"Biomedical applications based on magnetic nanoparticles"

M.R. Ibarra[1,2], R. Fernández-Pacheco[1], C. Marquina[2] , D. Serrate[2] and J.G Valdivia[1]

[1]*Instituto de Nanociencia de Aragón, Edificio Interfacultades II, Zaragoza (Spain)*

[2]*Instituto de Ciencia de Materiales de Aragón (CSIC/Universidad de Zaragoza), Facultad de Ciencias, Zaragoza (Spain*

The wide interdisciplinary world of Nanoscience has experienced a strong development during the last years. One exciting topic is the possibility of using nanoscale magnetic materials for biomedical applications. Many interesting problems regarding magnetic properties exist to be investigated from the fundamental point of view, and expectations are opened for their application as magnetic carriers and bioferrofluids.

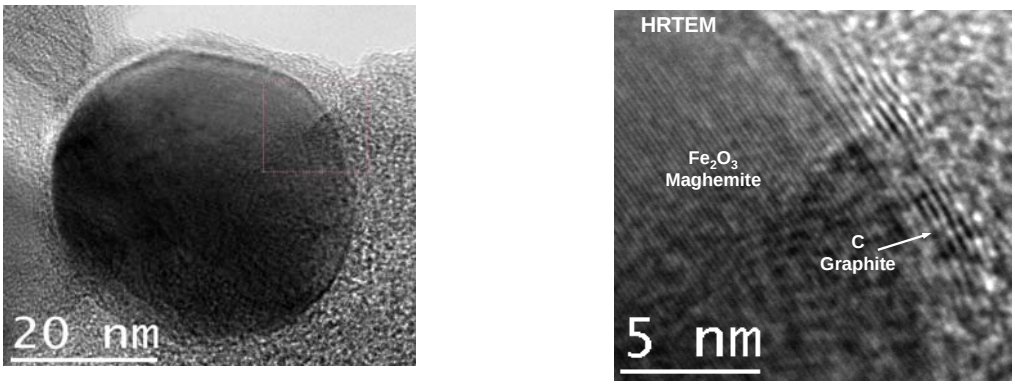
One of the important points in the use of magnetic nanoparticles for biomedical applications is the encapsulation of the magnetic material, in order to make it biocompatible, and to have the possibility of producing a bio-ferrofluid. Coating the nanoparticles with a suitable material offers the possibility of attaching them to antibodies, proteins, medical drugs etc. Therefore studies on surface adsorption, the possibility of functionalising and/or conjugating the particle coating with bioactive components are also a crucial issue. The election of the magnetic material as well as a detailed knowledge of its magnetic properties play an important role in the use of the nanoparticles in Biomedicine, as well as in the effectiveness of the desired application. In order to obtain the biocompatible ferrofluid, we have prepared carbon coated iron nanoparticles. In the following sections the adequacy of both carbon and iron as staring materials for the synthesis of biocompatible magnetic carriers is discussed.

The magnetic nanoparticles offer the possibility of being directed towards a specific target in the human body and remaining eventually localised, by means of an applied magnetic field. Obviously, when magnetic nanoparticles are going to be used for in vivo applications, very low values of applied magnetic field are desirable. Therefore the suitable materials are those with high magnetisation at the operation temperature, that is to say, at room temperature. As stated at the beginning of this section, standard starting materials for the production of magnetic particles are iron, cobalt and nickel. Nevertheless, the control of the particle size and shape, and the matrix or medium in which the particle is embedded, is also of crucial importance. In vivo applications (like drug delivery [1,2], magnetic resonance imaging [3,4], hyperthermia

[4,5], additionally require particles being biocompatible, stable and biodegradable. This is achieved by coating and embedding the particles in a suitable material. Polysaccharides (like dextran) [6], or polymers (as for example polyvinyl alcohol [7]) are used as typical coatings, whereas water-based ferrofluids are the most commonly used injection vehicle [8]. Coating the particles allows also the possibility of modifying the particle surface by attaching bioactive components, as antibodies, proteins etc [6], by means of chemical bonding or by means of adsorption, which broadens their possibilities for applications. Concerning the particle size, and particularly in the case of in vivo applications, the magnetic particles should not retain any remanent magnetisation once the magnetising field has been removed. This would avoid aggregation due to dipolar interactions between their respective magnetisations, favouring the biological absorption and eventual excretion of the particles by the body.

An optimum magnetic response has been achieved using iron nanoparticles. With respect to the coating, carbon has been used as biocompatible material. One of the advantages of using carbon is its high capacity of adsorption. Iron coated nanoparticles are therefore appropriate to be used as magnetic carriers of medical drugs, magnetic resonance imaging contrasts, biological labels etc, adsorbed into the carbon surface.

The production of carbon coated magnetic nanoparticles is accomplished by two methods: by the arc discharge method designed by Krätschmer-Huffman in 1990 [9,10] which uses a plasma furnace, with two graphite electrodes. One of them is a stationary anode, which consists of a drilled carbon cylinder where, in our particular case, several micrograms of 10 micron iron powders are introduced. The cathode is a moveable graphite electrode. An arc is created between the graphite electrodes in a helium atmosphere. The moveable electrode is sublimed and builds up a carbonaceous deposit or *collaret* around it, at the same time that soot is deposited on the inner surface of the chamber walls. The morphologic and structural characterizations have been performed by Transmission Electron Microscopy (TEM), Dynamic Light Scattering, and electron diffraction. The magnetic nanoparticles are encapsulated either in carbon nanotubes (this are mainly found in the cathode), in concentric graphitic shells (“onions”) or in amorphous carbon. In the case of samples obtained by high energy milling TEM shows that the final powder is composed by a mixture of carbon coated nanoparticles together with the free carbon and non encapsulated magnetic metallic particles.



HRTEM images of carbon coated magnetic nanoparticles produced by arc discharge. As corresponds to a lighter element, C appears with a lighter contrast compared to Fe.

Electron Filter (EF)-TEM has been also performed in order to get a better insight into the chemical nature of the obtained materials. EFTEM images have been taken selecting the Electron Energy Loss Spectroscopy (EELS) peaks separately for iron and carbon (iron at 708 eV, L_3 peak, and carbon at 284, K peak). Once both images were obtained, an elementary map can be drawn, showing a coloured distribution of each of the elements. The EFTEM confirms the presence of iron content in the core of the nanoparticles, which is completely surrounded by a carbon coating. The crystallographic structure of the samples has been analysed by electron diffraction. According to the patterns, the core of the magnetic nanoparticles contains iron oxide, crystallised in the cubic phase γ-Fe_2O_3, or maghemite (Fernández-Pacheco et al., to be published). The morphological and structural characterization shows that the samples prepared by arc discharge as well as the samples prepared by mechanical milling contain also non-coated or partially-coated nanoparticles, which are not biocompatible and therefore not suitable to be used as magnetic carriers. Moreover, all those carbon nanostructures that not contain magnetic nanoparticles have to be eliminated, in order to enrich the magnetic concentration of a future bio-ferrofluid. With this purpose a combined magnetic and chemical purification method as been developed. For magnetic purification, stable suspensions of the particles are prepared in a surfactant solution (2.5 g of SDS in 500 ml of distilled water). Magnetic separation (by means of a 3 KOe permanent magnet), yields only magnetic nanoparticles. Afterwards the purified magnetic material is washed with HCl 3M at 80ºC. All the non-completely coated magnetic material is dissolved and the remaining coating carbon forms carboxylic

groups, which, due to their hydrophobic nature, contribute to the stability of a future ferrofluid suspension. The sample is finally heated at 350ºC in order to evaporate remaining free amorphous carbon structures.

Magnetisation measurements on purified samples were performed in a Superconducting Quantum Interference Device (SQUID). The magnetisation isotherms at room temperature show no coercivity and no remanent magnetisation. This is characteristic of superparamagnetic behaviour, in good agreement with the nanometric size of the magnetic particles derived from TEM images. Therefore we can conclude that the synthesised magnetic nanoparticles fulfil all the requirements to be used as magnetic carriers for future in vivo applications. The experimental results can be compared to those predicted by the theory of Langevin for superparamagnetism, in which the magnetisation of a nanoparticle, *m*, is a function of the temperature, *T*, and of the applied magnetic field, *H*, and follows the Langevin's law [11]

$$m(T,H) = m_s\left(\coth\left(\frac{\mu H}{k_B T}\right) - k_B T \Big/ \mu H\right)$$

were m_s is the saturation magnetisation of the particle, μ is the magnetic moment of the particle and k_B is the Boltzman constant. Introducing the value of m_s for iron (1740 emu/cm^3), and fitting the experimental data to the Langevin's law the value of μ can be derived. As the magnetic moment is proportional to the particle volume V ($\mu = m_s$V) the values of the particle diameter can be derived, assuming spherical shape for the magnetic nanoparticles.

The magnetic characterisation has been completed by Mössbauer spectroscopy. The Mössbauer spectra allowed us to identify the presence different iron phases in the samples, even non-magnetic phases, and also to calculate the percentages of each of them. In the case of arc discharge produced samples taken from the *collaret* and the soot the Mössbauer spectra revealed the presence of non-magnetic γ-Fe in addition to α-Fe and Fe_3C [12].

Because of its biocompatibility, small size, superparamagnetic behaviour and big surface area, possible applications of these nanoparticles in biomedicine are nearly unlimited. At present we have focused in two main fields: drug targeting and conjugation to proteins. In the first case, the magnetic nanoparticles are suspended in water forming an stable bioferrofluid. Subsequently the drug is chemically bound to the carbon coating. This ferrofluid is directed to the target area in the human body by means of an applied magnetic field [13,14]. The magnetic carriers are concentrated there until the drug is released in a desorption process, at the end of which the magnetic field is switched off. This enables a very specific treatment and eliminates the limitations on the applied dose because of possible damage to healthy tissues. On the other hand, our synthesised magnetic nanoparticles have been conjugated to a number of antibodies and have been widely used for the development of lateral flow tests. These tests are available for pregnancy, ovulation, infectious disease, drug monitoring or bacterial contamination. The introduction of magnetic particles allows the immobilization, controlled delivery and release of molecules adsorbed to the surface of these particles by means of an external magnetic field, as well as the quantification of these tests by magnetization measurements. Carbon coated metal nanoparticles represent an alternative to polymeric coatings, and the first results obtained have been really satisfactory.

References

1 Alexiou C., Schmidt A., Klein R., Hulin P., Bergemann C., and Arnold W. (2002) "Magnetic drug targeting: biodistribution and dependency on magnetic field strength", *J. Magn. Magn. Mat.* Vol. 252 (1-3), pp. 363-366

2 Kuznetsov A.A., Filippov V.., Alyautdin R.N., Torshina N.L., Kuznetsov O.A. (2001) "Application of magnetic liposomes for magnetically guided transport of muscle relaxants and anti-cancer photodynamic drugs" *J. Magn. Magn. Mat.* Vol. 225 (1-2), pp. 95-100

3 Bulte J.W.M., de Cuyper M., Despres D. and Frank J.A. (1999) "Preparation, relaxometry, and biokinetics of PEGylated magnetoliposomes as MR contrast agent" *J. Magn. Magn. Mat.* Vol. 194 (1-3), pp. 204-209

4 Pardoe H., Clark P.R., St Pierre T.G., Moroz P., Jones S.K. (2003) "A magnetic resonance imaging based method for measurement of tissue iron concentration in liver arterially embolized with ferrimagnetic particles designed for magnetic hyperthermia treatment of tumors", *Magnetic Resonance Imaging* Vol. 21 (5), pp. 483-488

5 Jordan A., Scholz R., Maier-Hauff K., Johannsen M., Wust P., Nadobny J., Schirra H., Schmidt H., Deger S., Loening S., Lanksch W. and Felix R. (2001) "Presentation of a new magnetic field therapy system for the treatment of human solid tumors with magnetic fluid hyperthermia", *J. Magn. Magn. Mat.* Vol. 225 (1-2), pp. 118-126

6 Gruttner C., Rudershausen S. and Teller J. (2001) "Improved properties of magnetic particles by combination of different polymer materials as particle matrix", *J. Magn. Magn. Mat.* Vol. 225 (1-2), pp. 1-7

7 Pardoe H., Chua-anusorn W., St Pierre T.G. and Dobson J. (2001) "Structural and magnetic properties of nanoscale iron oxide particles synthesized in the presence of dextran or polyvinyl alcohol", *J. Magn. Magn. Mat.* Vol. 225 (1-2), pp. 41-46

8 Ziolo R.R (2002) "Self-stabilized aqueous ferrofluids. Properties and characteristics", *European Cells and Materials* Vol. 3, pp. 92-9

9 Kratschmer W., Lamb L.D., Fostiropoulos K. and Huffman D.R. (1990) " Solid C-60 - A new form of carbon", *Nature* Vol. 347, pp. 354-358

10 Cadek M., Murphy R., McCarthy B., Drury A., Lahr B., Barklie R.C., Panhuis M., Coleman J.N. and Blau W.J. (2002) "Optimisation of the arc-discharge production of multi-walled carbon nanotubes" *Carbon* Vol. 40 (6), pp. 923-928

11 Cullity B.D. (1974) *Introduction to Magnetic Materials*, Addison-Wesley, Reading MA, pff. 94

12 Rechenberg H.R, Coaquira JA.H., Marquina C., Garcia-Landa B., Ibarra M.R., Benito A.M., Maser W., Munoz E., Martinez M.T. (2001) "Mossbauer and magnetic characterisation of carbon-coated small iron particles", *J. Magn. Magn. Mat* Vol. 226, pp. 1930-1932

13 Fernandez Pacheco R., Ibarra M.R., Valdivia J.G., Marquina C., Serrate D., Romero M.S., Gutierrez M. and Arbiol J. "Carbon coated magnetic nanoparticles for local drug delivery using magnetic implants" Proceeding of the NSTI Nanotech 2005 (Anaheim, California) Vol 1 pag 144-147

14 De Teresa J.M., Marquina C., Serrate D., Fernandez-Pacheco R., Morellon L., Algarabel P.A. and Ibarra M.R., "From magnetoelectronics to biomedical applications based on the nanoscale properties of advanced materials" Int. J. Nanotechnology Vol 2 (2005) pp. 3-22