

# The electrochemical oxidation of troxerutin and its sensitive determination in pharmaceutical dosage forms at PVP modified carbon paste electrode

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## Abstract

The voltammetric responses of troxerutin were investigated at polyvinylpyrrolidone (cross-linked) (PVP) modified carbon paste electrode (CPE) in 0.1 mol/L KCl by several electrochemical techniques. A well-defined oxidation peak was observed at about 0.97 V. Compared with poor responses of troxerutin at bare electrode that at this modified electrode has been greatly improved. It is PVP that enhances the adsorption of troxerutin to electrode surface based on their hydrophobic property. Under some optimized experimental conditions, a simple and sensitive electroanalytical method was developed for the quantitative analysis of troxerutin. A very low detection limit of  $5.0 \times 10^{-9}$  mol/L was obtained for 5 min accumulation at open circuit ( $S/N = 3$ ). This proposed method was successfully applied to the detection of troxerutin in pharmaceutical dosage forms and satisfying results had been obtained.

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**Keywords:** Troxerutin; Polyvinylpyrrolidone (cross-linked) (PVP); Carbon paste electrode (CPE); Determination

## 1. Introduction

Troxerutin (2-[3,4-bis(2-hydroxyethoxy)phenyl]-3[[6-deoxy- $\alpha$ -(L-manno-pyranosyl)- $\beta$ -(D-glucopyranosyl)]oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4H-1-benzopyran-4-one), known as Vitamin P4, is a flavonoid present in tea, coffee, cereal grains and a variety of fruits and vegetables [1–3]. The structure was shown in Chart 1. It has aroused considerable interesting due to their broad pharmacological activities. Troxerutin shows a marked affinity for the venous wall [4]. It may act to improve capillary function, reduce capillary fragility and reduce abnormal leakage. Applications also exist for reducing the occurrence of night cramps and other circulatory problems. Its common usage is mainly in the treatment of varicose veins and haemorrhoids. Based on the discussed applications, the detection of troxerutin is of great significance. Currently several methods including spectrophotometric [5,6], capillary electrophoresis (CE) [7] and high-performance liquid

chromatography (HPLC) [8], are often used for determination of troxerutin. However, these methods were complicated and needed expensive instruments. In our work, a simple and sensitive electroanalytical method was developed for the determination of troxerutin at polyvinylpyrrolidone (cross-linked) (PVP) modified CPE. As one kind of carbon electrodes, carbon paste electrode (CPE) has been widely used in electrochemical and electroanalytical chemistry because of its low costs, low background current and convenience [9–11]. As for PVP mentioned here, it is a water-fast polymer and shows strong adsorption character to phenolic compounds, which is attributed to hydrogen bond between imide in the center of polymer and hydroxyl group in phenolic compounds. As a kind of surfactant PVP can endow the modified electrode/solution interface with different electrical properties. In the present work, the PVP modified CPE showed greatly enhancement effectiveness on the oxidation of troxerutin in 0.1 mol/L KCl according to experimental results. Several corresponding electrochemical parameters of troxerutin oxidation at PVP modified CPE was investigated by electrochemical techniques. After that the experimental conditions were optimized the proposed method was demonstrated by using troxerutin injection and the results

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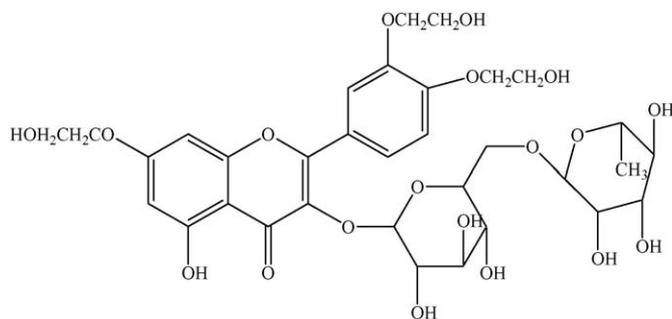


Chart 1. Chemical structure of troxerutin.

were satisfying. All the experiment data proved that this electroanalytical method was feasible and reliable.

## 2. Experimental

### 2.1. Apparatus and reagents

All the electrochemical measurements were performed on a CHI 650 B electrochemical analyzer (Shanghai Chenhua Co., China) in a three-electrode system. The working electrode was a PVP modified CPE. 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 (5  $\mu\text{m}$ , 150 mm  $\times$  416 mm) (Dikma Technologies, Diamonsil). The mobile phase consisted of 0.1% citric acid–acetonitrile–tetrahydrofuran (82.5:15:25, v/v/v); Determined-wave was 254 nm.

Graphite was obtained from Shanghai Regent Co., China (SP). Polyvinylpyrrolidone (cross-linked) (PVP) was purchased from Acros Chemical Reagent Co., USA. Troxerutin (98.15%, the National Institute for the Control of Pharmaceutical and Biological Products, China) was dissolved in water to form stock solution at a concentration of  $1.0 \times 10^{-2}$  mol/L stored at 4 °C. Potassium chloride (KCl, Shanghai Regent Co., China) was dissolved in water at a concentration of 0.1 mol/L. All the chemicals were used without further purification and all the solutions were prepared with doubly distilled water. Troxerutin injection was purchased by Yangzhou Pharmaceutical Corp. Ltd. and diluted using in 0.1 mol/L KCl.

### 2.2. Preparation of the PVP modified CPE

The PVP modified CPE was prepared by mixing 100 mg graphite, 10 mg PVP and 20  $\mu\text{L}$  paraffin oil in a small mortar to form a homogeneous graphite mixture. In succession, the mixture was pressed into the end cavity of a homemade poly (tetrafluoroethylene) (PTFE) cylindrical electrode body (diameter: 2.0 mm), and the electrode surface was polished manually on a piece of weighting paper. It is important that the amount of paraffin oil must be carefully monitored. Excessive paraffin oil can result in the decrease of conductivity of the electrode and

insufficient paraffin oil is not beneficial to the uniform mix of graphite.

### 2.3. Experimental procedures

A certain volume of 0.1 mol/L KCl was used as the supporting electrolyte in a conventional electrochemical cell. At the beginning of experiment, PVP modified CPE was scanned by successive cyclic voltammograms between 0.50 and 1.20 V at 100 mV/s to get a steady cyclic voltammograms. A certain volume of troxerutin stock solution was placed into the cell to make up 10 mL mixture solution. The accumulation was carried out at open circuit via stirring the solution for 5 min, and then kept quiet for 5 s. The voltammograms were recorded using a cyclic potential scan between 0.50 and 1.20 V at a scan rate of 100 mV/s. After each measurement the modified electrode was refreshed by polishing manually on a piece of weighting paper or renewal of fill to get a reproducible electrode surface.

## 3. Results and discussion

### 3.1. Voltammetric responses of troxerutin

Successive cyclic voltammograms of  $1.0 \times 10^{-6}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl was shown in Fig. 1. A well-defined oxidation peak appears at 0.97 V on the first cycle of multicyclic voltammograms when the potential sweeps from 0.50 to 1.20 V and no apparent peak is observed in the reverse scan. This shows that the oxidation of troxerutin is a totally irreversible process. During following successive cyclic sweeps, the oxidation peak appearing at 0.97 V greatly decreases, resulting from the fact that the electrode surface was blocked by the strong adsorption of the reaction products.

The electrochemical responses of  $1.0 \times 10^{-6}$  mol/L troxerutin were investigated in 0.1 mol/L KCl by linear sweep voltammetry (LSV) at two different electrode surfaces, bare CPE surface and PVP modified CPE surface. The results were illus-

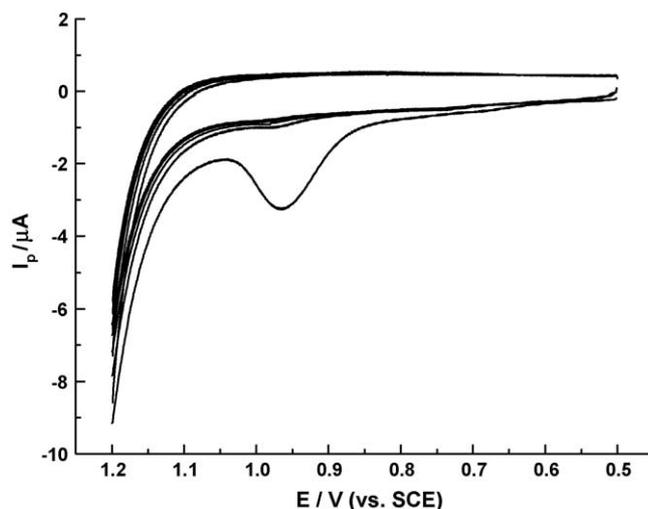


Fig. 1. Successive cyclic voltammograms of  $1.0 \times 10^{-6}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl. Scan rate, 100 mV/s.

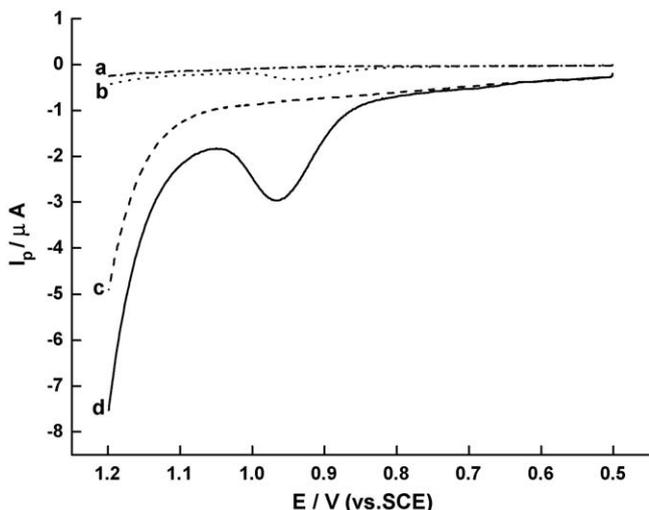


Fig. 2. Linear sweep voltammograms responses of bare CPE (a and b) and PVP modified CPE (c and d) in the absence of troxerutin (a and c) and in the presence of  $1.0 \times 10^{-6}$  mol/L troxerutin (b and d) in 0.1 mol/L KCl. Scan rate, 100 mV/s.

trated in Fig. 2. It is clear that no peak appears at both bare CPE (curve a) and PVP modified CPE (curve c) in absence of troxerutin. After the addition of  $1.0 \times 10^{-6}$  mol/L troxerutin, one well-defined oxidation peak can be observed. The oxidation peak potential ( $E_p$ ) of troxerutin at PVP modified CPE (0.96 V in curve d) is a little more positive than that at bare CPE (0.93 V in curve b), but the peak current ( $I_p$ ) at PVP modified CPE is much larger than that at bare CPE. As a polymeric sorbent, PVP had hydrophobic backbone and heterocyclic groups. Based on its hydrophobicity, PVP played an important role in this work. PVP embedded in carbon paste electrode surface could effectively facilitate the adsorption of troxerutin on the surface of the electrode, which lead to the enhancement of current responses of troxerutin. Additionally, PVP embedded in carbon paste electrode surface might alter the overvoltage of the electrode and make that the free energy changes of troxerutin oxidation reactions at two different electrodes were different, and then further influences the rate of electron transfer between troxerutin and electrode surface. The large difference of peak current at the bare CPE and PVP modified CPE shows that the enhanced oxidation of troxerutin may be based on the interaction of troxerutin with PVP. This is consistent with the results in Fig. 2 that the background current of PVP modified CPE is apparently higher than that of bare CPE.

### 3.2. The choice of supporting electrolytes

The types of supporting electrolytes and solution pH played a key role in the voltammetric responses of troxerutin. Different supporting electrolytes were used to estimate the current responses of  $5.0 \times 10^{-5}$  mol/L troxerutin by LSV at PVP modified CPE. Better current responses of troxerutin could not be obtained in several kinds of buffer solutions such as 0.1 mol/L phosphate buffer solution (PBS, pH 5.0–9.0), 0.1 mol/L sodium acetate–acetic acid buffer solution (NaAc–HAc, pH 3.5–5.6). And then other different acids and alkalis including 0.1 mol/L

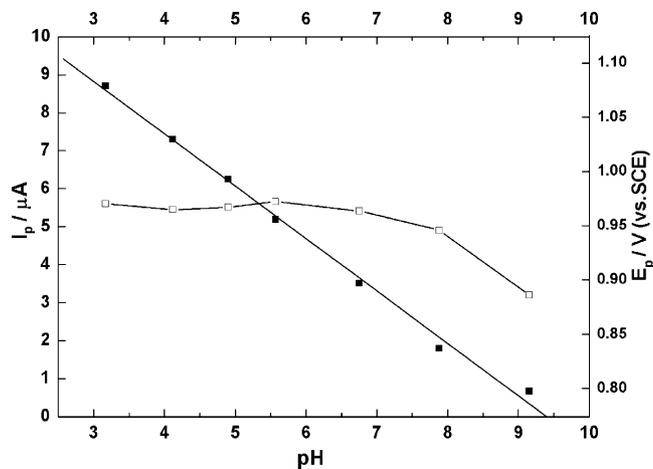


Fig. 3. Effects of solution pH on the peak potential (■) and peak current (□) of  $5.0 \times 10^{-5}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl.

$\text{HClO}_4$ , 0.05 mol/L  $\text{H}_2\text{SO}_4$ , 0.1 mol/L HCl, 0.1 mol/L HAc and 0.1 mol/L NaOH worked in the same way. The results showed that higher peak current and better peak shape could be obtained in 0.1 mol/L KCl. Consequently, 0.1 mol/L KCl was chosen as support electrolyte to determine troxerutin. Whereafter, the influences of solution pH on the peak current of troxerutin were also tested by cyclic voltammetry (CV) in 0.1 mol/L phosphate buffer solution, mixing  $\text{H}_3\text{PO}_4$  to adjust pH, and the results were described in Fig. 3. The relative standard deviation (R.S.D.) of each sample for five times parallel detections was less than 3.2%. It can be seen that the oxidation peak current of troxerutin has not remarkable changes in the range of solution pH from 3.0 to 7.0 (curve a). However, the peak current of troxerutin decreases greatly in the range of solution pH from 8.0 to 9.0 (curve b). Maybe the ionization of troxerutin in alkaline solution decreases the active groups on troxerutin molecules. The oxidation peak potential negatively shifts with increasing of solution pH and obeys the equation:  $E_p = 1.23 - 0.049\text{pH}$  ( $R = 0.9986$ ). The relative standard deviation (R.S.D.) of each sample for five times parallel detections was less than 0.3%. The slope of 0.049 mV/pH suggests that the number of the electrons transferred in the oxidation of troxerutin equal with that of protons.

### 3.3. Influences of accumulation conditions

Accumulation conditions play a key role in affecting the current responses of troxerutin and were estimated by LSV. In range of accumulation potential from +0.50 to  $-0.10$  V it has almost no influences on electrochemical oxidation of troxerutin in 0.1 mol/L KCl at PVP modified CPE. So an open circuit accumulation was optimal condition. On the contrary, the accumulation time significantly affects the current responses of  $1.0 \times 10^{-6}$  mol/L troxerutin as shown in Fig. 4. The relative standard deviation (R.S.D.) of each sample for five times parallel detections was less than 3.5%. As for the electrochemical oxidation of troxerutin, its peak current increases greatly within the first 300 s and then increases slowly. Maybe this was attributed to the saturated adsorption of troxerutin on modified electrode

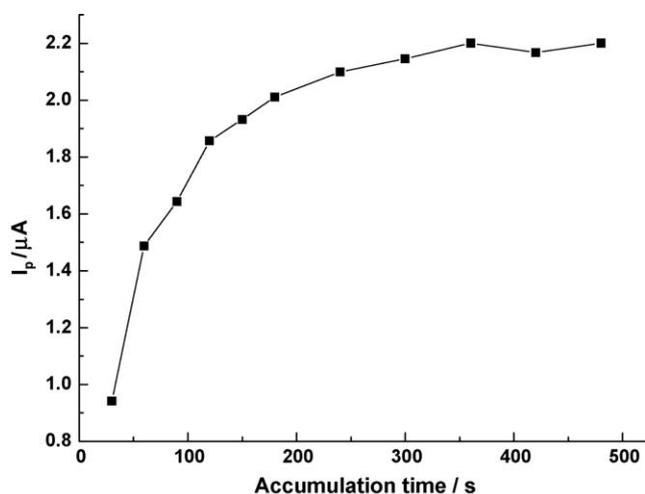


Fig. 4. Influences of accumulation time on the peak current of  $1.0 \times 10^{-6}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl.

surface. The accumulation time of 300 s was the best condition for quantitative detection of troxerutin.

### 3.4. Effects of scan rate

Scan rate can influence the current responses of troxerutin and corresponding electrochemical parameters could be deduced from the relationship between scan rate of potential sweep and current responses of troxerutin oxidation. The dependence of oxidation peak current of  $5.0 \times 10^{-5}$  mol/L troxerutin on scan rate at the PVP modified CPE in 0.1 mol/L KCl was illustrated in Fig. 5. As shown in Eq. (1), the peak current is linear with scan rate ( $\nu$ ) that ranged from 0.025 to 0.30 V/s, suggesting the adsorption-controlled process of the troxerutin oxidation:

$$I_p = 15.01\nu + 0.6289 \quad (R = 0.9984) \quad (1)$$

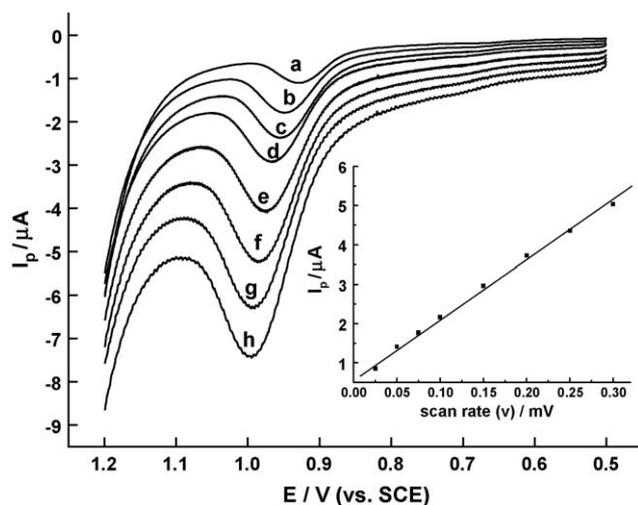


Fig. 5. Effects of scan rate on electrochemical oxidation of  $1.0 \times 10^{-6}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl: (a) 25 mV/s, (b) 50 mV/s, (c) 75 mV/s, (d) 100 mV/s, (e) 150 mV/s, (f) 200 mV/s, (g) 250 mV/s, (h) 300 mV/s. Inset shows the linear relationship between the peak current and scan rate.

In the range of scan rate from 0.025 to 0.30 mV/s relationship between peak potential ( $E_p$ ) of troxerutin oxidation and scan rate ( $\nu$ ) is consistent with following equations:

$$E_p = 0.02773 \ln \nu + 0.8392 \quad (R = 0.9945) \quad (2)$$

As for an irreversible electrochemical process in the case of adsorption system,  $E_p$  is defined by following equations [12]:

$$E_p = (2.3RT/(1 - \alpha)nF) \log(RTk_f^0/(1 - \alpha)nF) + (2.3RT/(1 - \alpha)nF) \log \nu \quad (3)$$

where  $k_f^0$  is the rate constant of the surface reaction for  $E=0$ ,  $\alpha$  the transfer coefficient of troxerutin oxidation, and  $n$  is the number of electrons involved in the oxidation of troxerutin. From Eqs. (2) and (3), we can obtain the value of  $(1 - \alpha)n$  is 0.93. The number of electrons  $n$  is 1.9 when  $\alpha$  in the irreversible electrode process is assumed as 0.5, i.e. troxerutin oxidation underwent a two-proton and two-electron process at the PVP modified CPE. This result is consistent with the oxidation of hydroxyl group on troxerutin and it is in agreement with a suggestion by Yang [13]. Differently Kapoor and coworkers [14] obtained one obvious oxidation peak with a shoulder in differential pulse voltammetric scan. They thought that the higher one-electrode oxidation potential value could be due to the phenolic OH and the lower one due to the glucose moiety.

### 3.5. Chronocoulometry

The method of chronocoulometry was used to characterize the oxidation of  $5.0 \times 10^{-5}$  mol/L troxerutin at the PVP modified CPE in 0.1 mol/L KCl and to determine the diffusion coefficient  $D$  and  $Q_{ads}$  according to the formula given by Anson [15]:

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

where  $A$  is the surface area of the working electrode,  $c$  the concentration of troxerutin,  $D$  the diffusion coefficient of troxerutin, and  $Q_{ads}$  is the adsorption charge. Other symbols have their usual significances. The effect of double-layer charge  $Q_{dl}$  could be eliminated by subtraction of the background charge in this experiment. The results were depicted in Fig. 6. 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 Fig. 6). According to Eq. (4) and on the assumption that the value of  $n$  is 2, it is calculated that the diffusion coefficient of troxerutin here is  $6.58 \times 10^{-7}$  cm<sup>2</sup> s<sup>-1</sup>.  $Q_{ads}$  can be obtained by the difference of the intercepts of the plot of  $Q$  versus  $t^{1/2}$  in the presence and absence of troxerutin. Here  $Q_{ads}$  is 1.69  $\mu$ C, according to the equation  $Q_{ads} = nFA\Gamma$ , the value of  $\Gamma$  is  $2.78 \times 10^{-10}$  mol/cm<sup>2</sup>.

### 3.6. Calibration graph

In the ranges from  $1.0 \times 10^{-8}$  to  $2.5 \times 10^{-6}$  and  $2.5 \times 10^{-6}$  to  $1.0 \times 10^{-4}$  mol/L, the oxidation current ( $I_p$ ) has a good linear relationship with troxerutin concentration as shown in Fig. 7 and inset of Fig. 7, respectively, which could be respectively

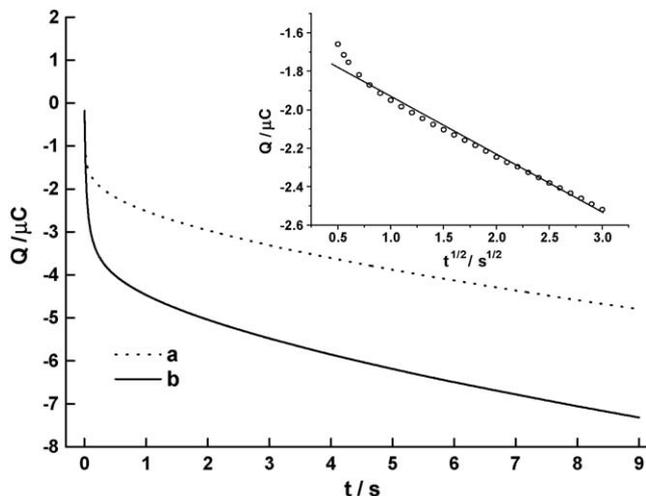


Fig. 6. Chronocoulometry of  $5.0 \times 10^{-5}$  mol/L troxerutin at PVP modified CPE in 0.1 mol/L KCl (curve b). Curve a stands for that in the blank solution. The inset shows the linear relationship between the charges ( $Q$ ) and the square roots of times ( $t^{1/2}$ ) for the oxidation reaction (background subtracted). Initial potential 0.70 V, final potential 1.05 V, and pulse width 9 s.

described as

$$I_p (\mu\text{A}) = 1.5944c + 0.4111 \quad (R = 0.9962) \quad (5)$$

$$I_p (\mu\text{A}) = 0.1192c + 4.3851 \quad (R = 0.9934) \quad (6)$$

A very low detection limit of  $5.0 \times 10^{-9}$  mol/L was obtained for 5 min accumulation at open circuit ( $S/N=3$ ). The relative standard deviation (R.S.D.) for 12 times parallel detections of  $1.0 \times 10^{-6}$  mol/L troxerutin is calculated as 4.8%, suggesting excellent reproducibility of PVP modified CPE.

### 3.7. Interferences

In real samples, there are many concomitant substances. In order to get better analysis results the interferences by these

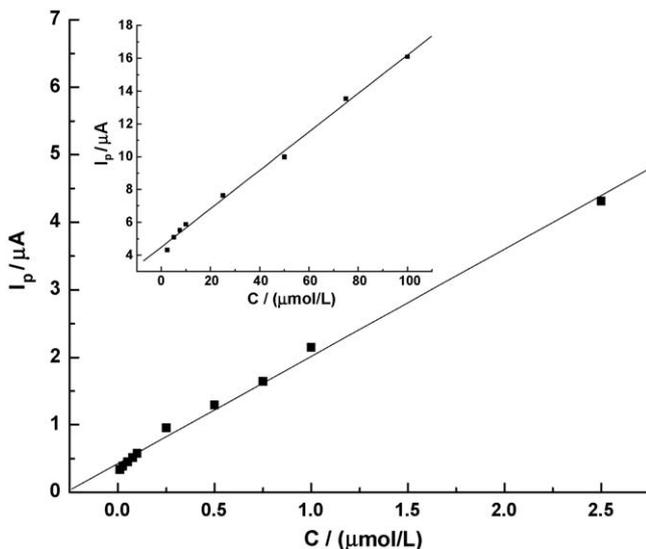


Fig. 7. Calibration graph for determination of troxerutin at PVP modified CPE in 0.1 mol/L KCl.

Table 1  
Determination of troxerutin in injection forms ( $n=5$ )

Sample no.	Declared (mol/L)	Detected by HPLC (mol/L)	Detected by this method (mol/L)	Recovery (%)
1	0.04039	0.03980	0.03979	100.8
2	0.04039	0.03991	0.03998	99.5
3	0.04039	0.03984	0.03977	101.9
4	0.04039	0.03973	0.03983	102.0
5	0.04039	0.03977	0.03989	99.3

compounds should be estimated. Firstly, under optimized experiment conditions the effects of some organic compounds on oxidation of troxerutin were evaluated. The experiment results showed that 1000-fold of glucose, 100-fold of ascorbic acid (AA), dopamine (DA), faecula, uric acid (UA), dextrin and 3-fold of rutin had not apparent effects on the current responses of  $1.0 \times 10^{-6}$  mol/L troxerutin. Then, influences of some inorganic ions such as 100-fold of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$ , 50-fold of  $\text{Fe}^{3+}$ , 15-fold of  $\text{Cu}^{2+}$ , 1000-fold of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  almost did not influence the current responses of  $1.0 \times 10^{-6}$  mol/L troxerutin (signal change  $<3\%$ ). All data indicated that this analytical method has excellent stabilization and anti-jamming ability.

### 3.8. Applications

The enhanced current responses of troxerutin oxidation at PVP modified CPE in 0.1 mol/L KCl was applied to the determination of troxerutin in injection forms. The concentration of troxerutin was calculated using standard additions method. The relative standard deviation (R.S.D.) of each sample for five times parallel detections was less than 4.5% and the recovered ratio on the basis of this method was investigated and the value is between 97.3% and 103.0% as shown in Table 1. These experimental data indicated that determination of troxerutin using PVP modified CPE was effective and sensitive. At last, in order to estimate the feasibility, precision and efficiency of this method, the method of HPLC were used for determination of troxerutin in the same samples. The good accordance between data from HPLC and those from this method we proposed indicated the reliability of the present electroanalytical method for troxerutin determination in injection samples.

## 4. Conclusions

A sensitive and selective electrochemical method for determination of troxerutin was established. This method was based on the enhanced current responses of troxerutin oxidation at PVP modified CPE. Compared with the poor response at bare CPE, the oxidation of troxerutin at PVP modified CPE was greatly improved. PVP embedded in CPE surface could effectively facilitate the adsorption of troxerutin on surface of the modified electrode and then lead to the enhancement of the oxidation peak of troxerutin. Under the optimized working conditions, this method has been successfully applied to the determination of troxerutin in injection forms. The result was consistent with that obtained by HPLC method, suggesting the reliability of this method.

## Acknowledgements

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