SYNTHESIS AND CHARACTERIZATION OF HYBRID NANOSTRUCTURED MATERIALS

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SYNTHESIS AND CHARACTERIZATION
OF HYBRID NANOSTRUCTURED
MATERIALS

Memoria que presenta Marcos Sanles Sobrido
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Que Marcos Sanles Sobrido, licenciado en Química, ha realizado en el Departamento de Química Física de la Universidad de Vigo bajo su dirección el trabajo descrito en la presente memoria, que lleva por título “SYNTHESIS AND CHARACTERIZATION OF HYBRID NANOSTRUCTURED MATERIALS”, y que presenta para optar al grado de Doctor.

Vigo, Enero de 2013

Fdo. Luis Manuel Liz Marzán
Fdo. Miguel Ángel Correa Duarte
Para mis amigos y familia
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The aim of the work presented in this thesis comprises the synthesis of new hybrid nano-structured materials, as well as the study of their optical, catalytic or magnetic properties. The interest on these materials stems from their unique nanosize-dependent properties, different from those observed in bulk materials. Such properties have rendered those structures as ideal objects for a considerable number of potential applications. This could be the case of integrating various dissimilar nanomaterials in a single device for the simultaneous sensing of multiple physical phenomena.

Thus, various hybrid nano-materials with optical, magnetic and/or catalytic properties have been developed in this work. Two different templates, carbon nanotubes (CNT) and polystyrene spherical colloids (PS), have been used as supports for the fabrication of hybrid nanostructures. In this task it was of great importance the synthesis of metallic nanoparticles and the layer by layer self-assembly technique. Such technique, based on the electrostatic interactions between different components, allowed us to integrate various components in a single nano-material.

So, we can divide this work in two main parts:

The first part covers the synthesis of hybrid nanostructured materials based on CNTs that were used as templates because of their excellent intrinsic structure-dependent properties. These nanotubes were use as templates for the deposition of metallic platinum and silver nanoparticles. In the first case we have doped CNTs with different types of platinum nanoparticles, spherical and dendritic, which allowed as to identify the high catalytic activity of the dendritic shape particles by using an electron transfer reaction as probe.

In the case of the CNTs coated with silver nanoparticles, we intend to develop a stable substrate for SERS-based applications in order to provide higher enhancement factors of the Raman signal. In this chapter we used this material to determine if a bio-analyte, in this case a derivate of cocaine, is or is not present in a sample and how much is present. First we use this substrate to perform the label-free indirect ultra-detection of the bio-
analyte in question through an antibody assembled onto the surface of the substrate. The comparison between this indirect detection and a direct detection using the same substrate revealed that, whereas the latter offers a higher detection limit than the specific indirect method, label-free indirect detection offers quantitative results at physiological levels, so that not only drug abuse can be demonstrated, but their consumption can also be quantified with a portable, non-invasive and fast technique.

The synthesis of new hybrid nanostructured materials, using spherical particles as support, has been explored in the second part of this work. Monodisperse Colloidal particles of polystyrene were chosen as templates because of their commercial availability in a wide range of sizes, and also because they can be easily removed by means of dissolution with organic solvents or thermal decomposition. In this regard, the deposition of metal nanoparticles onto spherical polystyrene colloids is a useful strategy for the fabrication of hybrid materials with unique properties, which are dependent on the nature of the nanoparticles deposited. Thus, in this work platinum and gold nanoparticles have been deposited onto the surface of polystyrene colloids. In a first example, the catalytic activity of platinum nanocatalyst previously deposited onto polystyrene beads has been exploited for the controlled deposition of a magnetic material onto them, thereby tuning the magnetic properties of the nanostructure.

The fact that these polystyrene colloids can be easily dissolved in organic solvents makes them very good candidates for obtaining hollow capsules. It is for that reason that polystyrene templates were coated with a homogeneous and porous silica shell, which allows removing the polystyrene while preserving the hollow structure. Moreover, porous nanocapsules may have different properties and applications attending to the nature of a material encapsulated in their inner space. As an example, the encapsulation of single crystal dendritic platinum nanoparticles renders highly active nanocapsules that were used for the confined deposition of a magnetic material. In a different work, gold nanoparticles encapsulated in silica capsules were shown as attractive optically active substrates for surface enhanced Raman scattering (SERS) - based applications, allowing the fabrication of SERS-encoded submicrometer particles with shape and size uniformity for use in antigen biosensing.

Finally, the deposition of CNTs onto the surface of polystyrene beads was attempted as strategy to increase the complexity and functionality of the hybrid material that resemble natural magnificent sea anemones. Thus, the nanotubes play two main roles, helping to reinforce the hollow structure as well as permitting to increase and tune their surface area. Additionally, the incorporation of magnetic nanoparticles into these structures provides them with an extra magnetic functionality, which allows this material to be manipulated by an external magnetic field.
The research described in this thesis was initiated in the context of the work carried out in the Colloid Chemistry Group, in which the research activity is focused on the synthesis, characterization, and optical properties of metal nanoparticles and their surface modification. Thus, the experience of the group has been crucial for the development of new nanostructured hybrid materials presented here in.

In this thesis the chapters are structured by published papers, and this structure allows us to develop in each chapter the protocol for the synthesis of the material and application. The application of hybrid nanostructured material is directly related to material properties.
Thesis Scope
CHAPTER 1

1 General introduction

1.1. Hybrid Nanostructured Materials: Interest
    1.1.1. Carbon nanotubes as templates
    1.1.2. Spherical colloidal as templates
1.2. Magnetic properties at the nanoscale
1.3. Catalytic properties of metallic nanoparticles
1.4. Optical properties of nanometals (Surface Plasmon Resonances and Surface Enhanced Raman Scattering)
1.1 Hybrid Nanostructured Materials: Interest

Materials in the nanometer scale,\textsuperscript{1a,b} such as colloidal dispersions and thin films, have been subject of intense study over the last decades due to their remarkable properties, which are dictated by their size. In particular, nanomaterials incorporating metal nanoparticles\textsuperscript{2} have raised a large deal of interest because of their special electronic, optical, magnetic and catalytic properties. These materials present properties that differ from those of any others because their properties are greatly affected not only by their size but also by their shape, making crucial the development of synthetic strategies to control the nanoparticle morphology. In this regard, several studies have pointed out the promising potential of metal nanoparticles in applications such as drug delivery, catalysis, nanomedicine or sensing devices\textsuperscript{3}. It is therefore necessary to develop effective strategies for the controlled, reliable and predictably preparation of nanomaterials so as to address the growing demand of nanotechnology in the synthesis and processing of materials.

Hybrid nanostructured materials\textsuperscript{4,5} can be defined as those with at least one dimension falling in the nanometer range, and which include nanoparticles, nanowires and nanotubes, bulk materials made of nanoscale building blocks or consisting of nanoscale structures. These materials include two types of functionalities at the molecular scale, and are usually composed of an organic and an inorganic part. Many hybrid materials exist in nature, in which the inorganic part provides stability while the organic part acts as a link to soft tissues. In this way, the combination at the nanosize level of inorganic and organic or even bioactive components in a single material has made accessible an immense new area of materials science, which may have extraordinary implications in the development of multifunctional materials.

The most common approaches for the fabrication of these materials are:

1. Template- based
2. Colloidal
3. Sol–gel
4. Electrodeposition

The “\textit{template method}” is one of the most relevant approaches for the fabrication of hybrid nanostructured materials. This category can be divided according to the nature of the template into hard-template and soft-template methods.

\textsuperscript{3} Guozhong Cao, “Nanostructured and Nanomaterials, synthesis properties and applications”, Imperial college press, 2004.
\textsuperscript{5} C. C. Koch "Nanostructured Materials: Processing, Properties, and Applications", William Andrew
In hard-template strategies, one material is deposited on the surface of another. If the template is organic, it may subsequently be washed away or burnt out, leaving a hollow center if desired. Alternatively another material can be grown inside, where the physical size of the cavity limits its growth. In soft-template strategies, the growth of nanostructured materials can be regulated within self-organized, biological and synthetic organic membrane assemblies (e.g. micelles, microemulsions, liposomes and vesicles). The molecules constituting these assemblies comprise a polar head group and a nonpolar hydrocarbon tail, which self-assemble into membrane structures in an aqueous environment. Aqueous phase reverse micelles have diameters in the range of 3–6 nm, whereas microemulsions possess diameters about 5–100 nm, though in some cases the morphology of the micelles may be irregular instead of spherical. From the structures of these self-organized membranes, mainly spherical nanoparticles with tunable size can be obtained.

Among the other three approaches, the “colloidal method” is convenient and inexpensive, and it has been extensively used for the production of nanostructured materials. This method is based upon the growth of nanometer-sized particles within an aqueous solution to form a colloidal sol. Generally speaking, a finely dispersed system is in a high free energy state. The colloidal material will therefore tend to aggregate due to attractive van der Waals forces, but this can be prevented by electrostatic stabilization mediated by surface adsorbed ions or by steric stabilization in the presence of bulky polymers or hydrophobic ligands.

The “sol-gel method” involves a set of chemical reactions that irreversibly convert a homogeneous solution of particle precursors (a sol) into an infinite molecular weight three-dimensional polymer (a gel), forming an elastic solid filling the same volume as the solution. Typically this involves a hydrolysis reaction followed by condensation polymerization, for example:

The main benefits of sol–gel processing are the high purity and uniform nanostructure achievable at low temperatures. The method is often used to produce metal-oxide nanomaterials. Further processing usually involves forming the gel using a number of techniques followed by gentle drying to remove the solvent (this often leads to
shrinkage and therefore cracks during this process should be prevented). Gels can be cast and molded to form a microporous preform, which is dried in order to produce a monolithic bulk material (e.g., a xerogel or an aerogel) commonly used for the fabrication of filters and membranes. They can also be spin coated or dipped to produce thin (typically 50–500 nm) films on substrates. These films are used for electronic thin film devices, for wear, chemical or oxidation protection, as well as for their optical properties (e.g., anti-reflection). Alternately, fibers can be drawn from the gel (e.g., silica fibers for light transmission).

Finally, the "electrodeposition method" involves inducing chemical reactions in an aqueous electrolyte solution by means of an applied voltage. This method, including electroless deposition, is widely applied in the synthesis of one-dimensional nanostructured materials, typically with the aid of templates.

The development of more complex characterization techniques, the potential for applications in many different fields, as well as the interest in imitating nature, has rendered the synthesis of hybrid nanostructured materials a busy area of research. In this regard, materials scientists continue to develop novel hybrid nanomaterials, which makes very hard to review all reported devices and applications that have been developed over the past few years. Moreover, it is even more difficult to predict which will be the new developments and applications of these new kinds of materials.

For the materials developed in this work, we used two different types of templates, namely multiwall carbon nanotubes (MWNT), and colloidal polystyrene spheres (PS). The different shape and dimensionality of these two different template systems have allowed us to develop a number of new hybrid nanosystems with a variety of potential applications.

1.1.1 Carbon nanotubes as templates

Carbon nanotubes have been produced and observed under a variety of conditions prior to 1991. A paper by Oberlin, Endo, and Koyama published in 1976 clearly showed hollow carbon fibers with nanometer-scale diameters using a vapor-growth technique. Additionally, the authors show a transmission electron microscope (TEM) image of a nanotube consisting of a single wall of graphene. Later, Endo has referred to this image as a single-walled nanotube. But, it was not until the early nineties when Iijima observed the structure of carbon nanotubes (CNTs) using a TEM, which can be considered as the start of their study. CNTs can be defined as graphene sheets rolled around a hollow cylinder. It is possible to differentiate between single wall carbon nanotubes (SWNTs), or multiwall carbon nanotubes (MWNTs), if they are composed of one or several concentric graphene sheets respectively (Fig 1). Since the discovery of

carbon nanotubes, they have been the subject of intense research because of their exceptional structure-dependent electrical, optical and mechanical properties\textsuperscript{8,9,10,11,12}. Their unique properties arise from the sp\textsuperscript{2} graphite hybridization, to which additional quantum confinement and topological constraints are imposed in the circumferential direction of the nanotube. CNTs may also contain different types of hybridization, sp, sp\textsuperscript{2}, sp\textsuperscript{3}, in their structure, which enables them to be more or less reactive.

![Fig1: Representation of a SWNT and a MWNT](image)

To describe the structure of the CNTs, the so-called Hamada indexes (n and m) are used, which determine both diameter and wrapping direction of the nanotubes.

According to the different ways in which the sheets of graphite can be rolled up, CNTs can be classified as follows:

- Armchair \( n = m \)
- ZigZag \( n = 0 \) or \( m = 0 \)
- Chiral any values of n and m

It has been shown that depending of the values of such indexes the CNTs can have metallic (when \( n-m = 3p \), p being an integer) or semiconducting (all other values of n and m) behavior. Additionally, the electrical properties of the nanotubes can be manipulated by using current-induced oxidation to break down systematically the outermost shells, in a layer-by-layer fashion. Moreover, the nanotube energy-band structure can be manipulated through both, doping or introducing defects or distortions in their structure.

In recent years considerable efforts have been made to prepare different carbon morphologies and explore their applications in various fields, including composite materials, electronic devices and nanoscale sensors, among others\textsuperscript{13,14,15,16,17,18}.  

\textsuperscript{13} Simmons, Trevor; Hashim, D; Vajtai, R; Ajayan, PM. J. Am. Chem. Soc. 2007, 129 (33), 10088–10089
Furthermore, recent research has shown that apart from their excellent structure-dependent properties, CNTs possess a high aspect ratio (length/diameter) and large surface area, which makes them ideal substrates for the deposition of different materials. The combination of nanotubes with different types of nanoparticles allows their integration in new hybrid nanomaterials that combine the properties of both components, thereby expanding their applications in various fields, such as catalysis and various areas of nanotechnology.\textsuperscript{19}

However, a major barrier for studying the properties and for the development of products made of CNTs is found in their poor solubility and processability. Thus, most of the applications proposed for CNTs have been shown to be strongly dependent on the development of strategies for functionalizing, processing and/or assembling the nanotube themselves, mainly because their surface is rather inert, rendering very difficult implementing any type of the proposed mechanisms or techniques. This is for example the case for the deposition of a certain material onto CNT walls, since their inertness makes it very difficult to control the final homogeneity. It is therefore very important to explore feasible techniques whereby surface modification would serve as a previous functionalization that guarantees the deposition of this material onto the surface of the CNTs.

The different approaches for functionalization of CNTs that have already been developed can be classified as defect-site chemistry, covalent side-wall functionalization and non-covalent functionalization (figure 2). For further information, the reader is directed to recent reviews\textsuperscript{20,21} that provide a very complete description over CNTs functionalization.

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\bibitem{17} Alberto Bianco, Kostas Kostarelos and Maurizio Prato, Current Opinion in Chemical Biology 2005, 9, 674–679
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General Introduction

Fig 2: Scheme showing the different types of SWCNT functionalization, which however can be generalized to MWCNTs: (A) Defect-site functionalization, (B) covalent side wall functionalization, (C) non-covalent functionalization, (D) wrapping, (E) endohedral functionalization. Copyright 2002 Wiley-VCH

- **Defect-site chemistry**

Defect-site chemistry exploits the intrinsic defect sites existing both at the ends and on the sidewalls of CNTs, as a result of the synthetic process. The purification process of CNTs commonly involves the use of strong acids to remove the catalytic particles necessary for the synthesis and this treatment helps generating such intrinsic defects due to their highly oxidative action. The process therefore results in the formation of holes with oxygenated functional groups like carboxylic acids or alcohol groups among others\(^\text{22}\), which are promising starting points for the attachment of particles, molecular moieties or for further coordination chemistry at these sites.

- **Covalent-sidewall functionalization**

Covalent-sidewall functionalization is based on the chemical reactivity of the CNTs, related with the pyramidalization of the sp\(^2\)-hybridized carbon atoms and the \(\pi\)-orbital misalignment between adjacent carbon atoms\(^\text{23}\). This pyramidalization and misalignment scale inversely with tube diameter, CNTs becoming more reactive as their diameter is decreased. This agrees with the fact that fullerenes have a higher surface reactivity\(^\text{24}\) (which depends strongly on their curvature) compared to SWNTs which have no strongly curved regions that could serve for direct additions. This statement also explains why side-functionalization of SWNTs by covalent-bond formation needs highly reactive reagents\(^\text{25,26}\). Some of the usual routes reported for functionalization are fluorination\(^\text{27,28}\), ozonolysis\(^\text{29,30,31}\), osmylation\(^\text{25}\), or organic functionalization\(^\text{18,26,32,33}\).

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\(^{24}\) A. Hirsch, Topics in Current Chemistry 1999, 1

• **Endohedral functionalization**

Endohedral functionalization comprises the use of the inner cavity of CNTs for the storage of molecules or compounds since their interaction takes place with the inner surface of the sidewalls, which is very convenient to carry out confined reactions inside the CNTs.

• **Non-covalent functionalization**

Non-covalent functionalization comprises the dispersion of CNTs in aqueous solution, by means of surface active molecules as sodium dodecylsulfate (SDS)\textsuperscript{34,35,36} or by wrapping them with polymers\textsuperscript{37,38,39}. While the first accommodate the CNTs in their hydrophobic interiors (sometimes by strong \(\pi-\pi\)-stacking interactions with the CNT sidewall if the hydrophobic part contains an aromatic group\textsuperscript{40}), the second one implies an association of the polymers with the sides of the CNTs, based on the hydrophobic thermodynamic preference of CNT-polymer interactions compared to CNT-water interactions, thereby suppressing the hydrophobic surface of the CNTs\textsuperscript{41}.

In summary, defect-side functionalization preserves the electronic structure of the CNTs, since they can tolerate a number of defects before losing their unique electronic and mechanical properties. Covalent-sidewall functionalization generates a high degree of functionalization rendering this method very useful for composite formation. However, the destruction of the \(sp^2\)-hybridized structure may result in a loss of thermal conductivity, reducing the maximum buckling force or changing their electronic properties, so that semiconductor rather than metallic behavior is displayed. Finally, non-covalent functionalization has the main advantage that it preserves both the

\textsuperscript{28} C. W. Bauschlicher, Chemical Physics Letters 2000, 322, 237.
\textsuperscript{29} Y. Lu, Y. Yin, Z.-Y. Li, Y. Xia, Nano Letters 2002, 2, 785.
\textsuperscript{31} S. Banerjee, M. G. C. Kahn, S. S. Wong, Chemistry--A European Journal 2003, 9, 1898.
\textsuperscript{32} C. A. Dyke, J. M. Tour, Journal of the American Chemical Society 2003, 125, 1156.
electronic properties and the structure of the CNTs by maintaining the intrinsic nanotube sp²-hybridization.

We can thus conclude that the main advantages of CNTs as supporting materials, in general, are:

- High surface area to volume ratio
- Thermal and electrical conductivity
- Chemical inertness
- Lack of porosity

### 1.1.2 Spherical Colloidal as templates.

The property dimension of colloidal nanoparticles is in the range of the nanometer scale.⁴²,⁴³ Since the pioneering work by Faraday and Graham⁴⁴ over 150 years ago, colloidal particles have been studied in different contexts due to their numerous applications in different fields such as chemistry, biology, materials science, condensed matter physics, applied optics, and fluid dynamics. Most of the studies on colloidal particles⁴⁵ require the preparation of monodisperse samples that are uniform in size, shape, composition, and surface properties. One of the reasons behind the use of spherical colloids as templates is that they represent a well-established and highly reproducible monodisperse system, and also because the spherical morphology helps to minimize the interfacial free energy⁴⁶ of the particles.

Therefore, spherical colloids are often used as supports for the synthesis of hybrid nanostructured materials because they allow the fabrication of monodisperse structures. Thus, the surface modification and functionalization of these colloidal nanostructures has opened a new avenue for the development and application of nanomaterials.

In recent years a large number of methods for producing colloidal particles with well-defined core-shell structure have been developed⁴⁷,⁴⁸,⁴⁹. The properties of such materials not only depend on the core material used but also on the nature of the shell. This offers a wide flexibility, since a single type of core material can be coated with many different

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⁴⁸ M. Ohmori; E. J. Matijevic, Colloid Interface Sci. 1993, 160, 288
⁴⁹ D. A. Antelmi; O. Spalla, Langmuir 1999, 15, 7478
shell materials, such as graphene oxide\textsuperscript{50}, magnetite\textsuperscript{51}, titanium dioxide\textsuperscript{52,53} or carbon nanotubes,\textsuperscript{54} among others. Similarly, the same type of material can be used to coat particles of different composition, size and shape. In this respect, the well-known Stöber method\textsuperscript{55} (base-catalyzed hydrolysis and condensation of alkoxy silanes) deserves particular attention, since it can be used to coat almost any type of colloidal nanoparticles with uniform silica shells, while allowing a tight control over shell thickness. However, application of the Stöber protocol to specific templates often requires the introduction of some modifications\textsuperscript{56}. One of the most common strategies used to obtain core-shell nanoparticles with dense silica coatings comprises on the surface functionalization of the cores with bifunctional molecules that can enhance the coupling (and thus deposition) of silica monomers or oligomers onto this surface\textsuperscript{57}. Another widely used approach comprises the use of charged polyelectrolytes to grow multilayers in the so called layer-by-layer assembly technique. This functionalization of the colloids contributes to modulate the surface charge through derivatization with appropriate functional groups\textsuperscript{58}. 

The growth of silica shells has been exploited to increase the complexity and diversity of colloidal systems in several ways.

1. Surface charge and surface reactivity of colloidal systems can be modified through silica coating, which in turn increases the stability and usefulness of these systems.\textsuperscript{24} The properties (optical, mechanical, electrical, rheological, catalytic) of core-shell particles can be tailored in a controllable fashion by independently altering the composition, dimension, and structure of the cores or the shells.

2. The formation of shells also provides a natural vehicle to incorporate different materials into the same structure and thus to obtain hybrid, multifunctional colloidal particles\textsuperscript{59}.

3. Core-shell particles have also been demonstrated as a new class of hybrid nanostructured materials to fabricate colloidal crystals with photonic properties different from those based on the conventional plain particles\textsuperscript{60}.

\textsuperscript{50} Jinkee Hong, Kookheon Char, and Byeong-Su Kim, J. Phys. Chem. Lett. 2010, 1, 3442–3445
\textsuperscript{51} Teruaki Fuchigami, Ryo Kawamura, Yoshitaka Kitamoto, Masaru Nakagawa, and Yoshihisa Namiki, Langmuir 2011, 27, 2923–2928
\textsuperscript{52} Mukesh Agrawal, Smrati Gupta, Andrij Pich, Nikolaos E. Zafeiropoulos, Jorge Rubio-Retama, Dieter Jehnichen, and Manfred Stamm, Langmuir 2010, 26(22), 17649–17655
\textsuperscript{53} Xiao-Feng Guo, Yong-Suk Kim, and Geon-Joong Kim, J. Phys. Chem. C, 2009, 113, 19
\textsuperscript{55} Stöber, W.; Fink, A.; Bohn, E. J. Colloid Interface Sci. 1968, 26, 62.
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\textsuperscript{58} F. Caruso, Chem.-Eur. J. 2000, 6, 413
\textsuperscript{59} K. P. Velikov; A. Moroz; Van Blaaderen, A. Appl. Phys. Lett. 2002, 80, 1, 49
\textsuperscript{60} Y. Lu; Y. Yin; Y. Xia, Nano Lett. 2002, 2, 785
Core-shell particles can also be used as precursors to obtain hollow structures upon removal of the core\textsuperscript{61}. As a result of their unique features, these structures can be exploited as nanoreactors, drug delivery systems or sensors\textsuperscript{62,63,64}. In this regard, hollow capsules can be generated using different kinds of templates, including hard (colloidal particles) or soft (micelles, vesicles, emulsions\textsuperscript{65,66} or bubbles\textsuperscript{67,68}) templates. The use of hard templates presents various advantages such as high monodispersity and the easy control over shell formation. Thus, the hybrid structures can be converted into hollow spheres by selectively removing the cores by etching or calcination depending on their nature. The void size of the hollow spheres is precisely determined by the diameter of the initial colloidal templates, whereas the wall thickness can be tuned through the shell growth conditions. Due to their monodispersity in size and shape, these core-shell and hollow particles can also serve as building blocks to form crystalline lattices exhibiting photonic properties that are different from those obtained through the assembly of either polystyrene or silica colloids.

1.2. Magnetic properties at the nanoscale

Recent advancement in the synthesis of hybrid nanostructured materials has greatly enhanced our ability to manipulate the magnetic properties of solid materials (particularly at the nanometer scale). Thus, the magnetization of a material can be expressed in terms of the density of net magnetic dipole moments “m” in the material. The vector $\mathbf{M}$ (magnetization) and the total magnetic field in the material $\mathbf{B}$ are expressed in terms of volume (V) by

$$\mathbf{M} = \frac{\mu}{V} \quad \text{(EQ. 1)}$$

$$\mathbf{B} = \mu_0 (H + M) \quad \text{(EQ. 2)}$$

where $\mu$ is the magnetic field inside the material, $\mu_0$ is the magnetic permeability of vacuum and H is the externally applied magnetic field (magnetic field strength). $\mu_r$ is called the relative permeability and is related to $\mu$ and $\mu_0$ by

$$\mu = \mu_r \mu_0 \quad \text{(EQ. 3)}$$

Another commonly used magnetic quantity is the magnetic susceptibility ($\chi_m$) which specifies how much the relative permeability differs from one.

$$\chi_m = \mu_r - 1 \quad \text{(EQ. 4)}$$

\textsuperscript{61} Z. Zhong; Y. Yin; B. Gates; Y. Xia, Adv. Mater. 2000, 12, 206
\textsuperscript{62} Yong Zhao and Lei Jiang. Adv. Mater. 2009, 21, 3621-3638
\textsuperscript{63} Yu Lu, Joe McLellan, Younan Xia. Langmuir 2004, 20, 3469-3470
\textsuperscript{64} Kaori Kamata, Yu Lu, Younan Xia. J. AM. CHEM. SOC. 2003, 125, 2384-2385
\textsuperscript{65} Wang, J. W.; Xia, Y. D.; Wang, W. X.; Poliakoff, M.; Mokaya, R.J. Mater. Chem. 2006, 16(18), 1751–1756
\textsuperscript{68} Ogawa, M.; Yamamoto, N. Langmuir 1999, 15(6), 2227–2229
According to the arrangement of their magnetic dipoles in the absence and presence of an external magnetic field, materials can be classified depending on the response to the action of an external magnetic field as follows$^{69,70}$.

- Diamagnetic
- Paramagnetic
- Ferromagnetic
- Ferrimagnetic
- Antiferromagnetic

![Figure 3: Schematic illustration of the arrangements of magnetic dipoles for five different types of materials in the absence or presence of an external magnetic field (H).](image)

- **Diamagnetic Materials**: are materials with no unpaired electrons (magnetic dipoles), and as a result they display no net magnetic moment in the absence of an external field and weak induced dipoles in the presence of a field, arising from the realignment of the electron orbits. The magnetization of a diamagnet responds in the opposite direction to the external field.

  \[ M = \chi_m H = C \cdot H / T \]  
  \[ \text{(EQ.5)} \]

  where $\chi_m$ is the magnetic susceptibility, $T$ is the temperature, and $C$ is a material-specific Curie constant.

- **Paramagnetic Materials**: Paramagnetic substances have unpaired electrons (magnetic dipoles), resulting in permanent magnetic moments, even in the absence of an applied field. These dipoles do not interact with each other, and are randomly oriented in the absence of an external field due to thermal agitation, resulting in a zero net magnetic moment. When a magnetic field is


applied, the dipoles will tend to align with the applied field, resulting in a net magnetic moment in the direction of the applied field.

- **Ferromagnetic Materials**: This kind of materials have unpaired electrons (magnetic dipoles) resulting in permanent magnetic dipoles (both in the presence and in the absence of an external field) and exhibit a long range order at the atomic level. Therefore, the unpaired electrons line up parallel to each other in regions called Weiss domains. In each domain, the magnetic field is intense, but the material is usually demagnetized because the different domains are randomly oriented. The transition area between two consecutive Weiss domains is called Bloch wall. This is the area where the orientation of the Weiss domains is gradually changing direction from one domain to another (Fig 4).

![Fig 4: Schematic representation of Bloch walls.](image)

When a small external magnetic field is applied, the magnetic domains align up along the field and the material is said to be magnetized. Strictly, a material is “ferromagnetic” in this narrower sense only if all of its magnetic dipoles add a positive contribution to the net magnetization. All ferromagnets have a maximum temperature where the ferromagnetic property disappears. This temperature is called the Curie Temperature ($T_c$). At $T_c$, a ferromagnetic material becomes paramagnetic, because the thermal energy becomes large enough to destroy the macroscopic magnetic ordering within the material.

$$M = \chi m H = C \cdot H / (T - T_c)$$  \hspace{1cm} (EQ.6)

- **Ferrimagnetic and Antiferromagnetic materials**: These materials have permanent magnetic dipoles, like ferromagnetic ones. In a ferrimagnetic material there are always weaker magnetic dipoles aligned antiparallel to the adjacent, stronger dipoles in the absence of an external magnetic field. For an antiferromagnetic material, the adjacent dipoles are antiparallel in the absence of an external field and cancel each other.
The loop is generated by measuring the magnetic flux of a ferromagnetic material while the magnetizing force is changed. A ferromagnetic material that has never been previously magnetized or has been thoroughly demagnetized will follow the dashed line as $H$ is increased. As the line demonstrates, the greater the amount of current applied ($H^n$), the stronger the magnetic field in the component ($B^n$).

At point "a" almost all of the magnetic domains are aligned and an additional increase in the magnetizing force will produce very little increase in magnetic flux. The material has reached the point of magnetic saturation. When $H$ is reduced to zero, the curve will move from point "a" to point "b". At this point, it can be seen that some magnetic flux remains in the material even though the magnetizing force is zero. This is referred to as the point of retentivity on the graph and indicates the remanence or level of residual magnetism in the material (some of the magnetic domains remain aligned but some have lost their alignment). As the magnetizing force is reversed, the curve moves to point "c", where the flux has been reduced to zero. This is called the point of coercivity on the curve.

The force required to remove the residual magnetism from the material is called the coercive force or coercivity of the material. As the magnetizing force is increased in the negative direction, the material will become magnetically saturated again but in the opposite direction (point "d"). Reducing $H$ to zero brings the curve to point "e". It will have a level of residual magnetism equal in order to that achieved in the other direction. Increasing $H$ back in the positive direction will return $B$ to zero. It should be noted that the curve did not return to the origin of the graph because some force is required to remove the residual magnetism. The curve will take a different path from point "f" back to the saturation point where it completes the loop.
It can be said that the hysteresis is the tendency that a material has to keep one of its properties in the absence of the stimulus that generated it. From the hysteresis loop, a number of primary magnetic properties of a material can be determined.

1. **Retentivity** - A measure of the residual flux density corresponding to the saturation induction of a magnetic material. In other words, it is a material's ability to retain a certain amount of residual magnetic field when the magnetizing force is removed after achieving saturation.

2. **Residual Magnetism** - the magnetic flux density that remains in a material when the magnetizing force is zero. Note that residual magnetism and retentivity are the same when the material has been magnetized to the saturation point. However, the level of residual magnetism may be lower than the retentivity value when the magnetizing force did not reach the saturation level.

3. **Coercive Force** - The amount of reverse magnetic field that must be applied to a magnetic material to make the magnetic flux return to zero.

4. **Permeability, m** - A material’s property that describes the ease with which a magnetic flux is established in the component.

5. **Reluctance** - Is the opposition that a ferromagnetic material shows to the establishment of a magnetic field. Reluctance is analogous to the resistance in an electrical circuit.

The magnetic behavior of magnetic materials depends on the temperature, but is also highly dependent on the volume of the material. This volume dependence arises from the fact that when materials with multiple magnetic domains become smaller, they can reach a certain critical value $d_0$, where the material becomes single-domain. If the size continues to decrease, it will reach a value $d_s$ where the thermal energy will be on the same order of magnitude as that needed to flip the spin orientation, so that the magnetic dipoles will become randomly oriented. These critical sizes, are in the range of a few nanometers and thus these effects are dominant for nanoparticles. Nanoparticles do not have permanent magnetic moments. However, if they can respond against an external magnetic field they are referred to as superparamagnetic colloids. The blocking temperature ($T_B$) is one of the main parameters used to characterize superparamagnetic colloids and is determined by temperature dependent magnetization measurements with a method called Zero Field Cooling –Field Cooling (ZFC– FC), in which the sample is first cooled down to a much lower temperature than the estimated $T_B$ (generally, to 5 K or lower) without an applied magnetic field (ZFC). Subsequently, a relatively weak external magnetic field (ca. 100 Oe) is applied to the sample while the system is heated up to measure the magnetization change with temperature. In the obtained curve, a maximum peak is observed; the temperature corresponding to this maximum is defined as the blocking temperature ($T_B$). The standard procedure to measure the blocking temperature ($T_B$) are temperature-dependent magnetization measurements, either in the absence (ZFC) or in the presence (FC) of an applied field. ZFC measurements show
how the magnetic moments start to align with the applied magnetic field and thus, how the magnetization increases. When \(T_B\) is reached, the magnetization is at its maximum value. Additional temperature increase over \(T_B\) will result in a decrease of the magnetization because the thermal energy will promote the fluctuation of the nanoparticles magnetic moments. In field cooling (FC) measurements, the sample is cooled down in the presence of a weak external field freezing the aligned nanoparticles net magnetic moments (Fig 6). The field is then removed and the magnetization is measured as the sample is slowly warmed up.

The magnetic behavior can be subdivided into four ranges:

1. **SPM: superparamagnetic:**

As particle size continues to decrease within the single-domain range, another critical threshold is reached, at which remanence and coercivity go to zero. When this happens, the grain becomes superparamagnetic.

A single-domain particle of volume \(v\) has a uniform magnetization directed along the easy axis of magnetization. If \(v\) is small enough, or the temperature is high enough, the thermal energy \((kT)\) will be sufficient to overcome the anisotropy energy separating the (+) and (-) magnetization states and thus cause a spontaneous reversal of magnetization.

For superparamagnetic particles, the net magnetic moment in zero field and at \(T > 0\)K, will average to zero. In an applied field, there will be a net statistical alignment of magnetic moments, which is analogous to paramagnetism, except that now the magnetic moment is not that of a single atom, but of a single-domain particle containing \(10^5\) atoms. Hence, the term superparamagnetism denotes a much higher susceptibility value than that for simple paramagnetism. In response to a change in the applied field or temperature, an ensemble of superparamagnetic particles will approach an equilibrium value of magnetization with a characteristic relaxation time, first derived by Néel:

\[
\frac{1}{t} = f_0 \exp\left(\frac{-K_u v}{kT}\right)
\]

(EQ.7)

where

- \(f_0\) -frequency factor \((10^9 \text{ sec}^{-1})\)
- \(K_u\) -anisotropy constant
- \(v\) -particle volume
- \(k\) -Boltzmann constant
- \(T\) -absolute temperature
The exponential nature of the relaxation time on $v$ and $T$ makes it possible to define a blocking temperature, $T_B$ (at constant volume), or blocking volume $v_B$, (at constant temperature) at which the magnetization goes from an unstable condition ($T \ll T_B$) to a stable condition ($T \gg T_B$).

2. **SD: single domain:**

As the grain size decreases, a critical size will be reached where the grain can no longer accommodate a wall. Below this critical size, the grain contains a single domain (SD). An SD grain is uniformly magnetized to its saturation magnetization. To change the magnetization of an SD grain, all you need to do is translate the domain wall, an energetically easy process, which can be accomplished in relatively low fields. Thus SD grains are magnetically soft with low values of coercivities and remanence.

3. **PSD: pseudo-single domain:**

The distinction between single domain and multidomain particles is straightforward. However, small multidomain grains exhibit a mixture of SD-like (high remanence) and MD-like (low coercivity) behavior.

There has been much theoretical and experimentally work on PSD grains. Some current thinking is that small MD particles that contain just a few domains may actually have difficulty nucleating domains. In some cases MD grains exist in metastable SD states. The transformation of one domain state into another, such as addition or loss of domains, is called transdomain transformation.

4. **MD: multidomain**

As size of a ferromagnet increases, the single-domain state incurs an increasing energy cost because of the demagnetizing field. This field tends to rotate the magnetization in a way that reduces the total moment of the magnet, and in larger magnets the magnetization is organized in magnetic domains. The demagnetizing energy is balanced by the energy of the exchange interaction, which tends to keep spins aligned. There is a critical size at which the balance tips pro the demagnetizing field and the multidomain state is favored.

The maximum coercivity for a given material occurs within its SD range. For larger grain sizes, coercivity decreases as the grain subdivides into domains. For smaller grain sizes, coercivity again decreases, but this time due to the randomizing effects of thermal energy. Domains constitute a fundamental concept in magnetism. A ferro- or ferrimagnetic material may be generally defined as one that possesses a spontaneous magnetization, $M_s$, dependent on temperature, but only slightly dependent on applied field. Ferromagnetism theory, based on electronic exchange forces, predicts the magnitude of $M_s$, but says nothing about the direction of $M_s$. Experimentally, it is observed that for a homogeneous specimen at constant temperature, the magnitude of $M_s$ is uniform but the direction of $M_s$ is in general not uniform from one region to
another (on a scale of microns to millimeters). Uniformity of direction is attained only by applying a large enough field to drive the domains out of the sample, or by reducing the particle's dimensions to small enough size to prevent domain formation.

**Fig 6**: (Left) Schematic illustration of the dependence of coercivity vs. particle size. The largest coercivity is observed at the particle size \( d_0 \), corresponding to the transition from multidomain to single domain structure. The coercivity falls to zero for super paramagnetic particles. (Right) Magnetization vs. temperature curves for \( \text{ZnFe}_2\text{O}_4 \) nanotubes measured under field cooling (FC) and zero-field cooling (ZFC) for a 50 Oe field.

### 1.3. Catalytic properties of metallic nanoparticles

Since a large fraction of the atoms of metal nanoparticles are present on their surface, their potential use in catalysis is obvious. In particular, transition metal surfaces are known to have very efficient catalytic properties for many relevant reactions\(^{71}\). The chemical and physical properties of a certain material depend on the number of electrons that the material has and the ability of these electrons to move in the material. In a metal, electrons are highly delocalized, but as we decrease the size of the metal and confine its electronic motion, the separation between the valence and the conduction bands becomes comparable to or larger than \( kT \), and the metal becomes a semiconductor.

The catalytic properties of metal nanoparticles depend on their shape\(^{72}\), which is directly related to the number of crystal faces that a particle may present. Many experimental studies on nanocatalysis have focused on correlating the catalytic activity with particle size\(^{72}\). While particle size is an important consideration, other factors such as geometry, composition, oxidation state, and chemical/physical environment can play a role in determining nanoparticle reactivity.

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\(^{71}\) Mostafa A. El-Sayed, Accounts of chemical research, 2001, 34, 4, 257-264  
\(^{72}\) Beatriz Roldan Cuenya, Thin Films, 2010, 518, 3127–3150
“Heterogeneous catalysis” has received a tremendous amount of interest, both from a scientific and an industrial perspective. This is demonstrated by the 2007 Nobel Prize in Chemistry awarded to Prof. Ertl, a pioneer in introducing surface science techniques to the field of heterogeneous catalysis and leading to a deeper understanding of how chemical reactions take place at surfaces.\textsuperscript{73,74} A related area of catalysis that is being developed at a rapid pace is nanocatalysis. Striking catalytic properties including greatly enhanced reactivities and selectivities have been reported for nanoparticle catalysts as compared to their bulk counterparts. In order to take advantage of the potential of these nanocatalysts, a detailed understanding of the origin of their enhanced performance is needed. While supported Au clusters provide the most dramatic example of nanocatalytic phenomena, palladium, platinum, and several other metal nanoparticles on oxide supports also exhibit high catalytic activity. There are indications that in addition to the reduced particle size\textsuperscript{75}, metal/oxide interactions are partially responsible for their high activity\textsuperscript{76}.

Therefore, the design of catalysts in a controlled and effective manner is crucial for the development of the chemical industry. Catalysis is also one of the scientific disciplines in which even small advances based on fundamental research can have a very significant impact on society. As an example, in order to obtain a further insight into the stability of nanocatalysts upon chemical adsorption, well-defined material systems\textsuperscript{77} with controlled and tunable size and interparticle distances are needed.

1.4. Optical properties of nanometals (Surface Plasmon Resonance and Surface Enhanced Raman Scattering)

Apart from the above mentioned properties that are characteristic of metals confined to the nanoscale, drastic changes in their optical properties are also observed as reported by Faraday as early as 1857. Already at that time he was able to prepare brightly colored colloidal solutions of gold with colors that were drastically differing from the typical golden color. However, the use of gold colloids dates back thousands of years before Faraday. One of the most popular examples is the famous Roman Lycurgus Cup, dated at the 4\textsuperscript{th} century. While under direct light the glass of the cup resembles jade with an opaque greenish-yellow tone, when illuminated from the inside and light it transmitted through the glass, it displays a translucent ruby red color. Transmission electron microscopy (TEM) and X-ray diffraction analysis of the glass revealed the presence of silver-gold alloy nanoparticles in a 7:3 ratio, also containing about 10% copper. These nanoscale alloy particles are responsible for this special color display, which we now understand as a consequence of surface plasmon resonances, which for

\textsuperscript{74} G. Ertl, Angew. Chem. Int. Ed. 2008,47, 3524
metals like gold, silver or copper take place in the visible region of the electromagnetic spectrum.

Fig 7: Left: Picture of the Rose Window of the Cathedral of Notre Dame. Right: Lycurgus cup

Like many other characteristics of metallic nanoparticles, the interaction with an electromagnetic radiation depends on the dimensions of the particles, thus allowing the design of tailor-made materials for novel optical components \(^{78,79}\). When an electromagnetic wave interacts with a metallic surface, the field close to the surface (near field) is different than that observed away from said surface (far field). In a metal nanostructure, the wave excites localized surface plasmon resonance (LSPR) on the surface, resulting in amplification of the nearby electromagnetic fields. Essentially, the LSPR can be defined as the collective oscillation of the conduction electrons in a metal nanostructure when this oscillation is in resonance with the frequency of the incident light. The LSPR depends strongly on the dielectric constant of the medium and the size, shape and chemical nature of the nanostructure. LSPR can be described using the classical electromagnetic theory \(^{80}\) by Mie theory \(^{81}\) or the Drude model \(^{82}\) for particles with spherical symmetry or by solving Maxwell’s equations using numerical methods such as the discrete dipole approximation (DDA) \(^{83}\) or the boundary element method (BEM) \(^{84}\), for anisometric geometries.

Mie explained this phenomenon by solving Maxwell’s equations for the particular case of spheres, in an analytical manner. For spherical particles that are very small compared to the wavelength of light (2R<<λ) Mie derived a simplified equation for the calculation of the optical extinction (scattering + absorption), as:

\(^{78}\) K. Lance Kelly, Eduardo Coronado, Lin Lin Zhao, and George C. Schatz, J. Phys. Chem. B 2003, 107, 668-677
\(^{79}\) Isabel Pastoriza-Santos and Luis M. Liz-Marzán, Nano Lett., 2002, 2, 8
\(^{81}\) G. Mie, Annalen der Physik 1908, 330, 377.
\(^{82}\) C. Kittel, Introduction to solid State Physics 1956.
\(^{84}\) F. J. H. García de Abajo, A., Physical review Letters 1998, 80, 11149.
\[
\sigma_{\text{ext}} = \frac{9 V \varepsilon_m^{3/2}}{c} \left( \frac{\omega \varepsilon_2(\omega)}{[\varepsilon_1(\omega) + 2\varepsilon_m]^2 + \varepsilon_2(\omega)^2} \right) \tag{EQ.8}
\]

Where \( V \) is the particle volume, \( \omega \) is the angular frequency of the exciting light, and \( c \) is the speed of light. \( \varepsilon_m \) and \( \varepsilon(\omega) = \varepsilon_1(\omega) + \varepsilon_2(\omega) \) are the dielectric functions of the surrounding medium and the metal, respectively.

![Fig 8: Schematic drawing of the interaction of an electromagnetic radiation with a metal nanosphere.](image)

The scattering from plasmonic nanoparticles is an ultra-fast process occurring in the femtosecond time scale\textsuperscript{85,86}. The percentage of the light extinction that is absorbed is dependent on the size and shape of the nanoparticle. As the particle size increases, the ratio of scattered light to absorbed light increases\textsuperscript{87}. This is an important fact for photothermal therapy using nanoparticles. The scattered light is elastically scattered from the particle, while the energy from the absorbed light is transferred to the hot electron gas that is oscillating in resonance with the light. At moderate intensities the hot electron gas cools via electron-phonon coupling (~1-10 ps) and transfers the energy to the positive nuclear lattice. The energy is then transferred to the surrounding medium (~100’s ps) as the hot positive lattice cools through phonon-phonon coupling with the environment\textsuperscript{86,88}.

These techniques have become significant because the strong fields that are induced around the nanoparticle upon irradiation at the plasmon frequency can be used to enhance the intensity of the observed spectroscopic response. Analytical methods such as SERS are truly a nanoscale event, occurring only when nanomaterials are present. Even on continuous metal films, where the phenomenon was first observed, the mechanism behind SERS was later discovered to be due to the roughness of the film which generates countless nanoscale features that focus the incident light into many LSPRs, which are responsible for the enhancement. In particular, SERS is important because of the wealth of information that can be obtained from the Raman scattering spectra of an analyte, and unlike FTIR absorption, water is not Raman active so measurements can be done in ambient conditions. The drawback to normal Raman

\textsuperscript{86} Link, S.; El-Sayed, M. A. International Reviews in Physical Chemistry, 2000, 19, 409-453.
spectroscopy is that Raman signals are very weak, with only one out of one million photons undergoing Raman scattering. For SERS, the strong plasmonic near-fields at the surface of the nanoparticle amplify the intensity of the incident and the scattered light, resulting in a surface enhanced Raman scattering signal.

Two types of scattering are readily identified. The most intense form of scattering, Rayleigh scattering, occurs when the electron cloud relaxes without any nuclear movement. This is essentially an elastic process and there is no appreciable change in the energy of the photon. Raman scattering\(^{89}\) (RS), on the other hand, is a much rarer event and involves only one in \(10^6\text{ to }10^8\) of the total photons scattered. This phenomenon occurs when the light and the electrons interact resulting in a movement of the nuclei. Since the nuclei are much heavier than the electrons, there is an appreciable change in energy of the molecule to either lower or higher energy depending on whether the process starts with a molecule in the ground state (Stokes scattering) or from a molecule in a vibrationally excited state (anti-Stokes scattering). Figure 9 shows a simple diagram illustrating Rayleigh and Raman scattering. Light shifted lower than the excitation energy \(\hbar \nu_0\) (Stokes scattering) has an energy of \(\hbar \nu_S = \hbar \nu_0 - \hbar \nu_m\); and light shifted higher (anti Stokes scattering) has an energy of \(\hbar \nu_{aS} = \hbar \nu_0 + \hbar \nu_m\); where \(\hbar \nu_m\) is the molecule’s vibrational energy. The wavenumber shown in a Raman spectrum represents the frequency shift between excitation photon and scattered photon. Therefore, the position of a Raman band will not change if a different excitation wavelength is applied.

![Figure 9. Diagram of the Rayleigh and Raman scattering processes.](image)

At room temperature, most of molecules are in the ground vibrational state (Boltzman distribution), and therefore the majority of RS will be Stokes RS. However, the intensity ratio Stokes / Anti-Stokes decreases with increasing temperature.

The intensity of the Raman radiation is directly related to the magnitude of the induced dipole moment of a molecule when it is under the influence of an external electromagnetic field. The induced dipole moment, in turn, depends directly on the polarizability, which measures the deformation parameter of the electron cloud with respect to vibrational coordinates of the molecule.

Raman spectroscopy is a technique widely used in materials analysis mainly due to their high specificity and simplicity in its implementation. However, it has two major disadvantages: the low cross section and the jurisdiction of that effect with the luminescent emission, much more intense than the Raman signal. The intensity of Raman scattering increases significantly when the external electromagnetic field energy applied is made to coincide or close to that of an allowed electronic transition of a molecule. Under these conditions there is an increase of the molecular polarizability in a process known as Raman Resonant. This process can increase the Raman cross section.

The SERS effect (Surface Enhanced Raman Scattering) was discovered in 1974 by Fleischmann et al\textsuperscript{90}. It consists of a huge enhancement of Raman emission from a molecule when it is in contact or near a nanostructured metal surface\textsuperscript{91}. Amplification of the SERS signal comes from the interaction of light with the conduction electrons on the surface of the metal. Upon the excitation of localized surface plasmon resonance, this interaction leads to an intensification of the electromagnetic field near the surface of the metal. The metals that have suitable optical properties for generating such intensification are mainly silver, gold and copper, the former two being more efficient and therefore more often applied in SERS experiments. The size of metal nanoparticles (NPs) involved in this phenomenon plays an important role. They must be smaller than the wavelength of the incident radiation to prevent excitation of quadrupolar modes in the metal. In general, the nanostructures with sizes between 10 and 100 nm are often effective in SERS.

It is now accepted that the SERS enhancement is due to two fundamental mechanisms: electromagnetic enhancement (EM) and chemical or charge transfer (CT) enhancement. It is considered that SERS effect does not occur in the absence of the EM mechanism. However, the charge transfer mechanism may participate, depending on the nature of the molecule, of the experimental conditions and requires a chemical bond between the molecule and the substrate.

1. Electromagnetic mechanism (EM)

It is generally accepted that the main contribution to the increase of the Raman signal arises from the intense local electromagnetic fields generated by an efficient coupling between the localized surface plasmon resonance of a nanostructure and the incident radiation\textsuperscript{92,93}. This enhancement, which is the origin of the SERS effect, depends on the nature of the metal, as well as the morphology of the nanoparticle.

![Diagram showing Raman scattering process in the presence (A) and in the absence (B) of spherical metal nanoparticles.](image)

The great enhancement that Raman radiation undergoes is due to the multiplicative effect produced by two processes, the intensification of the EM field incident on the molecule and the Raman radiation emitted by the molecule in the presence of the metallic nanoparticle (NP).

The EM field incident on the molecule, located at a position $r$ is the sum of the incident field ($E_i$) at a frequency ($\omega_0$) and the field emitted by the metal ($E_L$), the latter being predominant when the frequency $\omega_0$ matches the frequency of the localized surface plasmon resonances of the metal nanoparticle. In a second process, the Raman radiation emitted by the molecule ($E_{LR}$), with frequency $\omega_R$, is intensified again because it interacts with the localized surface plasmon of the nanoparticles. Therefore, it produces an enhanced Raman emission ($E_{LRS}$) compared with that obtained in the absence of metallic nanoparticles.

The SERS enhancement factor (G) conforms to the following expression:

\[
G = \frac{I_{SERS}}{I_{Raman}} = \left| \frac{\alpha_R}{\alpha_{R0}} \right|^2 |gg|^2
\]

\textsuperscript{92} Moskovits, M. Reviews of Modern Physics 1985, 57, 783.

Where $\alpha_R$ and $\alpha_{R0}$ are the Raman polarizabilities in the presence and in the absence of metal, respectively, $g$ is the amplification factor of the EM field for the presence of metal NP, and $g'$ is the intensification factor of the EM field to the Raman frequency ($\omega_R$).

According to the EM mechanism, several parameters influence the SERS enhancement, such as the morphology of the metal NPs, the distance and orientation of the molecule to the metal surface. Additionally it has been shown that aggregated NPs result in a greater SERS intensification. At the interstices between two NPs (at a distance less than 2-3 nm) the EM field increases substantially, leading to values of SERS enhancement of the order of $\sim 10^{11}$. These gaps are called "hot spots"\textsuperscript{94,95,96}. It is currently considered that most of the SERS enhancement is due to such hot spots. The distance of the adsorbate to the metal surface is another key factor involved in the SERS enhancement\textsuperscript{97,98}. The EM field outside a metal surface is strongly polarized in the perpendicular direction. Therefore, the vibrational modes of the adsorbate, which are perpendicular to the surface, exhibit a further intensification with respect to those that are parallel. Therefore, SERS spectra also provide important information to estimate the possible direction of the adsorbate with respect to the surface\textsuperscript{99}.

2. Charge transfer mechanism (CT)

The charge transfer mechanism (CT) arises from the need to explain certain phenomena related to the SERS enhancement that the EM mechanism cannot explain. If we assume only the existence of the EM mechanism, all adsorbates should show the same SERS enhancement, but experimental results show that this is not the case\textsuperscript{100,101,102}. Molecules can be adsorbed on the metal surface by either physisorption or chemisorption. When interaction energy is weak (physisorption), the modification of the polarizability of the molecule is small and the SERS spectrum profile is usually similar to the conventional Raman spectrum. However, the relative intensities of their bands may change due to orientation effects, as we saw in the previous case.

CT mechanism can occur from the HOMO orbital of the adsorbate to the Fermi level of the metal, or from there to the LUMO orbital of the adsorbed molecule, so that there are two possible processes of CT and it is depending of the system:

\textsuperscript{96} Steinigeweg, D., et al., Small, 2011. 7, 2443–2448
\textsuperscript{100} Lombardi, J.R., et al. Journal of Chemical Physics,1986, 84, 4174
from the molecule adsorbed to the metal or from the metal to the adsorbed molecule, although the latter mechanism is the most common\textsuperscript{103}. This mechanism has been revealed primarily through electrochemical measurements.

The CT mechanism is directly related to the nature of the adsorbate and the metal. For example, molecules with Π electrons in their structure, such as molecules with a higher aromatic character, it transfers the charge to the metal more easily. Also, the morphology of the metal surface substantially affects to the contribution of CT mechanism. The existence of an atomic-scale roughness\textsuperscript{104} is considered responsible, in large measure, in the process of charge transfer between the metal and the adsorbate.

\textbf{Fig 11.} Energy level diagram for a molecule-metal system showing a possible transfer charge process involving molecular stated (path (a)) and molecular and metallic states (paths (b), (c)).

\textsuperscript{103} Lombardi, J.R., et al., Journal of Chemical Physics, 1986, 84, 8, 4174-4180.

CHAPTER 2

Highly Catalytic Single-Crystal Dendritic Pt Nanostructures Supported on Carbon Nanotubes

Abstract

We describe in this chapter a one-step method for the preparation of single-crystal dendritic Pt nanoparticles with no need of organic solvents, templates, or seeded growth. It is shown that Pt nanoparticles with two different shapes, spherical or dendritic, can be efficiently supported onto the sidewalls of carbon nanotubes. Notably, the supported dendritic Pt nanostructures yield unprecedented catalytic activity, evidenced through the lowest activation energy for an electron-transfer reaction, as compared with those reported in the literature for Pt nanostructures.
2.1 INTRODUCTION

Catalysis is an essential tool for the development of modern society. More than 95% of the industrial activity, including the fabrication of medical drugs, fuels, or plastics, relies on catalyzed processes in at least one of their stages. Catalysts have as well a key impact on the environment since they are routinely used for the reduction of emissions and have thus become a crucial component in the development of future green technologies, for example, in hydrogen-based fuel cells. Therefore, the big challenge of catalytic activity, selectivity, and design has been brought up, offering a complete breakthrough when related to new, undiscovered properties of nanoparticles and clusters. Indeed, the catalytic activity and selectivity of nanoparticles are strongly dependent on their composition, size, and shape\(^1\). Platinum is by far the most widely studied catalytic nanomaterial, with an extraordinary projection toward potential catalysis-related applications. However, Pt is expensive and its availability in nature is limited. Thus, a great effort is being devoted to searching for optimized morphologies that would increase the Pt catalytic activity, while decreasing the required amount of material. Pt nanoparticles with several shapes, such as polyhedra\(^2\)\(^3\)\(^4\)\(^5\), nanowires\(^6\), nanotubes\(^7\)\(^8\), tetrahedra\(^9\), or dendritic structures\(^10\)\(^11\)\(^12\)\(^13\)\(^14\)\(^15\), have been synthesized by controlled growth on specific crystallographic facets or by templating methods. Mahmoud et al.\(^16\) reported a seeding method, based on the autocatalytic reduction of a Pt complex at the metal surface, for the controlled preparation of uniform, single crystal Pt nanostars. Interestingly, and in close agreement with Narayanan and El-Sayed\(^17\), the activation energy for the catalysis of electron-transfer reactions was shown to be shape-dependent. In fact, nanocatalysts that have more atoms on edges or corners (i.e., more valence unsaturated atoms) display a higher catalytic activity, as was found for these star-shaped colloids when compared to other, previously reported, active platinum nanoparticle (NP) shapes. An additional point that needs to be taken into account is the type of molecules that are adsorbed onto the surface of the nanocatalysts. Unfortunately,  

1 Bell, A. T. Science (Washington, D.C.) 2003, 299, 1688  
4 Ahmadi, T. S.; Wang, Z. L.; Green, T. C.; Henglein, A.; El-Sayed, M. A. Science 1996, 272, 1924  
amphiphilic polymers or surfactants, necessary for stabilizing high-energy surfaces of the different Pt nanoparticles, reduce the catalytic efficiency of the NPs because they tend to hinder the approach of the reactant molecules to the catalyst surface. Polyvinylpyrrolidone, one of the most widely used templating polymers for shape controlled NP synthesis, adheres strongly to the nanoparticles surface through a charge-transfer interaction between the pyrrolidone rings and surface Pt atoms, thereby blocking a significant number of active sites. An alternative route for this shape-controlled synthesis relies on altering the reduction kinetics with foreign ions of metals such as Fe or Ag, which allows for the preparation of Pt multipods or nanowires and polyhedra, respectively. Notwithstanding, it has been reported that the catalytic yield of Pt NPs decreases when they are deposited onto carbon supports. Different carbon supports for Pt NP deposition have been reported, including carbon black, carbon nanofibers, ordered activated carbon, and carbon nanotubes (CNTs). CNTs are considered to be ideal candidates due to their remarkable structure-dependent properties including high tensile strength and surface area, together with their high electric and thermal conductivity. Moreover, it has been reported that the use of CNTs as supports renders materials that are more resistant against corrosion than for example carbon black, permitting as well as better contact between reactant and NP surface than porous carbons (where NPs are trapped within the pores rather than on the outer surfaces). Thus, the main reason for the high activity of the Pt/CNT catalyst seems to be that reactant molecules easily arrive at the catalyst and the product molecules readily diffuse away from the Pt particles.

when supported on CNTs. The enhanced electrocatalytic properties of CNTs are expected, for example, to reduce the amount of precious metal catalyst increasing the commercial viability of proton exchange membrane (PEM) fuel cells. Unfortunately, the efficient attachment of Pt nanoparticles onto CNTs with a uniform distribution remains a challenge because of the high curvature, small size, and chemical inactivity of the nanotubes. A common strategy to deposit metal nanoparticles onto CNTs is the functionalization of their external wall by oxidative treatments, with a posterior deposition of the particles. However, during this treatment, the one-dimensional electronic structure and the desired properties of the nanotubes may be altered. Our group has developed a method for achieving size- and shape-controlled deposition of NPs without varying the CNT properties, through noncovalent functionalization of the nanotubes combining the polymer wrapping and the layer-by-layer technique. When this approach is used, the CNTs surface charge can be controlled preventing damage to their structure and permitting the assembly of charged nanoparticles, such as gold spheres and rods, quantum dots, or magnetic particles.

2.2 EXPERIMENTAL METHODS

**CNT polyelectrolyte functionalization.** CNTs were redispersed in ultrapure water (18 MΩcm) as previously described. Briefly, CNTs were dispersed in a 1 wt % aqueous solution of polyallylamine hydrochloride (PAH) up to a concentration of 150 mg/L. A combination of rapid stirring and sonication was used to ensure the presence of well-dispersed, individual nanotubes. Excess PAH was removed by repeated centrifugation and redispersion cycles.

**Spherical platinum nanoparticles.** Spherical Pt nanoparticles were synthesized as follows: to a solution containing 43 mL of ultrapure water, 2.5 mL of sodium citrate (0.1 M) and 2.5 mL of 0.05 M H₂PtCl₆, 2.45 mL of sodium borohydride (0.015 M) were added as reducing agents (citrate/H₂PtCl₆/NaBH₄ in a molar ratio of 2:1:0.3). The solution was stirred for 10 min.

**Dendritic platinum nanoparticles.** Dendritic Pt nanoparticles were synthesized as follows: to a solution containing 21.52 mL of ultrapure water, 1.25 mL of sodium citrate (0.1 M) and 1.25 mL (0.1 M) of K₂PtCl₄, 1.22 mL of sodium borohydride (0.0158 M) were added (citrate/K₂PtCl₄/NaBH₄ in a molar ratio of 1:1:0.15).

**NPs deposition onto CNTs.** CNT@PAH (0.6 mL; 0.5 mg/mL) was added to 50 mL of Pt seeds solution (0.5 mM). After 30 min, the solution was centrifuged (10 min, 8000 rpm) and redispersed in 20 mL of pure water to remove non-adsorbed nanoparticles.

**IR analysis.** Selected samples were characterized with transmission FTIR (Bruker, Equinox 55) on pressed KBr pellets (150 mg of KBr and 1 mg of sample). The transmission FTIR cell was flushed with N₂ gas for 10 min before scanning to remove atmospheric water vapor and CO₂ from the spectrophotometer. The spectral resolution was set to 1 cm⁻¹ and 32 scans were collected in each spectrum.

**ICP-AES analysis.** The sample (dispersion of CNT/Pts or CNT/ Ptd) was dried in an oven at 60 °C and calcined for 2 h at 600 °C to remove organic matter. Then, the solid residue was treated with 4 mL of aqua regia (50%) and finally diluted to 25 mL before proceeding with the ICP-OES analysis.

**Kinetic measurements.** The reactions were carried out at different temperatures in quartz cuvettes (thermostatted with an accuracy of 0.2 °C), placed in a diode-array UV/vis spectrophotometer (Agilent 8453). All solutions were previously deaerated, and the reaction mixtures were maintained at pH 13 to avoid NaBH₄ decomposition. Kinetic data were always satisfactorily fitted by the first-order integrated rate equations in hexacyanoferrate concentration. Experiments were reproducible to within 5%.

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2.3 RESULTS AND DISCUSSION

The one-pot synthesis of single-crystal dendritic platinum NPs was achieved by means of the combined reduction of K₂PtCl₄ by citrate and borohydride ions. The as-prepared colloids can be successfully assembled onto CNTs, thus rendering a homogeneously loaded hybrid material with clean Pt surfaces, whose catalytic activity was tested on a redox reaction and compared with analogous composites based on platinum nanospheres. Spherical and dendritic (Pt₄ and Pt₆, respectively) NPs of 3 and 20 nm, respectively (size defined as the longest distance between two points on a single particle), were successfully synthesized in the presence of citric acid using sodium borohydride as reducing agent. This new and simple approach allows the large-scale preparation of colloids with no need of using nanoparticle seeds or templates. Interestingly, the Pt₆ NPs were found to be single crystals, as recently reported for multiarmed Pt nanostructures by Mahmoud et al.¹⁶ but the two-step process was avoided which implies a seed-mediated approach. Additionally, the synthetic approach herein reported does not require the use of undesirable surfactants which could block the active sites on the Pt nanoparticles surface since the obtained Pt nanostructures are stabilized with a layer of citric acid which shows weak anchoring to the nanocrystals, thus favoring the catalytic activity.

Both negatively charged Pt₄ and Pt₆ nanoparticles (zeta potential, ζ = -20 mV) were electrostatically assembled onto the (positively) functionalized CNTs. Briefly, the nanotubes were individually functionalized and dispersed in water using polyallylamine hydrochloride (PAH) as a wrapping agent. PAH provides the tips and sidewalls of the nanotubes with a positively charged surface (ζ = +40 mV), rendering them suitable for the electrostatic adsorption of pre-synthesized nanoparticles.

![TEM micrographs at two different magnifications, of spherical (a) and dendritic (b) Pt nanoparticles assembled onto the CNT's surface. STEM (c) XEDS elemental mapping of C and Pt for a Pt₆-coated carbon nanotube, demonstrating the core-shell structure of the composite material.](image)
Importantly, both types of Pt NPs preserve their morphology and are homogenously distributed over the entire nanotube surface (Fig 1). Pt₈ nanoparticles, synthesized by reduction with NaBH₄ and stabilized through sodium citrate adsorption, have been widely reported and broadly studied. We carried out a complete characterization of CNT/Pt₈ for comparison with CNT/Pt₉, which could be considered a model system. A scanning transmission electron microscopy (STEM) analysis of the samples was performed to evidence the formation of the expected core-(CNT)/shell-(Pt) structure. STEM-XEDS elemental mapping of the hybrid structures (Figure 1c) showed the relative elemental distribution, with red areas corresponding to the CNT (C Kα line) and green areas corresponding to Pt₈ (Pt Lα line). The image clearly shows that the CNTs are located in the inner side while Pt covers the outer part, as expected for the proposed CNT-supported nanoparticle structure.

HRTEM images of the CNT/Pt₈ composites (Fig 2a-c) allow us to distinguish the unchanged multiwall structure of the CNTs used as substrates, with an intershell distance of 0.34 nm. The fast Fourier transform of the marked area in Fig 2c shows a spot pattern that can be readily indexed as face-centered cubic (FCC) Pt oriented in a [110] zone axis (ICSD No. 41525), evidencing the single-crystalline structure of the Pt₈ NPs. Notably, the SAED analysis on one CNT/Pt₈ composite (Fig 3) shows the Pt...
diffraction rings, as well a contribution of the CNT fringes corresponding to the (002) layers.

Fig 3. SAED analysis

Notably, the SAED analysis shows as well a contribution of the CNT fringes corresponding to the (002) layers. Additionally, the intensity distribution on all diffraction rings in the SAED indicates a non-preferred orientation of the Pt crystal structure with respect to the nanotube planes, as expected from an electrostatic assembly of the nanoparticles onto the CNTs template. The single-crystal nature of the Pt NPs suggests that their formation mechanism does not involve the aggregation of previously formed smaller NPs, which should lead to twin planes and/or other defects. Instead, we speculate about the formation of primary clusters which might act as seeds for further dendritic growth as the Pt(II) salt is reduced. We have observed that this dendritic morphology is determined by the molar ratio between Pt(II) salt, stabilizer, and reducing agent concentrations, with an optimal value of 1:1:0.15 (K₂PtCl₄/Na₃C₆H₅O₇/NaBH₄). The main advantage of this strategy stems from the highly desired NPs “clean” surface, free from strongly binding stabilizing molecules that would block the Pt active sites. The weak interaction between citrate ions and the Pt surface permits near-complete removal during the synthetic procedure, as indicated by the IR spectra (Fig 4).

In general, similarly prepared colloids show typical bands for citrate and citric acid (C=O (1715 cm\(^{-1}\)), asymmetric and symmetric COO- (1618 and 1385 cm\(^{-1}\), respectively), C-O (1230 cm\(^{-1}\)), C-C stretching (1064 cm\(^{-1}\)), and C-H deformation (1130 cm\(^{-1}\))\(^{50}\). In this case however, once the Pt NPs have been assembled onto the CNTs, the washing cycles (repeated centrifugation and redispersion) practically eliminate all the citrate as shown in the weak bands in the IR spectrum after washing, which mainly include the asymmetric and symmetric COO\(^{-}\) stretchings (at 1616 and 1387 cm\(^{-1}\), respectively). The catalytic activity of as-prepared CNTs/Pt (with Pt\(_{s}\) or Pt\(_{d}\) NPs) composites was tested using a model reaction where potassium hexacyanoferrate(III) is reduced by NaBH\(_4\) into potassium hexacyanoferrate(II)\(^{51}\). The concentrations of Pt\(_{s}\) and Pt\(_{d}\) NPs in the composites were similar (41.8 and 43.4 mg mL\(^{-1}\), respectively), as determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Since both types of nanostructures were prepared and stabilized in a similar way, we can safely compare the catalytic activity as a function of NP morphology. The catalytic efficiency of both composites was thus tested by monitoring the effect of the CNT/Pt\(_{s}\) and CNT/Pt\(_{d}\) hybrids on the reduction rate of potassium hexacyanoferrate(III) by NaBH\(_4\). In all experiments we used NaBH\(_4\) concentration in large excess so that, to a good approximation, it can be considered constant throughout the reaction (isolation method); the kinetics of the reduction process can be treated as a pseudo-first-order reaction. The progress of the reduction was monitored through changes in the UV-vis spectra of hexacyanoferrate(III) when using both Pt\(_{s}\) and Pt\(_{d}\) NPs supported onto CNTs (Figure 4). The characteristic hexacyanoferrate(III) absorption band at 420 nm decreases with time as being reduced into ferrocyanide, offering a good correlation with first-order kinetics, as shown in the inset of Fig 5 for various catalyst concentrations. Activation energies (E\(_a\)) for the selected reaction were determined by means of an Arrhenius plot.; i.e., the slope of the linear relationship between the natural logarithm of the temperature-dependent rate constant and the inverse temperature is \(-E_a/R\), where R


is the universal gas constant. The value of $E_a$ is used because it is independent of concentration and nanocatalyst surface area.

![Graph 1](image1)

![Graph 2](image2)

**Fig 5.** Spectral evolution of hexacyanoferrate in the presence of borohydride and CNT/Pt$_s$ (top) or CNT/Pt$_d$ (down) composites as catalysts. $[\text{K}_3\text{Fe(CN)}_6]$ $5.4 \times 10^{-4}$ M, $[\text{NaBH}_4]$ 0.011 M, $[\text{Pt}_s\text{-CNT}]$ 0.40 mg/L, $[\text{Pt}_d\text{-CNT}]$ 2.4 mg/L, pH 13. The insets show the good fit of the experimental results to first-order processes for different catalyst concentrations.

Analysis of the catalytic activity for CNTs loaded with Pt$_s$ and Pt$_d$ revealed a much lower activation energy when the reaction was catalyzed by the dendritic Pt NPs ($E_{a_d} = 10.34$ kJ mol$^{-1}$, $E_{a_s} = 27.0$ kJ mol$^{-1}$) (Fig 6), while both values were lower than that reported$^{52}$ for the same reaction in the absence of catalyst (31 kJ mol$^{-1}$). These results are in-line with those reported by Mahmud and co-workers$^{16}$ when using multiarmed single-crystal Pt nanoparticles, which offered increased catalytic activity for the reduction of hexacyanoferrate(III) by thiosulfate (from $E_a = 38.3$ kJ mol$^{-1}$ in the absence of catalyst down to $E_a = 14$ kJ mol$^{-1}$).

Fig 6. Activation energy ($E_a$) for the reduction of hexacyanoferrate(III) by sodium borohydride catalyzed by CNTs loaded with spherical nanoparticles $E_{a(s)}$ and dendritic single crystals $E_{a(d)}$. Both nanocatalysts are almost free of sodium citrate used as stabilizer.

### 2.4 CONCLUSIONS

In the present case, the exceptionally lower $E_a$ determined in the presence of CNT/ Pt$_d$ composites can be explained by an increase of Pt atoms at the edges and corners of the multiple arms due to the dendritic shape. In summary, we have developed a one-step method for the preparation of single-crystal, dendritic Pt nanoparticles with no need of organic solvents, templates, or seeded growth. It was shown that Pt nanoparticles with two different shapes spherical or dendritic can be efficiently supported on the sidewalls of CNTs. Additionally, their catalytic activity was studied, and a much lower activation energy was determined for the reduction of hexacyanoferrate(III) by sodium borohydride in the presence of the dendritic CNT/Pt$_d$ heterostructures. This exceptional catalytic behavior can be justified by the presence of many corners and edges in the particles with dendritic shape. Moreover, both the exceptional catalytic properties and synthetic nature exposed for the obtained CNT/Pt hybrid heterostructures make them ideal candidates for the preparation of CNTs composites for PEM fuel cells by means of the layer-by-layer assembly technique.$^{53}$

CHAPTER 3

Label-Free SERS Detection of Relevant Bioanalytes on Silver-Coated Carbon Nanotubes: The Case of Cocaine

Abstract

Surface-enhanced Raman scattering (SERS) spectroscopy can be used for the label-free determination and quantification of relevant small biometabolites that are hard to identify by conventional immunological methods, in the absence of labelling. The detection is based on monitoring the vibrational changes occurring at a specific biointerface (a monoclonal antibody, mAb) supported onto silver coated carbon nanotubes (CNT@Ag). Engineered CNT@Ag play a key role as they offer a stable substrate to support the biointerface, with a high density of hot spots. Proof of concept is demonstrated through the analysis and quantification of the main cocaine metabolite benzoylecgonine. These results open a new avenue toward the generation of portable sensors for fast ultradetection and quantification of relevant metabolites. The use of discrete particles (CNT@Ag@mAb) rather than rough films or other conventional SERS supports will also enable a safe remote interrogation of highly toxic sources in environmental problems or in biological fluids.
3.1 INTRODUCTION

Surface-enhanced Raman Scattering (SERS) is one of the most powerful analytical techniques with capacity for single molecule detection\textsuperscript{1,2}. Conventional SERS experiments rely on the interaction of a given analyte with a nanosized gold or silver structure, usually as a colloidal suspension or a nanostructured film\textsuperscript{3,4} which is able to generate a strong electromagnetic field when excited with visible or infrared light\textsuperscript{5,6}. However, this approach is sometimes limited, regarding the applicability of the method especially when complex mixtures are to be analyzed. In such cases, the target analyte vibrational features are very often screened by signals from other species present in the sample, in particular when the analyte is very dilute. Some strategies have been devised to overcome this screening effect, such as using codified SERS labels\textsuperscript{7,8,9} or coupling the SERS experiment to a prior purification step by using, for example, chromatography or electrophoresis\textsuperscript{10,11,12}. These strategies are powerful and reliable. However, the former requires the analyte to be able to complex to two different antibodies (for capture and detection), and thus is only useful for a medium-large molecular weight analyte, while the second is time consuming and requires the design of sophisticated specific instrumentation.

Other possibilities, such as the use of biointerfaces that may specifically bind certain moieties (i.e. antibodies and nucleic acids), are now being studied by several groups\textsuperscript{13,14,15}. By using SERS together with this strategy, we aim at monitoring changes in the enhanced vibrational signal of the receptor, as a result of a specific binding event with the analyte of interest. In line with this, SERS spectroscopy would be the method of choice as it brings together the sensitivity of Raman scattering spectroscopy toward small changes in the structure of macromolecules\textsuperscript{16,17,18,19}, magnified in this case by the

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surface selection rules\textsuperscript{20,21}, with the ability of SERS for ultra-detection of minute amounts of analyte. To succeed in this approach, however, it is essential to design hybrid materials that are able to covalently support large macromolecules as antibodies on optically active nanoparticles, without changes in their optical properties or colloidal stability, and that can be easily separated and cleaned from the biological fluid of interest\textsuperscript{22}.

3.2 EXPERIMENTAL METHODS

All the chemicals were purchased from Aldrich unless otherwise stated.

**Silver coated carbon nanotubes (CNT@Ag):** Silver seeds were produced by mixing 5 mL of 2.5 mM trisodium citrate, 0.25 mL of polystyrene sodium sulfonate (PSS, Mw \( \approx 100000; 500\text{mg/L} \)), and 0.3 mL of 10mM NaBH\(_4\), and then adding drop-wise 5 mL AgNO\(_3\) 0.5 mM under stirring\textsuperscript{23}. Seeds were assembled onto poly(allylamine hydrochloride) (PAH; Mw \( \approx 70000 \)) coated carbon nanotubes (NanoLab) by drop-wise addition of 15 mL of a suspension containing 7.5\( \times 10^{-2} \) mg/mL of PAH-coated CNTs to the seeds colloid under sonication\textsuperscript{26,27}. The silver seeds were subsequently grown *in situ* on the nanotubes (2 mL, 0.011 mg/mL) by adding 5 mL of destilled water, 75 \( \mu \text{L} \) of 10 mM ascorbic acid, 3 mL of 0.5 mM AgNO\(_3\), and 500 \( \mu \text{L} \) of 25 mM sodium citrate.

**Characterization:** Optical characterization was carried out by UV–Vis–NIR spectroscopy with an Agilent 8453 spectrophotometer, using 10 mm path length quartz cuvettes. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM 1010 transmission electron microscope operating at an acceleration voltage of 100 kV. **Antibody biocoupling onto CNT@Ag:** 100\( \mu \text{L} \) of thioglycolic acid was added to 1 mL of CNT@Ag and left for 12 h so that the thiol was adsorbed on the silver surface. The thiol-functionalized CNT@Ag was centrifuged twice at 3500 rpm for 15 min and redispersed in phosphate buffer solution (PBS). Coupling of the monoclonal mouse antibody anti-benzoylecgonine (ref: B1077-08; US Biological) to thioglycolic acid-functionalized CNT@Ag was achieved using carbodiimide chemistry to conjugate the primary amines of the antibody (Ab) with the carboxyl groups of thioglycolic acid. Briefly, the thioglycolic acid-functionalized CNTs@Ag (1 mL) and 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDAC) conjugation buffer (50\( \mu \text{L} \), 2\% w/v EDAC, 3\% w/v N-hydroxysuccinimide in PBS, pH 7.2) were mixed in an orbital shaker for 15 min at room temperature. The Ab (10 \( \mu \text{g} \)) was added to the reaction mixture.

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containing the CNT@Ag and incubated for 2 h with stirring, at room temperature. After 2 h, the reaction was quenched with 10 μL of 1M hydroxylamine to regenerate the original, non-reacted carboxyl groups. The antibody-conjugated CNT@Ag were centrifuged at 3500 rpm for 15 min and washed 3 times with 0.5 mL PBS.

**Surface-enhanced Raman scattering (SERS) spectroscopy:** The inelastic scattered radiation was collected with a LabRam HR system (Horiba-Jobin Yvon), equipped with Peltier charge-coupled device (CCD) detectors and an Olympus microscope. The spectrographs have 1800 g/mm gratings with additional band-pass filter optics. Samples were excited with three different laser lines at 532 (Nd:Yag), 633 (He-Ne) and 785 nm (diode). The power at the sample was set at 100 μW, with accumulation times of 10 s. The laser line was focused onto the sample in backscattering geometry using a 50x objective (numerical aperture 0.75) providing scattering areas of ca. 1 μm².

The SERS optical activity of the prepared CNT@Ag was tested with 1-naphthalenethiol (1NAT, Acros Organics), a well-studied SERS probe. Samples were prepared by adding 10 μL 10⁻⁵ M 1NAT solutions to 500 μL of CNT@Ag (0.011 mg/mL). After 30 min (to reach thermodynamic equilibrium), 10 μL aliquots were cast and air-dried on glass slides and studied with 3 laser lines: 532, 633 and 785 nm.

For benzoylecgonine detection, small amounts of BCG (10 μL from 10⁻⁵ to 10⁻⁹ M) were added to 100 μL of CNT@Ag (0.011 mg/mL) with and without antibody. Aliquots of the different samples (10 μL) were cast and air-dried on glass slides and SERS spectra were collected, exciting with a NIR (785 nm) laser line to avoid damaging the samples.

### 3.3 RESULTS AND DISCUSSION

We report in this chapter the design and fabrication of a colloidal and optically stable SERS substrate comprising silver coated carbon nanotubes (CNT@Ag), with activity in a wide window of excitation wavelengths, ranging from the visible to the near-infrared (NIR). A proof of concept of the suitability of this substrate for the label-free indirect ultra-detection of relevant bioanalytes is demonstrated by coupling a specific monoclonal antibody (mAb) directed against benzoylecgonine (BCG), a major cocaine metabolite, which is expressed in biological fluids such as saliva, blood, or sweat and which can therefore be used as an indicative of drug abuse.

Comparison of the results with those obtained from the direct SERS detection of BCG on the same substrate reveals that, whereas direct detection offers a higher detection limit than the specific indirect method, label-free indirect detection offers quantitative results at physiological levels, so that not only drug abuse can be demonstrated, but the consumption can also be quantified with a portable, non-invasive and fast technique.

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CNTs were selected as useful templates where the controlled deposition of Ag nanoparticles can be achieved\textsuperscript{26}, as required for an optimized SERS substrate. Additionally, CNTs possess a high physical and chemical stability, which together with a high aspect ratio have also led us to anticipate their suitability for the preparation of advanced SERS substrates.\textsuperscript{22} The fabrication of the CNT@Ag substrates used for this work comprised the homogeneous electrostatic adsorption of small silver nanoparticles (2 nm diameter) onto polyallylamine hydrochloride (PAH) coated CNTs\textsuperscript{27,28}. The small Ag nanoparticles were used as seeds and further grown with the intention of both increasing the effective cross-section and obtaining silver nanoparticles in close contact for an increased number of hot spots. The colloidal stability of these modified CNTs (CNT@Ag, Fig 1a) is crucial to allow adsorption of the target analytes and

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antibodies onto the metal nanoparticles. UV-Vis spectroscopy confirms the retention and high coverage of the Ag nanoparticles on the CNTs (Fig 1b). While a colloid containing silver nanoparticles with similar size and surface properties present a plasmon band centered at 412 nm, the particles grown onto the CNT external walls show a very broad surface plasmon band over the visible and NIR, similar to that of evaporated silver films. The optical-enhancing properties of the composite nanotubes were studied with SERS (Fig 1c). To this end, dilute solutions of 1-naphthalenethiol (1NAT, 10 μL 10^{-5} M) were added to 500 μL aliquots of CNT@Ag (0.011 mg/mL). After allowing 30 min for thermodynamic equilibrium to be reached, 10 μL aliquots were cast and air-dried on glass slides and their SERS-enhancing properties were studied by excitation with 3 laser lines: 532, 633 and 785 nm. The composite material presents strong SERS signals corresponding to 1NAT: ring stretching (1553, 1503, and 1368 cm\(^{-1}\)), CH bending (1197 cm\(^{-1}\)), ring breathing (968 and 822 cm\(^{-1}\)), ring deformation (792, 664, 539, and 517 cm\(^{-1}\)), and CS stretching (389 cm\(^{-1}\))\(^\text{30}\). Interestingly, a high signal to noise ratio was achieved for all three excitation laser lines.

which is related to the broad plasmon band and confirms the formation of a high density of hot spots on the CNT surface\textsuperscript{28,31,32}.

The selected receptor, a mouse mAb anti-benzoylcegonine (antib-BCG), is highly specific (affinity constant $5.8 \times 10^9$) to cocaine and to its main metabolite benzoylcegonine, but shows very low cross reactivity with other degradation metabolites of cocaine such as ecegonine or ecegonine methylester (recognition of 3.2\% and 0.1\%, respectively). The mAb antabi-BCG was conjugated onto the nanostructured silver surfaces by means of standard carbodiimide chemistry at low concentration. Small amounts of BCG (10 \mu L $10^{-5}$ M) were added to 100 \mu L solutions of CNT@Ag (0.011 mg/mL) with and without antibody, as illustrated in Fig 2. Aliquots of the different samples (10 \mu L) were cast and air-dried on glass slides and SERS spectra were collected using a NIR (785 nm) laser line for excitation, so as to avoid inducing damage on the samples.

The SERS spectrum of BCG (Fig 2A) is dominated by bands at 1581 cm$^{-1}$ (C=C stretching), 1440 cm$^{-1}$ (C-H bending), 1313 cm$^{-1}$ (NC-H stretching), 1031 cm$^{-1}$ (C-N stretching), 968 cm$^{-1}$ (ring breathing), 866 cm$^{-1}$ (pyrrolidine C-C stretching), 812 cm$^{-1}$ (C-H out-of-plane deformation), and 764 cm$^{-1}$ (piperidine C-C stretching). The SERS spectrum of the antibody (Fig 2B) is dominated by 1343 cm$^{-1}$ (C-H deformation), 1307 cm$^{-1}$ (amide III), 1085 and 924 cm$^{-1}$ (C-C stretching), 764 cm$^{-1}$ (C-C bending), and 693 cm$^{-1}$ (C-H bending)\textsuperscript{33}. Notably, the spectrum of the mAb-BCG complex is similar to that of the free antibody but contains significant and reproducible differences. The

![Fig 3.](image)

**Fig 3.** (A) Molecular structure of the Fab mAb fragment directed against benzoylecgonine before and after conjugation with BCG. (B) Overlapped molecular structure showing the slight structural differences between the free Fab antibody fragments (green) and the conjugated system (red). These structures can be consulted free of charge in the Protein Data Bank (accession numbers: 1RFD and 1QYG)\textsuperscript{34}


main difference is a drastic enhancement of the band at 693 cm\(^{-1}\) (C-H bending) for the antibody-BCG complex.

It is well-known that when an Ab binds specifically to its ligand, the interaction modifies the structure of the whole antibody\(^{34}\). The conformation change affects especially to the Fc fragment, which includes all heavy chain constant domains except CH1. In the case of a mAb anti-benzoylcegonine, modifications in the Fab region (antigen-binding fragment) after the interaction were also studied by single-crystal X-ray diffraction and computational modelling (Fig 3A)\(^{35}\). Notably, analysis of the overlapped structures before (green) and after (red) the interaction between the Fab and BCG (Fig 3B), shows that the positions of a significant number of atoms do not match between both structures.

![Image of Raman spectra](image)

**Fig 4.** Direct (A) and label-free indirect specific (B) SERS detection of BCG as a function of sample concentration.

Although the detection mechanism is conceptually different, indirect label-free detection based on changes in the coupled antibody SERS signal (Fig 4B) yields detection limits of about same order of magnitude. However, specific indirect detection presents several important advantages over the direct approach. First of all and most importantly, it is selective and it could be carried out within complex biological fluids such as saliva or urine, simply allowing some time for the BCG coupling onto the antibody receptor and then removing the remaining components of the sample by just washing the CNT@Ag@antibody tubes for further analysis. Second, the SERS spectra of the coupled system contain one intense band (764 cm\(^{-1}\); C-C in-plane bending) that hardly changes upon conjugation of BCG, as compared with changes recorded in the out-of-plane C-H bending (693 cm\(^{-1}\)). This different behaviour is related to smaller


changes in the orientation of the carbon chain as compared with that of the substituted hydrogen atoms and could be interpreted as follows. If we imagine the protein carbon chain extending perpendicular to the nanoparticle surface, the hydrogen atoms that saturate this chain are almost parallel to the surface. Thus, a small change in the antibody skeleton will yield a small orientation change in the carbon chain but a much more extended orientation change in the C-H bonds, with respect to the induced dipole normal to the surface. In full agreement with the SERS selection rules\textsuperscript{20,36}, vibrations with a deeper orientation change with respect to the surface will change more than those with a lesser orientational change. The specificity of these changes was confirmed by carrying out the same experiments but using polyclonal anti-BCG antibodies. Notably, no neat spectral changes were detected upon extended exposure to BCG. These may be explained in light of the nature of monoclonal and polyclonal antibodies. While monoclonal antibodies are monospecific and identical because they are produced by a single hybrid cell, the latter come from different cells. Thus polyclonal antibodies are actually a mixture of different immunoglobulin molecules secreted against a specific antigen, each one recognising a different epitope. This diversity in the molecular structures that constitute the polyclonal antibodies, renders its SERS spectra much more complex. They exhibit lower intensity at the same concentration than the mAb, and are not suitable for recording changes upon exposure to the antigen, because these will be much weaker and delocalised.

\[ \text{Fig 5. (A) Spectral deconvolution of the SERS spectra for free anti-BCG antibody and in the presence of decreasing concentrations of BCG. (B) Variation of the relative contribution of the C-C and C-H bending bands (ratio between corresponding areas) with BCG concentration. The solid line is a linear fit.} \]

Finally, it is interesting to compare the performance of the described method with other detection tools that have been developed for detection of cocaine and its metabolites. The detection limits for a variety of methods based on either separation (HPLC, LC or solid-phase extraction, coupled to fluorescence or mass detection\textsuperscript{37,38,39,40,41}), or immunological methods (ELISA, FIA or RIA\textsuperscript{42,43,44,45,46}) are in the range of 1 ng mL\textsuperscript{-1} (~1 nM), i.e. of the same order of the method presented here. However, one needs to bear in mind that the former (separation methods) require extensive sample preparation and are time consuming and not suitable for in situ detection, while the latter (immunological methods) require secondary detection antibodies, which not only notably increase the cost, but also reduce the reliability of the methods for the determination of small molecules, as the target structure needs to be bound by two different sites.

### 3.4 CONCLUSIONS

In summary, we have demonstrated the advantages of using selective monoclonal antibodies covalently attached to engineered silver coated carbon nanotubes for the determination of relevant small bioanalytes. This system profits from both the sensitivity of SERS spectroscopy toward small changes in the conformation of macromolecules and from the surface selection rules to evidence the changes taking place at the biointerface (the monoclonal selective antibody) upon complexation with the antigen (drug metabolite). This label-free, indirect detection method offers quantitative results at physiological levels as the bio-interface can also behave as an internal standard. The presented results pave the way toward the generation of portable sensors for fast ultradetection and quantification of relevant metabolites, which are important in diagnosis and detection. We see the use of discrete particles (CNT@Ag@mAb) instead of metal films or other conventional SERS supports as the basis for the safe remote interrogation of highly toxic sources in the environment or in biological fluids.

CHAPTER 4

Magnificent Sea Anemone-like Magnetic Silica Capsules Reinforced with Carbon Nanotubes

Abstract

This chapter reports a suitable strategy for the production of hollow capsules composed of silica-coated carbon nanotubes (CNTs). This approach is based on the combination of the layer-by-layer self-assembly technique which drives CNTs onto the surface of polystyrene spheres (PS) and a modified Stöber protocol for the silica coating of CNTs. The inorganic silica coating contributes to the formation of CNTs-based capsules with an anemone-like morphology. In a further improvement, previously synthesized Fe₃O₄ nanoparticles were first assembled onto the PS spheres, followed by the CNTs deposition and their silica coating, rendering the anemone-like hollow capsules magnetic. The magnetic response of these structures was evidenced by their electrophoretic deposition on a silicon substrate in the presence of an external magnetic field. Such hollow structures with this unique morphology present a high surface area together with a magnetic response and the presence of CNTs which reinforces them, inspiring potential applications in various different fields.
4.1 INTRODUCTION

Because of their exceptional mechanical strength, carbon nanotubes (CNTs) have a great potential as reinforcing materials\(^1\),\(^2\),\(^3\). Such applications may largely profit from their incorporation within more complex nanostructures, such as colloidal composites, or from coating with rigid materials as silica. In this respect, nature has served as an inspiring source of different SiO\(_2\)–based sophisticated morphologies such as hollow spheres with nano-macroholes resembling the hollow cell walls of diatoms (‘‘frustule’’\(^4\)), worm-like hollow silica shells encapsulating CNTs\(^5\), or silica-coated CdTe nanowires resembling bristled nanocentipedes\(^6\). We report in this chapter the fabrication of novel nanostructures that resemble magnificent sea anemones (heteractis magnifica), aiming at increasing the rigidity and the available surface of magnetic and reinforced CNT-based hollow capsules. The obtained final morphology comprises rigid hollow silica capsules reinforced with CNTs, which can be easily assembled by means of an external magnetic field upon loading with magnetic nanoparticles. We foresee potential applications for these structures such as reinforced capsules with increased mechanical stability, for use as nanoreactors, in drug delivery or as catalyst supports with increased surface area. The magnetic functionality additionally allows improved external manipulation of these anemone-like capsules for their assembly on surfaces or within tissues.

4.2 EXPERIMENTAL METHODS

**Chemicals:** Poly(diallyldimethylammonium chloride) (PDDA), poly(allylamine hydrochloride) (PAH, Mw~70 000 Da), and poly- (sodium 4-styrenesulfonate) (PSS, Mw~70 000 Da), were purchased from Aldrich. Sodium chloride was obtained from Merck. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system with a resistivity higher than 18.2M\(\Omega\)cm. Multiwall carbon nanotubes (MWNTs) 15±5 nm in diameter and 5–10 \(\mu\)m length, synthesized using plasma enhanced chemical vapor deposition (PECVD), were kindly provided by Nanolab (Boston) as a powder. Polystyrene colloidal particles were purchased from Microparticles GmbH, Berlin. The dynamic light scattering characterization of the final capsules was performed with a Zetasizer Nano S from Malvern Instruments operating with a 633 nm laser beam.

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Carbon nanotubes were oxidized by means of the following procedure\(^7\): 200 mg of MWNTs were sonicated for different times (2, 4, and 6 h) in 200 mL of a mixture of H\(_2\)SO\(_4\)/HNO\(_3\) (3:1) in order to obtain CNTs with different lengths. The sample was then washed with dilute NaOH aqueous solution and then three times with water by centrifugation/redispersion cycles. Finally, the MWNTs were dispersed in water, obtaining a stable dispersion of oxidized MWNTs with carboxylic groups on the walls, providing a negative surface charge.

Polyelectrolyte coated PS particles were prepared by diluting 1.5 mL of an ethanolic suspension of PS spheres (5 wt \%) with ethanol up to 5 mL, and adding this solution dropwise under weak sonication to 15 mL of PDDA solution (1 mg mL\(^{-1}\), containing 0.5 M NaCl), waiting for 1 h to allow PDDA adsorption, and then removing excess PDDA by three repeated centrifugation/wash cycles. 10 minutes of centrifugation at a speed of 3500 rpm was used. PSS (1 mg mL\(^{-1}\) containing 0.5 M NaCl) was then deposited onto the coated PS particles in a similar fashion and using the same conditions, the deposition of an additional layer of PAH, which forms the outermost layer, followed.

Fe\(_3\)O\(_4\) Nanoparticles: Aqueous dispersions of magnetite nanoparticles were prepared according to Massart’s method\(^8\), based on the co-precipitation of ferrous and ferric ion solutions (1:2 molar ratio). 20 mL of aqueous 1 M FeCl\(_3\) (97\%, Aldrich) and 5mL of 2M FeSO\(_4\)-7H\(_2\)O (99\%, Riedel-de-Haen) in 2 M HCl were added to 250 mL of 0.7 M NH\(_4\)OH (28–30\%, Aldrich) under rapid mechanical stirring. Stirring was allowed to continue for 30 min, and then the black solid product was allowed to precipitate. The sediment was redispersed in 50 mL distilled water, and subsequently three aliquots of 10 mL tetramethylammonium hydroxide solution (10\% in water, Aldrich) (1 M) were added, again with rapid stirring. Finally, water was added to the dispersion up to a total volume of 250 mL.

Fe\(_3\)O\(_4\)-coated PS spheres were obtained as follows: 1mL of the (PDDA/PSS/PAH)-coated PS particles (4 wt \%) were dispersed in 2.5 mL of a 0.5 M NaCl solution and added to 5mL of the as synthesized Fe\(_3\)O\(_4\) nanoparticles. An adsorption time of 30 min was allowed and the excess of magnetic nanoparticles was removed by three repeated centrifugation (3500 rpm, 10 min)/redispersion wash cycles.

Using the same approach CNT multilayer coatings were deposited on the PS and Fe\(_3\)O\(_4\)-coated PS spheres as follows: 1mL of the (PDDA/PSS/PAH)-coated PS particles (4 wt \%) were dispersed in 2.5 mL of a 0.5 M NaCl solution and added to 5mL of CNT dispersion (0.1 mg mL\(^{-1}\)). An adsorption time of 30 min was then allowed and excess of CNTs was removed by three repeated centrifugation (3500 rpm, 10 min)/redispersion wash cycles.

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In the first silica-deposition step, the hydrolysis and condensation of 3-aminopropyltrimethoxysilane (APS) and tetraethoxysilane (TEOS) were carried out under acidic conditions. 20 mL of ethanol containing 38.5 mL APS and 8mL TEOS was added to 5 mL of an aqueous dispersion of MWNTs-coated PS spheres containing citric acid (0.5 M, pH 2) under sonication. In the second step, the pH value of the solution was rapidly increased so that silane condensation was carried out under basic conditions. After 1.5 h, a solution of NH$_4$OH (1 M) was added dropwise until a pH value of 9–10 was reached. Subsequently, the solution was aged for 8 h and a compact, uniform, 30-nm-thick silica shell was obtained. Excess reactants were removed by three repeated centrifugation (3500 rpm, 10 min)/redispersion wash cycles.

Hollow capsules were prepared by exposing 1mL of the as-prepared PS–CNTs–SiO$_2$ structures to 1mL of CH$_3$Cl solution for 20 min. To ensure complete core removal this process was repeated two times. The hollow capsules were then washed three times by centrifugation at 4500 rpm for 10 min and redispersed in ethanol.

### 4.3 RESULTS AND DISCUSSION

The construction of these complex nanostructures involves an initial layer-by-layer (LbL)$^9,10,11$ sequential adsorption of polyelectrolytes and CNTs onto submicrometer-sized polystyrene (PS) spheres, which have been shown to retain the distinctive features of CNTs$^{12,13}$. Whereas PS-CNT composite particles have been converted into CNT-based hollow capsules by simply dissolving the spherical polymeric cores, the resulting flexible capsules readily collapse when dried, which largely hinders their practical use. However, further modification through subsequent coating with silica allowed us to create a new type of rigid branched capsule with a larger mechanical strength and an increased surface area that arises from their magnificent sea anemone-like structure. Additionally, further functionalization through adsorption of magnetic nanoparticles prior to CNTs deposition and silica coating leads to magnetic capsules, which can be assembled using an external magnetic field. A schematic representation of the synthetic processes followed in this work is provided in Scheme 1 for the sake of clarifying the different steps involved.

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Scheme 1. Schematic representation of the different steps followed in the synthetic process: polyelectrolyte coating of PS spheres (a), adsorption of Fe₃O₄ nanoparticles (b), adsorption of CNTs (c, d), and SiO₂ coating of the assembled CNTs.

**Fig 1.** Top: TEM images of PS–CNT composite spheres, before (a) and after (b) coating with an approximately 30 nm thick silica shell. Bottom: SEM image of PS–CNT composite particles before silica coating (c).

**Fig 1a** shows a typical transmission electron microscopy (TEM) image of the PS spheres (diameter~530 nm) coated with CNTs (diameter 15±5 nm, length~5–10 µm). The “hairy” morphology can be clearly seen in these images, with the CNTs protruding from the PS spheres surface. The extension of such “hairs” can be tailored through proper choice of the average length of the CNTs used for the assembly. As
Magnificent Sea Anemone-like Magnetic Silica Capsules Reinforced with Carbon Nanotubes

previously reported, the PS spheres were homogeneously wrapped, regardless of the CNTs length, and the composite particles form stable colloids with no need of further treatment, as evidenced by dynamic light scattering (DLS) measurements. The SEM image in Fig 1c (for silica coated particles) evidences the homogenous CNT coating over the entire PS surface.

The process used for silica coating of the PS–CNT nanocomposites was based on our previous work for the encapsulation of individual CNTs. This step is critical on the formation of these magnificent sea anemone-like hollow capsules because a controlled and selective silica deposition needs to be carried out to coat both the CNTs adsorbed onto the surface of PS template, as well as those partially protruding into solution, while avoiding silica nucleation in solution. Silica coating was performed by hydrolysis of tetraethyl orthosilicate, in two separate steps. A first silica deposition at lower (acidic) pH resulted in the formation of a thinner shell, whereas an outer shell was grown at a higher (basic) pH, allowing a tight control of the final thickness. The quality of the resulting coating and the smoothness of the silica surface are visible in Figure 1b,c with representative TEM and SEM images of silica-coated PS–CNT composite spheres. It is clear that the coating extends to partially desorbed CNTs, thereby maintaining the initial hairy morphology.

Upon silica coating, hollow structures were readily obtained through a washing process comprising the dissolution of the PS template in an ethanol/ chloroform mixture (1:1 volume ratio) and subsequent centrifugation and redispersion in ethanol. The TEM images shown in Fig 2 illustrate the gradual dissolution of the core, with PS particles partially dissolved after one washing cycle (a), and completely dissolved after two washing cycles. Although dissolution is expected to take place homogeneously from the shell inwards, upon drying the remaining PS forms approximately a hemisphere inside the CNT–SiO$_2$ shell. It is noteworthy that the outer morphology of the capsules was perfectly retained upon drying, as reflected in their spherical shape (see TEM image in Fig 2b, and SEM images in Fig 2c,d). Core dissolution is only possible because of the porous nature of the silica coating, as demonstrated in a number of previous works.

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Fig 2. Top: TEM images of partially (a) and completely (b) hollow CNT–SiO₂ capsules which remain rigid upon dissolution. Bottom: SEM images of hollow CNT–SiO₂ capsules fabricated with long (~5–10µm) (c), and short (~1µm) (d) CNTs.

The possibility of forming increasingly complex structures at the nanoscale, such as those with multi-level branching\textsuperscript{17}, opens the door to unique, three-dimensional materials and devices\textsuperscript{18}. The desorbed CNTs (like constituent arms) radiate in a branching fashion from a central core, creating a magnificent sea anemone-like structure whose specific surface area can be tailored according to specific requirements. Indeed, the physically stable silica-coated hollow capsules comprising CNTs acquire a much higher surface area when the “hair-like” structure due to the partially desorbed CNTs is enhanced. Therefore, by changing the length of the nanotubes used, the surface area can be controllably modified (Fig 2c,d). This increased surface area can be easily calculated as a function of the length of the desorbed CNTs and the sphere surface occupied by these attached CNTs. The following equation can be used to predict the available surface area of the branched capsules;

\[
S_{\text{capsule}} = S_{\text{sph}} - aS_{\text{sph}} + S_{\text{CNTs}} = S_{\text{sph}} - aS_{\text{sph}} + n(2\pi rL + 2\pi r^2)
\]

\(S_{\text{sph}}\) being the surface of the spherical substrate used, \(aS_{\text{sph}}\) (with \(a \in (0,1)\)) is the surface occupied by the \(n\) attached CNTs with a portion desorbed from the surface of the capsule. \(2 \pi rL\) is the surface of the CNT considered as a cylinder (\(r\) and \(L\) are the radius and the length of the desorbed portion, respectively) and \(2\pi r^2\) is the area of the outer tip.

that is considered to be a half-sphere. This increased surface area becomes available for specific functionalization and the great versatility of these end arms can be exploited for a range of purposes including sensing, catalysis, or biochemical activity.

In an attempt to increase the external addressability of the capsules, pre-synthesized Fe$_3$O$_4$ nanoparticles were assembled onto PS spheres prior to the LbL coating. These magnetic nanoparticles were adsorbed onto the PS templates using a modification of the work reported by Spasova et al.$^{19}$, which also comprises the use of the LbL self-assembly technique, in a similar way as for the CNTs deposition. The Fe$_3$O$_4$ nanoparticles were thus assembled between the sacrificial template and the CNTs shell, rendering the designed architecture magnetic. Once magnetically functionalized, the procedure described above was followed for CNTs adsorption, silica coating and hollow capsule production, obtaining an identical hollow morphology (Fig 3b). Furthermore, the magnetic nanoparticles do not desorb from the capsule into the inner cavity because they are held on the capsule wall by the inner polyelectrolyte layer.

![Fig 3. Top: SEM images of the one-dimensional structures formed by the magnetic, hollow and reinforced capsules under an applied magnetic field. Bottom: TEM image of a magnetic hollow silica capsule where the Fe$_3$O$_4$ nanoparticles can be appreciated as small dots.](image)

The magnetic response of these hollow capsules was demonstrated by drying a drop of the dispersion on a Si wafer under an external magnetic field. One-dimensional assemblies were formed through magnetophoretic deposition\textsuperscript{20} at 300 K. Figure 3a shows this typical magnetic behavior for capsules deposited on a substrate under a 0.2 T magnetic field. The alignment of these hollow structures in individual chains under the presence of an external magnetic field also shows that they are not aggregated in solution during the synthetic process. Additional dynamic light scattering (DLS) measurements of these systems with a 0.25 polydispersity index (PdI) (determined by a variant of the so-called cumulants method)\textsuperscript{21}, reveal a quite uniform dispersion with a Z-average diameter of 940 nm that agrees well with the size measured from the SEM and TEM images of these magnetic hollow structures (~900 nm).

4.4 CONCLUSIONS

In summary, we have devised a feasible and successful approach to the fabrication of SiO\textsubscript{2}-coated CNTs hollow capsules that resemble natural magnificent sea anemones. Such hollow structures with this unique morphology present a high surface area that can easily be tailored. Moreover, the reinforcement of the silica capsules with CNTs, together with incorporation of a magnetic functionality, makes them exceptional structures where the functionality of every intrinsic component can be exploited within the whole. Thus, while the silica shell acts as a porous membrane providing rigidity to the capsule (and allowing their bioconjugation for different applications), CNTs serve as reinforcement and template for the branched shape of the structure, and the magnetic

functionality from the Fe₃O₄ nanoparticles allows their manipulation under external magnetic fields. For all these reasons, these unique nanostructures offer interesting possibilities for use in potential applications such as controlled encapsulation and drug delivery, nanoreactors for catalytic reactions, biochemical activities or sensing, among others.
CHAPTER 5

Tailoring the Magnetic Properties of Nickel Nanoshells through Controlled Chemical Growth

Abstract

A wet-chemistry-based synthetic method has been devised for the preparation of core-shell particles with tuned magnetic properties. Tailoring of the morphology and properties was achieved through variations in the amount of magnetic material deposited. The fabrication of these superparamagnetic particles (with $T_B$ below or above room temperature), consequently permit us to envisage different applications, overcoming well-known drawbacks such as the superparamagnetic limit at room temperature.
5.1 INTRODUCTION

The study of magnetic nanoparticles and hybrid magnetic nanostructures has gained increased attention due to a considerable number of potential applications that range from data storage, catalysis to bio-related applications such as drug delivery, magnetic resonance imaging (MRI), bioseparation or environmental remediation. In order to fulfill the requirements of many of these applications, an accurate control over the magnetic properties is strongly demanded and the main strategies are based on the size dependence, as being one of the most influential parameters. Only in recent decades it has become clear that solid-state magnetism is to a large extent a nanostructural phenomenon and in order to face this challenge, colloid chemistry has proven a powerful tool to achieve a fine tuning, not only over particle size and morphology but also for designing hybrid nanostructures with the required magnetic properties (depending on the final application). However, it appears that most of the synthetic methods cannot achieve monodisperse particles within a broad range of sizes without critically modifying the nature of the stabilizing ligand and/or the reducing agent. This renders very difficult the direct comparison of the size influence on a particular magnetic property. Moreover, the ripening mechanisms have rarely been discussed even though they are highly relevant for the final magnetic properties of the particles.

In terms of hybrid nanostructures, the combination of dielectric organic cores with magnetic shells offers an increased interest since it yields particles with low average density, electrical conductivity and tunable magnetic behavior. However, there

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is again a lack of synthetic methods for the preparation of such (sub-)micron-sized core-shell spheres with a controllable magnetic behavior that would allow the extension or improvement of the related applications. There are only few reports in the literature related to this issue, among which it is worth pointing out the preparation of polystyrene/Ni\textsuperscript{21} or SiO\textsubscript{2}/Ni\textsuperscript{22} and the report by Tierno et al. about metallic shells of NiP, CoP or metal mixed alloys (CoNiP, NiFeP or CoFeP) deposited by electroless plating onto poly(methyl methacrylate) spheres\textsuperscript{23}. Some advantages of these amorphous alloys include their chemical stability (no oxidation), as well as their reduced magnetic moments due to the presence of non-magnetic atoms such as P, B or Si\textsuperscript{24}. However, synthetic methods allowing the tuning of the magnetic properties displayed by the typical transition metal-based magnetic materials are highly deficient.

Regarding such transition metal-based magnetic materials, Ni (particularly Ni nanoparticles) has been highly demanded in industry and has indeed put forward new applications in relevant areas such as catalysis\textsuperscript{22,25}, Ni-based batteries\textsuperscript{26}, carbon nanotubes growth\textsuperscript{27}, or nickel–ceramic complexes\textsuperscript{28}. Therefore, we have devised a wet-chemistry based synthetic method for the preparation of core-shell structured particles with magnetic properties directed by the Ni content. This approach is unique regarding the full control over the thickness of the shells (without variation in terms of ligands or reducing agents used), composed by this standard magnetic material onto diamagnetic spherical colloids. Consequently, the emphasis of this work is not only on the control of the shell thickness but also on the involved physics, because of the many relevant applications, including permanent magnets, soft magnets, magnetic recording media, sensors and structures and materials for spin electronics.

The deposition of Ni onto monodisperse sub-micrometer-sized polystyrene (PS) spheres was accomplished in several steps, starting from the growth of nano-islands of the magnetic material on the polymeric surface until the formation of a complete and homogenous metallic shell. This stepwise Ni deposition process becomes the key factor in the fine control over the thickness of the magnetic nanoshells and consequently, in the fine tuning of the magnetic properties displayed. It is very important to point out that the properties exclusively depend on the shell thickness and not on the surface chemistry or mechanism of formation since these are identical for all samples. This fine tuning leads to composite particles with a wide spectrum of magnetic behaviors (from dia- and superpara-magnetism to ferromagnetism). Interestingly, the magnetic properties of the final composites can be modulated while delimiting the final size

within the submicron range (600-700 nm), in which the diameter of the PS colloidal substrate is kept constant and only the magnetic shell thickness varies from zero up to ~40 nm.

These spherical magnetic nanostructures may become suitable as model systems where ensembles of interacting particles can be studied\textsuperscript{29}, or as magnetic tweezers to be manipulated using an external magnetic field\textsuperscript{30}. It is worth additionally pointing out that this synthetic approach is performed in aqueous solution, avoiding the use of expensive organic solvents which otherwise would render it impractical for industrial applications.

### 5.2 EXPERIMENTAL METHODS

All chemicals were of analytical grade and used without further purification.

#### Polystyrene bead functionalization:
600 nm polystyrene (PS) beads (Microparticles GmbH, 0.018 standard deviation) were coated with four alternating polyelectrolyte monolayers by using the electrostatic layer by layer self-assembly protocol\textsuperscript{1}. These four layers of polyelectrolytes were chosen instead of two, in order to improve the homogeneity of the coating shell and generate the positive surface charge (zeta potential, $\xi = +40$ mV) necessary for the electrostatic adsorption of 2-3 nm Pt seeds ($\xi = -20$ mV).

#### Platinum seeds:
The Pt nanoparticles to be deposited onto the PS surface were synthesized as follows. Sodium borohydride (1.22 mL, 0.02 M) was added as a reducing agent to a solution of sodium citrate (1.25 mL, 0.1 M) and $\text{H}_2\text{PtCl}_6$ (1.25 mL, 0.05M) in ultrapure water (21.5 mL) and the resulting solution stirred for 10 min. For Pt deposition on the PS surface, PS@PAH (5 mg/mL) was added to a solution of Pt seeds (25.2 mL, 2.48 mM), and 1h later the solution was centrifuged (20 min, 5000 rpm) and redispersed in pure water (20 mL) resulting in the removal of non-deposited and free Pt nanoparticles.

#### Ni growth:
The PS/Pt dispersion (0.25 mg/mL) was added to an aqueous solution (15 mL) containing a NiCl\textsubscript{2} stock-solution (0.25 M) and hydrazine (2.5 M), in the following Ni:PS weight ratios: 1:20, 1:10, 1:6, 6:1 and 12:1. The mixture was maintained at 40ºC for 2 h in all cases, then centrifuged (4000 rpm, 15 min) and washed with water and ethanol (twice for each solvent). In order to obtain the magnetic hollow structures, the PS cores were removed using a 50 % (v/v) chloroform/ethanol solution in which the core- shell structures were kept for 10 h. The solutions were subsequently centrifuged and the supernatant removed (the process was repeated 3 times). The samples were additionally washed with ethanol and water (twice for each solvent).

\textsuperscript{29} A. van Blaaderen, Science 2003, 301, 470-471.

\textsuperscript{30} S. B. Smith, L. Finzi and C. Bustamante, Science 1992, 258, 1122-1126.
5.3 RESULTS AND DISCUSSION

Fig. 1 summarizes the experimental procedure comprising two main steps. Starting from naked diamagnetic PS spheres, several layers of polyelectrolytes (PSS/PAH/PSS/PAH) were deposited onto the surface of these colloidal templates. This implies alternating the adsorption of PSS (poly(sodium 4-styrenesulfonate)) and PAH (poly(allylamine hydrochloride)) in order to smoothen their surface. Once more, exploiting electrostatic interactions available thanks to the presence of this priming multilayer assembly (which leads to homogeneous charging of the polymeric surface), Pt nanoparticles were adsorbed onto the surface of the PS colloids (Fig. 1aI)\textsuperscript{31,32}. This step is favored by the presence of citrate ions adsorbed onto the 2-3 nm Pt nanoparticles, and therefore responsible for their electrostatic attachment. These metallic Pt seeds provide the catalytic activity required for the subsequent deposition of nickel (Fig. 1aII)\textsuperscript{33,34}. This second step, that involves the controlled reduction of Ni\textsuperscript{2+} ions by hydrazine, permits a fine tuning over the thickness of the outer magnetic shell (illustrated in Fig. 1) through the use of different Ni precursor concentrations. The latter aspect renders the synthetic method very promising not only because of its mentioned simplicity but also because of the wide spectra of materials with which it can be used. One last characteristic that deserves to be pointed out is the fact that the synthetic strategy avoids the use of surfactants or other stabilizers in aqueous solution. This becomes particularly commendable in magnetism, where it is important to avoid surface chemistry perturbations of the magnetic properties, mainly in the nanosized structures characterized by an increased surface-to-volume ratio\textsuperscript{35,36}.

Fig. 1 Experimental synthetic procedure involving two main steps: functionalization of the PS colloids with four PSS/PAH/PSS/PAH polyelectrolyte layers and consequent attachment of negatively charged Pt nanoparticles onto their surface (I), and reduction of Ni²⁺ ions on their 40 surface favored by the Pt nanoparticles behaving as catalytic centers (II).

Fig. 2 Representative STEM (a) and TEM (b,c,d) micrographs at low (left) and high (right) magnification, and XPS (e, f) analysis of the spherical composites obtained with different PS/Ni ratios: (a) 1:20, (b) 1:10, (c) 6:1 and (d) 12:1.
The main goal of the second step focuses on the PS: Ni ratio, so as to modulate the relative content between diamagnetic and ferromagnetic materials within the composite nanostructures. Ni:PS weight ratios were quantified in five different samples, which were labeled as 1-5 (1:20(1), 1:10(2), 1:6(3), 6:1(4), 12:1(5)). These as-synthesized magnetic composites were thoroughly characterized by transmission electron microscopy (TEM). Fig. 2 (left column) shows TEM micrographs of PS/Pt spheres (≈ 600 nm average diameter) where different amounts of Ni were grown (Ni:PS ratio increases from top to bottom in the images displayed). When the lowest weight ratios were used (1:20(1), 1:10(2), 1:6(3)), Ni grows onto the PS surface forming nano-islands of magnetic material and, because of the insufficient amount, the outer shell is not completed. Even though the relative amount of Ni was clearly higher for the second and third samples (1:10(2), 1:6(3)), a uniform and entire magnetic shell was far from accomplished. Fig. 2b (right, higher magnification) shows a TEM image of sample 2 (10:1), on which Pt nanoparticles can clearly be observed. These homogeneously distributed Pt nanoparticles deposited on the surface of the polymeric substrates behave as the initial nucleation sites where the deposition of the magnetic material starts and thus assists the formation of the mentioned nano-islands, that can be appreciated in the TEM images, especially those of samples (1:20(1), 1:10(2)). Solid outer Ni shells were formed when the amount of magnetic material was significantly increased (6:1(4), 12:1(5)) and it is from this point on when shell thickness can be tuned. This was confirmed by TEM and an example of uniform magnetic shells (≈ 40 nm thick) onto PS/Pt nanocomposites from sample 5 (12:1) is shown in Fig. 2d (at lower (left) and higher (right) magnification). Further evidence of the steady deposition of the nickel shells was obtained by selected area electron diffraction (SAED). Fig. 3 shows that increasing the PS: Ni weight ratio the reflections of metallic nickel become more clearly defined, and no other reflections like nickel oxides are observed.

X-ray photoelectron spectroscopy (XPS) analysis of the as-synthesized samples (PS/Pt/Ni) was carried out to analyze the formation of nickel oxides (favored by the oxygen-rich environment given by the aqueous solution) (Fig. 2e,f). The binding energy of Pt4f detected for the PS/Pt/Ni samples is located at 71.42-71.57 eV, characteristic of metallic platinum. Since the amount of Ni varies in the different samples prepared, the Pt signal decreases and finally it cannot be identified for that with the highest Ni content (Fig. 2e). The XPS spectra (Fig. 2f) additionally show the binding energy of the Ni2p main peak at 853.1-853.8 eV, slightly shifted from standard values for Ni⁰ (852.1-853.2 eV) but also far away from energies attributed to Ni²⁺ (854.0-855.5 eV). It is interesting to note that although there is no clear evidence on the formation of nickel oxides, the water based synthetic process together with the minor shift of the main peak of metallic nickel towards higher binding energies presumable reveal the presence of a thin oxidized outer layer. This surface passivation would provide the samples with additional surface stabilization and would play a major role in preventing further oxidation of the inner metallic nickel shell.
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Fig. 3. SAED analysis of PS/Pt/Ni composite particles with different PS:Ni weight ratio, 6:1 (a) 10:1 (b), 1:6 (c).

Fig. 4. STEM elemental mapping through XEDS analysis, with C (K\(_\alpha\) line) in red, Pt (L\(_\alpha\) line) in green and Ni (K\(_\alpha\) line) in blue for PS/Pt/Ni composite particles with different magnetic content (samples 1:20 (1) (a), 6:1 (4) (b), and 12:1 (5) (c)). On the right column, magnified images are included.

The dielectric (core)/magnetic (shell) structure was confirmed through a detailed scanning transmission electron microscopy (STEM)-X-ray energy dispersion spectroscopy (XEDS) analysis. The STEM-XEDS elemental mapping of the obtained
heterostructures shows the relative distribution of the elements, with red, green and blue areas corresponding to C (C Kα line), Pt (Pt Lα line) and Ni (Ni Kα line) respectively. Fig. 4a shows the images obtained with this technique, corresponding to the hybrid PS/Pt/Ni nanocomposites with the lower Ni content (sample 1:20 (1)). Thus, only signals from C and Pt (red and green areas in the image) are observed and only few blue spots corresponding to Ni nanoislands can be appreciated. The formation of the complete magnetic shell was not accomplished either in the case of sample (3) (1:6), which corresponds to that in Fig. 4b. Indeed, blue areas corresponding to Ni can be observed in the elemental mapping but combined with green spots corresponding to the Pt from the previously deposited catalytic nanoparticles. As mentioned above, as the magnetic material to PS ratio is increased (samples 4 (6:1) and 5 (12:1)), the formation of thin but complete Ni shells is accomplished and evidenced using the STEM-XEDS technique. Thus, the observation of a complete blue area surrounding the particles can be appreciated in Fig. 4c (right). Additionally, SAED analysis (Fig.3) confirmed the formation of the characteristic fcc crystalline structure of metallic Ni.

Full evidence of the formation of homogenous and complete magnetic shells was obtained by treating the different PS/Pt/Ni hybrid composite particles with a solvent able to dissolve the PS core. Indeed, upon treatment with chloroform, the morphology of the samples 1 (1:20) and 2 (1:10) was strongly affected (Fig. 5a) while sample 4 (6:1) appears as a rigid magnetic shell (Fig. 5b) once the PS spherical templates were completely dissolved. On the contrary, sample 5 (12:1) evidenced a complete, compact and homogeneous magnetic shell since the dissolution of the polymeric core was hindered, even in the presence of chloroform in solution (Fig. 5c).

![TEM micrographs of hybrid composite particles with different Ni:PS ratio (samples 2 (1:10) (a), 4 (6:1) (b) and 5 (12:1) (c), after treatment with chloroform](image)

This experiment also underlines the porous nature of the magnetic shell (in the initial steps) which reflects the existence of defects in the compaction/conformation when Ni atoms are deposited to form the crystalline domains and the polycrystalline lattice (Fig. 5b) (very important in the formation of the magnetic domains).

Although many reports have been published on the synthesis of low-dimensional magnetic materials in high yields, it is still important to underline those strategies that enable control over the magnetic response. The DC magnetic properties of the nanocomposites were thus recorded in a SQUID magnetometer. Fig. 6a,b shows the
hysteresis curves collected at 300 and 5 K respectively, for the five samples studied (I-5). Fig. 6a underlines the room temperature transition from diamagnetic to superparamagnetic behavior as the thickness of the magnetic shell is increased. Since the Ni weight fraction is very low in sample I, at 300K the diamagnetic contributions from the polymer and polyelectrolytes become dominant (black line in Fig. 6a, see inset). However, when decreasing temperature down to 5K, the magnetic moments of the small amount of nickel deposited on the surface of the supporting colloids, become frozen (not affected by thermal fluctuations), therefore displaying an appreciable net value of magnetization. As the magnetic shell thickness is increased, we first encounter typical superparamagnetic behavior with an almost closed hysteresis loop at 300 K (displaying very low coercivity) for sample 2. If we further increase the magnetic material/PS ratio (samples 3, 4 and 5), we end up with open hysteresis loops presenting coercivities around 100 Oe even at room temperature (not appreciated in fig. 5a because of the low values of coercivity and the scale used to reflect the mentioned diamagnetic to superparamagnetic transition). So we can label these samples as ferromagnetic-like materials. At 5 K, coercivity increases as the magnetic material ratio is increased until reaching sample 3 and then decreases (for samples 4 and 5). There are indeed several reasons behind the decrease in coercivity when increasing size. In this case, besides the exchange bias contribution (directly dependent on the FM–AFM interface, which may decrease for the thicker shells), the formation of a multidomain structure is favored when the shell is thick, also leading to a decrease in the coercivity.

Fig. 6 Field (a, b) and temperature (c, d) dependence of the magnetization as a function of the Ni/PS ratio (labels correspond to sample names).

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**Fig. 6** Field (a, b) and temperature (c, d) dependence of the magnetization as a function of the Ni/PS ratio (labels correspond to sample names).
Fig. 6c, d shows the 100 Oe field-cooled (FC) and zero-field cooled (ZFC) magnetization curves as a function of temperature for the five samples analyzed. Fig. 6c includes samples 1, 2 and 3 ZFC-FC curves, while those belonging to samples 4 and 5 are shown in Fig. 5d, to better appreciate the shape of the curves depending on their different magnetic behavior.

Fig. 6c displays the characteristic superparamagnetic behavior of the ZFC/FC curves, with a maximum in the ZFC curve at 55 and 95 K for samples 2 and 3 respectively, and labeled as the blocking temperature \( T_B \) of the composites (Tab. 1). In these cases, both \( T_B \) and \( T_{irrev} \) (temperature at which FC and ZFC curves split) are very similar, which can be associated to a very low polydispersity within the samples (driven by the homogeneous average size of the PS spheres used as supports). There is an additional size-independent maximum located at 15 K (also in the case of sample 1) in the ZFC curves of the samples. This low-temperature peak can be associated to the presence of an antiferromagnetic phase \((\text{Ni(OH})_2\)) with a reduced Néel temperature \( T_N \). The reduced \( T_N \) can be explained by the finite size of the \( \text{Ni(OH})_2 \) crystallites formed due to the already mentioned oxidation process. Since sample 1 contains Ni nanoislands on the surface of the PS spheres, the oxidation process is more extended, leading to a magnetic behavior dominated by the nickel hydroxide at lower temperatures (at higher temperatures, the diamagnetic contribution of the PS substrates is dominant, as mentioned above). The ZFC and FC curves (Fig. 6d) corresponding to samples 4 and 5 display typical superparamagnetic behavior with blocking temperatures close to but above 300 K (ferromagnetic-like behavior). In both ZFC curves, the reduced \( T_N \) can also be appreciated, again reflecting the presence of \( \text{Ni(OH})_2 \) crystallites in the magnetic shell, in which dominates the metallic Ni content.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ni:PS (weight ratio)</th>
<th>Magnetic behavior</th>
<th>( M_s ) (emu/g)</th>
<th>( H_c ) (Oe) ( (T = 5 \text{ K}) )</th>
<th>( T_B ) (K)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>1:20</td>
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<td>15</td>
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<tr>
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<td>Superparamagnetic</td>
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<td>991</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>1:6</td>
<td>Superparamagnetic</td>
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<td>1297</td>
<td>95</td>
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<tr>
<td>4</td>
<td>6:1</td>
<td>Ferromagnetic</td>
<td>12</td>
<td>703</td>
<td>&gt;300</td>
</tr>
<tr>
<td>5</td>
<td>12:1</td>
<td>Ferromagnetic</td>
<td>35</td>
<td>304</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

Tab. 1 Summary of magnetic properties (magnetic behavior, \( M_s \), \( H_c \), \( T_B \)) as a function of the Ni:PS weight ratio

5.4 CONCLUSIONS

In summary, this work shows a feasible approach for the controlled and uniform deposition of a magnetic material onto colloidal templates. Tailoring of the magnetic properties of the final nanocomposites was successfully achieved as directly dependent on the amount of deposited magnetic material and neither on the surface chemistry (no ligands at the surface) nor on the formation process. This synthetic method thus renders viable fabrication of superparamagnetic particles (with $T_B$ below or above room temperature), dependent both on the specific template used and on the amount of magnetic material deposited. These characteristics consequently permit us to envisage different applications, overcoming well-known drawbacks such as the superparamagnetic limit at room temperature.
CHAPTER 6

Highly Active Nanoreactors: Encapsulation of Nanomaterials based on Confined Catalysis

Abstract

This chapter describes the development of spherical nanoreactors endowed with high catalytic activity. These systems are based on the previous encapsulation of singular dendritic Pt nanoparticles (Pt\textsubscript{d} NPs) located in the inner walls of porous silica nanocapsules. It is notable that this synthetic approach helps to preserve the catalytic activity of the catalyst, even after their encapsulation. Thus, the single-crystal Pt\textsubscript{d} NPs will catalyze specific reactions to take place inside the silica capsule. As it is the shell that allows the product exchange between the inner cavity and the bulk solution, the reaction becomes confined and controlled inside the nanoreactors. To verify this potential and the efficacy as a nanoreactor, an electron-transfer process was chosen as a test reaction, and additionally the system was successfully brought into play for the production of Ni/ NiO nanostructures with different magnetic behavior, in view of the controlled reactions carried out in the inner cavity.
6.1 INTRODUCTION

Considerable efforts have been devoted to the fabrication of nanomaterials with well-defined morphologies for specific applications. This is the case of nanocapsules that simultaneously provide the advantages of hollow and porous systems. They can consequently be used as storage spaces or reaction chambers while supplying the necessary paths for the design of controlled uptake/release systems\(^1\)\(^2\). A particular interest for the performance of chemical reactions in these confined environments has led to catalyst-containing hollow nanocapsules, so that a diffusional product/substrate exchange between the inner cavity and the bulk solution takes place in an efficient way. Clearly, the nature of the interface, as well as the inner and outer surface properties of these nanoreactors, appear as critical factors to modulate the catalytic activity of the whole system.

Nanocapsules synthetic strategies include self-assembly and subsequent cross-linking of pre-formed nanosized objects\(^3\), or direct reactive routes such as interfacial polymerization with reagents dissolved in different liquid phases\(^4\). This latter approach requires soluble precursors in both the dispersed and the continuous solvent phase. Thus, they can react at the interface and yield the desired shell but this condition also constrains the possible precursors to be employed. Additionally, because capsules should be permeable to numerous reagents, their applications as nanoreactors will depend on the controllable uniform porosity at the molecular scale, a feature that in fact would enable the fabrication of size-selective microreactors\(^5\). In order to fulfill this requirement we have chosen silicon oxide (SiO\(_2\))\(^6\). Indeed, depending on the method exploited for its precipitation, networks of uniform but different pores of molecular dimensions can be provided\(^7\)\(^8\).

The main driving force prompting the exploration of nanocapsule systems is based on their large and uniform porosity, underlying their remarkable potential in such diverse areas as biomedicine\(^9\)\(^10\)\(^11\), gas storage\(^12\), catalysis\(^13\), or adsorptive separation\(^14\)\(^15\). Their

\(^{6}\)V. Salgueirino-Maceira, M. A. Correa-Duarte, Adv. Mater. 2007, 19, 4131
\(^{8}\)G. Fei; Botella, Pablo; Corma, Avelino; Blesa, Jose; Dong, Lin, J. Phys. Chem. B, 2009, 113, 1796.
successful application depends on the availability of the synthetic methods to prepare defect-free capsules so that permeation does not occur as a result of uncontrolled porosity through gaps or cracks, but solely through the existing channels.

The nanocapsule activity and the properties of their permeable membrane have been widely reported but few of such reports were related to catalysts located inside the nanocavity\textsuperscript{16,17,18,19,20}. In fact, examples of catalyst-containing nanoreactors are scarce and no close attention has been paid to the degradation of the catalyst activity during their encapsulation. Thus, more efforts should be made in this direction.

6.2 EXPERIMENTAL METHODS

All chemicals were of analytical grade and used without further purification.

**Polystyrene beads functionalization:** 600 nm polystyrene (PS) beads (Microparticles GmbH, 0.018 standard deviation) were coated with four alternating polyelectrolyte monolayers by using the electrostatic layer by layer self-assembly protocol\textsuperscript{24}. Thus, PS nanoparticles were first grafted with polystyrene sulfonate (PSS), a negatively charged polyelectrolyte. Then a monolayer of polyallylamine hydrochloride (PAH) as positively charged polyelectrolyte was grafted onto the particles. This process was repeated in order to obtain PS grafted with four layers of polymers (PS/PSS/PAH/PSS/PAH). Four layers of polyelectrolytes were chosen instead of two, in order to improve the homogeneity of the coating shell and generate the positive surface charge (zeta potential, $\xi = +40$ mV) necessary for the electrostatic adsorption of 20-30 nm dendritic Pt nanoparticles ($\xi = -20$ mV).

**Dendritic Pt nanoparticles:** The Pt\textsubscript{d} nanoparticles to be deposited onto the PS surface were synthesized elsewhere\textsuperscript{26}. Briefly, sodium borohydride (1.22 mL, 0.02 M) was added as a reducing agent to a solution of sodium citrate (1.25 mL, 0.1M) and K\textsubscript{2}PtCl\textsubscript{4} (1.25 mL, 0.1M) in ultrapure water (21.5 mL) and the resulting solution stirred for 10 min. For Pt deposition on the PS surface, PS@PSS/PAH/PSS/PAH (5 mg/mL) was added to a solution of Pt seeds (25.2 mL, 4.96 mM), and 1h later the solution was centrifuged (20 min, 5000 rpm) and redispersed in pure water (20 mL) resulting in the removal of non-deposited and free Pt dendritic nanoparticles.

\textsuperscript{15} J. B. Fei, Y. Cui, X. H. Yan, W. Qi, Y. Yang, K. W. Wang, Q. He, J. B. Li, Avd.Mater. 2008, 20, 452.
Silica coating: PS@Pt@SiO₂: Silica coating was carried out following the method described by Graf et al. Briefly, 1.5 mL of 110 mg mL⁻¹ polyvinylpyrrolidone (PVP, MW=40,000) was added to 5 mL of PS@Pt dendritic suspension, 1.25 mg PS/mL. Two hours later, the suspension was washed three times by centrifugation at 3000 rpm, the sediment resuspended in 10 mL of a solution of NH₄OH in ethanol 4.2 % (v/v), and sonicated for 5 min. Then, 43.5 μL of a 10% (v/v) solution of tetraethoxysilane (TEOS) in ethanol was added to the suspension, centrifuged three times and washed with water.

Hollow capsules fabrication: Pt₄@SiO₂: Dissolution of PS cores was carried out by shaking the bead suspension in an ethanol/chloroform 1:1 mixture in an orbital shaker for three days. Hollow capsules were washed and centrifuged three times, the first two with ethanol and then with water.

Confined growth of metal NPs: Ni-Pt₄@SiO₂: In order to obtain the magnetic hollow structures, the Pt₄@SiO₂ dispersion (0.5 mg capsules/mL) was added to 10 mL of an aqueous solution containing NiCl₂ and hydrazine. Thus, we used 1.7x10⁻² M NiCl₂ and 0.35 M hydrazine for sample A, and 2.1x10⁻² NiCl₂ and 0.39 M hydrazine for sample B. The mixture was maintained at 40°C for 2 h, then centrifuged (4000 rpm, 15 min) and washed with water and ethanol (twice with each solvent).

Characterization: TEM images were obtained using a JEOL JEM 1010 transmission electron microscope operating at an acceleration voltage of 100 kV. HRTEM, STEM and elemental mapping by XEDS analysis were carried out with a JEOL JEM 2010F transmission electron microscope operating at an acceleration voltage of 200 kV. A Vibrating Sample Magnetometer (VSM) was employed for the magnetic characterization.

6.3 RESULTS AND DISCUSSION

Given the special relevance of each of the different features underlined, we have developed nanoreactors endowed with high catalytic activity. These systems are based on the previous encapsulation of singular dendritic Pt nanoparticles (Pt₄ NPs), located in the hollow empty space enclosed by porous silica nanocapsules. It is worth pointing out that this synthetic approach helps to preserve the catalytic activity of the catalysts, even after their encapsulation. Thus, the single crystal Pt₄ NPs will catalyze specific reactions taking place inside the porous silica shell. Since it is the shell that allows the product/substrate exchange between the inner cavity and the bulk solution, the reaction becomes confined and controlled inside the nanoreactors. In order to verify this possibility and the efficacy of the system as a nanoreactor, an electron transfer process was chosen as a test reaction. Additionally, the system was successfully brought into play for the production of Ni/NiO nanostructures with different magnetic behavior, in view of the controlled reactions carried out in the inner cavity. This finding opens the door to an engineered capsules fabrication to modulate the inclusion and possible isolation of different inorganic materials.
The hollow capsules were fabricated by means of an initial polymer functionalization in a similar way to that described in the literature\textsuperscript{21,22}. The method commonly comprises the coating of the surface template-particles (polystyrene (PS) in our case) with charged polyelectrolytes. The stepwise adsorption of these polyelectrolytes reverses the charge on the surface template and aids the deposition of subsequent layers of a wide range of charged components\textsuperscript{17,23,24,25}. Negatively charged Pt\textsubscript{d} NPs were therefore driven onto the modified and now positively charged PS templates surface (see experimental section) to form PS/Pt\textsubscript{d} composites. Subsequently, a controlled silicon oxide precipitation was forced to take place on the surface of the PS/Pt\textsubscript{d} composites, permitting the formation of a hybrid and inorganic shell surrounding the initial cores. Finally, the PS core was removed promoting the formation of hollow capsules (Scheme 1). This last step is also crucial for the availability of the Pt\textsubscript{d} NPs as catalysts in reactions confined in this evolved empty space, rendering the nanocapsules effective nanoreactors (Pt\textsubscript{d}/SiO\textsubscript{2}).

\textit{Scheme 1. Synthesis of Ni/NiO magnetic nanostructures, as a result of the controlled reactions carried out in the inner cavity of the previously formed nanoreactor: (a) silica coating of hybrid particles composed of dendritic Pt NPs deposited onto polystyrene (PS) colloidal templates, followed by polymer dissolution, (b) Ni\textsuperscript{2+} ions reduction using hydrazine and catalyzed with the dendritic Pt NPs, confined inside the silica hollow capsule.}

\textsuperscript{22} F. Caruso, R. A. Caruso, H. Mohwald, Science 1998, 282, 1111
\textsuperscript{24} M. Sanles-Sobrido, V. Salgueirio-Maceira, M. A. Correa-Duarte, L. M. Liz-Marzan, Small 2008, 4, 583
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Fig. 1. TEM images of Pt\(_d\) NPs deposited onto PS spheres (a) and of the hollow capsules obtained after silica coating of hybrid PS/Pt\(_d\) particles followed by the etching of the PS core (b, c, and d).

Fig. 1 shows the two-step strategy exploited for capsules fabrication. Fig. 1a includes a TEM image and a scheme (inset on top-right) of the Pt\(_d\)-coated PS templates. Fig. 1b,c and d display images of the obtained hollowed-out Pt-containing nanostructures and a representative scheme. A higher magnification image (Fig 1d) clearly shows the Pt\(_d\) nanoparticles, observed through the much lower contrast of the silica shell. These Pt\(_d\) nanoparticles, as already reported, are single crystal\(^{26}\). The silicon oxide has uniformly precipitated over the entire surface of the Pt\(_d\)-coated PS templates and no presence of defects or cracks is observed, even after dissolution of the PS core. This consistent and homogeneous silica shell acts as a membrane for the nanocapsules, therefore ensuring that the exchange of reactants and products takes place solely through the pores that its chemical disposition comprises.

Platinum-based nanomaterials are among the most widely used in catalysis. Their activity and selectivity are strongly dependent on composition, size, and shape, and consequently, much effort has been devoted in the search for new and more efficient Pt structures\(^{27,28,29}\). Accordingly, redox probe tests with a ferro/ferri-cyanide system in aqueous solution were conducted in the presence of the hollow (Pt\(_d\)/SiO\(_2\)) and non-hollow-core (PS/Pt\(_d\)) composites reported herein, in order to assess their catalytic activity. These reactions were run by first mixing the Pt-based catalysts and sodium

borohydride. In the case of hollow capsules, BH$_4^-$ ions diffuse through the pores of silica reacting with the Pt$_d$ NPs to form metal-hydrides on their surface. The ability of metal nanoparticles to act as electron acceptors has been previously reported in the literature$^{26}$. After that, hexacyanoferrate (III) is added. This complex also migrates through the silica shell to the negatively charged surface of the Pt$_d$ nanoparticles located on the internal wall of the silica membrane. Then, the reduction of the adsorbed Fe(CN)$_6^{3-}$ to Fe(CN)$_6^{4-}$ takes place. Once the final product is formed, it desorbs and diffuses out of the nanoreactor. In this case, the nanoparticle surface would operate as a source of electrons providing the necessary metal active sites for the reaction to proceed.

By plotting the logarithm of the pseudo first-order rate constant ($k_{obs}$) versus the inverse temperature (1/T), the Arrhenius equation was used to determine the activation energy for the Pt$_d$-catalyzed electron-transfer reaction. Thus, a value of 12.6±1.2 kJmol$^{-1}$ was obtained for the redox process in the presence of the PS/Pt$_d$ composites, while a value of 12.3±0.8 kJmol$^{-1}$ was achieved for the reaction catalyzed by Pt$_d$/SiO$_2$ capsules. As the activation energy for the noncatalyzed reaction is 30 kJmol$^{-1}$, the results obtained (Fig. 2) indicate a remarkable catalytic activity of these hybrid nanocomposites.

Accordingly, the catalytic efficiency of the proposed systems was seen when using similar nanostructures but employing 2–3 nm spherical Pt nanoparticles (Pt$_s$) instead of dendritic ones (for synthetic details, see the Supporting Information). The widely reported and broadly studied Pt$_s$ nanoparticles, synthesized by reduction with NaBH$_4$ and stabilized through sodium citrate adsorption, can be considered as the model or reference system. PS particles were consequently coated with Pt$_s$, and their catalytic activity was estimated using the reduction of ferricyanide with sodium borohydride. The energy barrier for this reaction is rather high (30 kJmol$^{-1}$) if uncatalyzed$^{30}$, but the presence of PS/Pt$_s$ composites lowers this value to 17.1±1.1 kJmol$^{-1}$. Nevertheless, it must be pointed out that the activation energy reported in the presence of the PS/Pt$_d$ composites (12.6±1.2 kJmol$^{-1}$) is significantly lower than that obtained through the widely used 2–3 nm spherical Pt morphology. This tendency is in good agreement with that reported in the literature$^{26,31}$.

$^{30}$ T. J. Freund, Inorg. Nucl. Chem. 1959, 9, 246
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Fig. 2. Arrhenius plot for the electron-transfer reaction of hexacyanoferrate (III) by NaBH$_4$ in the presence of PS/Pt$_d$ (●) and Pt$_d$/SiO$_2$ (■). [Fe(CN)$_6^{3-}$] = 2.0 × 10$^{-4}$ M; [NaBH$_4$] = 5.0 × 10$^{-3}$ M; pH = 13.

The high catalytic efficiency found for both systems can be attributed to the presence of Pt$_d$ NPs on the nanocomposites. The defects and under coordinated Pt atoms, commonly found in dendrite-like structures, are considered to be the real active sites in electron-transfer reactions$^{32}$. It must be also noted that the activation energy for the Pt$_d$/SiO$_2$ capsules is indistinguishable from the one obtained for the PS/Pt$_d$ composites and consistent with the values reported in the literature for dendritic Pt NPs$^{26}$. Accordingly, it can be assumed that the treatment to produce capsules through SiO$_2$ coating and PS core dissolution has no consequences on the Pt$_d$ NPs morphology and therefore, does not affect their catalytic efficiency.

These highly active nanoreactors favor therefore the confined fabrication of nanostructures of very different materials. Hydrazine has been reported to reduce nickel ions in the presence of Pt nanoparticles located on the surface of carbon nanotubes$^{33}$ or polymer beads$^{34}$. Therefore, we may assume that the Pt$_d$ NPs located in the cavities of the capsule can exert the same degree of catalytic activity giving rise to metallic nickel nanoparticles inside the reactor. Indeed, the porous nature of the silica shell that allows the reactants to reach the nanocavity also permits the Pt-catalyzed reduction to take place in a confined manner.

Fig. 3. (a) TEM image of the Pt/SiO$_2$ nanoreactor after growing nickel inside; (b) closer view of the edge of its Pt/SiO$_2$ membrane; (c) HRTEM image of Ni and Pt nanoparticles located in its inner surface; (d) EDS spectra; and (e) XEDS profiles (determined following the purple line trajectory indicated in the inset TEM image), demonstrating the composition and hollow nature of the capsules. Scale bar in inset: 100 nm.

Fig. 3a shows how the integrity of the nanoreactor remains intact after the formation of metallic Ni in its inner cavity. A higher magnification TEM image (Fig. 3b) over the edge of the hollow hybrid structure reveals: (i) the homogeneity of the silica shell (about 10 nm thick) and (ii) the growth of the magnetic material inside the nanoreactor. Additionally, a HRTEM analysis of the nanoreactor (Fig. 3c) shows the crystalline nature of the formed magnetic particles. Further evidences of the formation of Ni inside the reactor are provided by the EDS spectra (Fig. 3d) that clearly show the presence of Ni. Given that Ni$^{2+}$ reduction takes place on the surface of the Pt$_n$ nanoparticles, a layered morphology is obtained. The formation of this layered but still hollow structure was confirmed by XEDS measurements (Fig. 3e), with analogous profiles for SiO$_2$ (blue), Pt (orange) and Ni (green) that point out this kind of morphology. Selected area electron diffraction (SAED) pattern (Fig. 4) confirms the presence of metallic Ni, although does not discard the presence of small amounts of nickel oxide or hydroxide.
Fig. 4 Selected area electron diffraction pattern (SAED) from one capsule showing the interplanar distances (dhkl) measured on the pattern and the indexation of the most intense rings demonstrating the presence of metallic Ni in the capsules. The pattern also show a few number of spots out of the metallic Ni rings, which could be due to the presence of small amounts of nickel oxide or nickel hydroxide.

The amount of nickel reduced inside the capsule can be finely tuned by controlling the Ni$^{2+}$ precursor concentration employed. Thus, capsules with specific amounts of Ni loaded inside were prepared, highlighting their different magnetic behavior and consequently these hybrid nanostructures potential. The magnetic nanostructures contained in the hollow capsules were characterized using VSM magnetometry. Their magnetic response depends directly on the type of magnetic material formed but also on the size and interactions between the nanostructures attained inside the capsules. The hysteresis loops included in Fig. 5a and 5b (at T=5 and 300 K) reflect two main contributions in both samples; one responsible for the large magnetization values due to the ferromagnetic (FM) metallic nickel, which is superimposed on the antiferromagnetic (AFM) linear contribution ($\mathcal{M}_a = \gamma_a H$, clearly observed at low temperature) responsible for the non-saturation of the magnetization at high field values. This AFM contribution, more defined in sample A, corresponds to the nickel oxide and hydroxide magnetic phases identified.
The reason for a higher (or more visible) AFM contribution in sample A can stem from the fact that smaller magnetic nanoparticles are present, due to the lower amount of Ni\(^{2+}\) precursor employed. Smaller nanoparticles imply an increased surface to volume ratio and therefore an increase in the proportion of magnetic material that has become oxidized. This is also reflected in the saturation magnetization (\(M_0\)) values at high fields, that reached 5 emu/g for sample A (higher proportion of AFM NiO and/or Ni(OH\(_2\)), compared to the 15 emu/g of sample B (higher proportion of FM Ni). Despite these important values in terms of \(M_0\) for both samples, especially in the case of sample B, they keep the superparamagnetic character, in view of the very small almost null coercivity values observed at T=300K. This nearly superparamagnetic character of the magnetic hybrids underlines the individuality of the very small nanoparticles formed inside the cavities.
Zero-field-cooled (ZFC) and field-cooled (FC) magnetization curves (Fig. 4c) were recorded after cooling the samples in zero field and in a small field (H=100 Oe) respectively, from room temperature to 5 K. The ZFC curve corresponding to sample A displays a maximum around 121 K while sample B ZFC curve maximum is located close to but above 300 K. For sample A both curves coincide at high temperatures and may exhibit a paramagnetic-like decay with increasing temperature, reflecting the superparamagnetic response of the particles within the time window of the measurement technique. With decreasing temperature the curves split, the ZFC reaches the maximum at $T_B = 121$ K that roughly corresponds to the average blocking temperature of the nanoparticles and decreases after that, while the FC one keeps increasing until reaches a plateau.

The main reason for a markedly different $T_B$ is usually related to the final size of the nanoparticles, but in that case, very different coercivities would be observed. An assembly of noninteracting and identical nanoparticles with volume $V$ and magnetic anisotropy $K$ would have zero coercivity above the blocking temperature $T_B$ ($T_B(A)=121$ K, $T_B(B)>300$ K). In contrast, in the samples, the spread in $K$ and $V$ leads to a range of $T_B$ and the existence of a finite coercivity at 300 K. Thus, apart from or along with the fact that different sizes of the nanoparticles formed inside the capsules, we should take into account the oxidation at their surface (the Ni$^0$/Ni$^{2+}$ ratio in the nanoparticle composition, as already mentioned) and the distance in between (which defines the strength of interparticle interactions).

The magnetic interactions can be rather strong between the bare nanoparticles formed since this mechanism of formation lacks of any type of surfactants or molecules at the surface. This situation favors dipole-dipole interactions due to the proximity of adjacent particles and exchange interactions across grain boundaries can also be established, altering the superparamagnetic anisotropy energy barrier $E_B$ and causing a shift in the blocking temperature of the ZFC curve towards higher temperatures. Additionally, there is also a tendency toward increased broadening of the peak. The presence of strong magnetic interactions in both samples is indeed inferred from the ZFC-FC curves since the temperature at which irreversibility between them takes place at high temperature ($T_{irr} > 200$ K in sample A and $T_{irr} > 300$ K in sample B). This $T_{irr}$ is quite close to $T_B$, despite the fact of the very broad peak (generally associated to nanoparticles polydispersity) shown in the ZFC curve. Furthermore, in both cases, the FC curve reaches a plateau as decreasing temperature. Since both samples are composed of small primary nanocrystals, they retain the superparamagnetic behavior at room temperature (almost no coercivity), but in view of the magnetic interactions established they show much higher saturation magnetization than individual nanodots and a higher $T_B$.

36 J. Ge, Y. Hu, Y. Yin, Angew. Chem. Int. Ed. 2007, 46, 7428
6.4 CONCLUSIONS

In summary, we have reported the effective preparation of highly active nanoreactors based on the encapsulation of dendritic Pt nanoparticles inside hollow porous silica capsules. Especially relevant is the fact that the catalytic activity of these Pt nanoparticles is preserved after the encapsulation process. Furthermore, these nanoreactors are not just useful for the performance of chemical reactions in organic solvents by means of the easy functionalization of silica and its later dispersion in these solvents, but also for the fabrication of confined materials. Thus, making use of the catalytic activity of the Pt nanoparticles, different metals can be reduced inside the capsules, which may provide different synthetic advantages such as the formation of new catalysts inside the nanoreactor which otherwise will be difficult and/or the addition of extra functionalities. As a proof of concept, through these nanoreactors Ni nanoparticles have been synthesized in the inner cavity of the system leading to the formation of an interesting type of composites with tunable magnetic properties depending on the concentration of nickel precursor. The interest of these Ni-based composites lies in both their capability to be manipulated through an external magnetic field and the excellent catalytic properties of this metal\textsuperscript{37,38,39}. 

\textsuperscript{38} N. Cordente, M. Respaud, F. Senocq, M. J. Casanove, C. Amiens, B. Chaudret, Nano Lett. 2001, 1, 565
CHAPTER 7

Design of SERS-Encoded, Submicron, Hollow Particles
Through Confined Growth of Encapsulated Metal Nanoparticles

Abstract

The synthetic architectures of complex inorganic nanostructures, including multifunctional hollow capsules, are expected to play key roles in many different applications, such as drug delivery, photonic crystals, nanoreactors, and sensing. Implementation of novel strategies for the fabrication of such materials is needed because of the infancy of this knowledge, which still limits progress in certain areas. Herein we report a straightforward synthetic approach for the development of multifunctional submicron reactors comprising catalytic gold nanoparticles (2-3 nm) confined inside hollow silica capsules. Additionally, the confined growth of encapsulated metal nanoparticles was carried out to evidence the usefulness and functionality of these reactors in catalytic applications and as an approach for the development of novel complex nanostructures. Their potential and multifunctionality have been pointed out by fabrication of SERS-encoded submicrometer particles with shape and size uniformity for use in antigen biosensing; this was accomplished via codification of gold nanoparticle islands grown onto their inner surfaces.
7.1 INTRODUCTION

Precise diagnosis and proper therapeutic treatments for preventing and controlling pandemics and other biothreads are central to human health. Most of the established strategies for detection assays rely on specific receptor-ligand molecular recognition (for example, antibody-antigen or complementary oligonucleotide). In line with this, encoded supports are simple, cost-effective platforms amenable to multiplexing and high throughput screening assays\(^1\).\(^2\).\(^3\).\(^4\).\(^5\).\(^6\).\(^7\). Several strategies have been developed for the preparation of encoded particles\(^8\).\(^9\).\(^10\), among which those based on vibrational spectroscopy\(^11\) present advantages related to unambiguous classification of the encoded particles and the availability of thousands of different barcodes. Although Raman and IR spectroscopies are reliable, enhancement of the barcoded vibrational signal is required to improve the detection speed and sensitivity as well as to decrease the encoded particle size. In this context, surface-enhanced Raman scattering (SERS) spectroscopy offers a unique solution for amplification of the vibrational signal\(^12\) along with decreases in both read-out time and particle size. For SERS to be achieved, close contact between the molecular probe and the optical enhancer (typically silver or gold nanostructures) is necessary. Several synthetic strategies for the fabrication of micron-sized metal nanoparticle (MNP)-based composite beads have been developed, including chemisorption\(^13\), physisorption\(^14\), emulsion polymerization\(^15\), electron-beam evaporation\(^16\) and the versatile layer-by-layer (LbL) assembly technique\(^17\).\(^18\). These approaches were aimed at controlling the nanocomposite’s optical and chemical properties as well as exploring different product end uses in sensor design\(^19\), catalysis\(^20\).

CHAPTER 7

and spectroscopic labeling\textsuperscript{21}. However, MNPs produced by most of the currently available synthetic methods are located on the outer surface of the microparticle, which encompasses several problems, such as (a) a low MNP/supporting-material ratio; (b) possible side reactions due to the catalytic activity of the MNPs; (c) signal enhancement of the given analyte that overlaps the microparticle barcode; (d) difficult functionalization of the particle surface for the immobilization of biomolecules; and (e) leaching out of the vibrational label, thus decreasing the particle signal and promoting toxic reactions.

To circumvent all of these drawbacks, we have designed useful submicron-sized reactors composed of hollow silica capsules with a high density of metallic nanoparticles decorating their inner surfaces. A few examples of the use of active nanoparticles inside nano- and submicro-reactors for the design of complex inorganic nanostructures have been reported\textsuperscript{22,23}. In particular, these heterostructures were demonstrated to have great potential because of the catalytic activity of the metallic nanoparticles. On the basis of the great potential of such reactors, we report in this work on the development of a novel, specific, and robust synthetic strategy for the preparation of SERS-encoded submicrometer particles with shape and size uniformity; this approach is based on the generation and codification of gold nanoparticles on the inner surfaces of monodisperse, hollow silica capsules. The size of the resulting capsules is optimized to be small enough to maintain colloidal stability while carrying antibodies, yet big enough for a single particle to be readily resolved by conventional confocal Raman microspectroscopy. Proof of concept for biosensing applications of this material and its potential multiplexing capabilities are additionally demonstrated.

7.2 EXPERIMENTAL METHODS

Unless otherwise stated, all of the chemicals were purchased from Aldrich and used without further purification.

Polystyrene Bead Functionalization and Synthesis and Adsorption of Gold Seeds (PS@Au-Seeds) (Scheme 1a, Step I). Gold seeds (1-3 nm; [Au] \(\times 10^4\) M) were produced as described elsewhere\textsuperscript{24}. Polystyrene bead functionalization and subsequent adsorption of gold seeds were carried out as previously reported\textsuperscript{16}. In this case, 5 mL of functionalized PS beads (3.3 mg mL\(^{-1}\)) was added dropwise to 50 mL of an Au seed colloid and sonicated for 5 min. After 1 h, the sample was washed by three

centrifugation (3000 rpm)-redispersion cycles\textsuperscript{25}. The final concentration was 0.83 mg mL\textsuperscript{-1}.

**Silica Coating (PS@Au-seeds@SiO\textsubscript{2}) and Fabrication of Hollow Capsules (Au-seeds@SiO\textsubscript{2}-h) (Scheme 1a, Step II).** Silica coating was carried out following the method described by Graf et al\textsuperscript{26}. Briefly, 400 μL of 110 mg mL\textsuperscript{-1} PVP (MW = 10 000) was added to 5 mL of PS@Au-seeds suspension (0.83 mg mL\textsuperscript{-1}). After 2 h, the suspension was washed three times by centrifugation at 3000 rpm, and the sediment was resuspended in 10 mL of a 4.2% (v/v) solution of NH\textsubscript{4}OH in ethanol and sonicated for 5 min. Next, 43.5 μL of a 10% (v/v) solution of tetraethoxysilane (TEOS) in ethanol was added to the suspension, which was then centrifuged three times and washed with water. Dissolution of the PS cores was carried out by shaking the bead suspension in an 1:1 ethanol/chloroform mixture in an orbital shaker for 3 days. The hollow capsules were washed and centrifuged three times, first with ethanol and then with water.

**Confined Growth of Metal NPs (Au-NPs@SiO\textsubscript{2}-h) (Scheme 1a, Step III).** Au\textsuperscript{3+} was prereduced to Au\textsuperscript{+} by mixing 425 μL of 0.123 M H\textsubscript{3}AuCl\textsubscript{4} with 120 mL of 1.8 mM K\textsubscript{2}CO\textsubscript{3} for 1 h\textsuperscript{27}. Growth of gold seeds into nanoparticles was achieved by adding 100 mL of Au\textsuperscript{+} solution and 300 μL of formaldehyde to 10 mL of Au-seeds@SiO\textsubscript{2}-h under vigorous stirring. The extent of particle growth could be modulated through reaction time or amount of added Au\textsuperscript{+}. Samples were separated after 20 min of reaction (change in color from light-pink to purple-blue) to give Au-NPs@SiO\textsubscript{2}-h and after 18 h of reaction (purple-blue to gray-blue) to give Au-GNPs@SiO\textsubscript{2}-h (Scheme 1a, step III). Samples were centrifuged three times and washed with water.

**Characterization.** TEM images were obtained using a JEOL JEM 1010 transmission electron microscope operating at an acceleration voltage of 100 kV. HRTEM, STEM, and elemental mapping by X-ray energy dispersive spectroscopy (XEDS) analysis were carried out with a JEOL JEM 2010F transmission electron microscope operating at an acceleration voltage of 200 kV. UV-vis spectra were recorded on a Cary 5000 UV-vis-NIR spectrophotometer. Spontaneous Raman scattering, SERS, and electronic emission were measured with a LabRam HR system (HORIBA Jobin Yvon) equipped with a confocal optical microscope, highresolution gratings (1800 g mm\textsuperscript{-1}), and a Peltier CCD detector by using three excitation laser lines (488, 532, and 785 nm). Spectra were collected by focusing the laser line onto the sample using a 100 × objective (N.A. 0.95), providing submicrometer spatial resolution, with accumulation times of 0.1-10 s. The power at the sample was varied between 10 mW and 1 μW.

**Capsule Encoding (Scheme 1b, Step IV).** Each of three 500 μL aliquots of the capsule suspension (0.83 mg mL\textsuperscript{-1}) was mixed with 100 μL of 10\textsuperscript{-3} M solution of an aromatic thiol [either benzenethiol (BT), 4-nitrobenzenethiol (NBT), or 4-hydroxybenzenethiol (HBT)]. After 12 h, the thiol-functionalized capsules were centrifuged (2500 rpm, 15 min) and redispersed twice. NBT encoded particles were redispersed in ethanol to


continue the functionalization process, while BT- and HBT-codified capsules were redispersed in water. To test for possible thiol leaching, BT capsules were washed and centrifuged several times, and their SERS signals were recorded. The SERS activities of the material at different stages of the synthesis process were also monitored by using BT as probe analyte under conditions similar to those used for codification.

Biofunctionalization of Encoded Au-GNPs@SiO$_2$-h (Scheme 1b, Step V). Encoded capsules were primed with the silane coupling agent (3-aminopropyl)trimethoxysilane (APS) by stirring at 60-70 °C for 90 min. The amount of APS was calculated to provide the capsules with ~1 APS molecule nm$^{-2}$. The capsules were then centrifuged at 2500 rpm for 15 min and washed with ethanol and phosphate-buffered saline (PBS).

Carbodiimide chemistry was used to conjugate the APS primary amines with the carboxyl groups from dodecanedioic acid, providing the capsule surface with -COOH groups$^{27}$. Briefly, 3.5 mg of dodecanedioic acid (dissolved in 0.5 mL of ethanol with 50 μL of aqueous NaOH solution) and 50 μL of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) conjugation buffer [2% (w/v) EDAC, 3% (w/v) N-hydroxysuccinimide in PBS, pH 8.0] were mixed in an orbital shaker for 15 min at room temperature. After 2 h, the reaction was quenched with 10 μL of 1 M hydroxylamine to regenerate the original, nonreacted carboxylic groups. The carboxylic acid conjugated capsules were centrifuged at 5000 rpm for 15 min and washed with PBS (3 × 0.5 mL). For antibody (Ab) conjugation, carbodiimide chemistry was used as in the previous step. The amount of Ab [Alexa Fluor 594 rabbit anti-goat IgG (H+L), Invitrogen] was 10 μg. When coupling was concluded, the Ab-functionalized capsules were centrifuged at 8000 rpm for 5 min and washed with PBS (3 × 0.5 mL).

Biosensing Assay (Scheme 1b, Step VI, and Scheme 1c, Steps VII and VIII). Aliquots (1.5 mL) of capsules encoded with the three different benzenethiols were centrifuged at 7300 rpm for 10 min and redispersed in 1.5 mL of the buffer [50 mM Tris, 0.14 M NaCl, 0.05% (v/v) Tween 20, pH 8.0], incubated with 10 μg of antigen (Alexa Fluor 488 goat anti-guinea pig IgG (H+L), Invitrogen) in an orbital shaker for 1 h at room temperature, and then washed and centrifuged at 7300 rpm for 10 min with water (4 × 1.5 mL) to remove unbound antigen (Scheme 1b, step VI). The capsule suspension (10 μL) was then cast onto a glass slide, air dried, and studied within the Raman microscope with three different laser lines (785, 532 and 488 nm) (Scheme 1c, steps VII and VIII). The antigen (Ag) concentration was estimated by assuming that all of the Ab was covalently retained on the capsule surfaces during the bioimmobilization (Scheme 1b, step V).

$^{27}$ Nakajima, N.; Ikada, Y. Bioconjugate Chem. 1995, 6, 123–130.
7.3 RESULTS AND DISCUSSION

Scheme 1. Schematic Illustrations of the Multistep (a) Fabrication and (b) Codification, Biofunctionalization, and Biosensing Procedures (c) of the Biosensing Assay

(I) Polyelectrolyte coating of PS beads and Au seed deposition; (II) PVP wrapping, silica coating, and PS dissolution; (III) seeded Au NP growth; (IV) SERS probe diffusion; (V) antibody attachment; (VI) antigen biorecognition. (VII) Illumination of the capsule mixture with different laser lines; (VIII) spectral deconvolution.

Scheme 1a shows the overall procedure for the fabrication, codification, biofunctionalization, and biosensing proof of concept of the SERS-encoded silica capsules. Detailed procedures are described in the Experimental Section; briefly, homogeneous 530 nm polystyrene (PS) beads were coated with four alternating polyelectrolyte monolayers using the electrostatic self-assembly LbL protocol\(^\text{28}\). Four layers of polymer, rather than just two, were deposited in order to improve the homogeneity of the coating shell and generate the positive surface charge \(\zeta\) potential \((\zeta = +40 \text{ mV})\) necessary for the electrostatic adsorption of 1-3 nm gold seeds \((\zeta = -20 \text{ mV})\), yielding PS@Au-seeds [Scheme 1a, step I]. The PS@Au-seeds were then coated with a polyvinylpyrrolidone (PVP) layer and silanized with tetraethoxysilane, giving rise to PS@Au-seeds@SiO\(_2\) (Fig. 1a). Silica coating resulted in homogeneous shells with thicknesses of \(\sim 20 \text{ nm}\), as observed in high-resolution TEM (HRTEM) and

\(^{28}\)Decher, G. Science 1997, 277, 1232–1237
scanning TEM (STEM) micrographs (Fig. 2a,c). Hollow silica capsules containing Au seeds (Au-seeds@SiO$_2$-h; Fig. 1b) were obtained by dissolving the PS cores with an ethanol/ chloroform mixture (Scheme 1a, step II). Although during this step the seeds were slightly aggregated, they remained evenly distributed on the inner walls of the silica capsules. Aggregation was reflected in the UV-vis spectrum through the rise of a weak localized surface plasmon resonance (LSPR) band at 530 nm, in contrast with the featureless spectrum of the PS@Au-seeds (Fig. 3a). Growth of the Au seeds inside the capsules (Au-NPs@SiO$_2$-h, Fig. 1c) was achieved by in situ seed catalyzed reduction of gold ions with formaldehyde (Scheme 1a, step III). Selective reduction on the surface of the seeds, even when they were embedded in the polyelectrolyte multilayer, was confirmed through control experiments, as described in Fig. 4.

**Fig. 1.** (left) Low- and (right) high-magnification TEM micrographs of spherical particles at various stages of fabrication: (a) PS@Au-seeds@SiO$_2$; (b) Au-seeds@SiO$_2$-h; (c) Au-NPs@SiO$_2$-h; and (d) Au-GNPs@SiO$_2$-h.
Fig. 2. (a, b) HRTEM images of an Au-NPs@SiO$_2$-h capsule. (c) STEM image of the same capsule. (d) XEDS elemental mapping of Si and Au from the image in (c). (e) Combined XEDS mapping showing the elemental distribution. (f) XEDS profiles determined from (e) demonstrating the layered nature of the filled capsules.
Fig. 3. (a) UV-vis-NIR spectra of capsules at different growth stages, as indicated in the labels. (b) SERS spectrum of benzenethiol when excited with a 785 nm laser line. (c) Respective intensities of the ring breathing mode peaks at 999 cm$^{-1}$ for BT adsorbed on various capsules, as indicated.

Fig 4. TEM micrographs showing how Au NPs selectively grow on the surface of the beads only when the Au-seeds are present (a). Absence of seeds yields aggregated NPs outside the spheres when Au(I) solution is added to the growth medium (b).
HRTEM, STEM, and XEDS analyses (Fig. 2) provide clear evidence that Au NPs grow exclusively inside the capsules, as no trace of gold was detected on the outer surfaces. This NP growth method presents clear advantages over the adsorption of NPs on PS beads or in situ adsorption and reduction of metal salts with stronger reducing agents. First, the seeds are very small, and therefore, the morphology of the hollow silica spheres is not affected. Second, and more importantly, no metal particles are present on the capsule surfaces, thereby avoiding side effects such as catalytic reactions outside the capsules or optical contamination of the SERS code. Consistent with the NP growth, Au-NPs@SiO$_2$-h capsules show a notable change in their optical response (Fig. 3a), with enhancement and a red shift ($\lambda_{\text{max}} = 550$ nm) of the LSPR band. Gold reduction can be continued leading to extensive growth of the NPs until they apparently fill the entire inner hollow surface (Au-GNPs@SiO$_2$-h, Fig. 1d). In fact, the LSPR spectrum shows a dramatic absorbance increase at longer wavelengths, similar to that for goldisland films, which have been demonstrated to be one of the most efficient substrates for SERS$^{29}$.

SERS codification was achieved by immersion of the capsules into dilute solutions of selected SERS molecular probes (Scheme 1b, step IV). For the experiments reported here, the three aromatic thiols BT, NBT, and HBT were selected because they present huge SERS cross sections in the near-IR region, their vibrational spectra are clearly identifiable for each specific ring substitution (different nature and peak position), and their small sizes allow them to diffuse through the porous silica shells$^{30}$ and be chemisorbed onto the inner gold surfaces, giving rise to very stable surface complexes. The optical-enhancement properties of the particles obtained at different steps were characterized using BT as a spectroscopic probe. Figure 3b shows the SERS spectrum of BT when excited with a 785 nm laser line, spontaneous Raman spectra of the different probes are provided in Fig. 5.

$^{30}$ van Blaaderen, A.; Vrij, A. J. Colloid Interface Sci. 1993, 156, 1–18.
Fig. 3c shows the respective intensities of the ring breathing mode peaks at 999 cm\(^{-1}\). While the PS@Au\(_{\text{seeds}}\) gave no SERS signal, consistent with the absence of plasmon resonances from the small seeds (Fig. 3a), upon silica coating and removal of the PS core, weak BT SERS signals were obtained as a result of partial NP aggregation. The intensity of the signal notably increased when larger particles were grown inside the capsules, ultimately leading to a dramatic enhancement when capsules with a more compact inner metal particle layer were used. This behavior is easily understood by assuming the gold-filled core to be a compact group of nanoaggregates with a large mutual interaction and LSPR coupling (a collection of hot spots). Notably, after 18 h of gold growth, no extra enhancement was achieved. Another interesting feature is the disappearance of the S-H stretching band at 2567 cm\(^{-1}\) as a consequence of the complexation of the BT thiol group to gold surface atoms (Fig. 6)\(^{31}\). The stability of the thiol inside the capsule was confirmed by washing and centrifuging the last sample, Au-GNPs@SiO\(_2\)-h, several times with ethanol and water. Fig. 3c shows that after the sample was washed, the intensity of the signal remained unchanged. We may thus

conclude that the SERS label is stable and that no leaching occurs once it has been attached to the gold layer.

![Spontaneous Raman and SERS spectra of BT. The characteristic band of S-H stretching (2567 cm⁻¹) completely disappears in SERS as a consequence of the S covalent bonding to gold.](image)

An additional advantage of these materials is the versatility of their surface for subsequent functionalization with the desired functional groups. For the generation of surface functional groups (i.e., -COOH) that allow for the covalent conjugation of an antibody (Ab) at a later stage (see the Experimental Section for details), the capsule surfaces were modified with APS. A dicarboxylic aliphatic linker was then coupled within the amino group of the outer surface, following standard carbodiimide chemistry. Carbodiimide chemistry was used as well for the immobilization of the Ab onto the modified capsules (Scheme 1b, step V). It should be noted that this process was only carried out in one of the encoded capsules, those labeled with NBT. Following the conjugation of Ab to the NBT capsules, an aliquot of the suspension of these capsules was added to equal volumes of suspensions of the capsules containing the BT and HBT codes. The resulting suspensions were sonicated for several minutes to ensure complete and homogeneous mixing. Next, 10 μg of the Ab-specific Ag was added to the capsule mixture (Scheme 1b, step VI). The suspension was subsequently washed and centrifuged in PBS and water to remove the excess Ag and then resuspended in water. A 10 μL aliquot of the capsule suspension was cast onto a glass slide, air-dried, and then studied under the confocal microscope using three different laser lines (Scheme 1c, steps VII and VIII). Fig. 7 and Fig. 8 and 9a show the spontaneous fluorescence emission spectra.
of the Ab [labeled with Alexa Fluor 594 (AF 594)] and the Ag [labeled with Alexa Fluor (AF 488)]. Fig. 5b shows the specific SERS spectral fingerprints for the three encoded capsules\textsuperscript{15}.

Fig 7. Molecular structure and electronic absorption and emission of the AlexaFluor 488 and 594 labeling dyes.

Notably, a comparison of the signals for the SERS encoding probes and the two dyes excited with the three different laser lines shows no spectroscopic contamination. Upon excitation at 785 nm, only the SERS signals are observed, in agreement with Figure 8a. The molecular absorptions are completely out of resonance with the infrared laser; thus, no molecular emission is observed. Excitation with the green laser (532 nm) yields no SERS signal but does produce strong and weak emission of AF 594 and AF 488, respectively (Figure 8b). For Au nanoparticles with a plasmon peak located at \( \sim 520 \) nm or higher energies, the SERS signal is extremely weak or undetectable. This is mainly a result of the fact that the interband transitions severely depress the LSPR. The strong SERS signal can be obtained from Au NPs only for excitation wavelengths longer than 600 nm. The green laser is in resonance and preresonance with AF 594 and AF 488, respectively. Excitation with the blue laser (488 nm) gives rise to strong emission from AF 488 (in resonance), very weak emission from AF 594 (out of resonance), and no SERS (excitation is to the blue of the plasmon) (Fig. 8c).
Fig. 8. Comparison of the signals for the SERS encoding probes and the two dyes excited with three different laser lines.
Fig. 9. (a) Fluorescence emission spectra of AF 594 and AF 488, bound to the Ab and Ag, respectively. (b) SERS spectra of BT, NBT, and HBT (as labeled) upon excitation with a 785 nm laser line. (c) Images from a small area of the substrate with a random distribution of encoded capsules. The leftmost image is an optical micrograph, while the other three are SERS mappings obtained by illumination with the three different laser lines, as indicated.

Fig. 9c is a clear demonstration of the encoding capabilities of these particles. All of the images were obtained from the same area of the substrate, which contained a random distribution of spheres, as shown in the white-light image. Upon illumination with the 532 nm laser line, only the particles where the Ab was anchored were imaged (the fluorescence of AF 594 was excited), while upon illumination with the 488 nm laser line, the very same particles were observed, indicating the successful Ab-Ag biocoupling (AF 488 was selectively excited). Notably, the absence of a fluorescence background for the other codified particles demonstrates that the Ag was not retained in the absence of the coupling agent. Finally, when the SERS signal was measured over the same area under 785 nm illumination, deconvolution of all of the encoded capsules was achieved. It should be noted that all of the yellow capsules (corresponding to particles codified with NBT) perfectly fit with both fluorescence maps, demonstrating an accurate identification of the active particles with no signal of cross-reactivity even at such a small scale. The time required for complete analysis and deconvolution of the
images was less than 1 min, which clearly shows the high potential of this system for multiplexing and high-throughput biomedical screening applications.

7.4 CONCLUSIONS

In summary, these results demonstrate the efficiency of antibody conjugation and on-particle antigen recognition at a very low content (<2 \times 10^{-9} \text{ mg}, as estimated from the illuminated cross section of a single particle under the assumption that all of the Ab was covalently retained on the capsule surfaces) within short acquisition times (less than 1 s per particle) and with an unprecedented submicrometer spatial resolution, providing an excellent material for high-throughput screening. Furthermore, the readiness of the capsule surfaces to be functionalized with the desired functional group increases the scope of application to the design and solid-phase synthesis of combinatorial libraries for drug discovery\textsuperscript{32}, either when mounted onto patterned substrates or in real time using Raman flow cytometry\textsuperscript{33}. Additionally, many potential uses can be envisioned for the as-prepared submicreactors with active metallic nanoparticles inside, such as local overheating inside the reactor without affecting the solvent outside the particles. Moreover, this ability for confined growth of inorganic species provides a unique strategy for the synthesis of complex nanostructures with accurate size and shape control.


CHAPTER 8

General Conclusions
Conclusions

Although specific conclusions have been presented along with each of the chapters of this thesis, we present here a compilation of those we consider the most relevant, global conclusions of the work.

1. Stable dispersions of carbon nanotubes can be obtained through their functionalization with polyelectrolytes. This functionalization provides the nanotubes with a high surface charge, rendering them suitable candidates for further use as supports or for the reinforcement of other nanostructures.

2. Metallic nanoparticles can be uniformly assembled onto carbon nanotubes used as templates, by means of electrostatic layer-by-layer assembly. Platinum nanoparticles with dendritic morphology display a higher catalytic activity than that of their spherical counterparts, which can be explained by an increase of Pt atoms at the edges and corners of the multiple arms present in the dendritic shape.

3. Carbon nanotubes doped with Ag nanoparticles provide a highly efficient and stable platform for SERS, which could be useful for applications in portable sensors for fast ultrasensitive detection and quantification of relevant metabolites.

4. A method for making hollow and porous silica capsules was devised comprising a primer silica coating of polystyrene colloids, followed by controlled dissolution of the polystyrene cores.

5. The silica layer acts as a porous membrane, in turn providing stability to the capsule as well as allowing the exchange of substances between the interior and exterior of the capsules, which is a main pre-requisite for the controlled growth of different inorganic materials in their confined space.
6. Codification of the hollow capsules was achieved, assisted by the relatively easy diffusion of molecules through the porous silica layer.

7. The versatility of such silica capsules allows easy functionalization with a plethora of reactive groups. Thus, hollow structures were used to obtain encoded nanoparticles, which were functionalized for SERS-based biosensing applications.
CAPÍTULO 9

RESUMEN Y CONCLUSIONES GENERALES
RESUMEN

SÍNTESIS Y CARACTERIZACIÓN DE MATERIALES HÍBRIDOS NANOESTRUCTURADOS

En este resumen se pretende ofrecer una visión global del trabajo presentado en los distintos capítulos que conforman la presente disertación.
El tema central de la tesis ha sido la fabricación de materiales híbridos multifuncionales utilizando nanotubos de carbono y partículas coloidales de poliestireno como soporte, así como su caracterización morfológica, espectroscópica y magnética. Para el desarrollo de estos materiales han sido de gran importancia las técnicas de modificación superficial tanto de nanotubos de carbono como de partículas coloidales de poliestireno, lo que nos permite obtener nanoestructuras con múltiples funciones. Finalmente hemos comprobado la funcionalidad de estos materiales híbridos en diferentes aplicaciones como por ejemplo en catálisis, sensores, o nanoreactores.
Resumen y Conclusiones Generales

Objetivo

El objetivo del trabajo presentado en esta tesis comprende la síntesis de nuevos materiales híbridos nanoestructurados, así como el estudio de sus propiedades ópticas, catalíticas o magnéticas. El interés de estos materiales de tamaño nanométrico reside en que sus propiedades difieren de las propiedades observadas en los materiales macroscópicos, y además sus propiedades son función de su tamaño. Dichas propiedades, características de estas estructuras, hacen que se haya estudiado el uso de estos materiales en un gran número de potenciales aplicaciones.

En este trabajo hemos desarrollado dos modelos de materiales híbridos nanoestructurados con propiedades ópticas, magnéticas y catalíticas. Por una parte hemos desarrollado materiales híbridos basados en los nanotubos de carbono (CNTs) y por otro en coloides esféricos de poliestireno (PS), estos materiales los hemos utilizado como soportes para una posterior deposición de nanopartículas metálicas previamente sintetizadas.

Podemos dividir este trabajo en dos partes principales:

La primera parte abarca la síntesis de materiales híbridos nanoestructurados basados en nanotubos de carbono, los cuales se utilizaron como soporte debido a sus excelentes propiedades físicas (eléctricas, mecánicas, ópticas) y a su gran relación longitud/diámetro que les confiere una gran área superficial. Así, los CNTs fueron utilizados como soporte para la deposición de nanopartículas metálicas de platino y plata. Estos materiales híbridos han sido desarrollados para la mejora de aplicaciones basadas en el uso de nanopartículas metálicas, las cuales al estar soportadas en nanotubos de carbono mejorarán su comportamiento. Así, en el caso de las nanopartículas de platino (esféricas o dendríticas) soportadas sobre CNTs, los cuales le aportan a estas, estabilidad y la facilidad de ser recuperadas para su posterior reutilización, se ha conseguido demostrar una alta actividad catalítica frente a una reacción modelo de transferencia de carga. En el caso de los CNTs recubiertos con nanopartículas de plata, se ha desarrollado un sustrato que proporciona una gran intensificación de la señal Raman para aplicaciones basadas en SERS. Así, este material se ha utilizado para determinar si un bio-analito, en este caso un metabolito derivado de la cocaína, está o no presente en una muestra y en qué cantidad. En primer lugar, se ha
unido a la superficie de las partículas de Ag un anticuerpo específico para el bio-analito en cuestión, de forma que se produce un cambio en la señal espectroscópica del anticuerpo cuando tiene lugar su acoplamiento. De esta manera realizamos una detección indirecta del analito, que puede compararse con la detección directa del mismo, obtenida mediante el cambio de la señal Raman cuando el analito se deposita directamente sobre las partículas metálicas. Comparando ambos métodos se observó que la detección directa ofrece un límite de detección más bajo que el método indirecto específico, sin embargo este último ofrece resultados cuantitativos a nivel fisiológico, por lo que no solo se podría demostrar el consumo de drogas, sino que también sería posible cuantificarlo.

La segunda parte de este trabajo implica la síntesis de nuevos materiales híbridos nanoestructurados utilizando partículas esféricas como soporte. Se utilizaron partículas coloidales monodispersas de poliestireno de tamaño submicrométrico, que pueden ser fácilmente disueltas en medios orgánicos. Para aumentar la complejidad y funcionalidad de los materiales híbridos formados a partir de este soporte se han combinado estos soportes con los CNTs. Esta combinación nos proporciona un material híbrido nanoestructurado que imita una morfología que esta presente en la naturaleza, la de una anemona de mar, y además, los CNTs refuerzan la estructura de este material e incrementan notablemente su área superficial. La incorporación de nanopartículas magnéticas en esta estructura le aporta funcionalidad magnética, lo que permite que este material pueda ser manipulado mediante la aplicación de un campo magnético externo.

La deposición de nanopartículas metálicas sobre los coloides de poliestireno permite obtener materiales esféricos con distintas propiedades, las cuales son dependientes de la naturaleza de las nanopartículas depositadas. Se han depositado sobre la superficie de los coloides de poliestireno nanopartículas metálicas de platino y oro. En el caso de los coloides de poliestireno recubiertos con nanopartículas de platino, se ha utilizado la actividad catalítica de éstas para depositar un material magnético (en este caso níquel) de forma controlada sobre ellas, y obtener así materiales híbridos superparamagnéticos con propiedades características. El hecho de que estos coloides de poliestireno se puedan disolver con facilidad en disolventes orgánicos, los hace muy buenos candidatos para la obtención de cápsulas huecas. Sobre este soporte se crece una capa de sílice homogénea y porosa, y posteriormente se disuelve el soporte al transferir estas estructuras a un disolvente orgánico, lo que permite obtener de forma sencilla cápsulas
huecas de sílice. Dependiendo de la naturaleza de las nanopartículas metálicas depositadas en el interior de las cápsulas éstas pueden presentar propiedades y aplicaciones diferentes, en el caso de las cápsulas huecas de sílice con nanopartículas de platino confinadas en su interior se verificó su aplicación como nanocatalizadores, permitiendo llevar acabo reacciones confinadas en su interior.

En el caso de las cápsulas huecas de sílice con nanopartículas de oro confinadas en su interior, se ha desarrollado una aplicación para estas estructuras como biosensores basados en SERS, gracias a la codificación de las nanopartículas de oro confinadas en el interior.

La investigación descrita en este trabajo se inició en el contexto de trabajo en el grupo de química coloidal, en el que la actividad investigadora está centrada en la síntesis, caracterización, propiedades ópticas y también modificación superficial de nanopartículas metálicas. Por lo que la experiencia del grupo ha sido importante para el desarrollo de nuevos materiales híbridos nanoestructurados.

En esta tesis los capítulos están estructurados por artículos, y esta estructura nos permite desarrollar en cada capítulo el protocolo para la síntesis del material y la aplicación. La aplicación del material híbrido nanoestructurado está directamente relacionada con las propiedades del material.

**Introducción**

El interés del estudio de los materiales a escala nanométrica radica en que sus propiedades físicas y químicas son diferentes a las que presentan los materiales cuando se encuentra en un rango macroscópico. Esto se debe a que a escala nanométrica sus propiedades están directamente relacionadas con su tamaño, por lo que es necesario desarrollar nuevas rutas sintéticas que nos permitan controlar de forma eficaz el tamaño de estos materiales. Y son sus propiedades características las que hacen que estos materiales tengan un gran potencial en múltiples aplicaciones como son, la liberación de fármacos, catálisis, desarrollo de nuevos sensores, etc.

Podemos definir un material híbrido nanoestructurado como un material a escala nanométrica que está compuesto por materiales de diferente naturaleza, siendo lo más
CAPÍTULO 9

habitual que esté integrado por una estructura orgánica y una inorgánica. Los métodos más comunes usados para la fabricación de estos materiales son:

- Métodos basados en el uso de soportes
- Método coloidal
- Métodos sol-gel
- Métodos de electrodeposición

La estrategia basada en el uso de soportes consiste en depositar un material sobre la superficie de otro utilizado a modo de soporte, obteniendo de esta forma un material más complejo y con diferentes propiedades que el de partida. El método coloidal por su parte se basa en el crecimiento de partículas de tamaño manométrico dentro de una disolución acuosa para formar un sol coloidal, mientras que el método sol-gel involucra una serie de reacciones químicas irreversibles que convierten una dispersión homogénea de partículas en un sólido tridimensional. Por último, el método de electrodeposición se basa en inducir una serie de reacciones químicas en una disolución acuosa electrolítica aplicando un voltaje, con el objetivo de inducir una deposición de material en un electrodo.

Para desarrollar los materiales empleados en este trabajo hemos utilizado dos tipos de sustratos sobre los que depositaremos nanopartículas metálicas; los nanotubos de carbono multi-pared (MWCNTs) que se pueden definir como una serie de láminas de grafito enrolladas en torno a un cilindro hueco, y coloides esféricos de poliestireno (PS).

Las propiedades de los nanotubos de carbono hacen que sean materiales de gran interés para aplicaciones en diversas áreas. Sin embargo la falta de solubilidad en cualquier disolvente y su difícil manipulación son sus principales limitaciones, por lo que resulta muy importante el desarrollo de métodos prácticos que permitan su manipulación individual. En el contexto de los materiales desarrollados en esta tesis, nos hemos centrado en la metodología que propone su manipulación a través de la adsorción de moléculas funcionales (polielectrolitos), lo que nos permite una posterior funcionalización de los nanotubos con partículas metálicas.

Por lo que respecta a los coloides esféricos de poliestireno, su superficie es fácilmente modificable a través de la técnica de deposición capa por capa, lo que permite recubrir
su superficie con diferentes elementos obteniendo de esta forma estructuras del tipo núcleo-corteza con propiedades diferentes.

Las aplicaciones espectroscópicas que presentan nuestros materiales se deben al empleo de nanopartículas metálicas, las cuales presentan electrones libres en la banda de conducción. Dichos electrones oscilarán con una frecuencia característica al interactuar con el campo electromagnético de una radiación incidente, dando lugar a los modos de resonancia denominados plasmones superficiales. Esta característica puede observarse a simple vista en el caso de dispersiones de nanopartículas de metales nobles como el oro, la plata o el cobre, las cuales presentan colores característicos, que varían en función del tamaño y forma de las nanopartículas.

Los metales nobles son por tanto los materiales más utilizados para preparar sustratos ópticamente activos en SERS debido a que la resonancia de su plasmon de superficie localizado cae dentro del rango espectral del visible, donde se encuentran también la mayoría de los láseres más comúnmente utilizados. El efecto SERS consiste en la enorme intensificación de la emisión Raman procedente de una molécula, cuando ésta se encuentra en contacto o cerca de una superficie metálica nanoestructurada.

Las aplicaciones catalíticas de los materiales híbridos desarrollados están fundamentadas por el empleo de nanopartículas de metales de transición, que presentan una elevada actividad catalítica para un gran número de reacciones químicas de interés. La actividad catalítica de las nanopartículas metálicas depende del número de electrones que posea y de la habilidad de estos para moverse en el material. En el caso de los materiales híbridos que presentan propiedades magnéticas, estas son debidas a la presencia de nanopartículas magnéticas en su estructura. Cuando el tamaño de estas nanopartículas se reduce por debajo del volumen crítico, los materiales dejan de contener múltiples dominios magnéticos, por lo que la respuesta a un campo magnético aplicado corresponde a un único dominio magnético. Este tamaño crítico está típicamente en el rango de las decenas de nanómetros y se conoce como régimen superparamagnético. Las partículas superparamagnéticas no presentan remanencia o coercitividad, es decir, no hay histéresis en la curva de magnetización. Las propiedades de las nanopartículas magnéticas dependen, al igual que las propiedades ópticas de las nanopartículas metálicas, no solo del tamaño, sino también de la forma de las mismas, de modo que pueden presentar grados de magnetización diferentes a lo largo de los
diferentes ejes de la partícula, lo cual las hace muy interesantes para un gran número de aplicaciones.
• Nanopartículas monocristalinas de platino dendrítico altamente catalíticas soportadas sobre nanotubos de carbono.

En este capítulo hemos estudiado la forma de sintetizar nanopartículas de platino monocristalinas con morfología dendrítica, en un solo paso y en medio acuoso, y hemos comparado su actividad catalítica con la de nanopartículas esféricas. Para ello hemos decidido soportar estas estructuras sobre la superficie de nanotubos de carbono, los cuales le aportarán estabilidad y facilitarán su recuperación y reutilización. Además, estudios recientes han probado que al soportar las nanopartículas catalíticas sobre nanotubos de carbono su actividad catalítica se ve favorecida\(^1\). Para comprobar qué estructura es más activa, los nanotubos con platino dendrítico (CNT@Pt\(_d\)) ó los nanotubos con platino esférico (CNT@Pt\(_s\)), hemos evaluado mediante una técnica espectrofotométrica la reacción de reducción del hexaciano ferrato (III) en presencia de cada uno de los catalizadores híbridos. Mediante su seguimiento, se ha comprobado la alta actividad de las CNT@Pt\(_d\) frente a las CNT@Pt\(_s\) obteniendo una energía de activación considerablemente más baja para las dendríticas (E\(_a\)= 10kJmol\(^{-1}\)) que para las esféricas (E\(_a\)=27kJmol\(^{-1}\)).

![Fig. 1. (TEM) Imágenes tomadas con el microscopio electrónico de alta resolución a diferentes magnificaciones de nanopartículas de platino ensambladas en la superficie de los nanotubos de carbono (a) esférico y (b) dendrítico. STEM (microscopio electrónico de transmisión y barrido)(c) XEDS (espectroscopia dispersiva de energía de rayos X) muestra una distribución elemental de carbono y platino donde podemos comprobar la estructura núcleo-corteza de este material.]

- Detección indirecta de bioanalitos relevantes sobre la superficie de nanotubos de carbono recubiertos con nanopartículas de plata: el caso de la cocaína

En este capítulo hemos utilizado nanotubos de carbono (CNTs) recubierto de forma homogénea con nanopartículas de plata para determinar si un bio-analito, en este caso un metabolito de la cocaína, está o no presente en una muestra y en qué cantidad. En primer lugar, utilizamos este material para hacer una detección indirecta del bio-analito en cuestión a través de un anticuerpo que está ensamblado en la superficie de las partículas metálicas, y a continuación comparamos estos resultados con los obtenidos mediante la detección directa del mismo analito y sobre las partículas de plata. Esta comparación revela que mientras la detección directa ofrece un límite de detección más bajo que el método indirecto, este último es específico y ofrece resultados cuantitativos a nivel fisiológicos, por lo que no solo se puede demostrar el consumo de drogas, sino que también se puede cuantificar.

Fig. 1. (A) imagen representativa de TEM de los CNT@Ag. (B) espectro ultra violeta visible de las nanopartículas de plata ensambladas en los nanotubos y de nanopartículas coloidales de un tamaño similar. (C) espectro SERS del 1NAT sobre CNT@Ag calculados con tres líneas láser diferentes.
• **Capsulas de sílice magnéticas reforzadas con nanotubos de carbono, con morfología de anemonas de mar.**

En este capítulo hemos sintetizado un material híbrido nanoestructurado combinando los dos tipos de sustratos empleados para desarrollar todos los materiales descritos en esta tesis, y hemos comprobado como combinándolos se puede aumentar la complejidad de una estructura, pudiendo llegar incluso a imitar estructuras presentes en la naturaleza. Además se demuestra la versatilidad del método sintético, que permite aportar múltiples funcionalidades al material, como la funcionalidad magnética que permite manipular estas estructuras mediante la acción de un campo magnético externo.

![Fig. 1. Imágenes TEM de capsulas de nanotubos recubiertos con sílice parcialmente (a) y completamente (b) huecas, y (c) y (d) imágenes de SEM (microscopio electrónico de barrido) de capsulas huecas de nanotubos de carbono.](image-url)
• Control de las propiedades magnéticas de nanorecubrimientos de partículas mediante el crecimiento químico controlado de níquel.

En este capítulo se ha demostrado como partiendo de un soporte coloidal diamagnético se puede diseñar un material híbrido magnético y tener un control preciso sobre sus propiedades magnéticas.

En primer lugar se ensamblaron nanopartículas metálicas de platino sobre la superficie de coloides sub-micrométricos de poliestireno para catalizar la reducción de Ni$^{2+}$ en disolución, y formar películas nanométricas de Ni$^{0}$ sobre dichos soportes coloidales. Con esta estrategia hemos comprobado que podemos sintetizar materiales híbridos con propiedades magnéticas determinadas, controlando la relación entre el níquel que está presente en disolución y la cantidad de poliestireno recubierto de platino que añadimos.

![Imagen representativa de STEM (a) y TEM (b,c,d) a baja (izquierda) y alta (derecha) magnificación.](image)

*Fig. 1. Imagen representativa de STEM (a) y TEM (b,c,d) a baja (izquierda) y alta (derecha) magnificación.*
• **Nanoreactores altamente activos: encapsulación de materiales mediante catálisis confinada.**

Aquí hemos desarrollado un método para la obtención de cápsulas huecas de sílice porosas con actividad catalítica, que permiten la encapsulación de un material en su interior. En este caso también hemos partido de un soporte coloidal esférico, partículas de poliestireno, sobre las cuales hemos depositado nanopartículas de platino para a continuación crecer una capa de sílice porosa utilizando el método de Stöber. A continuación se procedió a retirar el núcleo polimérico a través de los poros de la sílice disolviéndolo en un medio orgánico, obteniendo de esta forma una estructura hueca y rígida con nanopartículas catalíticas en su interior. También hemos comprobado a través de la reacción de reducción del hexaciano ferrato (III), que la actividad catalítica del platino no se ve alterada durante el proceso sintético, lo que nos permitió reducir níquel en el interior de las cápsulas.

*Fig. 1. Esquema sintético del proceso de formación de los nanoreactores con níquel encapsulado en su interior.*
Diseño de sub-microcapsulas codificadas en SERS mediante el crecimiento confinado de nanopartículas metálicas encapsuladas

Como en el capítulo anterior hemos partido de coloides de poliestireno para sintetizar una estructura con forma de cápsula de sílice hueca que presenta una alta actividad óptica para aplicaciones basadas en SERS. En este caso hemos recubierto las partículas coloidales de poliestireno con nanopartículas de oro de 2nm, para a continuación crecer una capa de sílice porosa, y retirar posteriormente su núcleo polimérico. Así, las estructuras huecas obtenidas contienen nanopartículas de oro en su interior, que permitirán catalizar la reducción de Au⁺ y en consecuencia permitir el crecimiento de nanoislas de Au en el interior de las paredes de la cápsula. Esta estructura fue codificada con un una molécula activa en SERS (en este caso un tiol), que difunde a través de los poros de la capa de sílice para anclarse en las nanopartículas de oro crecidas que están en el interior de la cápsula.

Para verificar la aplicación de estos materiales híbridos como biosensores activos en SERS se ha planteado una prueba de concepto que consiste en la detección de un analito (antígeno) en una disolución problema, para ello, una vez codificadas las nanoislas de oro que están confinadas en el interior de las cápsulas con una molécula activa en SERS, se prosigue con la biofuncionalizado la capa de sílice para anclar sobre ella un anticuerpo, y detectar utilizando la técnica SERS si el analito está presente en la disolución a través de un acoplamiento específico antígeno-anticuerpo.
Fig. 1. Este esquema nos muestra los pasos seguidos durante el proceso sintético:

1. Funcionalización con polielectrólitos  
2. Deposición de nanopartículas de oro  
3. Recubrimiento con polyvinilpirrolidona  
4. Crecimiento de la capa de sílice  
5. Eliminación del núcleo polimérico  
6, 7. Crecimiento de las nanopartículas de oro  
8. Difusión de la molécula opticamente active en SERS  
9. Ensamblado del anticuerpo  
10. Reconocimiento del antígeno
Conclusiones

Aunque ya se han presentado conclusiones específicas a lo largo de cada uno de los capítulos de esta tesis, aquí vamos a resumir las conclusiones más generales que se han obtenido del trabajo presentado:

1. Se pueden obtener dispersiones estables de nanotubos de carbono a través de su funcionalización con polielectrolitos. Esta funcionalización les proporciona una carga superficial elevada lo que los hace buenos candidatos para su utilización como soportes o como refuerzo de diferentes estructuras.

2. Las nanopartículas metálicas se pueden ensamblar uniformemente sobre la superficie de los nanotubos de carbono debido a la utilización de la técnica de deposición capa por capa que se basa en interacciones de origen electrostático. Las nanopartículas de platino con morfología dendrítica presentan una mayor actividad catalítica que sus homólogas esféricas, y esto se puede explicar por un aumento del número de átomos de Pt en los bordes y esquinas de las nanopartículas con morfología dendrítica.

3. Los nanotubos de carbono dopados con nanopartículas de plata proporcionan un sustrato altamente eficiente y estable para SERS, que podría ser útil para aplicaciones como sensores portátiles que permitan hacer de forma rápida la ultradetección y cuantificación de metabolitos de interés.

4. Se ha diseñado un método para la fabricación de cápsulas huecas y porosas de sílice a través de un primer recubrimiento con sílice de los coloides de poliestireno, seguido de una disolución controlada de estos núcleos poliméricos.

5. La capa de sílice actúa como una membrana porosa, que a su vez, proporciona estabilidad a la cápsula, y permite el intercambio de
sustancias entre el interior y el exterior, que es un requisito fundamental para el crecimiento controlado de diferentes materiales inorgánicos en su interior.

6. La codificación de las cápsulas huecas ha sido posible debido a la relativamente fácil difusión de las moléculas a través de la capa de sílice porosa.

7. La versatilidad de las cápsulas de sílice permiten obtener una fácil funcionalización con gran número de grupos reactivos. Por lo tanto, estas estructuras huecas se utilizaron para obtener nanopartículas codificadas, que posteriormente fueron funcionalizadas para aplicaciones como biosensores basados en SERS.
Resumen y Conclusiones Generales
Lista de Publicaciones/List of Publications


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