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ORIGINAL PAPER

Electrochemical solid phase micro-extraction and determination of salicylic acid from blood samples by cyclic voltammetry and differential pulse voltammetry

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Abstract The electrochemical solid phase micro-extraction of salicylic acid (SA) at graphite-epoxy-composed solid electrode surface was studied by cyclic voltammetry. SA was oxidized electrochemically in pH 12.0 aqueous solution at 0.70 V (vs. saturated calomel electrode) for 7 s. The oxidized product shows two surface-controlled reversible redox couples with two proton transferred in the pH range of 1.0~6.0 and one proton transferred in the pH range of 10.0~13.0 and is extracted on the electrode surface with a kinetic Boltzman function of $i_p=3.473–4.499/[1+e^{(t-7.332)/6.123}]$ ($\chi^2=0.00285$ μA). The anodic peak current of the extracted specie in differential pulse voltammograms is proportional to the concentration of SA with regression equation of $i_p=-5.913+0.4843\ c$ ($R=0.995$, SD=1.6 μA) in the range of 5.00~200 μM. The detection limit is 5.00 μM with RSD of 1.59% at 60 μM. The method is sensitive and convenient and was applied to the detection of SA in mouse blood samples with satisfactory results.

Keywords Electrochemical solid phase micro-extraction · Cyclic voltammetry · Differential pulse voltammetry · Salicylic acid · Mouse blood

Introduction

Solid phase micro-extraction (SPME) was firstly developed by Pawliszyn and co-workers in 1989 in an attempt to redress inherent limitations in solid phase extraction and liquid–liquid extraction [1, 2], has been considered as a solvent-free green sample preparation method, and is well reviewed in analytical chemistry [3, 4]. It has successfully combined with chromatography such as gas chromatography-mass spectrometry [5–7] and high-performance liquid chromatography [8–10] and is used in the detections of inorganic and organic components in complex matrices. Combination of SPME with electrochemistry derives a new sample preparation method, called electrochemical solid phase micro-extraction (ESPME) [11]. It possesses some advantages over SPME in improving the extraction efficiency and selectivity and enhancing the sensitivity due to (1) potentially controlling the extraction processes according to electric-field-driven ions and molecules electrophoresis [12, 13], (2) electrochemically controlling the states or forms of the extracted species and (3) the separation and detection can be performed at the same electrode without other extra processes such as eluting or releasing. The ESPME with polypyrrole modified solid phase electrodes have been applied to the detections of metal ions or cations [12–14] and anions (chloride, nitrite, bromide, nitrate, sulfate, and phosphate), in which the extraction efficiency has been increased for about 210 times, and the detection limit has been down to 625 nM from 10 μM. Differential pulse voltammetry (DPV) is a sensitive electroanalytical method for the detection of redox components in chromatography and other separation eluants [15]. The combination of ESPME with DPV is a real integral technique on separation and measurement.

Aspirin is a very old therapeutic medicine, which slowly hydrolyzes in aqueous solution and produces salicylic acid (SA), one of the most widely used as pain relieving, anti-inflammatory and analgesic agents, preventer of colorectal cancer [16], and antibacterial or antifungal agent. The detection of SA is a very important target in pharmaceutical, clinical, and physiological studies, and many methods have been developed such as spectrophotometry [17], spectropho-

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tofluorometry [18], enzymatic assay [19], chromatography [20], and electrochemistry [21]. Most of these methods need the preconcentrations of organic solvent extractions or reversed phase extractions, which are environmentally unfavorable, inconvenient, and time consuming. The adsorption and electrochemical behaviors of SA on glassy carbon electrode [21] and activated carbon [22] have been studied as well as its direct electrochemical detections [23, 24].

Graphite-epoxy-composed electrode (GECE) is a three-phase (insulator/solution/electrode) electrode [25] with some unique adhesion properties [26–29] and important electroanalytical applications [25–27]. In our experiments, it was found that SA shows a novel ESPME behavior from mouse blood samples at GECE and can be determined by DPV with higher selectivity and sensitivity without the need of additional organic solvent pretreatments. The results were reported in this paper.

Experimental

Apparatus and reagents

The electrochemical experiments were carried out on CHI620B electrochemical equipment (made in USA). A conventional three-electrode cell was used with a piece of spiral platinum wire as counter electrode, a homemade GECE in a glass tube as working electrode (geometry area is about 0.13 cm^2), and a saturated calomel electrode (SCE) as reference electrode. All potentials reported here were with respect to this reference electrode.

Salicylic acid, phenol, 1,2-diphenol, 1,3-diphenol, 1,4-diphenol, and other chemicals (purchased from Beijing Chemical) are all analytically pure. All solutions were prepared with ultrapurified water (18.2 MΩ)from a Millipore A-10 water purification system (Billerica, MA, USA). KCl was used as the electrolyte. All detections were carried out in pH 12.0 aqueous solution with 0.01 M KOH as the buffer system. All the solutions were dearated with high-pure nitrogen gas for 20 min prior to the electrochemical experiments.

Mice used in the experiments and blood sampling

The ten healthy Kunming male mice (at 8 weeks age) with average weight of 25.68±1.81 g were supplied by experimental animals center of Shenyang medical college, where they were fed according to the national standard with standard solid feeds.

Before the experiments, the mice were fasting for 12 h, and then intraperitoneal injection of 5% sodium salicylate into the mice bodies at the dose of 50 mg/kg was performed. After the injections of SA, 0.2 mL blood from each mouse was taken by internal carotid vein blood collection method for five times at every other hour (that were at 1, 2, 3, 4, and 5 h), from which 0.5 mL of the collected blood samples was used for the electrochemical experiments and the rest for the other physiological experiments.

Preparation of GECE

Graphite powder (200#), epoxy resin, and polyamide resin were mixed into a paste with a weight ratio of 8:2:2, tightly pressed into a clean glass tube (inner diameter is about 4 mm) with a copper wire at the other end as an electrode lead, and solidified in air for 72 h as described in the literature [23–27].

Newly prepared working electrode (<20 Ω) was polished with 360#, 600#, 800#, and 1000# sandpapers and glassine paper sequentially, cleaned by ultrasonic wave for 1 min, and pretreated in supporting electrolyte solution by cyclic voltammetry in the potential range of −0.5~0.55 V until a stable signal was obtained.

Experimental procedures

ESPME and DPV experiments SA solution (10.0 mL) was mixed with 8 mL 1.0 M KCl solution as electrolyte and 2.0 mL KOH in conventional electrochemical cell (calculated pH= 12.0) and dearated with high purity nitrogen gas for 20 min; the electrodes were put into the cell and the initial potential set at 0.70 V (vs. SCE) for the oxidation of SA in 7 s. The oxidized product adsorbs on GECE surface for ESPME. After ESPME, DPV was performed at the same electrode with the experimental parameters as initial potential of E_i=0.00 V, final potential of E_f=0.40 V, potential increment of 0.004 V, pulse width of 0.05 s, sample width of 0.0167 s, pulse period of 0.2 s, quiet time of 2.0 s, and sensitivity (A/V) of 5 μA/V for the detection of SA.

Blood sample measurement procedure Mouse blood sample (0.50 mL) was mixed with 1.0 mL 1.0 M KCl, 0.2 mL 0.02 M KOH, and 0.3 mL ultra-pure water in 5.0 mL electrochemical cell, and ESPME was performed according to the procedure described about (in 2.4.1). After ESPME, the electrodes were carefully washed with ultra-pure water, transferred into the conventional electrochemical cell electrolyte aqueous solution including 0.50 M KCl and 0.01 M KOH (pH 12.0), and the DPV for SA detection was performed.

Results and discussion

Electrochemical behavior of SA at GECE

Figure 1 shows the cyclic voltammograms of 0.20 mM SA in 0.50 M KCl electrolyte solution including 0.01 M KOH

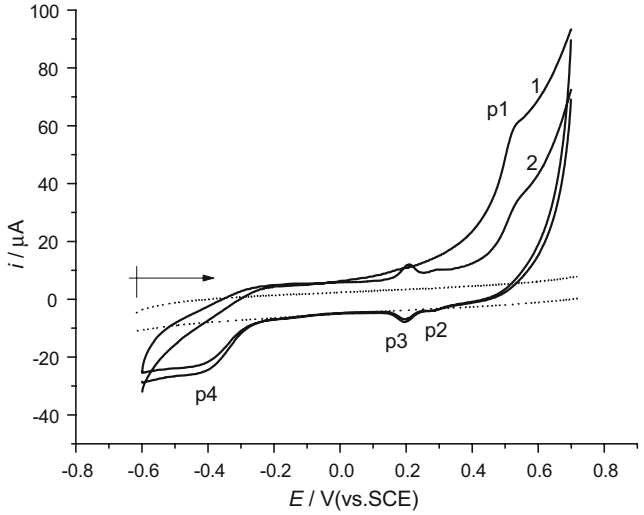


Fig. 1 Cyclic voltammograms of SA (*solid line*) and background (*dashed line*) at graphite-epoxy solid electrode in a 0.50-M KCl solution (pH=12) including 0.20 mM SA, quiet time 3 s at scan rate of 50 mV/s

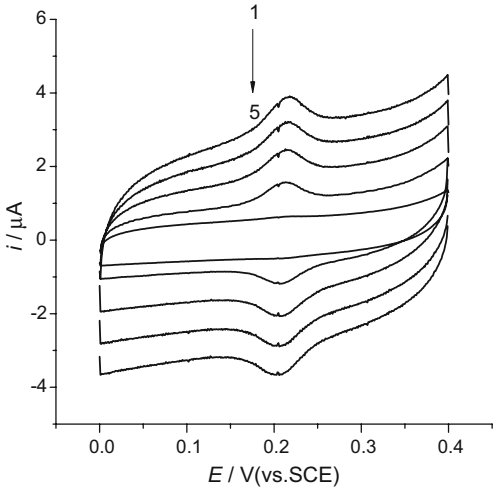


Fig. 2 CVs of oxidized SA on GECE in a 0.50-M KCl solution (pH=12) including 0.20 mM of SA, quiet time of 3 s, scan rate (V/s): *1* 0.04, *2* 0.03, *3* 0.02, *4* 0.01, *5* 0.005

(pH 12) at GECE working electrode. In the first scan, SA gives an irreversible anodic peak (p_{a1}) at 0.53 V and three cathodic peaks (p_{c2}, p_{c3}, p_{c4}) at 0.29, 0.20, and −0.42 V, respectively. In the second scan, p_{a1} is still there but decreases in peak height. There are two new anodic peaks (p_{a3}, p_{a2}) located at 0.32 and 0.21 V, combined with p_{c2} and p_{c3} composed of two reversible redox couples (labeled as p_2, p_3) [24] with the peak–peak potential differences of 15 and 29 mV, respectively. The small peak–peak potential differences indicate the redox processes belong to the adsorption of oxidation products.

After oxidation at 0.70 V with the quiet time of 3 s, cyclic voltammetric experiments were performed in the potential range of 0.00–0.40 V; the resulting CV curves are shown in Fig. 2. The p_2 is less sensitive than p_3. The p_2 alone shows in CV curves at shorter oxidation time (quiet time <3 s). The peak currents of p_3 increase proportional to scan rates with regression equations of $i_{pa3} = 0.918 + 75.7\,v$ ($R = 0.999$, $SD = 0.0555$) and $i_{pc3} = 0.417 + 82.5\,v$ ($R = 0.9999$, $SD = 0.0183$), respectively. Under the experimental conditions, the SA first irreversibly oxidized at 0.70 V for p_1. The oxidation product adsorbed at electrode surface, resulted in two reversible redox processes signed as p_2,and p_3, and was further irreversibly reduced for p_{4c}.

The p_{a1} is commonly used for direct electrochemical determination of SA [21, 23, 24] on glassy carbon electrode at 0.85 V. In the present work, it was reduced to E_{p1}= 0.53 V due to the pH effect and catalytic effect of GECE, the three-phase electrode. We also try to used p_1 for the detection of SA, but its shows less sensitivity (i_p(μA)= 0.3278+0.1639 c (μM) (R=0.9986, SD=0.8435) and narrow linear range of 20~200 μM. The ESPME behavior of SA might offer a new chance for the electrochemical detection of SA in complex matrix of samples).

The effects on the ESPME behavior of SA

The efficiency of ESPME has been affected by several experimental conditions such as solution pH, oxidation potential, oxidation time, quiet time, et al.

pH effects SA molecule is a hydroxyl benzoic acid with two dissociable protons (pK_{a1}=2.97; pK_{a2}=13.4 [30]) and shows several ionization states depending on solution pH, which greatly influences the electrochemical activity and ESPME of SA.

The solution pH was controlled in the range of 1.0~13.0 with conventional buffer systems such as potassium phosphate, ammonium, or KOH buffer systems in 0.50 M KCl electrolyte including 0.20 mM SA. After ESPME at 0.70 V for 7 s in different pH solution, the extracted SA was determined by DPV. The anodic current of p_3 goes up

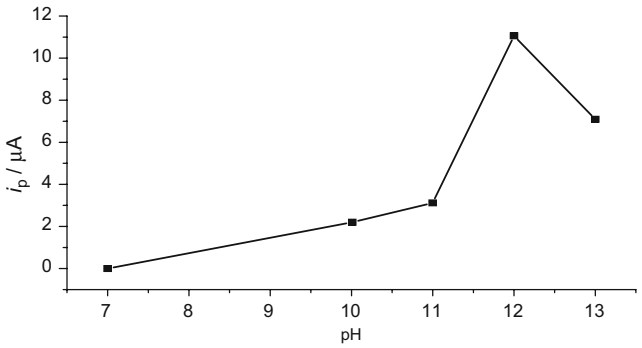
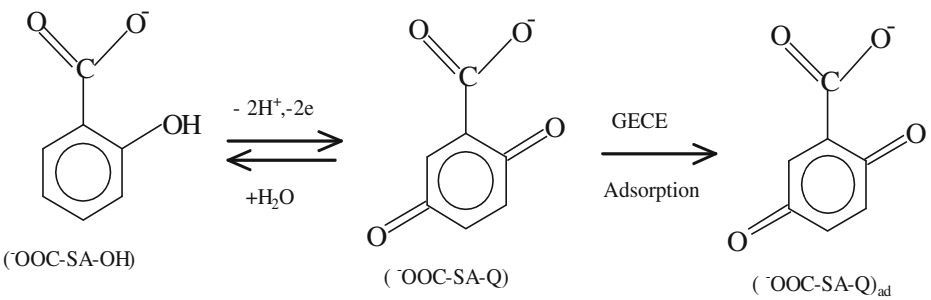


Fig. 3 The pH effects on oxidation peak current in electrolyte solution including 1.00×10^{-5} M SA after oxidation at 0.70 V for 7 s

linearly with the increase of pH in the range of 7.00~11, sharply goes up at pH=12.0, and then goes down at pH 13.0 as shown in Fig. 3. When the solution pH is near pK_{a2}=13.4, SA exists mainly in the form of $^{-}$OOC-SA-O^{-}, which is favorable for the oxidation process(p_1) and increases the amount of extraction.

KCl as electrolyte is barely used in alkaline solution due to the unstable chloride anion, which is easily oxidized into chlorine molecule and hypochlorite. But in our case, small amount of hypochlorite may be favorable for the SA oxidation into quinone for the ESPME and is responsible for the sharp increase of anodic current in pH≥12. With the increase of solution pH (>12), much amount of hypochlorite was produced with the help of oxygen in air, which not only oxidizes SA but also adsorbed quinone of SA into complex substance, which is responsible for red color change of solution and the sharp decrease of anodic peak current. In practice, pH 12.0 was chosen as the optimal condition in SA detection.

In the pH range of 1.0~6.0, the anodic peak potential is linearly changed according to the Nernst formulism with regression equation of E_p=1.078−0.03943 pH (R=0.9972, SD=0.01111), which indicates that there are two protons transferred in the p_3 redox process. In the pH range of 6.0~13.0, the anodic peak potential is linearly changed with pH with the regression equation of E_p=1.216−0.06141 pH (R=0.9969, SD=0.01256), which indicates there is one proton transferred during the redox processes. According to the above results and literatures [28], the mechanism of the ESPME of SA was summarized as the following:



(1)

At pH=12 aqueous solution, SA exists mainly in $^{-}$OOC-SA-O^{-} form, oxidized at 0.70 V, and forms a free radical (p_1), which further forms a quinoid form, Q-SA-COO^{-}, the Q-SA-COO^{-} adsorption at GECE electrode surface and reduction at returning scan, and becomes the hydroquinone and is responsible for the p_2 and p_3.

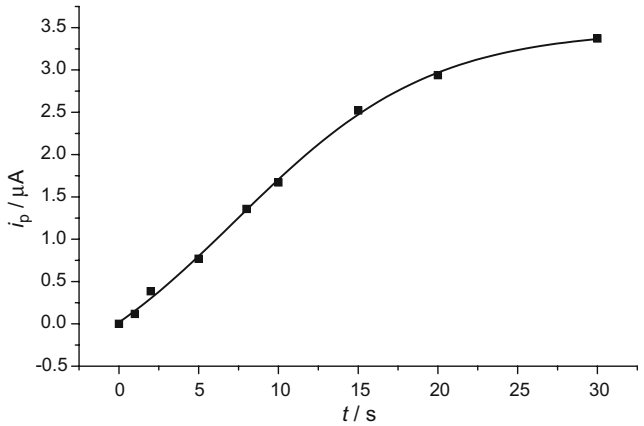


Fig. 4 The relationship between normalized peak current and oxidation time. Quit time, 7 s; oxidation potential, 0.70 V; other conditions were the same as before

Oxidation potential and oxidation time The oxidation potential is the potential for oxidation of SA in ESPME step. The amount of oxidation product of SA increases linearly with the increase of oxidation potential in the potential range of 0.45~0.90 V (not shown here), but too large positive oxidation potential will damage the GECE surface or increase the references from other substances. If the oxidation potential is lower than 0.40 V, the oxidation reaction does not occur at all. In practice, 0.70 V was chosen as the optimal oxidation potential in ESPME.

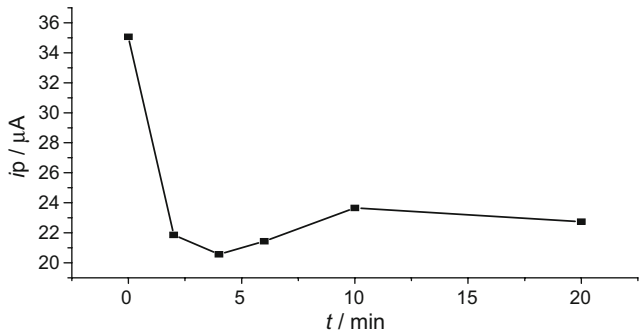


Fig. 5 The effect of quiet time on anodic current of SA

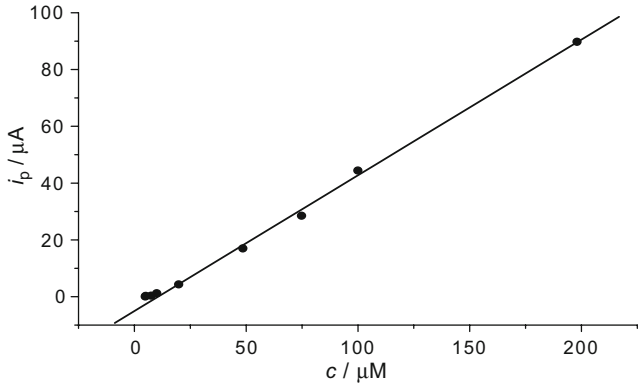


Fig. 6 The linear relation between anodic currents and concentrations. The experimental conditions were the same as described in experimental section

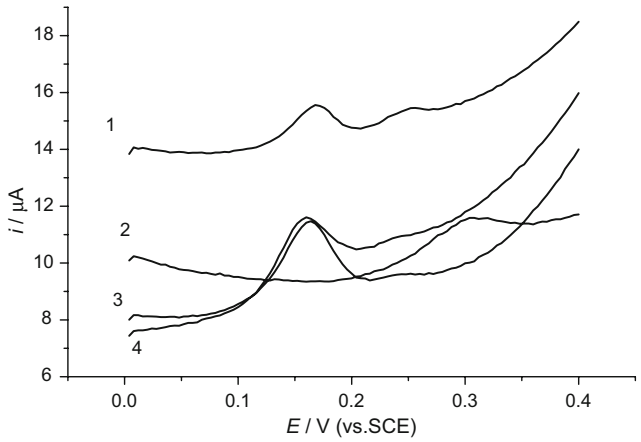


Fig. 7 DPV curves of SA at different conditions. *1* 20 μM SA in electrolyte solution, *2* directly measured in blood sample, *3* ESPME in blood sample then measure in electrolyte solution, *4* after addition of 5 μM of SA in blood

The oxidation time is defined as the time for the SA oxidation in ESPME process at the oxidation potential. The peak currents in DPV curves of SA with different oxidation time goes up firstly and then reaches the plateau value after 30 s. The peak current plotted against oxidation time is a Boltzman curve as shown in Fig. 4, which indicates the growth of adsorption membrane of oxidized SA on the electrode surface [24, 27].

The experimental data are fitted by general Origin software with regression equation as

$$i_p = 3.473 - 4.499 \Big/ \left[1 + e^{(t-7332)/6.123}\right] \left(R=0.9959, \chi^2=0.00285\right) \quad (2)$$

apparent rate constant of k=0.1633 (±0.017) s^{-1} and t_0= 7.33 s as the inflection point. In practice, the quiet time of 7 s for oxidation was set by CHI 620B instrument program. In practice, 7 s of oxidation time was chosen in order to get higher sensitivity for lower concentration of SA.

Accumulation time effects Accumulation time is defined as the period time putting the electrodes into sample solution before ESPME and waiting for the SA reaching the electrode surface, and establishes an equilibrium state at the surface of working electrode. The accumulation time can also be used to test the adsorption forms whether the SA or the oxidation product of SA. The anodic peak currents in DPVs sharply decrease with the increase of preconcentration time and then goes up and down slightly as shown in Fig. 5. This result indicates that the adsorption of SA occupies the active sites at electrode surface; the adsorbed SA cannot oxidized into quinine and is not favorable for the ESPME. It also indicates the oxidation before the adsorption in the case of SA. In practice, the electrochemical oxidation was performed just after setting up the electrodes in the solution without accumulation time.

Table 1 The allowed ratio of interference of phenols

Name	1,2-dp	1,3-dp	1,4-dp	Phenol
Allowed ratio of phenol	1.4	1.0	2.0	1.0

1,2-dp 1,2-diphenol, *1,3-dp* 1,3-diphenol; *1,4-dp* 1,4-diphenol

Detection of SA

Standard curve, detection limit and linear range After ESPME, DPV experiments were performed under optimal experimental conditions in 5.0~200 μM concentration range of SA. The anodic peak current is proportional to

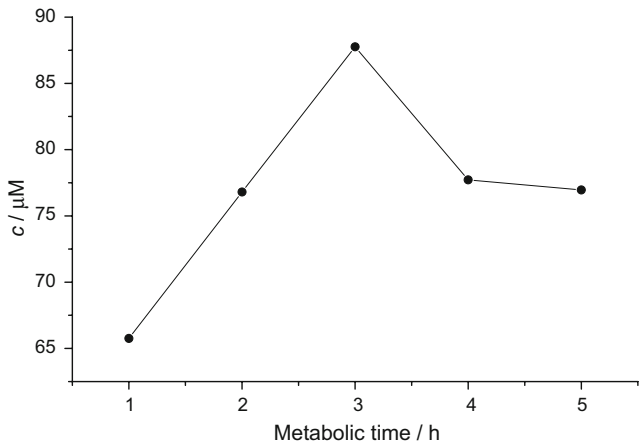


Fig. 8 The SA concentration changes with metabolic time in mouse blood

the concentrations of SA (Fig. 6) with regression equation of i_p (μA)=−5.913+0.4843 c (μM) (R=0.991, SD= 1.6 μA). The limit of detection is calculated as 5.0 μM according to the signal/noise ratio≥3, and 5.0 μM is also the low end limit of the linear range. The relative standard deviation was obtained as 1.7% at 60 μM concentration of SA for seven times, which is satisfactory for determination of trace SA. Compared with the direct detection with oxidation p_1, the sensitivity is increased three times, and the linear range is broadened about one amplitude.

Interferences The interferences from some electrochemically active phenols similar to SA on the electrochemical solid phase extraction and on the electrochemical detection of SA were tested under the optimal experimental conditions at 100 μM SA. The four phenols found some serious interference on the anodic peak current and peak shapes. Phenol is oxidized at 0.70 V on GECE electrode, adsorbs on the electrode surface, and shows an anodic peak at 0.06 V, which is 0.11 V more negative than that of SA. The oxidation products of SA and phenol competitively adsorb on the same electrode surface. With the increase of phenol, the phenol oxidation peak increased, but the oxidation peak of SA decreased. So the allowed ratio of phenol is no more than 1.0. The three diphenols (e.g., 1,2-diphenol, 1,3-diphenol, 1,4-diphenol) have no new oxidation peaks, but their existence will decrease the oxidation peak current of SA and influence the SA detection; the allowed ratios are 1.4, 1.0, and 2.0, respectively, as shown in Table 1.

Detection of SA in mouse blood samples According to the mice blood collection method (in experimental section), the mice blood samples were obtained. In the direct detection of SA in the sample by DPV, the oxidation potential shifts to 0.30 V from 0.17 V, and the peak broadened as shown in Fig. 7 (line 2) due to the matrix effects of blood. But we do the ESPME in blood sample solution and then carefully clean the electrode with ultrapure water and determined the SA in electrolyte solution as described above; we got an identical DPV curve (Fig. 7, line3, the concentration of SA was calculated as 16.17 μM) as that in electrolyte solution (Fig. 7, line 1). SA (5.0 μM) was added to the blood solution, the experiment was repeated, and we obtained DPV curve in Fig. 7 line 4 (the concentration of SA was calculated as 21.1 μM). The recovery of standard addition was calculated as 98.6%. Each sample was determined for three times, and average amounts of SA were plotted against the metabolic time as shown in Fig. 8. After the injection, the concentration of SA in mouse blood increases linearly, reaches the maximum value at 3 h, and then slowly decreases, which is similar to the general formula of drug metabolism in mouse body [31].

Conclusions

This paper described the ESPME behavior of SA at GECE and the direct electrochemical determination of SA in mouse blood samples; some conclusions are obtained as the following.

1. SA molecule can be easily oxidized in basic solution and separated from complex samples by ESPME.
2. The preconcentrated SA by ESPME offers a good chance for the determination of SA by DPV.
3. GECE electrode is a unique three-phase electrode for ESPME of quinone derivatives and can be used to analyze other phenol derivatives.
4. The combination of ESPME with DPV is a real integral technique and powerful for electroanalysis in a complex sample.
5. The determination of SA by DPV with ESPME is a successful example for organic drugs in blood samples, which offers lower detection limit and wide linear range.

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