

## Ultrasensitive SERS Nanoprobes for Hazardous Metal Ions Based on Trimercaptotriazine-Modified Gold Nanoparticles

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Trimercaptotriazine-modified gold nanoparticles exhibit strong SERS effects,<sup>1</sup> yielding vibrational profiles very sensitive to the presence of heavy metal ions. Because of the contrasting response observed for selected vibrational bands in the SERS profiles, they provide useful nanoprobes for Hg<sup>2+</sup> and Cd<sup>2+</sup> ions, allowing direct quantitative assays by employing relative peak intensity ratios instead of using internal standards.

Gold nanoparticles (AuNPs) exhibit enhanced optical interaction with visible light because of the so-called localized surface plasmon resonance, which can be conveniently employed in the development of novel chemosensors, incorporating many advantages such as long-term stability and easy surface functionalization.<sup>1,2</sup> For sensing purposes, two relevant aspects have been exploited: (a) the electronic spectral shifts induced by interparticle plasmon–plasmon coupling and (b) the SERS enhancement of specific molecular vibrational modes. The first one is based on the selective, induced flocculation of the AuNPs and has been extensively employed in colorimetric tests and optical detection of many organic and biological substrates.<sup>2,3</sup> The last one is primarily based on the intensification of the electromagnetic fields, which are critically dependent on the nanoparticles' size, shape, orientation, and agglomeration state<sup>1,4–8</sup> as well as on chemical or charge-transfer mechanisms. In fact, SERS has been successfully employed in

ultrasensitive trace detection methods.<sup>9–12</sup> It is known that under favorable resonance-enhanced conditions<sup>13,14</sup> the SERS effect on gold, silver, or copper metal nanoparticles or nanostructures can exhibit extremely high sensitivity, reaching the limit of single-molecule detection.

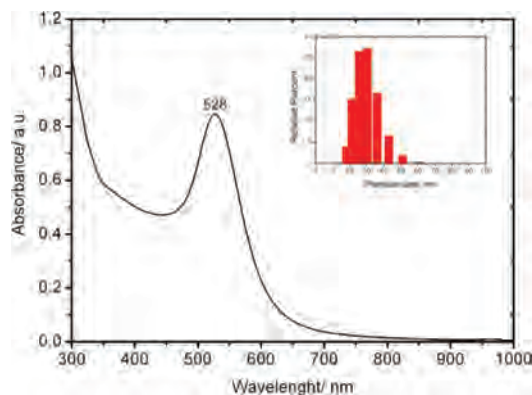
The current focus on the analytical applications of SERS has been the development of efficient nanostructured substrates where Raman scattering can be enhanced by more than 10 orders of magnitude. This has been ascribed to so-called hot spots<sup>15,16</sup> exhibiting exceedingly high electric fields originating from the coupling of the surface plasmon polaritons. Alternatively, metal nanoparticles can be converted into powerful nanoscale chemical probes by tailoring their surface with suitable molecules in order to introduce new functionality. Such molecules should strongly bind to the metal nanoparticle surface and also possess additional binding sites to interact with substrates, such as metal ions in solution. Systematic changes can be expected in the SERS profiles of the sensing molecules at the AuNP interface, reflecting their selective interaction with the analyte. In fact, vibrational modes of ligands are very sensitive to the metal ion coordination, and their SERS response when associated with AuNPs provides a powerful strategy to be explored in analytical chemistry. This is an interesting concept to be incorporated in SERS chemosensors, for conjugating the high sensibility of the SERS effect with the binding characteristics of multifunctional molecular species.

Along this line, we have chosen the 2,4,6-trimercapto-1,3,5-triazine species (TMT, Aldrich) as a convenient multibridging molecular probe associated with AuNPs. This species has been extensively used for the precipitation of

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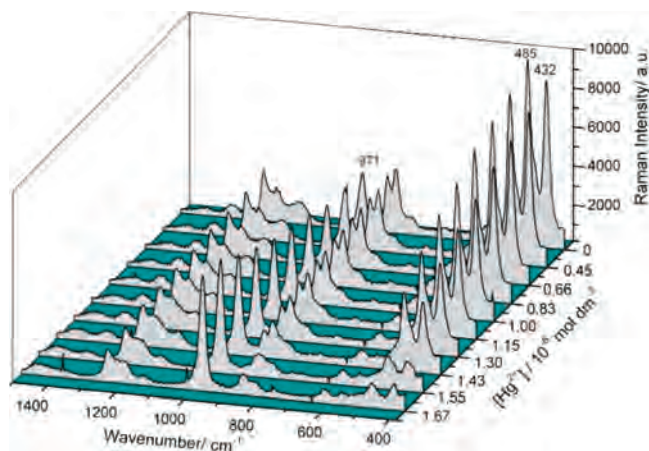
**Figure 1.** UV-vis spectrum of a TMT-AuNP aqueous suspension (pH 4.4). Inset: Histogram of AuNP size distribution.

hazardous metal ions from industrial wastewaters and polluted waters<sup>17–19</sup> and is suitable for interacting with AuNPs and heavy metal ions such as  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$ . In an aqueous solution, TMT undergoes three successive acid-base dissociation reactions. The corresponding equilibrium constants have already been determined<sup>20</sup> as  $\text{p}K_{\text{a}1} = 5.71$ ,  $\text{p}K_{\text{a}2} = 8.36$ , and  $\text{p}K_{\text{a}3} = 11.38$ . In this work, the pH was kept at 4.4, in order to ensure that TMT is predominantly in the neutral form.

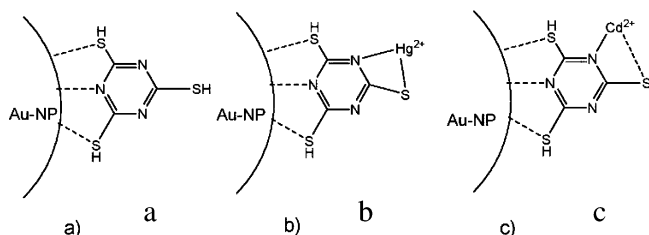
The TMT molecule exhibits five tautomeric forms, varying from trithiol to the mono-, bi-, and trithione species.<sup>20,21</sup> In fact, the presence of thiol and thione tautomers is evidenced by IR peaks at 3155 and 3039  $\text{cm}^{-1}$  ( $\nu_{\text{N-H}}$ ) and 2650 and 2625  $\text{cm}^{-1}$  ( $\nu_{\text{S-H}}$ ). This is corroborated by the N-H combination bands at 4502  $\text{cm}^{-1}$  as well as by the bands at 5977 and 5369  $\text{cm}^{-1}$  in the NIR spectra, ascribed to the first overtones of the N-H and SH stretching modes, respectively, in the FTIR and NIR spectra of the solid. The TMT IR and Raman vibrational spectra are provided as supplementary data (Supporting Information). The assignments were based on ab initio theoretical calculations previously reported for the trithiol and trithione tautomers.<sup>21</sup>

When AuNPs (25 nm,  $7 \times 10^{-10}$  mol  $\text{dm}^{-3}$ ) prepared by the Turkevich method<sup>22</sup> are mixed with TMT ( $1.7 \times 10^{-6}$  mol  $\text{dm}^{-3}$ ), there is a shift of the localized plasmon resonance band from 522 to 528 nm and an increase of the average size distribution from 25 to 30 nm. However, no evidence of agglomeration has been observed in the electronic spectrum and Nanotracer dynamic light scattering (DLS) profiles (Figure 1), showing that TMT is a good stabilizing agent for AuNPs in an aqueous solution.

Originally, under the conditions of this work ( $\lambda_{\text{exc}} = 785$  nm), the direct observation of SERS effects for the TMT-stabilized AuNPs (TMT-AuNPs) was not feasible because the resonance at the plasmon band at 528 nm could not be accessed using the excitation wavelength of the available



**Figure 2.** SERS spectral profiles for a TMT-AuNP aqueous suspension (pH 4.4), recorded at several concentrations of  $\text{Hg}^{2+}$  ions.



**Figure 3.** Schematic illustration showing the TMT binding mode on the AuNP surface (a) and the proposed binding mode of (b)  $\text{Hg}^{2+}$  and (c)  $\text{Cd}^{2+}$  ions on the TMT-AuNP nanoprobe.

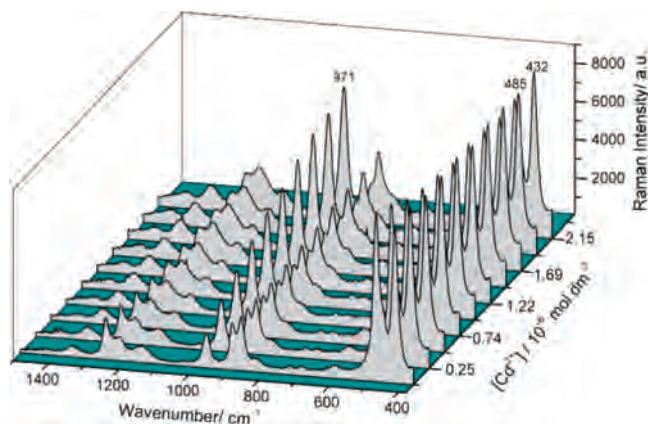
Raman spectrometer. However, in order to induce resonance, a good strategy is to change the ionic strength, e.g., by adding a small amount ( $<100 \mu\text{L}$ ) of a NaCl solution (1 mol  $\text{L}^{-1}$ ) into the TMT-AuNP solution (2.5 mL), just enough to start agglomeration. This process can be monitored by the rise of the characteristic plasmon coupling band around 700 nm and should remain stationary during the time of the experiments (1 h). This is an important point to be observed because if agglomeration proceeds in a high extent, precipitation can take place, decreasing the signals and increasing the noise.

In this way, strong, reproducible SERS signals have been obtained for the TMT-AuNPs (Figure 2), showing the characteristic TMT vibrational peaks modified by interaction with the AuNP surface.

The most strongly enhanced peaks can be associated with the vibrational modes exhibiting major contributions from the C-S and S-H groups, e.g., at 1259, 897, 872, 485, and 432  $\text{cm}^{-1}$ , except for the peaks at 1218 and 971  $\text{cm}^{-1}$ , which are associated with ring vibrations. This result is consistent with TMT bound to the AuNPs through the S and the heterocyclic N atoms. Because of the symmetry and close proximity of the thiol and nitrogen binding sites, the most probable mode of interaction of TMT with AuNP should involve a tridentate coordination (Figure 3a). In this way, the third S and two heterocyclic N atoms remain available for interaction with metal ions at the TMT/solution interface.

Because of its chemical nature, TMT-AuNP is expected to be selective for heavy metal ions, including the hazardous  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$  species. Such interaction cannot be detected from the electronic spectra and DLS profiles because the binding of metal ions prevents the occurrence of agglomera-

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**Figure 4.** SERS spectral profiles for a TMT-AuNP solution (pH 4.4), recorded at several concentrations of  $\text{Cd}^{2+}$  ions.

tion, because of their electric charges. For this reason, the addition of a very small amount (e.g., 10  $\mu\text{L}$ ) of a NaCl solution (1 mol  $\text{dm}^{-3}$ ) has been necessary in order to induce the formation of agglomerates, but just enough to produce stable, reproducible and intense SERS signals.

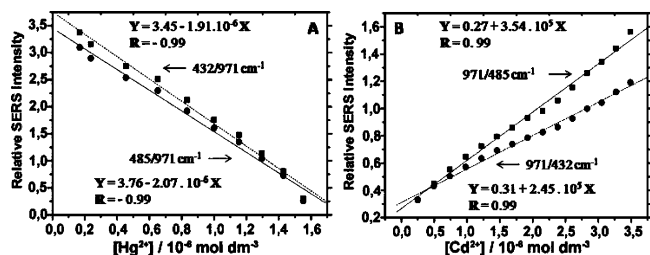
The SERS spectra recorded on an InPhotote portable instrument equipped with a Raman probe optical fiber and a 300 mW, 785 nm diode laser (350–2000  $\text{cm}^{-1}$  spectral range), after the addition of  $\text{Hg}^{2+}$ , at pH 4, are shown in Figure 2.

There is a systematic decrease of the  $\nu_{\text{C-S}}$  peaks at 485 and 432  $\text{cm}^{-1}$  upon the metal ion concentration increase in the range of  $2 \times 10^{-7}$ – $2 \times 10^{-6}$  mol  $\text{L}^{-1}$ , consistent with the strong binding of the  $\text{Hg}^{2+}$  ions to the thiol groups. Concomitantly, there is a gradual increase of the  $\beta_{\text{ring}}$  peak at 973  $\text{cm}^{-1}$ , suggesting the involvement of the heterocyclic N atom in a bidentate coordination mode to the  $\text{Hg}^{2+}$  ion, as illustrated in Figure 3b.

In contrast, the addition of  $\text{Cd}^{2+}$  ( $2.5 \times 10^{-7}$ – $3 \times 10^{-6}$  mol  $\text{L}^{-1}$  range) to the TMT-AuNP solution promotes strong enhancement of the ring vibrational peak at 971  $\text{cm}^{-1}$ , while the C–S stretching modes at 485 and 432  $\text{cm}^{-1}$  exhibited only a small decay as a function of the  $\text{Cd}^{2+}$  concentration, as shown in Figure 4.

Accordingly, this metal ion should be interacting primarily with the ring N atoms. However, a bidentate coordination seems also rather plausible because of the proximity of the S atom, as illustrated in Figure 3c, but involving a weaker  $\text{Cd}^{2+}$ –S bond.

Calibration curves can be readily obtained by plotting the peak intensities versus concentration. However, there is an



**Figure 5.** (A) Plots of the intensity ratios at 432/971 and 485/971  $\text{cm}^{-1}$  versus the concentration of the  $\text{Hg}^{2+}$  ions. (B) Plots of the intensity ratios at 971/485 and 971/432  $\text{cm}^{-1}$  versus the concentration of the  $\text{Cd}^{2+}$  ions.

interesting point to be noted in Figure 2, which is the multiple trends of the intensities of the peaks, such as those at 971 and 485 or 432  $\text{cm}^{-1}$  as a function of the  $\text{Hg}^{2+}$  concentration. In fact, on the basis of the linearity observed for the plots of the intensity ratios of the peaks at 432/971 and 485/971  $\text{cm}^{-1}$  versus the concentration of  $\text{Hg}^{2+}$  (Figure 5), one can propose a very convenient analytical strategy. Accordingly, the use of the relative peak intensity ratios is a straightforward procedure, eliminating most of the concerns on making absolute measurements under variable conditions, especially the need of introducing internal standards, as in most of the spectroscopic methods. The linear correlation was better than 0.99, and the slopes reflected a good accuracy and sensitivity for the analytical method, in the ppb range. Similar good analytical results have been obtained for  $\text{Cd}^{2+}$ , as can be seen in Figure 5.

In conclusion, the TMT-modified AuNPs exhibit strong SERS effects, yielding vibrational profiles very sensitive to the presence of heavy metal ions. This aspect allows one to extend the current application on the SERS detection of organic and biological substrates,<sup>1</sup> to the exploitation of the modified AuNPs as highly sensitive spectroscopic probes for substrates such as the heavy metal ions. Moreover, on the basis of the contrasting response observed for selected vibrational bands in the SERS profiles, a new quantitative analytical method for  $\text{Hg}^{2+}$  and  $\text{Cd}^{2+}$  ions has been devised, employing relative peak intensity ratios instead of using internal standards.

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**Supporting Information Available:** TMT IR and Raman vibrational spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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