March 2010 ISSN : 0974-7419 Volume 9 Issue 1

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 9(1) 2010 [86-92]

Enhanced oxidation of salbutamol sulphate at acetylene black-dihexadecyl hydrogen phosphate composite film electrode and its analytical applications

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Received: 3rd January, 2010 ; Accepted: 13th January, 2010

ABSTRACT

An electroanalytical method for determination of salbutamol sulphate was established on basis of enhanced oxidation of salbutamol sulphate at an acetylene black-dihexadecyl hydrogen phosphate composite film modified glassy carbon electrode (AB-DHP/GCE). Under optimal working conditions, this oxidation current was proportional to salbutamol sulphate concentration range from 4.0×10^{-7} to 7.5×10^{-6} mol L^{-1} and from 7.5×10^{-6} to 1.0×10^{-4} mol L^{-1} with a low detection limit of 1.0×10^{-7} mol L^{-1} for 2 min accumulation at open circuit (S/N = 3). The proposed method was applied to the determination of salbutamol sulphate in tablets and the results were satisfying.

KEYWORDS

Acetylene black;
Dihexadecyl hydrogen phosphate;
Salbutamol sulphate;
Chemically modified electrode;
Analytical applications.

INTRODUCTION

Salbutamol sulphate, also known as albuterol, is a β_2 adrenoceptor agonist and one of the most prescribed bronchodilators for the treatment of bronchospasm. It is also indicated in the prevention of exercise-induced bronchospasm and used in obstetrics for the prevention of premature labor and as a nasal decongestant. It would decompose in aqueous solution at elevated temperatures[1-5]. Different analytical methods for quantification of salbutamol sulphate have been described previously, such as high-performance liquid chromatography (HPLC)[6-8], capillary electrophoresis (CE)[9], spectrophotometric[10,11] and other methods[12,13]. Because most of these methods are complicated and expensive, and then the electrochemical method has been widely used based on its conveniency and high sensitivity[14]. To the best of our knowledge, a direct electrochemical method based on an acetylene black-dihexadecyl hydrogen phosphate (AB-DHP) composite film modified glassy carbon electrode (GCE) was not developed for its determination. Here, enhanced oxidation of salbutamol sulphate at AB-DHP composite film modified GCE and its analytical applications are reported.

As we know that many kinds of carbon electrodes with conventional dimensions are used in electrochemistry and electroanalytic chemistry, including diamond electrode[15], carbon paste electrode[16,17], pyrolytic

graphite electrode[18,19], GCE[20,21] and so on. During the last two decades, the focus on electroanalytical method turned to the functionaliztion and chemically modification of electrodes, which resulted in the enhancement of sensitivity and selectivity of electroanalysis subsequently[22-24]. Now more and more modified carbon electrodes appeared, such as carbon nanotubes or other nanoparticles modified electrode[25-29], polymers modified electrode[30,31]. In our previous work, acetylene black (AB) is successfully dispersed in a surfactant, dihexadecyl hydrogen phosphate (DHP), to form a homogeneous and stable suspension, which has been applied to the electrochemical analysis of organic molecules including drugs[32,33]. Herein, we prepare the AB-DHP composite film modified GCE by casting AB-DHP suspension on GCE surface and this modified electrode showed great enhancement effect towards electrochemical oxidation of salbutamol sulphate. The proposed method is feasible because of low detection limit, low cost, low background, and good reproducibility. Finally it was demonstrated by using salbutamol sulphate tablets and the results were satisfying.

EXPERIMENTAL

Apparatus and reagents

All the electrochemical measurements were performed on a CHI 650B electrochemical analyzer (Shanghai Chenhua Co., China) in a three-electrode system. The working electrode was an acetylene black-dihexadecyl hydrogen phosphate (AB-DHP) composite film modified glassy carbon electrode (GCE). A Pt wire and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. HPLC system consisted of a LC-10AT vp model pump (Shimadzu, Japan), SPD-10Avp detector (Shimadzu, Japan), JS-3050 chromatogram workstation (Dalian Jiangshen, China), C18 analytical column (5μm, 150mm×416mm) (Dikma Technologies, Diamonsil). The mobile phase consisted of sodium dihydrogen phosphate (adjust pH to 3.10 ± 0.05 with phosphoric acid)-methanol (85:15, v/v). Monitoring wavelength was 276nm.

Dihexadecyl hydrogen phosphate (DHP) was supplied by Fluka Chemical Reagent Corporation and stored at -18°C. Salbutamol sulphate was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China. Its stock solution was prepared by dissolving it in water to form a concentration of 1.0×10^{-2} mol L^{-1} and stored at 4°C. All the chemicals were used without further purification and all the solutions were prepared with doubly distilled water.

Preparation of the AB-DHP film coated GCE

5mg AB and 5mg DHP were dispersed together in 5ml redistilled water to give a 1mg mL^{-1} black AB-DHP suspension with the aid of ultrasonic agitation for several hours. Prior to modification, the GCE (A = 0.072 cm^2) was mechanically polished to a mirror finish with polishing microcloth containing 0.05μm Al_2O_3 slurry, and then carefully cleaned in 1:1 HNO_3-H_2O (v/v) and ethanol (C_2H_5OH), water in turn via ultra-sonication each for 3 min, and finally allowed to be aired. At last, 10μL of the AB-DHP suspension was cast on the GCE surface and air dried. Then a stable and uniform AB-DHP film was formed.

Procedure

A certain volume of 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 7.13) was used as the supporting electrolyte in a conventional electrochemical cell. At the beginning of experiment, AB-DHP film coated GCE was successively scanned between 0.20V and 1.00V at a scan rate of 100mV s^{-1} to get a steady cyclic voltammograms. Then salbutamol sulphate stock solutions were added into the cell to make up 10mL mixture solution. The accumulation was carried out at open circuit via stirring the solution for 2 min, and then kept quiet for 5 s. Finally the voltammograms were recorded between 0.20V and 1.00V. After each measurement the modified electrode was refreshed by successive cyclic voltammetric sweeps in blank supporting electrolyte to get a reproducible electrode surface.

RESULTS AND DISCUSSION

Electrochemical responses of salbutamol sulphate

The electrochemical responses of 5.0×10^{-5} mol L^{-1} salbutamol sulphate were investigated in phosphate buffer solution (PBS, pH 7.13) at AB-DHP film modi-

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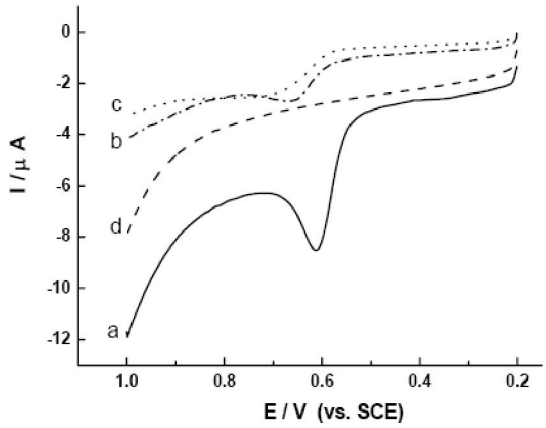

Figure 1 : Linear sweep voltammetric responses of AB-DHP film modified GCE (a), bare GCE (b), DHP film modified GCE (c) in the presence of 5.0×10^{-5} mol L^{-1} salbutamol sulphate and AB-DHP film modified GCE (d) in the absence of salbutamol sulphate at in phosphate buffer solution (PBS, pH 7.13). Scan rate, 100mV s^{-1}.

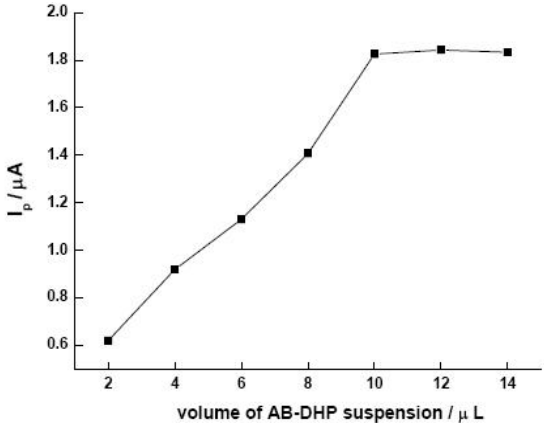

Figure 2 : Dependence of volume of AB-DHP suspension at GCE surface on peak current of 8.0×10^{-6} mol L^{-1} salbutamol sulphate in phosphate buffer solution (PBS, pH 7.13). Scan rate, 100mV s^{-1}.

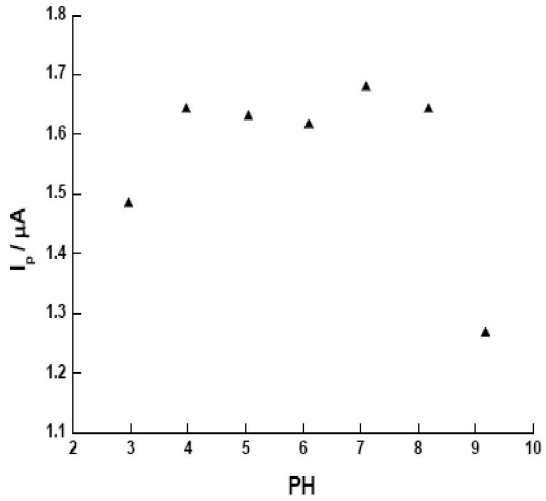

Figure 3 : Effects of solution pH on the peak current (-▲-) of salbutamol sulphate at AB-DHP film modified GCE in phosphate buffer solution. Scan rate, 100mV s^{-1}.

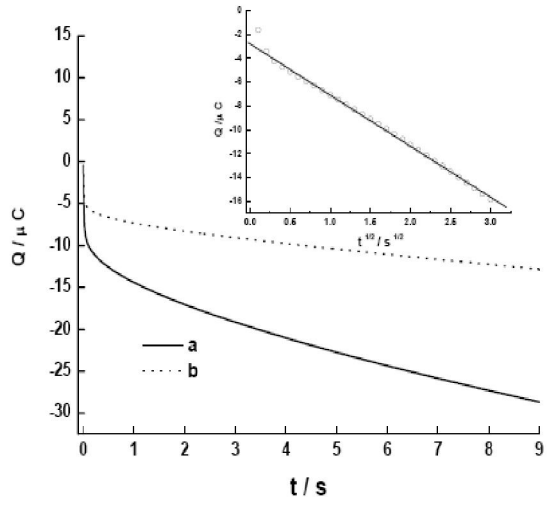

Figure 4 : Chronocoulometry of 5.0×10^{-4} mol L^{-1} salbutamol sulphate at AB-DHP film modified GCE in 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 7.13) (curve a). Curve b stands for that in the blank solution. The inset shows the linear relationship between the charges (Q) and the square roots of times ($t^{1/2}$) for the oxidation reaction (background subtracted). Initial potential 0.35V, final potential 0.70V, and pulse width 9 s.

fied GCE (AB-DHP/GCE). A well-defined oxidation peak with large peak current appears at 0.62V on the first cycle of successive voltammograms when the potential sweeps from 0.20V to 1.00V and no apparent peak is observed in the reverse scan, indicating that the oxidation of salbutamol sulphate is a totally irreversible process. During following successive cyclic sweeps, the oxidation peak at 0.62V greatly decreases with the increasing of scan number, resulting from the fact that the electrode surface was blocked by the strong adsorption of the reaction products.

Linear sweep voltammetry (LSV) was used to research the electrochemical responses of 5.0×10^{-5} mol L^{-1} salbutamol sulphate at different electrodes in PBS, including AB-DHP/GCE, DHP film modified GCE (DHP/GCE) and bare GCE as shown in figure 1. Curve d stands for the electrochemical responses of AB-DHP/GCE in the absence of salbutamol sulphate(Figure 1). Obviously, at bare GCE, a well-defined oxidation peak with low current can be observed at about 0.68V (curve b). When DHP was cast on the GCE surface, the formation of a DHP film blocks mass and electron transfer between salbutamol sulphate in solution and the electrode surface because DHP is hydrophobic and insulated. The oxidation peak current of salbutamol sul-

phate decreases lightly and peak potential shifts positively (curve c in Figure 1). On the contrary, the electrochemical responses of salbutamol sulphate at AB-DHP/GCE (curve a) are different from those poor responses at DHP/GCE and bare GCE. The oxidation peak current increases greatly and peak potential shifts negatively, suggesting that the oxidation of salbutamol sulphate was improved because the acetylene black in DHP layer could improve the conductivity of modified electrode and promote the electron transfer between salbutamol sulphate and electrode. At the same time, the porous AB-DHP film could increase the area of electrode surface and offer more active sites for oxidation of salbutamol sulphate, and then the oxidation peak current increased correspondingly. In fact, AB-DHP composite can be seen as a new type of carbon paste, in which AB acts as the carbon conductive materials and DHP as the adhesive. Similar to conventional carbon paste, AB-DHP composite has the virtues of wide potential windows, easy fabrication and modification, low cost, and so on. These, coupled with the properties of good reproducibility, variable surface properties by changing the adhesive and controllable film thickness, indicate the promising applications in electroanalytical chemistry.

Effects of thickness of film

The thickness of film cast on the electrode surface had greatly influenced the oxidation current of salbutamol sulphate. The key factor influencing thickness of film is the volume of solution used in making membranes. So we studied the effects of film thickness on oxidation current of 8.0×10^{-6} mol L^{-1} salbutamol sulphate by LSV(Figure 2). The results were described in figure 2. In general, the oxidation peak current of salbutamol sulphate increases quickly with the increase of the amount of AB-DHP dispersion cast on the GCE surface that ranged from 2 to 10μL. Further improving the amount of dispersion, there will be no further increase. This would be better explained as a mass transfer issue. If all incoming salbutamol sulphate reacted by the time it reaches a certain depth in the film, then a thicker film would not help unless the mass transfer rate could somehow be improved. As a result, considering the convenience of constructing modified electrode 10μL of 1mg mL^{-1} AB-DHP suspension was suitable to the development of AB-DHP film electrode for determination of salbutamol sulphate.

The optimum of the supporting electrolyte

Supporting electrolyte played an important role in the voltammetric determination of salbutamol sulphate. The current responses of 8.0×10^{-6} mol L^{-1} salbutamol sulphate were studied by cycle voltammetry (CV) at AB-DHP/GCE in several supporting electrolytes, including 0.1 mol L^{-1} PBS (pH 3.0~9.0), 0.1 mol L^{-1} sodium acetate - acetic acid buffer solution (NaAc-HAc, pH 3.5~5.6) and other different acids and alkalies such as $HClO_4$ (0.1 mol L^{-1}), H_2SO_4 (0.05 mol L^{-1}), HCl (0.1 mol L^{-1}), HAc (0.1 mol L^{-1}), KCl (0.1 mol L^{-1}) and NaOH (0.1 mol L^{-1}). It was found that PBS (pH 7.13) was the most favorable condition for determination of salbutamol sulphate and the electrode had good repeatability. Based on this result, PBS (pH 7.13) was chosen as supporting electrolyte to detect salbutamol sulphate.

LSV was used to examine the effects of solution pH on voltammetric determination of 8.0×10^{-6} mol L^{-1} salbutamol sulphate. The results were illustrated in figure 3. With the increase of pH the peak potential of salbutamol sulphate shifts negatively and obeys the equation:

$$E_p = 1.0408 - 0.05886\ pH\ (R = 0.9953) \quad (1)$$

The slope of 59 $mVpH^{-1}$ suggested that the number of the electrons transferred in the oxidation of salbutamol sulphate is equal with that of protons. It can be seen that the oxidation peak currents of salbutamol sulphate have not remarkable change in the pH range from 4.0 to 8.0 and decrease out of this pH range. It may be caused by the protonization or ionization of salbutamol sulphate in strong acidic or alkaline solution. In this system, pH 7.13 was used as optimized pH to determine salbutamol sulphate in standard solutions and real samples.

Influences of accumulation conditions on the determination of salbutamol sulphate

Firstly, influences of initial potential on oxidation current of 8.0×10^{-6} mol L^{-1} salbutamol sulphate were tested. In the range of initial potential from - 0.5 to + 0.2 V, it almost had no influence on the oxidation peak current. If the potential sweep started from anodic scan

at + 0.3 V then reversed at 1.0 V, the peak current became depressed. This fact indicated that excessive positive potential would influence the adsorption and oxidation of positive charged salbutamol sulphate in weak acidic medium and not be appropriate for its quantitative analysis.

Then, as to the accumulation potential, its effects on the oxidation of salbutamol sulphate were described as following sentences. In the range from -0.5 to + 0.5 V, the oxidation peak current changed slightly and was similar to that obtained at open circuits. In our system, all accumulation experiments were carried out at open circuits.

Lastly, the influences of accumulation time on the determination of salbutamol sulphate were studied. In the accumulation time range from 1 min to 2 min, the peak current increases greatly with the prolonging of accumulation time. Overrunning 2 min, the increase of the oxidation current becomes very slow due to the saturation of surface coverage of adsorbed salbutamol sulphate. So the 2 min was chosen as the best accumulation time.

Effects of scan rate and calculation of some other parameters

The effects of scan rate on the determination of salbutamol sulphate were tested by LSV. In the range of scan rate from 0.025 to 0.30V s^{-1}, peak current increased with the increase of scan rate obeying equation as follows:

$$I_p = 17.2813\, v^{1/2} - 0.0515 \quad (R = 0.9989) \qquad (2)$$

Thus it can be seen that the oxidation process of salbutamol sulphate is controlled by diffusion step. Although the peak current increases with the increase of scan rate the background current is enhanced too. In our system, high scan rate is not suitable for the measurement of peak current. And then the scan rate 100mV·s^{-1} was chosen for quantitative analysis of salbutamol sulphate. The relationship between the oxidation peak potential and scan rate is described by the following equation:

$$E_p = -0.01428 \ln v + 0.6500 \quad (R = 0.9953) \qquad (3)$$

The slope indicates that the value of αn_α is 0.91 where α is the transfer coefficient and n_α is the number of the electrons transfer. On the base of the assumption that α is 0.5 and then the value of n_α is calculated to be 2. The electrochemical oxidation of salbutamol sulphate at AB-DHP/GCE in PBS (pH 7.13) is an irreversible process involved two electrons and protons, which may be attributed to the oxidation of the phenolic group and the amino group at the same potential range under this experimental conditions referring to the references[13,34], one possibility is the formation of dimmer.

Chronocoulometry was used to characterize the oxidation of salbutamol sulphate at AB-DHP/GCE in PBS (pH 7.13). The results were depicted in figure 4. The diffusion coefficient D could be calculated according to following equation given by Anson[35].

$$Q = 2nFAcD^{1/2}\pi^{-1/2}t^{1/2} + Q_{dl} \qquad (4)$$

where A is the surface area of the working electrode, c is the concentration of salbutamol sulphate. Q_{dl} is double-layer charge, which could be eliminated by subtraction of the background charge in this system. Other symbols have their usual significances. After the subtraction of the background charge, the plots of Q against t in figure 4 are converted into the plots of Q against $t^{1/2}$. It is clear that the charges (Q) have linear relationships with the square roots of time ($t^{1/2}$) for the oxidation reaction (inset in Figure 4). Based on the calculated value of the electron number n involved in the oxidation of salbutamol sulphate, the diffusion coefficient of D in this system can be obtained as 1.64×10^{-6} cm^2s^{-1}.

TABLE 1 : Determination of salbutamol sulphate in tablets (n=5)

Sample No.	Declared (mg/tablet)	Detected by HPLC (mg/tablet)	Detected by this method (mg/tablet)	Recovery (%)
1	2.4	2.312±0.016	2.344±0.035	102.6
2	2.4	2.308±0.029	2.313±0.041	99.6
3	2.4	2.289±0.101	2.301±0.056	100.9
4	2.4	2.301±0.019	2.326±0.028	101.0
5	2.4	2.322±0.026	2.328±0.107	99.5

Calibration and interferences

The dependence of oxidation current on salbutamol sulphate concentration was tested. The peak current was proportional to the concentration range from 4.0×10^{-7} to 7.5×10^{-6} mol L^{-1} and from 7.5×10^{-6} to 1.0×10^{-4} mol L^{-1}, which could be respectively described as:

$$I_p\ (\mu A) = 0.1523 + 0.2063\, c\ (\mu mol\ L^{-1}) \quad (R = 0.9970) \qquad (5)$$

$$I_p\ (\mu A) = 1.2304 + 0.08299\, c\ (\mu mol\ L^{-1}) \quad (R = 0.9963) \qquad (6)$$

Different slopes of two curves suggested an adsorption process has been involved in the electrochemical oxidation of salbutamol sulphate.

Under the optimized experiment conditions, a detection limit of 1.0×10^{-7} mol L^{-1} was obtained. After each measurement the modified electrode was rinsed with water thoroughly, transferred to the blank electrolyte and refreshed by successive cyclic voltammetric sweeps in order to get a reproducible electrode surface. The relative standard deviation (RSD) of 3.8% for 12 parallel detections of 5.0×10^{-5} mol L^{-1} salbutamol sulphate suggested excellent reproducibility of AB-DHP/GCE.

If this proposed electroanalytical method would be applied to detect salbutamol sulphate in real samples, the interferences of some concomitant substances should be estimated. The interferences of some substances on the determination of 5.0×10^{-6} mol L^{-1} salbutamol sulphate were investigated. The experiment outcomes indicated that most of metal ions had almost no effects on the determination of salbutamol sulphate (signal change below 3%). For examples, 100 fold of Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Fe^{2+} and Fe^{3+}, combined with 1000 fold of SO_4^{2-}, NO_3^- and Cl^- all of them did not influence the determination of salbutamol sulphate. Additionally, the interferences from some common biomolecules were also examined. The results proved that they had also no effects on the determination of salbutamol sulphate including 1000 fold of glucose, 150 fold of saccharose, 100 fold of ascorbic acid (AA), dopamine (DA), uric acid (UC), lactose, amylum, dextrin and 50 fold of sodium tartrate. Based on experiment data, an electrochemical method was proposed and applied for the determination of salbutamol sulphate in tablet forms.

Drug analysis

The proposed electrochemical method for determination of salbutamol sulphate was demonstrated by using salbutamol sulphate tablets. The tablet samples were pretreated as follows: one tablet of Liusuanshadinganchun Pian (Changzhou Kangpu Pharmaceutics Co. Ltd., China, 2.4mg salbutamol sulphate/tablet) was accurately weighed and finely powdered. The precise amount of powder was dissolved in 2.4ml water and sonicated in water bath for 10 mins, and centrifuged at 3000rcf. The clear supernatant was collected and stored as stock sample solution at 4°C in dark. Prior to measurements, the sample solutions were diluted with supporting electrolyte.

The concentration of salbutamol sulphate was calculated using standard additions method. The relative standard deviation (RSD) of each sample for 5 times parallel detections was less than 3.8%, and the recovered ratio on the basis of this method was investigated and the value is between 99.1% and 103.0%. The results were illustrated in TABLE 1. These experimental data indicated that the determination of salbutamol sulphate using AB-DHP film modified GCE in phosphate buffer solution (PBS, pH 7.13) was effective and sensitive. At last, the method of HPLC was used for the determination of salbutamol sulphate to estimate the feasibility, precision and efficiency of this method (TABLE 1). The good accordance between data from HPLC and this method we proposed indicated the reliability of the present electrochemical method for salbutamol sulphate determination in tablet samples.

CONCLUSIONS

A simple and sensitive electrochemical method was established for determination of salbutamol sulphate and was well demonstrated by using salbutamol sulphate tablets. This developed method was based on the enhanced electrochemical responses of salbutamol sulphate due to its adsorption accumulation at an acetylene black-dihexadecyl hydrogen phosphate composite film (AB-DHP) modified GCE. This composite film modified electrode was easy to be prepared with low costs and it could greatly enhance the sensitivity of detecting salbutamol sulphate compared with bare GCE. According to all the experiment data this method we proposed for determination of salbutamol sulphate was sensitive and feasible.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 30770549 and 60571042).

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