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Adsorption Kinetics of Glutathione Monolayers on Platinum and Gold Surfaces used for Ca^{2+} Sensor Development

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Abstract: This work investigates the electrochemical study of glutathione self-assembled monolayers (SAMs) prepared from aqueous solution on gold and platinum surfaces. The deposit SAMs monolayers were characterized by cyclic voltammetry and electrochemical impedance spectroscopy methods. In addition, the interpretation of electrochemical impedance spectroscopy results was based on the charge-transfer reaction using potassium hexacyanoferrate (II/III) as redox-active probe. The structure and the properties of the deposit monolayers on the metal surfaces were obtained under optimal experimental conditions. It was shown that the adsorption kinetics is also influenced by some parameters such as the metal nature, the temperature, the pH and the immersion time in the thiol solution. The developed self-assembled monolayer (SAM-Glutathione) on the gold surface was applied to the detection of calcium ions. As a result, a stable and reproducible calcium sensor was obtained with a sensitivity of 58.4 KΩ/pCa in the linear range from 10^{-4} M to 10^{-1} M. A detection limit around 10^{-4} M for Ca^{2+} ions was obtained. *Copyright © 2014 IFSA Publishing, S. L.*

Keywords: Gold and Platinum Surfaces, SAM-Glutathione, Impedance Spectroscopy, Calcium Sensor.

1. Introduction

Adsorption of alkanethiols self-assembled monolayers (SAMs) on metal materials such as gold [1], platinum [2], silver [3], copper [4] and palladium [5] has been widely investigated to obtain functionalized organic surfaces. The study of organic monolayer systems has attracted a great deal of research attention because of their potential utility in the science of materials, chemical separation applications (electronic and optical devices, biosensors, protective layers). In addition, the self-assembled monolayers method can provide controlled and ordered organic films of few nm to several hundred-nm thickness. Moreover, SAMs containing functional head groups such as –COOH,–OH and $–NH_2$ that can easily be tuned by the bathing electrolyte solution are important and have been intensely studied. Thus, alkanethiols SAMs with several functionalities have been extensively investigated by many research groups [6-11]. Among many studied thiol molecules Glutathione

(L-γ-glutamyl-L-cysteinyl-L-glycine) plays various functions of biological processes [12]. It is a co-factor in several enzymatic reactions and plays an important role in intracellular cysteine storage, intra-organ cysteine transport, amino acid transport, disulfide bond reduction and detoxification of reactive electrophiles. Thus, GSH esters may be of practical importance in protection against radiation and various types of chemical toxicity produced during oxygen reduction. Therefore, the detection of GSH is of more and more significant importance. GSH contains three functional groups ($-NH_2$, $-COOH$ and a $-SH$ groups), of which $-SH$ group is involved in forming the surface monolayer with Au electrode and the polar $-COOH$ terminate heads are the key to interact with metallic ion [13]. Thus, Chow Edith and all have used voltammetric detection of cadmium ion using the glutathione-modified gold electrodes [14].

The present work describes the preparation and characterization of SAM of glutathione on gold and platinum surfaces with emphasis on application to chemical sensor. The adsorption of the GSH SAM was evaluated and analyzed by cyclic voltammetry and impedance electrochemical techniques in presence of $Fe(CN)_6^{3-/4-}$ as redox-active probe. Furthermore the developed Au-GSH SAM was applied to complexing the Ca^{2+} ions.

2. Experimental

2.1. Materials and Instrumentation

Glutathione (GSH reduced form) is purchased from Sigma chemical Co. without further purification. Glutathione solution and $CaCl_2$ were dissolved in phosphate buffer solution. Hexacyanoferrate (II/III) were obtained from Fluka and were used without further purification. All electrolytic solutions were prepared using ultra-pure water. All electrochemical measurements were performed using an Autolab (PGSTAT 302 N, Eco Chemie). A three-electrode cell was used with the glutathione SAM-modified electrodes (Au or Pt) as working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl/KCl as reference electrode. The geometrical area of the gold working electrode was 0.031 cm^2. Impedance measurements were performed in the frequency range from 0.1 to 100,000 Hz with signal amplitude of 10 mV. The calcium sensor response was recorded in 10 mM phosphate buffer solution (pH 7.4) containing 0.1 M NaCl. All measurements were performed in a dark Faraday cage.

2.2. Working Electrode Cleaning and SAM Formation

The gold and Pt working electrode surfaces (2 mm diameter) were polished with aqueous slurry of fine alumina powder (0.05 µm) on microcloth pads and rinsed with ultra-pure water. The working electrodes were then chemically cleaned in 1 M NaOH solution for 15 min. Then, the electrodes were rinsed with ultra-pure water and cleaned in 0.5 M sulfuric acid by cycling the potential from 0.2 to 1.3 V. The cleaning process end with dipping the working electrodes in piranha solution (H_2SO_4:H_2O_2 (30 %), 3:1) for 1 minute and rinsed with water immediately before immersion in GSH solution (10 mM). After the functionnalization process, the electrodes were rinsed with ultra-pure water to remove non-covalent attached glutathione molecules.

3. Results and Discussion

3.1. Influence of the Immersion Times

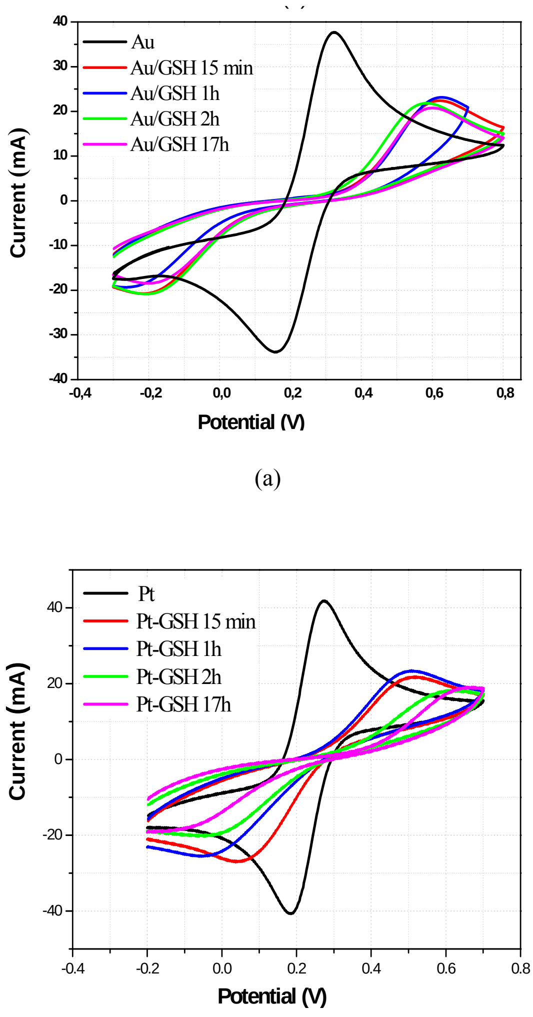
Cyclic voltammetry (CV) is an important technique to evaluate the blocking character of the monolayer coated electrodes using diffusion controlled redox couples as probes. This electrochemical method can be used effectively to understand the packing density and the distribution of pinholes and defects in nanostructured thiol monolayers [15]. It is well-known that a SAM can form a lipophilic barrier for electron transfer between the electrode surface and the hydrophilic electroactive probes in solution. Fig. 1a shows a comparison of voltammograms obtained from bare and SAM modified (Glutathione) gold surfaces after immersion in the glutathione solution during 15 min, 1 h, 2 h and 17 h using the redox couple $Fe(CN)_6^{3-/4-}$. When the immersion time increases the redox activity of the electroactive couple is inhibited because the monolayer becomes more compact. It can be seen that the bare Au electrode shows a reversible voltammogram for the redox couple explaining that the electron transfer reaction is completely diffusion controlled. In contrast, the decrease of the voltammogram peak of the monolayer-modified electrodes indicates that GSH was assembled on the Au electrode and acts to a blocking behaviour to the $Fe(CN)_6^{3-/4-}$ redox probe. A remarkable change in the cyclic voltammetry (Fig. 1b) of the modified platinum electrode was observed when the time of immersion alters leading to a decrease in the peak current and a larger ΔEp. This behaviour was less significant for the modified gold electrode in comparison to the modified platinum electrode treated in the same conditions.

This phenomenon can be attributed to the high affinity between the $-SH$ group and the gold surface resulting to a rapid adsorption and ordered glutathione monolayer in a few minutes.

However, in the case of the platinum surface we note that the glutathione layer becomes more densely and compact after a long immersion time.

Other electrochemical techniques are able to give quantitative analysis of surface such as impedance spectroscopy. This method is a powerful tool to determine the surface coverage of functionalized SAM glutathione electrode by evaluating the presence of pores and pinholes using the redox couple $Fe(CN)_6^{3-/4-}$ as probe molecule. Fig. 2 illustrates the impedance plots of the SAMs-modified Au and Pt electrodes in 5 mM $Fe(CN)_6^{3-/4-}$ for different immersion times.

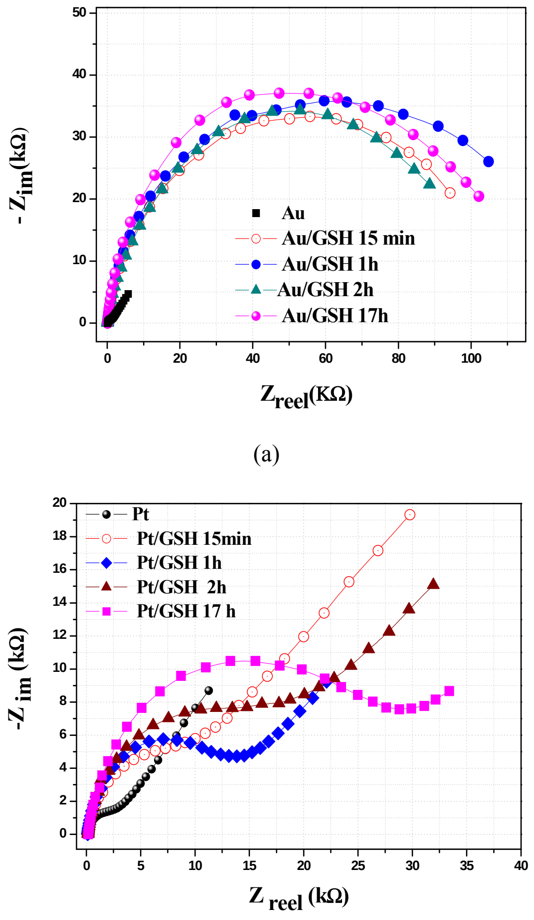
The corresponding impedance spectra are shown in Fig. 2a and 2b. It can be seen that the bare Au and Pt electrodes show a frequency straight line with a very small semicircle at high frequency region indicating a diffusion controlled process for the redox couple on the bare metal surfaces. However we observe that the impedance plots of Au modified electrode show large semicircles in the entire range of frequency signifying a blocking behaviour. The impedance values are fitted to standard Randle's equivalent circuit depicted in Fig. 3.



(a)

(b)

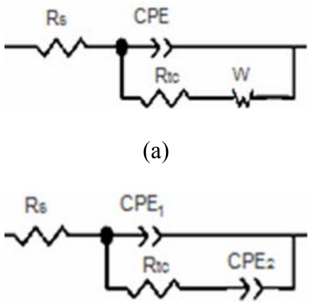
Fig. 1. Cyclic voltammograms at different immersion times, recorded in 10 mM PBS, 5mM $Fe(CN)_6^{3-/4-}$, 0.1 M NaCl at a scan rate 50 mVs^{-1}: (a) bare Au and Au-GSH modified electrodes; (b) bare Pt and Pt-GSH modified electrodes.



(a)

(b)

Fig. 2. Complex plane plots obtained for the faradic impedance measurements recorded in 10 mM PBS, 5 mM of $Fe(CN)_6^{3-/4-}$, 0.1 M NaCl at a scan rate 50 mVs^{-1}: (a) bare Au and Au-GSH modified electrodes; (b) bare Pt and Pt-GSH modified electrodes.



(a)

(b)

Fig. 3. Randles-type equivalent circuit of the Au-GSH (a) and Pt-GSH (b) electrodes.

Table 1, shows the charge transfer resistance values (R_{ct}) of bare and SAM-modified (glutathione) metal electrodes obtained from the numeric simulation of the impedance plots. As a result, it can be seen that the R_{ct} values of SAM-modified electrodes, as expected are higher in comparison with the bare electrodes. This result can be attributed to the inhibition of the electron transfer rate by the presence of the GSH monolayer on the electrodes surface. From the R_{ct} values, the apparent electrode coverage (θ) of GSH SAMs on the Au and Pt electrode surfaces can be approximately calculated according to the following equation (1) [16]:

$$\Theta = 1 - \frac{Rct}{Rct`}, \qquad (1)$$

where R_{ct} and $R_{ct'}$ are the charge transfer resistance of the bare and the modified metal electrodes, respectively. Based on the equivalent circuits presented in Fig. 3 many parameters were estimated such as the solution resistance (R_s), the charge transfer resistance (R_{ct}), the constant phase element (CPE), the frequency power (N), the diffusion impedance (W) and the surface coverage (θ). Al electrochemical parameters obtained from the numeric simulation of impedance spectra were gathered in Table 1 and Table 2.

Table 1. Electrochemical parameters of Au electrode modified after different immersion times, obtained from the analysis of impedance data with the Randles circuit in Fig. 3. The N1 values were around 0.9.

	Rs (Ω)	CPE_1 (µF)	R_{tc} (kΩ)	CPE_2 (µF)	N_2	θ (%)
15 min	128	2.283	107	4.555	0.029	99.3
1h	125	1.255	216	3.169	0.024	99.6
2 h	120	1.793	136	4.463	0.056	99.4
17 h	127	1.306	261	2.748	0.023	99.7

Table 2. Electrochemical parameters for Pt electrode modified after different immersion times, obtained from the analysis of impedance data with the Randles circuit in Fig. 3.

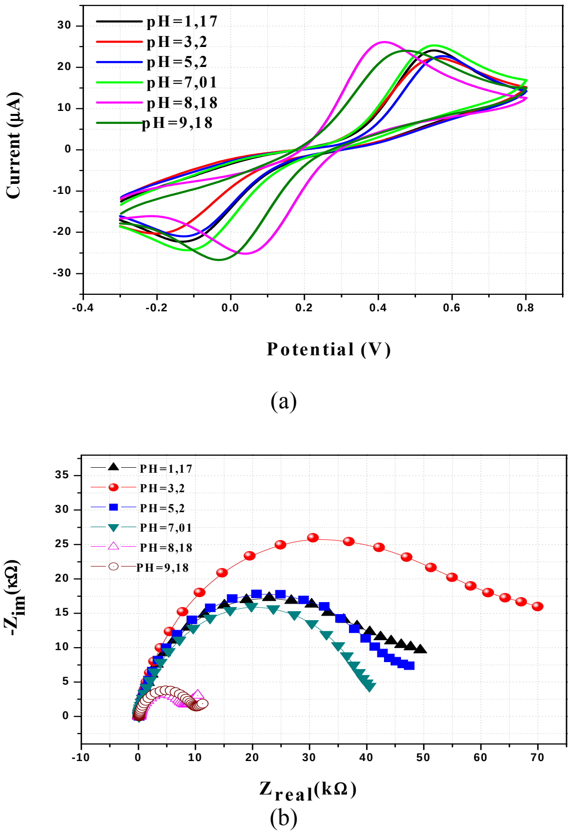
	Rs (Ω)	CPE (µF)	N	R_{tc} (kΩ)	W (µΩ)	θ (%)
15 min	156	1.609	0.899	18.078	53.357	79.6
1 h	153	1.8642	0.899	18.982	131.66	80.5
2 h	158	2.7803	0.756	20.084	63.185	81.6
17 h	160	2.124	0.903	24.772	100.82	85.1

As can be seen from the table values, the SAM formed on gold surface during 1 h presents a maximum coverage. This time may be sufficient to obtain a densely and packed monolayer. However, in the case of the SAMS-platinum electrode the coverage surface increases considerably with the immersion time. We conclude that glutathione molecules adsorb preferentially on the gold surface due to the high affinity between the –SH groups and the surface.

3.2. Influence of the Deposit Solution pH

The pH of the thiol solution used for the SAM preparation plays an important role on the electrochemical properties of the nanostructured layer. In our work, the deposit thiol contains reactive acid-base groups such as –SH, –COOH and $–NH_2$. These groups depend on the pH change and can alter the adsorption process of the thiol on the metal surface. For this reason, the effect of the pH deposition on the anchor thiol layer was also investigated. The GSH solutions were prepared in different pH phosphate buffer.

Fig. 4 shows the cyclic voltammogram and impedance spectra.



(a)

(b)

Fig. 4. Cyclic voltammograms (**a**) and Electrochemical impedance plots (**b**) of the Au-SAMs electrodes prepared at different pH recorded in 10 mM PBS, 5 mM of $Fe(CN)_6^{3-/4-}$, 0.1M NaCl at a scan rate 50 mVs^{-1}.

As can be seen in Fig. 4(a), the peak currents change significantly when the deposit pH varies. A large potential peak difference was obtained which indicates that the adsorption of glutathione layer decreases the reversibility of the redox process. The same behaviour was observed by impedance method as shown in Fig. 4(b). Indeed, a weak change of impedance was observed in the pH range from 5 to 7. When the deposit pH becomes around 3 we note a significant increase of impedance. For the pH values higher than 7 a better shift of impedance was obtained. This result indicates that the acid-base reactive groups have a considerable effect on the adsorption process. In a previous work, it was demonstrated that GSH molecule has four possibilities for acid dissociation [17]. This tripeptide is characterized by different pKa values: 2.1 (glu-COOH), 3.5 (gly-COOH), 8.7 (SH) and 9.6 (NH_2) [18]. The four acidity constants allow the glutathione molecule to adopt some different possible ionic forms such as protonated, deprotonated and two zwitterionic forms.

The protonation of GSH carboxyl groups leads to the absence of electrostatic repulsion interaction between the glutathione molecules during the adsorption process which confirms the high surface coverage (θ=99.4 %) as shown in Table 2. When the GSH carboxyl groups become deprotonated the -COO^- group can interact with the gold surface which minimize the adsorption of the glutathione molecules. For the basic deposit pH the presence of two deprotonated groups such as –COO^- and –S^- (=S) establish high repulsion between the glutathione molecules and leads to a less densely gold surface.

Table 2. Electrochemical parameters of Au-GSH SAMs obtained at different pH deposition, calculated from the analysis of impedance data with the Randles circuit shown in Fig. 3.

Parameters	**Rs (Ω)**	**CPE$_1$ (μF)**	**N$_1$**	**R$_{tc}$(kΩ)**	**CPE$_2$ (μF)**	**N$_2$**
pH = 1.17	112.61	1.363	0.927	21.555	127	0.056
pH = 3.2	120.2	1.793	0.905	136	4.463	0.056
pH = 5.2	113	2.670	0.843	39.918	559.84	0.503
pH = 7.01	118.38	2.3693	0.836	26.845	65.913	0.194
pH = 8.18	123.35	2.813	0.878	7.783	322.64	0.542
pH = 9.18	113.73	2.536	0.860	9.189	463.25	0.433

3.3. Influence of the Temperature on the Kinetic Adsorption

Temperature for the self-assembly process is another factor which influences the structural and the electrochemical properties of the SAM-GSH prepared on the Au electrode surface. The monolayers of glutathione were prepared at different temperature by immersing the fresh cleaned gold electrodes in the glutathione solution for 1 h. Fig. 5 illustrates the cyclic voltammograms and the impedance plots of the SAM-modified Au electrode prepared by varying the temperature of deposition from 16 °C to 90 °C. In Fig. 5(a) we observe a significant effect of temperature variation on the reaction kinetics of adsorbed glutathione molecules. Moreover, the potential peak of the $Fe\ (CN)_6^{3-/4-}$ redox couple was strongly shifted with the temperature variation.

The structure and the properties of the SAM-GSH at different pH has been discussed by several authors [19]. This result demonstrates that the SAM layer density and the structuration depend strongly on the pH variation which causes a rearrangement of the hydrogen bonding network within the adsorbate layer upon deprotonation [20].

This observation can be explained by the effect of temperature on the thiol layer organization. The same behaviour was observed by impedance measurements. Fig. 5(b) shows the impedance spectra of prepared SAMs for different temperatures. A critical temperature of 50 °C was obtained which corresponds to the maximal charge transfer resistance. For temperature values being lower or higher than this optimal temperature a decrease in R_{ct} was observed.

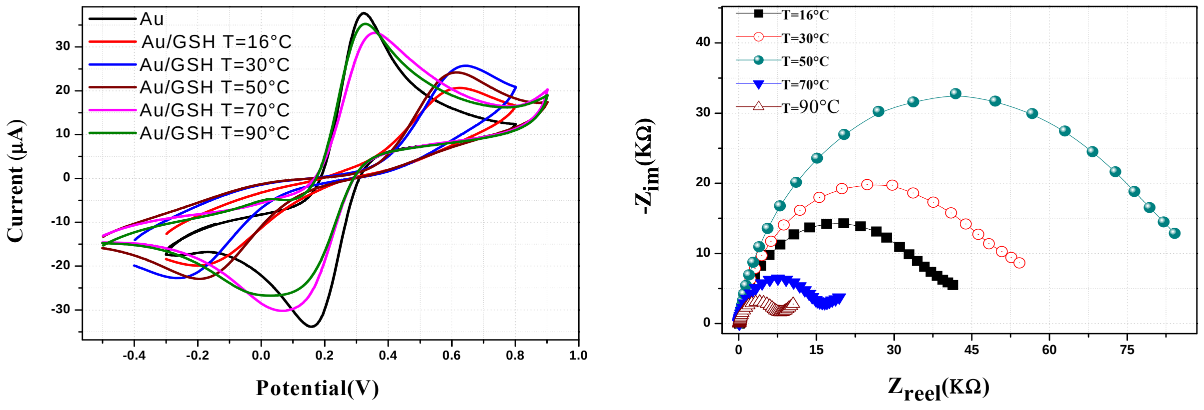
In Table 3, we report the numerical parameters obtained from the fitted impedance spectra. However, lower temperatures reduce the thiol molecule adsorption and the high temperature causes the desorption of the SAM which characterized by the decrease of the charge transfer resistance.

We conclude that the temperature preparation improves kinetics and reduces defects.

The temperature-dependent impedance can be modeled by the generalized Arrhenius equation (Eq. 2) for the extraction of the activation energy for adsorption (E_{ads}) and the activation energy for the desorption (E_{des}).

$$Z = Ae^{-Ea/RT}, \qquad (2)$$

where Z is the imaginary impedance, A is the constant, Ea is the activation energy for adsorption or desorption, R is the universal gas constant and T is the temperature. Using the Arrhenius plot type $Ln(Z_{im})$ vs. 1/T. The adsorption and the desorption energies can be deduced from the slope of the straight lines given by Fig. 6. For the adsorption and desorption of GSH we obtain E_{ads} = 18.76 kJ/mol and E_{des} = -54.07 kJ/mol, respectively. The important desorption energy value confirms that adsorbed thiols on Au surface do not desorb easily [21, 22].



(a)

(b)

Fig. 5. Cyclic voltammograms (a) and electrochemical impedance plots (b) of GSH modified electrode at different temperatures. Recorded in 10 mM PBS, 5 mM of $Fe(CN)_6^{3-/4-}$, 0.1M NaCl at a scan rate 50 mVs^{-}.

Table 3. Electrochemical parameters for Au-GSH modified electrode at different temperature, obtained from the analysis of impedance data with the Randles circuit in Fig. 3.

T (°C)	R_s (Ω)	CPE_1 (μF)	R_{tc} (KΩ)	CPE_2 (μF)	θ (%)
16	111	1.559	34.58	2.276	97.7
30	100	2.162	44.50	6.448	98.2
50	118	1.688	63.50	35.397	98.8
70	118	1.673	9.38	180.39	91.7
90	120	1.734	6.56	256.18	88.1

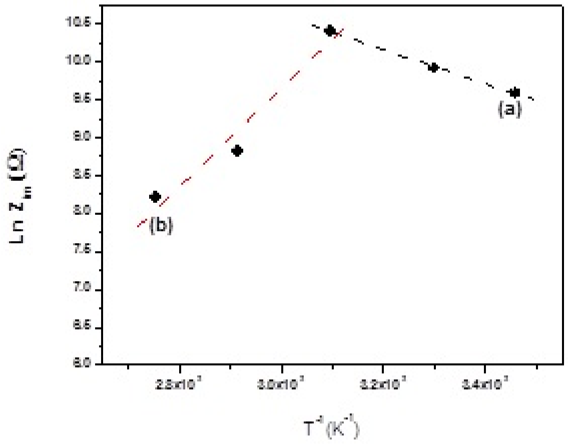


Fig. 6. Arrhenius type plot of the GSH layer deposit on Au surface at different temperatures. The adsorption and desorption energies can be obtained from the slope of the straight lines (a) and (b), respectively.

3.4. Desorption of the Deposit Glutathione Layer

Porter and co-workers found that n-alkanethiols adsorbed on gold surface can be both oxidatively and reductively desorbed [23]. The reductive desorption of glutathione from Au-GSH electrode was studied in 0.5 M NaOH. Fig. 7 shows the first scans of cyclic voltammograms corresponding to the bare Au, scan 1 and 10 consecutive scans for the Au-GSH electrode prepared for 1 h. The desorption voltammograme shows three peaks A, B and C at - 0.073 V, - 0.601 V and -1.049 V potentials, respectively. The existence of three peaks has been discussed in previous works by many researchers [24, 25]. The desorption of previously adsorbed glutathione is believed to occur according to the reaction (3) [26]. Furthermore, in this work the desorption of the adsorbed glutathione monolayer from the metal surface was studied in 0.5 M NaOH solution, cycling the potential between 0 and -1.3 V.

$$Au - SR + e^- \rightarrow Au + RS^- \qquad (3)$$

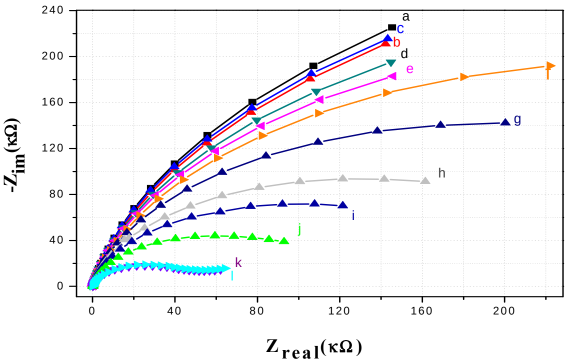
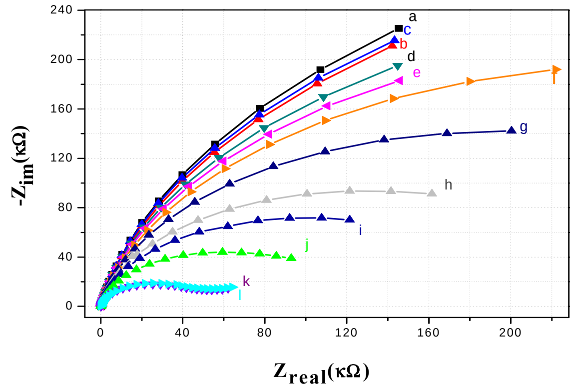


Fig. 7. Desorption spectra of glutathione from modified Au-GSH electrode (T_{im}=1h) in 0.5 M NaOH solution obtained in the potential range between 0 and -1.3 V vs. Ag/AgCl/KCl. Scan rate = 50 mV s^{-1}.

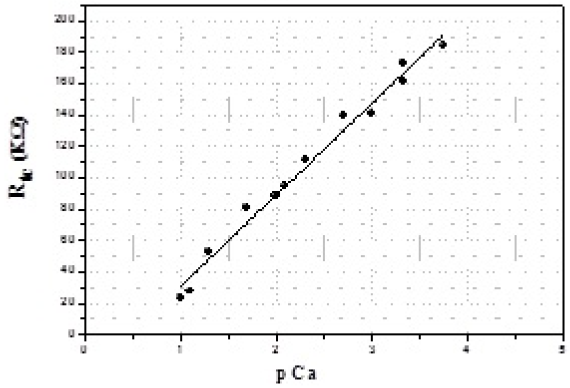
3.6. Interaction of Au-GSH Surface with Ca^{2+} Ions

«Ion-gating» phenomena of GSH SAMs modified electrodes by positively charged ions (Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Eu^{3+}) have been reported by previous cyclic voltammetry studies [27, 28]. There are eight active sites for bonding to metallic ions in the GSH ligand. For different metallic ions, multiple sites participate in the complex interaction, which means the complexes of GSH-metallic ions are formed with different molecular structural conformations. The developed Au-Glutathione SAM was applied for Ca^{2+} detection. Fig. 8(a) shows the impedance spectra of the glutathione modified electrode for different Ca^{2+} concentrations measured in 10 mM of phosphate buffer electrolyte at pH=7.4.

The increase of the Ca^{2+} concentration induces the decrease of the charge transfer resistance. The changes in glutathione configuration due to the –COOH and Ca^{2+} interaction may be the more critical reason. The significant shift of the impedance confirms that the terminal carboxyl groups of GSH coordinate strongly with Ca^{2+} [19]. The analytical response of the impedimetric calcium sensor can be represented by the variation of the charge transfer resistance versus the calcium concentration as shown in Fig. 8(b). A stable and linear response was obtained to calcium ion in the range from 0.1 mM to 0.1 M with a sensitivity of 58.4 KΩ/pCa. A lower detectable Ca^{2+} concentration was of 10^{-5} M was obtained.



(a)



(b)

Fig. 8. (a) Impedance spectra of GSH/Au sensor for different Ca^{2+}concentrations: (a) 0, (b) 10^{-5}, (c) 10^{-4}, (d) 5.10^{-4}, (e) 10^{-3}, (f) 2.10^{-3}, (g) 3.10^{-3},(h) 5.10^{-3}, (i) 10^{-2}, (j) 2.10^{-2}, (k) 6.10^{-2} and (l) 10^{-1}M, in a phosphate buffer solution, pH 7.4. The applied potential was 850 mV. (b) Variation of the b R_{tc} modified SAM electrode versus the Ca^{2+} concentration in a phosphate buffer solution, pH 7.4.

4. Conclusion

The electrochemical characterization of the self-assembled monolayers prepared by immersion of gold and platinum electrodes in glutathione solution was investigated using cyclic voltammetry and impedance spectroscopy methods. It was demonstrated that the kinetics adsorption of the GSH layer can be influenced by the metal type, the pH, the temperature and the immersion time in the thiol solution. In fact, the change of these parameters could alter the state of glutathione on the gold surface, leading to the corresponding change of voltammetry and impedance spectroscopy responses. As a result, we have observed that glutathione molecules present fast kinetic adsorption to be adsorbed preferentially at the gold surface than the Pt surface. The glutathione modified electrode was successfully applied to the detection of calcium ions and displays good linearity from 10^{-5} M to 10^{-1} M. Thus, a stable and reproducible calcium sensor was developed with a sensitivity of 58.4 KΩ/pCa.

Acknowledgements

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