Nanotechnology

Metallic Nanomagnets Randomly Dispersed in Spherical Colloids: Toward a Universal Route for the Preparation of Colloidal Composites Containing Nanoparticles**

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The fabrication of colloids with desired properties has been the aim of many recent investigations.[1] Their modification with metallic nanoparticles is a particularly suitable tool to modulate their optical, catalytic, and mechanical properties.[2] Of special interest for biotechnology and data storage applications, as well as for fundamental studies in the areas of physics and physical chemistry, is the modification of colloids with metallic nanomagnets (MNMs).[3] The properties of these composites in particular, and magnetic composites in general, strongly depend on the size of the nanoparticles, the distance between them, and the nature of the nanoparticle–colloidal matrix interaction.[4] Thus, the development of methods able to fine-tune the size of MNMs, their interparticle distance, and their particle composition (e.g., preparation of multicomponent alloys), while readily modifying the composition of the colloidal matrixes, could help us to produce materials with a highly predictable magnetic response and/or showing unusual magnetic phenomena of interest, for example, in recording applications.[4c] Herein, we report a synthetic route for the preparation of MNMs randomly dispersed in inorganic spherical colloids, which have potential interest in biotechnology.[5] Random distribution, instead of the typical confined distribution obtained in the widely used surface template methods, helps to screen magnetic dipolar interactions.[6] Even though we have focused on the preparation of MNMs randomly dispersed in spherical colloids, the adaptability of the synthetic strategy reported here could allow its use in the fabrication of spherical colloids containing nanoparticles of very different nature and properties.

Aerosol-based processes are convenient routes to prepare nanoparticles randomly dispersed in spherical colloidal matrixes.[7] In fact, we recently reported that α-Fe MNMs dispersed in spherical silica colloids can be produced by heating, in air and H_2, a powder obtained by aerosol pyrolysis of a methanol solution of iron(III) nitrate and tetraethoxysilane.[7c] However, the method fails when trying to obtain MNMs made up of more than one component (alloys) dispersed in colloidal matrixes, because of differences in the solubility of the metallic precursors. Compositional homogeneity at the particle level in aerosol methods is strongly dependent on the solubility of the precursors.[7,8] Specifically, the different solubilities of the metallic precursors during thermal drying (especially at low-volume-packing fractions) result in their sequential precipitation within the colloidal matrix. Thus, at the end of the process, the chemical composition of each individual MNM is not necessarily equal to the average of the alloy (compositional inhomogeneity at the nanoparticle level).

The approach we used to overcome the problem of compositional inhomogeneity, and simultaneously to better control the size of MNMs, was to generate an aerosol from an aqueous sol of ferritin and a matrix precursor (Figure 1). Our

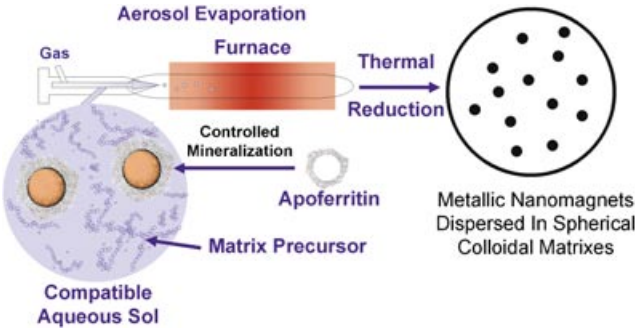

Figure 1. Stepwise fabrication of MNMs randomly dispersed in spherical colloids.

synthetic route is similar to that used for the preparation of mesoporous silica (undoped and doped with gold nanoparticles) and titania materials by aerosol evaporation of sols containing surfactant micelles,[7a,9] though logically we need to produce nanoparticles not "holes". In this sense, ferritin seems an ideal template for reaching our final goal and, more importantly, ferritin could serve as an ideal model to extend the synthetic strategy to other ferritin-like systems. For example, one can easily deduce that the ability of dendrimers to encapsulate metallic nanoparticles of very different composition (Au, Pt, Pd, Cu, Ag, Ni)[10] could be used for the direct fabrication of colloidal composites containing nanoparticles of very different nature and properties.

Nature has developed a variety of proteins that function as carriers or storage devices for metal components. Of these systems, the iron-storage protein ferritin is one of the most intensively studied.[11] Ferritin consists of a spherical polypeptide shell surrounding a ferrihydrite core. Ferritin can easily be demineralized (apoferritin), to leave an approximately 8-nm internal cavity that can be used as a template for the production of nanomaterials containing elements such as Fe, Mn, U, Co, Ni, and Cr.[11,12] The biological template of ferritin has also been used to prepare CoPt nanometallic alloys of interest in data-storage technology, nanoparticles of Prussian blue ferritin, and very recently to prepare metallic Pd nanoparticles of interest in catalysis.[13] In addition, Lan

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et al. demonstrated that it is possible to prepare a sol containing a silica inorganic component and the protein ferritin.[14] However, their final goal was to encapsulate ferritin in a sol–gel-derived silica glass, and not to prepare metallic nanoparticles dispersed in colloidal matrixes with a spherical shape.

Therefore, based on previous work carried out with ferritin, we expected that the internal cavity of ferritin would allow us to confine the various metallic precursors (in the form of inorganic nanoparticles), thus avoiding the problems associated with compositional inhomogeneity in aerosol processes. Besides, if the right matrix is chosen, the particle size can easily be controlled by the particle size of the inorganic component of ferritin. A necessary requirement for the success of the method is the preparation of an aqueous sol of ferritin and the matrix precursor. Simple arguments based on colloid science dictate that both ferritin and the matrix precursor must be equally charged to avoid heterocoagulation. An additional advantage of using ferritin is that its protein cage is remarkably stable, and able to withstand extremes of pH (2–10) and temperature (up to 70 °C).[12] Thus, the high stability of ferritin allows the use of a wide range of experimental conditions to prepare stable sols (that is, compatibility with different matrix precursors).

To probe the reliability of the method in obtaining MNMs of different size and composition dispersed in spherical colloids we used ferritin with its internal cavity fully or partially filled with Fe- and FeCo-containing species. An amorphous silica sol and a crystalline boehmite (AlOOH) sol were selected to probe the versatility of the method in changing the nature of the colloidal matrix. An amorphous silica sol was selected in the first instance because of the several benefits that amorphous colloidal silica matrixes with spherical shape impart to MNMs for applications in biotechnology (colloidal stability, functionality, biocompatibility, and corrosion resistance).[15] The criteria we followed to select a crystalline boehmite sol were based on the different colloidal behavior of boehmite and silica (isoelectric points of ca. 9 and 3, respectively) and the different nature of the initial sol (crystalline versus amorphous).

The aerosol evaporation of a stable aqueous sol containing commercial ferritin[16] and silica (see Experimental Section) led to powders that were heated in air (500 °C, 2 h) to eliminate residual organic matter and hydroxy groups, and finally reduced in H_2 atmosphere (700 °C, 10 h) to produce α-Fe MNMs randomly dispersed in silica colloids (Figure 2a). The presence of this phase was confirmed by X-ray diffraction (XRD, see the Supporting Information).[17] The crystallite size estimated from the width of the (110) α-Fe reflection was 5.3 nm, which is similar to the diameter obtained by transmission electron microscopy (TEM; 4–6 nm, Figure 2a). This value is consistent with the expected shrinkage when going from the ferrihydrite (average size of the ferrihydrite core of ferritin is about 7 nm)[11] to the α-Fe phase, by considering the amount of iron in a unit volume of these two phases (that is, no significant interparticle sintering between MNMs on heating).

A similar methodology was used for the preparation of spherical composites with α-Fe MNMs of smaller size (3–4 nm, Figure 2b), though in this case we started from an apoferritin reconstituted only with 1000 Fe atoms (for details of reconstitution, see the Experimental Section). The decrease in the size of the α-Fe MNMs was also detected by XRD (see the Supporting Information). Specifically, a decrease from 5.3 to 3.4 nm was estimated from the width of the (110) α-Fe reflection. The volume estimated from the experimental data for a spherical shape (10–35 nm^3, 3–4 nm diameter) is within the margins of experimental error expected for a cubic cluster of 1000 Fe atoms in the α-Fe phase (ca. 12 nm^3). However, the fact that the 1000-atom cluster volume is at the lower limit does not allow us to ignore the possible growth of some nanocrystallites. Finally, FeCo MNMs (Co content ca. 30 mol %; 3–4 nm) dispersed in silica colloids (Figure 2c) were also prepared by using a similar methodology. This particular Co/Fe composition was selected because it has the highest magnetic moment.[18] In this case, apoferritin was reconstituted with 670 Fe atoms and 330 Co atoms (see Experimental Section). Energy-dispersive X-ray (EDX) analyses carried out on the MNMs clearly indicated that the composition of individual nanomagnets was the expected one (Co content of ca. 30 mol % in the FeCo alloy).[19] Thus, we showed the versatility of the method in preparing spherical composites containing randomly distributed MNMs of controlled composition and size. An important point is that the colloids are polydisperse in size (70–400 nm); however, monodispersity can be achieved with aerosol techniques by using differential mobility analyzers.[20]

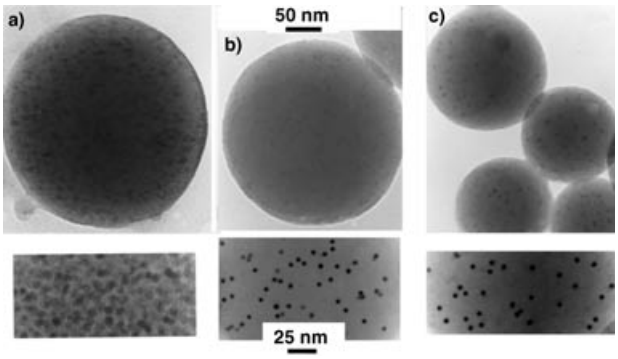

Figure 2. TEM pictures of powders showing random distributions of MNMs in spherical silica colloids: a) from ferritin; b) from Fe-reconstituted apoferritin; c) from FeCo-reconstituted apoferritin. The lower pictures are magnified to enhance the visual resolution of the MNMs (dark spots). The higher concentration of MNMs in (a) reflects the higher number of Fe atoms in commercial ferritin with respect to the reconstituted apoferritin. The pictures were computer-enhanced to improve the MNM contrast.

We also used a crystalline boehmite sol (see Experimental Section) to probe the versatility of the method in changing the nature of the colloidal matrix. Specifically, the use of a boehmite sol (no ferritin added) resulted in a porous matrix (Figure 3a), which was not ideal for stabilizing small MNMs (< 5–6 nm) against corrosion. Thus, the results for boehmite probe the benefit of the confinement effect of ferritin in controlling chemical composition at the nanoparticle level (that is, preparation of multicomponent MNMs) even in porous matrixes. The aerosol evaporation of an aqueous sol of

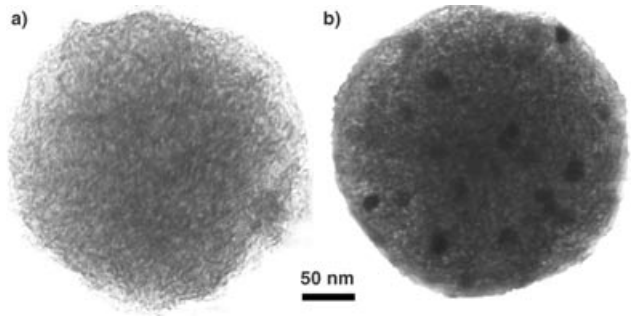

Figure 3. TEM pictures of powders obtained after aerosol evaporation of an aqueous sol of a) boehmite and b) FeCo-reconstituted apoferritin and boehmite with further heating in air (500 °C, 2 h) and H_2 (800 °C, 10 h).

apoferritin (after reconstitution with 670 Fe atoms and 330 Co atoms) and boehmite, followed by thermal annealing first in air and then in H_2, produced powders made up of FeCo MNMs dispersed in γ-Al_2O_3 colloids (Figure 3b). After the thermal treatment boehmite transformed to γ-Al_2O_3, as evident by XRD analysis. EDX analyses clearly indicate that we were successful in controlling the chemical composition at the nanomagnet level even in a porous matrix. An increase in MNM size was produced when silica was replaced by alumina (ca. 15 nm versus ca. 3–4 nm, Figure 3b and 2c). Thus, no temperature was found that reduced the porosity of the alumina matrix while avoiding sintering between MNMs (small MNMs cannot be stabilized against oxidation). Finally, similar results were obtained in the case of MNMs made up of Fe atoms, which additionally confirmed the benefit of the silica matrixes in providing protection against corrosion.

In summary, metallic nanomagnets (MNMs) of different size and composition randomly dispersed in spherical colloids have been prepared by the aerosol evaporation of an aqueous sol containing mineralized ferritin and a colloidal matrix precursor. Essential to the success of the method is the template effect provided by ferritin. Its internal cavity offers the ideal environment for the encapsulation of multicomponent MNM precursors with the simultaneous control of size. Moreover, the high stability of the ferritin cage allows compatibility with different matrix precursor sols (that is, the nature of the colloidal matrix can be changed). Our results indicate that this synthetic strategy could be extended to other ferritin-like systems, for example, metals encapsulated in dendrimers.

Experimental Section

Aerosol generation: The aerosol device used for the preparation of the spherical composites containing nanoparticles is described elsewhere.[8b] It essentially consists of two furnaces that could be set at different temperatures for drying (first furnace) and thermal decomposition (second furnace). In our experiments we set the temperature of the two furnaces at 400 °C (aerosol evaporation). The aqueous sol containing the ferritin and matrix precursor was atomized at 1.6 mL min^{-1} with an air pressure of 1.7 kg cm^{-2} to give different spherical colloidal composites. The particles obtained were collected with an electrostatic filter (8 kV). Experiments carried out with a freshly prepared sol and a sol aged for 6 h led to similar results, which confirms the reproducibility during the aerosol experiment (typically 2 h).

Ferritin reconstitution: A dispersion of apoferritin (Sigma; 75 mL, 1.46 μM) in NaCl (0.1M) and 3-(*N*-morpholine)propanesulfonic acid (MOPS) buffer (50 mM, pH 6.5) was reconstituted by the controlled addition of a deaerated $FeSO_4$ solution (25 mM, pH 2) at a rate of 0.1 μmol min^{-1} until completion of the calculated loading of 1000 Fe atoms per unit of protein. The method is similar to that described by Chasteen and co-workers.[11c,d] In our case the rate of addition was slower and reconstitution was restricted to 1000 atoms to assure complete mineralization inside the protein. No iron(III) hydroxide sediment or deposit was found after centrifuging at 4000 rpm ($r_{max} = 12$ cm, rotor angle = 30°) for 5 minutes and then filtering through a 0.1-μm Millipore filter, which further suggests that the formation of iron(III) hydroxide did not take place outside the protein.

To prepare the FeCo-reconstituted apoferritin, an apoferritin dispersion (75 mL, 1.46 μM) in NaCl (0.1M) and MOPS buffer (50 mM, pH 6.5) was reconstituted by the controlled addition of a deaerated $FeSO_4$ solution (25 mM, pH 2) at a rate of 0.1 μmol min^{-1} until completion of the calculated loading of 670 Fe atoms per molecule of protein. Again following this protocol, we did not find any evidence of the formation of iron(III) hydroxide outside the protein. The dispersion was dialyzed (12-kDa cutoff membrane) against aqueous NaCl solution (0.1M) to eliminate unwanted ions and then 330 Co atoms per protein unit were added by controlled addition of $Co(NO_3)_2$ (25 mM, 0.125 μmol min^{-1}) and H_2O_2 (25 mM, 0.125 μmol min^{-1}) to the partially filled apoferritin dispersion (75 mL, 50 mM 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO) buffer, pH 8.5). The method is a combination of the one reported by Chasteen and co-workers for iron mineralization[11c,d] and that reported by Douglas and co-workers for cobalt mineralization.[12d,e] Since ferritin has more affinity for iron than for cobalt, we added the iron atoms first. In this way, nucleation of cobalt atoms inside the ferritin core was favored by the presence of the Fe-containing nuclei. The high compositional homogeneity of the nanoparticles dispersed in the colloidal matrixes suggests that cobalt was indeed mineralized in the ferritin cores containing iron atoms. To avoid mineralization outside the protein, we only filled the apoferritin with a total of 1000 Fe atoms.

Before mixing with the matrix precursor sol both the Fe- and FeCo-reconstituted apoferritin dispersions were dialyzed against water to avoid excess electrolyte, which can induce coagulation.

Preparation of aqueous sol containing ferritin and silica: Horse spleen ferritin dispersion (2 mL, Sigma Aldrich, type I, 102 mg mL^{-1}) in NaCl (0.15M) was diluted with water to 96 mL and mixed with a silica sol (2 mL). The clear silica sol stock dispersion was prepared by mixing tetramethoxysilane (2.95 mL, Aldrich, 99%), water (1.4 mL), and HCl (0.04 mL, 0.04M) at 4 °C with strong agitation for 15 minutes. Use of this procedure to prepare the silica sol allows the amount of alcohol present in the system to be reduced as much as possible, thus avoiding the possible denaturation of the protein ferritin.[14] When reconstituted apoferritin was used instead ferritin, silica sol (2 mL, pH 5.5) was added to reconstituted apoferritin dispersion (75 mL). At this pH value both ferritin (isoelectric point 4–5) and silica (isoelectric point ca. 3) are negatively charged, so heterocoagulation is avoided. The order of addition of the protein, water of dilution, and silica sol was important for the stability of the system. Coagulation of the protein was observed if the silica sol (2 mL) was added to water (96 mL) and followed by ferritin (2 mL).

Preparation of aqueous sol containing ferritin and boehmite: A stable boehmite sol was prepared by following a standard methodology.[21] HCl (final concentration 0.02M) was added to an aqueous suspension of aluminum isopropoxide (0.25M, Aldrich) which had been previously heated at 80 °C for 1 h. The vessel was opened at 90 °C for 2 h to allow evaporation of the isopropanol generated from the alkoxide hydrolysis. Finally, the suspension was refluxed at 90 °C

for 16 h to produce a stable sol. For the preparation of the aqueous sol containing apoferritin and boehmite, the reconstituted apoferritin sol (60 mL) was added to boehmite sol (40 mL, 0.25 M). The pH value of the resulting sol was 3.5, which assures compatibility between apoferritin and boehmite. In this case both colloids are below their isoelectric points (that is, positively charged).

The morphology of the powders was examined by TEM (2000 FX2, Jeol). Chemical analyses at the particle level were carried out with an EDX spectrometry analyzer (QX 2000, Oxford Link) integrated in the transmission electron microscope. The different phases present in the solids were detected by X-ray diffraction (XRD, PW1710, Philips).

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[16] We did not try to eliminate the inorganic phosphate, because of the small amount of phosphate with respect to iron in native horse spleen ferritin (usually about 5 mol% of the Fe content, Ref. [11a]) and the fact that its presence could even help to stabilize the metallic phase against corrosion (phosphates are routinely used in metallurgy as anticorrosive agents). When the reduction, elimination, or even addition of inorganic phosphate is needed for other systems, protocols can be found in the literature (see for example reference [11b]).

[17] Apart from α-Fe, we expect the formation of a low-crystallinity Fe_2SiO_4 shell (not observable by XRD) surrounding the α-Fe MNMs, as happens in Fe/silica systems obtained in a similar way (see references [7c] and [15d]). This protective layer imparts stability against corrosion of the metallic component.

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[19] The Fe and Co atoms form a metallic alloy throughout the range of compositions and we expect, at the end of thermal treatment, the diffusion of Co atoms within the Fe metallic core. In fact, the formation of $Fe_{1-x}Co_x$ metallic alloys is reported in the literature by using methods such as ball milling (see for example M. Sorescu, A. Grabias, *Intermetallics* **2002**, *10*, 317 and E. C. Passamani, C. Larica, V. P. Nascimento, *J. Mater. Sci.* **2002**, *37*, 809).

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