disulfide. *Electrochim. Acta*, 1992, 37:403

4 Lin Z H, Huang S H, Luo J. Electrode reaction kinetics of cytochrome c at gold electrode. Book of Abstracts: 45th Annual Meeting of the International Society of Electrochemistry, Porto, Portugal. 1994:O1-10

5 Lin Z H, Huang S H, Luo J. In situ FTIR and UV/Vis study of the adsorption of cytochrome c on gold electrode. Book of Abstracts: 45th Annual Meeting of the International Society of Electrochemistry, Porto, Portugal, 1994: I-7

6 Luo J, Lin Z H, Huang S H, Wu J T. In situ FTIR and UV/Vis study of the adsorption of cytochrome c on gold electrode. *Chinese Chem. Lett.*, 1994, 5:971

7 Szucs A, Novak M. Stable and reversible electrochemistry of cytochrome c on bare electroeds Part Ⅰ: effects of ionic strength. *J. Electroanal. Chem.*, 1995, 383:75

8 Szucs A, Nouak M. Stable and reversible electrochemistry of cytochrome c on bare electrodes Part Ⅱ: effects of experimental conditions. *J. Electroanal. Chem.*, 1995, 384:47

9 Luo J, Lin Z H, Tian Z W. A combined measurement system for UV/Vis in situ spectroelectrochemical study. *Chinese Chem. Lett.*, 1991, 2:309

10 Laviron E. Adsorption, autoinhibition and autocatalysis in polarography and in linear potential sweep voltammetry. *J. Electroanal. Chem.*, 1974, 52:355

11 Southampton Electrochemistry Group. *Instrumental Methods in Electrochemistry*, Chapter 6. New York: Ellis Horwood Limited, 1985

12 Ogoshi H, Saito Y, Nakamoto K. Infrared spectra and normal coordinate analysis of metalloporphins. *J. Chem. Phys.*, 1972, 57:4 194

13 Srininasan S, Gileadi E. The potential-sweep method:a theoretical analysis. *Electrochim. Acta*, 1966, 11: 321

14 Marcus R A, Sutin Norman. Electron transfers in chemistry and biology. *Biochim. Biophys. Acta*, 1985, 811:265

# 细胞色素 c 在经预处理的金电极上电化学行为的研究①

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**摘要**　本文观察到了吸附态和本体态的细胞色素 c 在经预处理的金电极上的准可逆反应。采用现场 FTIR 光谱法、紫外可见反射光谱法、循环伏安法、交流阻抗法和电位阶跃法研究了细胞色素 c 吸附行为和反应动力学。结果表明,细胞色素 c 能够满单层、不可逆地吸附在金电极表面。在无促进剂存在情况下,吸附的细胞色素 c 层能够成为溶液相细胞色素 c 在电极上进行氧化还原反应的电子递体.

**关键词**　细胞色素 c, 金电极,电化学活性,现场光谱法

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# Studies of Electrochemical Behaviors of Cytochrome C on Pretreated Gold Electrode ①

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**Abstract** The quasi-reversible responses of both adsorbed and bulk cytochrome c at the pretreated gold electrode are observed. The adsorption behaviors and reaction kinetics have been studied using *in situ* FTIR spectroscopy, UV/Vis reflection spectroscopy, voltammetry, AC impedance and potential step methods. The results show that the cytochrome c can irreversibly adsorb on the gold surface in a complete monolayer and the adsorbed layer can mediate the redox reaction of the cytochrome c in the solution in the absence of promoter.

**Key words** Cytochrome c, Gold electrode, Electrochemical activity, *In situ* spectroscopies

## INTRODUCTION

The electrochemical processes of cytochrome c have been widely studied in order to understand electron transfer mechanisms in biological systems. In particular, electron transfer between electrodes and cytochrome c has received considerable atttention. Most research works were carried out in the presence of promoters. Usually, proteins can adsorb strongly on electrode surfaces from aqueous and the adsorbed layer plays an important role in the electrode reactions of these proteins from the bulk of solution [1]. However, previous works showed that cytochrome c was electroinactive at the thermodynamic potential at a gold electrode in the absence of promoters[2], and adsorbed cytochrome c could only mediate the reduction of cytochrome c in solution via electron transfer through the unfolded protein layer[3]. Recently, the quasi-reversible responses of both adsorbed and bulk cytochrome c were observed at the bare gold electrode with suitable pretreatment in the absence of promoters by our research group[4-6]. The research research results by A. Szucs and M. Novak also suggested that the adsorption of the protein molecules, and reversible electron transfer can be observed if it is the monomeric form of cytochrome c[7,8]. In this paper, the adsorption behaviors and reaction kinetics of cytochrome c at a bare gold electrode are studied using *in situ* FTIR spectroscopy, UV/Vis reflection spectroscopy, voltammetry, AC impedance and potential step methods.

① Received 16 May, 1996

## EXPERIMENTAL

Horse heart cytochrome c (type Ⅵ), which purity is 96 % cytochrome c based on molecular weight 12,384 and 4 % $H_2O$, was purchased from the Sigma Chemical Company. All chemicals used were of analytical reagent quality. A gold disk (apparent area: 0.28 $cm^2$) was as the working electrode, a platinum ring as the counter electrode and an aqueous saturated calomel electrode as the reference electrode. All the experiments were carried out at room temperature (around 20 ℃) and the electrode potentials are represented with respect to SCE in this paper.

Electrochemical measurement and AC impedance measurement were performed using the Electrochemical System (PARC M370) and AC Impedance System (PARC M378). For FTIR and UV/Vis spectroscopic measurments, the FTIR apparatus (Nicolet 730), UV/Vis Spectrometer (Shimadzu UV-240), and the combined spectroelectrochemical measurement system[9] were used, respectively.

## RESULTS AND DISCUSSION

### 1 Dependence of Electroactivity of Cytochrome c upon Electrode Pretreatment

The cyclic voltammograms of the gold electrode in a phosphate buffer solution without cytochrome c (blank solution) is shown in Fig. 1-1. For studying dependence upon electrode pretreatment, the gold electrode was pretreated under different conditions. The potential of the gold electrode was first cycled with 100mV/s sweep rate between the potentials of hydrogen evolution and oxygen evolution (- 0.9V ～ + 1.2V) in a phosphate buffer solution (pH7.0) (containing 0.1mol/L $NaClO_4$). After seven cycles (about five minutes), Condition Ⅰ at - 0.9V (potential of hydrogen adsorbing); Condition Ⅱ at - 0.3V (potential of double-layer charging); Condition Ⅲ at + 0.7V (start potential of oxygenic species adsorbing); Condition Ⅳ at + 1.2V (start potential of oxygen evolution).

The cyclic voltammograns of the gold electrode with different pretreatment in a phosphate buffer solution containing 5 mg/mL (388 umol/L) cytochrome c is showm in Fig. 1-2. The background current decreases with repetitive scanning in Fig. 1-2. A couple of peaks which are attributed to redox reactions of cytochrome c appear in Fig. 1-2(Ⅰ) and 1-2(Ⅱ). The results indicate that cytochrome c is electroactive at the gold electrode if the electrode is pre-polarized at the potential of hydrogen adsorbing (Condition Ⅰ) or at the potential of double-layer charging (Condition Ⅱ), and cytochrome c is electroinactive at the gold electrode if the electrode is pre-polarized at the potential of oxygenic species adsorbing (Condition Ⅲ) or oxygen evolution (Condition Ⅳ). It is shown that existence of oxygenic species on the gold electrode surface will cause inactivating of cytochrome c. The AC impedance spectra are also confirm the existence of oxygenic species on the electorde surfaces and the affecting to electroactive of cytochrome c.

The experiments also indicate that the cytochrome c can also be electroactive at the Condition

Ⅰ or the Condition Ⅱ if they are immersed at first in the phosphate buffer solution containing 5mg/mL cytochrome c for two hours. It is interesting that the cytochrome c will lose its activity if the electrode (Ⅰor Ⅱ), at which the cytochrome c is already electroactive, is controlled at +0.7V for ten minutes in the solution contining cytochrome c because of the effect of oxygenic species adsorbing.

Therefore the electrochemical treatmtnt for the bare gold electrode is crucial in the electrochemical activity of the cytochrome c, regardless of the adsorbed one or the bulk one. The cytochrome c will loss its electrochemical activity if the final potential is controlled at a more anodic potential than the one of the adsorption of oxygenic species.

## 2 Voltammetric Studies of Adsorbed Cytochrome c and Bulk Cytochrome c

In the following experiments, the gold electrode with adsorbed cytochrome c was prepared as follows: (1) the surface of gold electrode was polished mechanically with emery paper and 0.5μm alumina slurry (double-layer capacity $C_{dl}$, ~15μF/cm$^2$); (2) the surface was cleaned electrochemically by cycling the electrode potential between the potentials of hydrogen evlution and oxygen evolution (-0.9V ~ +0.2V) for five minutes and then was controlled at -0.9V for five minutes in the phosphate buffer solution (pH 7.0) ($C_{dl}$, 60~80μF/cn$^2$); (3) the gold electrode was immediately immersed in the phosphate buffer solution containing 5mg/mL cytochrome c for 2 hours or cycled the electrode potential in the redox potential range of the cytochrome c till occurring a stable voltammogram with the redox current peaks.

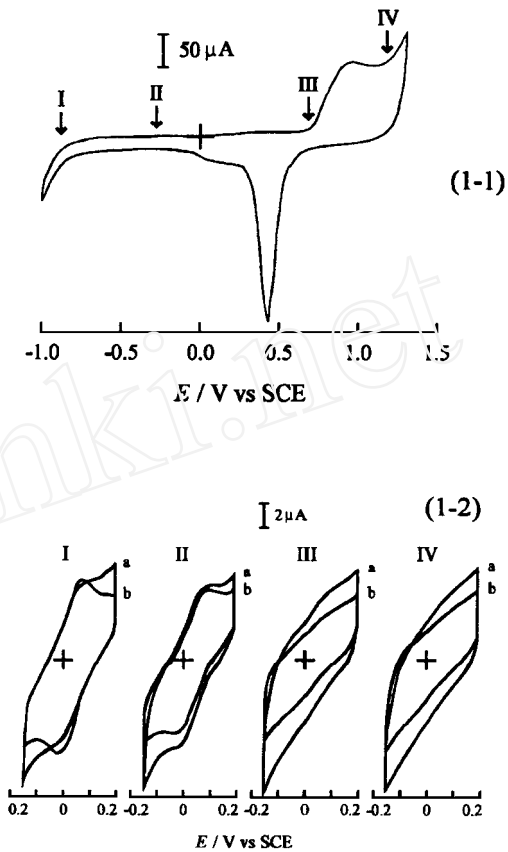


Fig. 1-1) Voltammogram of a gold electrode in the phosphate buffer solution containing 0.1 mol/L $NaClO_4$ (pH 7.0)

1-2) Voltammograms (a: the first cycle; b: the cycle cycling till ten minutes) of the gold electrode with different pretreatment (Condition Ⅰ, Ⅱ, Ⅲ and Ⅳ) in a phosphate buffer solution containing 5 mg/mL cytochrime c.

Sweep rate: 100 mV/s. Apparent area of gold electrode: 0.28 cm$^2$

The stable cyclic voltammograms of the gold electrode with adsorbed cytochrome c in the

phosphate buffer solution (pH7.0) without cytochrome c are shown in Fig. 2-1. The anodic and the cathodic peak potentials ( $E_{pa}$ and $E_{pc}$ ) are near 50mV and near 0mV respectively when the sweep rate $\upsilon$ is 100mV/s. Fig. 2-1 shows that the redox reaction of the adsorbed cytochrome c at the gold electrode is a quasi-reversible electron transfer reaction. The rate constant $K_s$ is estimated as $1.9 s^{-1}$ according to the kinetic equations

$$E_{pa} = -(2.3RT/\beta n_a F)\log_{10}(RTK_b/\beta n_a F\upsilon)$$

and

$$K_s = K_b \exp(\beta E^{0\prime} F/RT)$$

of the irreversible electron transfer reaction of adsorbed species from the voltammogram ( $E_{pa}$ = 0.29V vs NHE, $E^{0\prime}$ = 0.26V vs NHE, $\beta$ = 1 - $\alpha$ = 0.5, $n_a$ = 1 )[10]. This rate constant is slightly less than the real value. It is shown that electron transfer of the adsorbed species was rather fast.

The open circuit potential of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution (pH7.0) containing 5mg/mL cytochrome c was 60mV, and it indicated that the cytochrome c was in a state of oxidized. The cyclic voltammograms of the gold electrode with adsorbed cytodhrome c in the phosphate buffer solution containing 5mg/mL cytochrome c are shown in Fig. 2-2. For 100mV/s sweep rate, the anodic and the cathodic peak potentials are at +65mV and at -5mV, respectively. Their difference $\Delta E_p$ is 70mV, greater slightly than that ($\Delta E_p$ = 59mV) of reversible electron transfer. The shapes of the anodic peak and the cathodic peak are similar, and their peak current values are approximately equal. The peak current showm in Fig. 2-2 is directly proportional to the square root of the sweep rate at slow sweep rate, and the peak potential is almost independent of the sweep rate. The above results show that the redox reaction of bulk cytochrome c at the gold electrode with adsorbed cytochrome c is a quasi-reversible reaction. The diffusion coefficient $3.2 \times 10^{-7} cm^2/s$ for the cytochrome c in the buffer solution may be derived from the slope of the curve ($5.5 \times 10^{-7} A \cdot S^{1/2} \cdot mV^{-1/2}$), and is much lower than the value of $1.1 \times 10^{-6} cm^2/s$ from non-electrochemistry[2]. The formal standard rate constant $K_s$ is calculated as $7.1 \times 10^{-3} cm/s$ from the peak potential difference $\Delta E_p$ according to the working curve of $n \cdot \Delta E_p$ as a function of the vari-

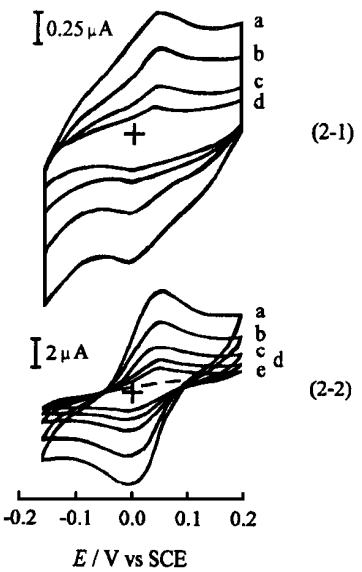


Fig. 2　Voltammograms of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution (pH 7.0) without cytochrome c (1) and containing 5mg/mL cytochrome c (2).
Sweep rate: (a) 100 mV/s; (b) 50 mV/s; (c) 20 mV/s; (d) 10 mV/s; (e) 5 mV/s. Apparent area of gold electorde: 0.28 $cm^2$.

able $\psi$ Defined by the equation

$$\psi = (RT)^{1/2} K_s / (n FD\pi\nu)^{1/2}$$

for quasi-reversible electron transfer reaction[11]. It is shown that the adsorbed cytochrome c can mediate the redox reaction of the cytochrome c in the solution via electron transfer.

## 3 In Situ FTIR and UV/Vis Spectroscopic Studies of Adsorbed Cytochrome c

The *in situ* FTIR difference spectra and the UV/Vis reflection spectra between the oxidizing potential and the reducing potential of the adsorbed cytochrome c at the gold electrode in the phosphate buffer solution were obtained. The positive of negative IR difference spectrum bands near 1 397 $cm^{-1}$, 1 454 $cm^{-1}$, 1 538 $cm^{-1}$ and 1 670 $cm^{-1}$ (Fig. 3) according to the oxidizing-minus-reducing or the reducing-minus-oxidizing potential can assign to the porphyrin ring vibrational modes in the cytochrome c molecule[12]. The UV/Vis spectrum band at 420 nm can ascribe to the characteristic Soret band for the cytochrome c, and it is a bipolar band due to Soret band shift of cytochrome c at different potentials (Fig. 4). The resulting FTIR and UV/Vis spectra confirm adsorption of the cytochrome c on the gold surface, redox reaction of the adsorbed cytochrome c at the gold electrode and effect of a potential on the spectroscopic behaviour of the absorbed cytochrome c.

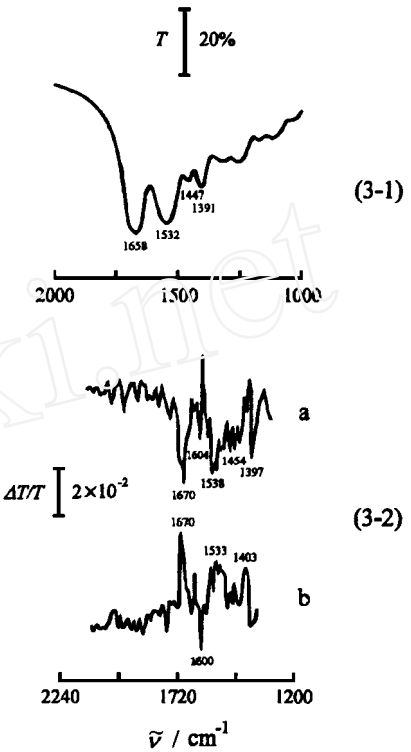


Fig. 3-1) Transmission IR spectrum of cytochrome c (KBr pellet).

Fig. 3-2) *In situ* FTIRdifference spectra of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution (pH7.0).
Step potentials: (a) $E1$ = 200 mV, $E2$ = - 200 mV; (b) $E1$ = - 200 mV, $E2$ = + 200 mV.

## 4 AC Impedance and Potential Step Measurement Studies of Adsorbed Cytochrome c and Bulk Cytochrome c

The AC impedance spectra were measured during adsorbing process of cytochrome c. The admittance spectra ($Y'\omega$-$Y''\omega$) with time are shown in Fig. 5-1, and the period of sampling a curve is seven minutes. Fig. 5-1 shows that the adsorption of the cytochrome c incurs decrease of double-layer capacity. The double-layer capacity decreases quickly at initial stage, and remains a certain value ($C_{dl}$, ~10 $\mu F/cm^2$) after two hours. It is shown that the adsorbing process of cytochrome c was slow. The adsorption charge 1.38 $\times 10^{-6}$ $C/cm^2$ is acquired from the peak current in the voltammogram of the adsorbed cy-

tochrome c (Fig. 2-1, $i_p = 2.5 \times 10^{-7}$ A, $\beta = 0.5$, $\nu = 100$ mV/s) according to the reference [13], which well corresponds to the complete monolayer adsorbed amount $1.43 \times 10^{-11}$ mol/cm$^2$. The maximum adsorbed amount would be $1.2 \times 10^{-11} \sim 1.56 \times 10^{-11}$ mol/cm$^2$ considering cytochrome c molecule as a sphere with a 3.5～4.0 nm diameter and assuming the most compact hexagonal packing on the surface[3]. Since above values is estimated using apparent electrode area and real electrode surface is uneven, it can be assumed that the cytochrome c adsorbed on the gold surface in the form of less unfolded protein layer.

The Nyquist diagram of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution containing 5 mg/mL cytochrome c shows a approximate Randles behaviour (Fig. 5-2). The results show that the redox reaction of bulk cytochrome c at the gold electrode with adsorbed cytochrome c can be carried out. The electrode process of the bulk cytochrome c is controlled by combining electron transfer and diffusion process. The equivalent circuit of the electrode impedance is assumed as the insert shown in Fig. 5-2.

In the potential step measurement, the current is directly proportional to the minus square root of time and the charge has linear relation to square root of time (Fig. 6). The intercept ($8 \times 10^{-7}$ C/cm$^2$) of the charge curve on the charge axis involves charges of both the electron transfer reaction of the adsorbed cytochrome c and the charging of double layer. This charge is smaller than the adsorption charge $1.38 \times 10^{-6}$ C/cm$^2$. Therefore, the adsorbed cytochrome c can mediate the electron transfer reaction of the cytochrome c in solution, but it is proposed that not all adsorbed molecules would play this role, because both the conformation and the micro-environment of not all adsorbed cytochrome c can match completely the ones of cytochrome c in solution. The fact that adsorbed cytochrome c can be a mediator agrees with the assumption that the electron-transfer reactions of cytochrome c proceed through the exposed edge[14].

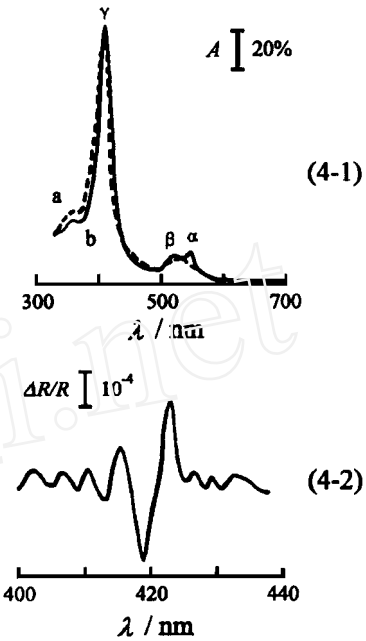


Fig. 4-1) UV/Vis absorption spectra of the solution containing 8 umol/L cytochrome c in a state of oxdizde (a) and in a state of reduced (b);
Fig. 4-2) *In situ* UV/Vis reflection difference spectra of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution (pH7.0).
Range of potential modulation: - 60～+ 100 mV.

## Conclusion

The cytochrome c can adsorb on surface of gold with less unfolded complete monolayer, and the adsorbed cytochrome c can mediate the quasi-reversible electron transfer reaction of the cy-

tochrome c in the solution on condition that the gold surface is treated accurately electrochemically. Existence of oxygenic species on surface would cause electroactivity loss of cytochrome c. Not all adsorbed molecules would mediate the electron transfer reaction of the bulk cytochrome c. It is more possible that the electron-transfer reactions of cytochrome c proceed through the exposed edge.

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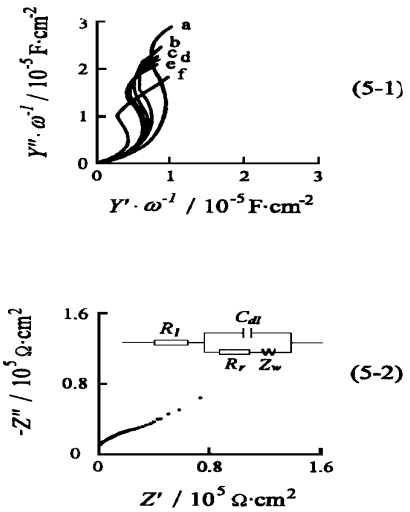


Fig. 5-1) Admittance spectra of the adsorbed cytochrome c at the gold electrode during adsorbing process in the phosphate buffer solution containing 5 mg/mL cytochrome c.
Adsorbing time: (a) 0～7 min; (b) 7～14 min; (c) 14～21 min; (d) 21～28 min; (e) 28～35 min; (f) after 2 hours.

Fig. 5-2) Nyquist plot of the gold electrode with adsorbed cytochrome c in the phosphate buffer solution containing 5 mg/mL cytochrome c.
Insert: equivalent circuit of the electrode impedance.
Range of AC frequency: $10^{-2}$ Hz ～ $10^5$ Hz.
Electrode DC potential: + 0.1V.

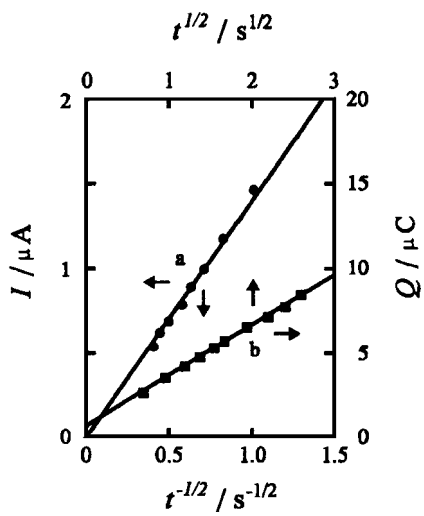


Fig. 6 Current curve as a function of minus square root of time (a) and charge curve as a function of square root of time (b) in potential step measurement of the gold electrode with the adsorbed cytochrome c in the phosphate buffer solution containing 5 mg/mL cytochrome c.
Apparent area of gold electrode: 0.28cm$^2$.

## References

1 Sagara T, Murakami H, Igaarashi S, Sato H, Niki K. Spectroelectrochemical study of the redox reaction mechranism of cytochrome c at a electrode in a neutral solution in the presence of 4,4′-bipyridyl as a surface modifier. *Langmuir*, 1991, 7: 3 190

2 Rodrigues C G, Farchione F, Wedd A G, Bond A M. Relationship of two electroactive forms of horse heart cytochrome c at gold and glassy carbon electrodes in water and methanol. *J. Electroanal. Chem.*, 1987, 218: 251

3 Szucs A, Hitchens G D, Bockris J O'M. Ellipsometry of cytochrome c on gold surfaces: effect of 4,4′-dipyridyl