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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 341–346

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Trace determination of shiomarin using a hanging copper/amalgam drop electrode

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Received 31 October 2001; received in revised form 5 December 2001; accepted 18 December 2001

Abstract

The electrochemical behavior of shiomarin (moxalactam; latamoxef sodium) was investigated at hanging copper/amalgam drop electrode (HCADE) in HCl–KCl solution (pH 1.1). It was found that shiomarin can form complex with Cu(II) and can be adsorbed at the electrode surface. The coordination number is 1 and the number of transfer electron involved in the reaction is 2. The peak current of the complex related to the concentration of shiomarin and was used to determine trace amounts of shiomarin in solution. The linear range of determination is from 1.0×10^{-9} to 8.0×10^{-5} M using differential pulse adsorptive cathodic stripping voltammetry (DPAdCSV). The detection limit is about 4.0×10^{-10} M ($t_a = 70$ s, S/N = 3). The method was also applied to the determination of shiomarin in injection powder and urine sample with satisfactory results.

Keywords: Hanging copper/amalgam drop electrode (HCADE); Shiomarin (Moxalactam Latamoxef sodium); Differential pulse adsorptive cathodic stripping voltammetry (DPAdCSV)

1. Introduction

Shiomarin (Latamoxef sodium), as a new parenteral semisynthetic β-lactam antibiotic [1] contains 1-oxyazine instead of 1-thiazine ring in the cephem nucleus (Scheme 1). It possesses two acidic functions: one on the side-chain, and the other on the cephem nucleus (as in cephalosporins). It is widely used in clinical therapy of severe infections [2].

Up to now, some methods have been used for the determination of shiomarin such as microbiological assay [3], HPLC with UV detection [4,5], capillary electrophoresis [6], and polarography [7]. However, in these methods, the detection limit of the more ideal polarography was also only 8×10^{-8} M.

It was reported that the compounds such as iodide [8], thiocyanate [9], penicillins [10] and penicillamine [11] couple with cupric ion could be adsorbed on the hanging mercury drop electrode (HMDE) surface and could increase the determination sensitivity of these substances. Cruz and coworkers [12] reported the polarographic study

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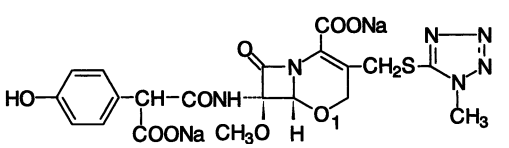
0731-7085/02/$ - see front matter
PII: S0731-7085(02)00062-6

of the Cu(II)–tannic acid complexes by using factor analysis and an alternating least squares optimization. This effect has also been observed at a copper-based mercury film electrode (MFE) [13]. But the addition of Cu(II) ions to the bulk solution may produce some unfavorable side-reactions. In order to eliminate the disadvantages, Bilewicz and Kublik [8,9] proposed that Cu(I) should be generated anodically from the hanging copper/amalgam drop electrode (HCADE). Tanaka and Yoshida [14] also used HCADE to determine cysteine by anodic stripping voltammetry (ASV). However, the investigation on its electrochemical behavior and mechanism on HCADE was hardly reported.

We have reported the electrochemical behavior and determination of 6-Mercaptopurine at HCADE [15]. The aim of this study was to develop simple, sensitive and validated electrochemical method for determining shiomarin. It was found that shiomarin could form complex with cupric ion, which was stripped from copper/amalgam at -0.1 V and adsorbed on HCADE. This behavior greatly increased the response of shiomarin on the electrode. The developed procedure based on differential pulse adsorptive cathodic stripping voltammetry (DPAdCSV) for determining shiomarin in pharmaceutical injection power and urine sample can be used. The results obtain by DPAdCSV were compared with the HPLC in the literature [5], the statistical difference was not observed. In pharmaceutical and biomedical analytical field, the proposed method was also validated by evaluation of the validation parameters, such as linearity ranges, equation of calibration curve, LOD/LOQ, sensitivity, selectivity, robustness, ruggedness and stability. In addition, the electrochemical behavior of shiomarin, its coordination number and electron transfer numbers were also investigated (scheme 1).



Scheme 1. Chemical structure of shiomarin.

2. Experimental

2.1. Apparatus

Polarography and cyclic voltammetry were performed with E506 Polarecord, E-612 VA-Scanner, E-608 VA-Controller and 663-VA polarographic stand (Metrohm, Herisau, Switzerland). Hanging mercury drop microelectrode (HMDE, $r = 130$ μm) or a hanging copper/amalgam drop electrode (HCADE, $r = 130$ μm) was used as a working electrode, an Ag/AgCl (3 M KCl) used as the reference electrode and a Pt wire used as the auxiliary electrode. The amalgam was prepared by dissolving 2.0 mg copper powder in 5.0 ml pure mercury for a few days.

2.2. Reagents

Shiomarin was obtained from Hainan Pharmaceutical Factory (P. R. China) without further purification. Copper powder is spectrographic pure grade. All the other chemicals were of A.R. grade. Twice-quartz-distilled water was used in all experiments.

3. Results and discussion

3.1. Electrochemical behavior of shiomarin at HCADE

The supporting electrolyte in the experiment was found to influence the shape, half-width and potential of the peak of shiomarin. 0.10 M HCl–0.05 M KCl (pH 1.1) was finally chosen as the supporting electrolyte with its satisfactory results (Fig. 1a).

Fig. 1b showed the cyclic voltammogram of HCADE in HCl–KCl solution containing 2.0×10^{-7} M shiomarin. There were two reduction peaks in the potential range from -0.2 to -0.9 V. The peak located at -0.67 V was the cathodic reduction peak of shiomarin at mercury dropping electrode [10]. The peak of -0.34 V was the adsorption stripping peak of the copper ion from the complex formed by shiomarin with the Cu(II) at the HCADE. It was more sensitive than the

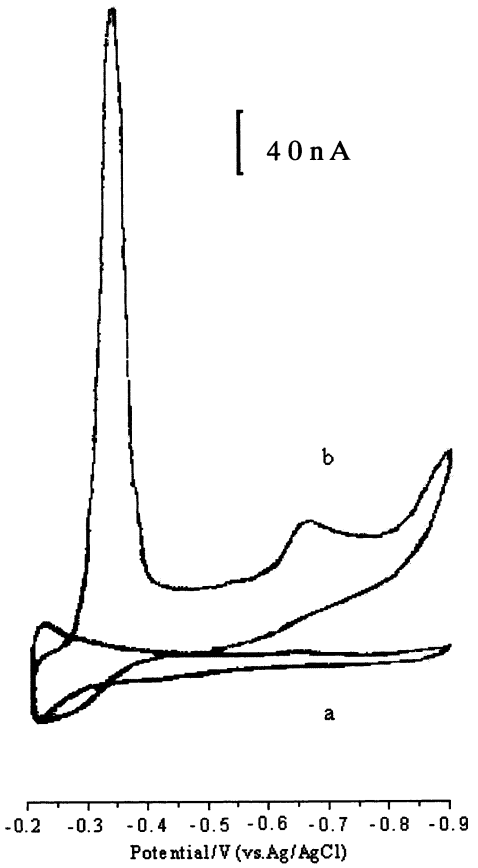


Fig. 1. Cyclic voltammogram of HCADE in (a), 0.10 M HCl–0.05 M KCl (pH 1.1); (b), $a + 2.0 \times 10^{-7}$ M shiomarin. Scan rate, 200 mV/s; preconcentration time (t_a), 70 s.

peak of shiomarin. The current of the peaks (I_{pc}) was found to be relevant with the concentration of shiomarin when accumulated at -0.1 V for a period of time. In the scan rate (v) range from 80 to 400 mV/s, a linear correlation between I_{pc} and v was found. It suggested that the electrode reaction belong to the kind of surface adsorption-controlled process.

3.2. Mechanism of the electrode reaction

3.2.1. Electronic numbers of electrode reaction (n)

From Fig. 1b, the half width of adsorption cathodic stripping peak of the complex is 52 mV. So it was a quasi-reversible reaction. According to the formula:

$$W_{1/2} = \frac{62.5}{\alpha n}(\text{mV},\ 25\ ^\circ\text{C})$$

[16], so $\alpha n = 1.2$. Suppose $\alpha = 0.5$, then $n = 2.4 \approx 2$.

3.2.2. Coordination numbers (p) of the complex of Cu(II) with shiomarin

At the HMDE, according to the formula [17]:

$$\frac{1}{I_p} = \frac{1}{I_{p,max}} + \frac{1}{\beta \cdot I_{p,max}} \frac{1}{C_{Sh}^p}$$

where $I_{p,max}$ represents the maximum reduction current of the Cu(II)-complex; C_{Sh} is the concentration of shiomarin; p is the coordination number; β is the stability constant of the complex. When $p = 1$, $1/I_p$ is linear with $1/C_{Sh}^p$, Thus Cu(II):Sh = 1:1.

Hence we proposed the following possible reaction mechanism of shiomarin and Cu(II) at the HCADE:

Stripping of Cu(II):

$$\text{Cu(Hg)} \rightleftharpoons \text{Cu}^{2+} + 2\text{e} + \text{Hg}$$

Formation of the complex:

$$\text{Cu(II)} + \rightleftharpoons \text{Cu(II)Sh}$$

Adsorption and cathodic stripping of the complex:

$$[\text{Cu(II)Sh}]_{sol} \rightleftharpoons [\text{Cu(II)Sh}]_{ad}$$

$$[\text{Cu(II)Sh}]_{ads} + 2\text{e} + \text{Hg} \rightleftharpoons \text{Cu(Hg)} + \text{Sh}$$

3.3. Trace determination of shiomarin

3.3.1. Determination of trace amount of shiomarin by DPAdCSV

From the cyclic voltammogram of shiomarin, it was shown the peak current is relative to the concentration of shiomarin. Differential pulse voltammetry (DPV) is a relatively sensitive new technique with better peak shape [18]. Fig. 2(b–h) showed the differential pulse adsorption of shiomarin in buffer solution at the HCADE. Compared with the cyclic voltammogram in Fig. 1(b), the shape of the peaks become symmetry and the reduction current was much higher. So the peak of the complex could be used to determining shiomarin by DPAdCSV.

3.3.2. Effect of the accumulation potential and the preconcentration time (t_a)

The accumulation potential affected directly the cupric stripping [15]. If the accumulation potential is too positive, such as from -0.06 to -0.08 V, excess of Cu(II) would interfere in the detection of the stripping of Cu(II)Sh. If the accumulation potential is too negative, such as from -0.12 to -0.15 V, no stripping peak of Cu(II) was observed. Therefore, the optimum accumulation potential was also -0.10 V. The peak current was found to depend on the preconcentration time (t_a) at -0.1 V. The current increased with increasing time and finally became stable. The time of adsorption equilibrium of Cu(II)Sh depended on the concentration of shiomarin. The higher its concentration was, the shorter the equilibrium time was. In Fig. 3, it could be seen that at first the peak current increased with t_a and then remained constant. Thus, we chose 70 s as the preconcentration time.

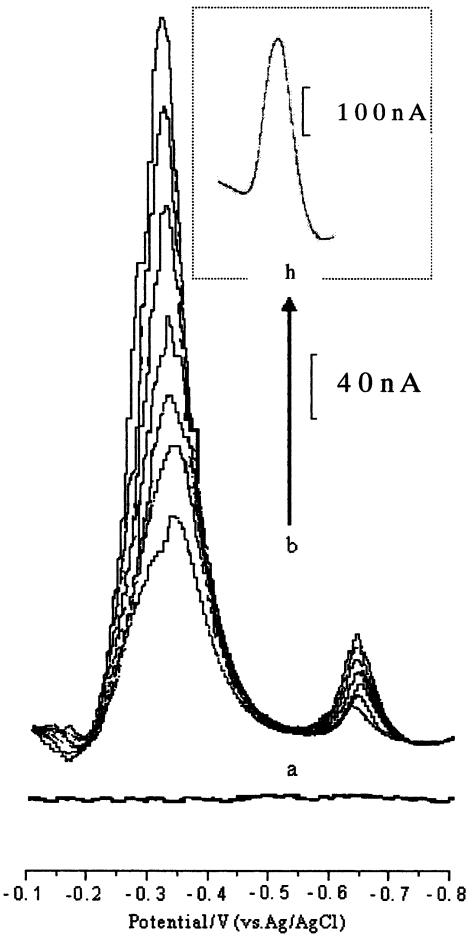


Fig. 2. Differential pulse adsorption cathodic stripping voltammogram in (a): 0.10 M HCl–0.05 M KCl (pH 1.1); (b–h): a + 3.0×10^{-8}, 4.0×10^{-8}, 5.0×10^{-8}, 6.0×10^{-8}, 7.0×10^{-8}, 8.5×10^{-8}, 1.0×10^{-7} M shiomarin, respectively, in buffer solution; (Inset): 8.2×10^{-8} M shiomarin in the electrolytic cell (1 ml urine + 9 ml electrolyte), which is 1/10 of shiomarin concentration in original urine sample. $\Delta E = -16$ mV; scan rate, 2.5 mV/s; preconcentration time (t_a), 70 s.

3.3.3. Linearity range, LOD/LOQ, sensitivity, and selectivity

At different t_a, the response current of shiomarin was different. When t_a is 70 s, the response was linear within the concentration range from 1.0×10^{-9} to 8.0×10^{-5} M. The linear regression equation was $y(i) = -1.502 + 4.977 \times 10^{9}$ x(C), with a correlation coefficient (r) of 0.9960, where $y(i)$ is the peak current in nA, $x(C)$ is the concentration in M. In the HPLC, peak height (h, mm) was linearly related to the concentration over the range from 5.6×10^{-7} to 4.0×10^{-5} M, $r = 0.9991$. The results of calibration curves for two methods were given in Table 1. The linearity range of the proposed method was wider than HPLC, but r was not very good as HPLC.

The limit of detection (LOD) was as low as 4.0×10^{-10} M (three times the ratio of signal to noise, $S/N = 3$) which was lower than the polarography in the literature [7] (8.0×10^{-8} M). The limit of quantitation (LOQ) was found as 8.5×10^{-10} M for the proposed method.

Selectivity is the ability of this method to determine shiomarin response in the presence of all the potential impurities. In this work, Br^- ($\leqslant 2.5 \times 10^{-4}$ M), I^- ($\leqslant 1.8 \times 10^{-4}$ M), 6-thioguanine ($\leqslant 1.0 \times 10^{-6}$ M), and azathiopurine ($\leqslant 1.0 \times 10^{-6}$ M) did not interfere the determination of 1.0×10^{-6} M shiomarin in pharmaceutical injection and urine samples.

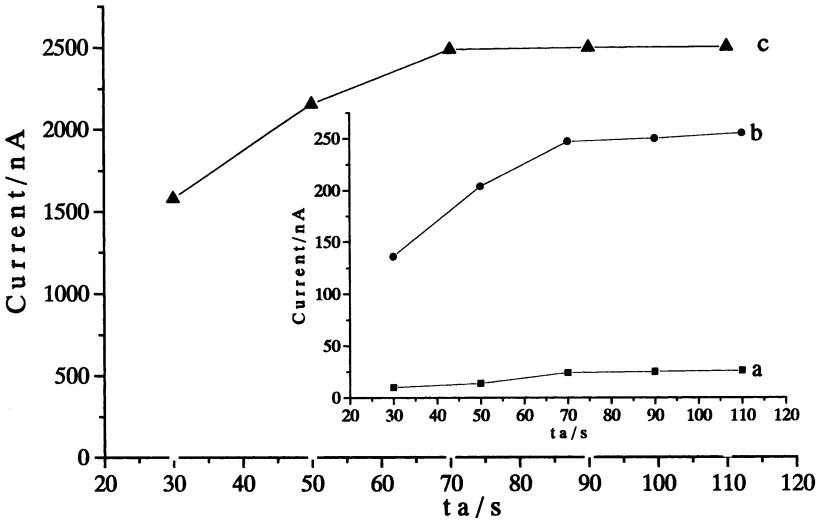


Fig. 3. The dependence of peak current on preconcentration (t_a). The concentration of shiomarin was (a), 5.0×10^{-9}; (b), 5.0×10^{-8}; and (c), 5.0×10^{-7} M.

3.3.4. Sample determination

Pharmaceutical injection of shiomarin weighing 10 mg was dissolved in 50 ml H_2O and diluted to various concentrations. Then the sample solution was put into electrochemical cell and was determined using the standard graph method. The results and the recovery were given in Table 2. The mean value of 9.61 ± 0.28 (mg) with variation coefficient of 3.4% was found for this method. The mean recovery of 96.6 ± 2.9 (%) with variation coefficient of 3.7% was found.

Fresh urine samples were diluted for ten times using HCl–KCl solution and then determined using this method (Fig. 2, inset). The contents of shiomarin were also compared with those obtained by applying HPLC [5]. The precision and validity of the results were shown in Table 3. Statistical analysis of the results revealed that this method was equally precise and accurate as HPLC.

3.3.5. Robustness, ruggedness and stability

The robustness of proposed method was tested by changing conditions, such as optimized parameters involving ΔE (pulse amplitude), v (scan rate), A (drop size), and t_a etc. were chosen for this study.

Table 1
The determined parameters for calibration curves of shiomarin obtained from proposed method (DPAdCSV) and comparison method (HPLC [5])

Method	DPAdCSV ($n = 8$)	HPLC ($n = 8$)
LOD (M)	4.0×10^{-10}	9.0×10^{-8}
Linearity range (M)	$1.0 \times 10^{-9} \sim 8.0 \times 10^{-5}$	$5.6 \times 10^{-7} \sim 4.0 \times 10^{-5}$
Regression equation ($y = a + bx$)	$y(i) = -1.502 + 4.977 \times 10^{9} \cdot \times$ (C)	$y(h) = 0.7200 + 2.828 \times 10^{7} \cdot \times$ (C)
R.S.D. of intercept (%)	0.8	0.4
R.S.D. of slope (%)	0.02	0.02
r	0.9960	0.9991
S_r	0.0003	0.0003
r^2	0.992	0.998

LOD, the limit of detection; $y(i)$, peak current; $x(C)$, concentration of shiomarin(M); $y(h)$, peak height(mm); r, correlation coefficient; S_r, standard error of correlation coefficient, r^2, determination coefficient.

Table 2
Determination of shiomarin in injection

Sample number	Value determined (mg)	Recovery (%)
1	9.13	94.5
2	9.62	94.7
3	9.38	96.4
4	9.96	96.1
5	9.44	93.6
6	9.38	93.9
7	9.96	99.9
8	10.0	104
Average values	9.61 ± 0.28	96.6 ± 2.9
R.S.D. (%)	3.4	3.7

Table 3
Statistical analysis determining shiomarin using the proposed method and HPLC

Sample	Amount found[a]			
	Proposed method	HPLC	t	F
Injection (mg)	9.61 ± 0.28	9.55 ± 0.27	0.36	1.01
Urine (10^{-7} M)	8.20 ± 0.24	8.38 ± 0.25	1.22	1.09

[a] Average $\pm$ S.D. of eight determinations; the t-and F-values refer to comparison of the proposed method with the pharmacopoeia methods. Theoretical values at 95% confidence limits $t = 2.145$, $F = 3.79$.

The ruggedness test of this method was also researched. The parallel shiomarin sample was analyzed by two analysts with this method using the same instrument. The results showed no statistical differences between different analysts.

In addition, in order to control the stability shiomarin stock solutions, injection and urine samples were kept at 4 °C during 20 days and were analyzed every day. It has been seen that repeatable peak currents of shiomarin solutions occurred and were stable.

4. Conclusion

Shiomarin can form complex with Cu(II) and be effectively accumulated from aqueous solution or urine samples onto the surface of HCADE. The DPAdCSV with hanging copper/amalgam drop electrode (HCADE) can be used to determine shiomarin at trace levels, its LOD was lower than microbiological assay [3], HPLC [5] and polarography [7]. The linearity range was wider than HPLC (Table 1). Analysis results determined shiomarin with this method was compared with the HPLC, no statistically significant difference was found between two methods. Obviously, this method was suitable and reliable like HPLC. Hence, this method was validated by evaluation of the validation parameters, such as, LOD, linearity ranges, equation of calibration curve etc.

In addition, this method is cheaper (such as chemicals, instrument etc.) and simpler than HPLC.

These results showed that the proposed method could be recommended for determining shiomarin in pharmaceutical injection and urine samples.

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