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Self-assembled multilayers of gold nanoparticles as versatile platforms for molecular sensing by Fourier transform-surface enhanced scattering (FT-SERS) and surface enhanced infrared absorption (SEIRA)

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A wet-chemistry strategy for fabricating multilayers of gold nanoparticles on solid substrates is proposed. The strategy is based on immobilization of discrete gold colloid arrays on aminosilane-functionalized substrate with subsequent immersion of the substrate in gold colloidal and p-aminothiophenol solutions. The formation of multilayers structures was confirmed by AFM images and uv-vis absorption spectroscopy. Both FT-SERS and SEIRA (in ATR mode) spectra of p-aminothiophenol absorbed onto as prepared gold films were successfully recorded. The result offers a valuable approach for chemical trace analysis and bio-sensing.

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1. Introduction

Nanostructure-based devices fabricated with a "bottom-up" strategy often rely on the layer-by-layer self assembly of functionalized nanoparticles on solid substrates. The assembly of nanoparticles in two and three-dimensional structures display new physical and chemical properties that are different from bulk properties of the same materials. The ability to tune particle size and shape, surface coverage and linking chemistry provides flexible routes to manipulate material properties and gives practical applications in optoelectronic devices, chemical sensors, biosensors and catalysis [1,2].

Colloidal gold nanoparticles are very attractive for developing biological applications due to their long-term stability and friendly biocompatibility with antibodies, proteins, DNA, and RNA [3]. Different methods have been used to form gold nanoparticle films on various substrates with high controlled film thickness and ease of fabrication. Musick *et al.* have used bifunctional cross-linkers to self-assemble gold nanoparticles onto (3-aminopropyl)trimethoxysilane (APTMS)-coated or mercaptosilane-modified glass substrate to form conductive films [4]. Doron *et al.* have demonstrated the organization of gold colloids as monolayers on indium tin oxide (ITO) surfaces using (aminopropyl)siloxane or (mercaptopropyl)nsiloxane as base monolayers for adhesion of the metal nanoparticles [5]. Zheng and co-workers has described a metal–molecule–metal nanosystem witch has been fabricated by the assembly of gold and silver nanoparticles interconnected with p-aminothiophenol (PATP) molecules [6].

In this work, we propose a wet-chemistry strategy for fabricating multilayers of gold nanoparticles on aminosilane-functionalized glass or silicon substrates. The strategy is based on subsequent and alternate immersions of the functionalized substrate in gold colloidal and p-aminothiophenol solution, respectively. The formation of multilayers structures was confirmed by atom force microscopy (AFM) and optical absorption spectroscopy.

Our aim is to investigate the prepared gold nanofilms as versatile spectroscopic enhancers for chemical or biochemical analysis *via* Raman and IR spectroscopy.

The work with biological objects, such as nucleic acids, proteins, lipids, imposes some restrictions on using traditional spectroscopic techniques and makes it necessary to use specific approaches and methods. The point with biological material is the restricted amount of the available substances under investigation. One of the most sensitive spectroscopic tools available for the detection of a wide range of adsorbate molecules down to the single molecule detection limit is the Surface Enhanced Raman Scattering (SERS) technique. Two mechanisms have been found to be responsible for the giant Raman enhancement. The first one is due to the excitation of localized surface plasmons on metal surface, while the second one (chemical) is a consequence of the resonant charge transfer. As regard to the capacity to register the IR spectra and analysis the samples of extremely small amounts of substance, a modified IR technique based on enhancement of infrared molecular absorption by rough metallic surface was proposed [7]. The Surface Enhanced Infrared Absorption (SEIRA) is due to the increase of local plasmon fields and specific increase of the dipole transition moments of the molecules adsorbed on the metallic nanstructured surface.

Here, we prove the ability of the same metallic substrate to enhance both Raman and IR spectra of very small amount of probe molecules.

The high enhancement of (some) IR and Raman bands of pATP molecules adsorbed on self-assembled multilayers of gold nanoparticles recommends these substrates as outstanding candidates for upcoming investigations of biological relevant molecule *via* SERS and SEIRA measurements.

2. Experimental

2.1. Sample and substrate preparation

$HAuCl_4 \bullet 3H_2O$, p-Aminothiophenol (p-ATP) and aminopropyltriethoxysilane (APS) were purchased from commercial sources (Aldrich) and used as received. Ultrapure water was used throughout the experiments. All other reagents employed for substrate and solutions preparation were of analytical grade.

The gold colloid was prepared by citrate reduction of $HAuCl_4$ [8]. The assembling protocol of the gold nanoparticles involves a few steps. First of all, the glass slides were sonicated in ethanol and maintained in *Piranha* solution (4 parts concentrated H_2SO_4, 1 part 30% H_2O_2) for 24 h to remove organic groups. Clean glass slides were rinsed in methanol and functionalized for a few minutes in aminopropyl)methoxysilane (APS) solution. Before immersing the silanized substrates into the gold colloidal solution, where were maintained for 120 min, the substrate were rinsed with methanol and water for several times. The slides were then removed and dried with nitrogen. For SERS measurements the p-ATP molecules were adsorbed on the Au particle surface by immersion for 24 hours. In the first experiment substrate was modified by immersion in p-ATP solution 10^{-3} M and multilayers were constructed by alternate immersion into colloidal solution of gold and p-ATP solution. A rinse with ultrapure water is necessary after each step. In the second experiment multilayers of gold nanoparticles was constructed by successive immersion of functionalized glass slide in gold colloidal solution and only the last step was the immersion in p-ATP solution 10^{-3} M.

2.2. Experimental measurements

The UV-VIS absorption spectra were recorded with a Jasco V-530 spectrophotometer.

The FT-SERS and FT-IR (SEIRA) spectra were recorded by using a Bruker Equinox 55 spectrometer with an integrated FRA 106 Raman module. A radiation of 1064 nm from an Nd-YAG laser was employed for excitation. The power incident on the sample was of 400 mW and the spectral resolution was of 4 cm^{-1}.

3. Results and discussion

The layer-by-layer self assembly of gold nanoparticles is illustrated in Fig. 1. A layer of gold nanoparticles (20 nm) was assembled onto the functionalized glass slide by immersion in gold solution for 120 min.

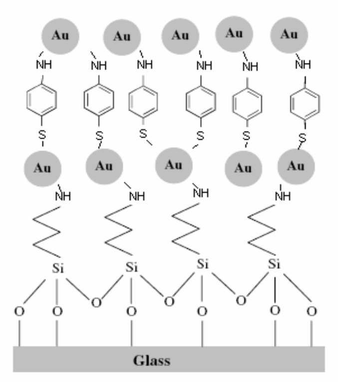

Fig. 1. Illustration of the assembly of gold nanoparticles and p-ATP molecule on glass slide.

Next, the p-ATP molecule was adsorbed on the surface of the assembled gold nanoparticles through the formation of Au-S bonds. A new layer of gold nanoparticles was assembled on top of p-ATP molecules. In this case the gold nanoparticles are assembled through formation of $Au\text{-}NH_2$ bonds. Multilayer films of gold nanoparticles result from repetitive exposure to the p-ATP molecule and gold colloidal solution. Deposition of each layer of gold nanoparticles results in a shift of the surface plasmon bands (SPR) to the higher wavelengths (Fig. 2, a).

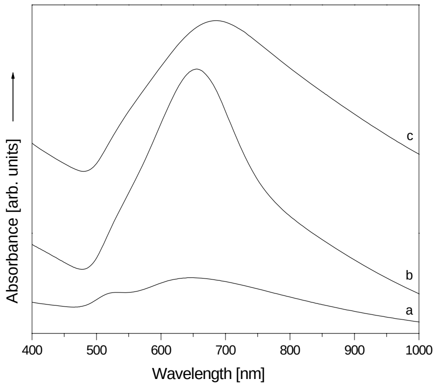

Fig. 2. Absorption spectra for multilayer of gold nanoparticles (a) monolayer (b) Au/Au/ Au/p-ATP (c) Au/p-ATP/Au/p-ATP/Au/p-ATP.

The absorption spectrum of the first layer of gold nanoparticles (a) presents two bands, first one is due to the isolated gold nanoparticles on the glass slide (520 nm) and the second one is due to the aggregates formed on the substrate (640 nm) [8]. After further successive immersions in p-ATP and gold colloidal solution the red shift of the λ_{max} to 701 nm and a broadening of the SPR band are observed (Fig. 2, b and c). Aggregation of the nanoparticles on the functionalized glass substrate is responsible for the shifted peak in the absorption spectra.

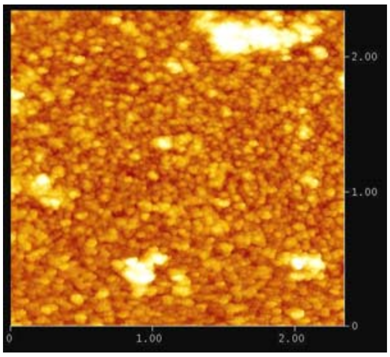

Fig. 3. AFM image for Au/p-ATP multilayer on glass substrate.

The surface morphology of the Au/p-ATP multilayers is shown in the AFM image (Fig. 3). It can be seen that for three successive layers of gold nanoparticles and p-ATP molecules the film topography is similar with a roughness film structure. It well known that the roughened gold and silver electrodes or metal nanoparticles clusters produce a very high signal enhancement [9, 10].

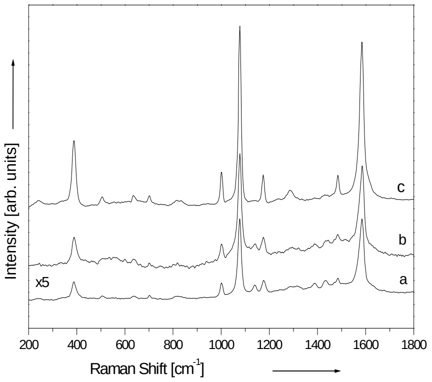

Fig. 4. FT-SERS spectra for p-ATP molecule adsorbed on: (a) monolayer of gold nanoparticles, (b) Au/p-ATP/Au/p-ATP/Au/p-ATP, (c) Au/Au/Au/p-ATP.

Metal nanoparticles associated with a spectroscopy-active analyte and surrounded by a (bio) molecule are useful as sensitive optical tags detectable by surface-enhanced spectroscopy. The FT-SERS and SEIRA (in ATR mode) measurements have been used to probe the signal enhancement of such nanostructures. The SERS and SEIRA measurements can provide useful information about molecular structure of interest molecule. The surface enhanced Raman spectra of the p-ATP molecule adsorbed on the gold nanoparticles film and cross-linked in the layers of the gold nanoparticles is shown in figure 4. There are two contributions to the surface enhancement in SERS: charge transfer enhancement (CT) arising from chemisorption of the adsorbate at the metal surface [11] and an electromagnetic enhancement (EM) [12, 13]. It can be seen from Fig. 4 (a) that the SERS spectra of the p-ATP molecule adsorbed on monolayer of gold nanoparticles is dominated by the three major bands at 389, 1078 and 1588 cm^{-1} (Table 1) which are the a_1 mode of a p-ATP molecule [2].

Previous investigations of the adsorption behavior of the p-ATP molecules on silver island films was performed [14] by using visible excitations (488 and 633 nm) have demonstrated that the CT mechanism considerably contributes to the SERS enhancement. The different enhancement patterns evidenced in the SERS spectra recorded with different excitation lines (532, 633, 830 and 1064 nm) were explained from the perspective of the different contributions of the mechanisms responsible for the total SERS enhancement [8]. Specifically, the enhancement of the band around 1432 cm^{-1} that intensity varied as function of the applied potential for three visible excitation wavelengths was well interpreted in terms of the CT mechanism. On the contrary, the CS stretching mode around 1083 cm^{-1}, which was almost insensitive to the potential change, was believed to be enhanced only by the EM mechanism [8, 14].

Tabel 1. SERS bands of p-ATP molecule.

SERS (cm^{-1})	***Vibrational assignment***
1078s	SC str + NH2 rock
1142m	CH bend
1175m	CN str + CH rock
1388	CC str in Ph ring + CH rock NH2 rock
1432	CC str in Ph ring + NH_2 rock
1588s	CC str in Ph ring + NH2 bend

The bands appeared at 1142, 1388, 1432 and 1588 cm^{-1} that were selectively enhanced in SERS through the vibronic coupling [14] and were assigned to the nontotally symmetric b_2 vibration modes of p-ATP molecule, which are closely related to the thiol group vibrations, was considered to be enhanced by the CT mechanism. The spectral characteristics of the SERS spectra demonstrate that the p-ATP molecules are adsorbed onto the gold nanoparticles through their sulfur atoms and are standing-up relative to the substrate surface [8].

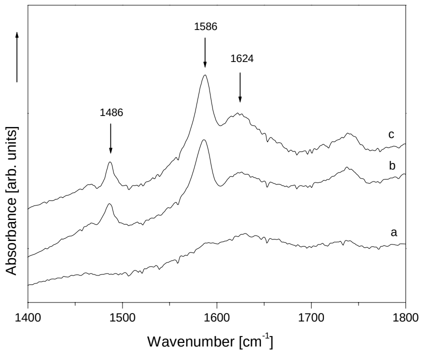

Fig. 5. SEIRA spectra for p-ATP molecule adsorbed on: (a) monolayer of gold nanoparticles, (b) Au/p-ATP/Au/p-ATP/Au/p-ATP, (c) Au/Au/Au/p-ATP.

With increasing the number of layers on the glass slide the intensity of the SERS bands also increases (Fig. 4, b). A high SERS enhancement of the interconnecting molecules is due to a strong local electromagnetic field produced in interstitial region between the neighboring nanoparticles. In this case the CT of the metal-molecule metal could occur through the interconnecting p-ATP molecule.

In the case of p-ATP molecule adsorbed on three layers of gold nanoparticles it can be seen that the a_1 mode of p-ATP molecule is dominated and only a few a_2 nontotally symmetric b_2 vibration modes are enhanced (Fig. 4, c).

In Fig. 5 are shown the SEIRA spectra of p-ATP molecule adsorbed on gold monolayer (a) and multilayer (b, c). It can be seen that from monolayer of gold nanoparticles no signal of p-ATP was observed. The SEIRA spectra of P-ATP molecule adsorbed on multilayers of gold nanoparticles presents three major bands 1486, 1586 and 1624 cm^{-1} (Tabel 2). The vibration modes of p-ATP molecule are referred to the corresponding bands of benzene.

Tabel 2. Vibration modes and symmetry species of some SEIRA bands of p-ATPmolecule.

Frequency(cm^{-1})	***Mode assignment***
1486s	CC stretching (a_1)
1586vs	CC stretching (a_1)
1624s	NH2 deformation

The active species that contribute to the SEIRA of p-ATP are the amino group and benzene skeleton. The band that appears around 1624 cm^{-1} is assigned to the bending band of NH group. The benzene skeleton and the in-plane bending of CH are presents at 1486, 1586 cm^{-1} with a_1 symmetries in the SEIRA spectra. The benzene ring plane is perpendicular to the gold surface and the a_1 modes are perpendicular to [15].

The presented results demonstrate that such substrates could be outstanding candidates for forthcoming investigations of biological relevant molecules by using SERS and SEIRA techniques.

4. Conclusions

In this work we obtained multilayers of nanoparticles produced by alternate immersion into a p-ATP and nanoparticle solutions to produce random arrays of nanoparticles of controllable thickness. The optical properties and morphology of our substrates were characterized by optical spectroscopy and Atomic Force Microscopy. By recording both FT-SERS and SEIRA (in ATR mode) spectra of the probe molecules we successfully demonstrate the potential applicability for sensitive optical tags detectable by surface-enhanced spectroscopy.

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References

[1] Weiqing Xua, Shuping Xua, Xiaohui Jib, Bo Songa, Hang Yuanc, Lan Mac, Yubai Baib, Colloids and Surfaces B: Biointerfaces **40**, 169 (2005).
[2] Nanotechnology, Gregory Timp Ed., AIP Press, (1999).
[3] Michael D. Musick, Christine D. Keating, L. Andrew Lyon, Steven L. Botsko, David J. Pen˜a, William D. Holliway, Todd M. McEvoy, John N. Richardson, and Michael J. Natan Chem. Mater. **12**, 2869 (2000).
[4] M. D. Musick, C. D. Keating, M. H. Keefe, M. J. Natan, Chem. Mater. **9,** 1499 (1997).
[5] Doron A, Katz E, Willner I Langmuir **11,** 1313 (1995).
[6] Qun Zhou, Xiaowei Li, Qiang Fan, Xingxia Zhang, Junwei Zheng, Angew. Chem. Int. Ed., **45**, 3970, (2006).
[7] D. Enders, A. Pucci, Appl Phys Lett. **88**, 184104 (2006),
[8] M. Baia, F. Toderas, L. Baia, J. Popp, S. Astilean, Chem. Phys. Letters **422**, 127 (2006).
[9] Zhong-Qun Tian, Bin Ren, De-Yin Wu, J. Phys. Chem B, **106,** 9465 (2002).
[10] D. L. Jeanmarie, R. P. Van Duyne, J. Electroanal. Chem. **84,** 1 (1977).
[11] Andreas Otto, J. Raman Spectrosc. **36,** 497 (2005),
[12] Hongxing Xu, Javier Aizpurua, Mikael Kall, Peter Apell, Physical Review E, **62**, 4318 (2000).
[13] Martin Moskovits, J. Raman Spectrosc. **36**, 485 (2005),
[14] M. Osawa, M. Matsuda, K. Yoshii, I. Uchida, J. Phys. Chem. **98**, 12702 (1994).
[15] Shou-Jun Xiao, Marco Wieland, Samuel Brunner, Journal of Colloid and Interface Science **290**, 172 (2005).

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