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CHITOSAN-COATED BIMETALLIC GOLD-SILVER NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND SERS ACTIVITY

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ABSTRACT. In this study we report a fast and non-toxic approach for the synthesis of bimetallic gold-silver spherical nanoparticles coated with chitosan biopolymer. The obtained hybrid nanoparticles were characterized by UV-vis-NIR extinction spectroscopy, transmission electron microscopy (TEM) and zeta potential measurements. In view of future applicability in molecular sensing, we tested the feasibility of chitosan protected bimetallic nanoparticles to operate as surface-enhanced Raman scattering substrates.

Keywords: *chitosan; plasmonic nanoparticles; localized surface plasmon resonance; surface-enhanced Raman spectroscopy; transmission electronic microscopy; zeta potential*

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INTRODUCTION

An important challenge of nanotechnology is the preparation of nanoparticles with desired properties and functions. In many cases the physical and chemical properties as well as the applications of nanoparticles are critically controlled by their dimension and shape [1]. This is also the case of noble-metal nanoparticles which exhibit extremely interesting optical response in interaction with light. When the incoming light couples with the oscillation frequency of the conduction electrons, a so-called localized surface plasmon resonance (LSPR) arises, which is manifested as an intense absorption band [2]. However, the precise spectral position of the plasmon resonance depends on several parameters, among which particle size and shape and their degree of assembling are the most important [3].

Due to their unique physical and chemical properties, noble-metal nanoparticles also called plasmonic nanoparticles are very attractive in a variety of biomedical applications such as: molecular sensing [4], molecular diagnosis [5], antibacterial action [6], drug delivery [7], bio-labeling [8], cancer therapy [9], etc. Besides the size and shape control, the usefulness of plasmonic nanoparticles in different applications strongly depends also on their structural and optical stability in the growth medium. For example, the plasmon-enhanced spectroscopies require stable and well-defined resonances and a shift of plasmon resonance can reduce the signal intensity by orders of magnitude.

The use of natural polymers to stabilize the plasmonic nanoparticles has received considerable attention recently [10], [11], especially for the safety point of view. Among them, chitosan shows very interesting biological, physical and chemical properties which make it possible to use chitosan-protected nanoparticles in biological applications [12]. Chitosan is a cationic polysaccharide obtained by partial deacetylation of chitin. It is an inexpensive polymer which possesses several favorable biological properties such as an excellent biocompatibility, low toxicity, biodegradability and adsorption ability [13].

The metal type is another parameter that influences the applicability of plasmonic nanoparticles. For example, gold nanoparticles exhibit higher chemical stability in comparison with the silver ones. However, silver is a more efficient plasmonic material than gold and can be excited with more energetic light due to a significantly reduced damping for interband transitions which make it an ideal candidate for sensing applications based on plasmon-enhanced spectroscopies [14]. In this context, bimetallic gold-silver nanoparticles could be a good option for sensing application.

In this work we report a facile, rapid, and inexpensive route to synthesize bimetallic gold-silver spherical nanoparticles. Due to its interesting biological, chemical and physical properties we selected chitosan biopolymer for the stabilization of the particles. The produced chitosan-coated bimetallic gold-silver nanoparticles were characterized by UV-vis-NIR extinction spectroscopy, transmission electron microscopy (TEM) and zeta potential measurements. The surface-enhanced Raman scattering efficiency of the fabricated nanoparticles was assessed with para-aminothiophenol (pATP) as a probe molecule.

EXPERIMENTAL DETAILS

Chemicals

Chitosan flakes (medium molecular weight), $HAuCl_4$, and paraamino-thiophenol (pATP) were purchased from Aldrich and used without further purification. Glacial acetic acid (99.8%), silver nitrate ($AgNO_3$) and ascorbic acid were obtained from Merck. Glacial acetic acid was diluted to a 1% aqueous solution before use. All reagents employed were of analytical grade and the solutions were prepared using ultrapure water with a resistivity of at least 18 MΩ cm. The glassware used was cleaned with aqua regia solution ($HCl:NO_3$ 3:1) and then rinsed carefully with ultrapure water. A solution of 2 mg/mL chitosan was prepared by dissolving the polymer in 1% acetic acid solution. The mixture was kept for a day until the solution became clear.

Preparation of chitosan-coated bimetallic gold-silver nanoparticles

Chitosan-coated bimetallic gold-silver nanoparticles (Au-AgNPs) were synthesized using a two-step approach. In the first step, spherical gold nanoparticles (AuNPs) were prepared by mixing a volume of 10^{-3} M $HAuCl_4$ with 18 ml chitosan solution and keeping this mixture at 50 °C under magnetic stirring until the solution turns red. In the second step, ascorbic acid, $AgNO_3$ and 5 ml of as-prepared spherical gold nanoparticles were combined and kept at room temperature for 30 minutes under continuous magnetic stirring. For SERS measurements, 990 µL of Au-AgNPs were incubated at room temperature for several hours with 10 µL solution of pATP, followed by centrifugation and re-suspension in ultrapure water.

Equipment and characterization methods

A 2 mm quartz cuvette was used to collect the LSPR spectra, using a Jasco V-670 UV-VIS-NIR spectrometer with 1 nm spectral resolution.

The transmission electron microscopy (TEM) images were collected using a high resolution electronic microscope FEI Tecnai F20.

The Raman spectra were recorded on a confocal Raman microscope (CRM alpha 300R from WITec GmbH, Germany) in backscattering geometry, employing 532 nm laser line as the excitation source directed through a 20x objective lens with a numerical aperture (NA) of 0.4.

The zeta potential of the AuNPs and Au-AgNPs was determined using a Malvern Zetasizer Nano ZS-900. The analysis was performed at a temperature of 25^{o}C, with a He-Ne laser (5mW).

The theoretical extinction spectra were calculated using the method Finite Difference Time Domain (FDTD) provided by the software FDTD Lumerical Solutions [15]. The FDTD method solves electromagnetics problems, Maxwell's differential equations to be more specific. We calculated the extinction spectrum for a 16 nm gold sphere enveloped in a silver layer of

2.2 nm and surrounded by water (n=1.33). In order to perform the simulation, we used a 0.2 nm mesh and an electromagnetic source with a domain between 300 nm and 700 nm.

RESULTS AND DISCUSSION

AuNPs are obtained using the reducing agent chitosan, which is also a stabilizer agent, to reduce the Au ions and form the nanoparticles as crystalline networks of Au atoms. The UV-Vis spectrum of the AuNPs (Fig. 1) shows a narrow band with a single peak at 524 nm which is typical for monodispersed spherical AuNPs. The lack of band in the near-infrared (NIR) region indicates that the AuNPs do not aggregate.

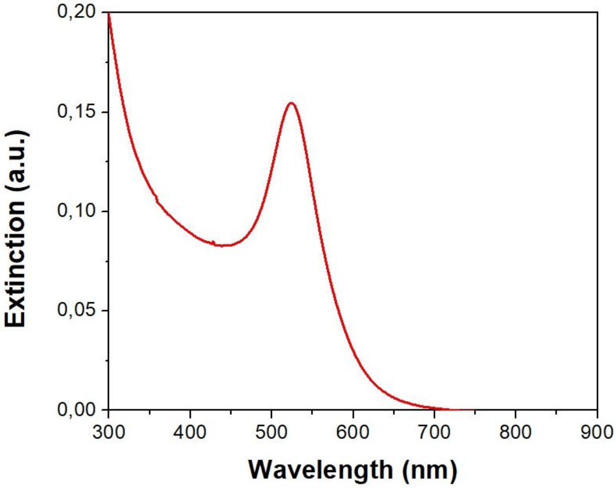

Fig. 1. The extinction spectrum of chitosan-coated AuNPs

In the interest of obtaining more information about the size and the shape of the AuNPs some TEM images were taken, a representative TEM image of the synthesized AuNPs being presented in Fig. 2. As can be seen from the TEM analysis, the NPs are spherical with the mean particle diameter of 16 nm (see histogram in Fig. 2(a) inset). The results were obtained by analyzing several TEM images.

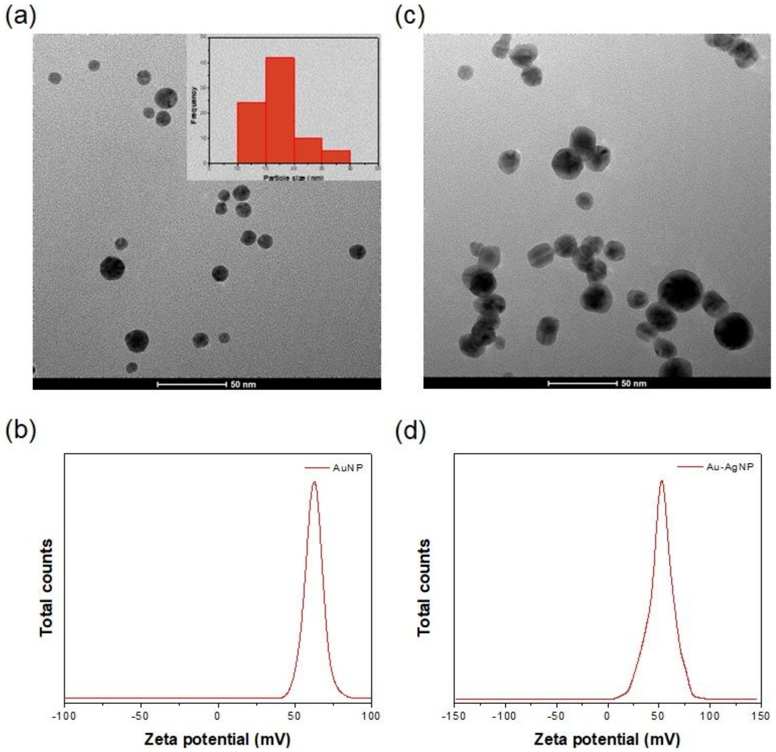

Fig. 2. (a) A representative TEM image of the chitosan-coated AuNPs, (b) the zeta potential of chitosan-coated AuNPs (c) A representative TEM image of chitosan-coated Au-AgNPs, (d) the zeta potential of chitosan-coated Au-AgNPs

Zeta potential measurements were further performed to evaluate the electrostatic repulsion between polymer-coated AuNPs. The value of the zeta potential gives us information about the stability of the colloidal solutions. Nanoparticles with a value of the zeta potential higher than ± 30 mV are considered stable. The zeta-potential analysis from Fig. 2(b) reveals that the particles are positively charged (+62.4 mV) due to the polymeric coating.

Au-AgNPs were obtained by gradual reducing silver ions with ascorbic acid into a solution of chitosan-coated AuNPs. Reduction of silver ions leads to the formation of bimetallic gold-silver nanoparticles. Fig. 2(c) illustrates a representative TEM picture of obtained Au-AgNPs which clearly reveals the formation of individual particles mainly of spherical shape. The strong positive surface charge of Au-AgNPs in Fig. 2(d) (+ 42.6 mV) indicates the presence of polymeric coating around Au-AgNPs and the good stability of colloidal suspension.

Fig. 3 (black line) shows the extinction spectrum of Au-AgNPs which features two LSPR bands resulting from the hybridization of individual surface plasmon resonance localized on gold nanosphere coated by silver layer and surface plasmon resonance localized on silver nanolayer. To understand the plasmonic response of Au-AgNPs a numerical simulation of hybridization was carried out using FDTD software (Lumerical). For simulation we considered a gold sphere of 16 nm diameter covered by a silver layer of 2.2 thickness surrounded by water (n =1.333). The simulated spectrum in Fig. 3 (red line) clearly exhibits two distinct bands indicating once more the bimetallic structure of the fabricated nanoparticles.

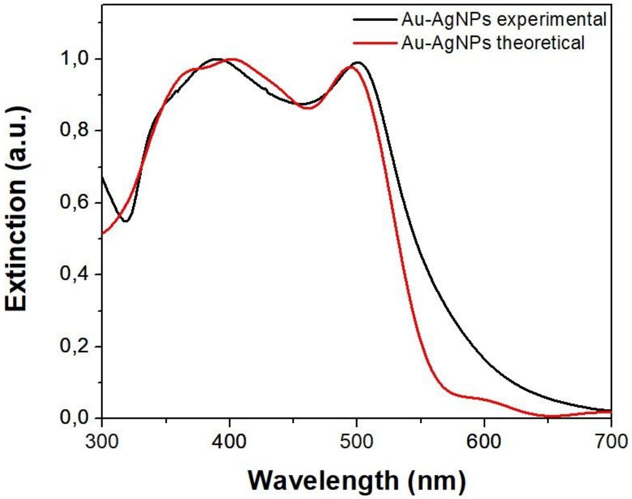

Fig. 3. The experimental and theoretical extinction spectra of chitosan-coated Au-AgNPs

Next, we investigate the SERS activity of Au-AgNPs using pATP as a probe molecule. Fig. 4(a) illustrates the extinction spectra of Au-AgNPs before (black spectrum) and after incubation with pATP (red spectrum). We noticed that the shape of extinction spectrum was preserved and no sign of aggregation is observed. This suggests that chitosan provide good stability to Au-AgNPs keeping nanoparticles far from approaching and preventing their aggregation. However, a 3 nm red shift of the plasmonic band at 500 nm was observed in the presence of pATP molecules. This result indicates that chitosan preserves the stability of nanoparticles, while allows the exchange with surrounding medium, keeping the ability of Au-AgNPs to sense the modifications of refractive index values. This is why we assume that pATP molecules can diffuse through the internal nanoporosities of chitosan and immobilize on the particles surface, leading to an increase of the refractive index of the medium surrounding the particles.

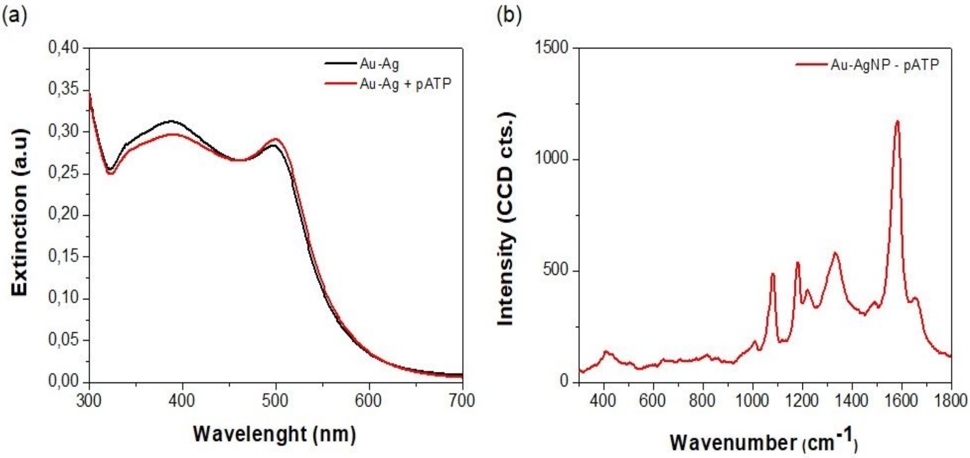

Fig. 4. (a) The extinction spectra of Au-AgNPs before (black spectrum) and after (red spectrum) the addition of pATP (b) SERS spectrum of pATP on colloidal suspension by laser excitation wavelength 532 nm

In Fig. 4(b) we recorded the SERS spectrum of pATP molecules in solution at a final concentration of 10 x 10^{-6} M. The illustrated spectrum clearly identifies the characteristic bands of pATP when is adsorbed onto the surface of Au-AgNPs. In particular, the most intense bands at 1080 cm^{-1}, 1582 cm^{-1}, and 1184 cm^{-1} are assigned to a_1 vibrational modes of p-ATP, namely, C−S stretching vibration, C−C stretching mode, and C−H bending mode [16]. Considering the final concentration of pATP in colloidal solution relatively low and no sign of aggregation observed after the addition of pATP, the signal detected from adsorbed molecules clearly demonstrates a good SERS activity of as prepared Au-AgNPs.

CONCLUSIONS

In conclusion, stable chitosan-coated bimetallic Au-AgNPs dispersed in aqueous solution were prepared through a rapid, environmentally friendly procedure. The presence of chitosan onto the surface of Au-AgNPs was evidenced by zeta potential measurements. The bimetallic structure of the fabricated nanoparticles was confirmed by comparing the experimental and theoretical extinction spectrum of Au-AgNPs. The morphology of Au-AgNPs was investigated by TEM analysis, which revealed the formation of individual nanoparticles, mainly of spherical shape. The obtained Au-AgNPs were found to be excellent SERS substrates.

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