1057

A Study on Adsorption of Electrogenerated Cystine Precipitate onto Au Electrode using Electrochemical Quartz Crystal Impedance System

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Abstract: An electrochemical quartz crystal impedance system has been applied to monitor generation of precipitate and adsorption of the precipitate onto Au electrode during electrochmical oxidation and $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ electrochemical catalytic oxidation of L-cysteine in phosphate aqueous buffer (pH 7.4). Significant decreases in the resonant frequency and increases in the motional resistance and the static capacitance were found during the precipitate adsorption, and the frequency shift found in solution was *ca.* 2 times that in air. Similar responses of quartz crystal impedance and a white precipitate were obtained during redox titration of L-cysteine solution using $K_3Fe(CN)_6$ solution. FT-IR analysis indicated that the white precipitate is cystine. The electrode collection efficiency of the electrogenerated cystine was estimated.

Keywords: Electrochemical quartz crystal impedance system, adsorption of electrogenerated precipitate, cystine/cysteine, $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$.

In situ quartz crystal impedance (QCI) measurement during electrochemical perturbations has found wide applications in investigating metal and polymer deposition *etc*[1-5], as it provides multidimensional *in situ* piezoelectric information. QCI analysis has been based on the Butterworth-Van Dyke (BVD) equivalent electrical circuit composed of a motional arm and a static arm in parallel. The motional arm contains three equivalent circuit elements in series, namely, the motional resistance R_1, the motional inductance L_1 and the motional capacitance C_1, while the static arm contains only the static capacitance C_0, and each equivalent circuit parameter is of certain physical meaning[6]. However, to the best of our knowledge, there are no reports on the monitoring of the crystallization process of solution species using this technique. In the present work, our goal is to study the generation of cystine precipitate *via* direct electrochemical oxidation or $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ electrochemical catalytic oxidation of L-cysteine in phosphate buffer (pH 7.4), and the adsorption of the precipitate onto Au electrode using an electrochemical quartz crystal impedance system (EQCIS). We found that, in vivid contrast to metal and polymer electrodeposition[1-3,5], the adsorption of the precipitate onto the electrode began only after a certain amount of cystine had formed and it never ceased immediately after the turnoff of electrochemical perturbations.

The EQCIS used included an HP 4395A Network/Spectrum/Impedance analyzer, an EG & G M283 potentiostat (under control of EG&G 270 software) and two personal

computers[3-5]. The conductance G and susceptance B of piezoelectric quartz crystal (PQC) resonance were measured synchronously on the HP 4395A equipped with an HP 43961A impedance test adapter and an HP 16092A test fixture. A user program was written in Visual Basic (VB) 5.0 to control the HP 4395A and to acquire admittance data *via* an HP 82341C high-performance HP-IB interface card for Windows 3.1/NT/95. This program was also applied to analyze the admittance data on time by fitting each group of the G and B data to the BVD model based on the Gauss-Newton non-linear least square fitting algorithm. R_1, f_0, $1/C_1$ and C_0 were used as estimation parameters during the fitting, where f_0 is defined as the resonant frequency at which the reactance of the motional arm vanishes, and $f_0=1/[2\pi(L_1C_1)^{1/2}]$ [1-6]. Equivalent circuit parameters were obtained at a time interval of *ca*. 2 s during QCI measurements. An AT-cut 9 MHz PQC (12.5 mm in diameter) was used. The gold electrode (6.5 mm in diameter) in contact with the solution was connected to the ground terminal of the HP 16092A and served as the working electrode, while another electrode of the PQC was placed in air. A saturated KCl calomel electrode (SCE) with a supporting electrolyte salt bridge served as the reference and all potentials in this work are referenced to it. A large-area platinum plate served as the counter electrode. The electrolytic cell had a volume of *ca*. 50 mL, and the volume of the investigated solution was fixed at 33.0 mL. Doubly distilled water and freshly prepared solutions were used throughout. All experiments were done at 26 ± 0.5^{o}C.

Figure 1 shows EQCIS responses in a potential cycling experiment for 40.0 mmol/L L-cysteine aqueous phosphate buffer (pH 7.4) containing 2.00 mmol/L $K_4Fe(CN)_6$ and 0.2 mol/L Na_2SO_4. Frequency decreases and increases in R_1 and C_0 were found after a certain electric quantity has been consumed, and such responses continued for a while after the turnoff of the potential cycling perturbation. Stirring of the solution could recover the initial values of f_0, R_1 and C_0. Increase in R_1 suggests energy dissipation of the PQC oscillation and indicates a viscous loading on the PQC electrode, and increase in C_0 implies changes in the dielectric properties between the two PQC electrodes. We found similar responses of f_0, R_1 and C_0 only for concentration of L-cysteine greater than 30 mmol/L, and the frequency decreases and increases in R_1 and C_0 in 80 mmol/L L-cysteine solution were more significant than those in **Figure 1**. It is well known that cysteine can adsorb on an Au electrode at the monolayer level *via* S-Au bonding[7]. However, this kind of adsorption should cause only *ca*. 20 Hz of frequency decrease for a 9 MHz PQC[8], suggesting that the much greater responses here should result from a different mechanism from the direct adsorption of cysteine *via* the S-Au bonding and its desorption.

Although L-cysteine itself could be electrochemically oxidized experimentally, the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ electrochmical catalytic oxidation of L-cysteine has been confirmed from the greater current found in the presence of $K_4Fe(CN)_6$ than that in its absence. In the similar potential cycling experiment to that shown in **Figure 1** for the 40.0 mmol/L L-cysteine solution but containing no $K_4Fe(CN)_6$, no similar decrease in f_0 and increases of R_1 and C_0 were found. However, such responses have been observed in double potential step experiments for L-cysteine solutions at concentration of 30 mmol/L or greater even containing no $K_4Fe(CN)_6$, as shown in **Figure 2**, and the initial values f_0, R_1 and C_0 could also be well recovered after the solution stirring. **Figure 3** shows simultaneous responses of Δf_0, ΔR_1 and ΔC_0 during redox titration of a stirred solution containing 40.0 mmol/L L-

cysteine with $K_3Fe(CN)_6$ solution. White precipitate formed after the addition of 480 µL of 1.00 mol/L $K_3Fe(CN)_6$ solution to the 33.0 mL L-cysteine solution, and decrease in f_0 as well as increases in R_1 and C_0 were simultaneously observed as well.

All the above results suggest that a precipitate formed after a certain amount of L-cysteine had been oxidized electrochemically or chemically, and its adsorption onto the electrode gave responses of equivalent circuit parameters as above. We think that the

Figure 1. EQCIS responses during potential cycling between –0.2 V and 0.5 V *vs*. SCE (**A**) and time-dependent EQCIS responses during and after the potential cycling perturbation (**B**) in 40.0 mmol/L L-cysteine + 2.00 mmol/L $K_4Fe(CN)_6$ + 0.20 mol/L Na_2SO_4 + 0.06 mol/L NaH_2PO_4 + 0.14 mol/L Na_2HPO_4 (pH 7.4). dE/dt = 20 mV/s. Arrows in **B**: 1 and 2 indicate the start and the cease of the potential cycling perturbation, respectively; 3 indicates the onset of the solution stirring with a magnetic stirring bar.
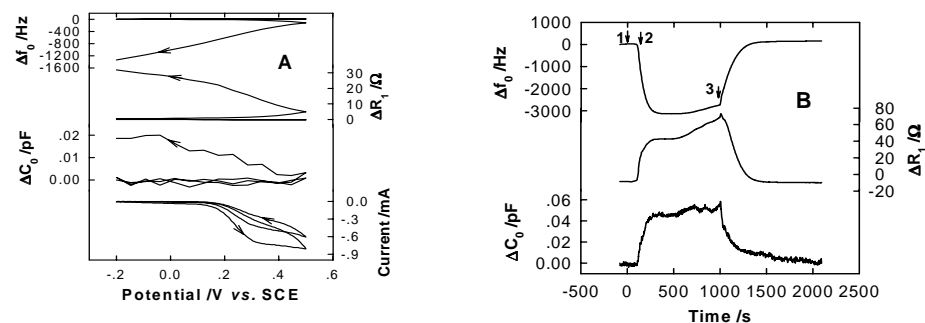

Figure 2. Simultaneous responses of Δf_0, ΔR_1, ΔC_0 and current during and after double potential step perturbation between –0.2 and 0.5 V *vs*. SCE in 80.0 mmol/L L-cysteine aqueous solution containing 0.20 mol/L Na_2SO_4 + 0.06 mol/L NaH_2PO_4 + 0.14 mol/L Na_2HPO_4 (pH 7.4). The arrow indicates the time when the solution stirring began.
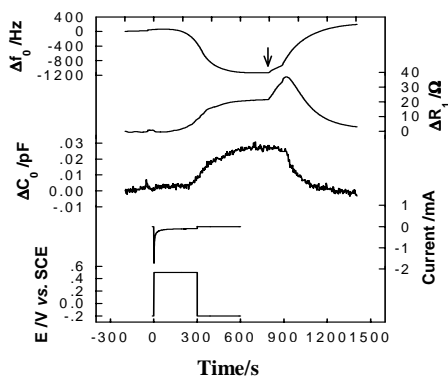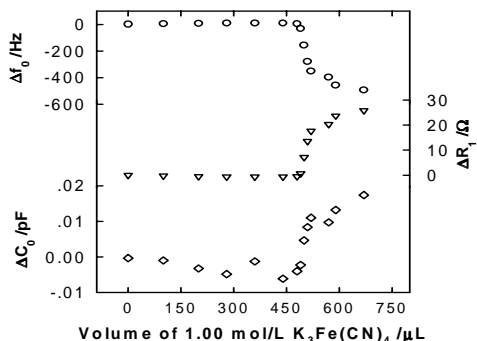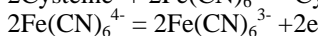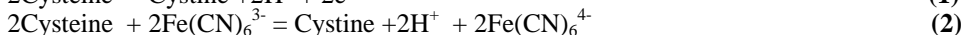
Figure 3. Simultaneous responses of Δf_0, ΔR_1, ΔC_0 during redox titration of 40.0 mmol/L L-cysteine + 0.20 mol/L Na_2SO_4 + 0.06 mol/L NaH_2PO_4 + 0.14 mol/L Na_2HPO_4 (pH 7.4) with 1.00 mol/L $K_3Fe(CN)_6$ + 0.20 mol/L Na_2SO_4 + 0.06 mol/L NaH_2PO_4 + 0.14 mol/L Na_2HPO_4 (pH 7.4). Data shown were recorded at the 2 min moment after each injection of $K_3Fe(CN)_6$ solution.

white precipitate may be cystine. To confirm this idea, we collected the white precipitates produced after the exhausted electrolysis of L-cysteine and after the redox titration of L-cysteine solution using $K_3Fe(CN)_6$ for FT-IR analyses, together with a

standard cystine reagent as a comparison. We found from FT-IR spectra perfect coincidence of IR peak positions of the precipitates with those of the standard cystine reagent, demonstrating undoubtedly that the white precipitate should be cystine and the responses of Δf_0, ΔR_1 and ΔC_0 must result from adsorption of the precipitate onto the PQC Au electrode.

By considering all the results given above, reaction schemes as follows are postulated (for simplicity, charge of the amino acids in the solution is not considered)

$$2\text{Cysteine} = \text{Cystine} + 2H^+ + 2e \tag{1}$$

$$2\text{Cysteine} + 2Fe(CN)_6^{3-} = \text{Cystine} + 2H^+ + 2Fe(CN)_6^{4-} \tag{2}$$

$$2Fe(CN)_6^{4-} = 2Fe(CN)_6^{3-} + 2e$$

$$2\text{Cysteine} + 2Fe(CN)_6^{3-} = 2Fe(CN)_6^{4-} + \text{Cystine} + 2H^+ \tag{3}$$
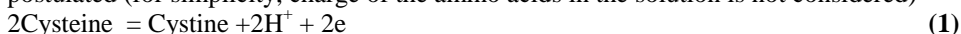
where **(1)** can be used for describing the process of direct electrochemical oxidation of L-cysteine (**Figure 2**), while **(2)** for the redox titration (**Figure 3**) and **(3)** plus **(1)** for the electrochemical process in the presence of $K_4Fe(CN)_6$ (**Figure 1**). The product cystine may become insoluble, depending on the solution condition and the amount of cystine. For example, according to **(2)** and results shown in **Figure 3**, precipitation of cystine occurred when the concentration of cystine produced in the solution exceeds 7.3 mmol/L.

For the present systems accompanied with large changes in R_1 (*i.e.* the viscous loading case), the mass of the adsorbed precipitate on the electrode can not be correctly estimated from values of Δf_0 found in solution with the Sauerbrey equation, due to its applicability only to the rigid loading case (*e.g.* film loading in the dry state)[6]. Hence, we measured the "dry" frequency shift (found in air for the dry precipitate films), Δf_{0g}, as a function of the "wet" frequency shift (in solution), Δf_{0L}. For 11 values of Δf_{0L} between 1000 and 20000Hz, we obtained $\Delta f_{0g} = 0.5208\Delta f_{0L} + 179.3$ (r=0.9973). Therefore, we may evaluate the electrode collection efficiency (ECE) of the electrogenerated cystine for each experiment, which is defined as the ratio of the final mass of the adsorbed precipitate, m_a, to the total mass of the cystine produced, m_t. Results are m_a=2.82 μg, m_t=34.6 μg and ECE= 8.15% for the experiment shown in **Figure 1**, and m_a=0.798 μg, m_t=58.8 μg and ECE= 1.36% for the experiment shown in **Figure 2**, respectively. Scattered ECE values should suggest that, as expected, ECE depends greatly on the production rate of cystine, the local concentration of cystine near the electrode surface and the solution condition.

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