Structural Characterization of 4-Aminothiophenol in Silver and Gold Colloids using Surface-Enhanced Raman Scattering

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Abstract: Surface-enhanced Raman scattering (SERS) spectroscopy was used for the structural characterization of 4-aminothiophenol molecules in silver and gold colloids. During the SERS experiment on silver nanoparticles, structural changes occurred in the molecules adsorbed on the metal surface. The observed changes were associated with the dimerization process of 4-aminothiophenol under the influence of laser radiation, leading to the formation of 4,4'-dimercaptoazobenzene. The use of excitation at 532 nm with radiation power that is quite low for traditional SERS measurements proved to be optimal for catalyzing the dimerization process, while using laser excitation at 785 nm less stimulated dimerization of the studied compound. Unlike silver, gold nanospheres proved to be less suitable substrates for catalyzing the dimerization process under the chosen experimental conditions. Unmodified, freshly prepared silver colloid proved to be an adequate substrate for stimulating the dimerization of 4-aminothiophenol during classical SERS measurements, even when an excitation radiation power of less than 5 mW was used.

Keywords: surface-enhanced Raman scattering, 4-aminothiophenol, dimerization, 4,4'-dimercaptoazobenzene, silver, gold, colloid.

INTRODUCTION

SURFACE-enhanced Raman scattering (SERS) spectroscopy is a vibrational technique that provides information about the structure of molecules located near or attached to a rough metal surface, called SERS substrate.[1] The most common metal substrates are silver or gold nanoparticles dispersed in colloidal suspensions or immobilized on a solid support.[1–6] The SERS effect can be explained by two mechanisms: an electromagnetic mechanism and a chemical mechanism. In electromagnetic mechanism, the amplification of the Raman radiation is the result of the excitation of localized surface plasmon resonance (LSPR), while in chemical enhancement, there is a charge transfer between metal and molecule electronic states.[1] Although the electromagnetic mechanism contributes the most to the overall scattering enhancement, the SERS effect is mostly a combination of both mechanisms, and therefore, inelastic scattering can be enhanced up to 10^{10} times compared to classical Raman spectroscopy. Thus, it is possible to detect micromolar but also significantly lower concentrations of analytes (even to the level of single-molecule detection) in aqueous solutions by choosing the appropriate metal substrate and measurement conditions for the SERS experiment.[3,7–9] Due to the fact that, under the influence of electromagnetic radiation, a local surface plasmon resonance occurs on the rough metal surface, it was found that metal nanostructures can also be used in photon-driven catalysis.[10–12] Thus, metal nanostructures can simultaneously act as enhancers of Raman scattering and catalysts as well. One example of photon-induced catalysis on a rough metal surface is the dimerization of 4-aminothiophenol (**4-ATP**) molecules[13,14] which are commonly used as probe molecules for testing the SERS potential of newly prepared metal substrates.[15–18] It was found that the SERS spectra of 4-aminothiophenol on solid SERS substrates contained new bands that were not observed in the Raman spectrum of solid 4-aminothiophenol. At first, such bands were attributed to new modes of vibration of analyte molecules due to the formation of a chemical bond between

the molecules and the metal surface, whereby the chemical enhancement significantly contributes to the overall scattering enhancement.[19–21] However, the following research indicated that the bands originated from a new compound, 4,4'-dimercaptoazobenzene (**DMAB**), which was the result of the dimerization of 4-aminothiophenol under the influence of laser radiation, where the metal substrate catalyzed the reaction.[22–24] According to available works, the dimerization process of 4-aminothiophenol was catalyzed by silver nanostructures, while this process was less detectable with gold.[25] Dimerization was mainly observed when solid metal substrates were used,[14,26] but it has also been found in colloidal suspensions after prior irradiation of 4-aminothiophenol molecules.[23] Also, the formation of dimers was noticed when more complex SERS substrates were used, in which increased SERS efficiency was the result of combining two metals during the substrate preparation.[23,24]

In this work, surface-enhanced Raman spectroscopy was used for detailed structural analysis of 4-aminothiophenol (Scheme 1.a) molecules in silver and gold colloidal suspensions. Since the **4-ATP** dimerization process was observed mostly on solid SERS substrates or when the sample was exposed to laser radiation before SERS measurements, our aim was to explore whether metal nanoparticles in a complex colloidal system can act as catalysts to promote the dimerization process of 4-aminothiophenol when the sample is irradiated with laser light only during the SERS experiment. To our knowledge, the chosen silver colloid has not yet been tested for catalysis of 4-aminothiophenol dimerization. For comparison, a gold colloidal suspension was also used as SERS substrate, since in some published work the 4-aminothiophenol dimerization was observed on gold substrate.[25–27] The colloidal spherical particles used in the analysis were very close in size and stabilized by anions (chlorides were used for silver nanoparticles and citrate anions for gold ones). The used metal colloids were not purified, concentrated, or treated in any way before the measurement. Spectra were recorded using two excitation wavelengths immediately after sample preparation, without prior incubation of the metal substrate and the investigated compound. Based on a detailed analysis of the bands in the SERS spectra, the ability of ordinary, freshly prepared

colloids to catalyze the dimerization reaction of **4-ATP** has been studied. In addition, the influence of the type of metal in the SERS substrate and how the energy and power of the excitation laser radiation affected the behavior of **4-ATP** molecules in the colloidal suspension was also researched.

EXPERIMENTAL

Chemicals and Solutions

Silver nitrate (Gram Mol), sodium hydroxide (Kemika), hydroxylamine hydrochloride (Kemika), gold(III) chloride trihydrate (Sigma Aldrich GmbH), trisodium citrate (Kemika), and methanol (Alkaloid) were of p.a. purity and used as received. 4-aminothiophenol was purchased from Sigma Aldrich GmbH. Water was purified by passage through Milli-Q (Millipore) deionization and filtration columns.

The main stock solution of **4-ATP** ($c = 1 \times 10^{-3}$ mol L^{-1}) was prepared by dissolving the appropriate amount of solid compound in methanol, while stock solutions for SERS measurements were prepared by dilution in methanol of the above-described main stock solution.

Colloids Preparations

All glassware used in the preparation of colloids was thoroughly cleaned with a detergent solution, then rinsed with 5% nitric acid and finally with water of Milli-Q purity.

Silver colloid was prepared according to the reduction method published by Leopold and Lendl.[28] Here, 17 mg of silver nitrate was dissolved in 90 mL of Milli-Q water. Also, 17 mg of hydroxylamine hydrochloride was dissolved in 10 mL of Milli-Q water, followed by adding 220 μL of sodium hydroxide solution ($c = 2$ mol L^{-1}). The alkaline solution of hydroxylamine hydrochloride was added to the silver nitrate solution under vigorous stirring, and the mixture was left to stir for another 10 minutes. The resulting colloidal suspension was grayish-yellow, characterized by an absorption maximum at 406 nm and a pH value of 7.67.

Gold colloidal nanoparticles were prepared according to modified Fren's method[29] where 50 mg of gold(III) chloride trihydrate was dissolved in 50 ml of Milli-Q water and brought to a boil under vigorous stirring in an oil bath under reflux. Then, 5.6 mL of trisodium citrate solution (1 % w/v) was added to the solution, and the mixture was left to boil for another 15 minutes. The resulting colloidal suspension was dark red in color, with an absorption maximum at 546 nm and a pH value of 3.37.

Samples Preparation

For the concentration-dependent SERS measurements, samples were prepared by mixing the silver or gold colloidal suspension (95 μL) with appropriate **4-ATP** methanol stock
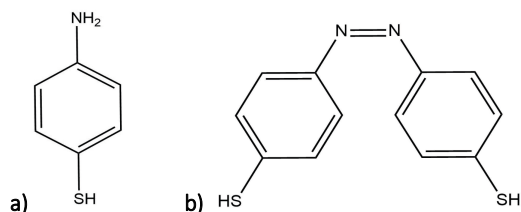
Scheme 1. Chemical structure of a) 4-aminothiophenol (**4-ATP**) and b) 4,4'-dimercaptoazobenzene (**DMAB**).

solutions (5 µL). The final concentrations of 4-aminothiophenol in working samples were 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5} and 1×10^{-4} mol L^{-1}. The share of methanol in measuring samples was $\varphi = 5$ %. For measuring the SERS spectra, 40 µL of the prepared mixture of **4-ATP** and colloid was placed in an aluminum pan, and spectra were recorded immediately after mixing the compounds. For recording blank spectra of colloidal solutions, the volume of analyte was replaced with a methanol solution.

Instrumentation

Raman and SERS spectra were measured on a Renishaw inVia Raman microscope equipped with lasers emitting at 532 and 785 nm. The Raman spectrum of the solid compound was recorded on an aluminum holder, while the SERS spectra of colloidal samples were recorded in aluminum pans (V = 40 µL, OD 5.4 mm × H 2.6 mm), using an ×5 microscopic objective (NA = 0.12). Exposure time was 10 s, and 3 accumulations were collected for a spectrum. The applied laser power depended on the measuring sample. Therefore, the Raman spectra of solid **4-ATP** were measured at laser powers of 1 mW (532 nm) and 15 mW (785 nm), respectively. SERS spectra in silver colloid were taken using 1 mW of a 532 nm laser and 3 mW of a 785 nm laser. For research with the gold colloids, the spectra were acquired at 50 mW when a 532 nm laser was used and at 3 mW using 785 nm laser excitation.

UV/Vis/NIR measurements of silver and gold colloids were performed on an Analytik Jena spectrometer (model SPECORD 200) using quartz cells of 2 mm path length.

A Mettler Toledo pH meter (model MP 220) with a Mettler Toledo InLab 413 combined glass calomel electrode was used for the pH measurement. The pH meter was calibrated with standard aqueous buffer solutions of pH 7.00 and 4.01.

RESULTS AND DISCUSSION

Raman Spectra of 4-Aminothiophenol

To obtain information about the structure of 4-aminothiophenol, Raman spectra of the solid sample and the methanol solution of **4-ATP** (c = 2 × 10^{-3} mol L^{-1}) were recorded using different laser wavelengths for excitation, namely 532 and 785 nm.

The Raman spectra of the solid 4-aminothiophenol were very similar, regardless of the excitation wavelength used, and several prominent bands characteristic of the vibrations of the investigated molecules were observed (Figures 1.a and 2.a). Therefore, in spectra recorded using laser emitting at 532 nm, the most intense band found at 1086 cm^{-1} was assigned to C–S stretching mode.[15,16,19] A

medium band attributed to aromatic ring CC stretching was located at 1594 cm^{-1}, while intensities of the bands at 636 and 466 cm^{-1} were the result of benzene ring CCC deformation. C–N stretching contributed to the band at 1288 cm^{-1}, while CH deformation gave rise to the band at 1176 cm^{-1}.[23] CC ring stretching and CH deformation contributed to the band at 1494 cm^{-1}. The weak band in low-wavenumbers region around 390 cm^{-1} was attributed to deformations of the C–S bond, while the band at 1005 cm^{-1} corresponds to deformations of the benzene ring backbone. Quite similar band positions for the described vibrations were found in the Raman spectrum of **4-ATP** obtained using a 785 nm laser for excitation. Detailed spectra analyses were shown in Table 1.

In the Raman spectra of the **4-ATP** solution obtained by excitation at 532 nm, the bands of the methanol solvent dominated, while the bands of the compound were mainly absent (Figure S1.a). Therefore, the spectrum of the **4-ATP** solution contained intense methanol bands at 2836 and 2945 cm^{-1} that originated from asymmetric and symmetric stretching of the C–H bonds of the methyl group, while a weak band at 1034 cm^{-1} was attributed to the stretching of the C–O bond from solvent molecules, and the one at 1456 cm^{-1} originated from the deformation of the CH$_3$ group.[30] Excitation at 532 nm is high-energy radiation and can stimulate the electronic transition process when irradiating a sample solution. This was manifested in the spectrum of **4-ATP** as the appearance of a very broad and intense band between 350 and 2500 cm^{-1}, which covered the SERS bands of the analyzed compound. Unlike the spectra obtained by excitation at 532 nm, the spectra at 785 nm showed several weak bands originating from the vibration of the **4-ATP** molecules. Laser radiation of a longer wavelength and lower energy does not cause electronic transitions; therefore, bands of the investigated compound were observed in the Raman spectrum (Figure S1.b). The bands at 1592 and 1082 cm^{-1} were attributed to CC stretching of aromatic unit and C–S stretching, respectively, while the one at 392 cm^{-1} originated from CS deformation. Apparently, the chosen concentration of 4-aminothiophenol stock solution was too low to obtain more characteristic bands in the Raman spectrum of the analyte solution.

SERS Spectra of 4-Aminothiophenol on Silver Colloidal Nanoparticles

To study in detail the changes that occur when laser radiation illuminates the 4-aminothiophenol molecules in metal colloidal suspensions, concentration-dependent SERS spectra of the **4-ATP** were recorded in silver and gold colloids using excitation wavelengths at 532 and 785 nm, respectively.

Using a silver substrate and laser emitting at 532 nm resulted in SERS spectra of 4-aminothiophenol with intense

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Raman bands, pointing to successful adsorption of analyte molecules on the metal surface. Compared to the Raman spectrum of a solid compound, in the SERS spectrum of the highest studied concentration of **4-ATP** ($c = 1 \times 10^{-4}$ mol L^{-1}), several new bands were observed (Figure 1). Therefore, the most intense band in the SERS spectrum matched the most prominent one in the Raman spectrum of the studied compound and originated from stretching vibrations of the C–S bond at 1078 cm^{-1}. In the SERS spectrum of **4-ATP,** there was also a medium band attributed to aromatic ring CC stretching around 1596 cm^{-1}, while a weak band associated with aromatic CC stretching and CH bending was found at 1490 cm^{-1}. The band corresponding to CH deformation settled at 1182 cm^{-1}. The appearance of new intense bands in the SERS spectrum, found at 1432, 1389, and 1143 cm^{-1}, indicated the changes in structure of molecules located on the metal surface. From previous studies, it is known that the observed bands originated from 4,4'-dimercaptoazobenzene (**DMAB**) (Scheme 1.b), a dimer of **4-ATP** molecules formed under the influence of laser radiation, where metal substrates catalyzed the dimerization process.[22,23,26] The exact mechanism behind the **4-ATP** dimerization has not yet been fully revealed because of the complexity of processes at the metal molecule interface, which are the subject of many studies.[31] According to some research, **4-ATP** dimerization is a plasmon-mediated catalytic reaction in which O$_2^-$ radicals are formed from the oxygen molecules adsorbed on the metal surface after transfer of plasmon-excited hot electrons to O$_2$ molecules.[23,32] The radicals participate in the oxidation process of **4-ATP,** whereby **DMAB** is formed. Another study

suggests that the dimerization process is induced by energy transfer from surface plasmon resonance to **4-ATP**.[33] In this mechanism, O$_2$ is essential and acts as an electron acceptor, while water molecules can accelerate the reaction and were used for deprotonation of intermediates. During the process, the –NH$_2^+$ radical cation is formed, which dimerizes into an intermediate, and further oxidation leads to the formation of **DMAB** molecules. Regardless of the dimerization mechanism for 4-aminothiophenol, monomeric molecules are connected via amino groups in dimer molecules, and therefore the stretching of the bond –N=N– resulted in bands at 1389 and 1432 cm^{-1} in the SERS spectrum.[34–36] The new band found at 1143 cm^{-1}, attributed to CN stretching and CH group deformation, indicated changes in the placement of molecules on the metal surface due to dimerization.[14] This is also supported by the appearance of a band at 1577 cm^{-1} that originated from ring CC stretching and CH deformation modes. According to the literature, the dimerization of 4-aminothiophenol was primarily detected on solid SERS substrates[14,24,34–36], while in this work it was observed in a freshly prepared, unmodified, widely used colloidal suspension using laser power of just 1 mW.

The decrease in the concentration of 4-aminothiophenol molecules in the measuring samples resulted in differences in SERS band intensities, while no new bands in the spectra were observed. Therefore, the SERS spectra of 4-aminothiophenol solutions showed a nonlinear dependence of band intensity on the concentration of the studied compound. If compared to the normal Raman spectrum, in the SERS spectrum of **4-ATP,** there was an increase in the intensity of the bands at 1078 and 389 cm^{-1}, which originated from C–S bond stretching and CS deformation vibration, respectively. Noticed changes pointed to the adsorption of molecules on the silver nanoparticles where the thiol group was the closest to the metal surface. The sample with the most intense bands in SERS spectra was the one containing **4-ATP** at a concentration of 5×10^{-6} mol L^{-1}. Figure 1 clearly shows that lowering the compound concentration down to 5×10^{-6} mol L^{-1} resulted with increase of the band intensities, particularly the ones originated from vibrational modes of **DMAB** structural moieties. Therefore, dimer bands were amplified compared to bands corresponding to vibrations of the thiol group and aromatic unit. For example, the intensity ratio of the band from stretching vibrations of the C–S bond (1078 cm^{-1}) and the dimer bands from CH deformation (1143 cm^{-1}) and NN stretching (1389 and 1432 cm^{-1}) were as follows: $r(I_{1143}/I_{1078}) = 0.79$, $r(I_{1389}/I_{1078}) = 0.93$, and $r(I_{1432}/I_{1078}) = 1.04$ in the sample with the highest concentration of 4-aminothiophenol, while the intensity ratio of the same bands in spectrum at a concentration of 5×10^{-6} mol/L was: $r(I_{1143}/I_{1075}) = 1.14$, $r(I_{1388}/I_{1075}) = 1.21$, and $r(I_{1434}/I_{1075}) = 1.60$. The observed
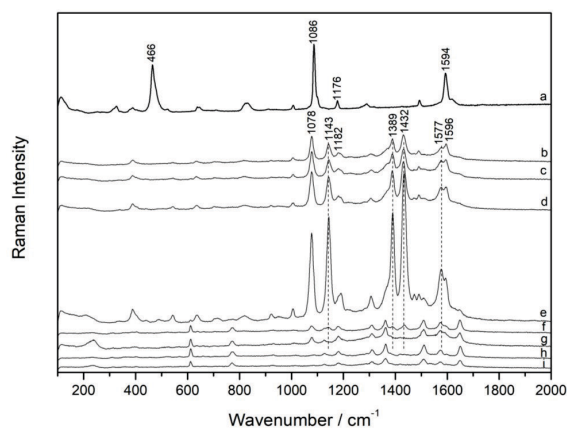

Figure 1. (a) The Raman spectrum of solid **4-ATP** and the SERS spectra of (b) c(**4-ATP**) = 1×10^{-4} mol L^{-1}, (c) c(**4-ATP**) = 5×10^{-5} mol L^{-1}, (d) c(**4-ATP**) = 1×10^{-5} mol L^{-1}, (e) c(**4-ATP**) = 5×10^{-6} mol L^{-1}, (f) c(**4-ATP**) = 1×10^{-6} mol L^{-1}, (g) c(**4-ATP**) = 5×10^{-7} mol L^{-1}, (h) c(**4-ATP**) = 1×10^{-7} mol L^{-1}, and i) methanol in silver colloid. Excitation at 532 nm. The spectra are displaced for visual clarity.

differences in band intensity ratio could be explained either by a change in the orientation of the dimer molecules on the silver surface by reducing the concentration of analyte molecules where **DMAB** molecules could take optimal perpendicular position toward the silver surface or by the fact that at a lower concentration there was a reduced number of **4-ATP** molecules, which could more optimally orient for a possible dimerization process, and therefore a larger number of dimer molecules was present in suspension. Decreasing the concentration of **4-ATP** molecules in measuring samples also resulted in the intensification of anomalous bands from the colloidal suspension at 612, 772, 1310, 1510, and 1649 cm⁻¹, which originated from species formed during the metal reduction.[37,38] The detection limit for the observed metal substrate in the chosen measurement conditions was 1×10^{-6} mol L⁻¹, where **4-ATP** bands were still visible in the SERS spectrum.

SERS spectra of 4-aminothiophenol solutions obtained by excitation at 785 nm (Figure 2) revealed less pronounced changes compared to the Raman spectrum of the solid, in contrast to the previously described spectra obtained by excitation at 532 nm. In the SERS spectra of **4-ATP** solutions obtained by excitation at 785 nm, bands specific for 4-aminothiophenol originated from CS and CC stretching vibrations appeared at 1079 and 1594 cm⁻¹, respectively (Table 1). The intensity of the mentioned bands was significantly higher than the intensity from the **DMAB** dimer bands found at 1144, 1390, and 1435 cm⁻¹. It can be assumed that using a laser with a longer wavelength, therefore with less radiation energy, resulted in the dimerization of fewer **4-ATP** molecules. Thus, on the silver surface, there were dimer and monomer molecules of 4-aminothiophenol, and the resulting spectrum was probably a combination of dimer and monomer bands. This was supported by the previously mentioned fact that monomer bands are more intense in the SERS spectrum of **4-ATP** than bands corresponding to dimer vibration modes, and, also, the lack of dimer vibration mode around 1580 cm⁻¹

Table 1. Assignment of the relevant vibrational bands in the Raman and SERS spectra of 4-aminothiophenol.

	Wavenumbers / cm⁻¹							
	532 nm			785 nm				
		SERS (AgNP)				SERS (AgNP)		SERS (AuNP)		Assignment
Raman	1×10^{-4} mol L⁻¹	5×10^{-6} mol L⁻¹	Raman	1×10^{-4} mol L⁻¹	5×10^{-6} mol L⁻¹	1×10^{-4} mol L⁻¹	5×10^{-6} mol L⁻¹	
1618			1620					δ NH₂
1594	1596	1593	1595	1594	1593	1587	1582	v CC
	1577	1574						v CC
						1522	1517	v CC + δ CH
1494	1490	1490	1493	1492	1492	1489	1488	v CC + δ CH
		1472						δ CH + v NN
	1432	1434		1435w	1433			v NN
	1389	1388		1390w	1392			v NN
1288			1289					v C–N
						1228	1232	δ CH
1176	1182	1190	1179	1180	1183	1177	1176	δ CH
	1143	1143		1144w	1145			δ CH + v C–N
1086	1078	1075	1089	1079	1079	1080	1078	v C–S
1005	1006	1001	1007	1006	1006	1007	1007	δ CC
828	816	817	834	817w	813	821	811	δ CH
636	633	637	637	638	639	638	643	δ CCC
466			466					δ CCC
390	389	385	380	391	392	391	393	δ CS
						237w	250	v Au–O, v Au–S
				228	228			v Ag–S

supports the thesis that monomers were also present on the silver surface after the sample was irradiated with a laser at 785 nm.[13] A band of medium intensity at 228 cm^{-1}, originating from the stretching of the bond between the sulfur atoms of the **4-ATP** molecule and the silver surface, was also observed in the spectrum. The very intense band at 391 cm^{-1} originated from the CS deformation vibration and indicated the orientation of the analyte molecules towards the nanoparticle in which the thiol part of the structure was very close to the metal surface. The intensity of bands in the SERS spectra obtained by excitation at 785 nm showed a nonlinear increase while the concentration of compound decreased, and the highest intensity was observed for the concentration sample of 5×10^{-6} mol L^{-1} (Figure 2). Lowering the analyte concentration in the SERS sample resulted in more intense bands, very likely due to the optimal rearrangement of analyte molecules on the metal surface. The detection limit was found to be 1×10^{-7} mol L^{-1}, as was also determined with 532 nm excitation.

SERS Spectra of 4-Aminothiophenol on Gold Colloidal Nanoparticles

Concentration-dependent SERS spectra of the **4-ATP** molecule in the prepared gold colloidal suspension were recorded at excitation wavelengths of 532 nm and 785 nm, as well.

The SERS spectra obtained by excitation at 532 nm contained just a few weak bands corresponding to the vibrations of the **4-ATP** model molecule (Figure 3). The
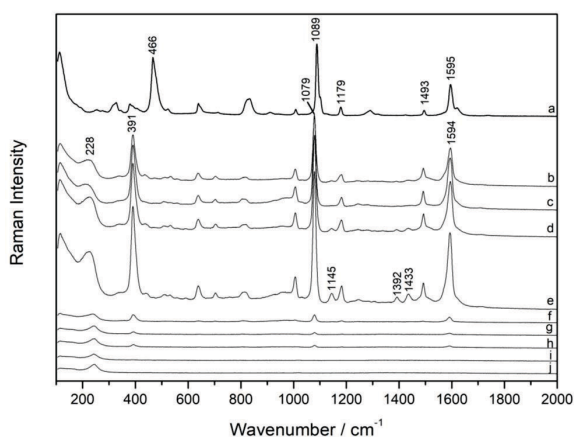

Figure 2. (a) The Raman spectrum of solid **4-ATP** and the SERS spectra of (b) c(**4-ATP**) = 1×10^{-4} mol L^{-1}, (c) c(**4-ATP**) = 5×10^{-5} mol L^{-1}, (d) c(**4-ATP**) = 1×10^{-5} mol L^{-1}, (e) c(**4-ATP**) = 5×10^{-6} mol L^{-1}, (f) (f) c(**4-ATP**) = 1×10^{-6} mol L^{-1}, (g) c(**4-ATP**) = 5×10^{-7} mol L^{-1}, (h) c(**4-ATP**) = 1×10^{-7} mol L^{-1}, (i) c(**4-ATP**) = 5×10^{-8} mol L^{-1}, and (j) methanol in silver colloid. Excitation at 785 nm. The spectra are displaced for visual clarity.

bands from C–S and C–C bond stretching were observed at 1080 and 1587 cm^{-1}, respectively. A broad band noticed at 1177 cm^{-1} originated from deformations of CH bonds. The weak **4-ATP** spectra obtained in the gold colloid upon 532 nm radiation might be due to the fact that the strongest enhancement of scattering occurs when the laser excitation wavelength is red shifted in regard to the local surface plasmon resonance of the metal substrate, which was not the case for the here used gold colloid, which had the plasmon resonance at 546 nm.[39,40] In the SERS spectra of 4-aminothiophenol solutions, there was an anomalous band at 1018 cm^{-1}, that was observed in pure colloidal gold suspension as well. The characteristic bands of methanol at 1018, 2484, and 2956 cm^{-1} were also visible, although the methanol volume share in the system was only 5 %. The highest enhancement of Raman scattering was recorded in the sample with the highest concentration, c = 1×10^{-4} mol L^{-1}, while the concentration, c = 1×10^{-5} mol L^{-1}, was the last one in the series whose SERS spectrum showed characteristic bands of 4-aminothiophenol and was considered as the limit of detection for the used metal substrate in the chosen measurement conditions.

In the concentration-dependent SERS spectra of **4-ATP** in gold colloid (Figure 4) obtained with laser emission at 785 nm, very sharp and intense bands of the analyte were observed, where the stretching bands of C–S and C–C bonds at 1080 and 1587 cm^{-1} stand out the most. There were also very clearly visible bands corresponding to other 4-aminothiophenol vibration modes, such as the ones corresponding to CH deformations at 1177 and 1489 cm^{-1} and CCC ring deformation at 638 cm^{-1} (Table 1). The medium-intense band at 391 cm^{-1}, originating from CS
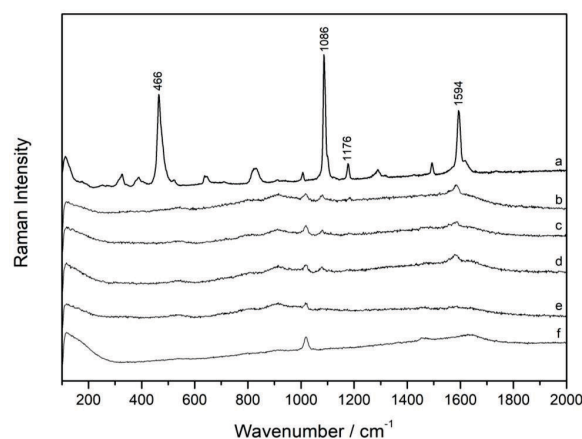

Figure 3. (a) The Raman spectrum of solid **4-ATP** and the SERS spectra of (b) c(**4-ATP**) = 1×10^{-4} mol L^{-1}, (c) c(**4-ATP**) = 5×10^{-5} mol L^{-1}, (d) c(**4-ATP**) = 1×10^{-5} mol L^{-1}, (e) c(**4-ATP**) = 5×10^{-6} mol L^{-1}, and (f) methanol in gold colloid. Excitation at 532 nm. The spectra are displaced for visual clarity.

deformation, referred to the binding of analyte molecules via the sulfur of the thiol group to the gold surface. The characteristic bands of **DMAB** at 1148, 1390, and 1440 cm^{-1} were not observed in the spectrum, indicating that dimerization of **4-ATP** molecules on the used gold surface did not occur. It can be assumed that the combination of gold colloid and excitation at 785 nm was not suitable for stimulating the chemical transformation of 4-aminothiophenol into 4,4'-dimercaptoazobenzene. The broad bands that appeared in the SERS spectra at 1384, 1323, and 1229 cm^{-1} could also be found in the Raman spectrum of colloid without **4-ATP** and most likely originated from species that remained in solution after nanoparticles synthesis.[37,38] Decreasing the concentration of analyte molecules in the SERS measuring samples resulted in an intensity change of individual bands in the spectra, pointing to a different orientation of the molecules with respect to the metal substrate at lower concentrations. The Raman spectrum of blank gold colloid contained a band around 250 cm^{-1}, which was attributed to stretching of the gold-oxygen bond from the citrate ions that surround and stabilize the gold nanoparticles. In SERS spectra, the band was found at 253 cm^{-1} and was much more intense and broader compared to one in the Raman spectrum of colloidal solution. It can be assumed that the stretching of the Au–S bond also contributes to the observed band intensity and that the thiol part of the **4-ATP** molecule was chemically bonded with the gold surface.[41] The detection limit for the observed gold substrate, while using 785 nm as the excitation wavelength, was c(**4-ATP**) = 5 × 10^{-7} mol L^{-1} in the described measurement conditions.
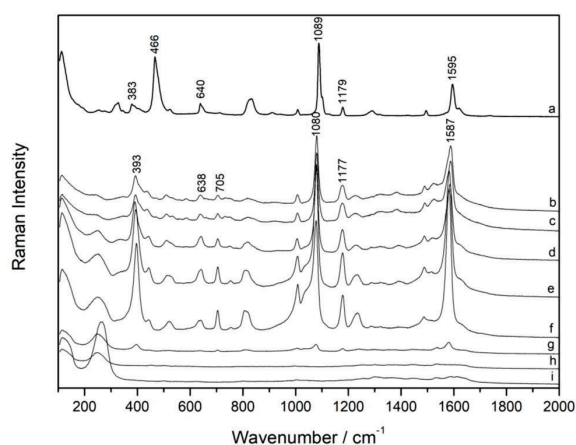

Figure 4. (a) The Raman spectrum of solid **4-ATP** and the SERS spectra of (b) c(**4-ATP**) = 1 × 10^{-4} mol L^{-1}, (c) c(**4-ATP**) = 5 × 10^{-5} mol L^{-1}, (d) c(**4-ATP**) = 1 × 10^{-5} mol L^{-1}, (e) c(**4-ATP**) = 5 × 10^{-6} mol L^{-1}, and (f) methanol in gold colloid. Excitation at 532 nm. The spectra are displaced for visual clarity.

CONCLUSIONS

In this work, a detailed structural analysis of 4-aminothiophenol in silver and gold colloidal suspensions was performed by surface-enhanced Raman scattering using laser excitation at 532 and 785 nm. When using silver colloid as SERS substrate, the spectra of **4-ATP** molecules, taken at both excitation wavelengths, contained bands that indicated successful adsorption of the analyte on the metal surface via the thiol group of 4-aminothiophenol molecules. Excitation at 532 nm resulted in intense bands from the 4,4'-dimercaptoazobenzene vibrations, proving that dimers of 4-aminothiophenol had been formed by the catalytic reaction on the silver surface of freshly prepared colloidal nanoparticles under the given measurement conditions. The mentioned bands were far less intense and poorly noticeable in spectra at 785 nm, which implied a strong influence of the excitation wavelength on the dimerization process of 4-aminothiophenol.

Concentration-dependent SERS spectra of 4-aminothiophenol in colloidal gold suspensions showed good enhancement of Raman scattering at 785 nm excitation, while using excitation at 532 nm, the enhancement was almost entirely absent due to the nature of the used metal substrate. The spectra of **4-ATP** molecules recorded in gold colloid were characterized by the absence of dimer bands, which were observed when silver nanoparticles were used, thus confirming the dependence of catalytic dimerization of 4-aminothiophenol molecules on the type of metal in the SERS substrate.

Here, it was shown that even the most commonly used, untreated colloidal silver nanoparticles could catalyze the dimerization reaction of 4-aminothiophenol, regardless of the complex composition of the used colloidal suspension. Therefore, the classical way of measuring SERS spectra in plain silver colloid, using excitation radiation power less than 5 mW, proved to be sufficiently stimulating for the dimerization process of 4-aminothiophenol molecules.

Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca4107.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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