

TUNING THE SP TO FM TRANSITION OF COBALT NANOPARTICLES IN VIEW OF BIOMEDICAL APPLICATIONS

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Magnetic nanoparticles are a powerful and versatile diagnostic tool in biology and medicine. Bound to a suitable antibody, they are used to label specific molecules, structures or microorganisms. Established techniques such as magnetic cell separation use magnetic field gradients to manipulate and isolate magnetically-labeled cells. More recently, magnetic immunoassay techniques have been developed in which the magnetic field generated by the magnetically-labeled targets is detected directly with a sensitive magnetometer. In addition, drug delivery and contrast enhancement in MRI would benefit from a simple reproducible chemical synthesis of monodisperse magnetic nanoparticles.

In general, the use of magnetic particles for biological and medical applications has been intense and focused on iron oxides.

At the present point, development of better techniques would require better-controlled nanoparticles. Two possible approaches are either improving synthesis of the iron oxide nanoparticles or changing the magnetic material. Synthesis of iron oxides often shows low reproducibility (due to the complex oxygen chemistry), polycrystallinity and low control. T. Hyeon *et al.* [1] recently showed the synthesis of monodisperse Fe₂O₃ nanoparticles. Even if the results are remarkable, still, Fe₂O₃ is a weak ferrimagnetic material, while Co (and Fe and Ni and their alloys) is ferromagnetic and has shown an outstanding capacity of shape control, perhaps thanks to the particular epsilon crystal structure [2], which is more complex than the conventional hexagonal and cubic compact faces, while keeping the magnetic properties almost unchanged.

The magnetic transition from the superparamagnetic (SP) to ferromagnetic (FM) state of the magnetic susceptibility and coercivity is determined at the synthesis. Depending on the desired properties, different magnetic behavior is requested. For example, Chemla *et al.* [3] recently showed a method for ultra-high sensitivity immunoassays where monodisperse SP nanoparticles are needed. Many tests reveal the presence of a molecule or disease-causing organism by detecting the binding of an antibody to the target. When antibodies labeled with magnetic nanoparticles bind to the target on a surface, brief

exposure to a magnetic field causes these probes collectively to give off a strong magnetic signal. Meanwhile unbound antibodies tumble about in all directions producing no net signal. For a 20 nm Fe₂O₃ particle, Neel relaxation (bound particles) is about 1 s while Brownian relaxation (unbound particles) is about 1 μs [3]. This last property makes it possible to read the results without first washing away any probes that fail to find their target.

Besides, weakly FM particles capable of self-assembly in solution would be useful for dynamically changing the local concentration of molecules linked (conjugated) to the nanoparticles surface.

The general requirements would often be of magnetic particles displaying a large magnetic susceptibility, while keeping interparticle interactions low so they do not agglomerate.

Progress has been made recently by Riffle and Rutnakornpituk (micelle-grown cobalt nanoparticles of 10 nm diameter). Other groups have also developed magnetic nanocrystal synthesis [4]. In this paper we present the synthesis of monodisperse cobalt ferrofluids with controlled size and shape. The synthesis is carried out in non-polar solvents, necessary for the size and shape control. Recent developments in the solubilization of this nanoparticles in aqueous solutions are commented.

By modifying the volume or the shape, and therefore the shape anisotropy, the SP to FM transition can be modified. In addition, by partially oxidizing the particles, coercivity may be increased (for passive sensor purposes) thanks to the exchange between CoO and Co. Surface chemistry, like absorption of CO may also modify magnetic properties.

The particles are obtained by rapid pyrolysis of Co carbonyl in dichlorobenzene in the presence (about 1%) of surfactants (carboxylic acids, phosphonic acids and amines), which controls the crystal growth, and therefore, the size and shape of the particles; solubilizes the particles; avoids agglomeration; and protects them (but not fully) against oxidation. In the presence of oleic acid and trioctyl phosphine oxide, spherical monodisperse particles are obtained (Fig.1).

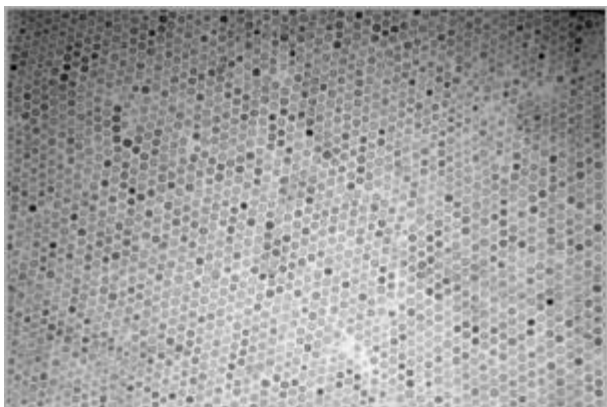


Fig.1: TEM picture of 10 nm Co nanoparticles

A concentrated solution of $\text{Co}_2(\text{CO})_8$ (0.40 to 0.80 g in 2-3 ml of *o*-dichlorobenzene anhydrous) was injected in an *o*-dichlorobenzene anhydrous refluxing bath (15 ml, $T=181^\circ\text{C}$). All reactions were conducted under Ar following standard airless procedures using commercially-available pure reactants. Macroscopic amounts (10^{16} particles/ml) of monodispersed nanoparticles were thus obtained. The decomposition and nucleation occurs instantaneously upon injection. The lifetime of atoms in solution is short leading to the simultaneous formation of many small metal clusters (nuclei). Control of the bath temperature and the surfactant composition modifies the strength of the metallic particle-organic molecule bonding. Thus, by controlling the precursor-to-surfactant ratio, the reaction temperature and injection time, the size of the spherical particles can be controlled and varied between 3 to 16 nm (Fig.2).

Small magnetic particles are single domain, i.e., all the atomic magnetic spins of the particle are coupled in the same direction and the particle behaves as a single magnetic dipole. Depending on anisotropy, size and temperature, the magnetic dipole of the particles will be free to rotate (superparamagnetism) or will be blocked in the anisotropy direction (ferromagnetism). In the first case, the time average of the magnetic moment of the particle is zero. The relation between anisotropic energy and thermal energy is $KV=k_B T$ (where K is the anisotropy constant, V is the particle volume, k_B is the Boltzmann constant and T is the temperature), determines the transition between the SP and the FM regime. Once the particles become FM, magnetic interactions among them start to play a crucial role following the relation between magnetic interaction and thermal energy $2\mu^2/a^3 = k_B T$ (where μ is the magnetic dipole moment and a is the particle diameter).

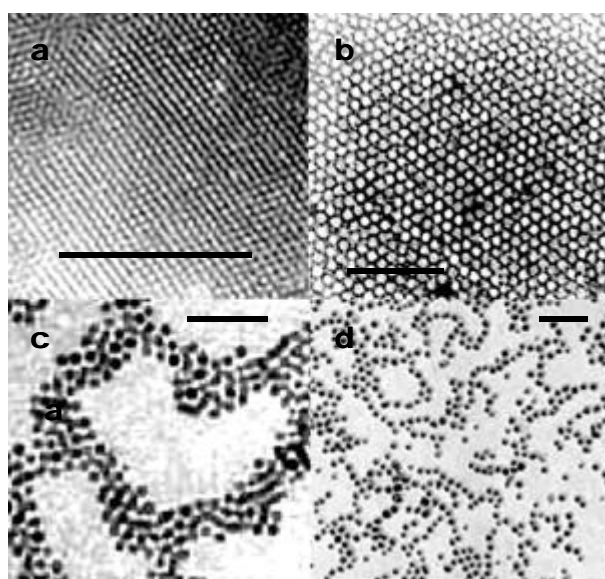
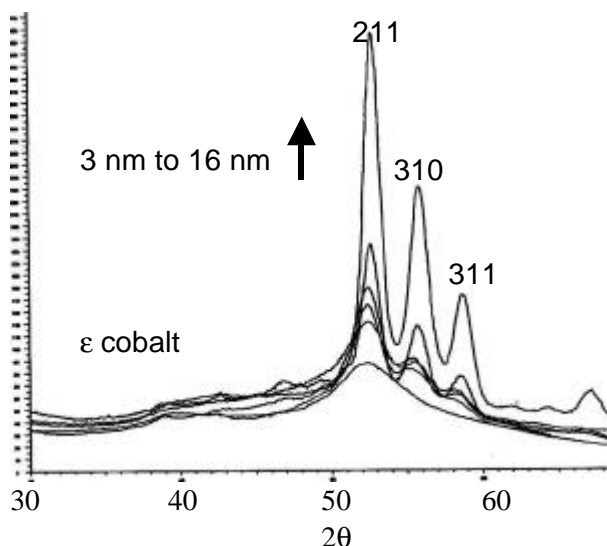


Fig.2: XRD and TEM as a function of particle size. The XRD peaks increase in intensity and become narrower as the size of the crystal domain increase. All bars are 100 nm.

Thus, due to strong dipole-dipole interactions, large nanocrystals form closed loops in order to minimize the magnetostatic energy (Fig.2d). In the transition range, a mixture of hexagonal monolayer and closed loops is observed (Fig.2c) suggesting that in this case, the SP-FM transition is taking place at room temperature for particles with sizes around 12 nm, as supported by low susceptibility magnetization measurements (not shown).

In addition to the volume, the shape may be modified to obtain objects with anisotropies up to an order of magnitude higher than the crystal anisotropy, shifting considerably the SP-to-FM transition to lower temperatures for a constant particle volume.

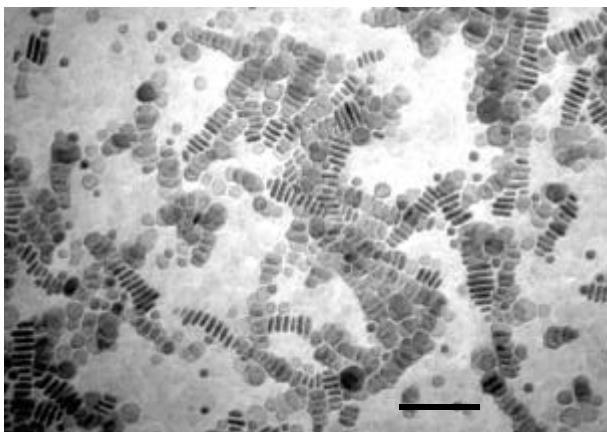


Fig.3: TEM picture of Co nanodisk-shaped crystals. The bar is 100 nm.

By mixing small amounts of amines to the surfactant mixture, Co nanodisks have been synthesized (Fig.3). And, as previously, the diameter may be modified (Fig.4). These crystals show a strong anisotropy and spontaneous self-assembly into large ribbons. Thus, when these particles are introduced in solution, they will find each other, and so will any molecule that is attached to them.

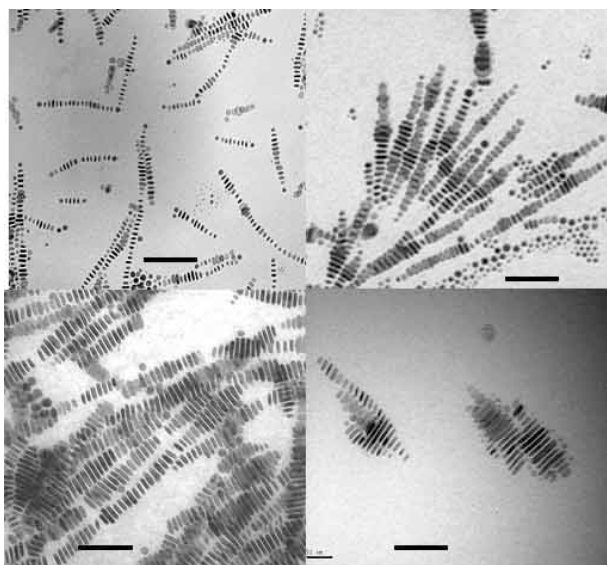


Fig. 4: Co nanodisks of different average diameters. The bars are 100 nm.

It is often desirable to have particles which interact strongly between themselves or with an external field, but not so much that they collapse and precipitate. Also desirable are large SP particles which give a large signal without interacting.

As an example of tuning the magnetic properties, small disk-shaped particles will show a FM behavior for much smaller magnetic moments than

the corresponding spheres, thus they will have a weak tendency for self-assembly, so they may be easily redispersed (by agitation, sonication or slight T increase). Once the particles are far apart, the recall interactions will be small, and independent tests may be performed before letting them slowly self assemble again. Assemblies of large disks will attach strongly and may be easily collectively transported by an external field. Large spherical particles may be useful for cell separation and drug delivery, and small ones may be useful for ultra-fast detection and labeling.

Proof of the quality and magnetic character of the samples yielded by this approach comes from observations of the spontaneous self-assembly of the nanocrystals, as well as from the formation of unique superstructures, such as chains and ribbons of disks. All sizes and shapes self-assemble into superstructures as a colloidal solution is allowed to evaporate slowly in a controlled atmosphere. A drop of the colloids (2% weight of particles) was placed on a carbon-coated TEM grid at room temperature and slowly evaporated (in a covered watch glass). The use of high boiling solvents (like o-dichlorobenzene), allows slow evaporation at RT, which permits the particles to diffuse to their lowest energy sites during evaporation, producing well-defined super-structures. The final arrangement of the nanocrystal assembly is driven by the balance of surface tension, van der Waals forces, and magnetic interactions among SP or FM particles.

Spherical particles self-assemble into hexagonal 2D superlattices (Fig.1). This assembly is determined by the size of the crystals and the thickness of the coating layer (~ 2 nm). Higher initial concentrations lead to 3D self-assemblies where the particles in the second layer occupy sites determined by the hexagonal arrangements.

As the particle diameter is increased, the magnetic moment - and thus, the interparticle interactions - increase. For 12 nm SP particles, their large moment leads to strong repulsive interactions that help to order the solid particles on the microscope grid, as the sample is dried. Thus, the largest and most ordered self-assemblies are found for the larger SP particles. However, when the volume further increases, particles become FM at room temperature and begin forming chains as previously commented.

In addition, particles oxidize progressively inwards when O₂ is bubbled through the solution. By controlling the oxygen rate, the thickness of the CoO layer may be regulated. CoO is antiferromagnetic and Co is FM. It has been repeatedly observed that as adjacent layers of FM

and AFM form, CoO-Co core-shell structures display an increase in coercivity (memory) and a shift of the hysteresis loop after cooling the sample under an applied field due to exchange interactions. This parameter may also be used to finely tune the properties of the desired particles.

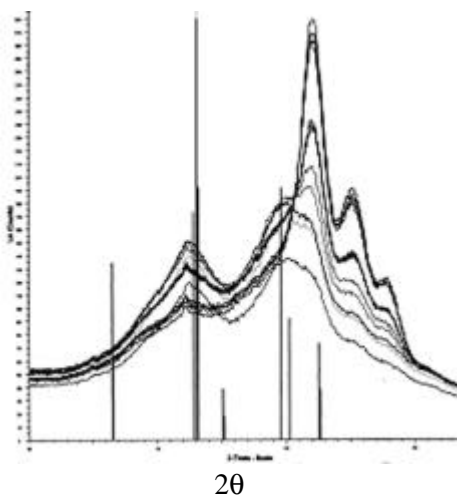


Fig. 5: XRD of a progressive Co nanoparticle oxidation. Vertical lines correspond to CoO peaks.

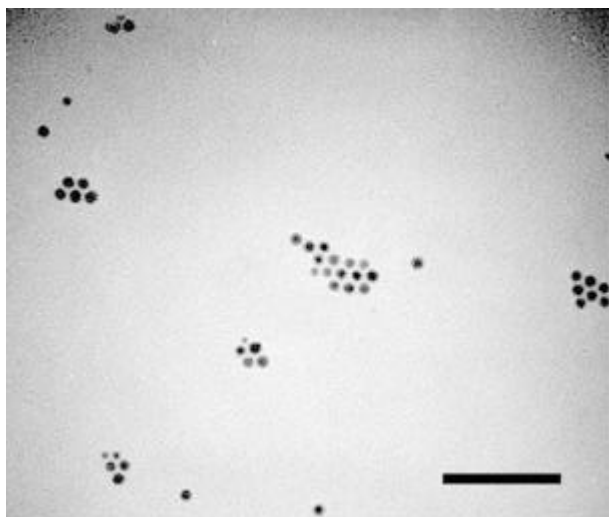


Fig. 6: TEM of CoO-Co particles. CoO appears as a lighter ring around a dark core (due to electron density differences).

These particles are completely insoluble in water and, moreover, they are attacked and rapidly transformed into $\text{Co}(\text{OH})_2$. There is an intense effort to solubilize such particles into aqueous solutions for biomedical techniques. Up to now there are four main approaches: i) growing epitaxially another metallic shell which will protect the particle and make it soluble (such as Au or Pt), ii) wrapping the particles with a polymer consisting of hydrophobic and hydrophilic sections, iii) growing amorphous silica around the particle and,

iv) attaching a surfactant molecule, like a sugar, that will make the particle water soluble.

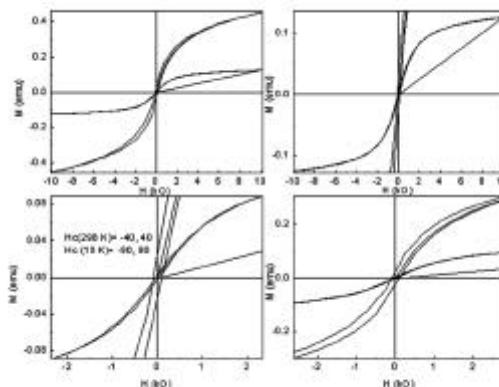


Fig. 7. Hysteresis loop at low T after field cooling at 1 Tesla, showing an increase of the coercivity and shift in the x-axis for samples which have been oxidized (by bubbling O_2 into the solution).

In conclusion, promising results in nanocrystal synthesis may assist the development of magnetic nanosensors and nanoactuators, first *in vitro* and then *in vivo*, for all the existing techniques applicable to magnetic carriers, as well as new ones to come.

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