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Electrochemical Behavior and Determination of Epinephrine at a Penicillamine Self-assembled Gold Electrode

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The electrochemical behaviors of epinephrine (EP) at the penicillamine (Pen) self-assembled monolayer modified gold electrode have been studied. The Pen/Au electrode is demonstrated to promote the electrochemical response of epinephrine by cyclic voltammetry (CV). The possible reaction mechanism is also discussed. The diffusion coefficient *D* of EP is 1.01×10^{-5} cm^2 s^{-1}. In 0.1 M phosphate buffer (pH 7.0), a sensitive oxidation peak was observed at 0.182 V and the peak current is proportional to the concentration of EP in the range of 1.0×10^{-5} ~ 2.0×10^{-4} M and 5.0×10^{-7}~ 1.0×10^{-6} M, the detection limit was 1×10^{-7} M. The modified electrode is highly stable and can be applied to the determination of EP in practical injection samples. The method is simple, quick, sensitive and accurate.

Keywords: Epinephrine; Self-assembled monolayer; Penicillamine; Cyclic voltammetry

1. INTRODUCTION

Epinephrine (EP) or adrenaline, a hormone in the catecholamine family, is an important neurotransmitter for the mammalian central nervous system. Many diseases are related to the change of catecholamine concentration, thus it is necessary to develop quantitative methods for catecholamine for studying its physiological function and diagnosing some diseases in clinical medicine field [1]. Usually, the determination of EP is carried out by using liquid chromatography [2], capillary electrophoresis [3], electrochemiluminescence [4] and flow injection analysis [5]. But they require expensive instruments, well-controlled experimental conditions and profound sample-making. Therefore, simple methods are expected and attention has been paid on them. Because of the simple procedure and high sensitivity of electroanalysis, studying and measuring EP with electrochemical

method was carried out [1,6-7]. As with other catecholamines, epinephrine has electroactive groups, the oxidation process to quinone has been widely studied from and electrochemical point of view [8-12]. But so many difficulties are existed to study its electrochemical behaviors, because, its electron transfer rates are so slow that they are often adsorbed on the surface of electrode, resulting in passivation [12]. On the other hand, the detection of EP in bio-systems is still facing the difficulties of the irreversible redox reactions in normal conditions, and the serious interference from the coexisting ascorbic acid (AA). Recently, research has been devoted to the development of new modified electrode for monitoring EP [12-14].

Self-assembled monolayer (SAM) of organosulphur compounds on metal surfaces comprise a wide field of potential applications due to their versatility in modifying surfaces in a controllable manner. It has been shown that organothiol molecules upon adsorption at gold lose the hydrogen from the thiol group and that a S-Au bond is formed [15-16]. The well-characterized self-assembled monolayer on metal electrodes has been widely used as a new strategy for the immobilization, orientation and molecular organization of biomolecules at interfaces. The stability on the bond between the specific functional group of a reagent and the electrode surface over a wide range of applied potentials and the well-defined microenvironment mimicking biological membranes make such a system suitable for orienting proteins without denaturation and facilitating the electron transfer of biomolecules. Such chemically modified electrodes to improve the selectivity and sensitivity of the electrochemical behavior of some biomolecules have been widely studied [17-22].

In this paper, the compound used to modify the gold electrode is penicillamine (Pen), $(CH_3)_2C(SH)CH(NH_2)COOH$. Pen is the main product of penicillin decomposition [23]. Because Pen involves the terminal SH groups, it can be self-assembled on the gold electrode surface as a new chemically modified electrode to study electrochemistry properties of biomolecules and the interaction between the biomolecules and the drug Pen. Most of the research of epinephrine is done in acid systems which are far from the normal value of human bodies [22, 24]. Since the electrocatalytic mechanism to EP is different in various pH conditions, we studied the electrochemical behavior of EP at the Pen self-assembled monolayer modified gold electrode in phosphate buffer (pH 7.0) which features a pH value much closer to human physical conditions. The Pen/Au electrode is demonstrated to promote the electrochemical response of epinephrine by cyclic voltammetry. Tow couple redox peak can be observed which is much different from the previous literature proposed by Li and co-workers based on the same modified method [22]. The possible two-step reaction mechanism is also discussed. The proposed Pen SAM modified electrode has been applied to the determination of EP in practical medicine with satisfactory results.

2. EXPERIMENTAL PART

2.1. Reagents

Penicillamine and epinephrine were purchased from Sigma and they were used as received. All other chemicals were of analytical grade and were used without further purification. A 0.1 M phosphate buffer solution was used to control the pH. The drug sample, Adrenaline Hydrochloride

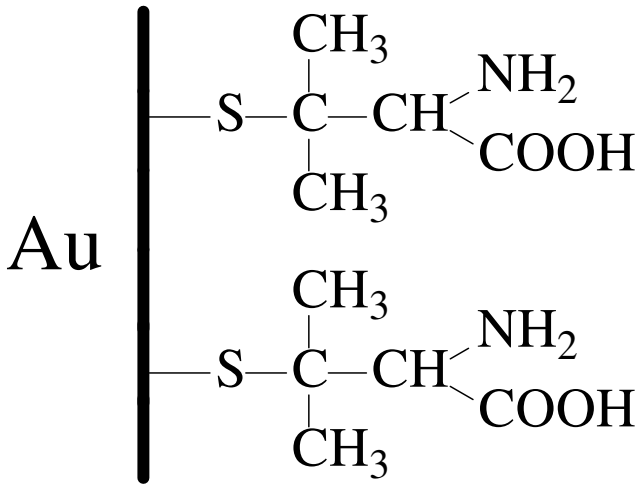
Injection, came from Nuojia Pharmaceutical Company (China). All solutions were prepared with deionized water treated in a Millipore water purification system (Millipore Corp.). All experiments were carried out at room temperature.

2.2. Apparatus

Voltammetric measurements were performed with a CHI 440 electrochemical analyzer (CH Instruments, Chenhua Co. Shanghai, China) controlled by a personal computer. A conventional three-electrode cell was used, including a saturated calomel electrode (SCE) as reference electrode, a platinum wire counter electrode and a bare or modified gold working electrode. The pH values were measured with a PB-10 pH meter (Satorius). Unless otherwise stated, the electrolyte solutions were thoroughly degassed with N_2 and kept under a N_2 blanket.

2.3. Preparation of the Pen-SAM modified gold electrode

Monolayer was formed by the self-assembling technique on gold substrates (scheme 1). The working electrode was a Au disk electrode with a diameter of 2 mm. Prior to each measurement, the electrode was polished with diamond pastes and an alumina slurry down to 0.05 μm on a polishing cloth (Buehler, Lake Bluff, IL), followed by sonicating in water and ethanol. Then, the Au electrode was electrochemically cleaned by cycling the electrodes potential between 1.6 V and -0.4 V (*vs.*SCE) in 0.5 M H_2SO_4 until a stable voltammogram was obtained. After it was washed with sonication and dried with a stream of high purity nitrogen, the electrode was immersed in an aqueous solution of 20 mM Pen for about 36 h at 4 ^{0}C. Upon removal from the deposition solution, the substrate was thoroughly rinsed with water to remove the physically adsorbed species. According to the reference of [20], the advancing contact angle was of Pen SAM is 14°. The scheme of the resulting self-assembling configuration at the gold electrode is shown as:



Scheme 1. Organization of Pen-SAM.

2.4. Procedure

The determination of EP was performed in phosphate buffer solution employing cyclic voltammetry from -0.5 V to 0.5 V. The sample was determined directly after diluting with buffer and water.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Pen self-assembled monolayer with electrochemical method

The redox behavior of a reversible couple can be used to probe the packing structure of the monolayer [25]. Fig.1 shows the cyclic voltammograms of the bare gold electrode (Fig. 1a) and the Pen/Au electrode (Fig. 1b) in 1 mM $Fe(CN)_6^{3-}$solution containing 0.1 M KCl. For a bare gold electrode, a couple of well-defined waves of $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ should appear, and the peak-to-peak separation (ΔE_p) should be 60 mV. However, it can be seen that the peak current was decreased and ΔE_p was increased for the Pen/Au electrode. Because Pen is a short mercaptan molecule, there are many pinhole defects and collapsed sites in the Pen monolayer, and the electron transfer rate constant at pinhole defects is the same as that at the bare gold electrode. So the redox couples can reach the gold surface through pinhole defects in the Pen monolayer.

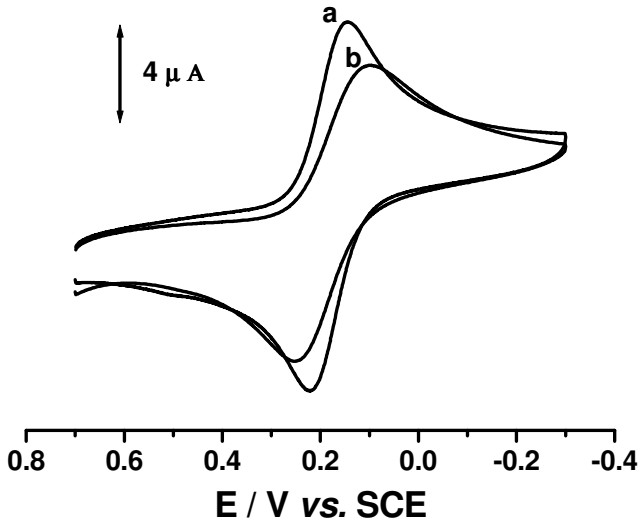


Figure 1. Cyclic voltammograms of 1.0×10^{-3} M $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ at a bare gold electrode (a) and Pen/Au electrode (b). 0.1 M KCl; scan rate, 100 mV s^{-1}.

From the reductive desorption of Pen monolayer from the Au electrode, the surface coverage of Pen at the Au electrode was calculated. First, the charge attributed to the desorption of sulfur atoms of Pen form the Au surface [26] was 1.386 μC which was obtained by integration of the cathodic peak in

the cyclic voltammogram of the Pen/Au electrode in 0.5 M KOH. The effective area of the electrode was calculated to be 0.085 cm^2 according to the cyclic voltammogram of 0.5 M sulfuric acid at the bare electrode [27]. Then the surface coverage of Pen at Au electrode was found to be 1.69×10^{-10} mol cm^{-2}.

3.2. The electrochemical response of EP at the Pen SAM-modified Au electrode

Fig.2 shows the Cyclic voltammograms at a Pen/Au electrode (Fig. 2a) and a bare gold electrode (Fig. 2b) in presence of 0.1 mM EP in phosphate buffer of pH 7.0. At the bare gold electrode, the cyclic voltammogram of EP demonstrated very poor and irreversible waves with the anodic peak potential (E_{pa}) at 0.346 V and the cathodic peak (E_{pc}) at -0.240 V. It was not suitable for the determination of EP. But, a well-defined redox wave of EP was observed at the Pen/Au electrode and the peak current increases significantly. According to Refs.[11-12,28], the peak A, appeared at 182 mV, can be interpreted as the oxidation of epinephrine to the open-chain quinone. The quinone, the first product in the electrochemical oxidation, is known to be proceeded deprotonation in the solution of pH > 3. Peak B, appeared at 131 mV, is the rereduction of this quinone, while peak C, appeared at -217 mV, is the reduction of the cyclized product adrenochrome to leucoadrenochrome.

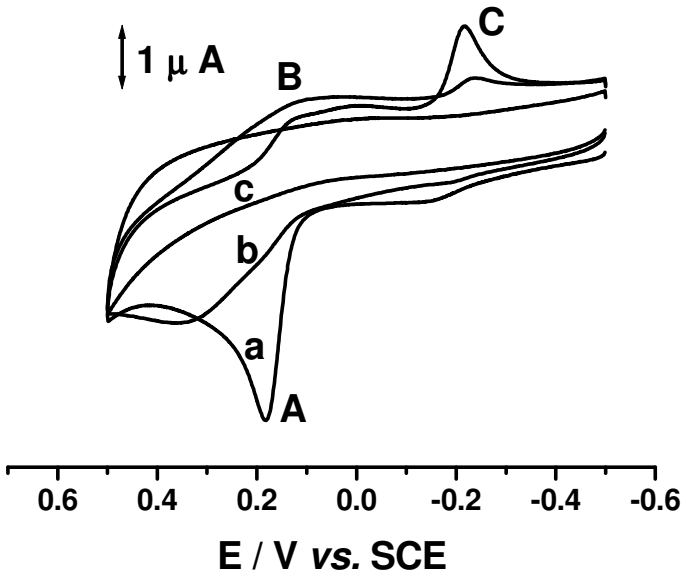


Figure 2. Cyclic voltammograms at a Pen/Au electrode (a, c) and a bare gold electrode (b) in presence of 1.0×10^{-4} M EP (a, b) and in the absence of EP (c) in phosphate buffer (pH 7.0), scan rate, 100 mV s^{-1}. See text for explanation of peaks A-C.

Two continuous cyclic voltammograms were shown in Fig.3. Peak D, appeared at -188 mV, is corresponded to the reoxidation of leucoadrenochrome to adrenochrome. These show that the electrochemical response of EP is promoted at Pen/Au electrode.

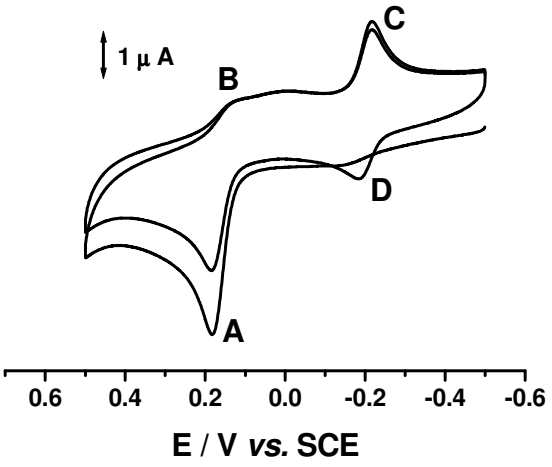
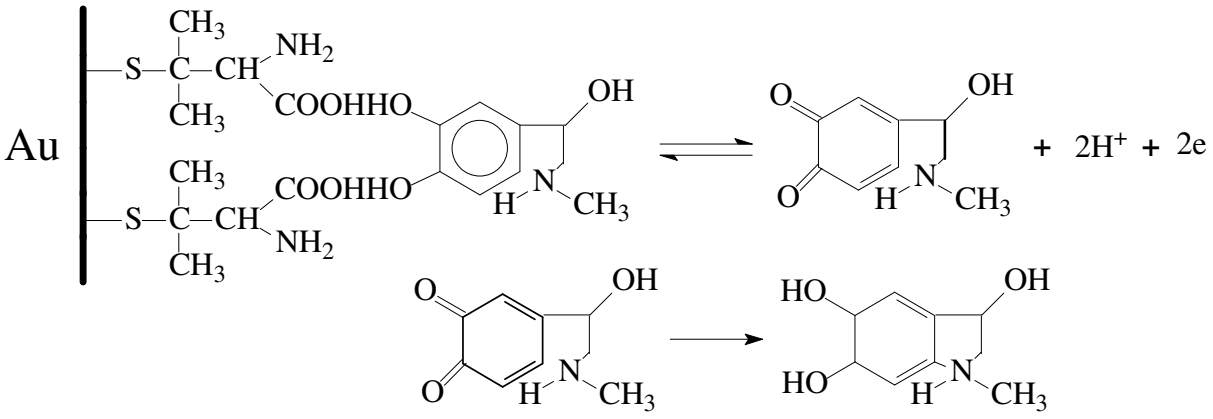


Figure 3. The two cycles of continuous cyclic voltammograms at Pen/Au electrode for 1.0×10^{-4} M EP. 0.1 M pH 7.0 phosphate buffer; scan rate 100 mV s^{-1}. See text for explanation of peakes A-D.

The RSH compound is a good nucleopile and therefore, it will preferentially add to the ring [11,29]. Since -OH group is the electroactive group in EP and is present in close of the carboxylic acid modified electrode this might lead to much fast kinetics of EP redox at the Pen/Au SAM [30]. The probable schemes of the reactions may be expressed bellows.



Pen/Au SAM shows no electrochemical response in phosphate buffer (pH 7.0) without EP (Fig. 2c). Compared with the bare gold, its background current drops greatly. This is because, Pen formed monolayer at gold surface; the diffusion coefficient between electrode surface and buffer solution was much slower. The peak currents of 0.1 mM EP solution remained the same after three continuous cyclic scanning at the Pen/Au electrode.

3.3. Effects of pH on the peak current and peak potential of EP

The effects of the pH value of phosphate buffer on the anodic peak potential and peak current were investigated, and the results are shown in Fig.4. It can be seen that as the solution pH increase, the anodic peak potential shifts to the negative and the potential of E_{Pa} *vs.* pH in phosphate buffer has a good linear relation in the range of pH 5.14 ~ 9.04. The linear regression equation E_{pa}/V=0.6007-0.0589pH (correlation coefficient r=0.9967) was obtained, which showed that the uptake of electrons is accompanied by an equal number of protons.

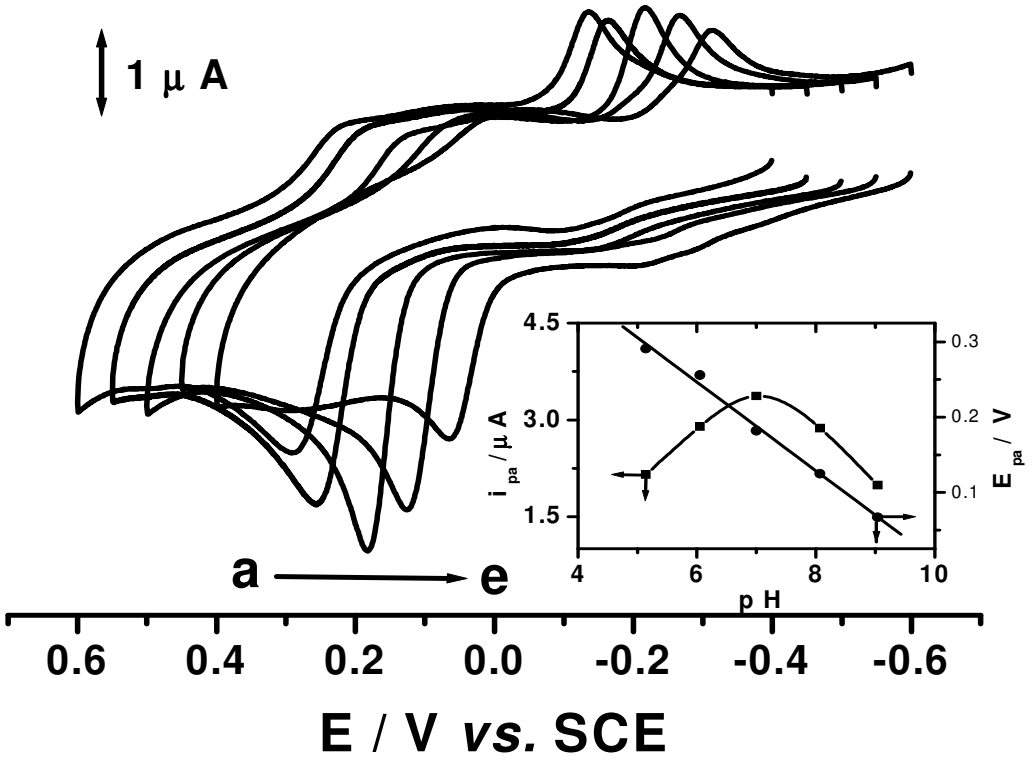


Figure 4. Cyclic voltammograms at Pen/Au electrode for 1.0×10^{-4} EP in 0.1 M phosphate buffer solutions at pH values of (a) 5.14, (b) 6.05, (c) 7.00, (d) 8.07 and (e) 9.04. The inset shows effects of pH on the anodic peak potential and anodic peak current in phosphate buffer solution. Scan rate 100 mV s^{-1}.

Fig.4 also shows the dependence of the anodic peak current (i_{pa}) on the pH of the solution. It is found that the anodic peak current increased with increasing the pH up to 7.0, after that the peak current decreased. Therefore, the pH value of 7.0 was selected for further studies.

3.4. Effect of Scan rate on the peak current and determination of the diffusion coefficient (D)

As shown in Fig.5, the anodic peak current (i_{pa}) of EP increased with increasing scan rate on Pen SAM modified electrode in pH 7.0 phosphate buffer and exhibited a linear relation to the square root of the scan rate, $v^{1/2}$, with the linear regression equation i_{pa} / μA = 0.6598 + 0.2465 $v^{1/2}$ /mVs^{-1} (correlation coefficient r=0.9966). The result indicates that the electron transfer reaction is controlled by the diffusion of EP.

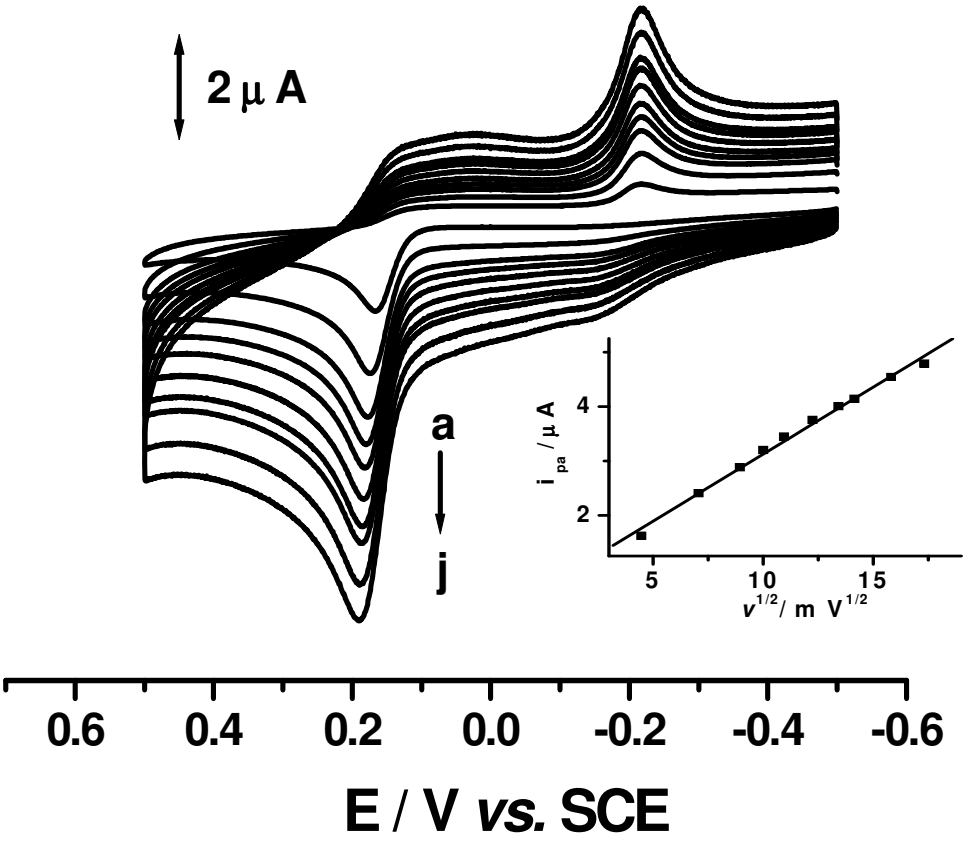


Figure 5. Cyclic voltammograms of 1.0×10^{-4} M EP at Pen/Au electrode in phosphate buffer (pH 7.0) at different scan rates (a) 20 mV s^{-1}; (b) 50 mV s^{-1}; (c) 80 mV s^{-1}; (d) 100 mV s^{-1}; (e) 120 mV s^{-1}; (f) 150 mV s^{-1}; (g) 180 mV s^{-1}; (h) 200mV s^{-1}; (i) 250 mV s^{-1}; (h) 300 mV s^{-1}. The inset is a plot of the oxidation peak current (i_{pa}) and the scan rate.

The diffusion coefficient *D* of EP was determined by chronocoulometric method [31]. According to the formula given by Anson:

$$Q = \frac{2nFAC(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl}$$

Where Q_{dl} is double-layer charge (integration of charging current). From the slope of the linear relationship between Q and $t^{1/2}$, D can be determined if C (concentration), A (surface area of the electrode), and n (electron transfer number) are known. Here n=2. From the slope of the plot between Q and $t^{1/2}$ (Fig. 6), D was calculated as 1.01×10^{-5} cm^2 s^{-1}.

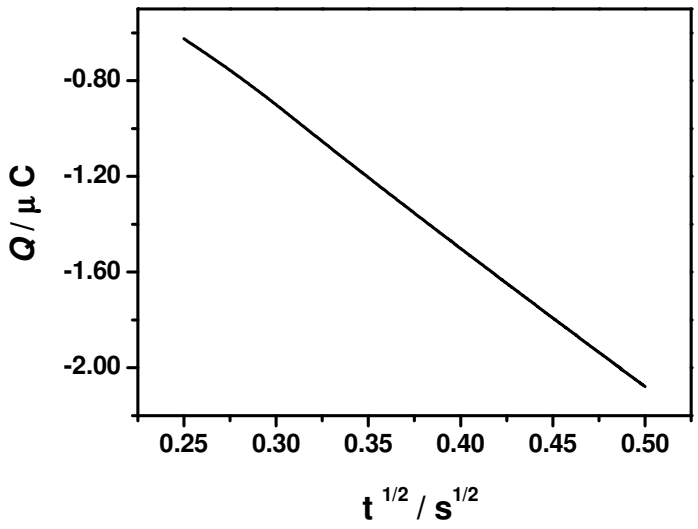


Fig. 6. Chronocoulometric dependence of charge on the square root of time for 1.0×10^{-4} M EP. Potential step: 0.0 ~ 0.5 V.

3.5. The response of EP in the presence of ascorbic acid (AA)

In biological environments, EP and AA always exist together and they can not be determined, respectively, at the conventional electrode [12]. As shown in Fig.7, AA exhibited an anodic peak at Pen/Au (Fig. 7a). The anodic waves of EP at Pen SAM could be observed obviously in the presence of AA, but, the CV has not high sensitivity and the anodic waves of AA and EP can not be distinguished perfectly when EP mixes with 5-fold AA (Fig. 7b). Only when EP mixes with 1-fold AA, the CV of EP was not interfered by AA. The concentration of AA is several orders of magnitude higher than EP in biological environments, so determining EP in the presence of 1–fold AA is not applicable. Therefore, at analytical applications we determine the concentration of EP in the absence of AA.

3.6. Stability of the self-assembled electrode

Stability tests were carried out by repetitive scans in the potential of -0.5 and +0.5 V in phosphate buffer of pH 7.0. The peak currents of EP did not obviously change after 100 cycles of repetitive scans for the Pen/Au electrode which was modified in an aqueous solution of 20 mM Pen for about 36 h at 4 ^{0}C. When placing the electrode in phosphate buffer of pH 7.0 for 3 h, the relative error of the peak current values is within the limit of 2.1 %. EP of 0.1 mM was determined 10 times, and the relative standard deviation was 1.3 %. The results indicate that the Pen/Au electrode is highly stable.

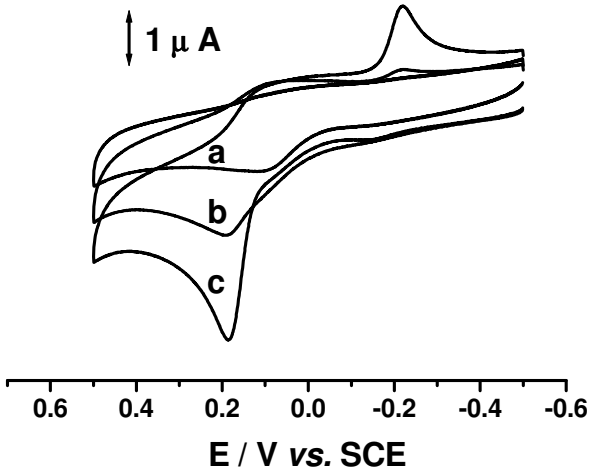


Figure 7. Cyclic voltammograms of Pen/Au electrode in 1.0×10^{-4} M AA (a), 1×10^{-4} M AA+ 2.0×10^{-5}M EP (b) and 1×10^{-4} M AA+ 1.0×10^{-4} M EP (c). 0.1 M pH 7.0 phosphate buffer; scan rate 100 mV s^{-1}.

3.7. Calibration plot

Under the optimum analytical conditions, the linear range of EP at the Pen/Au electrode was studied using cyclic voltammetry. The anodic peak current was linear to EP concentration in the range of 1.0×10^{-5} ~ 2.0×10^{-4} M and 5.0×10^{-7}~ 1.0×10^{-6} M. The linear regression equations are expressed as i_{pa}/μA =0.5562+0.0233C /μ M (correlation coefficients r = 0.9972) and i_{pa}/μ A =0.4092+0.0517C /μ M (correlation coefficients r = 0.9965), respectively. The detection limit was 1×10^{-7} mol L^{-1}.

3.8. Tolerance of foreign substances

The influence of various foreign species on the determination of 0.1 mM EP was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances which caused a

relative error of approximately ±5 % in the determination. The tolerated ration of foreign substances in 0.1 mM EP was 1000 for K^+, Na^+, Cl^-, $H_2PO_4^-$, HPO_4^{2-}; 15 for glycin, glucose, Mg^{2+}, Zn^{2+}, NO_3^-, CH_3COO^-; 4 for ferulic acid, respectively.

3.9. Analytical application

The Pen/Au has been used to determine the EP content in injection samples. The experimental procedure and conditions were the same as mentioned above. The average result of EP in injection by Pen/Au SAM is 1.003 mg mL^{-1}, which is quite correspond to standard content (1.000 mg mL^{-1}). The analytical results are summarized in Table 1. The recovery is 96.1 % to 102.5 %. This method is simple, sensitive and cheap in comparison with some other methods.

Table 1. Results for the determination of EP in injection

Sample (1×10^{-5} mol L^{-1})	**Added** (1×10^{-5} mol L^{-1})	**Found** (1×10^{-5} mol L^{-1})	**Recovery** (%)
5.0	2.0	7.05	102.5
5.0	3.2	8.21	100.3
5.0	5.4	10.38	99.6
5.0	6.5	11.38	98.2
5.0	7.1	11.82	96.1

4. CONCLUSIONS

In this work, we have prepared and characterized a penicillamine (Pen) self-assembled monolayer at gold electrode. The electrochemical behaviors of EP at the Pen self-assembled Au electrode were studied with cyclic voltammetric technique. The diffusion coefficient *D* of EP was determined by chronocoulometric method. From the analysis of currents and concentrations, the good linear relationship between them can be used for the determination of EP in practical injection, with quick and accurate results.

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References

1. B.Z. Zeng, Y.X. Yang, F.Q. Zhao, *Electroanal.*, 15 (2003) 1054
2. M.A. Fotopoulou, P.C. Ioannou, *Anal. Chim. Acta*, 462 (2002) 179
3. P.B. Mckibbin, J. Wong, D.D.Y. Chen, *J. Chromatogr. A*, 853 (1999) 535

4. Y.Y. Su, J. Wang, G.N. Chen, *Talanta*, 65 (2005) 531
5. T. Kamidate, T. Kaide, H. Tani, E. Makino, T. Shibata, *Anal. Sci*., 17 (2001) 951
6. J.M. Gong, X.Q. Lin, *Electrochim. Acta*, 49 (2004) 4351
7. W. Cheng, G. Jin, Y. Zhang, *Russ. J. Electrochem.*, 41 (2005) 940
8. S. M. Chen, M. Liu, *J. Electroanal. Chem.*, 579 (2005) 153
9. E. Dempsey, A. Kennedy, N. Fay, T. Mccormac, *Electroanal.*, 15 (2003) 1835
10. J. M. Gong, X. Q. Lin, *Electrochim. Acta*, 49 (2004) 4351
11. S. F. Wang, D. Dan, Q. C. Zou, *Talanta*, 57 (2002) 687
12. H. M. Zhang, X. L. Zhou, R. T. Hui, N. Q. Li, *Talanta*, 56 (2002) 1081
13. H. S. Wang, D. Q. Huang, R.M. Liu, *J. Electroanal. Chem.*, 570 (2004) 83
14. S.M. Chen, K.T. Peng, *J. Electroanal. Chem.*, 547 (2003) 179
15. A. Ulman, *Chem. Rev.*, 96 (1996) 1533
16. Y. Li, J. Huang, R. G. Melver, J. C. Hemminger, *J. Am. Chem. Soc.*, 114 (1992) 2428
17. H.M. Zhang, N.Q. Li, Z.W. Zhu, *Microchem. J.*, 64 (2000) 277
18. Q. Wang, J. Nan, N.Q. Li, *Microchem. J.*, 68 (2001) 77
19. X. H. Zhang, S. F Wang, Q.H. Shen, *Microchim. Acta*, 149 (2005) 37
20. Q. Wang, D. Dong, N. Q. Li, *Bioelectrochem*., 54 (2001) 169
21. B. Z. Zeng, Y. X. Yang, X. G. Ding, F. Q. Zhao, *Talanta*, 61 (2003) 819
22. L. M. Niu, H. Q. Luo, N. B. Li, *Microchim. Acta*,150 (2005) 87
23. J.P. Greenstein, M. Winitz (Ed.), Chemistry of the Amino Acids, vol. 3, Wiley, New York, 1961, pp. 2644
24. S. H. Kim, J. W. Lee, I. H. Yeo, *Electrochim. Acta*, 45 (2000) 2889
25. C. J. Miller, C. Pierre, M. Gratzel, *J. Phys. Chem.*, 95 (1991) 877
26. C. A. Widrig, C. Chung, M. D. Porter, *J. Electroanal. Chem.*, 310 (1991) 335
27. L. H. Duboise, R. G. Nuzzo, *Annu. Rev. Phys. Chem.*, 43 (1992) 437
28. M. D. Hawley, S. V. Tatawawadi, S. Piekarski, R. N. Adams, *J. Am. Chem .Soc*., 89 (1967) 447
29. P. T. Kissinger, K. Bratin, G. C. Davis, L. A.Pachla, *J. Chromatogr. Sci*., 17 (1979) 137
30. A. Dalmia, C. C. Liu, R. F. Savinell, *J. Electroanal. Chem.*, 430 (1997) 205
31. F. R. Anson, *Anal. Chem.*, 38 (1966) 54