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Synthesis and Characterisation of Functional Magnetic and Plasmonic Nanomaterials

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Contents

1	Introduction	5
1.1	Preface	5
1.2	Phenomenology of magnetic materials	8
1.2.1	Magnetic hysteresis	8
1.2.2	Size dependence of the magnetic behaviour	11
1.2.3	Magnetic Hyperthermia	13
1.2.4	Iron Oxides	17
1.2.5	Potential applications of Fe oxides nanoparticles in biomedicine	20
1.3	Surface Plasmon Resonance	24
1.3.1	Phenomenology of plasmon resonance . .	24
1.3.2	Simple model and resonance conditions .	29
1.3.3	Potential applications of plasmonic nanoparticles	31
1.4	Heterosupramolecular chemistry - Calixarenes on nanoparticles	35
1.4.1	Magnetic NPs	37

1.4.2	Plasmonic NPs	40
2	Experimental techniques	55
2.1	Transmission Electron Microscopy (TEM)	56
2.2	UV – visible spectroscopy	58
2.3	Magnetometry - AGFM	59
2.4	Magnetic Hyperthermia	61
2.5	X-ray photoelectron spectroscopy (XPS)	62
2.6	Dynamic Light Scattering (DLS)	63
3	Controlled Oriented Aggregation for the Synthesis of Novel Magnetic Nanostructures	65
3.1	Introduction	66
3.2	Results and Discussion	68
3.3	Conclusions	83
3.4	Experimental	84
4	Host-Guest Driven Controlled Aggregation of Plasmonic NPs	91
4.1	Introduction	91
4.2	Results and Discussion	93
4.2.1	Synthesis of the thiolate ligands	93
4.2.2	Synthesis and characterisation of Au NPs	94
4.2.3	Synthesis and characterisation of the Ag NPs	103
4.2.4	Aggregation studies	107
4.3	Conclusion	121
4.4	Experimental	122

5	Magnetic Hyperthermia in Au - Fe Oxide Heterodimeric Nanostructures	135
5.1	Introduction	136
5.2	Results and Discussion	138
5.3	Conclusions	150
5.4	Experimental	151
6	Magnetic Characterization and <i>in-vitro</i> Hyperthermia of Au - Fe Oxide Heterodimers	159
6.1	Introduction	160
6.2	Results and Discussion	161
6.2.1	Possible application in nanomedicine . . .	172
6.3	Conclusions	173
6.4	Experimental	175
7	Conclusions	183

Chapter 1

Introduction

1.1 Preface

Research and development of materials at the nanoscale has become one of the most expanding fields in material science in the last decades. Miniaturisation is one of the most stringent problems of modern technology. To have an idea of the great potential advantages deriving from the creation of nanometric objects, it is sufficient to think about electronics and informatics field, where the reduction of the dimensions leads to the construction of smaller and, at the same time, more powerful devices. However, the interest in the construction and properties of nano-objects and nanoparticles-based devices is multidisciplinary, and regards, among the others, physicists, chemists,

engineers and biologists. Materials at the nanoscale, thanks to the high surface / volume ratio, are characterised by properties that are very different from the macroscopic material. In the last years, much attention is given, by the scientific community, to nanocomposite materials. This has been leading to the creation of nanometric multicomponent materials, which allow the combination of the properties of two or more components in a single object. In this frame, high control of the dimension and morphology of the materials at the nanoscale is a key feature for the obtainment of the desired physical properties. Synthesis by chemical methods often fulfil these requirements. Great attention has to be done also to the surface of material. Nanoparticles are often a metastable phase, and nanocrystals usually tend to aggregate into a macroscopic material, to reduce the surface area, which is a defect of a crystal. A surface coating of molecules represents an important task in nanochemistry, because they can protect nanoparticles from aggregation, but may also afford an active functionality. The role of the coating can vary from the simple dispersion in solvents different from the one used for the synthesis to a real active one, which can be chosen and exploited. The combination between the properties of the inorganic core and of the organic shell can represent another step in the creation of nanocomposite materials. This thesis is focused on the preparation and study of magnetic iron oxide and noble metal nanoparticles. Both these classes of materials are characterised by peculiar properties, *e.g.* superparamagnetism or surface plasmon resonance, obtainable only at the nanoscale. To better control the magnetic properties of iron oxide nanoparticles, a new synthetic approach was

developed and studied. In the frame of plasmonic nanoparticles, a particular interest is given to the surface functionalisation of Au and Ag based nanomaterials, to obtain a controlled aggregation and tune the plasmonic properties. Finally, a combination between gold and iron oxide, to yield a nanocomposite material characterised by both plasmonic and magnetic functions, is described. A thorough study of their magnetic properties was performed, together with *in vitro* magnetic hyperthermia tests, to evaluate its capability as hyperthermia mediator.

1.2 Phenomenology of magnetic materials

1.2.1 Magnetic hysteresis

Ferromagnetic materials, from a phenomenological perspective, present a characteristic hysteretic behaviour of the magnetisation, *i.e.* an irreversible non-linear response to an external magnetic field. This behaviour is temperature-dependent, and exists below the Curie temperature, T_C . Above this characteristic temperature, the material becomes a paramagnet, characterised by a linear response to the field. Below the T_C , the action of an increasing external field (H) originates an increasing magnetisation of the material. When this magnetisation process is completed, a saturation value of magnetisation (M_S) is reached, that is the maximum magnetisation value reachable for the material. At the removal of the external field, a residual magnetisation, called *remanence* (M_r), remains. To move back to zero the magnetisation of the system, an opposite magnetic field, called coercive field (H_C), is needed.[1, 2] This hysteretic behaviour of the magnetisation with respect to the applied field, including the first magnetisation curve, is represented in figure 1.1. This particular response to an applied field can be explained considering the different energetic terms that sum up to give the material free energy. These terms are: exchange, magnetic anisotropy, magnetostatic, magneto-elastic and Zeeman energies, each originating from a different kind of microscopic interaction.

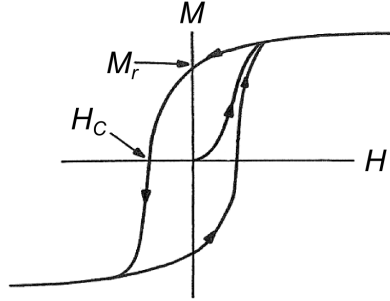


Figure 1.1: A typical M vs H hysteresis loop of ferromagnetic materials

$$E_{tot} = E_{exch} + E_{anis} + E_{magnetost} + E_{magnetoel} + E_Z \quad (1.1)$$

The competition between these terms is at the base of the hysteretic behaviour and of the formation of magnetic domains in ferromagnetic materials.[3] The term that, among the others, has a great influence on the magnetic hysteresis is the magnetic anisotropy energy. Magnetisation, in a ferromagnetic crystal, tends to be preferentially oriented along determined crystallographic axes. This property, called *magnetocrystalline anisotropy*, can be easily explained considering the local symmetry of the chemical environment of the "magnetic" atoms. It is due to the spin-orbit coupling of the electrons in the atoms responsible of magnetism. It depends on the shape of the elec-

tronic clouds of these atoms and on their orientation in the lattice. As a consequence, there is an *easy* axis of magnetisation, along which the saturation magnetisation can be reached applying relatively low fields, and one or more *hard* axes, depending on the geometry of the system. Along these axes, the magnetisation has a lower field dependence, and reaches the saturation value at a higher field, called *anisotropy field*. Following a phenomenological approach, magnetic anisotropy can be expressed as a power sum of trigonometric functions of the angles between the magnetisation and the crystallographic axes. This means that its expression depends on the symmetry of the crystal lattice considered. For a uniaxial system with hexagonal symmetry, the anisotropy energy density is:

$$E_{anis} = K_1 \sin^2 \theta + K_2 \sin^4 \theta + K_3 \sin^6 \theta + K'_3 \sin^6 \theta \cos^6 \phi + \dots \quad (1.2)$$

where θ is the angle between the magnetisation vector and the c-axis, ϕ is the angle identifying the magnetisation projection in the plane perpendicular to the c-axis and K_i coefficients are magnetic anisotropy constants and are temperature dependent.[4] In the presence of cubic symmetry, it is instead generally represented in terms of direction cosines of the magnetisation along the crystallographic axes (α_i):

$$E_{anis} = K_0 + K_1(\alpha_1^2\alpha_2^2 + \alpha_2^2\alpha_3^2 + \alpha_1^2\alpha_3^2) + K_2(\alpha_1^2\alpha_2^2\alpha_3^2) + \dots \quad (1.3)$$

In this phenomenological approach, the anisotropy field can be easily determined by minimise an energy term including

anisotropy and Zeeman. In the simplest case, cylindrical symmetry, *i.e.* no ϕ -dependent anisotropy in formula 1.2, it results to be:

$$H_A = \frac{2K_1}{M_S} \quad (1.4)$$

in the case of an easy-axis crystal. On the other hand, with an easy-plane system, the anisotropy field is:

$$H_A = -2 \frac{(K_1 + 2K_2 + 3K_3)}{M_S} \quad (1.5)$$

The value of this field is of particular relevance for the possible applications of magnetic materials, representing the maximum limit of the coercive field. Besides magnetocrystalline anisotropy, there are other terms contributing to the anisotropy energy, such as *shape anisotropy*, *exchange anisotropy* or *stress anisotropy*. They are not arising from the peculiar characteristic of the material at the atomic scale, but depends on the magnetic, thermal and mechanical processing of the sample.[3]

1.2.2 Size dependence of the magnetic behaviour

The reduction of the dimensions of a magnetic material brings to drastic changes of the magnetic behaviour, and allows the obtainment of materials which can have different applications. Below a critical dimension, the reduced size of the crystal implies an energetic cost to form domain walls bigger than the energetic advantage given by domains. As a consequence, no walls are formed and crystal is characterised by a single domain.[1] In

a hard magnetic material, characterised by a high anisotropic constant, this critical size, often in the nanometric scale, is given by:

$$r_C = 9 \frac{\sqrt{AK}}{\mu_0 M_S^2} \quad (1.6)$$

being K the magnetic anisotropy energy coefficient, A an exchange constant, which depends on the nature of the material and μ_0 the vacuum permeability. In materials with low anisotropic constant, however, also a cost in exchange energy has to be considered, because the magnetisation vector is not everywhere aligned along the easy axis.[4] By further decreasing the size of a magnetic particle, another critical dimension is met, at which the thermal energy of the system ($k_B T$) is comparable with its magnetic anisotropy energy KV , where k_B is the Boltzmann constant and V is the particle volume.[5] Below this size, the anisotropy energy can be overcome simply by the thermal energy of the system. As a consequence, magnetisation is continuously reversed from one direction to another without external field, as depicted in figure 1.2. This condition is defined as *superparamagnetism*. It is a time-dependent phenomenon, because the observation or not of the magnetisation reversal depends on the observation time. For an observation time of 100 s, and a spherical particle, we have a critical radius r_0 of:[6]

$$r_0 = \left(\frac{3}{4\pi} \frac{25k_B T}{K} \right)^{1/3} \quad (1.7)$$

A superparamagnet is characterised by an intense saturation

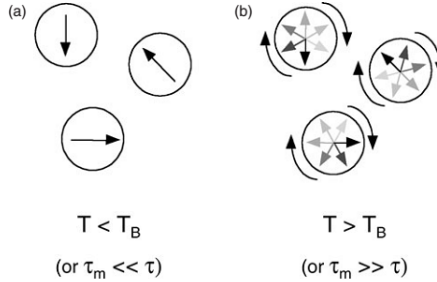


Figure 1.2: Schematic representation of the two possible regimes for superparamagnetic NPs: (a) blocked state, (b) superparamagnetic state. Adapted with permission from ref. [5]

magnetisation in the presence of an external field, which completely disappear at the field removal.

1.2.3 Magnetic Hyperthermia

An interesting feature of magnetic nanoparticles (NPs) is the possibility to heat up when exposed to an alternating magnetic field, with frequencies in the range of radiofrequencies (more specifically in the frequency range 1-1000 kHz). This property, called *magnetic hyperthermia*, can be exploited to induce a localised temperature rising, and it is very promising for biomedical applications, as adjunctive therapy for cancer treatment.[7] It consists in a thermal relaxation of magnetised nanoparticles. Rosensweig developed an analytical study of the power dissi-

pated during the application of an external field on a magnetic fluid. This power dissipation, for single-domain superparamagnetic nanostructures, can be expressed as:[8]

$$P_{SPM} = \pi\mu_0\chi_0 H_0^2 f \frac{2\pi f\tau}{1 + (2\pi f\tau)^2} \quad (1.8)$$

being H_0 and f the applied field strength and frequency, respectively, χ_0 the magnetic susceptibility of the material, assumed to be constant, and τ the relaxation time. This latter is a characteristic value of the material. In a typical magnetic nanoparticles suspension, the magnetic relaxation occurs mainly by two different mechanisms: the Néel relaxation, related to the fluctuations of the magnetic moment in the crystal lattice, and the Brown relaxation, which consists in a physical rotation of the particle in the dispersing medium. Related times are combined to form the total relaxation time by this expression:

$$\frac{1}{\tau} = \frac{1}{\tau_B} + \frac{1}{\tau_N} \quad (1.9)$$

being τ_B and τ_N the Brown and Néel relaxation times, respectively. Néel relaxation time is related to the magnetic anisotropy of the material, that tends to orient the magnetisation in a given direction, and is given by:

$$\tau_N = \tau_0 e^{\frac{KV}{k_B T}} \quad (1.10)$$

where τ_0 is the *attempt time*, a constant related to the nature of the material. On the other hand, Brown relaxation, being

a physical movement, is strictly related to the features of the dispersing medium, and the relative time is:

$$\tau_B = \frac{3\eta V_H}{k_B T} \quad (1.11)$$

where η is the viscosity of the medium and V_H is the hydrodynamic volume of the NP. For very small NPs the Néel relaxation occurs much faster than the Brown one, so it is the main responsible of the heat production. On the other hand, bigger NPs will present a larger magnetic anisotropy and the Brown mechanism become faster and hence dominant. In many systems both mechanisms are present at the same time. Indications about the dependence of the hyperthermia mechanism on crystal size are present in literature, but this phenomenon has to be studied material by material.[7–9] For magnetite, one of the better known magnetic Fe oxides, relaxation times vary with particle size as indicated in figure 1.3.

In the multi-domain ferromagnetic regime, on the other hand, the heat generation finds its origin in the hysteresis losses, which are proportional to the area of the hysteresis loop. The power dissipation, in this case, is given by:

$$P_{FM} = \mu_0 f \oint H dM \quad (1.12)$$

where the integral gives the area of the hysteresis loop. The experimental measurement of magnetic hyperthermia, in a colloidal suspension of magnetic nanoparticles, is carried out by measuring the temperature variation of the dispersing medium

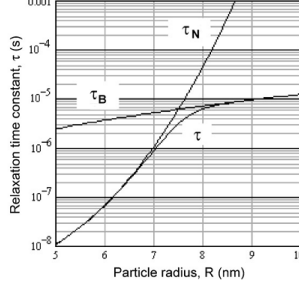


Figure 1.3: Time constants as functions of particle size in magnetite NPs. Adapted with permission from ref.[8]

during the alternate field application, usually in adiabatic conditions. Such temperature increase depends on the hyperthermia properties of the materials, but also on its concentration and on the parameters of the solvent. For these reasons, the Specific Absorption Rate (*SAR*) is widely used. It is expressed in W/g of magnetic material and calculated with this approximate formula:

$$SAR = \frac{c \cdot \rho}{C_{NP}} \cdot \frac{dT}{dt} \quad (1.13)$$

where c and ρ are respectively the specific heat capacity per mass unit and the density of water, $C_{Fe_3O_4}$ is the concentration of magnetite in the sample and slope of the curve dT/dt was calculated by taking into account only the first few seconds of the experiment, when the temperature shows a linear trend in

time.[10] This equation can be applied only with non-interacting particles and considering the mass of nanoparticles negligible if compared with the mass of solvent, approximation perfectly reasonable considering a NPs concentration in the range of mg/mL.

Thanks to these properties, among all the superparamagnetic NPs, nanometric Fe oxides have found great interest in the scientific community for their potential applications in the biomedical diagnostics and therapeutics. Nowadays they represent one of the most studied classes of nanomaterials for biomedical applications.

1.2.4 Iron Oxides

Iron oxides represent the most important and studied class of magnetic nanomaterials for biomedical applications. Among all the iron oxides, magnetite (Fe_3O_4) and maghemite ($\gamma-Fe_2O_3$) are definitely the most attractive, due to their known biocompatibility and the possibility to control nanoparticles size and shape.[11, 12]

Magnetite

Magnetite is characterised by a Fe_3O_4 composition and an inverse spinel crystal structure. The anions packing (cubic close packing, ccp) gives rise to both tetragonal and octahedral interstitial sites, as indicated in figure 1.4. The spatial crystal group is $Fd3m$ and the unit cell dimension is 0.8369 nm.[13, 14] The tetrahedral sites are occupied by Fe^{3+} cations, while

the octahedral ones by both Fe^{2+} and Fe^{3+} . Below the Curie temperature ($T_C = 850$ K) the magnetite is ferrimagnetic. This means that it has populations of atoms with opposing magnetic moments, as in antiferromagnetism; however, in ferrimagnetic materials, the opposing moments are unequal and a spontaneous magnetisation remains. This makes ferrimagnetic materials similar to ferromagnets, from a phenomenological point of view. In magnetite, the coupling between ions in each sublattice is ferromagnetic, while the coupling between ions of one lattice and the other is antiferromagnetic and stronger. At about 125 K, magnetite crystals undergoes a typical phase transition. This transition is called Verwey transition (and the corresponding temperature Verwey temperature T_V) in honour of E. J. W. Verwey, the first who evidenced a change of properties due to this structural change. At room temperature, the cubic structure correspond to the first-order magnetocrystalline anisotropy constant of $K_1 = -1.35 \cdot 10^4 J/m^3$ along the diagonal of the cube. Below the T_V , the structure changing from cubic to triclinic yields a change to uniaxial anisotropy.[15]

Maghemite

Maghemite has a Fe_2O_3 composition, and a crystal structure similar to magnetite. The main difference consists in the absence of Fe^{2+} cations. Referring to the cell depicted in figure 1.4 for magnetite, maghemite is characterised by the presence of only Fe^{3+} ions, which occupies the tetrahedral interstitial sites and a part of the octahedral sites. Vacancies take the place of Fe^{2+} . The space group is the same of magnetite, $Fd3m$ and the cubic

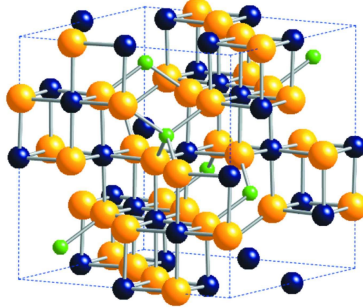


Figure 1.4: Magnetite crystal structure. ccp of oxygen anions (yellow) with iron ions in the tetrahedral sites (green) and in the octahedral sites (blue).

cell parameter was determined as 0.834 nm. Under the Curie temperature $T_C = 863 - 945$ K [3] the material is ferrimagnetic and has an anisotropy constant $K_1 = -4.7 \cdot 10^3 J/m^3$ along the $\langle 110 \rangle$ axis.[14]

Wustite

Worthy of note is also wustite, sometimes present, together with magnetite and maghemite, in magnetic nanoparticles. Wustite is antiferromagnetic below its Néel temperature, the analogous of Curie temperature for antiferromagnetic materials, which is $T_N = 203 - 211$ K. At room temperature, above the T_N , it is paramagnetic. For this reason, if present, it will likely decrease the magnetic properties of the material. FeO structure is not

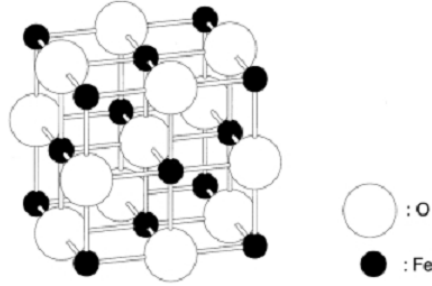


Figure 1.5: Structure of wustite. Adapted with permission from ref.[14]

stable at low pressure and at pressure higher than 10 MPa, but a phase stable deficient cations exists as $Fe_{1-x}O$ (where $x = 0.83 - 0.95$). FeO has a NaCl defective structure, that can be seen as an interpenetrating face center cubic structure of Fe^{2+} and O^{2-} , as shown in figure 1.5. The cell parameter changes as a function of the present vacancies in the range of $= 0.428 - 0.431$ nm.[14]

1.2.5 Potential applications of Fe oxides nanoparticles in biomedicine

Biological samples represent one of the most complicated matrices to be analysed. Isolation of the biological targets from the environment is a basic task before the analysis.[16, 17] Magnetic separation is an easy and non-destructive technique for the ex-

traction of biological analytes such as proteins or peptides.[18] Opportunely functionalised Fe oxide NPs can potentially reach and selectively bond the target. Thanks to the superparamagnetic response to an external magnetic field, the analyte can be extracted from the matrix.[19, 20] Fe oxide NPs can also be used for the preparation of electrochemical biosensors, exploiting the redox activity of magnetite.[21] This function can be coupled with magnetic separation to obtain, with a single material, the preconcentration and electro-analysis of a target analyte.[22]

Magnetic Resonance Imaging (MRI) is actually the most important diagnostic tool available, extensively used for three-dimensional, non-invasive scans of patient's bodies.[23] It utilises magnetic field gradients to obtain images, exploiting the relaxation of protons excited by a radiofrequency in a magnetic field. Biocompatible compounds able to modify the relaxation times are used as contrast agents. In this frame, magnetic iron oxide NPs have been found great interest due to their ability to shorten T_2^* relaxation times.[24, 25]

Site-specific drug delivery can provide a safe and reproducible pharmacological treatment of diseases in defined local positions, and may avoid overdosing and corresponding side effects as much as possible. Drug delivery using magnetic NPs is a constantly expanding field.[26] The possibility to remotely drive magnetic NPs inside an organism by the action of an external magnetic field is known for decades. Drugs loaded onto the NPs surface can be driven by the same principle.[27, 28] Moreover, this method can be useful also for the site-specific release of the

drug. The most used approach to achieve the site-specific drug release is the physiologically triggered release. Some examples are the release of the drug from the vector by a change of pH, in a particular tissue, or a time-controlled release, or also an enzymatic release.[29] This approach, however, is weak and not well controllable, and for this reason the possibility to remotely control the drug release is a fascinating subject in this frame. Magnetic NPs can be exploited to obtain a magnetically triggered drug release.[23]

Another promising field of application of magnetic NPs is thermal cancer therapy.[30] In a temperature range between 42 and 44 °C, cell necrosis occurs. Whole body hyperthermia is an already established treatment to be applied in combination with classical pharmacological therapy.[31, 32] As already explained, Fe oxide NPs are capable to produce heat if exposed to an alternate magnetic field. Magnetic hyperthermia can be exploited to obtain a localised heating inside an organism, avoiding the damages to healthy tissues far from the diseased cell. The heat generated by the magnetic field may also be used for purposes different from the simple thermal therapy. Interesting recent examples were provided by Amstad et al, with a drug release from liposomes achieved by magnetic hyperthermia,[33] and by Kakwere and co-workers with a thermo-responsive coating on Fe oxide nanocubes for a heat triggered release.[34]

Due to all these possibilities of diagnosis and therapy united in the same structure, research on Fe oxide NPs grows fast and many in vitro and in vivo experiments have been published in

the recent years. For a real application in theranostics, also bio-distribution and toxicity of these NPs have been studied.[35]

1.3 Surface Plasmon Resonance

Surface Plasmon Resonance (SPR) is one of the most interesting features of metallic nanoparticles.[36] It consists in a collective oscillation of the conduction electrons of the material, excited by the electromagnetic field of light. This occurs at the interface of the metal with a dielectric in nanostructures like NPs, but also films or nanowires.[37] With respect to these latter kinds of structures, however, the SPR of NPs is localised, because of their 3D structure, which don't allow the oscillation propagation. Historically, the scientific study of SPRs begins in the early 20th century, when Gustav Mie published his theory explaining the optical properties of metal colloids.[38] However, the application of metallic NPs finds its origin much earlier, especially for glass colouring. The Lycurgus Cup (400 AC, British Museum, illustrated in fig 1.6) is probably the most famous example of application of plasmonic NPs in the ancient times. It exhibits different colours when observed upon illumination inside or outside of the cup.[39] There are also many other examples of application of NPs in glass and pottery.[40–42]

1.3.1 Phenomenology of plasmon resonance

To explain easily the phenomenology of the SPR, it is possible to describe the NPs as a ionic lattice in which conduction electron move almost freely inside (the Fermi sea). When the particle is hit by the radiation, the oscillating electric field of the light moves the electrons inside the NP. Considering the charge neutrality, this produces an accumulation of negative and positive



Figure 1.6: the Lycurgus Cup, 400AC, British Museum, London.

charges at the opposite sides of the particle. This generates an electric dipole, which gives rise to an internal electric field opposite to the one of the exciting radiation. This field will force the electrons to return to the initial position. The system is similar to a linear oscillator, in which the restoring force determines a characteristic resonance frequency, in our case called plasmonic frequency. The electronic oscillation implies an increase in kinetic and electrostatic energies, associated with the electric field of the dipole. Since the energy must be conserved, its increase must be provided by the illuminating radiation. This results in a partial extinction of the light at the resonant frequency.

This description is reasonably valid only when applied to NPs characterised by a size much smaller than the incident light wavelength. In this condition, the electric field results homoge-

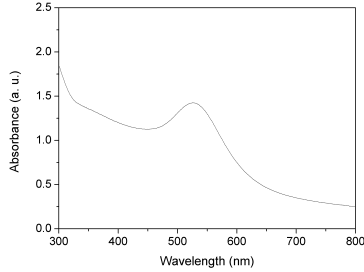


Figure 1.7: UV - visible spectrum of a chloroform suspension of 6 nm Au NPs

neous inside the particle (see figure 1.8). As soon as the particle size increase and become similar to the light wavelength, the field is not homogeneous, and multipoles can be generated at the surface, modifying the resonance conditions. Moreover, a delay between the electric field of the exciting light and the polarisation of the particle can occur, which is a new absorption mechanism.

The resonance of SPs is strongly affected by the particle shape, because the restoring force for SPs is related to the charge accumulated at the particle surface, which is in turn influenced by the geometry.[43] The easiest example of the shape effects are the nanorods,[44] in which the charge accumulation at the NP surface is different for electron oscillations along the rod axis (longitudinal plasmons) or along a perpendicular direction

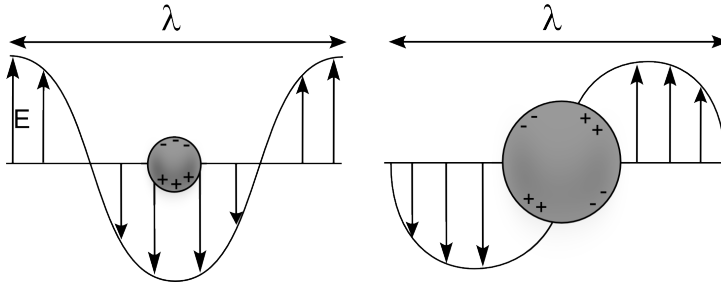


Figure 1.8: Schematic representation of NPs with small (left) and large (right) radii, in comparison with the wavelength of the incident light.

(transversal plasmons), as schematised in figure 1.9. Following the dipole model, the charge accumulation of the generated dipole will be maximum for the transversal oscillations and minimum for electron displacement along the rod axis. The restoring force is proportional to the dipole, and, as a consequence, the resonant frequency will be larger (and thus the resonant wavelength smaller) for the transversal oscillations than for the oscillations along the rod axis. In this case, two plasmonic modes are observed. Other geometries, as triangular prisms, shells or cubes give rise to more complicated effects, but in general deviation from sphericity shifts the resonance towards larger wavelengths.

The excitation of SPRs does not depend only on the characteristic of the plasmonic material. The surrounding medium,

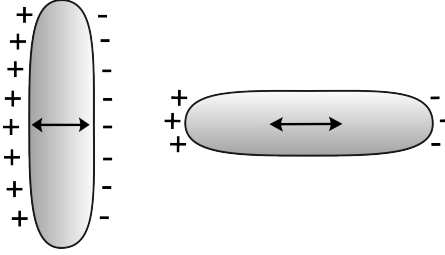


Figure 1.9: Schematic representation of electronic oscillation perpendicularly to the rod axis (on the left) or along it (right) during SPs excitation in nanorods.

which can be the dispersing solvent, a shell of a different material coating the plasmonic "core" in an hybrid, or even a self-assembled monolayer of molecules, may induce significant modifications on the excitation process.[43] There are two main effects on the SP resonance. First, the dielectric function of the surrounding medium determines the light wavelength at the NP vicinity and alters the geometry of the electric field at the surface of NPs. The most important effect, however, is related to the polarisation of the medium during SP excitation. The charge accumulation induces the polarisation of the dielectric medium at the interface between the dielectric and the NP. This polarisation will partially compensate the charge accumulation, and depends on the dielectric function of the media. If the charge at the NP surface decreases, the restoring force is consequently reduced, and this leads to smaller resonant frequencies. Thus,

a surrounding medium with larger dielectric constant will show a larger polarisation when the SPR is excited and will shift the SP resonant band towards larger wavelengths.[43, 45, 46]

Finally, if plasmonic NPs are close enough, excitation of SPs will be modified by the interactions between them. The net field generated in a particle by the SP excitation is strongly influenced by the electric field of NPs in its immediate surroundings. Consequently, there is a change in the resonance conditions. Generally, interparticle interactions induce a damping and red-shift of the plasmonic band. Controlling the aggregation of NPs, it is possible to tune the band shift inducing a controlled interference of the plasmonic electric fields.[47–49]

1.3.2 Simple model and resonance conditions

Plasmonic resonance cannot be considered as a normal electronic process in which light can promote electrons from the ground level to an excited one, because of its outstanding absorbing efficiency. The absorbing efficiency of a particle is given by its absorption cross section. To define it, we have to introduce the concept of the ideal opaque particle, which is defined as a particle that absorbs all photons reaching its surface. The section of an opaque particle that absorbs the same number of photons as our real NP represents its absorption cross section. In addition, light interacting with matter can be scattered, and for this process, it is possible to define the scattering cross section as the geometrical section of an ideal scattering particle (that scatters any photon reaching its surface) with the same

scattering efficiency as the real particle. The sum of absorption and scattering cross sections is defined as the extinction cross section, which represents the efficiency of the particle to remove photons from an incident beam. Following this model, the maximum possible value of the extinction cross-section for perfect opaque particles is the particle section (πR^2). However extinction cross sections larger than the 10% of the geometrical section are scarcely found for processes different from SPR.[50, 51] In noble metal NPs, the extinction cross section can be up to 10 times their geometrical section. This means that NPs are capable of absorbing and scattering photons even away from its physical position.

Following the dipole approximation and the Mie theory, the absorption cross-section corresponds to:

$$\sigma_{ext} = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon_2(\omega)}{(\varepsilon_1(\omega) + 2\varepsilon_m(\omega))^2 + \varepsilon_2^2(\omega)} \quad (1.14)$$

being λ the wavelength and ω the angular frequency of the incident radiation, ε_m the real dielectric function of the medium, and ε_1 and ε_2 respectively the real and imaginary part of the dielectric function of the metal. The Mie resonance condition for a maximum cross-section is obtained when the denominator is null, that is when:

$$(\varepsilon_1 + 2\varepsilon_m)^2 + \varepsilon_2^2 = 0 \quad (1.15)$$

This condition is satisfied when ε_m and ε_1 have opposite sign.

Since ε_m is always positive, ε_1 needs to be negative, condition often fulfilled by noble metals. This is the general condition for the plasmonic resonance using the simplest model available, but it is clear that the surrounding medium has an important effect for the plasmon excitation. To analytically understand the effects of shape of the particle or interactions between them, more complete models have been studied, like the Gans theory or effective medium theory.[44, 52]

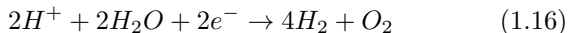
1.3.3 Potential applications of plasmonic nanoparticles

The outstanding plasmonic properties of metallic NPs, joined with their facile preparation and many potential applications, allowed them (especially Au NPs) to find great interest in the scientific community.

One of the most explored applications is the possibility to fabricate plasmonic NPs - based devices for sensing. Being the absorption spectrum of NPs dependent on many conditions, like their size and shape, dispersing medium or aggregation degree, the variations of the plasmonic resonance may be exploited to detect analytes by UV - visible spectroscopy. Many reviews are actually present on this topic.[53–55] Moreover, the electric field generated by the excitation of the SP may promote a strong enhancement of the Raman scattering of the molecules in the proximity of the NPs surface. The so-called SERS effect (Surface Enhancement Raman Scattering) is a powerful analytical tool, very promising in the field of sensing.

The charge accumulation due to the plasmon excitation can be also exploited to catalyse many chemical reactions.[56–58] This is a very attractive application and still a relatively open topic. In fact, plasmonic catalysis can be applied also to degradation of pollutants, very interesting in the field of environment.[56] Two mechanisms have been proposed for plasmonic catalysis. The first is the photo-induced electron transfer from the plasmonic NPs, excited at the SPR, to an unoccupied molecular orbital of a molecule next to the NP surface, which, if excited, results more reactive (see figure 1.10). The other one is the so-called plasmonic heating: the energy accumulated in the NPs during the SP excitation, if electrons are not transferred to other species, is released as thermal energy, due to the thermal relaxation of the excited electrons. This results in a heating of the NPs surface, exploitable to increase the kinetic of the chemical reactions.[59]

Principles of plasmonic catalysis could also be applied to the energy storage and conversion. Many studies have demonstrated the possibility to obtain a photocatalysis, using plasmonic NPs and solar energy, of the following water-splitting reaction:



In the frame of energy harvesting and storage, other applications of plasmonic NPs are possible. The incorporation of metallic NPs in photovoltaic devices may increase the efficiency of the cells, because of the high efficiency of SPs to convert solar energy

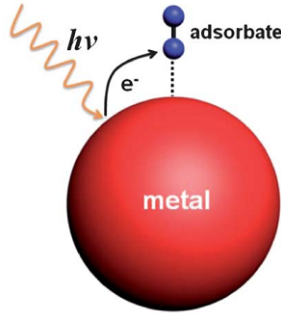


Figure 1.10: schematic representation of the photo-induced electron transfer

to electrons excitation.[60–62] Moreover, the possibility to apply the photothermal effect of plasmonic NPs as a heat source for thermoelectric devices has been recently demonstrated.[60, 63] The relative efficiency of conversion of absorbed and scattered light to thermal energy can be quantified through the so-called photothermal efficiency. Tuning the plasmonic NPs features (by changing material, size or shape), is possible to obtain a material with good photothermal efficiency absorbing visible (solar) light.

In the frame of information technology light manipulation is of extreme important, and is commonly achieved by dielectric materials, which allows good light penetration, but hinder to focus a beam below the diffraction limit. The use of plasmonic

NPs allows new methods of light manipulation, to read and write information in sizes smaller than the light wavelength. SP excitation can be used to design subwavelength optical devices as antennas, lenses and resonators with very low energy consumption and large amplification factors.[64]

The unique possibilities offered by the plasmon resonance make plasmonic NPs one of the most interesting nanomaterials at all also in the biomedical field of research. In regard to diagnostic methods, plasmonic NPs may be particularly useful for the development of biosensors or for imaging.[65] Au NPs were demonstrated to be capable, if opportunely functionalised, to target selectively cancer cells and be detected by optical microscopy. Excitation of SPs can also be exploited to amplify other processes, using other imaging techniques, like photoacoustic imaging or SERS.[66, 67] The photothermal effect described before, for catalytic and energy applications, can be successfully applied also in cancer therapy, exploiting the heat generated by excitation of SPs to kill cancer cells. In this frame, the limit is constituted by the fact that the human body is impermeable by visible light. This can be overcome by the construction of plasmonic NPs whose plasmon resonance can be excited by infrared light, usually anisotropic NPs like Au nanorods or nanostars.[60, 68] Finally, the heat generation is potentially useful for a triggered drug release, making plasmonic NPs a great material for drug delivery.[69] All these features allow plasmonic NPs to be a powerful and versatile tool in both diagnostic and therapeutic field of nanomedicine.

1.4 Heterosupramolecular chemistry - Calixarenes on nanoparticles

Ligand-stabilised nanoparticles (NPs) have been representing one of the most promising functional materials for the last decades. [70–76] These NPs are typically constituted by an inorganic core either of metals, metal oxides or semiconductors with a size range from 1 to *ca.* 100 nm, stabilised by a shell of organic molecules arranged like a monolayer around the core. This monolayer gives to the inorganic bulk a steric protection, very effective in preventing aggregation phenomena, making these nano-objects stable in several conditions and for relatively long time. But this is not the only feature provided by the self-assembled monolayer, because organic ligands often have their own physical and chemical functionality. The nature of the monolayer determines their colloidal properties in solution and, when coupled to the unique nanoscale-properties of the NPs, enable the manufacturing of nano-devices with potential applications in many applied fields such as optics, electronics, energy, environment, biology and medicine. [70–72, 77–79]

Functional molecules can be inserted on the surface of the NPs during the synthesis, as, for example, using the facile biphasic method developed by Brust and Schiffrin for the preparation of functionalised Au NPs. [80–82] But the most common strategy used to modify the NPs' organic shell is the displacement of one ligand for another, after the NPs formation. These so-called “ligand-exchange” methods are particularly useful if

the ligand to be inserted on the core is not compatible with the NPs synthetic conditions, or if the desired ligand is particularly valuable, or simply not commercially available. Moreover, this approach allow the possibility to insert ligands with different functionalities on the same inorganic surface.[83] Mixed-monolayer systems greatly enhance the versatility of these nanomaterials, allowing the construction of even more complicated and promising devices. In the frame of supramolecular chemistry, in the last two decades, NPs functionalised with receptor-like ligands have been largely employed either to study the supramolecular aspect of the monolayers and as new building blocks for self-assembly processes.[84–86] In this field, defined by Fitzmaurice as *hetero-supramolecular chemistry*,[85] calixarene derivatives are assuming an increasing role as functional protecting layers of NPs.

Calixarenes are a class of synthetic macrocyclic compounds derived from the condensation of phenol and formaldehyde, that owe their name to the characteristic three-dimensional ‘cup’ shape that they assume in one of their conformations.[87] They are characterised by a π -rich aromatic cavity that can be exploited, if properly functionalised and rigidified, for self-assembly processes through recognition of neutral and charged species.[88, 89] These compounds have found extensive use as active coatings of noble metals surfaces and NPs.[90, 91] In this section, an introduction on the potential of a hybrid material obtained by the combination of calixarenes and magnetic or plasmonic nanoparticles will be presented.

1.4.1 Magnetic NPs

A hybrid nanosystem based on a magnetic inorganic core opens to a series of possible applications, thanks to the possible interaction between the hybrid NPs and an external magnetic field. It would allow the construction of hybrid material with many potential applications, like sensing, heterogeneous catalysis, water purification and, in the biomedical field, drug delivery, magnetic resonance imaging and magnetic hyperthermia.[21, 92–95] In this frames, calixarenes are a very promising class of molecules to be inserted in the organic monolayer.

For example, water purification is an interesting potential application of calixarene-functionalised magnetic NPs. The idea is to exploit the calixarene cavity anchored on the NPs' surface as a receptor for pollutants, and an external magnetic field to remove the system from the purified solution (see scheme 1.11). The possibility to use a system based on calix[4]arene-functionalised magnetite NPs to remove dichromate ions from water has recently been demonstrated.[96] Magnetic NPs were coated by a 3-aminopropyltrimethoxy silane (APTMS) shell, and the amino groups on the surface of NPs were used to bind a calix[4]arene derivative bringing two ester functionalities at its lower rim. The binding efficiency of the system was tested in aqueous solutions, and it was found very efficient in extracting sodium dichromate at pH between 2.5 and 4.5, much more than the simple APTMS-coated NPs, which did not show remarkable extraction. The system was also found to be recovered (85–95%) after saturation, using a HCl solution. Following this route, in

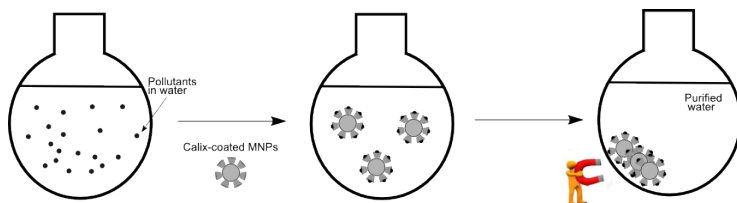


Figure 1.11: Scheme of the water purification by calixarene-coated magnetic NPs.

the subsequent years, many other similar systems for water purification from pollutants like chromate, arsenate and uranyl anions were constructed.[97–99] Another interesting example of this application was provided by Urban et al, who used magnetoferritin as magnetic core, functionalised with a calixarene-crown-6 derivative for the extraction of radioactive Caesium ion $^{137}\text{Cs}(I)$ from water.[100] Magnetoferritin is a particular variety of the Fe storage protein ferritin; it is easily dispersed in aqueous media and its magnetic response to an external field is sufficient to make it a good alternative to the classical synthetic magnetic NPs for this kind of applications.

In the frame of heterogeneous catalysis, the advantage to use a catalyst included in the organic shell of a magnetic NP-based hybrid material is represented by the possibility of an easy recovering of the material with an external magnet.[95] An interesting example is an organocatalyst for aldol reaction based on L-proline-functionalised calix[4]arene ([101], see scheme in



Figure 1.12: Molecular structures of calixarene derivatives **1** (ref [101]) and **2** (ref [102])

figure 1.12) grafted onto the surface of NPs of magnetite. Functional calixarene was anchored on the surface of [3-(2,3-epoxypropoxy)-propyl]-trimethoxysilane-modified - Fe_3O_4 nanoparticles in the presence of sodium hydride. As a model reaction to test the catalytic activity, the aldol reaction between cyclohexanone and 4-nitrobenzaldehyde was chosen. The system show good catalytic activity, diastereoselectivity and enantioselectivity, quite similar to the free L-proline-calix[4]arene derivative, and was also tested on a variety of substituted benzaldehydes. This catalyst was found to be magnetically recyclable up to five times without significant loss of activity.

Finally, an interesting example on the use of calixarene-coated magnetic NPs is the construction of bifunctional magnetic-optical nanomaterials. Khan et al used the calixarene cavities forming the monolayer on the surface of magnetite NPs as receptors to incorporate rare earth cations, in particular Tb^{3+} and Eu^{3+} . [102] Calix[4]arene derivative (depicted in figure 1.12), is anchored to the NPs' surface by the phenolic functionalities at the lower rim, and carboxymethyl groups present at the upper rim allow the formation of chelated complexes with the rare earth cations. A detailed description of magnetic and photoluminescent properties is present in the paper. All of the nanoma-

terials are superparamagnetic at room temperature and exhibit red (Eu^{3+}) and green (Tb^{3+}) photoluminescence emission.

1.4.2 Plasmonic NPs

The great potential of plasmonic NPs in many fields of the research has been introduced in the previous chapter. Opportunely functionalised Au NPs can found many application, like sensing, catalysis, energy or, in the biomedical field, imaging, drug delivery photothermal therapy.[58, 60, 103]

As already introduced, the coupling of the plasmon resonances strongly modify the shape and the wavelength of the absorption band.[104] Hence, the plasmon resonance of NPs results strongly dependent on the distance between NPs. For this reason, calixarene-coated plasmonic NPs can potentially be used for the construction of colorimetric sensors. A ditopic guest, selectively recognised by the calixarene cavity, may induce the aggregation of NPs with consequent colour change of the solution. For example, a calix[4]arene-coated Au NPs based system can be used as colorimetric sensor for lysine, arginine and histidine.[105] p-sulfonato-calix[4]arene (pSO3-CX4) bringing thiol functions at the lower rim (see figure 1.13) was anchored to previously prepared Au NPs. Aminoacids with an additional amine function, like lysine, histidine and arginine, are capable to act as guests for two calix[4]arene cavities at the same time. This results in aggregation of the NPs clearly visible by a colour change of the suspension, which passes from red to purple. The authors evidenced the aggregation also by UV-visible titrations:

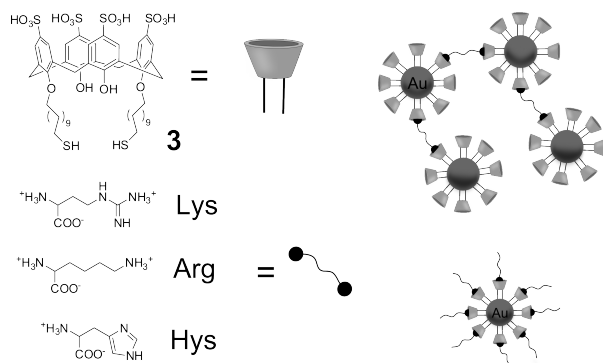


Figure 1.13: molecular structures of calixarene **3** and basic amino acids (left), from ref [105]; schematic aggregation of nanoparticles (right up); **3**-coated NPs interacting with amino acids bearing only one aminic function.

the addition of increasing amounts of the aminoacid solution to a suspension of the functionalised Au NPs generates a wavelength red shift (from 524 nm to 550 nm) and broadening of the surface plasmon band. Such approach can be potentially followed, with the opportune calixarene receptor, for the sensing of many diamine compounds, as evidenced by Chen and co-workers in 2015 using calix[4]arene crown ether-coated AuNPs.[106] Other colorimetric sensors based on such approach can be found in the literature.[107]

Applications can also be found in sensing by SERS effect.

An interesting example is a system based on a self-assembled monolayer of a dithiocarbamate calix[4]arene derivative (figure 1.14) on Ag NPs for pyrene sensing.[108] Pyrene is recognised by the calixarene and identified by its characteristic Raman bands. The efficiency of the system grows as the concentration of calixarene is increased, because of the higher number of host cavities available for the pyrene complexation. Thanks to the versatility of the technique, this calixarene-coated NPs can be used to detect, by Raman spectroscopy, other polycyclic aromatic pollutants (PAHs), like benzo[c]phenanthrene, triphenylene, and coronene, recognised by the cavity of the macrocycle.[109] In this type of sensing, selectivity is not as important as in other sensors, because the complexed compound can, in principle, be identified by Raman spectroscopy. The affinity constants and the limits of detection of PAHs were determined.

The high affinity of calix[4]arene-coated Au NPs for alkyl pyridinium salts in low polar media can also be exploited to obtain a guest-induced controlled aggregation of calixarene-functionalised AuNPs.[47] The ability of the calix[4]arene-coated NPs to give rise to a superlattice of NPs through the complexation of bifunctional guests was evaluated in chloroform solution. Guests with dipyridinium diiodides units connected by alkyl chain of different length were employed, in particular two guests, with alkyl chains of 10 and 6 carbon atoms (figure 1.14, up right) were compared. Using the shorter difunctional guest a more effective aggregation was obtained, with the formation of a larger 3D network, than with the longer one. This is probably due to the possibility, for the longer guest, to be complexed by calixarene cavities present on the same particle. Viologen ditosylate (fig-

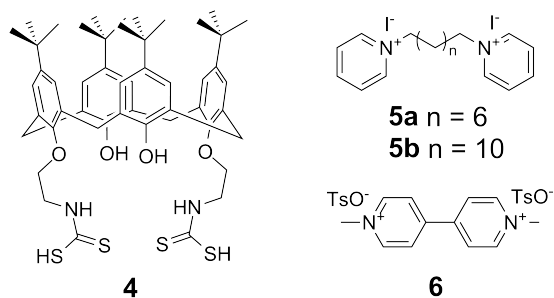


Figure 1.14: molecular structures of calixarene derivative **4** from ref [108] (left) and alkyl pyridinium iodide derivatives **5** and viologen ditosylate **6** used in ref [47] (right).

ure 1.14, down right) difunctional guest was found to lead to an irreversible aggregation and precipitation of NPs.

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Chapter 2

Experimental techniques

In this thesis, the synthesis and functionalisation of nanoparticles was always carried out by wet chemical methods. For Fe oxide-based nanosystems, the methods employed usually consist in thermal decomposition of an iron-organometallic precursor. For the syntheses of gold and silver nanoparticles, all processes, different from one specific material to the others, are specified and discussed in each chapter.

A simplified explanation of the characterisation methods mainly used during this thesis is herein presented, with a particular emphasis to the instruments used and the physical principles at their bases. More details about the preparation of samples and

the resulting data are present on the Results and Experimental sections of each chapter.

2.1 Transmission Electron Microscopy (TEM)

Transmission electron microscopy is the most important characterisation technique for nanomaterials. It allows, first of all, the characterisation of the morphology of the product, essential for the development of a synthetic method. It is also used to gather information about structure and composition of nanostructures. An electron beam is the investigation probe and its wavelength depends on electron acceleration. When the electron beam hits a sample, many different interactions are possible, either regarding electrons reflexion or transmission. A typical TEM apparatus is schematised in figure 2.1. An electron gun generates the electron beam, whose divergence and collimation is set by two or three condenser lenses. The beam reaches the sample holder, which is usually able to rotate, and the sample must be thin enough to permit the transmission of electrons downward to the objective, intermediate and projector lenses, that create the image.

The typical TEM micrograph is generated by the transmitted electrons. However, we are interested also in elastic scattering processes: small angle elastic electrons give information about the structure, while the high angle elastic electrons are

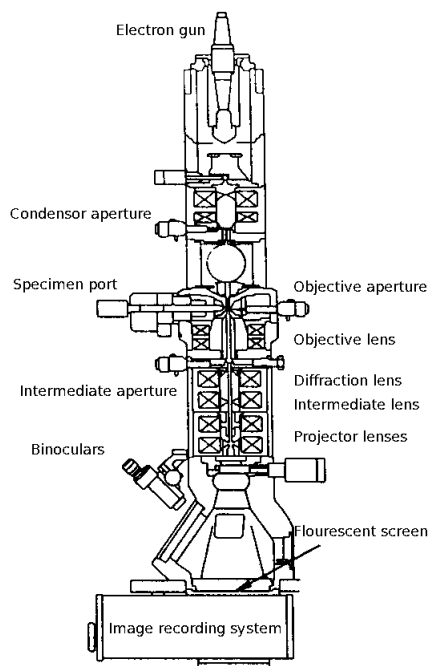


Figure 2.1: Schematic representation of a transmission electron microscope.

proportional to the atomic mass Z and give information about the composition. Also inelastic electrons are very important because give complementary information. The characteristic X-rays are produced from the decay of the excited electrons and are used by the X-ray energy-dispersive spectroscopy (XEDS or EDS). The High Resolution TEM (HRTEM) images can be obtained by means of phase contrast and in particular one transmitted beam with several diffracted beams are analysed. HRTEM is a very sensitive technique and needs a high performance microscope to have good imaging. High stability of the column and low spherical aberration are essential requirements.

2.2 UV – visible spectroscopy

One of the most important characterisation methods for plasmonic nanoparticles is the UV - visible spectroscopy. The UV - visible absorption spectrum depends on and gives information about the plasmon resonance. Basically, as illustrated in figure 2.2, a lamp generates light beam. Usually tungsten lamps are employed for visible light and deuterium lamps for light in the UV range. The monochromator mechanically selects narrow bands of the desired wavelength. After the sample-holder, a system detects the intensity of the light transmitted. Absorbance (A) is given by:

$$A = \log \frac{I_0}{I} \quad (2.1)$$

where I_0 and I are the intensities of the incident and transmit-

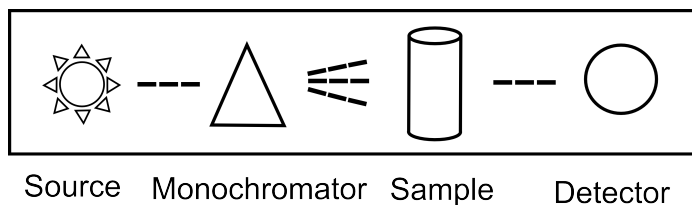


Figure 2.2: Block diagram of a classical single beam spectrophotometer.

ted light, respectively. The monochromator can move along all the spectrum covered by the lamp, and, in this way, this technique allows to determine the absorbance at each wavelength, registering the so-called absorption spectrum.

2.3 Magnetometry - AGFM

The alternating gradient force magnetometer (AGFM) is a very sensitive magnetometer, based on a force measurement. The measurements are carried out on solid samples. In our case, the sample is obtained drying a colloidal suspension of NPs on the surface of a suitable substrate, usually Si, and placed on an opportune probe. This probe has the sample holder at the end of a fibre, and is connected to a piezoelectric element. During the measurement, it is subjected to a fixed dc (direct current) field plus an alternating field gradient, produced by an appropriate coil pair, as indicated in figure 2.3. The magnetic field, up of 2 T, is applied by means of an electromagnet and measured by

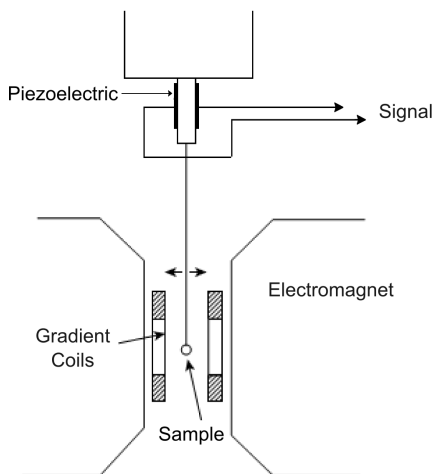


Figure 2.3: Schematic representation of the main components of an AGFM.

a Hall probe. The sample undergoes a magnetic force, which causes a deflection of the probe, detected by the piezo. In this way an electric signal is generated, proportional to the magnetic answer of the sample. To have a higher signal-to-noise ratio, a lock-in amplifier is used and the field frequency is fixed near the resonance frequency of the system. Through this kind of measurements, the magnetic behaviour, *i.e.* the presence of an hysteresis loop and the saturation magnetisation, can be characterised.

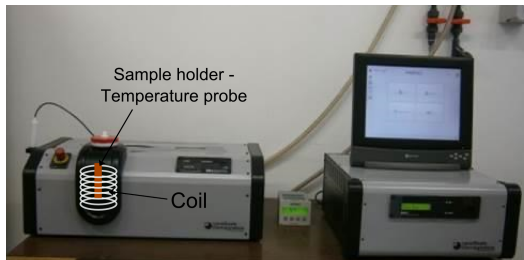


Figure 2.4: DM1 applicator for magnetic hyperthermia in colloidal suspension of nanoparticles (left). Coil and sample holder are evidenced; on the right, the controller (nanoScale Biomagnetics).

2.4 Magnetic Hyperthermia

A magnetic hyperthermia measurement is a measurement of temperature in a solution, during the application of an alternate magnetic field (in the range of radiofrequencies). The field is applied by means of a coil placed around a Dewar flask, inside which the sample is placed. This cryostat allows an adiabatic measurement, is made of glass and has an internal sleeve in which a vacuum of at least 10^{-6} mbar is applied. The temperature probe is a fibre optical thermometer without metallic parts, to avoid the coupling of signal with the radiofrequency.

All measurements reported in this thesis are carried out by the instrument in figure 2.4, nanoScale Biomagnetics DM100-series. Application of the alternate field to biological samples is performed with the different applicator indicated in figure

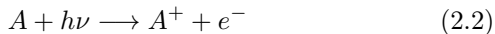


Figure 2.5: DM2 applicator for magnetic hyperthermia (nanoScale Biomagnetics).

2.5. It is similar to the former, but it is equipped with a much larger sample holder, capable to contain biological samples. This sample holder is not adiabatic, so no accurate measurements of solution heating are possible.

2.5 X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy is a very useful technique for the investigation of the nanoparticles surface coating, based on the photoelectric effect, *i.e.* the emission of electrons by a substance hit by an electromagnetic radiation. In general, with an A atom, we have:



Due to the conservation of energy, energetic terms must be equal

before and after the process.

$$E(A) + h\nu = E(A^+) + E_k(e^-) \quad (2.3)$$

$$h\nu - E_k(e^-) = [E(A^+) - E(A)] = E_b \quad (2.4)$$

$E_k(e^-)$ is the kinetic energy of the photoemitted electron and E_b is the binding energy, defined as the difference between the energetic states of A^+ and A . When the incident radiation is in the range of X-Ray, this effect regards the electrons in the inner shells of the atoms, and can be exploited to obtain information about the identity of atoms involved and their oxidative state. Photoemitted electrons, in a typical XPS apparatus, are directed to an analyser, schematised in figure 2.6. Here, by passing through a predetermined pathway, obtained with opportunely placed magnetic fields, are selected and revealed by their kinetic energy. The difference between the energy of the radiation $h\nu$ and this kinetic energy is the binding energy (BE).

2.6 Dynamic Light Scattering (DLS)

DLS is a very useful technique to have information about the colloidal properties of nanoparticles. DLS is used in many different fields from chemistry, to physics, to biology. The sample must be a much diluted colloidal dispersion of NPs, which are considered as non-interacting and subjected only on Brownian motion. The measurement is performed by measuring the rate at which

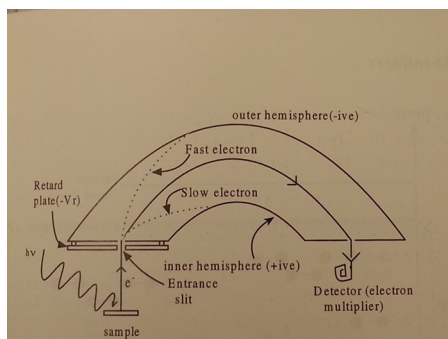


Figure 2.6: Schematic representation of the pathway followed by the photoemitted electrons in the analyser of the XPS instrument.

the intensity of the scattered light fluctuates. The autocorrelation function, given by the intensity at two different times, is measured and depends on the size distribution of nanoparticles. Small particles cause the intensity to fluctuate more rapidly than the large ones so there is a dependence of signal correlation on particles size. A typical dynamic light scattering apparatus is made up of a laser, providing a light source to illuminate the sample contained in a cell, an attenuator used to reduce the intensity of the laser source and hence reduce the intensity of scattering, and a detector, used to measure the scattered light that can be positioned at typically 173 or 90 degree.

Chapter 3

Controlled Oriented Aggregation for the Synthesis of Novel Magnetic Nanostructures

3.1 Introduction

Growth via particle attachment is an already known pathway for nanocrystals growth.[1] However, several fundamental aspects of

this growth mechanism are still not fully recognised and matter of discussion by the scientific community. The classical crystal growth by monomer addition is mainly driven by two energetic terms: the stabilizing free-energy of the ‘bulk’ of the crystal and the energetic cost of the crystal surface, at the interface between solid and liquid phase.[2, 3] This usually results in a free-energy landscape, as a function of crystal size, characterised by a maximum (the free-energy barrier to nucleation), followed by a progressive decrease of free-energy by increasing the crystal size, with a trend depending on the material.[4–6] However, in some cases, the free-energy landscape exhibits minima, and several growth pathways are possible, giving rise to morphologies that can be eventually very different from the thermodynamically stable final phase. Some of these possible growth pathways involves particle oriented aggregation. In general, all these types of mechanism are very difficult to observe, and there are very few systems in which this mechanism has been unequivocally demonstrated.[1]

The control over the dimension and morphology of Fe oxides NPs is essential for the optimisation of their magnetic and hyperthermia properties, as already introduced.[7, 8] In the synthesis of Fe oxides NPs by thermal decomposition of an iron precursor, the growth occurs by monomer addition, and the clusters dimension is controlled by the presence of surfactants, usually oleic acid and oleylamine.[9] Our aim was to apply an oriented aggregation-based approach on the synthesis of Fe oxides NPs, to obtain new morphologies in a simple and controllable way. We based our new approach on the hypothesis that, during the

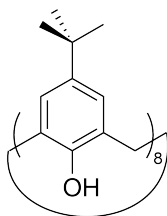


Figure 3.1: Molecular structure of *p-tert*-butyl calix[8]arene.

synthesis, the presence on the NPs' surface of an organic monolayer less packed than the oleic acid makes the growing surface more accessible and more reactive toward aggregation. As organic monolayer we used a synthetic macrocycle: the *p-tert*-butyl calix[8]arene, that is stable at the temperatures reached during the NPs synthesis and it has been discovered it can make chelate complexes with Fe(III).[10] Moreover, calix[4]arene and calix[6]arene derivatives were already found capable to functionalise iron oxides surfaces by the hydroxyl groups present on their lower rim [11, 12] and, as already introduced, may provide interesting functionalities.

3.2 Results and Discussion

Iron oxides nanostructures are obtained by a thermal decomposition of iron(III) acetylacetonate ($\text{Fe}(\text{acac})_3$) with oleic acid (OLAC) in benzyl ether in the presence of the *p-tert*-butyl calix[8]arene (CX8). Accordingly to a well-known procedure,

developed by our research group modifying the method by Park et al.,[13] synthesis involves an initial 2 hours nucleation step, induced by heating the solution at 200°C, is followed by a crystal-growth step promoted by refluxing the reaction mixture for one hour. Using a CX8 : Fe(acac)₃ : OLAC molar ratio of 0.5 : 1 : 2, it is possible to obtain NPs characterised by a multi-core morphology (MC-NPs), as evidenced by the TEM micrograph illustrated in figure 3.2b and STEM-HAADF (scanning transmission electron microscopy – high angle annular dark field) image in figure 3.3 (top left panel). Statistical analysis, carried out using Image J program,[14] indicates that the MC structures are characterised by a mean size of 40 nm (see figure 3.2b). Small aggregated NPs constituting the MCs were roughly estimated of 7 nm. Increasing the CX8 : Fe(acac)₃ ratio to 1 : 1, the morphology of the nanostructures changes substantially (figure 3.2c and 3.3, bottom left): NPs with a nearly octahedral shape (Oct-NPs) are obtained, with a mean size of 50 nm (diagonal). On the other hand, the synthesis carried out in the same conditions but without CX8 affords quasi-spherical single-core nanoparticles (SC-NPs), already well known in the literature,[13] with a mean size of 6 nm (see figure 3.2a). The evolution of the NPs morphology as a function of the CX8:Fe ratio is depicted in figure 3.4.

High Resolution Transmission Electron Microscopy (see figure 3.3) confirms that the MC-NPs are the result of the oriented aggregation [15] of several small NPs. Indeed, since adjacent small NPs of the aggregate share an identical crystallographic orientation, the MC-NPs exhibit an almost single crystalline

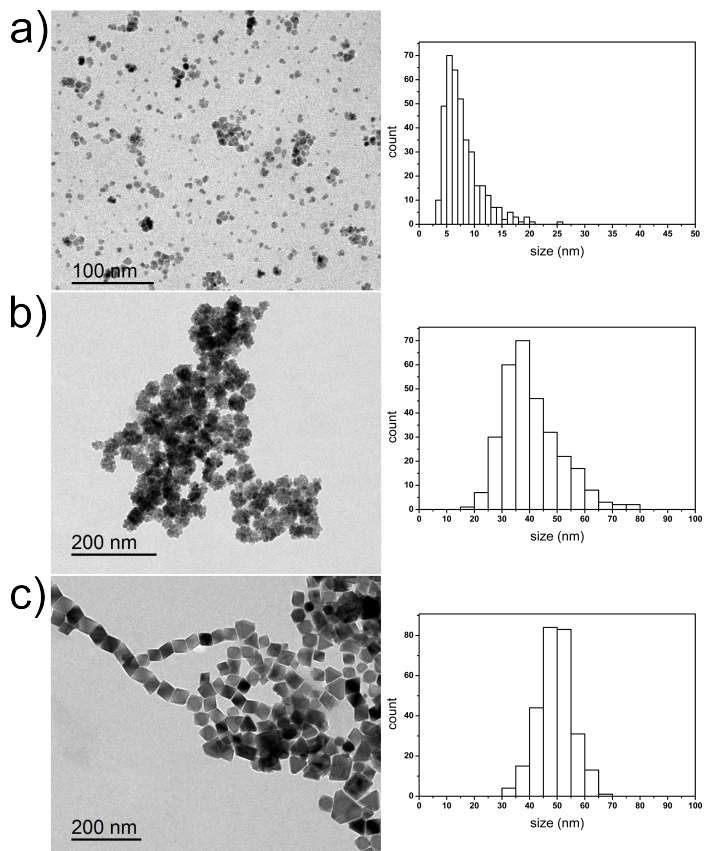


Figure 3.2: TEM micrographs and statistical analysis of a) single-core 6 nm NPs, b) multi-core and c) octahedral NPs.

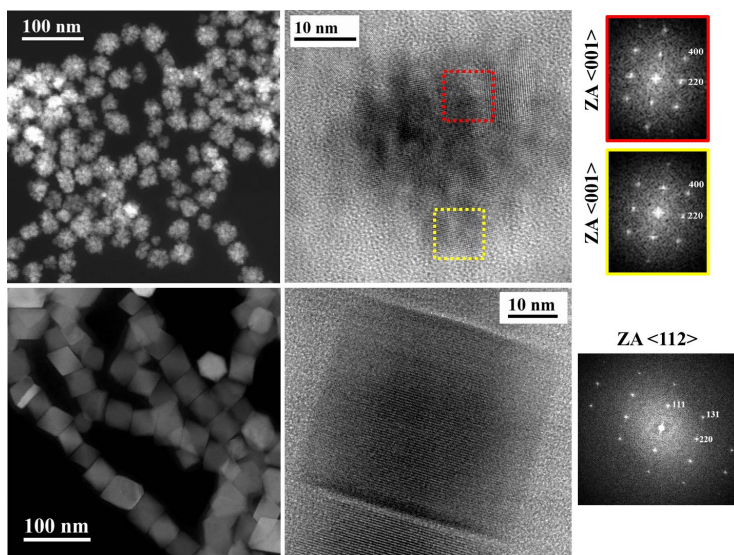


Figure 3.3: STEM-HAADF (left panel) and HRTEM (right panel) images, with the corresponding fast Fourier Transform in the inset, of MC (top line) and octahedral (bottom line) NPs.

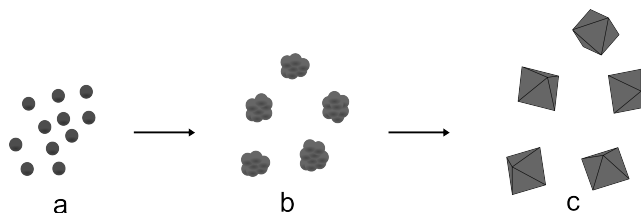


Figure 3.4: schematic representation of the evolution of the morphology of the NPs using a CX8 : Fe(acac)₃ ratio of: a) 0; b) 0.5; c) 1

diffraction pattern. As an example, the top right panel of figure 3.3 shows a representative HRTEM image of a single MC-NP, which is built up of *sub-particles* (as those in the red and yellow squares) with the same fast Fourier Transform (FFT in the red and yellow insets, corresponding to Fe₃O₄ imaged along zone axis $\langle 001 \rangle$). Increasing the CX8 : Fe ratio to 1 : 1, the MC structure is no longer observed and octahedral NPs are obtained. Figure 3.3, bottom reports a representative STEM-HAADF image of a NP ensemble (on the left) and a detail of a single Oct-NP, with the corresponding FFT showing the single crystalline structure of the Fe₃O₄ imaged along zone axis $\langle 112 \rangle$.

IR characterisation was performed on 6 nm SC-NPs, MC- and Oct-NPs. The corresponding IR spectra, together with the spectrum of CX8, reported as reference, are gathered in figure 3.5. The spectra of the three types of NPs are very similar, showing the characteristic band relative to the Fe-O bonds in magnetite at 581 cm⁻¹, and bands reasonably ascribable to oleic

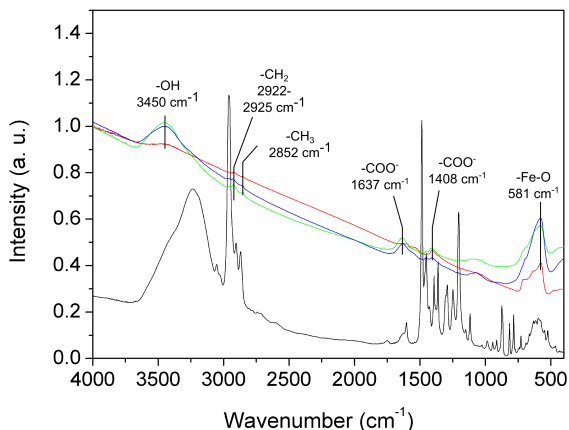


Figure 3.5: IR spectra of: CX8 (black line); single-core NPs (green line); multi-core NPs (blue line); octahedral NPs (red line).

acid, like the $\nu(\text{COO}^-)$ at 1637 and 1408 cm^{-1} , the single bond $\nu(\text{C-O})$ at 1070 cm^{-1} , $\nu(\text{CH}_2)$ and $\nu(\text{CH}_3)$ at 2922-2925 cm^{-1} and 2852 cm^{-1} . The broad band at 3450 cm^{-1} indicate the presence of hydroxyl groups in all the NPs samples. From all these considerations, it is reasonable to hypothesise that there is no calixarene on the NPs surface, which result totally coated by OLAC.

The absence of CX8 on the surface of NPs supports the hy-

pothesis that its role is mainly during the synthesis. The size of SC-NPs, prepared with a classical thermal decomposition of $\text{Fe}(\text{acac})_3$ with OLAC, is centred at 6 nm. The fact that similar sizes (the same, if we consider the experimental error) were found in the aggregated NPs constituting the "cores" of the MC structures, leads us to speculate that the calixarene derivative affects a synthetic step immediately after the nucleation and growth till 7 nm. It probably activates some crystal faces of the just-formed NPs towards the aggregation, while the OLAC, on the other side, maintains the size controlled to the final dimension of 7 nm. It is useful to note that calixarene derivatives are known to be capable to functionalise iron oxides NPs simply by their phenolic hydroxyl groups.[11, 12] This competition between OLAC and CX8 is crucial for the aggregation degree reached in the final product (see scheme in figure 3.4). Another possible reaction pathway, likely to occur together with the previous one, is the capture of $\text{Fe}(\text{III})$ ions from the reaction environment by the calix[8]arene, providing a new starting substrate for the nucleation of Fe oxides NPs already activated at the surface. The possibility for calix[8]arenes to form complexes with $\text{Fe}(\text{III})$ was already demonstrated.[10]

To gain a better control over the aggregation mechanism, two other experiments were devised, both keeping constant the CX8 : $\text{Fe}(\text{acac})_3$ ratio of 0.5. The first was carried out increasing the time of the refluxing step (2 hours instead of 1), to verify if the aggregation of NPs towards the octahedral morphology can take place, more slowly, with less calixarene. TEM micrograph and relative statistical analysis of the product are shown in figure

3.6b. The other experiment was performed increasing the total concentration of the system, in particular with concentration of $\text{Fe}(\text{acac})_3$ of 0.02 M instead of 0.01 M and a refluxing time of one hour, because reasonably with a higher concentration the oriented aggregation process is favoured (see figure 3.6a). Both reactions led to defected NPs, with a quasi-octahedral shape. In this way we have reasonably verified our hypothesis, and we have found that the reaction can be driven also changing other variables, like time and reactants total concentration, as well as the CX8 : $\text{Fe}(\text{acac})_3$ ratio. All these data are in agreement with the oriented aggregation mechanism.

Nanoparticles were then functionalised with a particular ligand, 2,3-dimercaptosuccinic acid (DMSA) by a ligand exchange reaction, following a well-known procedure.[16] DMSA allow the dispersion of nanoparticles in water, thanks to its double carboxylic function. Purification by ultracentrifugation and dialysis allow the removal all reaction sub-products. (see Experimental for details) Colloidal properties (hydrodynamic size and zeta potential) of water dispersed NPs were characterised by DLS measurements. The size distribution, the ξ -average diameter (ξ -ave) and the polydispersity index (PDI) were obtained from the autocorrelation function using the “general purpose” mode for magnetite in water (refractive indexes of respectively 2.42 and 1.33). All NPs, kept in water suspension by the DMSA coating, are prevented from aggregation by the electrostatic repulsion of the deprotonated carboxylate groups. Accordingly, zeta potential values obtained are always negative, and are shown in table 3.1, together with the ξ -ave and PDI values. Measurement

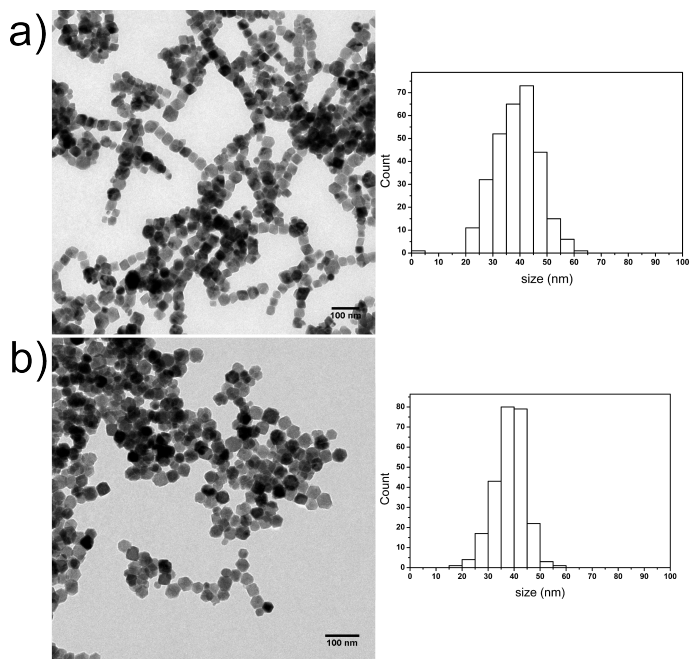


Figure 3.6: TEM micrographs and statistical analysis of octahedral NPs obtained increasing a) the total reagents concentration (0.02 M of CX8 and $\text{Fe}(\text{acac})_3$) and b) the reaction time at reflux (2h).

	ξ -potential (mV)	Intensity Mean (nm)	Number Mean (nm)	Polidispersity Index (PDI)
SC-NPs	-31	57 / 4375	25.7	0.206
MC-NPs	-63	134 / 4810	32.9	0.331
Oct-NPs	-49	70.7 / 432 / 5122	52.8	0.476

Table 3.1: Colloidal properties of SC, MC and Oct-NPs measured by DLS.

problems occur with the Oct-NPs sample, whose intensity size distribution indicates clearly a multimodal size distribution, although in the number distribution profile it is easy to note that the great majority of the NPs in the sample has a diameter of 52 nm.

Magnetisation curves as a function of field, shown in figure 3.7, indicate the ferrimagnetic behaviour for MC and Oct-NPs, with coercivity at room temperature respectively of 0.0022 and 0.0123 T, and saturation magnetisation M_S value of 70 Am²/kg of magnetite. SC-NPs show a superparamagnetic behaviour with a M_S of 55 Am²/kg, significantly lower than the other samples. In the zero-field cooling / field cooling (ZFC/FC) magnetisation vs temperature curves, shown in figure 3.8 and 3.9, the absence of a maximum M indicates clearly that MC- and Oct-NPs are blocked at room temperature. Hysteresis loops measured at 5 K (reported in figure 3.10) show a coercivity of 0.023 and 0.0315 T respectively for MC and Oct NPs.

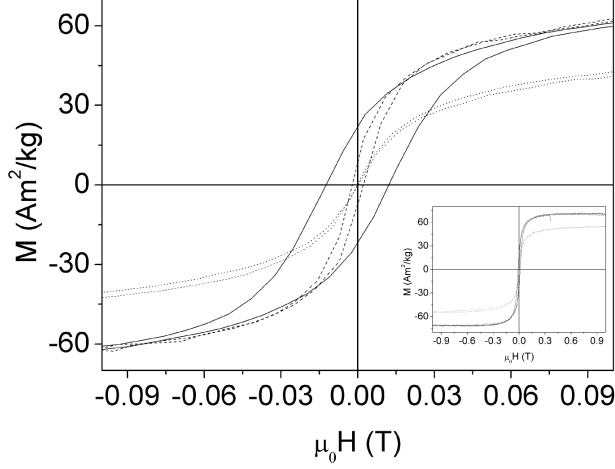


Figure 3.7: Magnetisation vs field curves for Oct (solid line), MC (dashed line) and SC-NPs (dotted line); in the inset the curves in a larger field range to evidence the saturation.

For the evaluation of the hyperthermia of nanoparticles, the temperature increasing of water suspensions of NPs was measured, in adiabatic conditions, during the application of an alternate field. Experiments were performed at frequencies of 150, 234, 335.5 and 429 kHz, with magnetic field values of 12, 16 and 20 kA/m. SAR values were calculated with the equation 1.13 and are reported in as a function of applied frequency (figure 3.11) and field (figure 3.12).

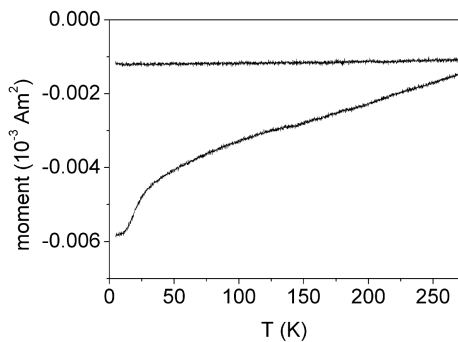


Figure 3.8: Zero-field cooling / field cooling (ZFC/FC) magnetisation vs temperature curves for multi-core NPs.

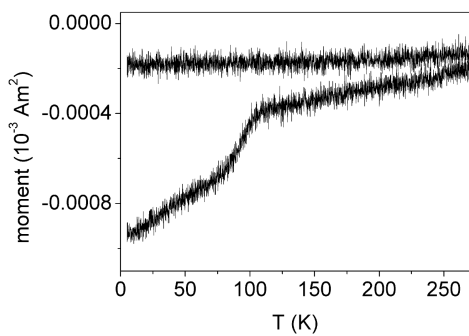


Figure 3.9: Zero-field cooling / field cooling (ZFC/FC) magnetisation vs temperature curves for octahedral NPs.

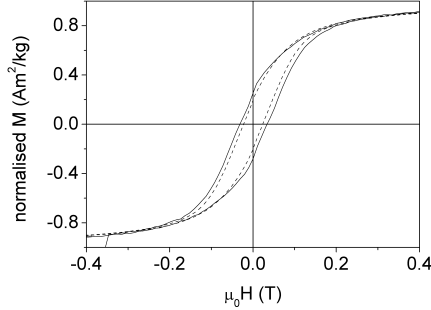


Figure 3.10: Magnetisation vs applied field at 5 K for multi-core NPs (dashed line) and octahedral NPs (solid line)

Maximum SAR value for SC-NPs resulted of 201 W/g (with $H = 20$ kA/m and $f = 429$ kHz). As widely discussed in the literature, SAR value show in general a strong dependence on particle size and magnetic anisotropy of the material, and the optimal size of Fe oxides NPs for hyperthermia was found to be about 22 nm.[17, 18] Despite this critical limit, MC-NPs exhibit a great increase of SAR , up to 567 W/g in the same conditions of field and frequency, even with the relatively big size (40 nm). This enhancement of the hyperthermia, occurring at any condition of field and frequency, is strongly bond to the increasing of M_S and to the presence of a small hysteresis loop in the MC-NPs, and can be reasonably ascribed to a cooperative behaviour between the 7 nm *sub-particles* constituting the MC structures. On the other side, larger NPs, especially in a size do-

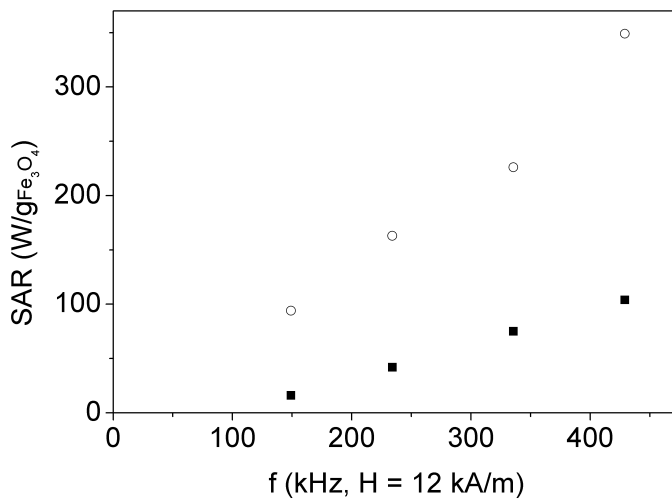


Figure 3.11: Plot of SAR as a function of frequency for SC (black squares) and MC-NPs (white circles).

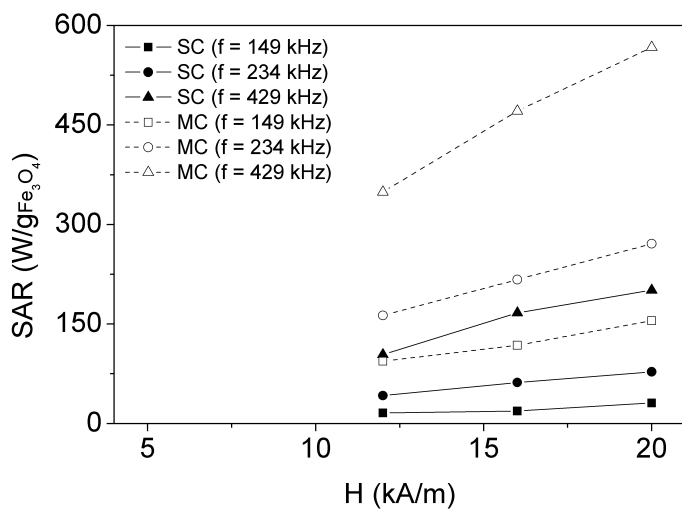


Figure 3.12: Plot of SAR as a function of the applied field.

main of more than 40 nm, exhibiting ferromagnetic behaviour at room temperature, usually show low hyperthermic properties, at the field and frequency values normally applied. As demonstrated by Guardia et al,[8] magnetite nanocubes showing highest heating properties are of about 19 nm (edge length), while nanocubes of 38 nm are characterised by decisively lower SAR values. Oct-NPs obtained with all the conditions described before, with a mean size of 50 nm, show no hyperthermia response in the frequency and field range of respectively 150 - 429 kHz and 8 - 24 kA/m, in agreement with the literature data. [8] A material with a potential application in magnetic hyperthermia for cancer treatment needs to show high *SAR* values at field and frequency conditions low enough to avoid an unwanted heating of both cancerous and healthy cells, due to generation of eddy currents. Accordingly to the work by Hergt and Dutz, a condition with a $H \times f = C$ product less than $5 \cdot 10^9 Am^{-1}s^{-1}$ is safe.[19] A remarkable *SAR* value of 271 W/g was found for MC-NPs at $H = 20$ kA/m and $f = 234$ kHz (within the safe conditions, $C = 4.68 \cdot 10^9 Am^{-1}s^{-1}$).

3.3 Conclusions

In this chapter, a new synthetic method for Fe oxides NPs, based on particle oriented aggregation, was presented. It was developed through the action of a calixarene macrocycle during the synthetic process. This easy and reproducible method allows the production of magnetic NPs with either a multi-core or octahedral morphology by simply changing the reaction con-

ditions, such as the Fe : CX8 ratio, the reaction time and the total reagents concentration. A thorough structural and magnetic characterisation of the obtained materials was performed, demonstrating that MC-NPs are characterised by remarkable hyperthermic properties.

This new approach, based on the surface activation by a macrocycle during the synthesis, can be potentially exploited to obtain the oriented particle aggregation of other nanomaterials, leading to a variety of new morphologies and allowing the tuning of their functional properties.

3.4 Experimental

Synthesis of Calix[8]arene: Calix[8]arene was synthesised using a very well-known procedure.[20]

Synthesis of Iron Oxides Nanoparticles: In a 100 mL round bottomed flask 53 mg of $\text{Fe}(\text{acac})_3$, 0.095 mL of oleic acid and the opportune amount of calix[8]arene was dissolved in 15 mL of benzyl ether. Under nitrogen flux and mechanical stirring, temperature was raised up to 200 °C. The system was maintained for 2 h and then heated up till the boiling point of the solvent (298 °C). The mixture was refluxed for one hour, then gently cooled to room temperature and purified by ultracentrifugation.

Water-dispersed nanoparticles: in a 20 mL reaction tube, 10 mL of toluene dispersion of Fe_3O_4 nanoparticles (5 mg) and 20 mg of DMSA, dissolved in 1 mL of dimethylsulfoxide, were

added. The mixture was left under mechanical stirring overnight. After precipitation of nanoparticles, supernatant was removed and product was redispersed in ethanol and washed several times by ultracentrifugation. NPs were then dispersed in water, pH was adjusted to 10 using NaOH and the dispersion was placed in a cellulose membrane and dialyzed for 4 days in distilled water, to remove unreacted DMSA and other impurities present. Finally the pH of the dispersion was adjusted to 7.

TEM characterisation: Transmission electron microscopy was carried out using a JEOL JEM 1010, with 100 KeV of acceleration voltage. Samples were prepared by dropcasting the sample on a carbon coated copper grid and subsequent evaporation of the solvent. The mean size was evaluated from the electron micrographs by counting around 300 particles.

DLS measurement: For hydrodynamic diameter and zeta potential measurement, Fe oxides NPs were diluted in distilled water to ca. 0.5 mg/ml and parameters measured by dynamic light scanning (DLS; Malvern NanoZS).

VSM characterisation: Magnetic properties of samples were determined using a vibrating sample magnetometer (MagLab VSM, Oxford Instruments). Magnetisation loops were measured at 290 K and normalised to the Iron Oxide content. 100 μL were deposited in a piece of cotton, dried and packed in a capsule.

IR spectroscopy: infrared spectra were measured with a Bruker iFS 66VS, with a resolution of 2 cm^{-1} . Preparation of the sample

was conducted by dispersing the powder in potassium bromide, and applying a 5 Ton pressure to obtain pellets.

Hyperthermia: hyperthermic heating in the presence of an alternate magnetic field was measured using the nanoScale Bio-magnetics DM100 apparatus, directly on colloidal nanoparticles suspension at about 1 mg/mL concentration.

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Chapter 4

Host-Guest Driven Controlled Aggregation of Plasmonic NPs

4.1 Introduction

As already introduced in the first chapter, plasmonic NPs may offer great possibilities in the frame of information technology. A very promising example can be the exploitation of the propagation of the SPR present in mono-dimensional arrays of neigh-

bouring plasmonic NPs, which can result in light transport below the diffraction limit (plasmonic waveguides) allowing for the construction of nanosized optoelectronic devices.[1–3]

The handling of these physical/chemical properties requires reliable procedures to assemble suitable nanometre-sized objects in a controllable manner. Among the possible strategies employed to assemble NPs,[4–7] slow evaporation of the solvent from their mother solutions has been the most extensively used.[8–11] A complementary and more promising approach is represented by the exploitation of the principles of supramolecular chemistry. By following this route, homo-aggregates of either gold (Au NPs) or silver nanoparticles (Ag NPs) were obtained either by inserting in their organic layers some effectors capable of yielding specific complementary noncovalent interactions such as hydrogen- and halogen-bonding, metal coordination, $\pi - \pi$ stacking or by the formation of host-guest inclusion complexes.[12–20] Relatively few groups have explored the aggregation of NPs of different metals to yield hetero-aggregates endowed with properties that are able to match and combine those of the single metals.[21, 22] Indeed, a supramolecular-assisted aggregation of metal NPs of different nature could, in principle, guide the fabrication of networks with properties that can be interpreted as the result of plasmon coupling between individual NPs of different nature in close proximityf.[23, 24] Opportunely functionalised calixarene derivative, as already introduced, would allow the possibility to achieve a controllable and reversible aggregation of nanostructures.

In this chapter, the specific recognition of the pyridinium moieties loaded on Au NPs by the calix[4]arene units immobilised on Ag NPs is presented and used for a controlled aggregation of the materials.

4.2 Results and Discussion

4.2.1 Synthesis of the thiolate ligands

The ω -thiolate N-undecanyl pyridinium bromide (**3**) was synthesised as depicted in figure 4.1. An S-acetyl protected thiolate moiety was placed on the termini of a 11-bromoundecanyl chain through the radical anti-Markovnikov addition of thioacetic acid to 11-bromoundec-1-ene. The resulting compound **1** was then heated to reflux with pyridine in acetonitrile to afford the pyridinium salt **2** in almost quantitative yield. The thiol group of **2** was safely cleared of its acetyl protecting group through ethanolysis. The pure salt **3** was recovered in 65% yield after its precipitation from ethyl acetate. The ^1H NMR spectrum of **3**, taken in CDCl_3 (see figure 4.2) shows the characteristic resonances of a pyridinium ring at $\delta = 9.37$ (d), 8.54 (t), and 8.15 ppm (t). As expected, the N- and S-methylene groups of the alkyl chain give rise to a triplet and a quartet at $\delta = 4.95$ and 2.51 ppm, the latter being diagnostic of the outcome of the deprotection reaction carried out on **2**. The thiolate calix[4]arene ligand **6** was synthesised from calix[4]arene **5** by following a reported procedure.[25] Ligand **6** was chosen as the supramolecularly active unit of the organic layer of the NPs. In fact, its

two ω -thiolate undecyl chains make it a very efficient bidentate ligand that is capable to favouring the formation of very stable Au NPs.[25] In this monotopic host, the ω -thiolate undecyl chains were introduced at the phenolic groups of the lower rim of the calix[4]arene. This structural arrangement makes the binding site, that is the π -rich aromatic cavity, exposed toward the bulk. It should be observed that the partial functionalisation of the lower rim contributes to increase the preorganisation of the calix[4]arene skeleton, which thus retains good recognition properties toward organic cations in weakly polar solvents.[26]

4.2.2 Synthesis and characterisation of Au NPs

The preparation of Au NPs having a mixed organic layer composed by thiolate ligands **3** and 1-dodecanethiol (C12SH) was accomplished as follows. First, approximately 5 nm Au NPs stabilised by tetraoctylammonium bromide (NP-TOABr) were synthesised by following the well-known method described by Schiffrin et al.[28]. The freshly prepared NPs suspension was then treated overnight with a large excess of C12SH. After solvent concentration, the dodecanethiol-capped Au NPs (NP1) were isolated from the unbound thiolate ligand and from the displaced ammonium salt through their precipitation, achieved by repeated ultracentrifugation cycles with a 9:1 CH₃OH/CH₂Cl₂ solution. A batch of these purified and rather monodisperse NPs was dispersed in CH₂Cl₂ and treated with a solution of **3** in the same solvent. After 5–6 h stirring at room temperature, the solvent was removed and the residue was purified by following the procedure reported previously for NP1. The Au NPs recov-

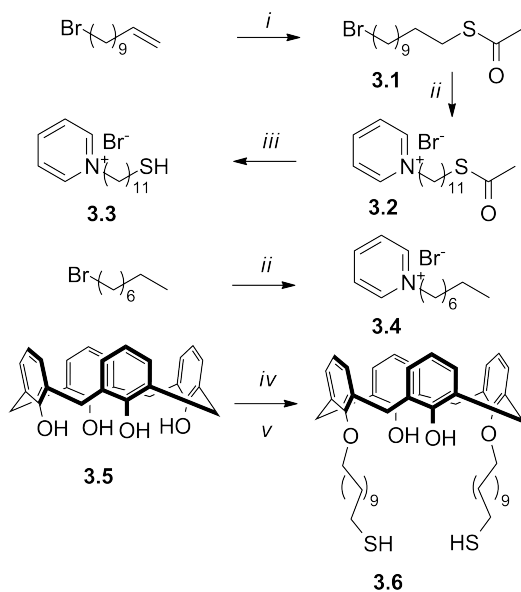


Figure 4.1: Organic Synthesis of ligands: i) CH_3COSH , AIBN, toluene, reflux, 5 h, 90%; ii) Py, CH_3CN , reflux, 12 h, 90%; iii) EtONa , EtOH , RT, 4 h, 65%; iv) **1** (2 equiv); CH_3CN , reflux, 48 h; v) EtONa , THF/ EtOH , RT, 4 h. Adapted with permission from ref [27].

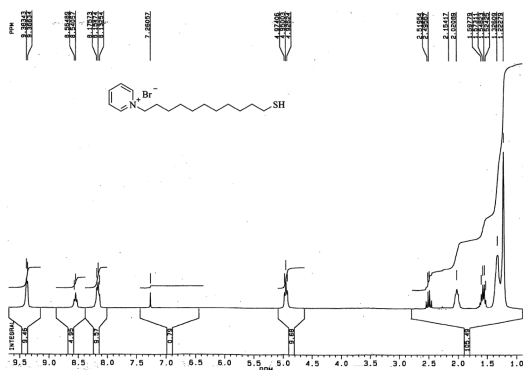


Figure 4.2: ¹H NMR (300 MHz) of compound **3** in CDCl₃. Adapted with permission from ref [27].

ered by following this synthetic procedure are indicated as NP2 (figure 4.3). These Au NPs could be easily dispersed in most weakly polar solvents, such as dichloromethane, chloroform, and toluene. For better control of the insertion of pyridinium units in the NP monolayer, an alternative synthetic procedure was devised. In separate experiments, toluene solutions of Au NPs NP-TOABr were reacted at room temperature with mixtures of ligands **3** and C12SH in 1:1 (NP3) and 1:20 (NP4) stoichiometric ratio, respectively. For comparison, an exchange reaction was also carried out using exclusively pyridinium ligand **3**; This sample is designated as NP5 (scheme in figure 4.3).

The size distribution of the synthesised NPs NP1–5 was de-

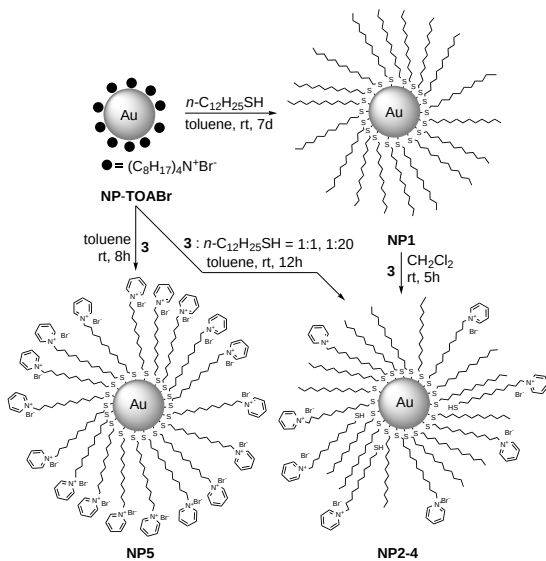


Figure 4.3: schematic representation of the preparation of NP1-5. Adapted with permission from ref [27].

terminated by analysing several HAADF-STEM images. In figure 4.4 are reported some of the corresponding images and the relative statistical analysis, accomplished with the ImageJ program.[29] The mean core diameter (d_{TEM}) calculated for each sample is reported in table 4.1, and representative STEM images together with their corresponding size distribution diagrams are gathered in figure 4.4. Statistical analysis revealed that the insertion of the pyridinium ligand **3** in the capping layer of NP1, to give NP2, has a little effect: NP2 mostly retain the morphology and size dispersity of their parent NPs. In contrast, the reaction of **3** with tetraoctylammonium-stabilised NPs, yielded the small and rather polydispersed NPs NP5. Mixing **3** with a large excess of C12SH (1:20) to control the insertion of the thiolate salt in the NPs monolayer resulted in NPs (NP4) that, with respect to NP2, have a smaller mean core diameter (4 vs. 5 nm; see table 4.1) but similar size dispersion (figure 4.4B,D). As seen for NP5, the equimolar mixture of the two thiolate ligands (1:1) produced NPs with a large size dispersion (sample NP3). Similarly to the dodecanethiol-capped NPs NP1, samples NP2 and NP4 also gave hexagonal close-packed (hcp) arrays of Au NPs on the TEM grid.[28, 30] However, it is worth noting that the mean distance between the metallic cores in this motif increases from NP1 (ca. 1.5 nm) to NP2 (ca. 2 nm) to reach its maximum in NP4 (ca. 3 nm). Such trend supports the hypothesis that the loading of **3** in the NPs organic layer adversely affects the interdigitation of the dodecanethiol chains.

The samples were submitted to XPS analysis to determine the elemental composition and the extent and nature of ligands

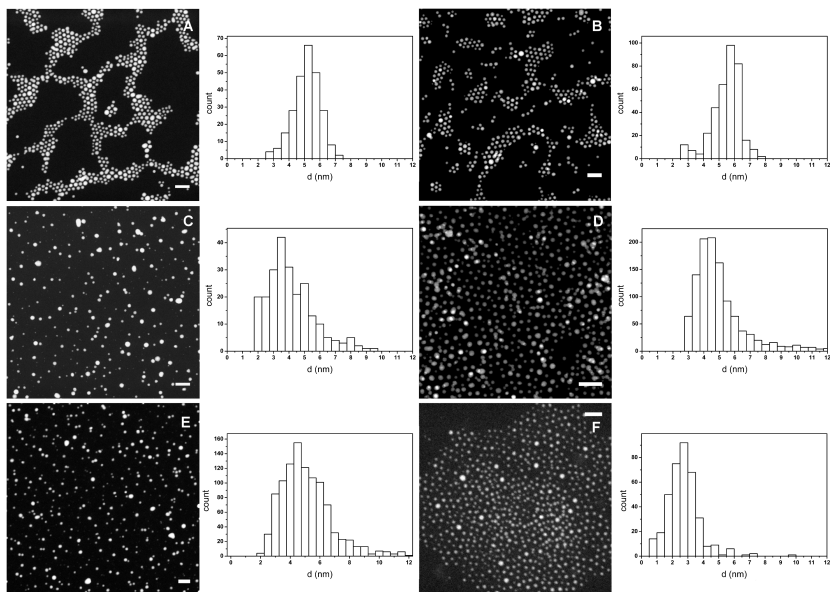


Figure 4.4: HAADF-STEM images (left) and size distribution profiles (right) of AuNPs NP1 (A), NP2 (B), NP3 (C), NP4 (D), NP5 (E), and AgNPs NP7 (F); scale bars are 20 nm. Adapted with permission from ref [27].

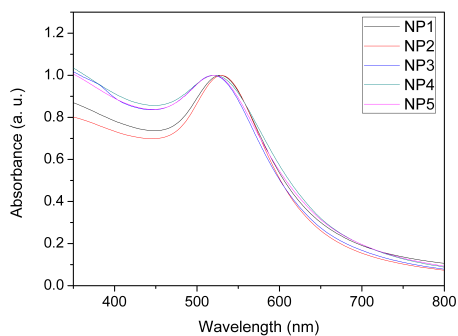


Figure 4.5: UV-visible spectra of Au nanoparticles NP1-NP5 taken in CH_2Cl_2 (NP1 and NP2) and in a 95:5 CH_2Cl_2 : MeOH mixture (NP3-NP5). Spectra are normalized taking the absorbance of NP1 as the reference. Adapted with permission from ref [27].

bonded to Au from the ratio of the thiolate ligands present in their organic layer; the results are summarised in table 4.1. The spectral regions of interest are Au 4f, S 2p, and N 1s. Experimental curves and theoretical reconstructions of Au 4f and S 2p spectra for AuNPs NP1–5 are shown in 4.6. Au 4f data show the presence of two main gold species: Au(0) at 83.4–83.7 eV and Au(I) at 83.9–84.2 eV, plus a very low Au(III) contribution at binding energies (BEs) higher than 85.0 eV from nonreduced aurate. The curve fitting of S 2p spectra reveals the presence of sulfur in two different states: the main component at approximately 162.0 eV is due to thiolate groups, and the obvious assignment is to sulfur bound to the gold surface atoms (S-Au), whereas minor amounts of sulfur either as free thiol or disulfide (RS-SR) were located at approximately 163.5 eV.[31] The N 1s signal is a single component for each sample of NPs in the series, and falls in the range experimentally determined for ammonium groups. The contribution from Au(I) can be assigned to the fraction of surface Au atoms reacted with the thiol groups of the ligands, on the basis of its positive shift with respect to Au(0), and because of the unitary value of the ratio S-Au/Au(I), found for the full series investigated, within the experimental error. As expected for NPs having a core-size significantly larger than the Au 4f photoelectron escape depth (ca. 2 nm, at the photon energy used), and at variance with the molecular Au clusters with low nuclearity,[32–34] the relatively high values for the Au(I)/Au_{tot} ratio reflect the fact that only an outer volume of the full NP can be explored with the photon energy used here. The S_{tot}/Au_{tot} ratio for NP5 falls in the range expected for Au NPs of this size. Such a ratio, which

does not vary significantly along the series, may indicate an upper limit to the amount of sulfur bound to Au NPs through organic thiol ligands, almost independent from the actual ligand. In further support of this last point, we recall here that such ratio was already reported, within the experimental error, in the case of Au NPs functionalised with either 1-dodecanethiol or calixarenes.[25, 35, 36] We can make the assumption that the value for N_{tot}/Au_{tot} ratio in NP5 is the reference value for Au NPs with the highest surface coverage by pyridinium ligands, and we use this value to compare the estimate of the actual extension of NPs functionalisation in the series. We may justify this position by considering the following three points: 1) the diameters of NPs are comparable all through the series; 2) the value experimentally found for N_{tot}/S_{tot} ratio in NP5 is, within experimental error, equal to 1; all through the series of NPs investigated here, only in the case of NP5 can this value be associated with the presence of one single type of ligand. and 3) the simplest conditions for pyridinium functionalisation in the series NP2–5 are those operating for NP5, because of the absence of competing ligands either already adsorbed (NP2) or in solution (NP3–4). With reference to the case of NP1, the value for N_{tot}/S_{tot} ratio for NP5 can clearly be interpreted as indicating a much easier functionalisation by the pyridinium ligand when the Au NPs is not covered with dodecanethiol. Such value does not change significantly for NP3, (1:1 mixture of 1-dodecanethiol and **3**). The NP4 sample shows approximately 2/3 the value for NP2, which hints at roughly 2/3-py-covered NPs, within this simplified approach. We note that N_{tot}/S_{tot} ratios in these N-containing molecules, which are bound through a terminal thiol

group, are overestimated because of the attenuation exerted by the long C chain on the S-related photo-electrons. For this reason this value cannot be used to estimate the real pyridinium : C12SH ratio in the monolayer of each sample.

The XPS measurements afforded an estimated surface coverage γ of the NPs, from the ratio of thiolate ligands to surface Au atoms. Similarly to the approach introduced by Fabris and co-workers,[37] this parameter was calculated by assuming the Au NPs having a truncated octahedral shape with a face-centred cubic (FCC) structure and by considering the S-Au/Au_{tot} ratio and the NPs mean core diameter (d_{TEM}) determined experimentally by XPS and STEM measurements (see the Experimental section for further details). The deduced values of γ for NP2–5 (0.40–0.58) were higher than those found for Au NPs of similar core size,[38] but they were very similar to the value calculated for NP1 (0.58). This result suggests that the insertion of **3** does not affect the packing of the organic layer of the NPs substantially.

4.2.3 Synthesis and characterisation of the Ag NPs

Calix[4]arene-functionalised Ag NPs NP7 were synthesised by mixing a dichloromethane solution of dodecanethiol-capped Ag NPs NP6, prepared by using the biphasic method reported by Fitzmaurice,[39] with a solution of the thiolate calix[4]arene ligand **6** in the same solvent (see scheme in figure 4.7). The ligand

NPs:	NP1	NP2	NP3	NP4	NP5
Ligands:	C12SH	3 /C12SH	3 /C12SH 1:1	3 /C12SH 1:20	3
λ_{SPB} [nm]	528	527	520	520	520
d_{TEM} [nm]	5 ± 1	5 ± 1	4 ± 2	4 ± 1	4 ± 2
Au 4f _{7/2} [eV]	Au ⁰	83.6	83.7	83.4	83.5
	Au ^I	84.1	84.2	84.0	83.9
	Au ^{III}	85.6	85.5	85.4	85.5
	S-S	163.7	163.4	not detected	not detected
	S-H				
S 2p _{3/2} [eV]	S ^{IV}	168.7	169.3	not detected	not detected
N 1S [eV]		not detected	402.0	402.0	402.0
S_{tot}/Au_{tot}		0.26	0.27	0.27	0.24
Au_{tot}/Au^I		5.2	5.4	5.9	5.0
$S-Au/Au_{tot}$		0.18	0.18	0.14	0.18
$S-Au/Au^I$		0.93	0.96	0.97	0.89
$S-Au/S_{tot}$		0.68	0.78	0.73	0.69
N_{tot}/S_{tot}		not applicable	0.67	0.89	0.81
N_{tot}/Au_{tot}		not applicable	0.18	0.23	0.20
γ		0.58	0.58	0.40	0.52

Table 4.1: Characterisation data for Au NPs NP1-5

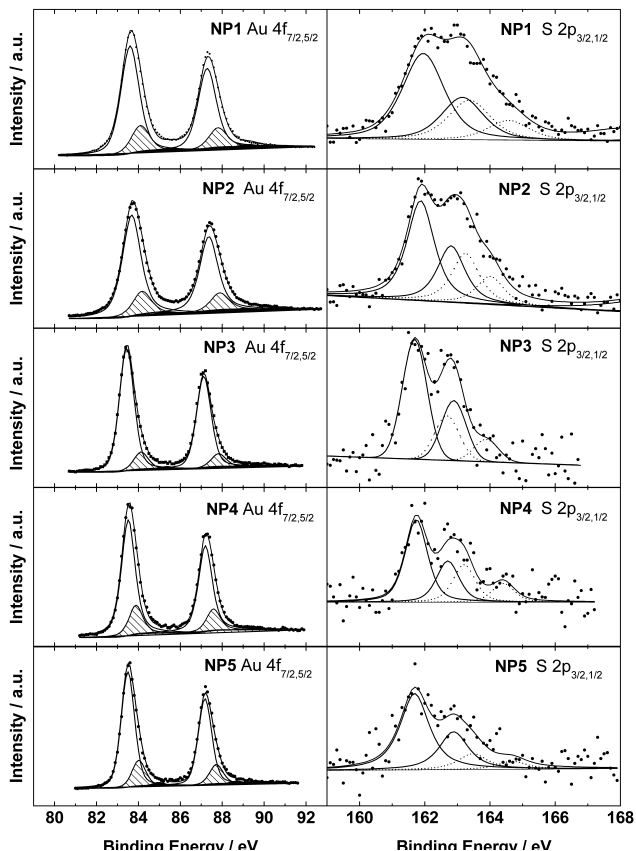


Figure 4.6: Au 4f (left) and S 2p (right) XPS regions of AuNPs NP1–5. Fitting of the experimental data and peaks deconvolution evidence the presence of three species of Au 4f: Au^0 (main component, continuous black lines), Au^I (cross-hatched area) and, if present, Au^{III} (black area), and two for S 2p: S-Au (continuous black lines) and S-S, S-H (dotted lines). Adapted with permission from ref [27].

exchange reaction was allowed to proceed for seven days, then Ag NPs NP7 were precipitated through the addition of methanol to the reaction mixture followed by ultracentrifugation. These NPs were characterised by UV- visible, STEM, and XPS measurements. The UV/Vis spectrum of a dichloromethane solution of NP7 shows a SPB centred at $\lambda = 450$ nm. The STEM measurements evidenced a rather monodispersed sample of Ag NPs having a mean core diameter $d_{TEM} = 31$ nm. On the TEM grid, these Ag NPs tend to form a quasi-ordered 2D lattice with a hcp motif already observed for the Au NPs (reported in figure 4.4). On average, the observed interparticle distance of approximately 4 nm was consistent with grafting of the bulkier calix[4]arene **6** on the surface of the NPs.

More useful insights on the effective insertion of the calix[4]arene units on the surface of NP7 were obtained from XPS analysis; the results are gathered in table 4.2 together with those related to the dodecanethiol-capped NP6, listed for comparison. The spectral regions of interest are Ag 3d and S 2p. Within experimental error (10%), the S-Ag/ S_{tot} and S-Ag/Ag(I) ratios are unitary, indicating that, in both samples, all thiolate ligands present are bonded to the Ag NPs. To evaluate the presence of calix[4]arenes in NP7, an accurate analysis of the C 1s spectral region was carried out. Several components contribute to this signal, including an unavoidable environmental contamination. Nevertheless, through its theoretical reconstruction it was possible to quantify a high-energy component (287.2 eV), which was assigned to carbon atoms linked to hydroxy phenolic groups (C_{PhOH}), thus exclusively belonging to the calix[4]arene macro-

cycle. The ratio between the intensity of this component with respect to the total intensity of the carbon signal C_{PhOH}/C_{tot} yields a value of 0.06, which, combined with the C_{tot}/S_{tot} experimental ratio of 27.6, may return an estimate of the contribution of the phenolic carbon atoms with respect to the total amount of sulfur found in the sample. We devised the following simple expression: $C_{PhOH}/S_{tot} = (C_{PhOH}/C_{tot})(C_{tot}/S_{tot})$ that for the sample of NPs NP7 yielded a value of 1.7. By following a similar approach for the components of the O 1s signal (see table 4.2), it was possible to determine a O_{PhOH}/S_{tot} ratio of 2.2 from $O_{PhOH}/O_{tot} = 0.7$ and $O_{tot}/S_{tot} = 3.2$. Within the experimental errors, both ratios suggest that the dodecanethiol-based capping layer of Ag NPs NP6 was totally displaced in NPs NP7 by calix[4]arene **6**. The structure of this ligand (indicated in figure 4.1) would indeed yield a theoretical ratio of 2 for both C_{PhOH}/S_{tot} and O_{PhOH}/S_{tot} . This result is not surprising considering that the large affinity of this bidentate thiolate ligand for the surface of other noble metal NPs and surfaces has already been documented.[25, 40]

4.2.4 Aggregation studies

Recognition-induced aggregation experiments between the Au NPs loaded with the pyridinium units (NP2–5) and the Ag NPs functionalised with the calix[4]arene units (NP7) were carried out in a 95:5 CH_2Cl_2 : MeOH solution and monitored by UV/Vis spectroscopy. The aggregation process between plasmonic NPs may in fact induce a large red-shift of their LSPR be-

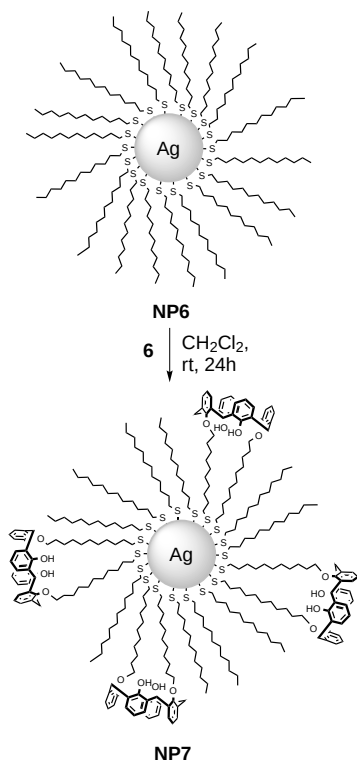


Figure 4.7: Schematic representation of the preparation of NP7. Adapted with permission from ref [27].

NPs:		NP6	NP7
Ligands:		C12SH	6 /C12SH
λ_{SPB} [nm]		540	540
d_{TEM} [nm]		not measured	3 ± 1
Ag 3d _{5/2} [eV]	Ag ⁰	368.0	368.2
	Ag ^I	368.7	368.8
S 2p _{3/2} [eV]	S-Ag	161.7	161.7
	S-S	not detected	not detected
C 1s [eV]	C(graph)	not measured	285.0
	C-S	not measured	285.5
	C(adv)	not measured	286.2
	C _{PhOH}	not applicable	287.2
O 1s [eV]	O(adv)	not measured	531.7
	O _{PhOH}	not applicable	532.7
S_{tot}/Ag_{tot}		0.15	0.14
Ag^I/Ag_{tot}		0.14	0.15
$S-Ag/S_{tot}$		1	1
$S-Ag/Ag^I$		0.9	1.1
C_{PhOH}/S_{tot}		not applicable	1.7
O_{PhOH}/S_{tot}		not applicable	2.2
γ		not measured	0.3

Table 4.2: Characterisation data for Ag NPs

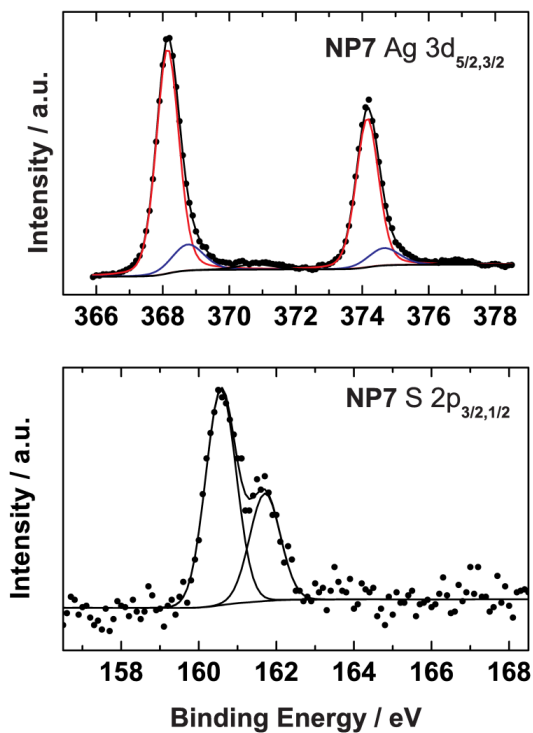


Figure 4.8: XPS spectral regions of Ag 3d (top) and S 2p (bottom) in calix[4]arene-loaded Ag NPs NP7. Adapted with permission from ref [27].

cause of changes to the interparticle spacing, coupling between plasmonic modes, and prevailing scattering phenomena.[41] In a first titration experiment, a 2 mg/mL solution of Ag NPs NP7 was added to a 2 mg/mL solution of Au NPs NP2. The UV/Vis spectra recorded after each addition are gathered in figure 4.9A, together with those of the free NPs NP7 (spectrum a, $\lambda_{SPB} = 450$ nm) and NP2 (spectrum b, $\lambda_{SPB} = 530$ nm), reported for reference. After addition of the first aliquot of the NP7 solution, a red-shift of approximately 30 nm was observed for the Au NPs SPB (spectrum c). Further additions of the NP7 solution did not promote any further shift of this absorption band (cf. spectra d and e). Nevertheless, this supramolecularly driven assembly process gives rise to interesting features in the near-IR region of the spectra (1.1 – 1.5 eV, see inset of 4.9A), which, although not fully disclosed, could be tentatively ascribed to near-field effects derived from the formation of discrete aggregates between the NPs of these two metals.[1, 42] A control experiment was devised to confirm that the aggregation of NPs was driven by complementary supramolecular interactions between the pyridinium units and calix[4]arene cavities present, respectively, in the organic layers of NP2 and NP7. The experiment consisted of adding increasing amounts of a 2 mg/mL solution of the dodecanethiol-capped Au NPs NP1 in a 95:5 CH₂Cl₂ : MeOH mixture to the above solution of Ag NPs NP7. The corresponding collection of UV/Vis spectra (indicated in figure 4.9B) evidences the appearance of the Au NPs SPB at $\lambda = 530$ nm which is not, as expected, accompanied by any appreciable shift at lower energies. At the end of the titration, two SPBs relative to the two types of NPs can indeed be easily

distinguished at $\lambda = 450$ and 530 nm (figure 4.9B, spectrum h). A final experiment was carried out to evaluate whether the aggregation between NP2 and NP7 is controlled by the diffusion of these nanometre-sized objects in solution. The two NPs solutions were mixed together in a 1:1 ratio and UV/Vis spectra were recorded at increasing times (see figure 4.9C). Immediately after mixing (figure 4.9C, spectrum c), the SPB of the Au NPs is barely recognisable as a shoulder of the more absorbing SPB of the Ag NPs. Notably, the position of the peak maximum of the latter band is not shifted appreciably with respect to the isolated Ag NPs (cf. spectra a and c). Over time, the two SPB merged in one broad band centred at $\lambda = 500$ nm and a new high-energy band appeared at $\lambda = 350$ nm, reasonably deriving from the pyridinium units present in the organic layer of NP2 (figure 4.9C, spectrum j). At $t = 24$ h, the SPB was red-shifted at $\lambda = 550$ nm and its intensity reduced because a black solid separated from the solution as a result of extensive NPs aggregation (spectrum k). These findings support the hypothesis that the recognition process between pyridinium units and calix[4]arene cavities present in the organic layers of NP2 and NP7 is a slow process. To confirm the occurrence of aggregation, a few drops of the solution obtained at $t = 200$ min were drop-casted onto a TEM grid. After evaporation of the solvents, HAADF-STEM measurements showed the formation of wire-like super-aggregates in which both type of NPs are present (figure 4.9D). Indeed, because of the higher atomic number of gold, the larger Au NPs NP2 appear brighter on the dark background than the smaller Ag NPs NP7 (see inset of figure 4.9D).

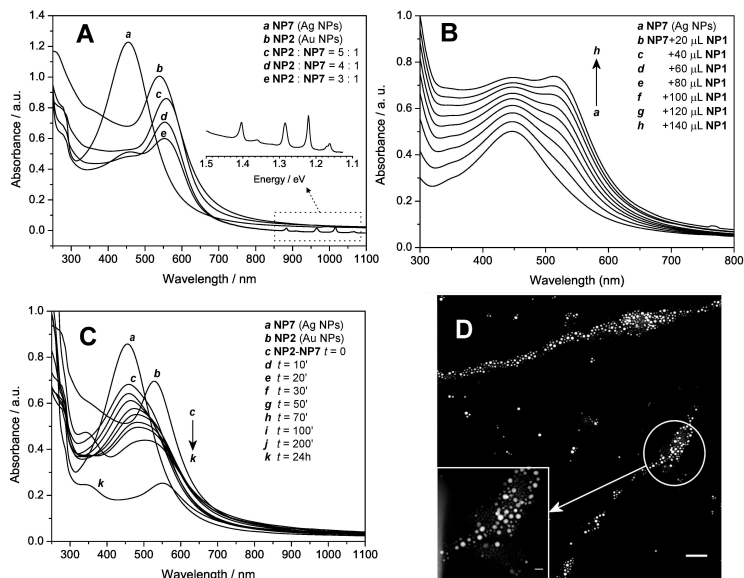


Figure 4.9: Collection of UV/Vis spectra in a 95:5 CH_2Cl_2 : MeOH mixture showing: A) the titration of pyridinium-loaded Au NPs NP2 with calix[4]arene-functionalised Ag NPs NP7 (in the inset is shown the 1.5 - 1.1 eV region, relative to the spectra showing small and narrow resonances appearing after mixing the two NPs solutions); B) the control experiment, carried out using dodecanethiol-capped Au NPs NP1 in place of NP2; C) the kinetic of formation of aggregates between NP2 and NP7; and D) HAADF-STEM image showing the wire-like structure of the aggregates (scale bar is 50 nm); in the inset a magnification evidencing the presence of both type of NPs in the aggregates (NP7, pale grey; NP2, bright white, inset is 10 nm). Adapted with permission from ref [27].

The aggregation of the Au-Ag NPs was also studied with NP3, NP4 and NP5 which, as previously discussed, differ in the loading of pyridinium units in their organic layer. Titration experiments can be carried out without incurring the precipitation of black solid aggregates of NPs as instead observed with NP2. The titrations were carried out by using the same procedure reported above, in a 95:5 CH_2Cl_2 : MeOH mixture of solvents, by adding increasing amounts of NP7 to the solution of Au NPs, and recording the corresponding UV/Vis spectrum after each addition (figure 4.10). In all of the titrations, the addition of NP7 led to an appreciable enhancement of the extinction of the SPB along with its expected red-shift. The enhancement of the SPB extinction of NPs has been discussed in several theoretical works,[3] and assigned to the coupling of plasmon modes of nearby metallic cores. The magnitude of the SPB red-shift was higher for NP3 and NP5 than for NP4. Indeed, from the full series of Au NPs, according to the XPS measurements, NP4 is the least loaded with pyridinium units in the monolayer and is the least favoured in the aggregation process in the series NP3–5.

A further insight into the growth and the size of the aggregates of NPs was obtained from dynamic light scattering (DLS) measurements.[43–45] The experiments were carried out by adding increasing amounts of NP7 solution to the Au NPs solution, and recording the decay of correlation of the scattered light from the possible aggregates, owing to diffusional motion, after each addition. Such decay can be related to the size of the aggregates in terms of hydrodynamic diameter d_H . From the size distribution profiles of the aggregates (reported in fig-

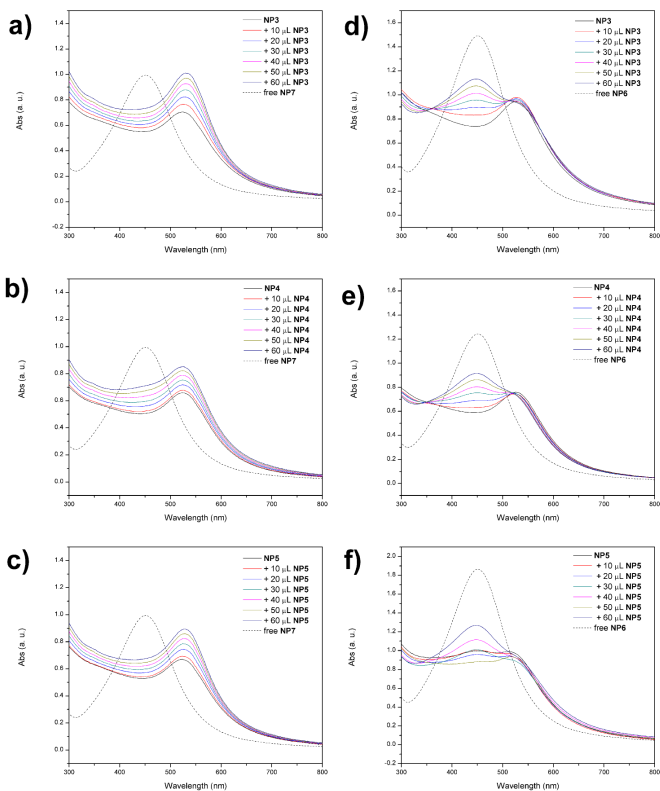


Figure 4.10: UV-visible aggregation experiments between the pyridinium-loaded Au NPs NP3-5 (respectively a - c) and the calix[4]arene-loaded Ag NPs NP7; the experiments were accomplished by adding a 2 mg/ml solution of NP7 in a 95:5 CH_2Cl_2 : MeOH mixture to a 2 mg/ml solution of each Au NPs sample. In figure d - f were reported the corresponding “blank” titration experiments carried out by adding a solution of dodecanthiol-capped Ag NPs NP6 to the samples of Au NPs. In each collection of spectra the extinction of the free Ag NPs NP6 and NP7 was reported for reference as a dashed line. Adapted with permission from ref [27].

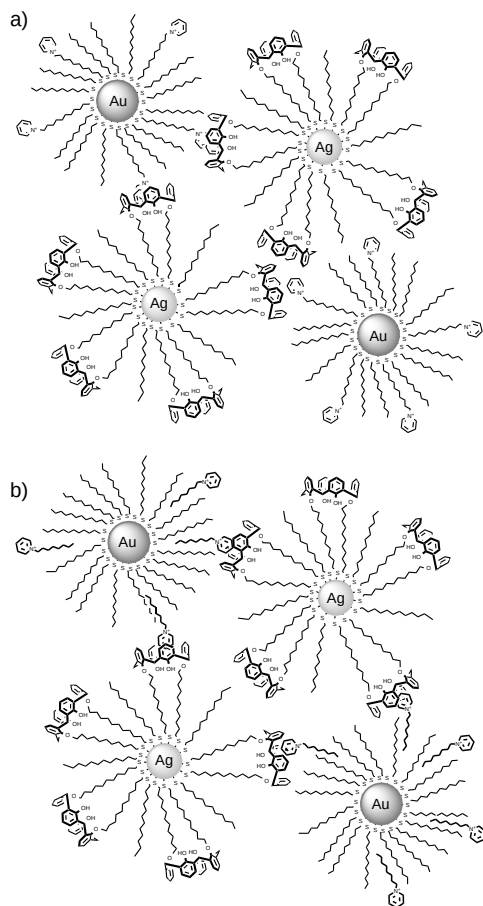


Figure 4.11: Representations of the assembly between Au and Ag NPs driven by supramolecular interactions with: a) the two complementary binding sites are covalently linked on the NPs surface; b) one binding site (that is, the N-octyl pyridinium bromide **4**), is interdigitated in the dodecanethiol layer of the Au NPs. Adapted with permission from ref [27].

ure 4.13) it is evident that the addition of increasing amounts of NP7 determines an increase in the size of the aggregates. However, in the case of NP3 and NP5, which present the highest loading of pyridinium units in this series, the formation of larger aggregates ($d_H > 120\text{nm}$) was observed when the maximum amount of NP7 was added. Under the same conditions, the reticulation reaction experienced by NP4 yielded aggregates with a mean size of approximately 65 nm. Such low aggregation behaviour was reasonably explained by considering the lower loading of pyridinium units in the organic layer of NP4. As already reported by other authors, it should be noted that the detection of small NPs in the presence of several percentages of bigger ones could in fact be very difficult because of large scattering of the largest NPs.[46, 47]

The UV/Vis and DLS titration experiments showed the feasibility of extensive NPs networking induced by the formation of several host-guest supramolecular interactions between complementary host and guest units covalently grafted on the surface of NPs of different metals. With the aim of gaining better control over the extent of aggregation, we considered a different aggregation strategy, based on the already reported bilayer surfactant assemblies, that consists of the formation of a secondary interdigitated monolayer in alkylthiolate-capped Au NPs.[48–51] For our system, this secondary monolayer would be made by one of the two constituents of the host-guest ensemble previously exploited. We selected the N-octyl pyridinium iodide **4** (see scheme 4.1) as the secondary unit because: 1) its long octyl chain could potentially interdigitate in the organic layer

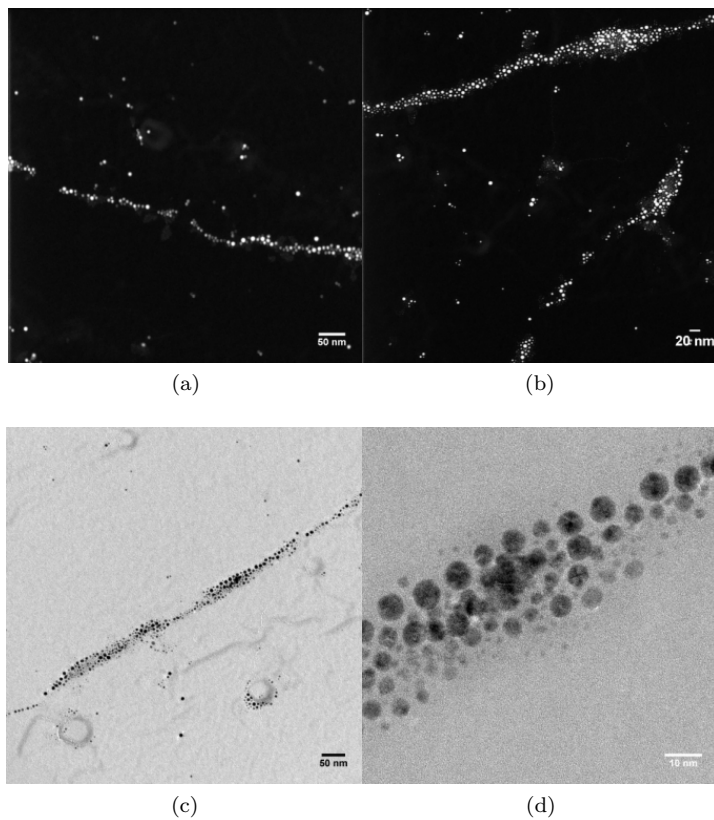


Figure 4.12: STEM and TEM images of the aggregates obtained by mixing solutions of Au NPs NP4 and Ag NPs NP7 in CH_2Cl_2 : MeOH = 95:5. Adapted with permission from ref [27].

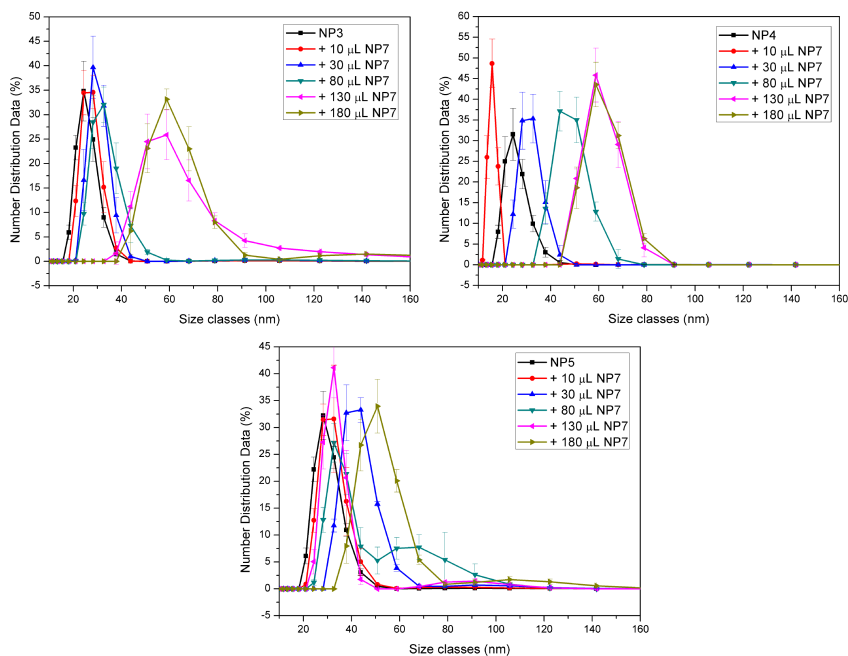


Figure 4.13: Size distribution profiles of the aggregates deriving from the DLS titrations of Au NPs NP3, NP4 and NP5 with increasing amounts of solution of Ag NPs NP7 in 95:5 CH_2Cl_2 : MeOH. Adapted with permission from ref [27].

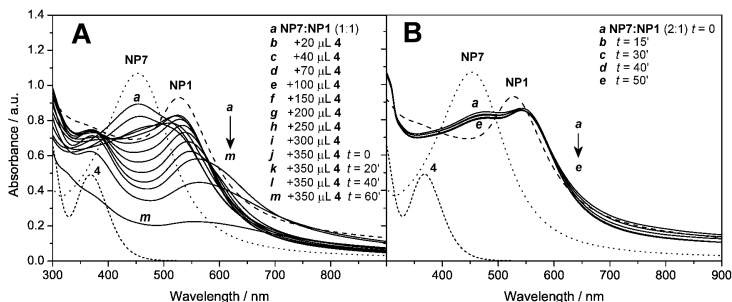


Figure 4.14: A) Collection of UV/Vis spectra recorded upon titration of a 1:1 mixture NP1 (dashed line) and calix[4]arene-functionalised Ag NPs NP7 (dotted line) in CH_2Cl_2 with a 10^{-3} M solution of octyl pyridinium iodide **4** (short-dashed line) in the same solvent; B) control experiment, carried out by stirring the same mixture for 50 min in the absence of the aggregating guest. Adapted with permission from ref [27].

of the dodecanethiol-capped AuNPs NP1; 2) the high affinity of its pyridinium moiety for the π -rich aromatic cavity of analogues of calix[4]arene **6** was already documented;[26, 52, 53] 3) it experiences good solubility in weakly polar solvents such as dichloromethane and chloroform in which particles NP1 and NP7 can be also easily dispersed. We also considered that, in contrast to pyridinium-loaded Au NPs NP2–5, the calix[4]arene-functionalised Ag NPs NP7 showed good durability even when dispersed in chlorinated organic solvents.

Several UV/Vis titrations between NP1, NP7, and **4** were carried out to investigate the formation of the aggregates. Solutions of the particles in CH_2Cl_2 were mixed in 1:1, 2:1, and 1:2 ratios and a $10^{-3}M$ solution of **4** in the same solvent was added. As a general result, a marked attenuation of the SPB of the Ag NPs was observed, whereas the corresponding band for the Au NPs underwent a minor intensity decrease but with a substantial red-shift (see figure 4.14A). Upon the addition of 350 mL of the solution of **4**, the mixture was allowed to standing for 60 min and UV/Vis spectra were taken at regular time intervals. After 60 min, only a broad band centred at approximately $\lambda = 580$ nm was observed, indicating the formation of large aggregates. A control experiment was also carried out by mixing the solutions of NP1 and NP7 in CH_2Cl_2 without the addition of the pyridinium guest: a small attenuation of both SPB band was observed (figure 4.14B), although no appreciable changes of their maximum was detected. This last experiment indirectly confirmed that the salt **4** drives the aggregation between the NPs.

4.3 Conclusion

Nanoparticle assemblies are of particular interest for fundamental research and applications, and aggregation between plasmonic NPs of different metals is a relatively new topic in the field. In this study, we have shown that supramolecular interactions among complementary moieties introduced as functional binding sites at the surface of gold and silver NPs is the key phe-

nomenon implied in their aggregation in a solution. Lipophilic 4-5 nm Au NPs having a mixed protecting organic layer consisting of 1-dodecanethiol and N-alkyl pyridinium units were synthesised and used as guests for ω -alkylthiolate calix[4]arene-functionalised Ag NPs as hosts. All the prepared NPs were dimensionally characterised by TEM, and structurally exploited by means of UV/Vis and XPS spectroscopy. Specific recognition of the cationic pyridinium moieties of the gold NPs by the calix[4]arene-functionalised silver NPs has been demonstrated in solution by UV/Vis titrations and DLS measurements. The extent of aggregation of the gold-silver NPs, evidenced through a bathochromic shift of their surface plasmonic bands, was found to depend strongly on the loading of the pyridinium moieties present in the organic monolayer of the gold NPs.

4.4 Experimental

Synthetic methods: All of the solvents were dried using standard procedures; all other reagents were of reagent-grade quality obtained from commercial suppliers and were used without further purification. NMR spectra were recorded at 400 and 300 MHz for ^1H and 100 and 75 MHz for ^{13}C . Melting points are uncorrected. Chemical shifts (δ) are expressed in ppm using the residual solvent signal as internal reference. Mass spectra were recorded in ESI mode. Calix[6]arenes **5**,^[54] **6**,^[25] N-octyl pyridinium iodide **4**,^[52] tetraoctylammonium bromide-stabilised Au NPs NP-TOABr,^[26] and dodecanethiol-capped Ag NPs NP6^[39] were synthesised according to published pro-

cedures.

S-11-Bromoundecyl ethanethioate (1): A spatula-tip of AIBN was added to a solution of 11-bromoundec-1-ene (7 g, 30 mmol) and thioacetic acid (4.3 g, 56 mmol) in degassed toluene (250 mL). The resulting mixture was heated to reflux for 6 h, then cooled to RT and the reaction was quenched by addition of H₂O (250 mL). The organic phase was separated, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The oily residue was purified by column chromatography (n-hexane/ethyl acetate, 97:3) to afford **1** (90% yield) as a yellowish oil. ¹H NMR (300 MHz, CDCl₃): δ = 3.38 (t, J=7 Hz, 2H), 2.84 (t, J=7 Hz, 2H), 2.30 (s, 3H), 1.9–1.8 (m, 2H), 1.6–1.4 (m, 2H), 1.4–1.2 ppm (m, 14H); ¹³C NMR (75 MHz, CDCl₃): δ = 196.0, 164.4, 34.0, 32.7, 30.6, 29.4, 29.3, 29.1, 29.0, 28.7, 28.6, 28.1; MS-ESI: m/z: 311 (100) [M+2], 309 (100) [M]⁺; elemental analysis calcd (%) for C₁₃H₂₅BrOS: C 50.48, H 8.15, S 10.37; found: C 51.28, H 8.14, S 10.07.

1-[11-(Acetylthio)undecyl]pyridinium bromide (2): A solution of pyridine (0.4 g, 4.9 mmol) and **1** (1 g, 3.3 mmol) in CH₃CN (50 mL) was poured in a small glass autoclave vial. The reaction mixture was heated to reflux under stirring for 12 h. After cooling to RT, the solvent was evaporated to dryness under reduced pressure. The desired salt **2** was obtained in 90% yield as a pure yellowish solid by dissolving the crude residue with the minimum amount of ethyl acetate and precipitation from cold n-hexane. M.p. 61–63°C; ¹H NMR (300 MHz, CDCl₃): δ = 9.46 (d, J=6 Hz, 2H), 8.48 (t, J=7.5 Hz, 1H), 8.11 (t, J=7.5

Hz, 2H), 5.04 (t, J=7.5 Hz, 2H), 2.86 (t, J=7.5 Hz, 2H), 2.33 (s, 3H), 2.1–2.0 (m, 2H), 1.6–1.5 and 1.4–1.2 ppm (2 m, 16H); ^{13}C NMR (75 MHz, CDCl_3): δ = 145.2, 144.9, 128.5, 61.9, 31.8, 30.6, 29.3, 29.2, 29.1, 29.0, 28.9, 28.6, 25.9 ppm; MS-ESI: m/z: 308 (100) $[\text{M}+\text{H}^+]$.

1-(11-Mercaptoundecyl)pyridinium bromide (3): Salt **2** (0.2 g, 0.52 mmol) was added to a freshly prepared solution of sodium ethoxide (0.05 g, 0.7 mmol) in absolute ethanol (20 mL), kept under nitrogen atmosphere. After 4 h stirring at RT, the reaction was quenched by addition of few drops of aqueous HBr (48% w/v) and the solvent was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 (4x20 mL), and the combined organic fractions were evaporated to dryness under reduced pressure to give **3** (0.12 g, 65%) as a white solid. M.p. 64–66°C; ^1H NMR (300 MHz, CDCl_3): δ = 9.37 (d, J=5 Hz, 2H), 8.54 (t, J=6 Hz, 1H), 8.15 (t, J=7 Hz, 2H), 4.95 (t, J=7 Hz, 2H), 2.51 (q, J=7 Hz, 2H), 2.1–2.0 (m, 2H), 1.6–1.5 (m, 2H), 1.4–1.2 ppm (m, 15H); ^{13}C NMR (75 MHz, CDCl_3): δ = 145.4, 144.9, 128.5, 61.9, 33.8, 31.8, 30.9, 29.3, 29.2, 29.1, 28.9, 28.8, 28.2, 25.9, 24.5 (ppm). MS-ESI: m/z: 266 (100) $[\text{M}+\text{H}^+]$.

Dodecanethiol-capped AuNPs (NP1): 1-Dodecanethiol (0.025 g, 0.13 mmol) was added to a solution of TOABr-stabilised Au NPs (NP-TOABr) in toluene (40 mL), prepared as reported in ref.[28] The resulting homogeneous mixture was stirred for 8 h at RT, and then ethanol (80 mL) was added to promote the precipitation of the capped NPs. To remove the residual TOABr from the NPs, the suspension was centrifuged at 5000 rpm for

10 min. Au NPs NP1 was isolated as a black solid sediment that could be dispersed in CH_2Cl_2 .

Pyridinium-loaded AuNPs (NP2): Ligand **3** (5 mg, 0.015 mmol) was added to a solution of dodecanethiol-capped Au NPs (NP1, 20 mg) in dichloromethane (100 mL). The resulting reaction mixture was stirred for 6 h at RT, and then the solvent was evaporated to dryness under reduced pressure (no heating). To remove the unbound ligand **3**, the sticky residue was taken up with ethanol (20 mL) and the resulting suspension was centrifuged at 5000 rpm for 10 min. AuNPs NP2 was isolated as a black solid sediment that could be dispersed in either CH_2Cl_2 or a 95:5 CH_2Cl_2 : MeOH mixture.

General procedure for the preparation of pyridinium-loaded Au NPs (NP3-NP5): To a solution of tetraoctylammonium bromide stabilised Au NPs (NP-TOABr) in toluene (20 mL), synthesised as reported in ref. [28], a mixture of thiolate ligand **3** (0.01 g) and 1-dodecanethiol (NP3: 0.06 g; NP4: 0.12 g) was added; no 1-dodecanethiol was used for the synthesis of NP5. The resulting reaction mixture was stirred for 8 h, then ethanol (40 mL) was added to promote NPs precipitation. To remove the residual TOABr, the resulting suspension was centrifuged for 10 min at 5000 rpm. The exchanged Au NPs NP3–5 were isolated as black solid sediments that could be dispersed in a 95:5 CH_2Cl_2 : MeOH mixture.

Calixarene-functionalised Ag NPs (NP7): To a solution of dodecanethiol-capped Ag NPs (NP6, 0.02 g), synthesised as

reported in ref. [39], in CH_2Cl_2 (30 mL), calixarene **6** (0.05 g, 0.06 mmol) was added. The resulting mixture was stirred at RT for 7 days, and then centrifuged at 5000 rpm for 10 min. After removal of the supernatant solution, the sediment was taken up with a 2:1 *EtOH*/*CH₂Cl₂* mixture (10 mL), and the resulting suspension was subjected to a further centrifugation step to remove the unbound ligands. Ag NPs NP7 was isolated as a black solid sediment that could be dispersed in either CH_2Cl_2 or a 95:5 CH_2Cl_2 : MeOH mixture.

XPS measurements: The solid compounds were homogeneously spread over a graphite tip attached to an XPS sample holder. Photoelectron spectra were acquired with a modified Omicron NanoTechnology MXPS system equipped with various photon sources and with an Omicron EA-127-7 energy analyser. The experimental conditions adopted were as follows: excitation by Mg $K\alpha$ photons ($h\nu = 1253.6\text{eV}$), generated operating the anode at 14 kV, 16 mA. XPS atomic ratios for the investigated compounds were estimated from experimentally determined area ratios of the relevant core lines, corrected for the corresponding theoretical atomic cross-sections and for a square-root dependence of the photoelectrons kinetic energies. The ratio of ligands to surface atoms (γ) was calculated by dividing the S-Au/ Au_{tot} ratio, experimentally determined through the XPS measurements, with the percentage of superficial gold atoms (%) of the NPs. The percentage of superficial gold atoms used in the calculation was 31.2 and 34.8 for NP1-2 and NP3-5, respectively. These values were inferred from the gold magic numbers ($n = 8$ for NP3-5 and $n = 9$ for NP1-2) of FCC structures with

truncated octahedral shape.[55]

TEM and HAADF-STEM measurements: TEM and high-angle annular dark field (HAADF) measurements were carried out at CNR-IMEM of Parma with a Jeol 2200FS field-emission microscope working at 200 kV in STEM mode. The probe size was minimised to approximately 0.2 nm. The NPs size distribution was determined with the ImageJ software [29] by statistical analysis of more than 300 NPs taken from at least three images for each sample.

UV/Vis and DLS aggregation experiments: The aggregation experiments were carried out by titrating solutions of the Au NPs NP1–NP5, obtained by dissolving 20 mg of the NPs in 10 mL of a 95:5 CH₂Cl₂ : MeOH mixture, with small aliquots of a solution of the Ag NPs NP7 obtained in the same way. Typically, a UV/Vis assembly experiment was carried out in a 1 cm path cuvette by adding increasing amounts of the NP7 solution to a 1 mL solution of the Au NPs NP1–NP5. The variation of the maximum of the SPB of the NPs was monitored after each addition (up to a maximum of 200 mL added). The DLS measurement were taken with a Malvern Zetasizer nano Z-690 using a 1 cm path glass cuvette, by titrating solutions of the AuNPs NP3–NP5 obtained by dissolving 20 mg of NPs in 10 mL of 95:5 CH₂Cl₂ : MeOH mixture, with small aliquots of a solution of Ag NPs NP7 obtained in the same way (up to 200 mL of NP7 were added).

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Chapter 5

Magnetic Hyperthermia in Au - Fe Oxide Heterodimeric Nanostructures

5.1 Introduction

Hybrid magnetic-plasmonic nanostructures [1] are now of great interest in the applied research, in particular in catalysis [2–5]

and in the biomedical field.[6–8] Combining Au and iron oxides moieties in a single nanostructure gives the possibility to obtain a material in which is possible to exploit simultaneously the properties of these materials. For these reasons, many potential applications in the biomedical field are possible,[9–13] like combined magnetic hyperthermia and photo thermal therapy,[14, 15] or dual magnetic and optical probe for cell imaging.[16] To gain a control over the properties of the hybrid nanosystem, many kinds of dimeric nanostructures were developed, with morphologies varying from dumbbell-like to flower-like or core-shell.[17–20]

Despite all the studies found in the literature regarding the synthesis and characterisation of Au - iron oxides hybrid nanomaterials, in view of their possible biomedical application, very few works have been published on characterisation of their magnetic hyperthermia properties. Di Corato and co-workers measured the hyperthermia properties of 2-nm Au - core / 14-nm iron oxide shell nanostructures in a cellular environment, demonstrating the possibility of their exploitation as hyperthermia mediators.[21]

In this chapter, a much clearer insight on the magnetic hyperthermia of these materials is illustrated, and its correlation with the magnetic properties is presented. This study aims to investigate how the presence of the Au domain contributes to the heating capability of the material. Several studies have been presented demonstrating that the interface between Au and Fe oxide is a critical factor for the magnetic properties of the mate-

rial.[18, 22] The heteroepitaxial linkage between the units, obtained by a lattice strain in the iron oxide domain, which itself change the magnetic properties, may induce an interaction between the individual components across their interface. All these feature reasonably disappear when Au is removed. For these reasons, Au - Fe oxide dimeric and coreshell NPs are compared with the ones obtained after a selective removal of gold, which are hollow iron oxide NPs.

5.2 Results and Discussion

Gold - Iron oxides dumbbell-like (DB) and core-shell (CS) heterodimeric NPs are synthesised by the thermal decomposition of $\text{Fe}(\text{CO})_5$ in the presence of Au seeds.[23] In a first step, Au NPs of *ca.* 10 nm are synthesised by a thermal reduction of HAuCl_4 with oleylamine in 1-octadecene. TEM micrograph and statistical analysis are shown in figure 5.1. After a purification step, Au NPs are dispersed in octadecene and added in a octadecene solution of $\text{Fe}(\text{CO})_5$, oleic acid and oleylamine. The resulting reaction mixture is heated at 300°C for 45 minutes, to obtain the DB-NPs. In the same conditions, but with a double amount of $\text{Fe}(\text{CO})_5$ and a 2 hours reaction time, core-shell NPs are obtained. A selective removal of Au from the dimeric structures was then achieved by an Iodine etching [24] to obtain hollow iron oxide NPs (see Experimental for details).

TEM characterisations show that the monodisperse Au seeds have an almost spherical shape with a mean size of 10 ± 2 nm

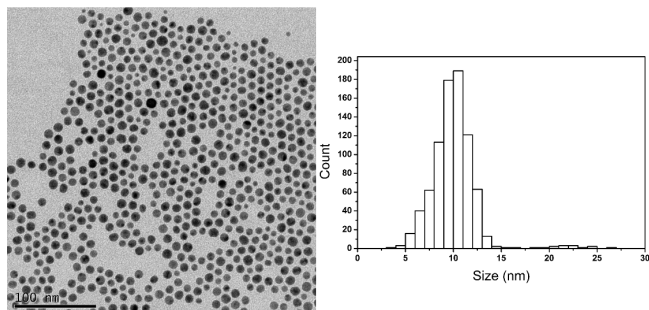


Figure 5.1: TEM micrograph and relative statistical analysis of Au seeds.

(see figure 5.1). Morphology of the Au - Fe oxide NPs is clearly evident in the TEM micrographs (figure 5.2 and 5.3) by the different contrast between the two parts. DB-NPs have a mean size of 17 ± 3 nm, while the higher amount of $\text{Fe}(\text{CO})_5$ and the longer reaction time in the CS-NPs provide the formation of a nearly complete shell, with an average particle size of 25 ± 5 nm. Regarding the hollow structures, this morphology is evidenced in TEM micrographs by the lower contrast of the spherical regions inside the nanoparticles, where Au was placed in the original dimeric nanostructures. Comparing the particle size distribution of the dimeric and relative hollow nanoparticles (figure 5.2 and 5.3), we observe that the size and dispersion of the Fe oxide moiety are conserved after the etching treatment.

To better understand the mechanism of Au removal we have

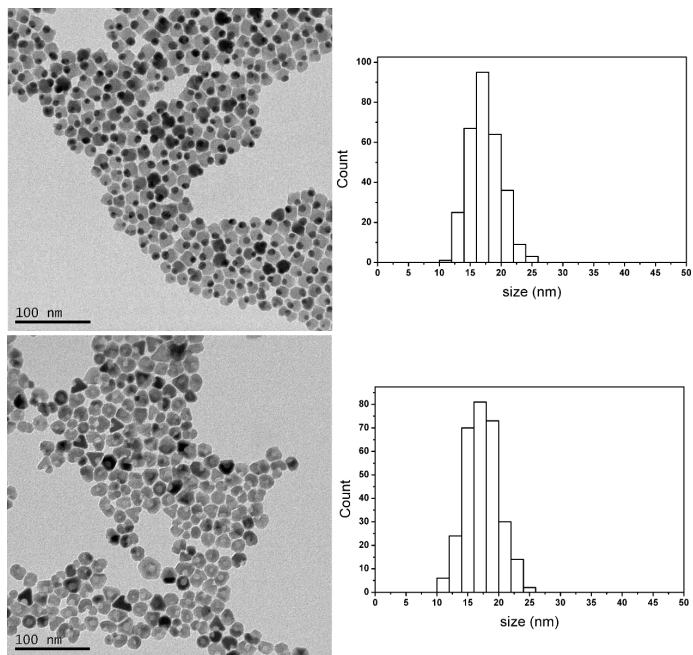


Figure 5.2: TEM micrographs of dumbbell-like (top) and 17-nm hollow NPs (bottom), and relative statistical analysis.

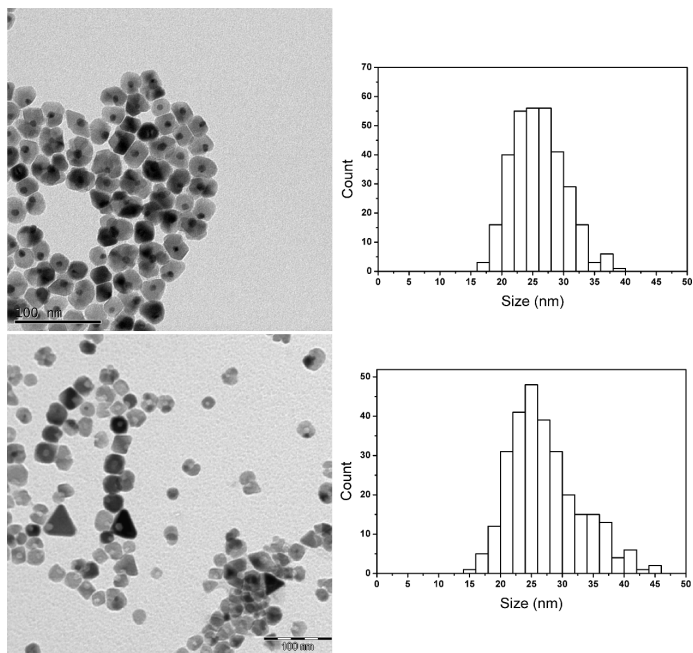


Figure 5.3: TEM micrographs of core-shell (top) and 25-nm hollow NPs (bottom), and relative statistical analysis.

performed experiments with DB-NPs using lower amounts of iodine than the one used for the complete etching. In figure 5.4 are gathered the TEM micrographs of samples obtained by the etching reaction carried out, for 16 hours, with 100, 200, 300 and 400 μL of 10^{-2} M solution of iodine in chloroform, respectively. Note that the complete etching is regularly achieved, in the same conditions, with 500 μL of the cited solution. In the two firsts samples (figure 5.4a and b), dumbbells are approaching one to each other through their Au domain. The aggregation of the Au domains becomes more important in the third sample, leading to the release of the iron oxide part from the dimeric structure. It has been proved that a growth of the Au part in the heterodimer can lead to its detachment from the rest of the structure.[25] Large aggregates of Au are the by-product of the reaction, and quickly precipitate from the suspension. For this reason they can be easily separated from the hollow NPs by decantation. From the observed trend it is possible to speculate that iodine interacts strongly with the Au surface, displacing the organic ligands forming the original monolayer (oleic acid or oleylamine). Being it not capable to protect the surface from ripening, aggregation occur. In the core-shell structures Au seed is placed heterogeneously from one particle to another, and generally not in the center of the structure. In particular, there are regions where the iron oxide is not reasonably capable to protect Au from aggregation, and this results in a well achieved etching of these NPs.

The optical absorption spectrum of Au seeds (figure 5.5) exhibit a peak at 521 nm corresponding to the surface plasmon

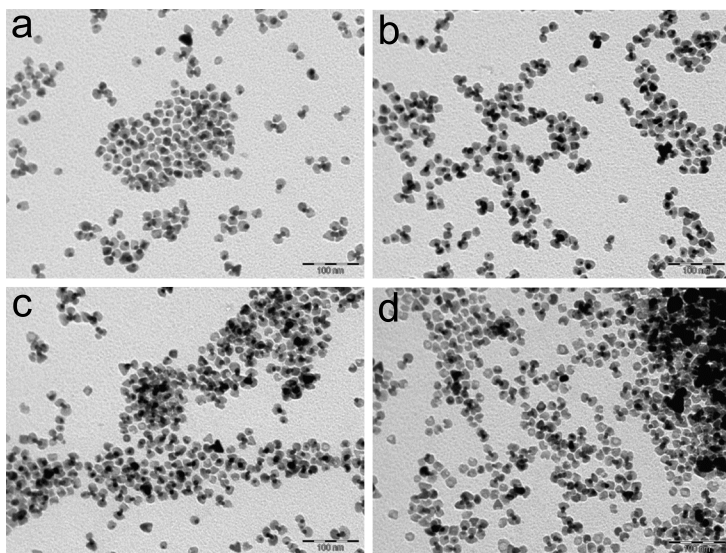


Figure 5.4: TEM micrographs of the partial Au etching obtained on DB-like NPs using: a) $100\mu\text{L}$; b) $200\mu\text{L}$; c) $300\mu\text{L}$ and d) $400\mu\text{L}$ of a 10^{-2} M solution of I_2 in CHCl_3 .

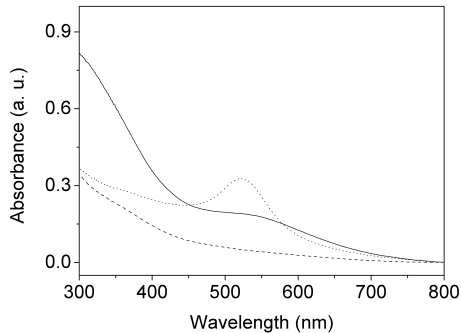


Figure 5.5: UV/Vis spectra of Au seed (dotted line), dumbbell (solid line) and core-shell NPs (dashed line).

resonance (SPR), as reported in the literature.[26] In Au - Fe oxides heterostructures, in general, a red shift accompanied with a reduction of the SPR intensity have been widely observed. This phenomenon is ascribable to the large optical absorption and refraction index of the Fe oxide shell or domain. [19, 27–29] Dumbbell NPs show this behaviour, while in core-shell structures no band related to the SPR is observed (see figure 5.5): here the oxide layer is thick enough to completely damp the resonance. On the other hand, considering the TEM micrographs, the lack of the SPR band in spectrum of the hollow particles, shown in figure 5.6, excludes also the presence of free Au NPs.

Hyperthermia was evaluated by measuring the temperature of a colloidal suspension of NPs during the application of an

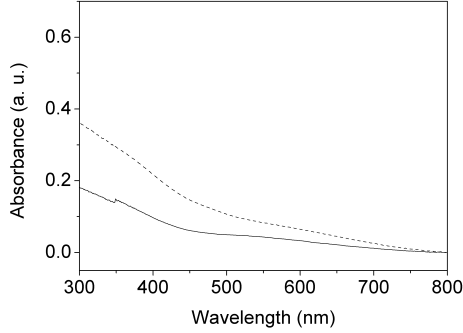


Figure 5.6: UV/Vis spectra of 17-nm (solid line) and 25-nm hollow NPs (dashed line).

external alternate magnetic field, and SAR (Specific Absorption Rate) values calculate using the equation 1.13. Figure 5.7 depicts the plot of the temperature increasing as a function of time, as obtained in these measurements, for three different frequencies applied. The obtained SAR values, calculated with the formula 1.13 are represented as a function of frequency (figure 5.8) and field (figure 5.9) for all samples. Such values are very different for the two kinds of structure, *i.e.* before and after the Au etching.

In particular, SAR values for 17-nm and 25-nm hollow NPs, up to respectively 84.4 and 225 W/g- Fe_3O_4 for $f = 335.5$ kHz and $H = 20$ kA/m, are much higher than the values of the DB and CS-NPs, measured in the same conditions (3 and 16 W/g respectively). To better understand these differences, the

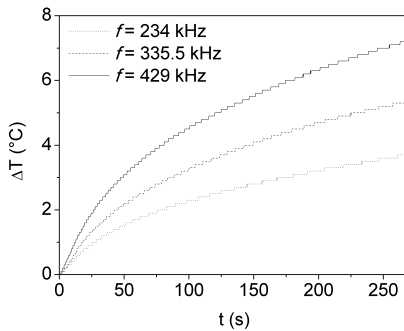


Figure 5.7: heating curve of dumbbell-like NPs at various frequency conditions and an applied magnetic field of 16 kA/m in a typical experiment of hyperthermia.

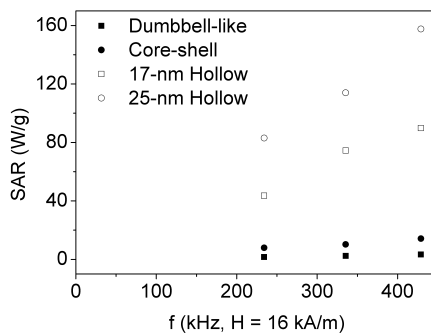


Figure 5.8: Plots of SAR values as a function of frequency, with a fixed field of 16 kA/m.

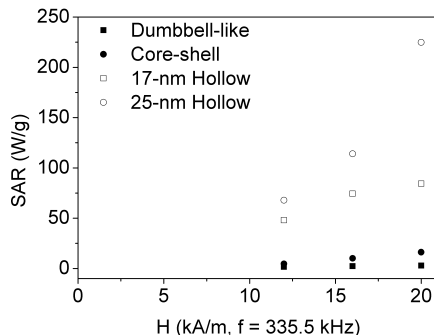


Figure 5.9: Plots of SAR values as a function of the applied field, with a frequency of 335.5 kHz.

magnetic properties at room temperature of the samples have been investigated. In figure 5.10 and 5.11 are reported the magnetisation vs field curves. The saturation magnetisation of the dimeric structures results smaller than that of the hollow NPs in both cases. In the system dumbbell-like - 17-nm hollow NPs, M_S values are respectively 9 and 72 Am^2/kg , normalised to the Fe oxide weight. Smaller difference is exhibited by the core-shell - 25-nm hollow NPs system (figure 5.11), where M_S values are, respectively, 20 Am^2/kg and 29 Am^2/kg . Moreover, the 25-hollow NPs have a small coercive field (3 mT), while no hysteresis is present in the core-shell NPs. These results clearly indicate different magnetic properties of the two samples.

The great difference in M_S between DB and the derived 17-

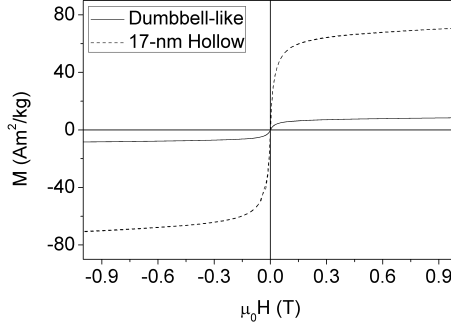


Figure 5.10: M vs H curve for dumbbell and relative 17-nm hollow NPs.

nm hollow NPs (see figure 5.10), both in the superparamagnetic regime, is likely to be attributed to a different amount of superparamagnetic volume in the samples. This strongly influences the relaxation time τ , as described in formulas 1.9 and 1.11 and the initial susceptibility χ_0 of the system, at the base of the dissipate power, as indicated in the equation ???. On the other hand, the difference in M_S between CS-NPs and 25-hollow NPs is smaller 20 and 29 Am²/kg, respectively, but here the hollow NPs M vs H curve shows a hysteresis loop. In this case, indeed, there is a significative population of NPs above the critical size for superparamagnetism [30] which are ferromagnetic. The absence of any hysteresis loop in the CS-NPs curve is coherent with a change of magnetic volume, which increases after the etching process and induces an enhancement of the dissipate power.

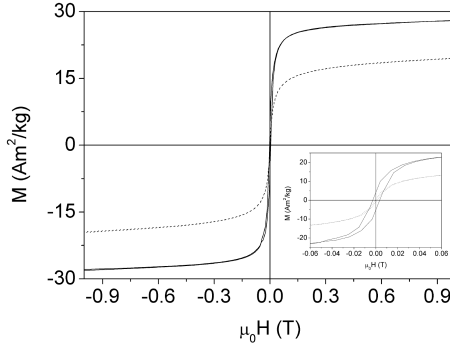


Figure 5.11: M vs H curve for core-shell (dashed line) and 25-nm hollow NPs (solid line). In the inset the magnification in a $(-0.06 - +0.06)$ T field range, to better evidence the hysteresis loop of the 25-nm hollow NPs.

Actually, several studies are present in literature about the possibility of the presence of two iron oxides coexisting inside the magnetic part of Au - Fe oxide heterodimeric NPs, magnetite and wustite.[17, 20] Wustite, as introduced in section 1.2.4 can reasonably change the magnetic volume.[31] Wustite is known to be a metastable iron oxide, that is slowly oxidated by oxygen of the air, and in the presence of I_2 a partial oxidation is reasonable to occur. DB heterodimers, after the etching process, show a M_S of 72 emu/g (figure 5.10), next to the values widely found for 17 nm magnetite NPs. This great increase in the M_S and SAR values can be indicative of a complete oxidation of the

wustite in the sample. Oxidation of wustite would increase the magnetic (magnetite) volume inside the structures, contributing decisively to the *SAR*, which finally results, for either 17 and 25-nm hollow NPs, in line with results for iron oxide spherical NPs with similar dimensions.[32–36]

5.3 Conclusions

In conclusion, we have investigated the hyperthermic properties of Au - Fe oxide heterodimeric nanostructures with two different morphologies. A confrontation with the respective hollow nanostructures was done, and the correlation of these result with the magnetic and morphological characterisation allowed us to conclude that, in the dimeric structure, two types of iron oxide, wustite and magnetite, growth together during the synthesis.

An oxidation of the wustite present that preserve the Au domain can potentially afford a material with all the magnetic and hyperthermic properties of magnetite NPs but also the plasmonic potential of Au NPs. A complete magnetic characterisation and a more thorough study on the hyperthermia of dimeric Au – iron oxide nanostructures is presented in the following chapter.

5.4 Experimental

All syntheses were carried out using a standard Schlenk line setup. Stock solutions of precursors were prepared and stored

under N₂ in glovebox.

Synthesis of Au NPs: Synthesis of Au seeds was carried out following an already known procedure.[17] 40 mg of HAuCl₄ (ca 0.12 mmol) were dissolved in 2 mL of 1-octadecene by the addition of 0.4 mL of oleylamine (0.12 mmol) and added to a degassed solution of oleylamine (2 mL, 6 mmol) and 1-octadecene (8 mL). Temperature was raised to 120 °C and kept for 20 minutes. The colour change from yellow to colorless to a final characteristic purple-violet is clearly noted.

Synthesis of dumbbell and coreshell NPs: following a reported procedure, [23] 190 mg of Fe(CO)₅ (ca 1 mmol) (390 mg for coreshell NPs) are added to a degassed solution of 6.7 mmol of oleic acid in 50 mL of 1-octadecene. After 15 min of equilibrating time, Au seed solution was added and the temperature raised to 300 °C and maintained for 45 min. Coreshell NPs were then purified by several ultracentrifuge cycles.

Synthesis of hollow NPs: to a chloroform suspension of the coreshell nanocrystals, 0.5 mL of a 10⁻² M solution of I₂ in chloroform were added. The system was allowed to stir mechanically for 16 hours. Decanted macroscopic Au was removed and hollow NPs were purified by at least 2 cycles of ultracentrifugation.

Transmission Electron Microscopy (TEM): Low-resolution TEM images were recorded with a Jeol Jem 1011 microscope, operating at an accelerating voltage of 100 kV. Statistical analysis was carried out using the imageJ software, and by counting

at least 300 NPs, to have a sufficiently large pool.

UV/Vis spectroscopy: UV/vis absorption spectra were recorded using a Cary 5 Varian UV – visible – near-IR spectrophotometer.

Magnetometry: Room-temperature magnetisation curves have been measured by an alternating gradient force magnetometer (AGFM).

Hyperthermia measurements: hyperthermia heating in the presence of an alternate magnetic field was measured using the nanoScale Biomagnetics DM100 apparatus, directly on colloidal nanoparticles suspension at concentration from 1 to 10 mg/mL.

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Chapter 6

Magnetic Characterization and *in-vitro* Hyperthermia of Au - Fe Oxide Heterodimers

6.1 Introduction

The properties and the great possibilities offered by Au / Fe oxide hybrid nanosystems have been already introduced in the

previous chapter.

Here, an improved and safer method for the synthesis of dimeric and hollow NPs is presented. A post functionalisation process is then used to transform the synthesised nanostructures in water dispersable and biocompatible. Magnetic and hyperthermia properties of the heterodimeric NPs are thoroughly characterised, with a particular attention on the differences in hyperthermia behaviour after the functionalisation. To discriminate between the Néel and Brown hyperthermia mechanisms, an evaluation of hyperthermia in a high viscosity medium is presented. Finally, the *in vitro* performances of both dimeric and hollow NPs were assessed with hyperthermia experiments on a MCF-7 breast cancer cell line.

6.2 Results and Discussion

Au - Fe oxide dimeric nanostructures (D-NPs) are prepared by a synthetic strategy involving the nucleation of the iron oxide domain, obtained via thermal decomposition of iron acetylacetonate on the surface of pre-existing Au NPs, used as "seeds" (see Experimental for details). To obtain hollow Fe oxide nanoparticles (H-NPs), a selective etching of Au from dimers was achieved by the same iodine treatment described previously (see chapter 5).[1]

Au seeds were prepared as described in the previous chapter. TEM imaging clearly evidences the dimeric morphology of

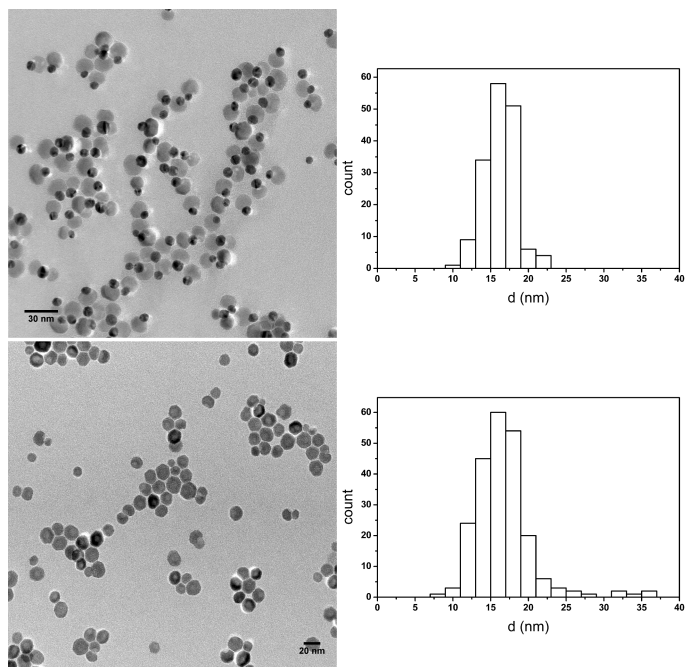


Figure 6.1: TEM micrographs and relative statistical analysis of Au - Fe oxides heterodimeric NPs (top) and hollow Fe oxides NPs (bottom).

the NPs, thanks to the different contrast between the Au and iron oxide domains (see figure 6.1). The TEM micrograph of the hollow nanostructures shows the characteristic cavity inside the iron oxide NPs. From the statistical analysis we observe that, in both samples, the iron oxide NPs have maintained their morphology after the iodine treatment, and they are still characterised by a mean core size of 17 ± 3 nm. With the aim to obtain a water-dispersed and biocompatible material, both dimeric and hollow NPs were functionalised through a ligand-place exchange reaction, with 2,3-dimercaptosuccinic acid (DMSA, see Experimental for details). To have an in-depth analysis of the properties of the material, we have to consider that all the transformations undergone after the synthesis may have effects on these properties. To evaluate all these effects, we characterised the material "as-prepared" and after functionalisation with the hydrophilic ligand.

Absorption spectrum of Au seeds (see figure 6.2) shows the typical surface plasmon band (SPB) at 521 nm, characteristic of Au NPs.[2] In D-NPs spectrum, we observe a damp, accompanied with a red-shift of the plasmon band, due to the presence of a material with high refractive index, like Fe oxide, at the interface. This behaviour has been widely observed and discussed.[3–6] The disappearance of the SPB in the spectrum of hollow NPs, together with the result of the TEM imaging, allows us to affirm that no residual nanometric Au is present in the H-NPs sample. In figure 6.3 is possible to observe the spectra of dimeric NPs before and after the functionalisation with DMSA. It is evident a change in the wavelength of maximum absorption

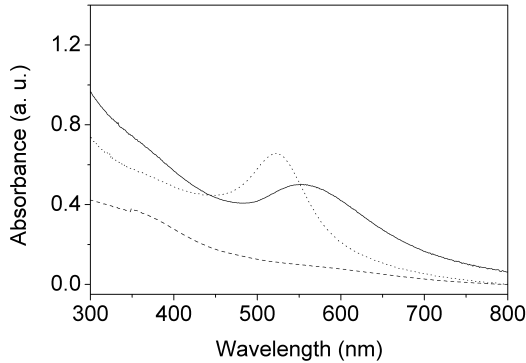


Figure 6.2: UV/vis spectra of Au seeds (dotted line), D- (solid line) and H-NPs (dashed line).

of the SPB of the system, ascribable to the change of dispersing medium, being the dielectric constants of chloroform and water respectively of 4.81 and 80.1 (at 20°C). It is well known that, in general, dispersing media with large dielectric constants will exhibit a larger polarisation when the SP is excited, leading to SPB with longer wavelength.[7]

The hyperthermia properties of the NPs samples were evaluated by measuring the temperature in a colloidal suspension of the NPs placed in an adiabatic holder during the application of an alternate external magnetic field (see Experimental and section 1.2 for details). Hyperthermia of NPs was evaluated at

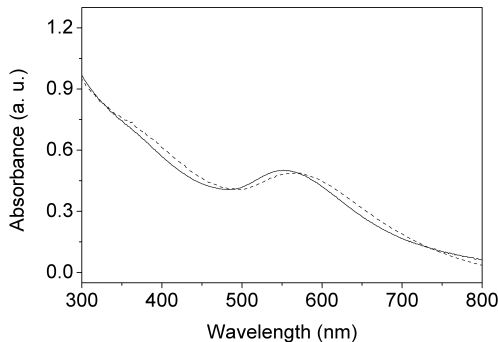


Figure 6.3: confrontation between chloroform dispersed (solid line) and water dispersed (dashed line) dimeric NPs.

several of field strengths, with a fixed frequency of 335.5 kHz. For the chloroform dispersion of NPs (see figure 6.4) it is possible to observe a great difference in SAR values between the dimeric and hollow NPs. In particular, the SAR of the latter result up to 8 times larger. This behaviour is similar to the one observed with dimeric and hollow structures described in the previous chapter. On the other hand, DMSA-coated water-dispersed D- and H-NPs exhibit much larger hyperthermia, as depicted in figure 6.5. SAR for chloroform dispersed D-NPs results up to 250 times lower than the same sample after the DMSA functionalisation. The water dispersed sample presents also very similar SAR between dimeric and hollow structures.

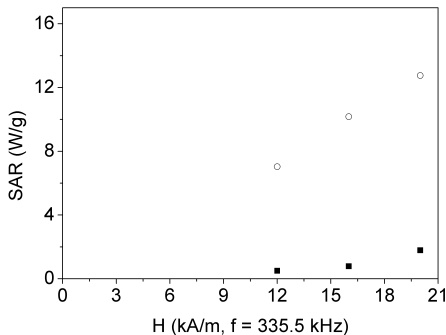


Figure 6.4: SAR values as a function of applied field, with a fixed frequency of 335.5 kHz, for dimeric (black squares) and hollow (white circles) NPs in chloroform suspension.

To better understand the different hyperthermia observed in the samples, we investigated the magnetic properties of dimeric and hollow NPs. The magnetisation vs applied field curves (reported in figure 6.6) evidence that both samples are superparamagnetic, with M_S values of 32 and 37 Am^2/kg , respectively for the dimeric and hollow NPs. Such values are far from the M_S value of bulk magnetite, 85 emu/g , [8] thus we hypothesised the presence of wustite together with magnetite in the Fe oxide part of the samples, as illustrated in the previous chapter. As already explained, wustite has been widely observed in dimeric Au - iron oxide systems prepared by similar synthetic methods. [3, 9–11] To verify this hypothesis, we measured the zero-field cooling - field cooling (ZFC/FC) magnetisation as a

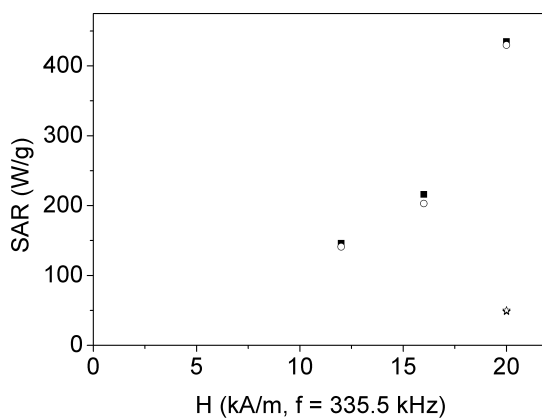


Figure 6.5: *SAR* values as a function of applied field, with a fixed frequency of 335.5 kHz, for DMSA-coated dimeric and hollow NPs (black squares and white circles, respectively) in water suspension. The black star and the white star indicate respectively the dimeric and hollow NPs in an agar gel suspension.

function of temperature, and the results are shown in figure 6.7. In particular, at T between 190 and 200 K, both D- and H-NPs curves exhibit a decrease in M , that can be ascribed to the transition between antiferromagnetic and paramagnetic regime for the wustite (T_N between 203 and 211 K, see section 1.2.4). It is well known that wustite can be easily oxidised to magnetite or maghemite, by leaving the sample in contact with air or water,[12] and an oxidation that preserve unchanged the Au domain would afford a dimeric material with the magnetic properties of magnetite. With this motivation, we decided to characterise the water-dispersed materials. The magnetic characterisation of the water dispersed NPs is shown in figure 6.8 and 6.9. M vs H curves show, for both samples, a M_S value of $90 \text{ Am}^2/\text{kg}$, and, in the ZFC/FC curves, the pattern previously described, ascribable to the presence of wustite, is not present anymore. This indicates the absence of wustite in the samples, due to a complete oxidation, hypothesis supported also by the high M_S value, typical of the superaparamagnetic magnetite or maghemite.

Our study aims to evaluate how the magnetic and hyperthermia properties do change after each step of the material preparation. An evaluation of the hyperthermia properties in a cellular-like environment is also required. To simulate this, we measured the hyperthermia of the NPs suspended in an agarose gel. In many cases, this gel was used to simulate real tissues,[13–15] especially to mimic the heat-transfer properties.[16–18] Moreover, the high viscosity of the agar gel can block the physical rotation of NPs, avoiding the Brown mechanism of magnetic

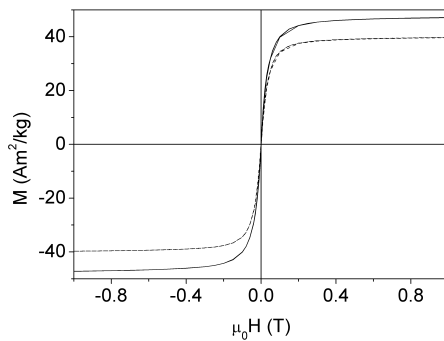


Figure 6.6: Magnetisation vs applied field curves of dimeric (dashed line) and hollow (solid line) NPs, measured at room temperature.

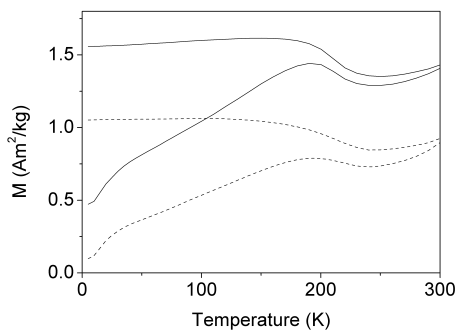


Figure 6.7: Zero-field cooling and field cooling magnetisation vs temperature curves, of heterodimeric (dashed line) and hollow NPs (solid line).

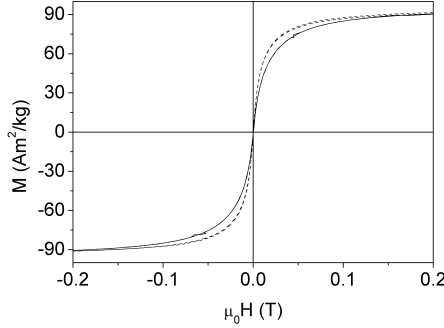


Figure 6.8: Magnetisation vs applied field curves of dimeric (dashed line) and hollow (solid line) NPs after the DMSA functionalisation, measured at room temperature.

hyperthermia. In such medium the Brown relaxation time τ_B is about 10^3 times smaller than the Néel one τ_N . [19] For this reason, hyperthermia measurement in agar gel is also useful to gain a better understanding of the mechanisms at the base of hyperthermia effects, *i.e.* Brown vs Néel contributions to the total heating. From recent studies exploiting this methodology reported in the literature, it was found that the optimum NPs size which gives a heating efficiency independent on the medium viscosity is 12 nm. [20] In this size range the heat generation is only due to the Néel relaxation, being the Brown contribution negligible. We tested hyperthermia in agar gel - suspended NPs with a magnetite concentration of about 1 mg/mL, $H = 20$ kA/m and $f = 335.5$ kHz. The results are depicted in figure 6.5

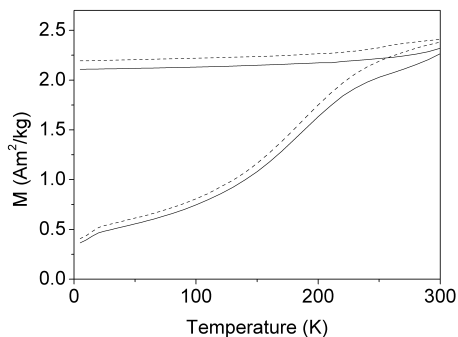


Figure 6.9: Zero-field cooling and field cooling magnetisation vs temperature curves, of heterodimeric (dashed line) and hollow NPs (solid line) after the DMSA functionalisation.

(black and white stars) and demonstrates that SAR , in high viscosity medium, decrease of more than 8 times, being the values of 48.4 and 50.1 W/g for respectively D- and H-NPs. Our study demonstrates that, for a particle size larger than the optimal one, there is a great difference between SAR values measured in water and in high viscosity media. Brown contribution to the total hyperthermia heating, in both dimeric and hollow NPs, can be roughly estimated in about 8 times larger than the Néel one. This result emphasises a problem almost overlooked in the literature, in which often NPs of size ranges giving the maximum hyperthermia contribute in water are indicated as the optimum ones, without taking into account the viscosity of the biological environment.

6.2.1 Possible application in nanomedicine

To evaluate the interaction between the dimeric and hollow NPs and cancer cells, the NPs were administered to MCF-7 breast cancer cells and both cellular uptake and cell death by magnetic hyperthermia were analysed. The NPs uptake was studied by TEM, and significant micrographs are reported in figure 6.10. The imaging of the fields considered showed NPs internalisation as a frequent event, in fact almost all the cells considered were involved. Low magnification analysis shows the presence of both extracellular agglomerates and intracellular membrane-bound vacuoles containing numerous NPs. At high magnification the appearance of these intracellular vacuoles is approximately spheroidal in shape with a size of 200 to 500 μm in diameter and light at the staining characteristics of endosomes. Multiple endosomes for cells with engulfed NPs were present within the cytoplasm. No lysosomes containing NPs were detected. Regarding the internalisation mechanisms, plasma membrane invagination in proximity of NPs was frequently detected, sign of macropinocytosis mechanism. As morphology, both the lamellipodia-like and blebs macropinocytosis were observed. Less frequently some NPs were detectable in the cytoplasm without membrane-bound vacuoles, indicating an escape from the vesicles or an alternative uptake mechanism. The structure of both kinds of dimeric and hollow NPs within the endosomes was checked with the NPs structure after cell treatment. No differences between the two NP types were detected in the internalisation and compartmentalisation mechanisms. No morphological signs of apoptosis were detected during the ultrastructural

analysis.

In vitro hyperthermia experiments were performed at $H = 14.3$ kA/m and $f = 396$ kHz on a pellet of MCF-7 breast cancer cells incubated with NPs, both dimeric and hollow. Control experiments on the cells incubated without NPs have been carried out to analyse possible effects of the magnetic field application. Figure 6.11 shows the histograms of cell viability, assessed by Trypan blue staining, 24 hours after the application of the magnetic field. Histograms shows that there is a cell death of about 30% induced by magnetic hyperthermia for either dimeric and hollow NPs. Negligible cell death is observed in control experiments, and NPs cytotoxicity is excluded by a measurement of the cell viability on MCF-7 incubated with NPs, but without any magnetic field application.

6.3 Conclusions

In conclusion, in this chapter a thorough characterisation of the magnetic properties of the dimeric and hollow NPs is presented. Wustite was demonstrated to be present in the dimeric NPs "as-prepared" by the ZFC/FC measurements. Functionalisation of NPs with DMSA provide, at the same time, a water dispersability of the system and a complete oxidation of wustite, with a consequent large increase of the magnetic properties and of the hyperthermia. In high viscosity media these nanoparticles do not behave as well as in water in terms of heat generation, but anyway they show remarkable performances *in vitro*.

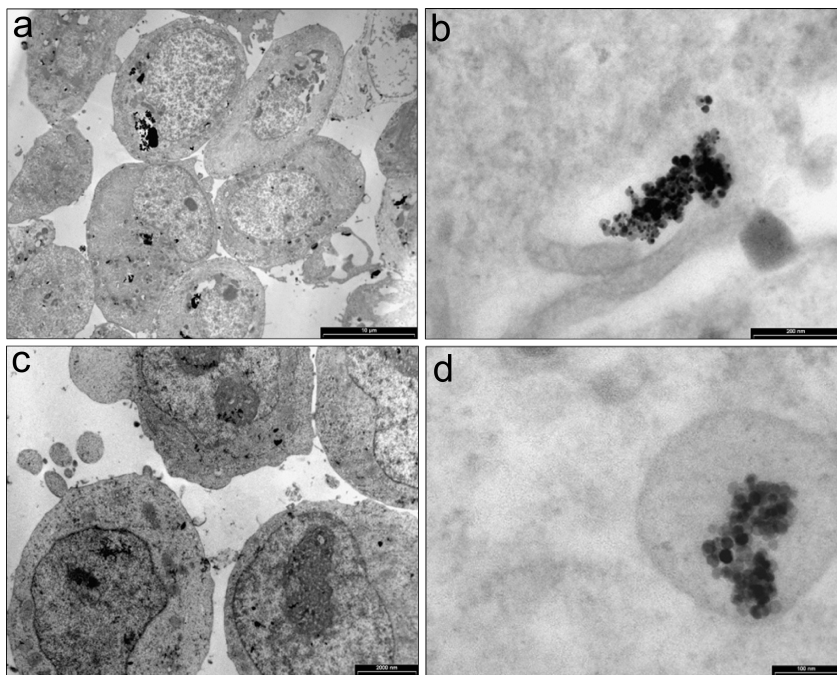


Figure 6.10: Internalisation of dimeric (a,b) and hollow (c and d) NPs in cells.

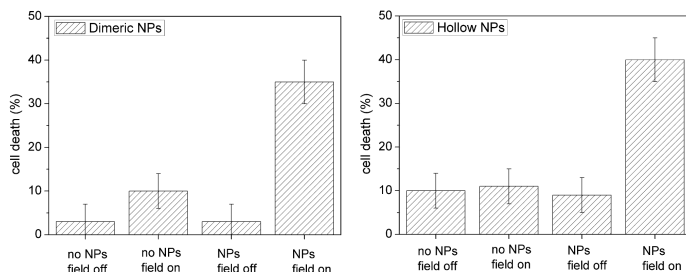


Figure 6.11: cell death evaluation for magnetic hyperthermia with dimeric (left) and hollow (right) NPs internalised in cells.

6.4 Experimental

Synthesis of Au NPs: Synthesis of Au seeds was carried out following an already known procedure.[10] 40 mg of HAuCl_4 (ca 0.12 mmol) were dissolved in 2 mL of 1-octadecene by the addition of 0.4 mL of oleylamine (0.12 mmol) and added to a degassed solution of oleylamine (2 mL, 6 mmol) and 1-octadecene (8 mL). Temperature was raised to 120 °C and kept for 20 minutes. The colour change from yellow to colorless to a final characteristic purple-violet is clearly noted.

Synthesis of dimeric NPs: In a round bottomed flask 0.96 mL of oleylamine (ca 3 mmol) and 350 mg of $\text{Fe}(\text{acac})_3$ was heated to 140 °C in 30 mL of 1-octadecene, under a blanket of nitrogen and magnetic stirring. The Au seeds solution was

then added, together with 0.16 mL of oleylamine (ca 0.5 mmol) and 1.15 g of 1,2-tetradecanediol (5 mmol) and the temperature was raised to 300 °C. After 45 minutes, the mixture was gently cooled to room temperature and the crude product was centrifuged at least two times with the addition of acetone as a counter solvent to eliminate any residue of the reaction. Precipitated nanoparticles were then redispersed in chloroform or toluene.

Synthesis of hollow NPs: to a chloroform suspension of the core-shell nanocrystals, 0.5 mL of a 10^{-2} M solution of I_2 in chloroform were added. The system was allowed to stir mechanically for 16 hours. Decanted macroscopic Au was removed and hollow NPs were purified by at least 2 cycles of ultracentrifugation.

Water-dispersed NPs: in a 20 mL reaction tube, 10 mL of toluene dispersion of nanoparticles (5 mg) and 20 mg of DMSA, dissolved in 1 mL of dimethylsulfoxide, were added. The mixture was left under mechanical stirring for 4 hours. After precipitation of nanoparticles, supernatant was removed and product was redispersed in ethanol and washed several times by ultracentrifugation. NPs were then dispersed in water and pH was adjusted to 10 using NaOH.

Agarose gel-dispersed NPs: 1 mL colloidal suspension of NPs was introduced in a 1.5 mL glass vial (Nanoscale BioMagnetics), and 20 mg of agarose (Seakem LE Agarose - Lonza) were added. The system was heated to 100°C and then cooled to room temperature in a ultrasonic bath, to allow a good dis-

persion of NPs in the suspension during the gelation process.

UV – visible spectroscopy: UV/vis spectra were collected by an Evolution 260 Bio spectrophotometer, by Thermo Scientific, using quartz cuvettes with an optical path of 1 cm.

Magnetometry: Room-temperature magnetisation curves have been measured by an alternating gradient force magnetometer (AGFM) and a Quantum Design MPMS SQUID magnetometer, operating in the 1.8 - 350 K temperature range.

Hyperthermia: magnetic hyperthermia heating in the presence of an alternate magnetic field was measured using the nanoScale Biomagnetics DM100 apparatus, directly on colloidal nanoparticles suspension at about 1 mg/mL concentration. The same apparatus was used to apply the alternate magnetic field for in vitro experiments. For hyperthermia experiments in cells, alternate field of $f = 396$ kHz and $H = 14.3$ kA/m was applied for 50 minutes, keeping a stable temperature of 37 °C.

Cell culture for in vitro experiments: For in vitro magnetic hyperthermia experiments, MCF-7 breast cancer cells were seeded into a cell culture flask. NPs suspension was added at concentrations of 250 $\mu\text{g}(\text{Fe}_3\text{O}_4)/\text{mL}$. Cells were incubated overnight at 37 °C. Next day, cells were collected, washed four times with fresh medium to remove the non-incorporated NPs and re-suspended in the medium to obtain a final amount of $2 \cdot 10^6$ cells in a volume of 300 μL . After hyperthermia experiment, the cell viability was assessed by Trypan blue staining.

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Chapter 7

Conclusions

This thesis aims with the control of the properties of materials at the nanoscale, mainly by developing new synthetic methods. In the first chapter, a tuning of morphology and magnetic properties of magnetite nanoparticles is presented. It was achieved thanks to a completely new approach, which involves the oriented aggregation of NPs during the synthesis. This method may be surely applicable to the preparation of other metal oxide NPs by thermal decomposition of the opportune precursors. Products obtained, in particular multi-core nanoparticles, show remarkable magnetic and colloidal properties, making them very interesting for biomedical applications.

The synthesis and functionalisation of plasmonic materials was faced in the second chapter. Here, a supramolecular approach was exploited to achieve an aggregation between Au and Ag

NPs followed by UV - visible spectroscopy.

In the final chapter, the conjugation of plasmonic and magnetic functionalities was faced by the preparation of dimeric nanostructures. Au - Fe oxide heterodimeric nanoparticles were prepared and their magnetic properties characterised. The results demonstrate the formation of FeO (wustite), together with magnetite, during the thermal decomposition of the iron precursor, even if it is a Fe(III) complex. This is in agreement with a stabilisation of the wustite phase by the presence of gold, already hypothesised in the literature. By an oxidation process that preserves Au in the dimer, wustite completely disappeared, with the formation of either magnetite or maghemite, better from the magnetic point of view. Hyperthermia was monitored after each post-synthetic step, and experiments *in vitro* demonstrated remarkable properties for these structures.

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