reproducibly obtain spheres of that material, in the colloidal size range, and considerably monodisperse. In addition, magnetite is a well-known material, properly characterized in many aspects. The polymer shell chosen was poly (DL-lactide), a completely amorphous polymer widely used in medical and pharmaceutical applications, either as such or co-polymerized with glycolide. These polymeric materials have been applied in hormonal or cancer therapies, and even in anesthetic medication (3–6, 8–13). Their degree of crystallinity has been shown to have a profound effect on the rate of degradation (14), which is also influenced by the molecular weight. Thus, amorphous polymers are more vulnerable to degradation because they are more accessible to water (15); higher molecular weights are associated with longer chain lengths, so that more bonds must be broken for the polymer to reduce to low-molecular-weight oligomers or monomers, a necessary step in the biodegradation of the polymer. The chemical degradation of these polymers (and, in general, polymers containing a hydrolyzable group in their backbone) includes a number of stages that always begin by water absorption from the environment. This is most important because, since the

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polymer must be only temporarily in contact with tissues, it is essential that the material be gradually degraded into cell-compatible components that, after being absorbed by the cell and used by it, should be secreted by the body mechanisms (16).

A number of authors (see, e.g., Refs. (17–19)) have described the preparation of particles or liposomes containing a certain amount of magnetite or other ferrites. Some of them have focused on the field of drug transport and release: thus, the use of albumin with entrapped magnetite has been checked for the release of anti-cancer drugs like mitomicin and adriamicin (20, 21). However, albumin has the disadvantage of provoking an immune response (22), so interest has been progressively focused on magnetite particles covered with a shell of polylactide/glycolide. However, the possible adverse effects of the presence in the body of inorganic particles as magnetite must also be taken into consideration. Experiments performed in vivo on the toxicity of magnetite or magnetite-loaded polymeric particles have demonstrated that the latter have rather low citotoxicity (22, 23), and magnetite itself has also little adverse effects (24). Nevertheless, care must be taken with the particle size of the carriers: any fraction larger than 5 μm must be avoided in order to prevent capillary blockade.

In this work we describe a technique for the synthesis of spherical mixed particles, consisting of a magnetite nucleus and a poly(m-lactide) (also known as poly(DL-lactic acid)) shell. The quality of the polymer coating will be analyzed by comparing the electrical, thermodynamic, and chemical surface properties of the composite particles to those of the pure magnetite and polymer colloids.

**EXPERIMENTAL**

**Materials**

All chemicals used were of analytical quality from Panreac, Spain, except for poly(m-lactide), provided by Sigma-Aldrich Quimica, Spain. The poly(m-lactide) has a catalog denomination LACTEL BP-0500, with average molecular weight 106,000, and inherent viscosity 0.66 dL/g. Water used in the experiments was of Milli-Q quality (Milli-Q Academic, Millipore, France).

**Methods**

**Synthesis of spherical magnetite particles.** The method followed to obtain the colloidal magnetite was originally proposed by Sugimoto and Matijević (7): it is based on the crystallization of a gel of amorphous ferrous hydroxide. The preparation of the latter involved mixing two solutions that were previously flushed with pure N₂ in order to eliminate dissolved oxygen to the greatest possible extent. The first solution was 0.14 M in KOH and 0.23 M in KNO₃; 1225 ml of it was used in the procedure. The second solution was prepared by mixing 0.2 ml of 1:1 96% H₂SO₄ in water with 55.6 g FeSO₄ · 7H₂O and 200 ml of water (1 M concentration of ferrous sulfate). A total of 88.8 ml of this solution was used.

Immediately upon mixing, the gel was formed. It was placed in an oil bath preheated at 90°C, and aged at such temperature for 4 h. After completion of this aging, magnetic particles were already visible and their cleaning was achieved by magnetic separation: the solids were repeatedly separated from the liquid medium by using a permanent magnet, and redispersed in pure water. While in the first repetitions, nonmagnetic dispersed material (elongated goethite particles, as shown by transmission electron microscope and X-ray diffraction) was present, after about 35 cycles, the conductivity of the supernatant was ≈2 μS/cm, and no other solids but magnetite were in the suspension. The particles were then dried at 60–70°C in a vacuum oven and stored until their use.

**Preparation of poly(m-lactide) microspheres.** Although with slight variations, most authors have used the double-emulsion method for obtaining colloidal spheres of poly(DL-lactide) or PLA. The technique includes the formation of a w/o emulsion of an aqueous solution (of a pharmaceutical drug in most instances) in an organic solution of the polymer. This emulsion is used to prepare a new emulsion in an aqueous surfactant solution (so as to finally obtain a w/o/w double emulsion). In our case, a 1.5% w/v solution of poly(DL-lactide) in dichloromethane was first prepared. To 40 ml of it we added 20 ml water and mixed with a Diax 900, Heidolph (Germany) homogenizer at 14,000 rpm for 5–10 s. A whitish, slightly transparent emulsion was thus obtained. This was added to 250 ml of a 1% w/v poly(vinyl alcohol) (PVA) solution, and homogenized as above to obtain the second emulsion, with white color. The next step was organic solvent evaporation; this was achieved by stirring the solution at room temperature with a magnetic stirrer set at 900 rpm. The polymer suspension was then subjected to a cleaning procedure that included repeated cycles of centrifugation (12,000 rpm) and dispersion in Milli-Q water, and finally serum replacement in a stirred filtration cell with a membrane of 0.2 μm pore size. In order to ensure that the suspension was sufficiently clean, both the conductivity and surface tension of the supernatant were measured. The polymer particles were dried at room temperature and stored in dry polyethylene containers.

**Preparation of magnetite/PLA core/shell particles.** The procedure followed was very similar to that described for the polymer spheres, except that the first water-in-oil emulsion was substituted by an (aqueous suspension of magnetite)-in-oil one. We first prepared a 5% w/v solution of poly(m-lactide) in dichloromethane. To this solution we added one-fourth its volume of a 20% w/v aqueous suspension of magnetite particles, and the mixture was homogenized at 14,000 rpm as described. Finally, a 1% w/v PVA solution (20 times the volume of emulsion) was added and again homogenized. The cleaning routines described for magnetite nuclei were also used for the mixed particles; after approximately 30 cycles, conductivity and surface tension of supernatants indicated that the suspensions were clean.
Characterization methods. The size and shape of the different particles synthesized were deduced from TEM pictures using a Zeiss EM 902 (Germany) transmission electron microscope. X-ray diffraction (Philips powder diffractometer, The Netherlands) and FTIR spectra ( Nicolet 20SXG, USA) were used for characterization of the bulk chemical structure, and XPS (Perkin–Elmer Corporation, USA) for the analysis of the surface chemistry. Given the peculiar magnetic properties of magnetite, they were also used as a tool for the analysis of the particles: a Manics DSM-8 (Germany) magnetometer was used to obtain the magnetization at $H = 2.09 \times 10^4$ A/m (approximately the saturation zone) of magnetite as a function of temperature, as well as the hysteresis cycle of both magnetite and composite particles at $20^\circ$C for $H$ ranging between $-16 \times 10^5$ and $16 \times 10^5$ A/m.

The electrical characteristics of the different particles were analyzed by electrophoresis measurements as a function of pH using a Malvern Zetasizer 2000 (England) electrophoresis device. Measurements were performed at $25.0 \pm 0.5^\circ$C. Finally, the differences between the surface properties of the three kinds of particles were ascertained by performing a surface thermodynamics analysis. Our starting point is the model developed by van Oss and co-workers (25–29), according to which the total surface free energy of any material $i$ is the sum of two contributions,

$$
\gamma_i^{\text{TOT}} = \gamma_i^{\text{LW}} + \gamma_i^{\text{AB}} = \gamma_i^{\text{LW}} + 2\sqrt{\gamma_i^{+}\gamma_i^{-}},
$$

one of which, $\gamma_i^{\text{LW}}$, is the nonpolar Lifshitz–van der Waals component, and the second one, $\gamma_i^{\text{AB}}$ or acid–base component, is related to the electron-donor ($\gamma_i^{-}$) and electron-acceptor ($\gamma_i^{+}$) characters of the material. A similar relationship can be established between the interfacial solid/liquid free energy, $\gamma_{\text{SL}}^{\text{TOT}}$, and the LW and AB components of the surface free energies of both the solid (subscripts S) and the liquid (subscripts L):

$$
\gamma_{\text{SL}}^{\text{TOT}} = \gamma_{\text{SL}}^{\text{LW}} + \gamma_{\text{SL}}^{\text{AB}} = \gamma_{\text{SL}}^{\text{LW}} + 2\sqrt{\gamma_{\text{S}}^{+}\gamma_{\text{L}}^{-}} + 2\sqrt{\gamma_{\text{S}}^{-}\gamma_{\text{L}}^{+}}
- 2\sqrt{\gamma_{\text{S}}^{+}\gamma_{\text{L}}^{-}} - 2\sqrt{\gamma_{\text{L}}^{+}\gamma_{\text{S}}^{-}}.
$$

Using the Young’s equation (30), these quantities can be related to the contact angle $\theta$ that a liquid forms on the solid:

$$
(1 + \cos \theta)\gamma_{\text{L}}^{\text{TOT}} = 2\sqrt{\gamma_{\text{SL}}^{\text{LW}}\gamma_{\text{L}}^{\text{LW}}} + 2\sqrt{\gamma_{\text{S}}^{+}\gamma_{\text{L}}^{-}} + 2\sqrt{\gamma_{\text{L}}^{+}\gamma_{\text{S}}^{-}}.
$$

If one measures the contact angles of three liquids of known components, the three unknowns ($\gamma_{\text{S}}^{+}$, $\gamma_{\text{S}}^{-}$, and $\gamma_{\text{L}}^{+}$) can be obtained by solving the resulting system of three equations. In our case, we used water ($\gamma_{\text{L}}^{\text{LW}} = 21.8$, $\gamma_{\text{L}}^{+} = \gamma_{\text{L}}^{-} = 25.5$ mJ/m$^2$), formamide ($\gamma_{\text{L}}^{\text{LW}} = 39.0$, $\gamma_{\text{L}}^{+} = 2.28$, $\gamma_{\text{L}}^{-} = 39.6$ mJ/m$^2$), and diiodomethane ($\gamma_{\text{L}}^{\text{LW}} = 50.8$, $\gamma_{\text{L}}^{+} = \gamma_{\text{L}}^{-} = 0$ mJ/m$^2$; all data taken from Ref. (26)). Measurements were performed with a Ramé–Hart 100-00 goniometer (USA).

RESULTS AND DISCUSSION

Particle Geometry

Figure 1a shows TEM pictures of the PLA particles; as observed, they are definitely spherical but rather polydisperse (diameter $1.7 \pm 0.5 \mu m$), and their surface area was found to be $2.0 \pm 0.2 m^2/g$. This value was obtained by multipoint B.E.T. nitrogen adsorption in a Quantasorb Jr. of Quantachrome (USA). Figures 1b and 1c correspond to the composite particles, with average diameter $0.18 \pm 0.05 \mu m$: the pictures clearly show that the magnetite particles are covered by a polymer shell $\approx 14$ nm thick. However, the ease of aggregation of the magnetite particles appears to make difficult the formation of individual PLA-covered magnetite units, and it seems more likely that a single PLA shell encloses more than one magnetite particle (see, however, Fig. 1c: a single magnetite nucleus is clearly observable in this figure). Note that they have a reasonably narrow size distribution (average diameter $0.16 \pm 0.06 \mu m$), and the shape of the particles is roughly spherical, although they are actually polyhedra with rounded edges. Their small size and the fact that they are formed by the aggregation of tiny (a few nanometers) particles (31) explain that the specific surface area of the particles is rather high, $8.2 \pm 0.3 m^2/g$, much larger than that of composite particles ($2.76 \pm 0.16 m^2/g$). The strong decrease in this quantity can thus be related also to the less porous structure of the PLA layer.

Bulk and Surface Chemical Structure

X-ray diffraction of the magnetite nuclei demonstrated that the crystal structure coincided perfectly with the magnetite standard (International Center for Diffraction Data, Newtown Square, PA, USA). The grain size ($\approx 300$ Å) suggests a high crystallinity of the sample.

The surface composition of the samples, as deduced from XPS spectra is detailed in Table 1. Appreciable and physically reasonable differences are observed between the various kinds of samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak</th>
<th>Percent surface concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetite</td>
<td>C 1s</td>
<td>29.7</td>
</tr>
<tr>
<td></td>
<td>O 1s</td>
<td>46.9</td>
</tr>
<tr>
<td></td>
<td>Fe 2p</td>
<td>23.2</td>
</tr>
<tr>
<td>PLA spheres</td>
<td>C 1s</td>
<td>68.0</td>
</tr>
<tr>
<td></td>
<td>O 1s</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>Fe 2p</td>
<td>40.2</td>
</tr>
<tr>
<td>Magnetite/PLA</td>
<td>C 1s</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>O 1s</td>
<td>40.2</td>
</tr>
<tr>
<td></td>
<td>Fe 2p</td>
<td>7.7</td>
</tr>
</tbody>
</table>

TABLE 1: Surface Composition of the Particles Synthesized, as Obtained from XPS Spectra
of particles: the high-carbon content in PLA microspheres and mixed colloids must proceed from the organic nature of the polymer material. Note also that magnetite has a measurable carbon concentration (most likely due to contamination from the room environment during sample manipulation), but considerably lower than that displayed by either pure PLA or core/shell particles. Finally, iron, although mostly present on magnetite, is not absent from magnetite/PLA composites, this fact suggesting that the coverage of magnetite by PLA is not complete, or that the polymer layer is very thin in some places.

It must be noted that the surface carbon of mixed particles could also be due to the presence of free PVA directly adsorbed on the inorganic material. With the aim of analyzing this possibility, infrared spectra of the three materials were obtained, as shown in Fig. 2. As observed, the spectrum of the composite particles is very similar to that of PLA spheres: note, however, that the single PLA band at 1758 cm\(^{-1}\) (C=O stretching) appears as a double one in the spectrum of the composite material, suggesting a slight contribution of the 1729 cm\(^{-1}\) band of PVA. Hence, the presence of small amounts of free PVA on magnetite cannot be discarded.

### Magnetic Characterization

The effect of temperature on the magnetization, \(M\), for magnetite particles is shown in Fig. 3, for an applied field \(H = 2632\) Oe (2.09 \(\times\) \(10^5\) A/m). Essentially, a parabolic decrease of \(M\) with temperature is observed, as expected from the fact...
that thermal motion tends to oppose the order created by the molecular field responsible for the parallel orientation of the magnetic moments of a domain (32), but the main source of information about the magnetic properties of a ferrimagnetic material as magnetite is the evaluation of the field dependence of the magnetization at a constant temperature, and, in particular, the characteristics of the hysteresis cycle (32, 33). This is done in Fig. 4 for both magnetite and composite particles. It can be seen that the two types of material exhibit similar overall magnetic behavior, characteristic of soft magnetic particles, with a narrow hysteresis cycle, and a small coercive field and remanent magnetization ($H_c \approx 6500$ A/m, $M_R \approx 77$ A m$^2$/kg for magnetite; and $H_c \approx 6900$ A/m, $M_R \approx 4.5$ A m$^2$/kg for the composite colloids). The saturation magnetization of Fe$_3$O$_4$ particles is around $M_s \approx 88$ A m$^2$/kg, or using $\rho = 5180$ kg/m$^3$ for their density (34), $\mu_0 M_s = 0.573$ T, in good agreement with magnetic data reported on magnetite (35). The presence of the nonmagnetic PLA shell is evidenced by the fact that $M_s$ of core/shell particles is about one half of that of the pure magnetite ($M_s \approx 40$ A m$^2$/kg), and the initial magnetization-field dependence is steeper in the latter case. Nevertheless, the composite spheres still show sufficient magnetization for their magnetic properties to be useful from the point of view of magnetic carrier technology.

**Electrokinetic Characterization**

Given that the surface properties of oxides are extremely sensitive to pH variations in the dispersion medium, a behavior not to be expected in the case of a polymer like PLA, we focused our study on the effect of pH on the electrophoretic mobility, $\mu_e$, and zeta-potential, $\zeta$, of the particles. The results are shown in Fig. 5, where both quantities are plotted as a function of pH for an ionic strength of $10^{-3}$ M KNO$_3$. The theory of O’Brien and White (36) was used to convert $\mu_e$ data into $\zeta$-potentials. As observed, magnetite particles show a distinct isoelectric point (or pH of zero zeta-potential) in the vicinity of pH 7, in agreement with previous determinations from other authors (37). In contrast, PLA microspheres always bear a net negative surface charge, and our data suggest an isoelectric point below pH 2. If an efficient coverage of magnetite by PLA is achieved, it is reasonable to predict that composite particles will have an isoelectric point lower than pH 7, and also that pH should have a lesser effect on either $\mu_e$ or $\zeta$ than it has in the case of magnetite. This is in fact found, as Figs. 5a and 5b demonstrate: the composite particles display an isoelectric point around pH 5.2, and appear to be less positive (negative) than magnetite at acid (basic) pH values. This is a consequence of the lesser extent to which PLA surface is pH-sensitive, as compared to magnetite. It should be mentioned, however, that the electrokinetic properties of composite particles do not coincide with those of pure PLA, as one would predict in case of an optimum coverage. A rather intermediate electrokinetic behavior is found: this can be explained as before simply by assuming that the polymer shell is
not homogeneous, or that some magnetic particles remain partially uncovered. Nevertheless, the presence of a layer of PLA is clear, and modifies significantly the surface electric properties of magnetite.

**Surface Thermodynamics**

The surface free energy components of the three types of particles constitute a set of physical quantities that can also be analyzed to ascertain the efficiency of PLA coverage. Table 2 shows

| Contact Angle $\theta$ ($^\circ$) of the Probe Liquids Indicated on Magnetite, Poly(lactide) (PLA), and Magnetite/PLA Core/Shell Particles |
|-----------------|--------------|-------------|-------------|
| Solid           | Water        | Diiodomethane | Formamide   |
| Magnetite       | 37 $\pm$ 7   | 16 $\pm$ 4   | 27 $\pm$ 6  |
| PLA             | 100 $\pm$ 4  | 43.4 $\pm$ 1.4 | 55.4 $\pm$ 1.5 |
| Magnetite/PLA   | 39 $\pm$ 3   | 26.1 $\pm$ 1.4 | 26.4 $\pm$ 2.1 |

**FIG. 5.** Electrophoretic mobility (a) and zeta-potencial (b) of magnetite, PLA, and mixed particles as a function of pH in the presence of $10^{-3}$ M KNO$_3$. 
the contact angles of water, diiodomethane, and formamide on the three types of surfaces. From these experimental data, the values of $\gamma_{SW}^L$, $\gamma_{SW}^C$, and $\gamma_{SW}^S$ could be calculated, and the results are detailed in Table 3. This table confirms to a great extent our estimations based on electrokinetic properties: in particular, whatever the component considered, its values for magnetite/PLA are intermediate between those of the pure surfaces. Thus, although the Lifshitz–van der Waals component is the least affected (as is usually the case, see, e.g., Ref. (38)), its value for the mixed particles is in between those of the pure materials. Concerning the electron-acceptor component, $\gamma_{SW}^C$, although small in the three cases, is strictly zero for magnetite, and takes finite values for PLA and composite colloids. The C=O groups of lactic acid molecules must be responsible for the mixed electron-acceptor and electron-donor character, both in PLA and in the shell of magnetite/PLA. Concerning $\gamma_{SW}^S$, it shows large values in magnetite (like in many other inorganic oxides, for instance hematite and yttria, as found in Ref. (38)), which is thus essentially a monopolar, electron-donor material. Its value of $\gamma_{SW}^S$ is much larger than that found for PLA (almost zero); when magnetite is covered by a layer of PLA, $\gamma_{SW}^S$ is significantly reduced, another proof of the presence of the polymer on the oxide particles. Although neither of the components of $\gamma_{SW}$ of mixed particles coincide exactly with those corresponding to PLA, changes due to the polymer layer are significant, and demonstrate that the surface of magnetite has been sufficiently modified by the presence of the biodegradable polymer.

### CONCLUSIONS

We have described a procedure that appears promising in the field of production of mixed colloidal particles with approximately spherical geometry, and composed of a magnetite nucleus and a biodegradable polymer, poly(3-hydroxypropionate-co-lactic acid). Both the electron microscopy and surface (chemical, electrokinetic, and thermodynamic) analyses of the synthesized material demonstrate that the objective of obtaining such composite particles can be achieved using rather simple methods. This kind of research is open to improvement, since the conditions for providing the particles with a thicker, homogeneous polymer layer must be still investigated, concerning, mainly, the relative amounts of magnetite and polymer in the starting emulsion, the type and concentration of surfactant that must be used, and the avoidance of magnetite aggregates before carrying out the coating.

### ACKNOWLEDGMENTS

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