

VILNIUS UNIVERSITY
INSTITUTE OF BIOCHEMISTRY

Arūnas Bulovas

**SELF-ASSEMBLED MONOLAYERS OF BIOMIMETIC
(METHYL)NAPHTHOQUINONE ω -MERCAPTO DERIVATIVES ON GOLD
AND SILVER SURFACES: ELECTROCHEMICAL AND SPECTROSCOPIC
STUDIES OF REDOX CONVERSION AND STRUCTURE**

Summary of doctoral dissertation
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VILNIAUS UNIVERSITETAS
BIOCHEMIJOS INSTITUTAS

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**BIOMIMETINIAI (METIL)NAFTOCHINONO ω -MERKAPTO DARINIŲ
SAVITVARKIAI MONOSLUOKSNIAI ANT AUKSO IR SIDABRO PAVIRŠIŲ:
ELEKTROCHEMINIAI IR SPEKTROSKOPINIAI REDOKSO VIRSMŲ IR
STRUKTŪROS TYRIMAI**

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INTRODUCTION

Natural naphthoquinones are synthesized in plants and some bacteria. Purified compounds are yellow, orange, red, and purple crystals soluble in organic solvents, and can be used like dyes. Among physiologically active naphthoquinones the most important are vitamins of group K taking part in maturation of blood coagulation factors and some other proteins (Koagulation in German and Scandinavian languages). Vitamin K₁ (2-methyl-3-phytyl-1,4-naphthoquinone or phyloquinone) takes part in electron transfer in Photosystem I of plant chloroplasts and cyanobacteria, vitamin K₂ (2-methyl-3-prenyl-1,4-naphthoquinone or menaquinone) transports reduction equivalents in bacterial respiration chains. Proton gradient across the biological membrane is built up in these important processes. Some other plant naphthoquinones express antimicrobial and antitumor activity, and are used in medicine. Therefore, redox activity and interfacial structural characteristics of naphthoquinone derivatives are worthy of thorough investigation.

Self-assembled monolayers (SAM) of long-chain alkylthiols on a metal surface are suitable biomimetic systems for a study of electron transfer across the biological membrane. Alkylthiols bind chemically to metals through sulfur atom of the terminal SH group. The most suitable often used metals are gold and silver. Electroactive SAM is formed from compounds with redox active group in the end opposite to SH. Structure of the monolayer is stabilized by the interaction between hydrophobic alkyl chains. Electrochemical and spectroscopy methods are the most commonly used for investigation of redox activity and structure of the monolayer. Surface-enhanced Raman scattering is especially suitable for study of the fine structure of SAM.

Studies of SAM functionalized with naphthoquinone (NQ) groups are not numerous. Moreover,, studied compounds have substituents in the NQ ring other than methyl (Cl), or their alkylthiol chains are linked to NQ rings through the NH group or S atom. Such compounds with substituents strongly affecting redox properties of NQ poorly mimic vitamins K and other natural NQ-based substances.

Purpose of this work was to study interfacial redox processes and fine structure of SAM formed from newly synthesized biomimetic (methyl)naphthoquinone ω -

mercapto derivatives (Fig. 1) on gold and silver by cyclic voltammetry (CV) and surface-enhanced Raman spectroscopy (SERS).

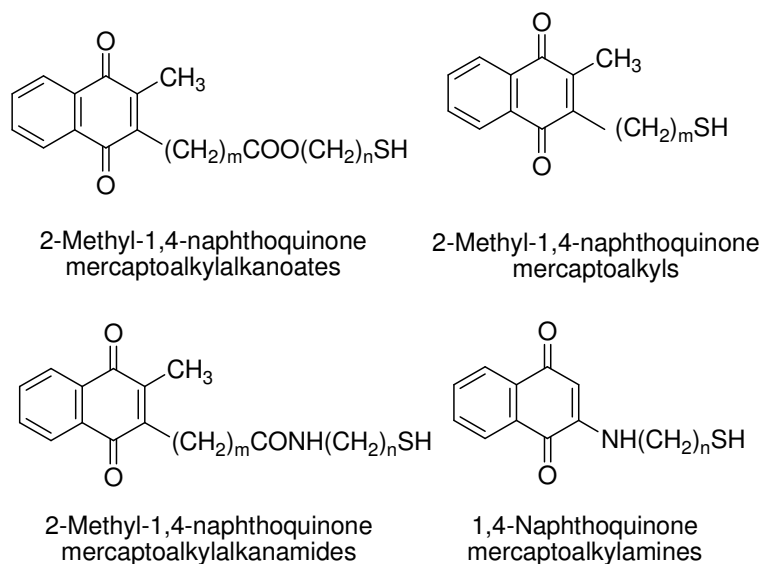


Figure 1. Structure of newly synthesized biomimetic (methyl)naphthoquinone ω =mercapto derivatives.

Novelty and significance of the work.

1. A large set of 1,4-naphthoquinone derivatives, structurally more closely related to vitamins K, and varying in the side-chain lengths and intrachain functional groups, that determine different types of intermolecular interactions in SAM, was synthesized.

2. Compared to earlier related studies, features of interfacial structure and redox conversion of new SAM were elaborated in far more detailed manner.

3. Structure-functioning characteristics of the mixed SAM of newly synthesized NQ derivatives with different redox inactive alkylthiols were studied. Such studies of biomimetic 2D-structure were performed earlier only with hydroquinone- and anthraquinone-based derivatives.

4. Interfacial structure and redox conversion characteristics of low-density NQ-based monolayers were studied more thoroughly.

5. In this work, formation, structural and functional properties of new NQ-based SAM on gold and silver were compared. Earlier, such study was performed only with the use of anthraquinone derivatives. Furthermore, double-layered Ag/Au electrodes (silver coated with thin layer of gold) were used in the work under discussion.

6. Structure of all pure and mixed monolayers in oxidized and reduced states of NQ head group was studied with the use of surface-enhanced Raman spectroscopy, previously applied only in the studies of anthraquinone-based monolayers.

MATERIALS AND METHODS

Synthesis of 2-methyl-1,4-naphthoquinone-3-(ω -mercaptoalkyl)alkanoates

The strategy used to obtain the title derivatives was based on direct functionalization of the quinone ring via radical substitution reaction using dicarboxylic acids. In contrast to Jacobsen and Torsell we used a slight excess of dicarboxylic acid and increased the quantities of AgNO₃ (2-fold) and ammonium persulfate (1.6-fold) in the reaction mixture. A controlled rate of persulfate addition and optimal ratio of CH₃CN/H₂O was selected in order to avoid precipitate formation and a satisfactory yield of the product was achieved. Methyl-naphthoquinone acids were isolated from the reaction mixture by transforming them into salts with potassium carbonate, and extracting the side-products with methylene chloride. The following step of the synthesis was the esterification of acids with ω -bromoalkanols to give the corresponding esters. Finally, ω -mercapto derivatives were obtained via isothiuronium salts using thiocarbamide in acetone and conducting the decomposition of the isothiuronium salt in water/chloroform heterogeneous phase containing Na₂S₂O₅. The structures of the synthesized compounds were confirmed by spectral and elemental analyses.

Synthesis of 2-methyl -1,4-naphthoquinone-3-(ω -mercaptoalkyls)

The method of direct free-radical alkylation of naphthoquinones described by Jacobsen and Torsell was used. To this end, 2-methyl-naphthoquinone was reacted with ω -bromoalkanoic acid in the presence of ammonium persulfate and silver nitrate in the

mixture of acetonitrile/water. Then bromides were transformed to the isothiuronium salts by reacting with thiocarbamide, and the salts were hydrolyzed to 2-methyl-3-(ω -mercaptoalkyl)-naphthoquinones according to previously developed procedure. The structure of the new compounds was confirmed by spectral and elemental analysis.

Synthesis of 2-methyl-1,4-naphthoquinone-3-(ω -mercaptoalkyl)alkanamides

The starting 2-methyl-3-carboxyalkyl-1,4-naphthoquinones were obtained by free-radical alkylation of 2-methyl-1,4-naphthoquinone with the appropriate dicarboxylic acids using the method of Jacobsen and Torsell adopted previously. Next, these compounds were converted to their ω -bromoalkylamine salts by the addition of triethylamine to a CH_3CN solution containing an equimolar amount of ω -bromoalkylamine hydrobromide and 2-methyl-3-carboxyalkyl-1,4-naphthoquinones. Shortly after the addition a fine yellow precipitate formed in a good yield, and the salts were isolated by filtration and used in the next step without further purification. The condensation reaction between salts leading to amide bond formation was successfully performed using DCC as coupling reagent at ambient temperature. Salts were dissolved in methylene chloride, then treated with a CH_2Cl_2 solution of DCC (30% excess) for 48 h at ambient temperature. The *N*-(ω -bromoalkyl)alkanamides obtained were isolated by standard methods. Next, they were transformed into isothiuronium salts and, after decomposition in a water/chloroform heterogeneous phase containing $\text{Na}_2\text{S}_2\text{O}_5$, the target 2-methylnaphthoquinone derivatives with a terminal mercapto group were obtained.

Synthesis of 1,4-naphthoquinone-3-(ω -mercaptoalkyl)amines

The strategy used to obtain the title derivatives of naphthoquinone was based on direct functionalization of the quinone ring with the corresponding terminal substituted alkylamines. 2-Bromo- or 2-chloro-substituted alkylamines were obtained by direct functionalization of 1,4-naphthoquinone with the corresponding bromo- and chloroalkylamines in acetonitrile. Yields of these reactions were moderate due to the side reactions. Formation of isothiuronium chloride from chloro derivative proceeded very

slowly, therefore this compound was not used for further synthesis. The subsequent steps include synthesis of isothiouonium salts, which were obtained by refluxing bromoalkylamines in ethanol with an excess of thiourea. Decomposition of the isothiuronium salts in water/chloroform heterogeneous mixture containing $\text{Na}_2\text{S}_2\text{O}_5$ under argon smoothly afforded thiols in good yields. The ordinary procedure of the decomposition of isothiuronium salts with aqueous sodium hydroxide could not be used in this work due to the instability of naphthoquinone ring under these conditions. The structure of the synthesized compounds was confirmed by ^1H and ^{13}C NMR, IR and FT-Raman spectroscopies.

Electrochemical measurements

Electrochemical studies were carried out on a computerized EG&G Versastat potentiostat system (Princeton Applied Research) in a conventional three-electrode cell. A saturated sodium calomel electrode (SSCE) was used as a reference ($E = 239$ mV vs. SHE). The platinum wire served as an auxiliary electrode. The cell was purged with argon for about 20 min. Measurements were performed in 0.1 M HClO_4 or in 0.01 M citrate (pH 3-7) and borate (pH 7-11) buffers with 0.1 M Na_2SO_4 , under argon atmosphere at 25°C .

The pretreatment of Au electrode for CV and SERS measurements was performed according to the following procedure. Polycrystalline Au electrode, made by pressing the gold wire into Teflon, was polished with $0.05\ \mu\text{m}$ alumina slurry, rinsed with Millipore water, sonicated twice for 10 min. in water, and cleaned electrochemically by E cycling in 0.1 M H_2SO_4 between 0.4 and 1.6 V at $100\ \text{mV s}^{-1}$ scan rate for 12 min (30 cycles). The real surface area of the electrode was estimated from the gold oxide reduction peak in 0.1 M H_2SO_4 . The Ag electrode was only polished with alumina slurry and twice sonicated in Millipore water. In some experiments Ag electrode was plated with a layer of Au by keeping it immersed in the plating solution in a refrigerator (about 5°C) for the appropriate time. The solution was prepared just before plating from the 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 experiments. The process of Au layer formation and the state of the electrode was monitored by cyclic voltammograms (CV) of Tl and Pb. These CV measurements were performed in deaerated solutions of 0.1 M HClO_4 or 0.1 M NaClO_4 containing 5 mM of TlNO_3 or in a deaerated solution of 10 mM of $\text{Pb}(\text{CH}_3\text{COO})_2$ under argon atmosphere at 25°C. The potential was scanned between 0.3 and -0.7 V (Tl) or 0 and -0.45 V (Pb) vs. SSCE at a rate of 100 mV s^{-1} . The sixth cycle was analyzed.

The SAMs were formed by incubating Au or Ag electrodes in the compound (or mixture of the compounds) solution of the appropriate concentration in 95% ethanol for about 20 h. After the incubation, the electrodes were thoroughly washed with 95% ethanol followed by water, and were immediately transferred to the working solution.

Spectroscopic measurements

NMR spectra of the compounds were recorded on Variant Unity Inova NMR spectrometer at 300 MHz in CDCl_3 solutions. IR spectra were recorded in KBr pellets on a Perkin-Elmer FT-IR System Spectrum GX spectrometer.

Raman spectroscopic measurements were carried out with Perkin-Elmer Model Spectrum GX FT-Raman spectrometer equipped with a InGaAs detector operating at room temperature. An air-cooled diode-pumped Nd-YAG laser provided the excitation with an emission wave-length of 1064 nm. In general, the laser power at the sample was 300 mW. The laser beam was focused to a spot of ca. 1 mm^2 in area. FT-SERS measurements were carried out in a spectroelectrochemical cell equipped with working Au or Ag electrode (circular electrode of 6 mm in diameter, pre-fitted into Teflon rod), Ag/AgCl reference electrode and an auxiliary Pt wire electrode. All potentials are reported vs. a SSCE. The Au electrode for SERS was electrochemically roughened by *E* scanning (50 cycles) in 0.1 M aqueous KCl solution between -0.30 and 1.35 V vs. Ag/AgCl at 300 mV s^{-1} scan rate. Roughening of the Ag electrode performed by cycling in 0.1 KCl between -0.3 and 0.3 V vs. Ag/AgCl at 10 mV s^{-1} for 20 min (10 cycles).

The cell body was a glass cylinder with a flat quartz window. The working electrode was placed at ca. 2 mm distance from the cell window. In order to reduce photo- and thermoeffects, the cell together with the electrodes was moved linearly with respect to the laser beam with the rate of about 15-25 mm/s. The spectroelectrochemical cell was connected to a holder consisting of two-axis manual stage, which features aluminum construction with ball-bearings running along polished stainless-steel rods giving precise ball-bearing motion. The movement of the cell holder was provided by specially constructed driver connected to the DC engine. The spectral resolution was set at 4 cm^{-1} , and the wavenumber increment per data point was 1 cm^{-1} . In order to enhance the signal-to-noise ratio, 200 scans were co-added. To determine integrated intensities, the overlapped bands were digitally decomposed into the components of mixed Lorentzian-Gaussian shape.

RESULT AND DISSCUSION

Formation of self-assembled monolayers from 2-MeNQ-3-(ω -mercaptoalkyl)alkanoates varying in the side-chain length

The typical cyclic voltammogram of the 2-methylnaphthoquinone-terminated SAM exhibit well-defined redox waves (Fig. 2). Assuming the two-electron electrochemical conversion of terminal NQ, the estimated surface concentration (Γ) of the redox-active 2-MeNQ groups in the monolayer was found to be only slightly dependent on the spacer length, and are about 1.5 times lower than the expected value of 3.4×10^{-10} mol cm^{-2} for the close-packed assembly, considering that one vertically oriented 2-MeNQ group occupies ca. 0.49 nm^2 . This result indicates that the monolayer is sufficiently compact even for the shortest side-chain (five atoms) compound. The formal redox potential, $E^0 = (E_p^c + E_p^a)/2$, where E_p^c and E_p^a are, respectively, cathodic and anodic peak-potentials, shifts to the negative region with a slope of ca. 15 mV per chain atom as the overall number of side chain atoms ($m + n + 2$) increases (Fig. 3a). At the same E scan rate, the separation E_p^c and E_p^a (ΔE_p) increases with increasing ($m + n$), indicating a decrease of electrochemical reversibility, and slowing of the redox process.

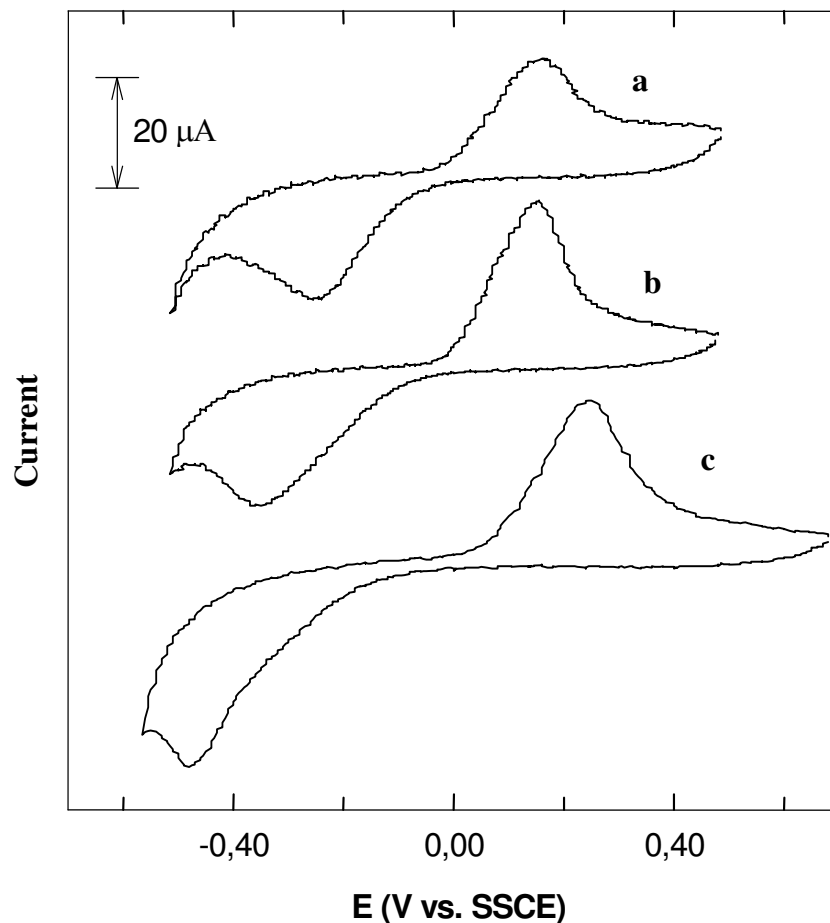


Figure 2. Cyclic voltammograms of the gold electrode modified by 2-MeNQ-3-(ω -mercaptoalkyl)alkanoates with different length side-chains (a – five, b – eight, c – eleven atoms). Measurements performed in anaerobic 0.1 M HClO₄ solution at 25°C. Potential scan rate 0.1 V s⁻¹.

It should be noted that, with the increase of space length, E_p^a shifts toward the positive values much more slowly than E_p^c towards negative values implying that the oxidation process at the SAM's head-group is more favourable than its reduction. Also the heterogeneous electron-transfer rate constant (k_{app}) value for the overall two-electron reaction were determined by Laviron's method from the dependencies E_p versus $\ln(E$ scan rate). The general decrease of k_{app} with increasing distance between the electrode and the redox moiety indicates that the electron transfer rate is controlled by the length of the spacer, as expected for the compact and ordered SAM. The exponential decrease

of k_{app} with increasing number of chain atoms between sulfur and 2-MeNQ is demonstrated in Figure 3b. From the slope of the plot $\ln(k_{app})$ versus distance (number of chain atoms) the exponential decay factor for tunneling through the SAM was obtained, assuming that the free and reorganization energies are comparable for all the studied SAMs. The calculated value $\beta = 0.89 \pm 0.16$ per chain atom.

Further the FT-SERS technique was applied with the aim of determining some structural features of new SAMs, and also the particular characteristics of their redox conversion. Figure 4 compares the bulk FT-Raman spectrum of solid 2-MeNQ(CH₂)₄COO(CH₂)₃SH and the FT-SERS spectra of SAM formed by this compound on gold. Two spectral features unambiguously demonstrate that formation of the SAM proceeds through the bonding of the sulfur atom with gold. The intense S-H stretching vibrational band at 2569 cm⁻¹ observed in the spectrum of bulk compound (Fig. 4a) disappears upon formation of the monolayer (Fig 4b and c). Consequently, a new broad low-frequency mode at 279-285 cm⁻¹ appears in the SERS spectra. This band was previously attributed to the stretching vibrational mode $\nu(\text{Au-S})$, directly indicating formation of the Au-S moiety due to the self-assembly of compound on the surface. The intense peak in the vicinity of 1654 cm⁻¹ (Fig. 4a) was assigned predominantly to the symmetric stretching vibration of C=O group of the NQ ring. The frequency of this vibration is sensitive to the interaction of C=O with environmental molecules. As can be seen from Figure 4b, the formation of the SAM does not noticeably affect the position of the C=O band, implying that the group does not interact directly with the surface. The FT-SERS spectrum of the compound provides direct evidence of the complete reduction of the NQ moiety as E is swept to -0.5 V. The intense $\nu(\text{C=O})$ peak disappears and three strong bands characteristic to naphthalene ring vibrations emerge at 1372, 1434 and 1566 cm⁻¹. These features indicate that the 2-MeNQ-terminal group converts to a 2-methyl-1,4-dihydroxynaphthalene moiety due to a reduction process. The FT-SERS method was also used for the evaluation of stability and integrity of new SAMs after a particular time of electrode polarization. Thus, it was found that the integrated intensity of the $\nu(\text{C=O})$ band at 1655 cm⁻¹ changes only by ca. 1% during 6 h of polarization (3 h at -0.6 and 3h at 0.5 V). The relative intensities and frequencies of other bands also remained practically unchanged after polarization experiments. These facts demonstrate the exceptional stability of the new SAMs.

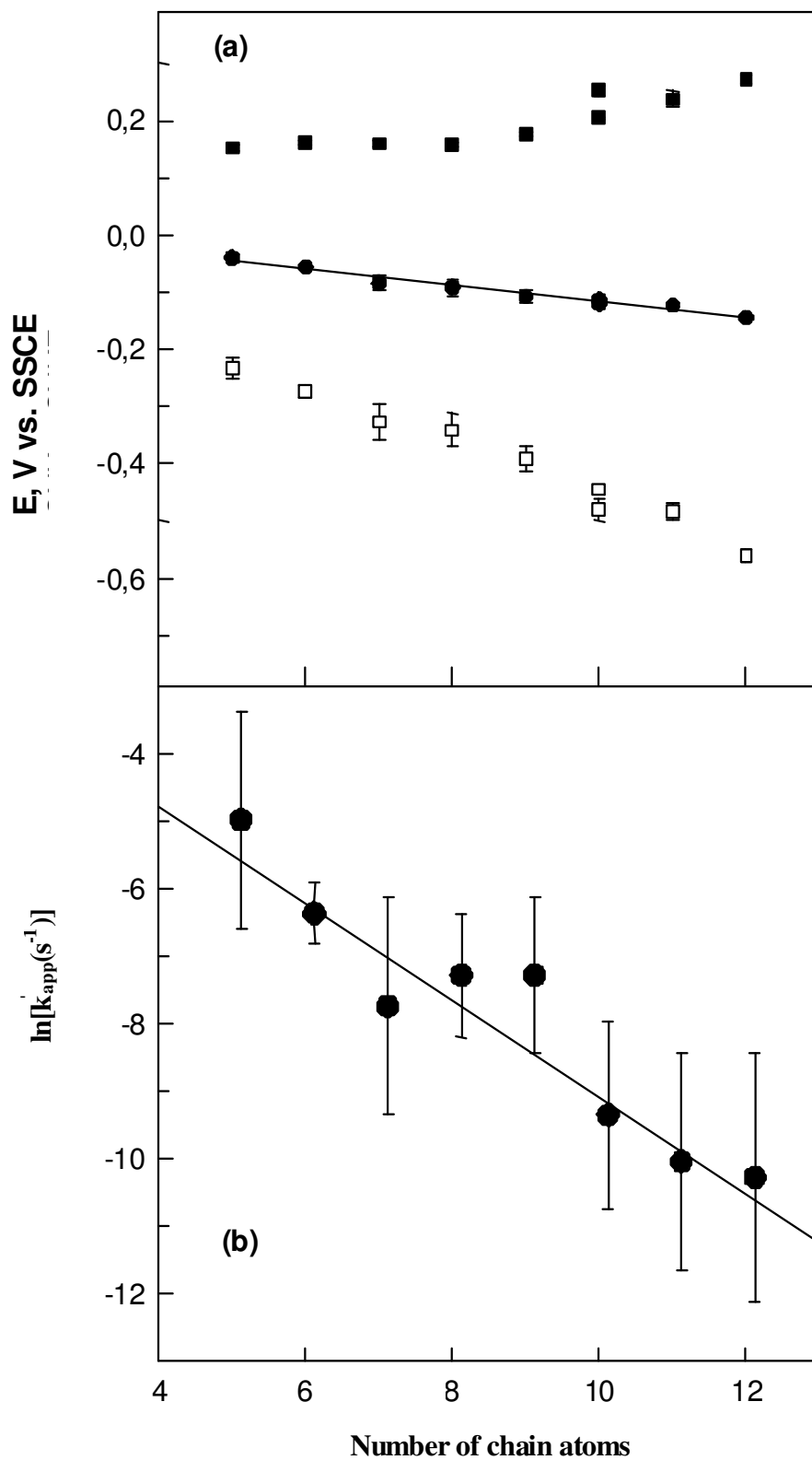


Figure 3. Dependence of (a) potentials (E_p^c , E_p^a , $E^{0'}$) and (b) $\ln[k_{app}(s^{-1})]$ on the number of side-chain atoms ($m + n + 2$) in 2-MeNQ(CH₂)_m COO(CH₂)_nSH SAMs on gold. Parameters determined in anaerobic 0.1 M HClO₄ at 25°C with an E scan rate of 0.1 V s⁻¹.

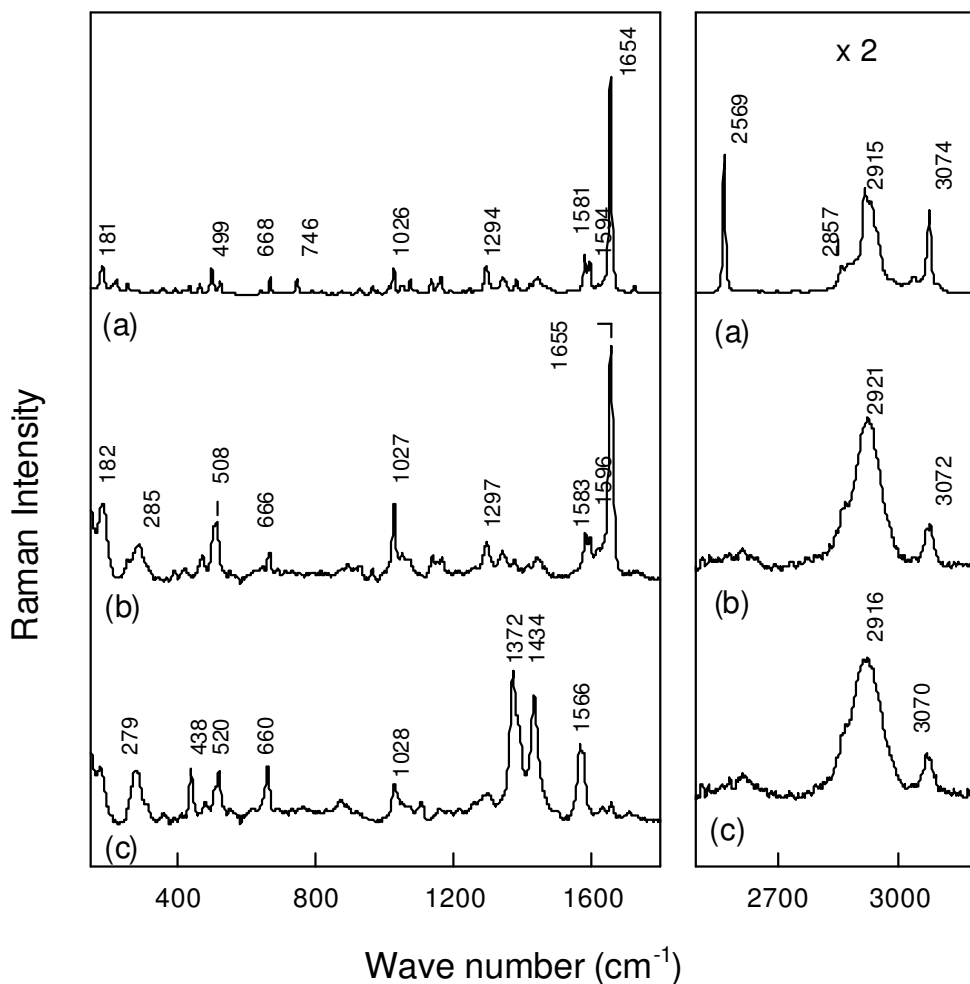


Figure 4. Comparison of (a) the FT-Raman spectrum of solid compound 2-MeNQ(CH₂)₄COO(CH₂)₃SH and (b, c) the FT-SERS spectra of SAM formed by compound on gold at (b) 0.6 and (c) -0.5 V versus SSCE. The excitation wavelength is 1064 nm. Laser power at the sample is 200 mW for (a) and 300 mW for (b, c).

Effect of the 2-MeNQ-3-(ω -mercaptoalkyl) concentration in the coating solution on the properties of self-assembled monolayers

The presence of ester linkage in the spacer may have an effect on self-assembling properties of the compounds due to the interaction between the dipoles of the ester linkage. With this in mind, the aim of the present study was to examine 2-MeNQ derivatives, containing hydrocarbon linker, and compare them with the above-mentioned ester analogues.

Among other factors, the structure of redox active monolayers depend on the

concentration of electroactive thiols in the coating solution. Figure 5A compares a set of CV curves of 2-MeNQ-terminated monolayers self-assembled from ethanolic coating solutions containing from 0.05 to 100 μM of compound 2-MeNQ(CH₂)₇SH. The electrochemical parameters extracted from curves do not substantially change until

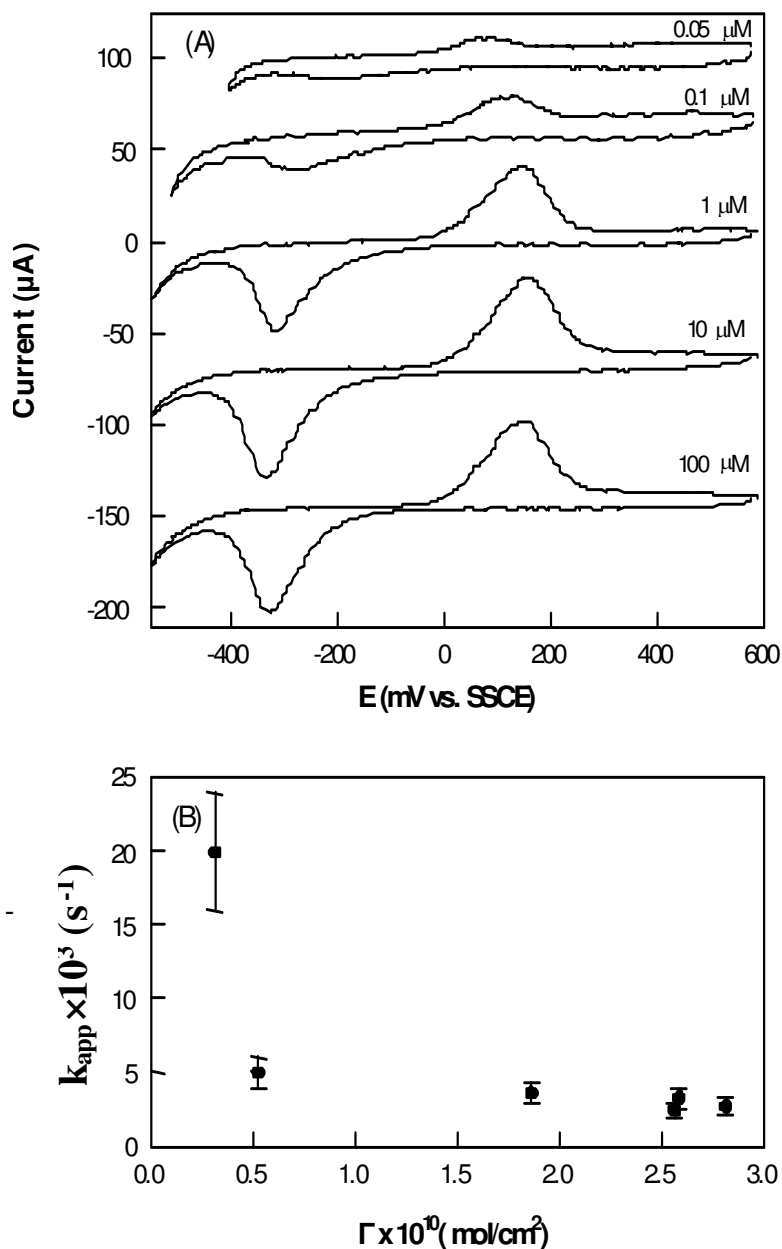


Figure 5. Cyclic voltammograms of compound 2-MeNQ(CH₂)₇SH self-assembled for 18 h on the Au electrode from various concentrations of substance ethanolic solutions in 0.1 M HClO₄ at 25°C and at the potential sweep rate 0.1 V s⁻¹ (a), the dependence of the apparent heterogeneous electron transfer rate constant, k_{app} , on the surface concentration Γ (b).

the concentration of compound in the coating solution decreases from 1 000 to 10 μM . However, when the concentration decreases to ca. 1 μM , Γ and ΔE_p start to decrease, $E^{0'}$ shifts towards more positive values, whereas the k_{app} value markedly enhances. The positive shift of $E^{0'}$ at lower concentration in the coating solution might be associated with destabilization of the oxidized form of SAM (for example, due to the decreased stacking between 2-MeNQ rings) and stabilization of reduced form (for example, due to the increased hydrogen bonding interaction in a less dense monolayer). Support for the weakening of interaction between the 2-MeNQ groups comes from the analysis of the width value of voltammetric waves. Thus, ΔE_{FWHM} decreases from 138 to 122 mV as the concentration of coating solution decreases from 10 to 0.05 μM , indicating weakening of the repulsive interaction between the redox centers. Figure 5B shows the dependence of apparent rate constant on the compound surface concentration. The sharp enhancement of k_{app} at submonolayer coverage indicates highly disordered structure of the surface layer, so that the average distance between the redox centers and surface becomes shorter (as for the compound with shorter side-chains), and some of the 2-MeNQ groups might even directly interact with the electrode surface. The tendency of NQ ring to adsorb on the metal surfaces is well documented.

The frequencies and relative intensities of FT-SERS signals of 2-MeNQ terminated monolayers self-assembled from the diluted (0.05 μM) ethanolic solution of compound 2-MeNQ(CH₂)₇ obtained at 0.5 V differs significantly as compared to the spectrum of the monolayer self-assembled from 1 mM solution. The prominent $\nu_s(\text{C}=\text{O})_{\text{Q}}$ band shifts higher frequencies by 3 cm^{-1} (1657 cm^{-1}) and considerably decreases in relative intensity. It is known that the frequency of $\nu_s(\text{C}=\text{O})_{\text{Q}}$ band depends slightly on solvent in which the naphthoquinone derivative is dissolved. Figure 6 compares the influence of the solvent and aggregation state on the parameters of $\nu_s(\text{C}=\text{O})_{\text{Q}}$ mode of the model compound, 2-methyl-1,4-naphthoquinone. Dissolution of solid 2-MeNQ in CCl₄ (0.64 M) results in the noticeable blue-shift (by 8.7 cm^{-1}) of the C=O stretching band. In order to test the influence of hydrogen-bonding on the position of $\nu_s(\text{C}=\text{O})_{\text{Q}}$ band, 2-MeNQ was dissolved in ethanol at 0.1 M concentration (Fig. 6c). In this case, the frequency increases further by 0.9 cm^{-1} , and the peak becomes considerably broader (FWHM increases from 9.1 to 12.2 cm^{-1}) as compared with mode characteristics in CCl₄.

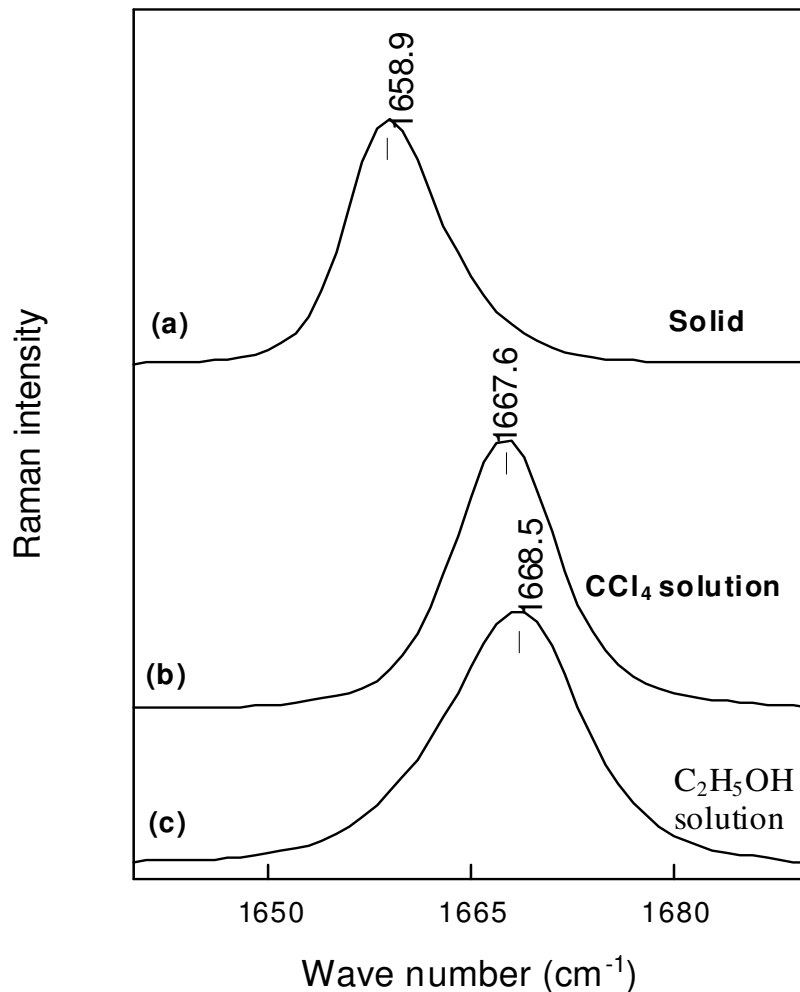


Figure 6. FT-Raman spectra of the model compound, 2-methyl-1,4-naphthquinone, in the solid state (a), and dissolved in CCl_4 (0.64 M) (b) and ethanol (0.1 M) (c) in the spectral region of C=O symmetric stretching band.

Observed spectral changes might be explained as superposition of two effects: stacking interaction between the extended ring systems, and hydrogen-bonding interaction of $\text{C}=\text{O} \cdots \text{H}$ type. In the solid state, the stacking interaction between the rings prevails, resulting in the lower $\nu_s(\text{C}=\text{O})_Q$ frequency as compared to the CCl_4 solution, where interactions are minimized. On the other hand, the hydrogen-bonding interaction tends to increase both the frequency and width of the band. Thus, when compared with the 1 mM solution, the increase in C=O stretching mode frequency by 3 cm^{-1} for the

monolayer self-assembled from the 0.05 μM solution definitely indicates the loss of stacking interaction between the rings.

Properties of mixed monolayers of 2-MeNQ-3-(ω -mercaptoalkyl) and redox inactive alkylthiols

In an effort to determine the importance of lateral interaction between the 2-MeNQ groups on their electrochemical properties in more detail, mixed monolayers of compound 2-MeNQ(CH₂)₇SH and various redox inactive thiols were constructed. Mixed monolayers consisting of compound and *n*-heptanethiol (HT) (Fig. 7a), dodecanethiol (DDT) (Fig. 7b) or mercaptoundecanoic acid (MUA) (Fig. 7c) in different proportions, taking the total concentration of the modifying solution equal to 1 mM, were composed. The nature of diluent crucially affects the electrochemical response of 2-MeNQ group in the monolayer. Well-defined anodic and cathodic waves are detected for the monolayer prepared with HT (Fig. 7a). The width of the anodic peak decreases from 144 to 105 mV upon diluting compound with HT (for the 1:3 ratio ratio of compound and HT in coating solution), indicating more homogeneous distribution of redox centers and decreased repulsive interaction between the 2-MeNQ groups. Simultaneously, the apparent rate constant of electron transfer increases by ~ 3.2 times. This might be associated with the particular structure of the monolayer allowing easier attainment of redox center by protons during the electrochemical transformation of the monolayer.

In contrast to HT monolayer, the adlayers prepared with the longer alkyl chain DDT results in the suppression of electrochemical activity (Fig. 7b), although the FT-SERS data indicate the presence of 2-MeNQ groups in the mixed SAM. These results could be explained by assuming that the C=O groups of 2-MeNQ moiety are “buried” in the DDT layer, and become inaccessible for protons.

Surprisingly, the mixed monolayer self-assembled from the 1:3 coating solution containing diluent with the same alkyl chain length as DDT, but having the COOH-terminal group (MUA) instead of CH₃, exhibits well-defined voltammetric waves (Fig. 7c). This observation illustrates the importance not only the diluent alkyl chain length, but also the nature of terminal group. Most likely, the hydrogen-bonded water molecules serve as proton source during the redox transformation of 2-MeNQ group. Alternatively,

protons from the COOH groups might participate in the electrochemical process. It should be noted that, for the MUA-containing SAM, the ΔE_{FWHM} value strongly increases as compared with the mixed with HT or even pure monolayers. The heterogeneity in hydrogen bonding strength between the COOH (or H₂) and redox active group most likely is responsible for this effect of wave broadening in the mixed with MUA monolayer.

For all mixed monolayers we were able to observe confidentially only the $\nu_s(\text{C=O})_Q$ feature in the FT-SERS spectra at 0.6 V. Based on the FT-Raman studies of model compound 2-MeNQ in various solvents and in solid state (Fig. 6), it is clear that the $\nu_s(\text{C=O})_Q$ mode sensitively probes the stacking interaction between the rings as well as hydrogen bonding of C=O · · · H-type. Figure 8 compares the FT-Raman spectrum of solid compound 2-MeNQ(CH₂)₇SH and the FT-SERS spectra of surface-confined compound in pure monolayer and in various mixed SAMs in the frequency region of symmetric stretching C=O vibrational mode. The wavenumber values for solid compound and pure monolayer self-assembled from 1 mM ethanolic solution coincides (1654 cm⁻¹), indicating that stacking interaction between the 2-MeNQ groups dominates in the monolayer. The frequency of $\nu_s(\text{C=O})_Q$ mode for the monolayer mixed with HT is considerably higher as compared with solid compound or the pure monolayer obtained from 1mM coating solution (Fig. 8). Such clear up-shift in frequency evidences absence of stacking interaction between the 2-MeNQ rings in the mixed monolayer and the involvement of rings in the C=O · · · H-O-H hydrogen bonding at the interface. Similar increase in frequency is detected for the model compound 2-MeNQ dissolved in ethanol (Fig. 6). For monolayers mixed both with MUA and DDT, the $\nu_s(\text{C=O})_Q$ peak shifts slightly to lower frequencies as compared with monolayer mixed with HT, indicating the decreased hydrogen bonding interaction strength for head-group in these SAMs. It will be recalled that no electrochemical response is detected for the monolayer mixed with DDT (Fig. 7b), although spectrum (e) in Figure 8 clearly shows the presence of 2-MeNQ groups in the mixed monolayer.

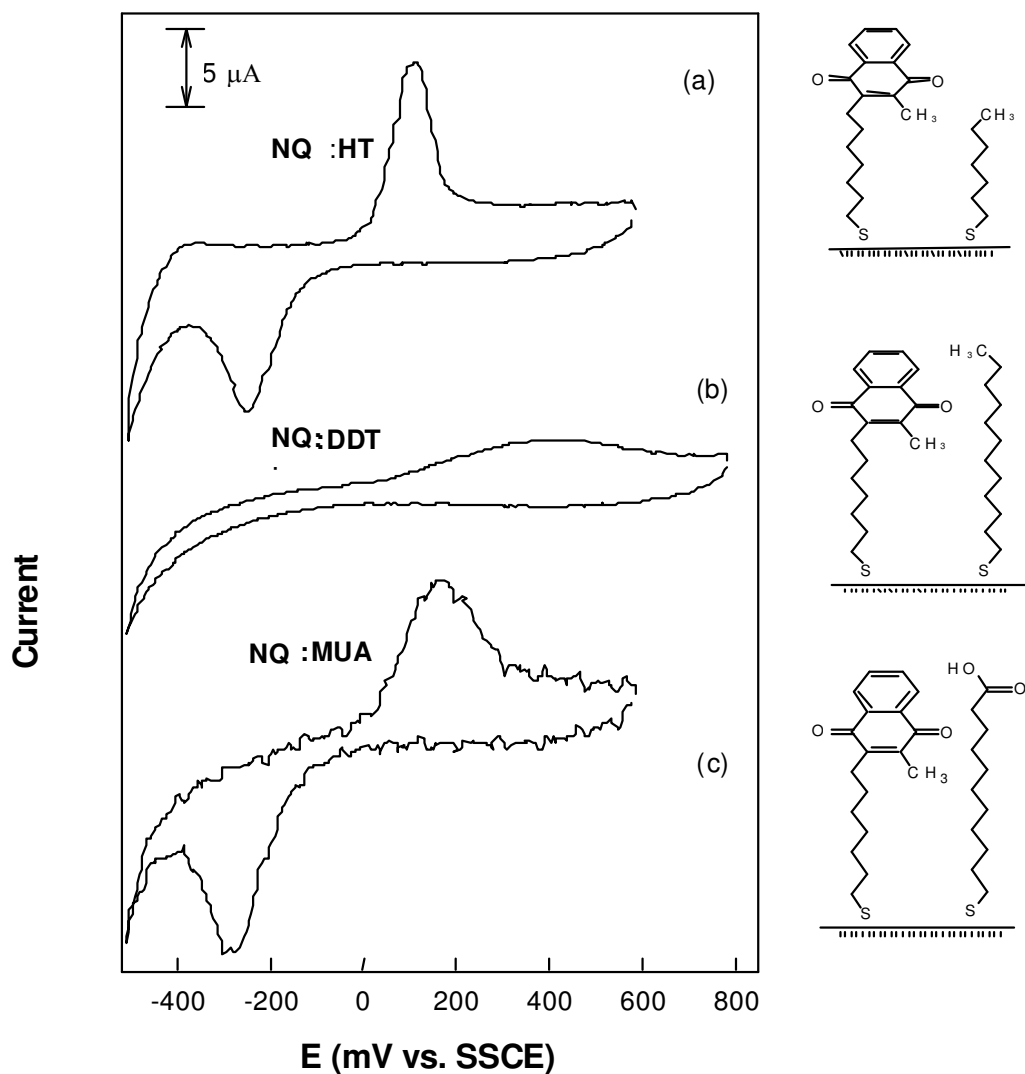


Figure 7. Cyclic voltammograms of compound 2-MeNQ(CH₂)₇SH (NQ) self-assembled on Au electrode from 1:3 (molar ratios) ethanolic solutions of NQ:HT (a), NQ:DDT (b), and NQ:MUA (c) recorded in 0.1 M HClO₄ at 25°C and the potential scan rate 0.1 V s⁻¹. Chemical structures of compounds forming mixed monolayers are also shown.

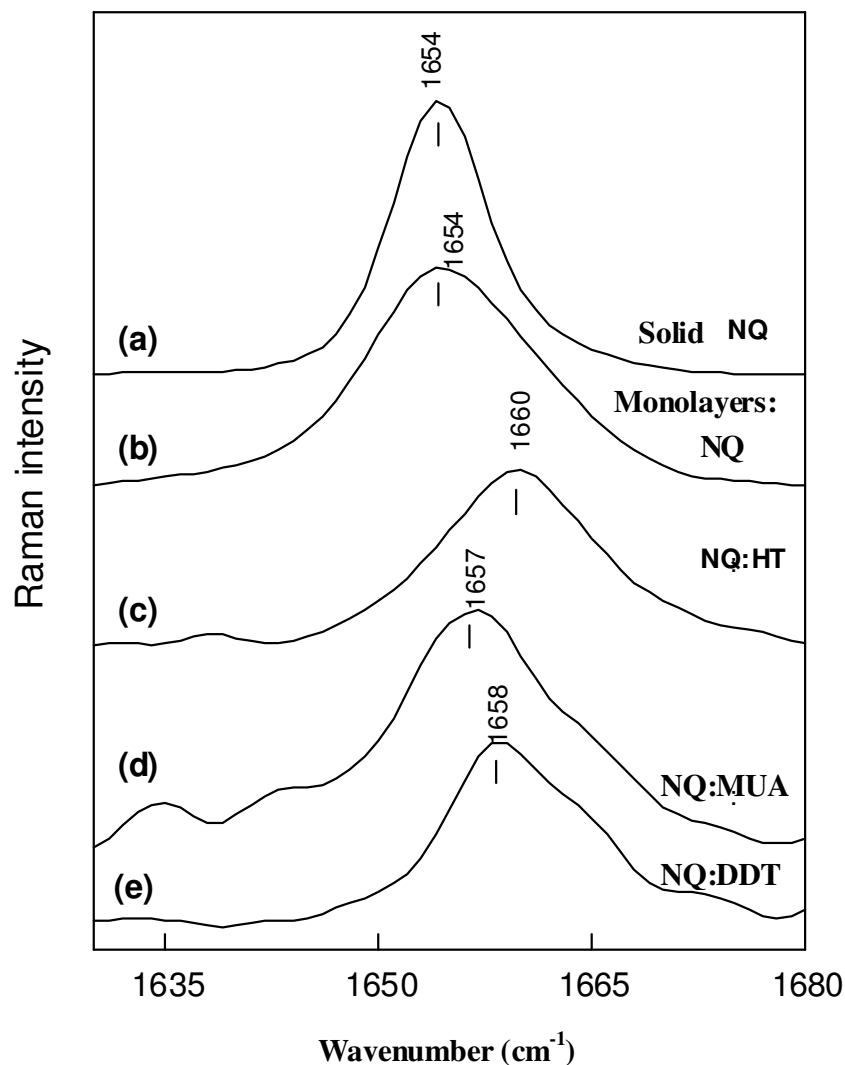


Figure 8. FT-Raman spectrum of solid compound 2-MeNQ(CH₂)₇SH (NQ) (a), FT-SERS spectra of Au electrode modified by pure NQ from 1 mM coating ethanolic solution (b), and 1 mM ethanolic solutions of mixtures (1:3 molar ratio) of NQ:HT (c), NQ:MUA (d), and NQ:DDT (e) in the spectral region of C=O symmetric stretching band. Surface spectra were recorded at 0.5 V (b, d and e) and 0.6 V (c) vs. SSCE. Intensities of the bands are scaled for clarity.

Comparison of self-assembled monolayers formed from 2-methyl-1,4-naphthoquinone ω -mercapto derivatives with different internal functionalities (“bridge”) in the side chains

The most important bridge in biological systems is an amide group, which is able to form hydrogen bonds inside the SAM. However, investigation of SAMs with attached

redox groups containing an amide bridge in the linker are scarce. The structure and electrochemical properties of the SAMs formed from derivatives 2-MeNQ(CH₂)_mCONH(CH₂)_nSH on the Au electrode were studied by FT-SERS and CV.

In order to determine the impact of the intrachain amide group on the electrochemical and structural parameters of the SAMs, the $E^{0'}$ and k_{app} values there compared with the corresponding data for previously studied compounds containing an ester group or an unfunctionalized hydrocarbon linker (Table 1). The $E^{0'}$ values of amides and the corresponding esters with $m = 1$ or 2 are shifted positively in comparison with hydrocarbons of the same length. This effect could be explained by the inductive action of these polar groups to the redox center. The difference in $E^{0'}$ disappears when $m + n + 2 \geq 7$. As concerns k_{app} , this parameter for amides is much higher than for the corresponding esters, but almost approaches the value for compounds with hydrocarbon linkers.

Table 1.

Comparison of formal reduction potential ($E^{0'}$, mV vs. SSCE) and heterogenous electron-transfer rate constants [$k_{app}(s^{-1})$] of SAMs obtained from of 2-methyl-3-[(CH₂)_m-X-(CH₂)_nSH]-1,4-naphthoquinones on Au measured in 0.1 M HClO₄ at 25 °C.

m, n	X = -CONH-		X = -COO-		X = -CH ₂ CH ₂ -	
	$E^{0'}$, mV	$-\lg[k_{app}(s^{-1})]$	$E^{0'}$, mV	$-\lg[k_{app}(s^{-1})]$	$E^{0'}$, mV	$-\lg[k_{app}(s^{-1})]$
1, 2	-	-	-40±7	2.0±0.5	-79±4	1.92±0.11
2, 2	-19±19	1.3±0.1	-56±4	2.8±0.2	-	-
2, 3	-73±5	2.8±0.3	-	-	-92±4	2.8±0.22
3, 2	-90±7	2.5±0.1	-84±12	3.4±0.7	-92±4	2.8±0.22
4, 2	-104±4	2.7±0.2	-92±14	3.2±0.4	-	-
4, 3	-	-	-108±11	3.2±0.5	-107±5	3.28±0.18
6, 3	-90±12	3.24±0.11	-123±4	4.5±1.1	-	-

In an attempt to explain the observed differences in the electron transfer rate of the above-mentioned compounds, containing the different intrachain bridges, the structure of the monolayers was studied in more detail by comparing the FT-SERS spectra of amides with the spectra of appropriate ester analogues. To this end, the CH₂ stretching region, which is known to be sensitive to the lateral interaction between *n*-alkyl chains, was used. The data obtained show that, in the SAM spectra, the wavenumbers of the asymmetric CH₂ stretching band ($\nu_{\text{asym}}(\text{CH}_2)$; in the vicinity of 2900 cm⁻¹) of the oxidized amides are 5-7 cm⁻¹ lower than those of the corresponding alkanoates, 2-MeNQ(CH₂)₂COO(CH₂)₂SH or 2-MeNQ(CH₂)₄COO(CH₂)₂SH, whereas the values are 9-10 cm⁻¹ lower for the reduced substrates. This implies that SAMs containing amide linkages have a higher degree of order than those of alkanoates.

Comparison of self-assembled monolayers formed from 1,4-naphthoquinone-3-(ω -mercaptoalkyl)amines on gold, silver and double layered Ag/Au surfaces

A complex electrochemical behavior is expected for biologically relevant quinones possessing a bulky aromatic NQ group because of π - π stacking interactions and distortions in the monolayer packing induced by large cross-sectional area of the terminal group. Objective of this part of the work was to study NQ derivatives bearing the redox-active moiety attached to the NQ ring via nitrogen atom and containing thiol-functionalized alkyl chains of short length for SAM-forming.

Cyclic voltammograms of the Au electrode modified by compound NQNH(CH₂)₂SH clearly show reduction and oxidation waves the parameters of which depend on the potential (*E*) sweep rate (ν). Cathodic and anodic peak-currents depend linearly on ν confirming irreversible coupling of the compound to gold. The estimated Γ values are about 2.2 times lower than the expected value of 6.1×10^{-10} mol cm⁻² for the close-packed assembly, considering that one vertically oriented NQ group occupies ca. 0.27 nm². This result shows that the monolayers are not close-packed, as expected for such short-chain thiol molecules. The $E^{0'}$ values are more negative by about 100 mV as compared with those reported previously for monolayers self-assembled on Au from short-chain 2-MeNQ derivatives containing (ω -mercaptoalkyl)alkanoate groups.

SERS spectra from Au are usually excited with a radiation longer than 600 nm, whereas spectra from Ag can be observed when shorter excitation wavelengths are used. Surface-enhanced resonance Raman scattering (SERRS) spectroscopy is particularly valuable for the studies of chromophoric groups in complex compounds, e.g. proteins, containing chromophoric cofactors. In this case, only the vibration of chromophoric group are probed without a perturbation by the vibration of protein backbone. However, electronic absorption bands of co-factors 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 electrode covered with a thin layer of Au.

The CV of the redox process of the SAMs obtained from NQNH(CH₂)₂SH on Au, Ag, and Ag/Au electrodes are given in Figure 9, and clearly demonstrate the similarity of Ag/Au and Au electrodes. The determined formal redox potential was found to be -0.53 V vs. SSCE at pH 7.

As one could expect, SERS spectra of NQNH(CH₂)₂SH SAMs obtained with the 1064 nm excitation, strongly depend on the electrode potential and only slightly on the nature of the metal. The disappearance of high frequency peak at 1675 cm⁻¹ characteristic of C=O stretching in quinone ring and the development of new bands located at 1380 and 719 cm⁻¹ characteristic of a substituted naphthalene ring unambiguously indicate that compound adsorbed on all three studied electrodes transformed to the reduced form. 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, it was suggested that the formation of the monolayer on the Ag surface results in a strengthening of the interaction between NQNH 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. The spectra observed from Ag/Au and Au electrodes are fairly alike.

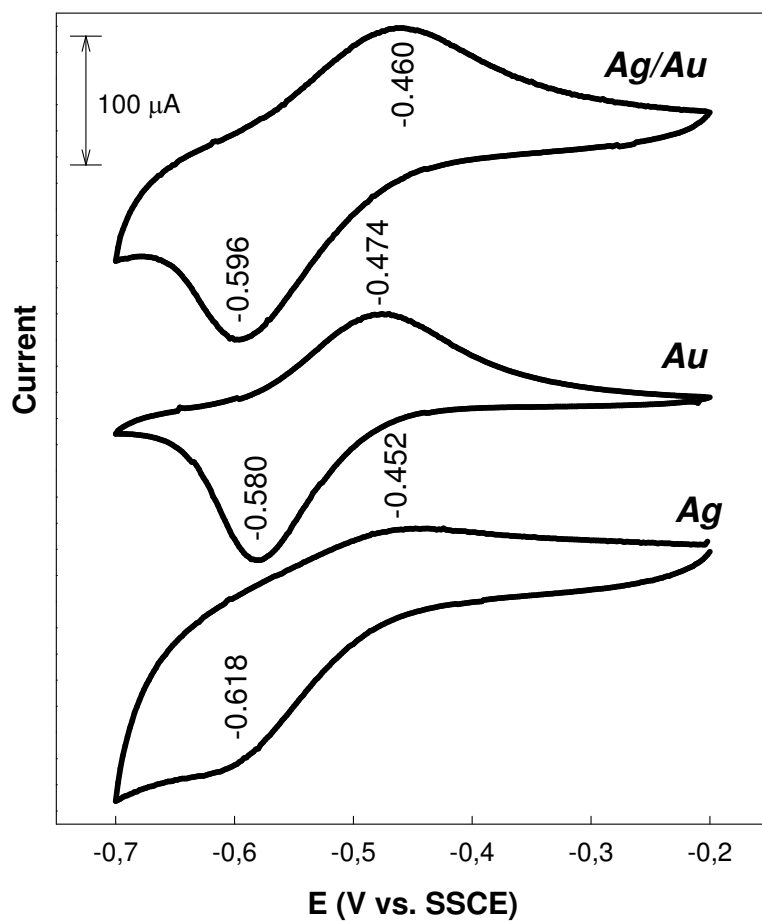


Figure 9. Cyclic voltammograms of redox of SAMs obtained from 1,4-naphthoquinone-3-(2-mercaptoethyl)amine on net Ag, double-layered Ag/Au and net Au electrodes (0.1 M Na_2SO_4 and 0.01 M FB, pH 7; potential scan rate 0.1V s^{-1}).

CONCLUSIONS

1. Electrochemical and spectroscopic studies demonstrate that newly synthesized biomimetic (2-methyl)-1,4-naphthoquinone ω -mercapto derivatives with 3 to 12 apparent carbon atoms in the side-chain form sufficiently compact and stable redox active self-assembled monolayers (SAM) on gold and silver surfaces bonding chemically through sulfur atom. Side-chains of the SAM are stretched towards solution, but have *gauche* defects in comparison with compounds in the solid state.
2. In the monolayers of compounds varying only in the chain length, the heterogenic electron transfer constant decreases with increasing chain length (the value of tunneling constant β slightly depends on the chemical structure of side chain and varies in the narrow range [0.8; 0.9]), and the formal redox potential shifts towards the negative values implying that the oxidation is more favourable than the reduction. This observation is associated with differences in the solvation of functional groups. The surface concentration (Γ) of SAM is practically independent on the chain length ($\Gamma \in [2,0; 3,1] \times 10^{-10}$ mol cm⁻²), but is from 2.2 (for the short-chain derivatives) to 1.5 times (for the long-chain derivatives) lower than the theoretical maximal value.
3. Ester (COO) and amide (CONH) groups in the side-chains of the 2-methyl-1,4-naphthoquinone derivatives shift the redox potential of SAM towards the positive values due the negative induction effect. In comparison with the amide group able to form intermolecular hydrogen bonds, the ester group decreases the order of SAM molecular organization, the surface concentration and the electron-transfer rate constant of these naphthoquinone derivatives.
4. SAM with the lower surface concentration formed from diluted coating solutions of 2-methyl-1,4-naphthoquinone derivatives undergo changes in the interfacial molecular orientation: redox active groups move closer to the gold surface and change the orientation from perpendicular to flat. The electron-transfer constant increases, and the formal redox potential shifts towards the positive side. This shows the decrease of stability of the oxidized form due to the decreased stacking

- interaction, and/or the increase of the stability of the reduced form due to the strengthening of hydrogen bond interaction.
5. In mixed monolayers of 2-methyl-1,4-naphthoquinone compounds and shorter simple alkanethiols, the repulsive interaction between redox active groups decreases, and the electron-transfer rate constant increases due to the particular structure of the SAM allowing easier attainment of redox centers by protons during electrochemical transformation. In mixed monolayers with longer alkanethiols, the redox conversion is not observed, but proceeds, if the terminal-group of diluting thiol is changed from CH₃ to COOH since the latter is able to provide protons for the redox process.
 6. SAMs of synthesized 1,4-naphthoquinone (ω -mercaptoalkyl)amines on Ag surface are more compact than on the Au surface due to the smaller radius of Ag atom. The structure-function properties of monolayers on Ag coated with the thin layer of Au are almost the same as on Au.

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SANTRAUKA

Sintezuotas didelis rinkinys naujų biomimetinių (2-metil)-1,4-naftochinono-3- ω -merkpto darinių, ir ištirta jų savitvarčių monosluoksnių ant aukso, sidabro ir dvigubo sluoksnio (sidabras, dengtas plonu aukso sluoksniu) paviršių struktūra ir redokso virsmai. Naudoti ciklinės voltamperometrijos (CV) ir paviršiaus sustiprintos Ramano spektroskopijos (PSRS) metodai. Junginių struktūra kietame būvyje ir tirpaluose palyginimui ištirta Ramano spektroskopijos metodu. Nustatyti elektrocheminiai parametrai: formalusis redokso potencialas E° , smailių plotis ties puse maksimalaus aukščio, apibūdinantis sąveiką tarp molekulių monosluoksnyje, paviršinė koncentracija Γ , elektrocheminės pernašos koeficientai αn ir $(1 - \alpha)n$, ir heterogeninės elektrono pernašos greičio konstanta k_{app} . PSRS metodu ištirti monosluoksnių struktūros ypatumai, sąlygojantys funkcinis parametrus. Palyginti junginių, besiskiriančių tik šoninės grandinės ilgiu, monosluoksniai. Iš elektrono pernašos greičio konstantos priklausomybės nuo grandinės ilgio nustatytas tuneliavimo eksponentinio slopimo faktorius β . Palyginti junginių, turinčių grandinėse įterptas skirtingas funkciškai aktyvias grupes (COO, CONH ir CH₂CH₂), sąlygojančias skirtingas tarpmolekulines sąveikas, monosluoksniai. Ištirta monosluoksnio paviršinės koncentracijos ir kitų parametrų priklausomybė nuo modifikacinių tirpalų koncentracijos. Palyginti mišrūs 2-metil-1,4-naftochinono darinių ir skirtingų alkantiolių, neturinčių redokso aktyvumo, monosluoksniai, įvertintas tiolių-skiediklių ilgis, galinės grupės ir komponentų koncentracijų santykis. Palyginti monosluoksniai ant Au, Ag ir Ag/Au paviršių.

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