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Synthesis, characterization and biofunctionalization of magnetic gold nanostructured particles

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ABSTRACT

Magnetic Fe_3O_4@Au nanocomposite, nanostructured particles were prepared by depositing gold onto seeds of superparamagnetic iron oxide nanoparticles (SPION). The particles were characterized by SEM, TEM, XRD, visible and near infrared spectroscopy, and magnetic measurements. They were also functionalized and employed as enzymatic carriers for electrochemical biosensing. The nanostructured particles showed an urchin-like, roughly spherical morphology whose surface was covered with nanometric protuberances. The average diameter of the particles could be controlled in the 100–500 nm range by changing the concentration of surfactants (oleylamine and oleic acid) employed in the synthesis. XRD showed a preferential growth of {1 1 1} facets. 3-Mercaptopropionic acid was used to chemically modify the surface of the nanostructured particles and horseradish peroxidase was immobilized onto the gold surface. Attached enzymes showed good activity even after several cycles of magnetic collection, drying, and redispersion.

1. Introduction

Nanocrystalline materials have been investigated intensively in the last years, due to their catalytic, optical, electronic, magnetic, and biosensing applications [1–9]. In particular, great efforts have been devoted to obtaining metallic particles with well defined characteristics of size, shape, and structure. So far, several approaches have been developed aiming at the systematic control of crystal size and morphology. The shape-controlled synthesis of particles has been reviewed in Ref. [2] and references therein. The production of highly branched nanoparticles has been reported by several groups [10–16]. However, the controlled preparation of nanostructured particles (NSP) in the submicrometric range with morphological features in the nanoscale remains to be a challenging task. NSP such as nanoflowers, nanourchins and nanostars show potential applications in fields like sensors, biology, catalysis, optics, and surface enhanced Raman spectroscopy (SERS). So far, gold NSP have been obtained mainly deposited onto substrates. Duan et al. [17] reported the fabrication of flowerlike gold nanoparticles electrodeposited onto indium tin oxide as substrates for SERS. Kim et al. [18] combined photolithography with electrodeposition to obtain arrays of flowerlike gold nanostructures. On the other hand, synthesis of such nanostructures in solution has proved to be difficult. Guo et al. [19] used a wet chemistry approach to obtain gold nanocorallines with average size of 500 nm, composed of nanorods sized in the 10–100 nm range.

Although gold flower-like particles have been grown onto magnetite nanoparticles [20], the controlled production of highly branched NSP with magnetic core has not been yet reported. In this work we present the synthesis, characterization, and chemical modification of magnetic NSP, consisting of a gold urchin-like shell grown onto a magnetic core. The potential application of the NSP as protein carriers for biosensing is shown by electrochemically detecting the horseradish peroxidase enzymatic activity.

2. Experimental

2.1. Preparation of nanostructured particles

Magnetite nanoparticles were synthesized by the co-precipitation of ferric and ferrous ions. 25 ml of 25% solution of NH_4OH were added at room temperature to 100 ml of a solution containing 12 g of $FeCl_3$ and 6 g of $FeCl_2$ in 0.1% HCl. The precipitate, composed by Fe_3O_4, was collected with the aid of a permanent magnet, rinsed with distilled water to eliminate the excess of ferric ion, and dried at 65 °C for an hour. Then, 30 mg of Fe_3O_4 were added to 80 ml of a solution of toluene containing x ml of oleic acid (Olac) and x ml of oleylamine (Olam), with $x = 0.25$, 1.25, 2.5 and 5.0, and redispersed with ultrasonic agitation during 15 min. A solution of

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gold in toluene was obtained by dissolving 53 mg of $KAuCl_4{\cdot}xH_2O$ (49% Au, Aldrich) in 20 ml of 2-propanol and then added to 100 ml of toluene. The magnetite dispersion was added to the organic phase, and 150 mg of hydroquinone dissolved in 20 ml of 2-propanol were added. After 12 h, particles were collected with a permanent magnet, rinsed twice with toluene under ultrasonic agitation, and re-dispersed in a solution of Olac and Olam. By varying the Olac + Olam concentration, NSP particles with sizes in the 100–500 nm range were obtained.

2.2. Characterization

Magnetite nanoparticles were observed by transmission electron microscopy (TEM) using a Jeol HARP 3010 microscope (point resolution 1.7 Å), operating at 300 kV. Samples were prepared by placing a drop of a nanoparticles dispersion onto a 400-mesh copper grid coated with carbon film.

SEM images of NSP were acquired with a Carl Zeiss Supra 40 scanning electron microscope. Visible and near infrared absorption spectra were registered with a Shimadzu 1610-PC UV–VIS diode array spectrophotometer using quartz cells of 10 mm path length in aqueous media. X-ray diffraction (XRD) patterns were recorded on a Philips XPert diffractometer equipped with a monochromator, using Cu Kα radiation of a wavelength of 1.54060 Å. Magnetic properties were measured with a Quantum Design PPMS magnetometer.

2.3. Immobilization of enzymes onto nanoparticles

HRP was immobilized onto NSP employing 3-mercaptopropionic acid as a molecular linker between the gold surface and the enzyme. NSP were immersed overnight in a solution containing 40 mM 3-mercaptopropionic acid dissolved in a mix of 75% ethanol + 25% water. Particles were recovered with a permanent magnet and rinsed with distilled water.

NSP were treated during 30 min with 20 μl of a solution containing 0.1 M 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride and 25 mM *N*-hydroxysuccinimide in 0.1 M PBS buffer of pH 7, and then 20 μl of HRP 2.4 U μl^{-1}, also in a 0.1 M PBS buffer of pH 7, were added. The HRP-coated particles (NSP-HRP) were collected with a permanent magnet and transferred to an electrochemical cell, where the enzymatic activity of the enzyme was determined.

2.4. Electrochemical measurements

Thick film Au electrodes (Ø 1 mm, see Fig. 1) were printed onto α-Al_2O_3 substrates by conventional screen printing technology. Commercial Au paste (Heraeus D5789) and 96% α-Al_2O_3 substrates were employed. Electrode layout was transferred by means of photolithography to a stainless steel mesh (400 wires per inch) with a negative photosensitive film (Ulano CDF-2). Au ink printing was performed by an EKRA Microtronic-II printer, dried at 125 °C during 15 min and finally fired at 850 °C. The electrodes were integrated in an electrochemical cell, constructed in PMMA using a numeric control device from a CAD layout.

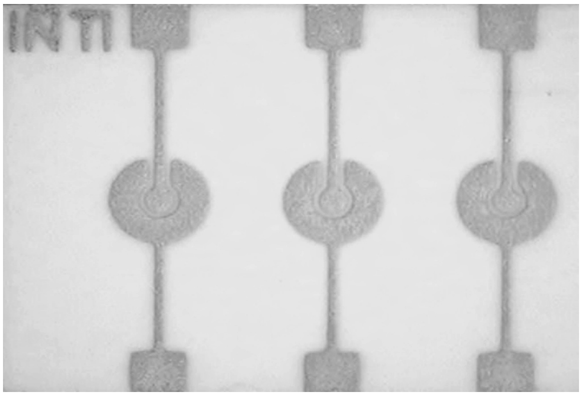

Fig. 1. Photograph of thick film Au electrodes deposited onto an alumina substrate.

The enzyme-modified NSP where concentrated onto the working electrodes with permanent magnets placed underneath the printed electrodes. By removing the magnets the particles could be easily re-dispersed when needed.

Electrodes were cleaned with 98% H_2SO_4 + 30% H_2O_2 (in a 2:1 volume ratio) and thoroughly rinsed with water (miliQ quality). Potentials were measured and referred to in the text against a Ag|AgCl|0.1 M KCl reference electrode. Electrochemical measurements were carried out at 25 °C in a 0.1 M PBS buffer of pH 7 + 0.1 M KCl + 4 mM hydroquinone (redox mediator), prepared from analytical grade reagents (Merck) and three-fold distilled water. The potential of the working electrode was controlled with a potenciostat EG&G PAR 273A.

3. Results and discussion

3.1. Synthesis and characterization of NSP

Magnetic NSP were obtained in a two stages process in which gold was deposited onto seeds of superparamagnetic Fe_3O_4 nanoparticles (SPION) by reduction of $AuCl_4^-$ in non-aqueous media in the presence of Olac and Olam as growth modifiers. In Fig. 2a, the TEM image of SPION seeds is shown. The particles have an average diameter of 15 nm. Fig. 2b and c shows the SEM images of the NSP. The particles present a roughly spherical symmetry and their surface is covered by rod-like nanostructures of an average length of 30 nm and a width of 10 nm. Fig. 2d shows the HR-TEM image of a single tip. The interplanar distance measured is 2.4 Å, which corresponds to the {1 1 1} crystal orientation of pure Au.

The size of the NSP could be controlled by changing the Olac + Olam concentration. In Fig. 3, SEM images of the NSP obtained at three values of Olac + Olam concentration are shown. As depicted in Fig. 4, the diameter of the particles decreases as the Olac + Olam concentration increases. The mechanism of the NSP formation is not clear and further studies are being carried on with this aim. The overall process is slow and several stages must be involved. A possible reaction path is proposed. Initially, SPIONs are strongly stabilized by the oleic acid [21] and $AuCl_4^-$ must compete and eventually displace Olac from the surface of the magnetite seeds by a ligand exchange mechanism [22,23], thus forming SPION-Au particles. However, ligand displacement is a slow reaction and the simultaneous formation of gold clusters following a homogeneous nucleation mechanism cannot be ruled out. In this case, aggregation of SPION-Au particles due to an oriented attachment (OA) of gold clusters to magnetite can also be envisaged [24,25]. These newly formed SPION-Au particles agglomerate to form larger aggregates [12]. Finally, Olam-controlled Au anisotropic deposition continues onto these aggregates, giving rise to the development of branched NSP structures [27].

The obtained XRD patterns were consistent with that of face centered cubic Au, with peaks at 2θ of 38.185° (relative intensity: 100%), 44.393° (52%), 64.578° (32%), 77.549° (36%), which correspond to diffraction at planes (1 1 1), (2 0 0), (2 2 0), and (3 1 1) respectively (Fig. 5). All the obtained XRD patterns were very similar, and no significant dependence on the size of the NSP particles was found. For instance, for NSP particles grown using 3.33% Olam + Olac, peaks at 2θ equal to 38.25 (100%), 44.42° (32.31%), 64.67° (23.92%), 77.63° (24.54%),