

Double-layered Ag / Au electrode for SERS spectroscopy: preparation and application for adsorption studies of chromophoric compounds

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The method for preparation of SERS-active Ag electrodes plated with a thin layer of gold has been elaborated by using the electroless deposition technique based on the reduction of $\text{Na}_3\text{Au}(\text{SO}_3)_2$ to metallic Au by formaldehyde. The electrodes were used for taking Raman spectra of the SAMs formed from 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone, and for the adsorbed hemin and protein alcohol dehydrogenase. The possibility to obtain surface enhancement from the lower layer (Ag) and, thereby SERRS spectra of the chromophoric compounds, have been shown. These double-layered electrodes are especially suitable for studying the structure of chromophoric cofactors of proteins.

Key words: double-layered Ag / Au electrode, SERRS spectroscopy, 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone, hemin, alcohol dehydrogenase

INTRODUCTION

Surface-enhanced Raman scattering (SERS) spectroscopy is a powerful analytical tool for obtaining information on the structure of species adsorbed on metallic substrates [1]. In general, the SERS effect is observed on roughened surfaces or colloidal Ag, Au or Cu nanoparticles. In order to achieve a strong enhancement of Raman signal from these metals, different excitation wavelengths (λ_{ex}) should be employed. Thus, SERS spectra from Au and Cu are usually excited with a radiation longer than 600 nm, whereas spectra from Ag can be observed when shorter excitation wavelengths are used. When λ_{ex} coincides with the absorption band of the adsorbate, an enormous enhancement of the signal takes place because of additional resonant enhancement yielding the surface-enhanced resonance Raman scattering (SERRS) effect. SERRS spectroscopy is particularly valuable for the studies of chromophoric groups in complex compounds, e.g., proteins, containing chromophoric cofactors [2, 3]. In this case, only the vibrations of a chromophoric group are probed without a perturbation by the vibrations of the protein backbone. However, electronic absorption bands of the cofactors are

usually in a region shorter than 600 nm. Therefore, for the studies of such chromophoric compounds adsorbed on Au it would be desirable to use λ_{ex} appropriate for Ag. In that case, both the surface enhancement effect and the electronic resonance enhancement effect would be explored. One can expect to solve this problem by using SERS-active Ag electrodes covered with a thin layer of Au. Earlier, Weaver's group at Purdue University has demonstrated the possibility to obtain high quality SERS spectra from a thin layer of Pt-group transition metals electrodeposited onto a roughened gold substrate [4]. On the other hand, it has been found recently [5] that bimetallic Au / Ag nanoparticles composed of Au core and Ag shell exhibit a very strong enhancement effect.

Considering the above, in this work we have explored the possibility to obtain double-layered Ag / Au electrodes for the SERRS studies of adsorbed chromophoric compounds with electronic absorption band in the vicinity of 532 nm.

EXPERIMENTAL

The Millipore purified ($18.3 \text{ m}\Omega \cdot \text{cm}$) water was used throughout all the experiments. Perchloric acid, other inorganic acids and salts (ASC reagent grade), organic solvents and hemin (bo-

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vine, crystalline) were purchased from Sigma-Aldrich Chemie GmbH and used without further purification. The solution of $\text{Na}_3\text{Au}(\text{SO}_3)_2$ (0.081 M) and Na_2SO_3 (0.4 M) was kindly supplied by Dr. Benjaminas Šebeka (Institute of Chemistry, Vilnius), 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone (SNQ) was synthesized by the previously described procedure [6].

Quinohemoprotein alcohol dehydrogenase IIG from *Pseudomonas putida* HK5 (ADH) was isolated from *Pseudomonas putida* HK5. The cells were grown at 30 °C in 750 ml shaking flasks containing 200 ml of the basal medium supplemented with 1,2-propanediol (0.5%) to an early stationary phase [7]. The enzyme was isolated and purified employing the method published previously [8].

Pre-treatment of Au and Ag electrodes was performed according to the procedures described previously [9, 10]. Au and Ag electrodes were polished with 5 μm alumina slurry, rinsed with water and sonicated in water twice for 10 min. Then the Au electrode was electrochemically cleaned by potential cycling in 0.1 M H_2SO_4 between 0.4 and 1.6 V vs saturated sodium calomel electrode (SSCE) at potential scan rate 0.1 V/s for 12 min (30 cycles), and roughened for the SERS measurements by cycling in 0.1 M KCl between -0.3 and 1.35 V vs Ag / AgCl at 0.3 V/s scan rate for 9 min (50 cycles). Roughening of the Ag electrode was performed by cycling in 0.1 M KCl between -0.3 and 0.3 V vs Ag / AgCl at 0.01 V/s for 20 min (10 cycles).

The roughened Ag electrode was plated with a layer of Au by keeping it immersed in the plating solution in a refrigerator (at about 5 °C) for the appropriate time. The solution was prepared just before plating from the above mentioned concentrated solution of $\text{Na}_3\text{Au}(\text{SO}_3)_2$ (0.081 M) and Na_2SO_3 (0.4 M) by mixing 100 μl of the cold concentrate, 100 μl of Na_2SO_3 solution (0.87 M), and 100 μl of formaldehyde (18–20%) and diluting the mixture with 700 μl of cold water. The final composition of the plating solution was: 8.1 mM of $\text{Na}_3\text{Au}(\text{SO}_3)_2$, 0.127 M of Na_2SO_3 and about 0.63 M of formaldehyde. The obtained Ag electrode plated with a thin layer of Au (Ag / Au electrode) was rinsed with water and used for electrochemical or SERS experiments.

The process of Au layer formation and the state of the electrode was monitored by cyclic voltammograms (CV) of underpotential deposition (UPD) of Tl and Pb. These CV measurements were performed in deaerated solutions of 0.1 M HClO_4 or 0.1 M of NaClO_4 containing 5 mM of TlNO_3 or in a deaerated solution of 0.1 HClO_4 containing 10 mM of $\text{Pb}(\text{CH}_3\text{COO})_2$ under argon atmosphere at 25 °C. The electrode potential was scanned between 0.3 and -0.7 V (Tl) or 0 and -0.45 V (Pb) vs SSCE at a rate of 0.1 V/s. The sixth cycle was analyzed.

Self-assembled monolayers (SAMs) of SNQ on Au, Ag and Au / Ag electrodes were formed by incubating the electrodes in 1 mM solution of this compound in ethanol for 14–16 h. After the incubation, the electrodes were thoroughly washed with 95% ethanol followed by water and immediately transferred to the working solution.

The adsorption of hemin and ADH on Ag or Ag / Au electrodes was carried out in a spectroelectrochemical cell by immersing the electrodes into 20 mM phosphate buffer (PB) solution, pH 7.5, containing 10^{-7} M of these adsorbates.

The electrochemical measurements were carried out on an EG & G Versastat computerized potentiostat system (Princeton

Applied Research, Princeton (NJ), U.S.A.) in three-electrode cells. A platinum wire served as an auxiliary electrode, SSCE or Ag / AgCl electrode as the reference electrode, and polycrystalline Au or Ag disc electrodes as the working electrodes.

Absorption spectra of SNQ, hemin and ADH were recorded on a Perkin-Elmer UV-VIS spectrophotometer Lambda 35.

Raman spectroscopic measurements were carried out with a Perkin Elmer Model Spectrum GX FT-Raman spectrometer equipped with an Nd-YAG laser providing the emission wavelength of 1064 nm. The spectral resolution was set at 4 cm^{-1} . In order to enhance the signal-to-noise ratio, 200 scans were co-added. The SERS measurements were performed in a spectroelectrochemical cell equipped with the working electrode (Au, Ag or Ag / Au), Ag / AgCl reference electrode and the auxiliary Pt wire electrode. In order to reduce photo- and thermal effects, the cell with the electrodes was moved linearly in respect of the laser beam with a rate of 15–20 mm/s [11].

In order to obtain the SERRS spectra excited with 532 nm radiation, a dispersive Raman spectrometer was used. The measurements were carried out in 90° geometry. The 532 nm beam of a diode pumped solid-state laser (Viasho Technology Co. Ltd.) was used as the excitation source. The incidence angle of the laser beam was 60° and the laser power at the sample was typically 30–50 mW. The Raman scattering light was analyzed with a 400 mm focal length, f/2.5 aperture ratio spectrograph equipped with 1200 lines/mm grating, and recorded by a thermoelectrically cooled (-70 °C) CCD camera (Princeton Instruments, Model: Spec-10:256E). A long-wave-pass-edge filter (Semrock, Inc.) was placed in front of the entrance slit of the spectrograph to eliminate the Rayleigh scattering from the sample. The Raman frequencies were calibrated using the toluene spectrum. The integration time was 1 second. Each spectrum was recorded by an accumulation of 200 scans.

RESULTS AND DISCUSSION

Preparation of Ag electrode plated with thin layer of Au

The double-layered Ag / Au SERS electrode should meet the following requirements: the layer of Au must be as thin as possible, the coating has to be compact, and the electrode should remain sufficiently rough to support the surface enhancement of Raman scattering. Therefore, the Ag electrode was firstly roughened according to the known procedure [9], and subsequently, the thin Au layer was deposited. For this purpose the procedure of electrodeless plating suitable for Au coating on the surfaces of glass or polymeric material was employed [12]. The SERS-active Ag electrode was immersed into the solution containing 8.1 mM of $\text{Na}_3\text{Au}(\text{SO}_3)_2$, 0.127 M Na_2SO_3 and about 0.63 M of formaldehyde, and the reaction was carried out at a temperature of 3–5 °C. In searching for convenient methods to monitor the formation of the Au layer, CV of UPD of thallium and lead has been explored [13–16]. It was established that the most expressed difference between the Au and Ag electrodes corresponds to CV of UPD of Tl from acidic (0.1 M HClO_4 and 5 mM TlNO_3) electrolyte. It should be noted that CV of UPD of Pb from 0.1 M HClO_4 and 10 mM $\text{Pb}(\text{CH}_3\text{COO})_2$ electrolyte can also be used.

Considering the shape of the CV upon the plating time, one could see that both anodic and cathodic positive waves of Tl UPD

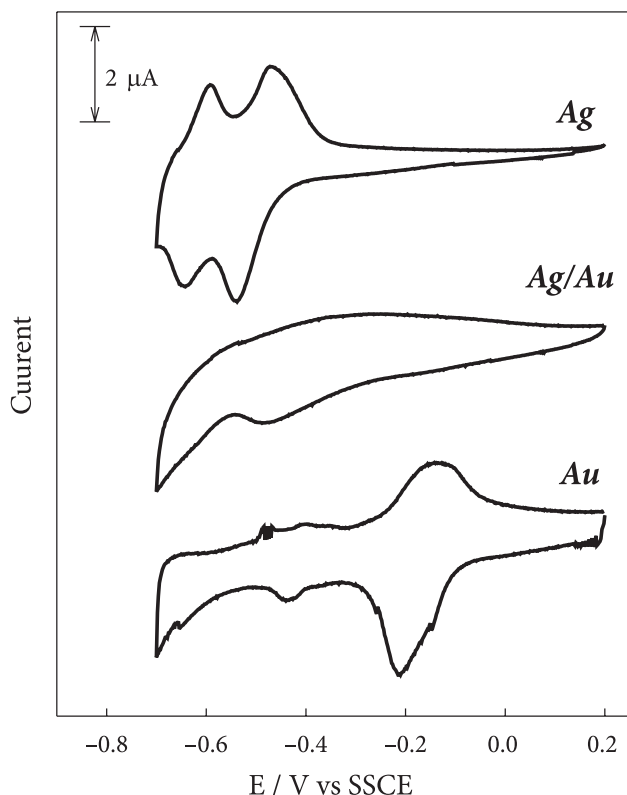


Fig. 1. Cyclic voltammograms of thallium underpotential deposition (0.1 M HClO₄ and 5 mM TlNO₃ electrolyte, potential scan rate 100 mV/s) of net Ag, double-layered Ag / Au electrode after 4 h of electroless deposition in a solution containing 8.1 mM of Na₃Au(SO₃)₂, 0.127 M Na₂SO₃ and about 0.63 M of formaldehyde, and net Au electrodes

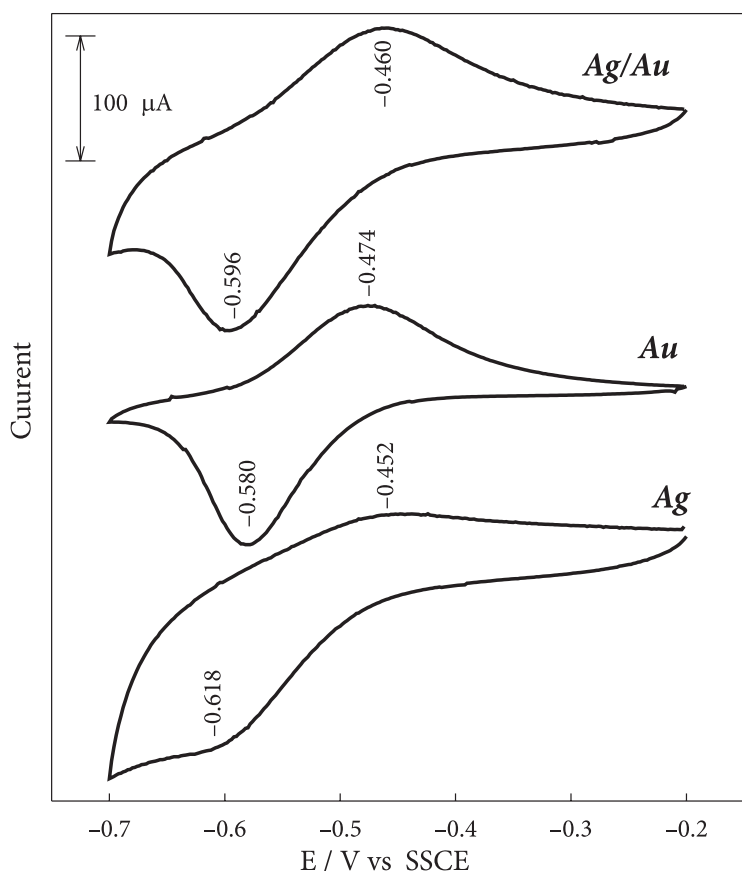


Fig. 2. Cyclic voltammograms of oxidation–reduction of SAMs obtained from 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone of net Ag, double-layered Ag / Au and net Au electrodes (0.1 M Na₂SO₄ and 0.01 M PB, pH 7; potential scan rate 100 mV/s)

on Ag completely disappeared after 4 h (Fig. 1), and the CV form broadened in the region of the expected peaks due to the Au surface. By using the UPD of Pb, after 4 hours of plating, the waves of Au became visible (data not shown). Therefore, this plating time was selected for the preparation of SERS-active Ag / Au electrodes used in the subsequent spectroscopic studies.

We have examined on the SAMs of SNQ and on the adsorbed hemin and ADH the possibility to use the double-layered electrodes for the studies of SERRS by employing 532 nm excitation. The data were compared to the SERRS spectra from individual Ag and Au electrodes and non-resonant SERS data obtained with 1064 nm excitation.

The use of the Ag / Au electrode for the SERRS study of chromophoric compounds

SERS and SERRS spectra of SAMs obtained from 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone

As we have shown previously [6], SNQ is a redox-active compound that is able to form stable and compact SAM on Au surface. The CVs of the redox process of the SAMs obtained from SNQ on Au, Ag / Au, and Ag electrodes are given in Fig. 2, and clearly demonstrate the similarity of Ag / Au and Au electrodes. The determined formal redox potential was found to be -0.530 V vs SSCE.

As one could expect, SERS spectra of SNQ SAMs obtained with the 1064 nm excitation, strongly depend on the electrode potential and only slightly on the nature of the metal (Fig. 3). The disappearance of high frequency peak at 1675 cm⁻¹ characteristic of C=O stretching in quinone ring [17] and the development of new bands located at 1380 and 719 cm⁻¹ characteristic of a substituted naphthalene ring [18] unambiguously indicate that SNQ adsorbed on all the three studied electrodes transforms to the reduced form, i. e. 2-[(2'-sulfanylethyl)amino]-1,4-dihydroxynaphthalene. SERS spectra of the oxidized and reduced forms of the SAM from Au and Ag electrodes are quite similar, except the fact that in the SERS spectrum from the Ag electrode, an additional band at 1595 cm⁻¹ arising from C–C stretching vibration of NQ ring was present. Based on the position of the band, we suggest that the formation of the SNQ monolayer on the Ag surface results in a strengthening of the interaction between NQN groups. This may result from the decreased distance between the molecules of the adsorbate due to a smaller radius of the Ag atoms as compared to Au. The other important difference between the SERS spectra of Au and Ag electrodes was observed in the 200 – 300 cm⁻¹ spectral region, where the bands due to Au–S (at about 280 cm⁻¹) and Ag–S (at 213 – 220 cm⁻¹) stretching modes were detected [19]. The spectra observed from the Ag / Au and Au electrodes are fairly alike.

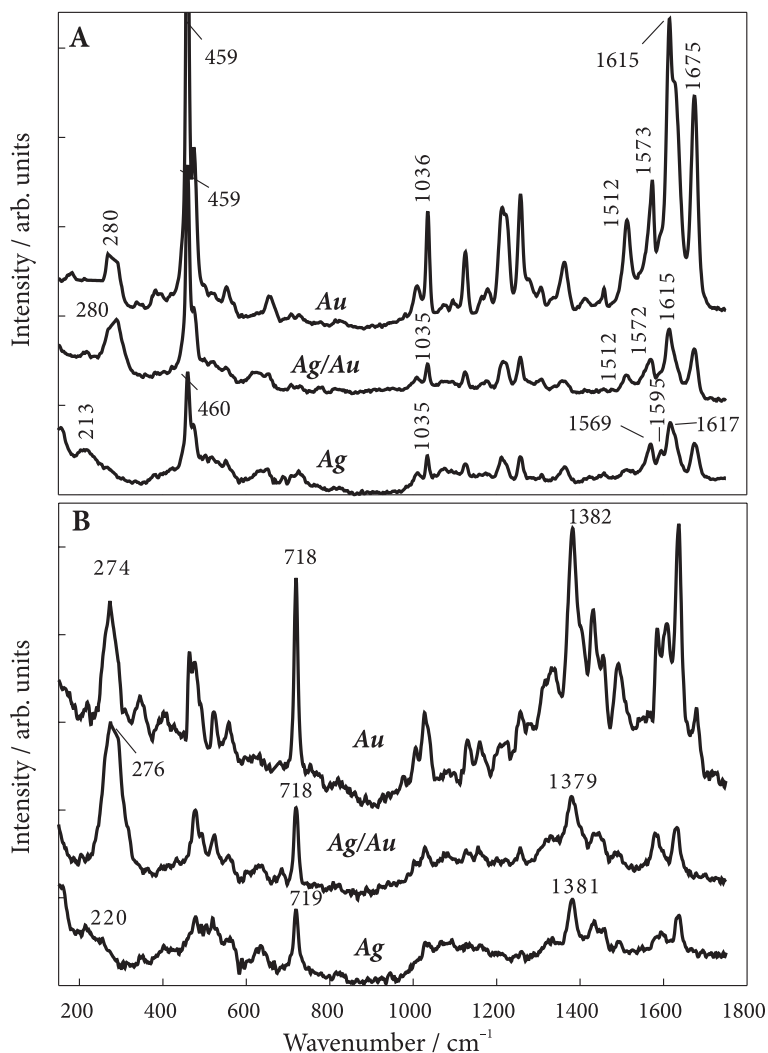


Fig. 3. SERS spectra of SAMs formed from 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone at -0.2 V electrode potential (A) and -0.7 V electrode potential (B) excited by 1064 nm radiation

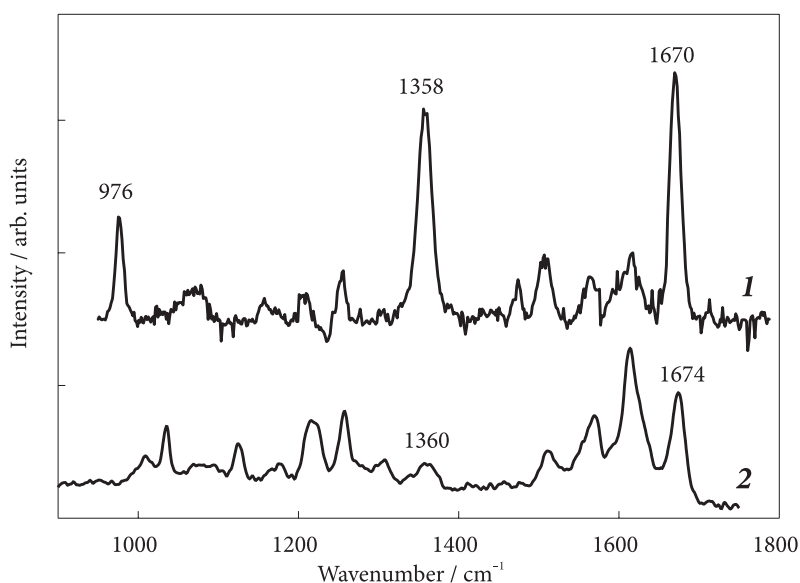


Fig. 4. SERS spectra of SAMs formed from 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone from Ag / Au electrode at -0.2 V electrode potential excited by 532 nm radiation (1), and with 1062 nm radiation (2)

When comparing the intensities of the spectra from these three electrodes, it was observed that the enhancement of the SERS spectra excited by 1064 nm radiation from the Ag / Au electrode was less than that from the Au electrode, but larger than the one from the Ag electrode.

However, a different picture was observed by using an excitation of 532 nm (Fig. 4, trace 1). Thus, relative intensities of the SNQ bands in its ox-form adsorbed on the Ag / Au electrode are clearly different comparing the 532- and 1064-nm excited spectra (Fig. 4). Furthermore, we were not able to observe the

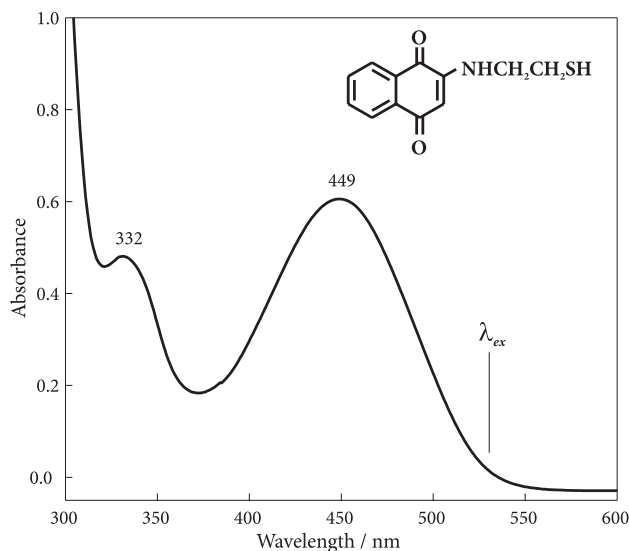


Fig. 5. UV-Vis spectrum of 2-[(2-sulfanylethyl)amino]-1,4-naphthoquinone in C_2H_5OH solution

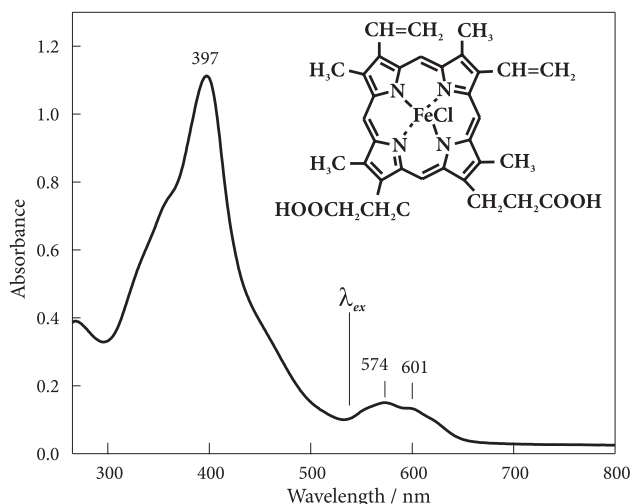


Fig. 6. UV-Vis spectrum of hemin in DMSO solution

spectrum of the reduced form of SNQ by using the 532 nm excitation. Such phenomenon might be explained by the operation of a resonant enhancement effect, because the 532 nm radiation falls into the region of electronic absorption band of the studied compound (SERRS effect). Indeed, the electronic absorption band of SNQ is observed in the 400–550 nm spectral interval (Fig. 5), whereas 1,4-dihydroxy-3-aminonaphthalene does not absorb in the visible region [20].

SERRS spectra of hemin and alcohol dehydrogenase adsorbed on Ag and Ag / Au electrodes

Hemin (iron(III) protoporphyrin IX chloride) is often used as a model compound for the investigation of heme c cofactor that is present in hemoproteins. The compound is prone to adsorb on Ag, and has intensive electronic adsorption bands in the visible region (Fig. 6), therefore, a strong resonant enhancement of the Raman signal is expected by using a 532 nm laser line for excitation. Indeed, we were able to observe the SERRS spectra of hemin from both Ag and Ag / Au electrodes, but in the latter case, the

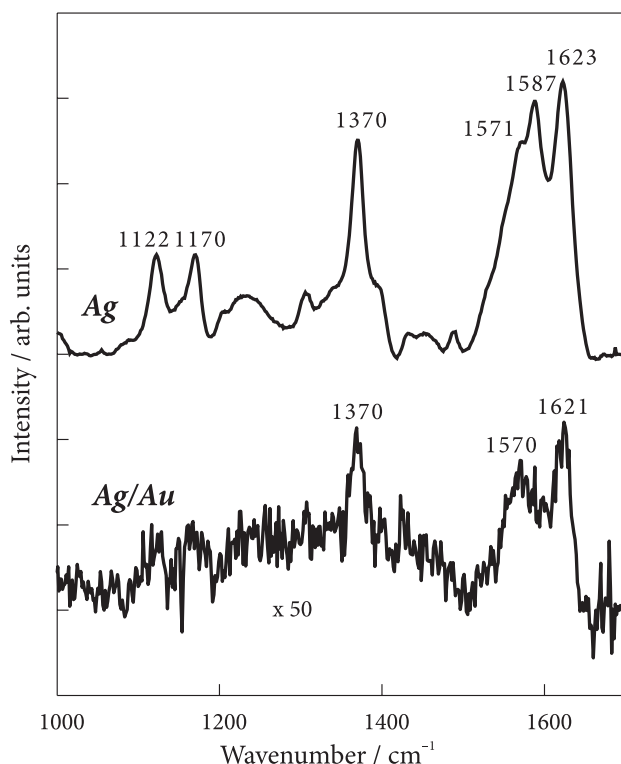


Fig. 7. SERRS spectra of hemin ($c = 2 \times 10^{-7}$ M) from Ag and Ag / Au electrodes at the -0.1 V potential, excited by 532 nm radiation

spectrum was much weaker (Fig. 7). The main difference of the SERRS spectrum observed in the case of Ag / Au as compared to Ag, is the absence of the intense feature at 1587 cm^{-1} assigned to ν_{37} mode of E_{1u} symmetry [23–24], which is forbidden in the Raman spectra if the porphyrin ring is flat. This implies that the porphyrin ring is strongly deformed on the Ag surface, whereas on the surface of Au it is much flatter.

ADH is a hemoprotein containing two cofactors: heme c and pyrroloquinoline quinone (PQQ). In the visible region, it has a very strong electronic absorption band (Soret band) at 417 nm and two other intensive bands near 523 and 552 nm arising from γ -, β - and α -transitions, respectively (Fig. 8) [25]. PQQ adsorbs at about 350 nm. Therefore, by using the 532 nm excitation, one might expect to obtain the vibrational information only from the heme group. Certainly, the intensive SERRS spectrum was detected from the Ag electrode kept in a buffer aqueous ADH solution ($c = 10^{-7}$ M) for 1 hour (Fig. 9). Under identical conditions, the SERRS spectrum from the Ag / Au electrode is comparatively weak, but the prominent bands are still detectable. The frequency of the most pronounced ν_{15} mode of B_{1g} symmetry [21–24] is the same in both spectra. Interestingly, when the Ag / Au electrode was kept in the same solution for 17 h at the stationary potential, a rather intensive SERRS spectrum was obtained even after washing the electrode (Fig. 9, trace 3). Some changes of band positions and relative intensities were observed. These data clearly indicate that ADH is irreversibly adsorbed on the Au layer.

Thus, in principle, by using the double-layered Ag / Au electrode it is possible to study the process of adsorption and interaction of hemin and ADH with the Au surface by the very sensitive SERRS method. Nevertheless, the Ag / Au electrode de-

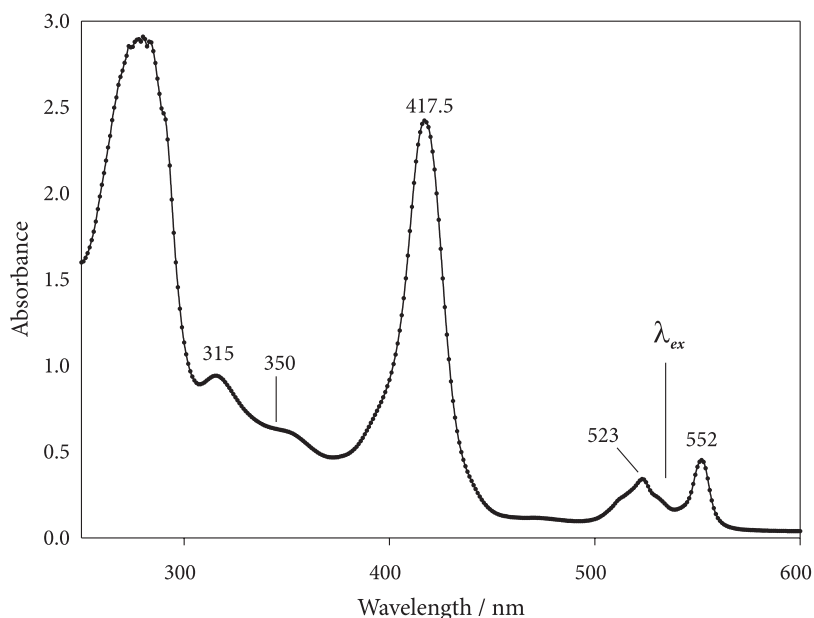


Fig. 8. UV-Vis spectrum of alcohol dehydrogenase solution in 5 mM TRIS, 10 mM CaCl_2 and 0.1 M NaCl buffer, pH 8.0

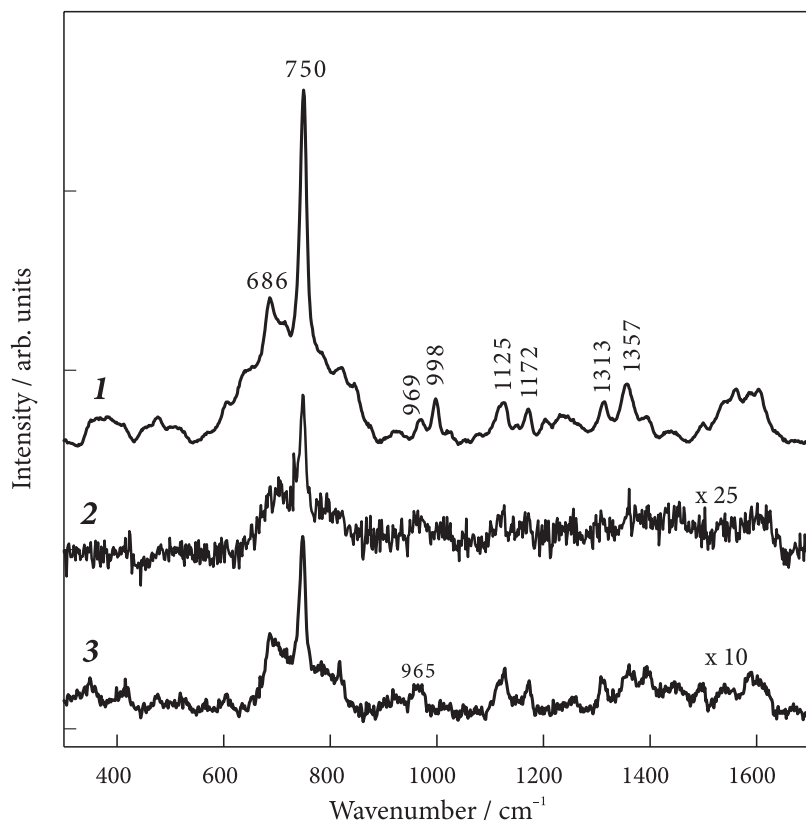


Fig. 9. SERRS spectra of alcohol dehydrogenase (ADH) excited by 532 radiation at -0.6 V electrode potential (in 20 mM phosphate buffer, pH 7.5, concentration of ADH 10^{-7} M): 1 – Ag electrode, 2 – Ag / Au electrode after 1 h from the injection of ADH, 3 – Ag / Au electrode kept for 17 hours in ADH solution under stationary potential and washed with buffer (the spectrum recorded in buffer solution)

scribed above is not enough effective. Among the reasons there may be an insufficiently thin layer of Au and a decrease of the roughness of the pre-treated Ag electrode in the process of plating. Therefore, the method requires further elaboration.

CONCLUSIONS

1. The plating technique of Ag electrodes with a thin layer of Au was elaborated based on the reduction of $\text{Na}_3\text{Au}(\text{SO}_3)_2$ to metallic Au by formaldehyde. The reaction was carried out in a solution

containing 8.1 mM of $\text{Na}_3\text{Au}(\text{SO}_3)_2$, 0.127 M of Na_2SO_3 and about 0.63 M of formaldehyde at a temperature of 2–4 °C for 4 hours.

2. It was established that the enhancement of SERS spectra excited by 1064 nm radiation from Ag / Au electrode was less than that from the electrode of Au, but larger than the one from the electrode of Ag. When a radiation of 532 nm is used, it is possible to obtain the enhancement from the under-layer of the electrode.

3. Double-layered Ag / Au electrodes are especially suitable for obtaining structural information of chromophoric co-factors of proteins adsorbed on Au surface from their SERS spectra.

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DVIEJŲ SLUOKSNIŲ AG / AU ELEKTRODAS PSRS SPEKTROSKOPIJAI: PARUOŠIMAS IR TAIKYMAS CHROMOFORINIŲ JUNGINIŲ ADSORBCIJAI TIRTI

Santrauka

Panaudojant $\text{Na}_3\text{Au}(\text{SO}_3)_2$ redukciją formaldehidu į metalinį Au, sukurta paviršiumi sustiprintos Ramano sklaidos (PSRS) spektrams gauti tinkamų Ag elektrodų padengimo plonu Au sluoksniu metodika. Redukcijos reakcija buvo vykdoma 8,1 mM $\text{Na}_3\text{Au}(\text{SO}_3)_2$, 0,127 M Na_2SO_3 ir 0,63 M formaldehido tirpale 2–4°C temperatūroje 4 val. Ištirtas šių dviejų sluoksnių Ag / Au elektrodų tinkamumas savitvarčių monosluoksnių, gautų iš 2-[(2-sulfanyletil)amino]-1,4-naftochinono, struktūrai bei hemino ir alkoholdehidrogenazės adsorbcijai ant Au paviršiaus tirti, panaudojant PSRS spektrus, žadinamus 1064 nm ir 532 nm spinduliuote. Nustatyta, kad šio dviejų sluoksnių elektrodo duodamas stiprinimas yra mažesnis nei gryno Au, bet didesnis nei gryno Ag elektrodo, jeigu žadinama 1064 nm spinduliuote. Jeigu spektrui žadinti yra naudojama 532 nm spinduliuotė, galima gauti stiprinimą nuo apatinio (Ag) sluoksniu. Tai atveria galimybę gauti adsorbuotų ant Au paviršiaus chromoforinių junginių paviršiaus sustiprintos rezonansinės Ramano sklaidos spektrus, kurie yra ypač tinkami baltymų chromoforiniams kofaktoriams tirti.