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Irreversible Biamperometry Determination Of Penicillamine Based On A Flow-Injection Analysis

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ABSTRACT

An original flow-injection coupling irreversible biamperometric method is developed for the direct determination of penicillamine. The method depends on electrocatalytic oxidation of penicillamine at a gold electrode and the reduction of permanganate at a platinum electrode to form an irreversible biamperometric detection system. Under the applied potential difference of 0 V, in the 0.05mol/L sulfuric acid, penicillamine can be determined over the range 6.00×10^{-5}-1.00×10^{-3} mol $L^{-1}$with a sampling frequency of 80 samples per hour. The detection limit for penicillamine is 8.0×10^{-6} mol L^{-1} and the RSD for 19 replicate determinations of 4.0×10^{-4} mol/L penicillamine is 2.89%. The proposed method was applied to the analysis of penicillamine in penicillamine tablets with satisfactory results.

KEYWORDS

Penicillamine;
Flow injection;
Coupling irreversible biamperometry;

INTRODUCTION

Penicillamine(3,3-dimethylcysteine) has been extensively used in the treatment of many diseases, including rheumatoid arthritis and Wilson's disease, it is also used in treatment of Pb poisoning as chelating agent[1,2]. Different direct and indirect methods based on spectrophotometry[3,4], chromatography[5,6], capillary electrophoresis (CE)[7] and titrimetry[8] have been proposed for the analysis of penicillamine in pharmaceutical preparations. The official methods for penicillamine, listed in the Pharmacopoeia of P. R.China(Part II) and US Pharmacopoeia, potentiometric titration or ion-pairing HPLC method[9,10].

A novel flow-injection irreversible biamperometry has been introduced[11,12]. The new method inherits the conventional advantage of biamperometry for reversible couples but differs from classic biamperometry in operation. In the scheme, two independent, inverse and irreversible electrode processes which half-wave potentials $E_{1/2}$(or peak potential E_p) are close to each other, were chosen and coupled to

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establish an electrochemical systems similar to a single reversible redox couple. With the potential difference ΔE existing in nature between two electrodes, the coupled system can work spontaneously without any external potential difference. In respect that the applied ΔE is very small, the method shows high selectivity and S/N, and has been successfully applied to the determination of vitamin C, hydroxylamine, cysteine, low mass molecule alcohol, morin, calcium dobesilate, phenlo ethamsylate , iodide, vitamin B_1 etc[13]. Through the review on the application of gold, platinum, glass-carbon electrode in reversible biamperometry, Sacchetto G A. point out that the gold electrodes can also be applied to biamperometry[14], moreover it has even smaller background current and satisfactory sensitivity. Gold was found to be the most suitable material in the biamperometry detector. Similar to the platinum electrodes which were usually used in the irreversible biamperometry, gold electrodes have even wider reduction potential, and easily to acquire suitable construction of irreversible biamperometry. But there is little report on the study of gold electrodes used in biamperometry.

As demonstrated in this paper, an irreversible biamperometry detection scheme for the direct determination of penicillamine was established. The irreversible biamperometric detection scheme is established by coupling an oxidation of penicillamine on the gold electrode and the reduction of permanganate on the platinum electrode. With the advantages of the applied potential difference ΔE of 0 V, high selectivity and high S/N are obtained. The proposed method is suitable for automatic and continuous analysis.

EXPERIMENTAL

Apparatus

A CHI660 Electrochemical Workstation(CH Instrument, USA) equipped with a personal computer was used throughout present work to impose the potential difference and to record the resulting current. Additionally it was used to perform the cyclic voltammeter experiments. The cyclic voltammograms were obtained with a three-electrode system, namely a platinum wire or a gold wire working electrode, a platinum auxiliary electrode and a saturated calomel electrode(SCE). Unless otherwise mentioned, potentials in this work were referred to the potential of the saturated calomel electrode.

A model IFIS-C intelligent flow injection sampler (Xi'an Remex Analyze Instrument CO., LTD, China) which consisted of two peristaltic pumps and a eight-way injection valve, controlled by a microcomputer.

The dimensions and assembly of the homemade biamperometric detector, which was made by ourselves and made from a Teflon rod, was introduced previously[15].

Pretreatment of the two electrodes

The electrodes were pretreated electrochemically by alternating polarization between +1.0 and -1.0V in 0.05 mol L^{-1} H_2SO_4 solution after soaked with concentrated nitric acid for 5 min and rinsed with water. Such pretreatment was repeated prior to every measurement to allow for a maximum sensitivity of response.

Reagents and material

All reagents were prepared from analytical reagent grade chemicals unless specified otherwise and doubly distilled water was used for the preparation of solutions and all dilutions throughout the measurements.

Standard stock solution of penicillamine(1.0×10^{-3}mol L^{-1}) was prepared by dissolving 0.0149 g of penicillamine(Tokyo Chemical Industry, Tokyo, Japan) 100ml of 0.05mol L^{-1} H_2SO_4 solution. The stock solution was kept in an opaque brown glass bottle and stored in the refrigerator. The standard working solutions were prepared daily from the stock solution by appropriate dilution with 0.05mol L^{-1} H_2SO_4 solution.

$KMnO_4$ solution(2.0×10^{-3} mol L^{-1}) was prepared by dissolving 0.0316g $KMnO_4$(Guang hua Chemical Plant, Shantou, China) in 100ml of 0.05mol L^{-1} H_2SO_4 solution.

Procedure

A potential difference(ΔE) of 0 V was kept across the two electrodes and the cell current was

recorded by the CHI660 workstation. By keeping the valve of model IFIS-C automatic sampling system in the sampling position, 2.8ml min^{-1} of 0.05mol L^{-1} H_2SO_4 solution was continuously pumped into the gold electrode room of the biamperometric detector and the auxiliary solution of $KMnO_4$ solution(2.0×10^{-3} mol L^{-1}) in the platinum electrode room at the same rate. When baseline was established on the recorder, 120 μl of working standard or sample solution was injected into the detector. Calibration graphs were obtained by plotting the current versus standard concentration of penicillamine, and the content of each sample was determined.

RESULTS AND DISCUSSION

Cyclic voltametric studies

Voltametric behaviors of penicillamine at gold wire electrode and MnO_4^- at platinum wire electrode were examined in the potential range from 1.4 to -0.2 V, respectively(Figure 1a). In 0.05mol L^{-1} H_2SO_4 solution, a reduction wave P_2 appeared at about 0.854 V, which was attributed to the reduction of gold oxide. And an irreversible oxidation wave P_2 appeared at about 1.052 V, which is due to the oxidation of penicillamine on the gold electrode.

As shown in figure 1b, the potential difference between the oxidation potential of penicillamine and the reduction potential of gold oxide is about 0.198 V. If a biamperometric detection system was established by these two couples, a large potential difference(ΔE) imposed between two electrodes would have to be applied to obtain current response. Undoubtedly, the increasing of the applied ΔE will lead to the decrease of selectivity and S/N level. In order to obtain current response when the applied ΔE was very smaller even zero, another irreversible couple with opposite electrode process and close $E_{1/2}$ to that of penicillamine was needed. Among various alternatives, the reduction of permanganate was a good choice in present work. Figure 2b showed that the adding 2.0×10^{-3} mol L^{-1} $KMnO_4$ led to two reduction waves P_3 and P_4. The irreversible wave P_3 appeared at about 0.875 V corresponds to the reduction of MnO_4^- to Mn^{2+} on the platinum electrode.

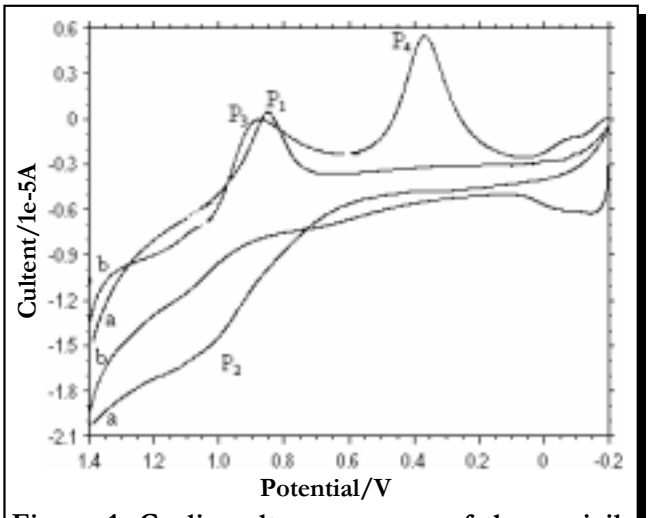

Figure 1: Cyclic voltammograms of the penicillamine/permanganate biamperometric system. a: The oxidation of penicillamine; b: The reduction of permanganate; Scan rate υ=100 $mV\cdot s^{-1}$; Initial potential: 1.4V; Reversal potential: -0.2V

Figure 2b showed that the oxidation wave P_1 and the reduction wave P_3 were separately in anodic and cathodic polarized curves, they were from two independent and irreversible couples with the potential difference of only 0.177 V. According to the irreversible biamperometry, the biamperometric detection scheme was established by coupling the oxidation of penicillamine and the reduction of permanganate. In this case, the determination can be carried out with a ΔE of 0 V. Moreover, the two reactants were separated into two electrode rooms by means of a salt bridge when performing the flow injection determination, which avoids the influences from the homogeneous reaction of penicillamine and MnO_4^-.

Selection of the wire electrodes

Penicillamine has been found to be electrocatalytically irreversible oxidized on the platinum and gold electrode at the close oxidation potential. The biamperometric detection scheme is established by coupling irreversible reduction of permanganate and irreversible oxidation of penicillamine on platinum electrode and gold electrode respectively. Penicillamine can be absorbed on the surface of the platinum electrode which account for the current decrease. But the gold electrode has no this phenomenon. Therefore, the gold electrode was chosen in this work.

Effect of the applied potential difference

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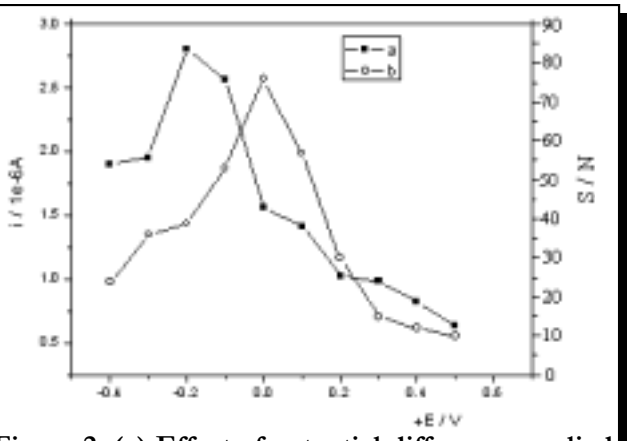

Figure 2: (a) Effect of potential difference applied on current. (b) Effect of potential difference applied on signal to noise (S/N). $C_{penicillamine}$=4.0×10^{-4} mol L^{-1}; Flow rate r=2.8 mL min^{-1}

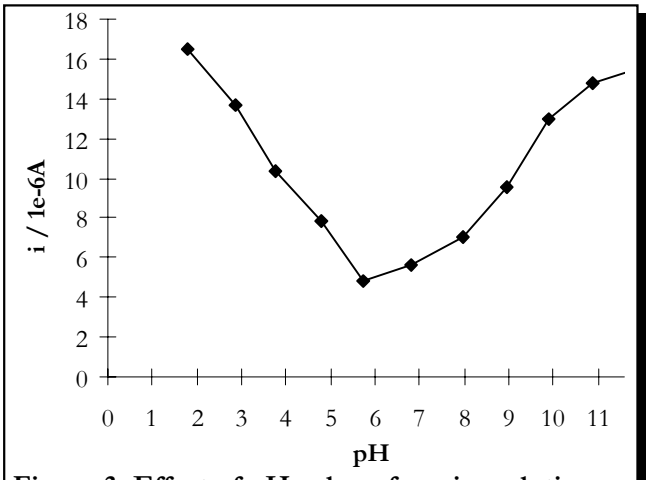

Figure 3: Effect of pH value of carrier solution on cell current. $C_{penicillamine}$=4.0×10^{-4}mol L^{-1}; ΔE=0V; Flow rate r=2.8ml Min^{-1}

Figure 2 showed that, in this work, when the applied ΔE was 0 V, the true potentials of two electrode processes were between 0.87 V and 1.05 V. The proposed biamperometry can spontaneously work, i.e., it can work without imposing any potential difference ΔE between two electrodes. This differs from such biamperometry as I_2/I^-, $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ reversible couple systems. The potentials of two electrodes are in the same level and the cell currents flowed though two electrodes are equal to each other, therefore, the work often occurs in an under potential situation. The effect of the imposed potential difference ΔE between two electrodes on cell current is examined. Experiments showed that the increase of noise current value with the ΔE increasing, which resulted in the reduction of S/N ratio. As shown in figure 2, a maximum S/N value was achieved when the ΔE was 0 V. So the 0 V of the ΔE was chosen.

Selection of carrier solution for penicillamine

The carrier solution was selected based on the effect of pH value of carrier solution both on peak height and peak width of the current response, which was investigated using 4.0×10^{-4}mol L^{-1} penicillamine standard solution with the applied ΔE of 0 V. In operation, a series of Britton-Robinson buffers (H_3PO_4-HOAc-H_3BO_3-NaOH) with various pH values and 0.05 mol L^{-1} H_2SO_4 solution were tested. As shown in figure 3, the cell current decreased with the increasing of pH value of the carrier solution in low pH value(1.81-6.80), and the cell current increased with the increasing of pH value of the carrier solution in high pH value(7.96-11.98). The carrier solution with low pH value and high pH value were, therefore, favorable to obtain high sensitivity. But the S/N of the work was decreased with the increasing of pH value of the carrier solution. The detector showed more stable response and well-defined peak shape in H_2SO_4 solution than that in B-R buffers solution. So there was a tradeoff, in present work, 0.05 mol L^{-1} of H_2SO_4 solution was selected as the carrier solution.

Selection of $KMnO_4$ solution concentration

In order to obtain the linearity between current and penicillamine, the $KMnO_4$ concentration should be kept enough high according to the irreversible biamperometry theory[15]. A 2.0×10^{-3} mol L^{-1} $KMnO_4$ solution was selected since the penicillamine concentration in this work was less than 2.0×10^{-3} mol L^{-1}.

Optimization of flow injection system

Such parameters of the FIA operation as flow rate, injection volume and others had been optimized by using 4.0×10^{-4} mol L^{-1} penicillamine standard solution.

The flow rate defined not only the change in the peak shape but also the rate of the return to the baseline. In this system, the peak height was found decreasing with the flow rate increasing from 0.5 to 6mL min^{-1}, whereas the peak broadened at lower flow rate. Thus, a compromised value of 2.8mL min^{-1} was selected. The injection volume also had a significant

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effect on the peak shape. The increase of injection volume induced a higher peak height and a boarder peak width. When injection volume was above 120 μl, the peak height reached a maximum value. The more consumption of sample should not be paid. Consequently, a sample volume of 120 μl was chosen as the peak width was still acceptable at this volume. The distance between detector and valve had no obvious effect on detector response and a value of 30cm was used.

Interference study

The effects of various inorganic ions and organic compounds commonly existed in pharmaceuticals on the determination of 1.0×10^{-4} mol L^{-1} penicillamine were studied. The tolerance limit was defined as the molar ratio of additive to penicillamine causing less than ±5 % relative error. Since the applied potential was 0 V, the detector was free from interference induced by direct oxidation or reduction of the additives. The tolerance limit of additives to 1.0×10^{-4} mol L^{-1} penicillamine was 3500-fold of Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, NO_3^-, 100-fold of glucose, lactose, starch, sucrose, L-cystine, benzoic acid, citric acid, tartaric acid, and 1-fold of uric acid, vitamin C, respectively.

Calibration curve, detection limit and precision

Under the optimized conditions, the biamperometric detector used had good response to penicillamine standard solutions(Figure 4). The linear relationship between the cell current and the concentration of penicillamine in the range of 6.0×10^{-5} to 1.0×10^{-3} mol L^{-1} was obtained. The linear regression equation was i (nA)=900+3.4×10^{6}C (mol L^{-1}) with a regression coefficient r=0.9989(n=9). The detection limit estimated was 8.0×10^{-6} mol L^{-1}(S/N =3). The precision of the method shown by RSD of 2.89 % for 19 replicate determinations of 4.0×10^{-4} mol L^{-1} penicillamine was good.

Sample analysis and recovery test

To validate the present method, it was applied to the determination of penicillamine tablet. The determination was conducted according to the above-mentioned experimental procedure. The results obtained are summarized in TABLE 1.

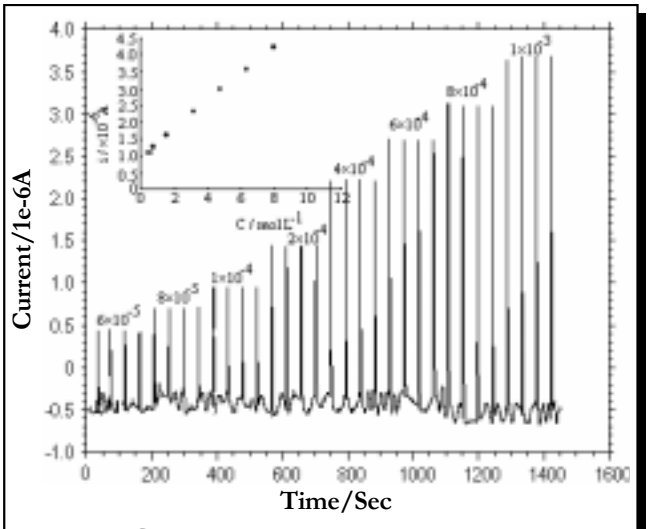

Figure 4: Current responses to penicillamine standard solutions ΔE=0 V, Flow rate r=2.8mL min^{-1}

TABLE 1: Determination of penicillamine in pharmaceutical formulations

Sample	Labeled	Proposed method	Average	RSD
Tablet	125	128.4	127.5	1.49
		127.6		
		130.2		
		125.4		
		126.1		

TABLE 2: Recovery results in samples(n=5)

Sample	Added (10^{-5}mol L^{-1})	Found (10^{-5}mol L^{-1})	Recovery (%)	Average (%)	RSD (%)
1	6.00	6.12	102.0	100.09	1.34
2	8.00	7.93	99.13		
3	10.00	9.87	98.70		
4	20.00	20.17	100.85		
5	40.00	39.90	99.75		

To study the accuracy of the proposed method, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. Each recovery was calculated by comparing the results obtained before and after the addition of penicillamine standard solution. It was shown that the recovery was good(TABLE 2). Therefore, the proposed method is reliable for the quantitative analysis of penicillamine in pharmaceutical preparations.

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