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# Voltammetric determination of ethinylestradiol at a carbon paste electrode in the presence of cetyl pyridine bromine

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## Abstract

Electrochemical behaviors of ethinylestradiol at a carbon paste electrode (CPE) in the presence of cetyl pyridine bromide (CPB) are investigated by electrochemical techniques. Compared with that at a CPE without CPB, the oxidation peak potential of ethinylestradiol shifts negatively and the peak current is increased significantly, due to the enhanced accumulation of ethinylestradiol via electrostatic interaction with CPB at the hydrophobic electrode surface. It is verified by the influences of different kinds of surfactants on the electrochemical signals of ethinylestradiol. Some parameters such as pH, scan rate, accumulation potential and accumulation time on the oxidation of ethinylestradiol are optimized. Under optimal conditions, the oxidation peak current is proportional to ethinylestradiol concentration in the range of $5.0 \times 10^{-8}$ to $2.0 \times 10^{-5}$ mol $L^{-1}$ with a detection limit of $3.0 \times 10^{-8}$ mol $L^{-1}$ for 150 s accumulation by linear sweep voltammetry (LSV). The proposed procedure is successfully applied to determine ethinylestradiol in pharmaceutical formulation (Levonorgestrel and Etinylestradiol tablets) and the results are satisfying compared with that of high-performance liquid chromatography (HPLC).

*Keywords:* Ethinylestradiol; Cetyl pyridine bromide; Carbon paste electrode; Electroanalysis

## 1. Introduction

Ethinylestradiol is a semisynthetic estrogen female sex hormone that is widely used as oral contraceptives. The determination of synthetic estrogen has attracted much attention since these compounds are included in the endocrine disrupting chemicals and able to cause the deleterious reproductive dysfunction of animals and humans [1].

Primarily because of its high estrogenic potency, ethinylestradiol is also included as a test compound in a series of studies designed to evaluate the developmental, reproductive, and chronic toxicities of a series of hormonally active compounds with estrogenic activity [2].

Many analytical methods have been established for its determination with a variety of sample matrices involved in surface water, underground water, waste water, pharmaceutical formulations and biological fluids. Spectrophotometry [3–6], liquid chromatography [7,8], gas chromatography [9–13], high-performance liquid chromatography [14–17], micellar electrokinetic capillary chromatography [18,19], isotope dilution mass spectrometry [20], electrophoresis [21], immunoassay [22,23] and chromatographic separation with amperometry detection [24] have been employed for the determination of ethinylestradiol. Although sensitive and selective, they are time-consuming, expensive and require skilled personnel and therefore are unsuitable for on-line or field monitoring. The versatility of electrochemical techniques and the smallest detection limits as well as its low acquisition costs made them widely applied in the various fields, especially in the determination of biologic/chemical materials which possess electroactive groups. However, few experiments have been carried out to assay ethinylestradiol using any of electrochemical techniques except that the voltammetric assay of the estrogen drug ethinylestradiol in pharmaceutical formulation and human plasma at a mercury electrode with adsorptive cathodic stripping voltammetry [25]. Although lower detection limit can be obtained for the determination of ethinylestradiol in pharmaceutical formulation and in human serum and plasma without the necessity for sample pretreatments and/or time consuming extraction or evaporation steps prior to the analysis, the pollution of mercury towards environmental protection and human health can not be

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neglected. Therefore, a rapid, sensitive, accurate and low cost electrochemical procedure is desired for the determination of ethinylestradiol and testing content uniformity of its dosage form.

In the presented paper, electrochemical behaviors of ethinylestradiol are investigated at a carbon paste electrode in the presence of cetyl pyridine bromide. Experimental results indicate that the oxidation peak potential of ethinylestradiol shifts negatively and its peak current is enhanced significantly with the cooperation of CPB. Base on this, an electrochemical measurement is proposed for the determination of ethinylestradiol in pharmaceutical formulation. The procedure possesses a lower detection limit, a rapid response, excellent reproducibility, simplicity and low cost, and has a potential application in the successive detection of ethinylestradiol in environmental conditions.

## 2. Experimental

### 2.1. Instruments and reagents

Electrochemical data are obtained with a three-electrode system using a CHI 660 A electrochemical workstation (CH Instruments, Texas, USA). A carbon paste electrode is used as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. HPLC system consists of a LC-10AD model pump (Shimadzu, Japan), SPD-10AV UV–Vis detector (detect at 250 nm), Kromasil ODS analytical column (5 μm, 4.6 × 250 mm), N2000 chromatographic workstation (Zhida, China) and 20 μL injection loop. The mobile phase is prepared with methanol and distilled water (70:30, V/V) and is delivered at a constant flow-rate of 1.0 mL·min$^{-1}$.

Ethinylestradiol stock solution of $5.0\times10^{-3}$ mol L$^{-1}$ is prepared by dissolved in ethanol and keeps at 4 °C until used. Then, they are diluted to required concentration using phosphate buffer solution. Phosphate buffer (PB) is prepared from $KH_2PO_4$ and $Na_2HPO_4$ with distilled water. CPB (purchased from Shanghai Reagent Corporation, China) is dissolved in double-distilled water to form $1.0\times10^{-2}$ mol L$^{-1}$ homogeneous solutions. Other chemicals used are of analytical grade and are used without further purification. All electrochemical experiments are carried out at room temperature.

### 2.2. Preparation of a carbon paste electrode

Carbon paste electrode is prepared as following procedure: 500 mg graphite and 200 μL paraffin oil are mixed in a small mortar to form a homogeneous mixture. The mixture is pressed by hand into the end cavity of a home-made polytetrafluoroethylene (PTFE) cylindrical electrode body and the electrode surface is polished manually on a piece of weighing paper. The freshly prepared CPEs are activated in blank supporting electrolyte by using successive cyclic scans from −0.10 V to 1.00 V until stable voltammograms are obtained.

### 2.3. Electrochemical measurements

Electrochemical experiments are carried out in a conventional electrochemical cell, containing 10 mL 1/15 mol L$^{-1}$ PB solution (pH = 8.04) and a certain concentration of ethinylestradiol and CPB. After accumulating at 0.30 V for 150 s with stirring the solution and keeping quiescent for 10 s, the voltammograms are recorded in the potential range from −0.10 V to 1.00 V at the scan rate of 100 mVs$^{-1}$. The oxidation peak currents are measured at 0.56 V for the quantification of ethinylestradiol. After each measurement, in order to remove the previous deposits completely, the electrode surface is refreshed by cyclic voltammetric sweeps from −0.10 V to 1.00 V in a blank supporting electrolyte solution to produce a reproducible electrode surface. The same procedure is applied in the samples analysis and all electrochemical experiments are carried out at room temperature (20 ± 2 °C). Removal of oxygen is achieved by using high purity $N_2$.

### 2.4. Sample preparation

Samples of Levonorgestrel and Etinylestradiol tablets (Triphasic) are obtained from Schering Gmbh and Co. Produktions KG (Weimar, Germany). Sugar-coat of the sample (three pieces) is peeled off by hand and weighed, then is extracted with 10 mL ethanol for 24 h. After centrifugation (5 min at 10000 rpm), the extract is filtered with 0.45 μm membrane filters, then the residues are washed twice with ethanol. The extracts and washings are combined and then diluted in a volumetric flask with ethanol. The extracts are further diluted with supporting electrolyte solution prior to their analysis.

## 3. Results and discussion

### 3.1. Electrochemical behavior of ethinylestradiol at a carbon paste electrode

The electrochemical behaviors of ethinylestradiol in the absence or presence of $5.0\times10^{-5}$ mol L$^{-1}$ CPB are investigated by cyclic voltammetry (CV) (Fig. 1). It can be seen that no apparent redox peaks appear in the phosphate buffer in the presence of CPB within the potential window from −0.10 to 1.00 V (curve a). When in the absence of CPB, a weak oxidation peak is observed at 0.592 V during the CV scan (curve b). After the addition of CPB, the oxidation peak current of ethinylestradiol are markedly enhanced and the anodic peak potential shifted negatively to 0.543 V (curve c). It suggests that the electrochemical process of ethinylestradiol is facilitated by the addition of cation surfactant CPB.

It is well known that surfactants can be adsorbed on hydrophobic surface to form surfactant film, which may alter the overvoltage of the electrode and influence the rate of electron transfer. When in the presence of CPB, the electrode surface may form a hydrophilic film with positive charge. Nevertheless, in a basic solution (pH 8.04), ethinylestradiol is in the form of phi-O$^-$. The anions can be adsorbed to the surfactant film through electrostatic interaction and the concentration of ethinylestradiol at the electrode surface is therefore increased. The conclusion is well demonstrated by the effect of various surfactants on the electrochemical behaviors of ethinylestradiol at the carbon paste electrode.

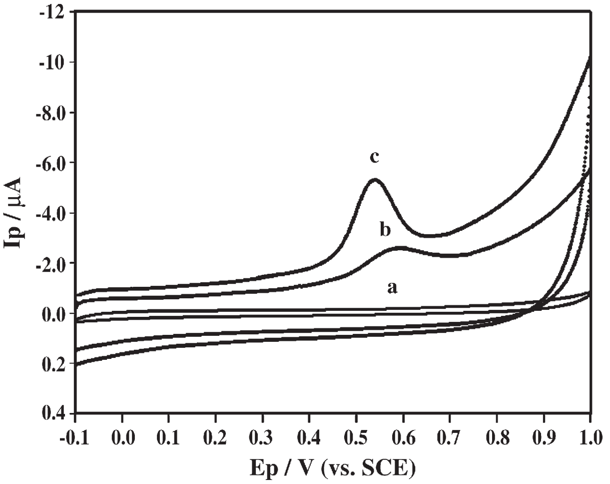

Fig. 1. Cyclic voltammograms of ethinylestradiol in 1/15 mol $L^{-1}$ phosphate buffer (pH = 8.04) at a carbon paste electrode: (a) in the presence of CPB; (b) in the presence of ethinylestradiol; (c) in the presence of ethinylestradiol and CPB. Scan rate: 100 $mVs^{-1}$; Accumulation time: 150 s; ethinylestradiol concentration: $2.0 \times 10^{-5}$ mol $L^{-1}$; CPB concentration: $5 \times 10^{-5}$ mol $L^{-1}$.

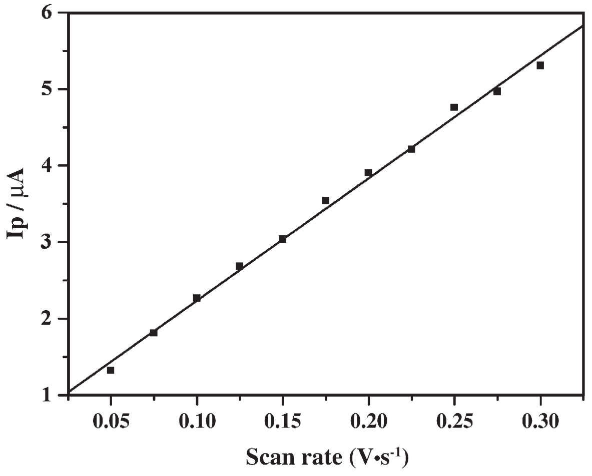

Fig. 2. Dependence of the oxidation peak current on the scan rate.

The influence of different kinds of surfactants including anionic sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS), neutral polyethylene glycol (2000) (PEG-2000), cationic cetyltrimethyl ammonium bromide (CTAB) and CPB on the oxidation of ethinylestradiol at the carbon paste electrode are investigated, and the specific values of Ip and Ep in the presence of different surfactants were summarized in Table 1. The results indicated that the oxidation peak current and potential are almost not improved in the presence of PEG-2000, indicating weak adsorption of ethinylestradiol to the electrode surface. Whereas, after the addition of anionic surfactants (SDS and SDBS), the peak current is found to decrease greatly. However, in the case of CPB and CTAB, the peak potential shift negatively and peak current increase greatly with diverse degree. The enhancement of peak current in the presence of CPB is larger than that in the presence of CTAB, thus, CPB is chosen for the further determination of ethinylestradiol. Above results indicate that the enhancement of the peak current mainly come from the increase of the amount of surface adsorption via electrostatic interaction.

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the effects of scan rate ($v$) on the oxidation of ethinylestradiol at the carbon paste electrode are investigated by linear sweep voltammograms. From Fig. 2, we can see that the oxidation peak current increased linearly with the scan rate in the range of 10–275 $mVs^{-1}$, and can be expressed as following: $I_{pa}$ (μA) = 0.0167 $v$ ($mVs^{-1}$) + 0.557, $r = 0.999$. Thus, the electrochemical reaction is rather an adsorption-controlled step than a diffusion-controlled process.

Electrooxidation of ethinylestradiol at the carbon paste electrode in the presence (curve a) and absence (curve b) of CPB is characterized by chronocoulometry which is an electrochemical measuring technique used for electrochemical analysis or for the determination of the kinetics and mechanism of electrode reactions. As shown in Fig. 3, after the background subtraction, the plot of the charges ($Q$) against the square root of time ($t^{1/2}$) shows a linear relationship. According to the integrated Cottrell equation [26], the diffusion coefficient of

Table 1
Electrochemical data for the oxidation of ethinylestradiol in presence of different surfactants

| Surfactants | Ethinylestradiol concentration (mol $L^{-1}$) | Peak potential (V) | Peak current (μA) |
|---|---|---|---|
| SDS | $1.0 \times 10^{-5}$ | 0.595 | 0.274 |
| SDBS | $1.0 \times 10^{-5}$ | 0.603 | 0.287 |
| PEG-2000 | $1.0 \times 10^{-5}$ | 0.588 | 0.470 |
| CTAB | $1.0 \times 10^{-5}$ | 0.545 | 1.356 |
| CPB | $1.0 \times 10^{-5}$ | 0.543 | 1.493 |
| None | $1.0 \times 10^{-5}$ | 0.592 | 0.451 |

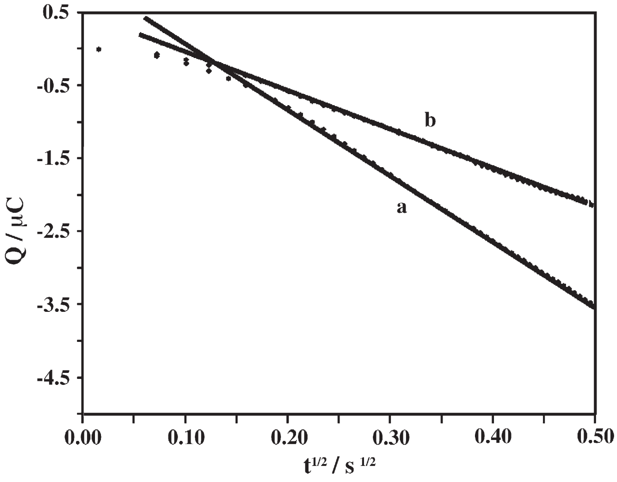

Fig. 3. Plots of $Q$ vs. $t^{1/2}$ for $1.0 \times 10^{-4}$ mol $L^{-1}$ ethinylestradiol at the carbon paste electrode in the present of $5.0 \times 10^{-5}$ mol $L^{-1}$ CPB (a) and the absence of CPB (b). Initial potential 0.30 V, final potential 0.70 V, and pulse width 0.25 s.

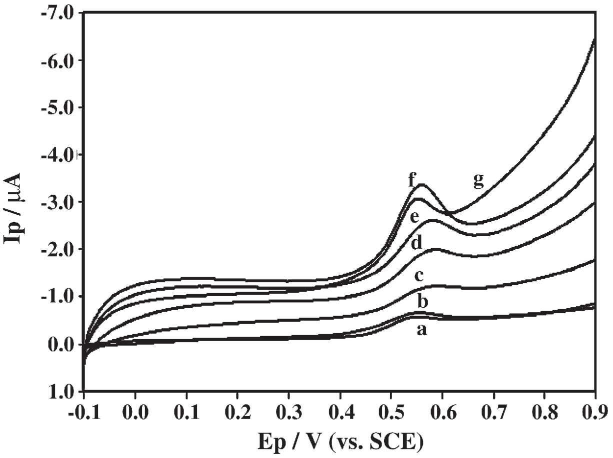

Fig. 4. Voltammograms for ethinylestradiol at the carbon paste electrode in the presence of different concentration of CPB (from a to g): $5.0\times10^{-8}$, $2.0\times10^{-7}$, $1.0\times10^{-6}$, $5.0\times10^{-6}$, $1.0\times10^{-5}$, $5.0\times10^{-5}$, $1.0\times10^{-4}$ mol $L^{-1}$.

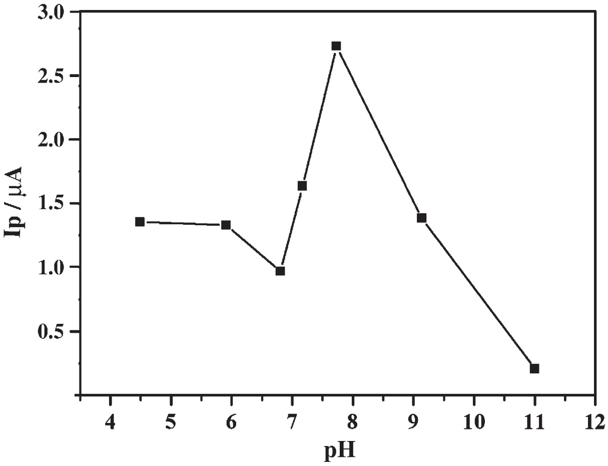

Fig. 5. Dependence of the oxidation peak current on the solution pH.

ethinylestradiol can be estimated from the slope of the plot of $Q$ versus $t^{1/2}$.

$$Q = 2nFAcD^{1/2}t^{1/2}\pi^{-1/2} + Q_{\mathrm{dl}} + Q_{\mathrm{ads}}$$

Where $A$ is the area of the carbon paste electrode, $c$ is the concentration of ethinylestradiol, $Q_{\mathrm{dl}}$ is the double layer charge, $Q_{\mathrm{ads}}$ is the adsorption charge. Other symbols have their usual significance. According to the investigation of the electrochemical oxidation of ethinylestradiol [27], the number of transfer electrons involved in this process should be two and $A=0.00785$ cm$^2$ (geometric area of the used electrode), $c=1.0\times10^{-4}$ mol L$^{-1}$. The values of slope are 3.368 μCs$^{-1/2}$ in the absence of CPB and 9.055 μCs$^{-1/2}$ in the presence of CPB, respectively. It is calculated that $D=3.87\times10^{-6}$ cm$^2$ s$^{-1}$ without CPB, which increased to $2.80\times10^{-5}$ cm$^2$ s$^{-1}$ after the addition of CPB. From the intercepts of plots ($Q$ vs. $t^{1/2}$), the excess charge ($Q_{\mathrm{ads}}$) corresponding to the adsorbed component (after background charge correction from the backward step) can be obtained, and the results showed that $Q_{\mathrm{ads}}$ of ethinylestradiol at the carbon paste electrode in the absence and presence of CPB are 0.0665 μC and 0.998 μC, respectively. Under identical conditions, the value of $Q_{\mathrm{ads}}$ of ethinylestradiol at the CPE in the absence of CPB is much lower than that in the presence of CPB, suggesting that CPB offers more effective accumulation of ethinylestradiol. There is no doubt that the carbon paste electrode in the presence of CPB can give more sensitive signals to ethinylestradiol.

Meanwhile the surface coverage $\Gamma$ of ethinylestradiol at the carbon paste electrode can be evaluated from the following equation:

$$Q_{\mathrm{ads}} = nFA\Gamma$$

$Q_{\mathrm{ads}}$ can be obtained by the difference of the intercepts of the plot of $Q$ versus $t^{1/2}$ in the presence and absence of ethinylestradiol when $Q_{\mathrm{dl}}$ is assumed unchanged. In the absence of CPB, $Q_{\mathrm{ads}}$ is 0.0665 μC and the value of $\Gamma$ is $2.20\times10^{-11}$ mol cm$^{-2}$. In the presence of CPB, $Q_{\mathrm{ads}}$ increased to 0.998 μC and the value of $\Gamma$ reached $3.29\times10^{-10}$ mol cm$^{-2}$. It reveals that the adsorption amount of ethinylestradiol on the electrode surface greatly increased in the presence of CPB.

### *3.2. Effect of cetyl pyridine bromide concentration*

The effects of CPB concentration on ethinylestradiol oxidation peak current are shown in Fig. 4. The oxidation peak current increases gradually in the beginning with the increase of the concentration of CPB and reaches maximum at $5.0\times10^{-5}$ mol L$^{-1}$. It may be interpreted that the adsorptive behavior of CPB changes from monomer adsorption to monolayer adsorption with the increase of the concentration of CPB, resulting in the increase of ethinylestradiol adsorbed to the electrode surface. The CPB concentration of $5.0\times10^{-5}$ mol L$^{-1}$ may have reach the critical micelle concentration (CMC), but the reaction process is cooperated by the adsorption and the accumulation of CPB. However, the peak current decreases as further increasing the CPB concentration. It may be caused by the micelle effect. Namely, the electron transfer between

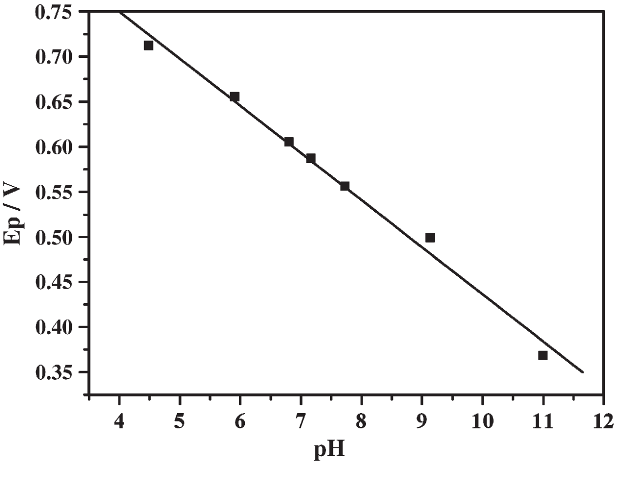

Fig. 6. Dependence of the oxidation peak potential on the solution pH.

ethinylestradiol and the electrode surface would be inhibited since aggregates of micelles. To sum up, the CPB concentration of $5.0\times10^{-5}$ mol $L^{-1}$ can furthest enhance the electrochemical signals of ethinylestradiol.

### 3.3. Influence of pH

Influences of solution pH on the oxidation of ethinylestradiol at the carbon paste electrode in the presence of $5.0\times10^{-5}$ mol $L^{-1}$ CPB are investigated with linear sweep voltammometry in the pH range from 4.42 to 11.00. The effect of the pH value on the oxidation peak current is demonstrated in Fig. 5. In the pH range of 4.42–6.24, the influence of pH on the peak current almost can be neglected. A lower peak current is obtained in the pH 6.98, nevertheless, an increase can be observed above pH 6.98, and a maximum is reached at pH 8.04. With the further improving solution pH value, a great decrease of the oxidation peak current can be observed. Therefore, a pH of 8.04 is chosen for the subsequent analytical experiments. As shown in Fig. 6, a linear shift of the oxidation peak potential ($Ep_a$) towards negative potential with an increase in pH indicated that protons are directly involved in the oxidation of ethinylestradiol, and that it obeys the following equation: $Ep_a$ (V)$=-0.0523$ pH$+0.959$ ($r=0.995$). A slope of 0.0523 V per pH, from which m/n value of 1 was estimated, suggested that the proton transfer number ($m$) is equal to the electron transfer number ($n$).

### 3.4. Influence of accumulation potential and accumulation time

The influence of accumulation potential ($E_{acc}$) on the oxidation peak current of ethinylestradiol is investigated at different potentials from −0.20 V to 0.50 V at a potential interval of 0.10 V. When the accumulation potential shifts from −0.20 V to 0.30 V, the peak current of ethinylestradiol increases slightly. Then, a great decrease in the peak current is observed (Fig. 7). A potential of 0.30 V is chosen as the accumulation potential which gives well-defined peak and a more developed peak current.

The effects of accumulation time ($t_{acc}$) range from 0 to 250 s on the peak current following accumulation at 0.30 V is demonstrated in Fig. 8. The peak current is found to increase linearly with the accumulation time up to 120 s. The longer the accumulation time, the more the CPB-ethinylestradiol coexist system is adsorbed to the electrode surface. Afterwards, the peak current tends to be stable with further increasing accumulation time beyond 150 s. This may be due to the saturated adsorption of ethinylestradiol at the electrode surface. Therefore, an accumulation time of 150 s is found reasonable for the present analytical study.

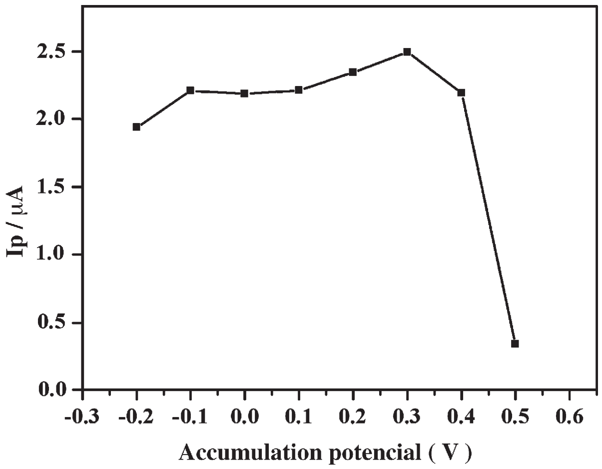

Fig. 7. Effect of accumulation potential ($E_{acc}$) on the oxidation peak current of ethinylestradiol at the carbon paste electrode in the presence of CPB.

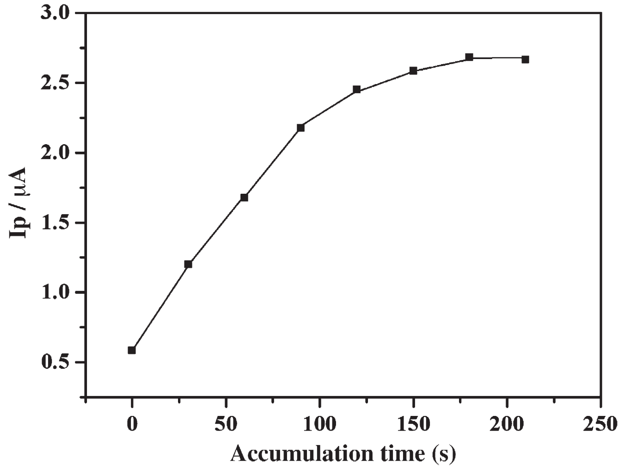

Fig. 8. Effect of accumulation time on the oxidation peak current of ethinylestradiol in the presence of CPB.

### 3.5. Interference and reproducibility

Under optimal conditions, the interference of some metal ions and organic compounds on the oxidation of ethinylestradiol has been evaluated. The tolerance limit of interferents is taken as the maximum concentration that gives a relative error less than ±5% at a concentration of $5.0\times10^{-6}$ mol $^{-1}$ ethinylestradiol. The results show that 100-fold of NaCl, KCl, $NH_4Cl$, $ZnSO_4$ and glucose, L-serine don't interfere with the electrochemical response of ethinylestradiol. However, 10-fold $Fe_2(SO_4)_3$, dopamine, acetaminophen, adrenaline, uric acid and vitamin C has

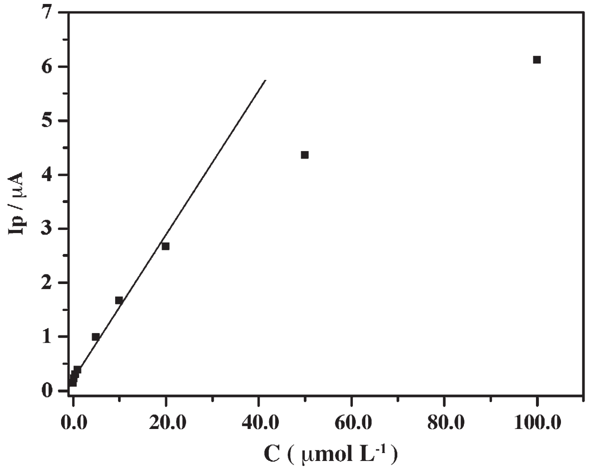

Fig. 9. Calibration curve for the determination of ethinylestradiol at the carbon paste electrode in presence of CPB.

apparent influence on the determination of ethinylestradiol. The interference of $Fe^{3+}$ may come from the formation of complexes with ethinylestradiol. While, a similar structure, ph-OH, which can be oxidized near the oxidation potential of ethinylestradiol will be responsible for the interferences of these compounds.

The stability of the carbon paste electrode is evaluated by measuring the oxidation peak current of $5.0 \times 10^{-6}$ mol $L^{-1}$ ethinylestradiol in the presence of CPB over a period of one week. The electrode is used daily and stored in the air. The deviation of 3.86% from its original current response indicated that the sensor possesses long-time stability. Performing seven successive measurements for $5.0 \times 10^{-6}$ mol $L^{-1}$ ethinylestradiol solution demonstrates the reproducibility of the results obtained by the proposed procedure, and a relative standard deviation of 3.74% is obtained for seven parallel measurements. The fabrication reproducibility of seven electrodes, independently made, give an acceptable reproducibility with a relative standard deviation of 4.24% for the determination of $5.0 \times 10^{-6}$ mol $L^{-1}$ ethinylestradiol.

### 3.6. Analytical characterization

Linear sweep voltammetry was used to investigate the current response of ethinylestradiol at the carbon paste electrode in the concentration range from 0.05 μmol $L^{-1}$ to 100 μmol $L^{-1}$. It is found that the oxidation peak current increase linearly with the increase of ethinylestradiol concentration, moreover, the oxidation peaks shift gradually towards more positive potential. The calibration curve for the bulk ethinylestradiol is established using the proposed voltammetric procedure after its accumulation on the surface of the electrode (Fig. 9). Variation of peak current (Ip) with the concentration of bulk ethinylestradiol gives a straight line following the equation: Ip (μA) = 0.125 C (μmol $L^{-1}$) + 0.263 ($r = 0.995$). The linearity is observed in the concentration range from $5.0 \times 10^{-8}$ mol $L^{-1}$ to $2.0 \times 10^{-5}$ mol $L^{-1}$ with a detection limit of $3.0 \times 10^{-8}$ mol $L^{-1}$. For the concentration of ethinylestradiol higher than $2.0 \times 10^{-5}$ mol $L^{-1}$, the peak current increase only slightly and approaching a constant value due to the saturate adsorption of ethinylestradiol on the electrode surface.

In order to confirm the sensitivity and generality of the proposed method, the voltammetric procedure is applied for the determination of ethinylestradiol in a pharmaceutical formulation (Levonorgestrel and Etinylestradiol tablets). The prepared sample solution is added to 10 mL supporting electrolyte solution by micro syringe, and then is analyzed with the proposed method. An acceptable reproducibility with a relative standard deviation of 4.16% is obtained for four parallel measurements. The calculated average content of ethinylestradiol in the analyzed sample is 0.0786 g $g^{-1}$. The result obtained from voltammetric determination is consistent well with that obtained from the analysis of high-performance liquid chromatograpy (0.0791 g $g^{-1}$, an average value for three determinations), suggesting that the proposed procedure is very accurate, selective and sensitive enough for practical application.

## 4. Conclusion

In the presented paper, electrochemical behavior of ethinylestradiol has been investigated at a carbon paste electrode in the presence of cetyl pyridine bromide. The results indicate that the electrochemical response of ethinylestradiol can be facilitated by the cationic surfactant CPB via electrostatic/hydrophobic interaction in the basic electrolyte solution. Advantages of the method such as simple, sensitive, accurate and rapid have been demonstrated by the successful application in the voltammetric determination of ethinylestradiol in a pharmaceutical formulation.

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