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A sub-nanomole level electrochemical method for determination of prochloraz and its metabolites based on medical stone doped disposable electrode

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ABSTRACT

A ultrasensitive, simple and convenient electrochemical method was firstly developed for the determination of prochloraz and its metabolites as 2,4,6-trichlorophenol (2,4,6-TCP) using nano-aperture medical stone. Compared with the undoped disposable electrode (UDE), nano-aperture medical stone doped disposable electrode (MSDDE) not only significantly enhances the oxidation peak current of 2,4,6-TCP but also lowers the oxidation overpotential, suggesting that the nano-aperture MSDDE can remarkably improve the sensitivity of 2,4,6-TCP. The experimental conditions such as pH values of buffer solution, the content of nano-aperture medical stone, accumulation potential and time were optimized for the determination of 2,4,6-TCP. At optimal conditions, the oxidation peak current is proportional to the concentration of 2,4,6-TCP over the range from 6.0×10^{-9} to 8.0×10^{-5} mol L^{-1}. Finally, this novel method was successfully employed to detect prochloraz and its metabolites in orange rind with the detection limit of 8.4×10^{-10} mol L^{-1} (0.3 ng g^{-1}) and the method was validated by gas chromatography.

1. Introduction

Prochloraz [N-propyl-N-[2-(2,4,6-trichlorophenoxy)-ethyl]imidazole-1-carboxamide PCZ] is a broad-spectrum protectant and eradicant fungicide belonging to the class of imidazoles which was widely used in agriculture. It is especially active against eyespot, leaf spot, powdery mildew in the treatment of cereal crops, oilseed rape, citrus, beets, and mushrooms [1]. PCZ and its metabolites are harmful to health. Several analytical methods for determining PCZ in feed, fruits and vegetables have been reported. Most of them were developed to analyze PCZ and another fungicides separately. Such methods include liquid chromatography [2–5], gas chromatography [6–8], micellar electrokinetic chromatography [9] and capillary zone electrophoresis [10]. However, most of the published methods require long and laborious sample preparation as well as large sample amounts due to their low sensitivity and selectivity. In addition, all the mentioned instrumentations are fairly expensive. Therefore, it is necessary to explore a convenient, fast and ultrasensitive way to detect total residue of PCZ and its metabolites.

Disposable electrode was often modified with different materials for determining many kinds of analytes [11–18]. Medical stone is a kind of compound mineral with innocuous and neutral characteristics, which contains Ca, Mg, Si, Al, Fe, K, Na, lanthanon and radioelement. Medical stone is widely used in medical care, food, antisepsis, decontamination and so on. It can absorb or dissolve substances and adjust pH values of solution. However, even about 10 nm aperture medical stone (see Fig. 1) has not been used as sensitized electrode material at present.

PCZ itself has no response to electrochemistry. But after hydrolyzation, it can transform into 2,4,6-trichlorophenol (2,4,6-TCP) with electrochemistry activity (chemical structures of PCZ and its mainly metabolites are presented in Fig. 2). In plants, the primary metabolic step is a breaking of the imidazole ring with the formation of N′-formyl-N-propyl-N-[2-(2,4,6-tri-chlorophenoxy)ethyl]urea (PCZ-FU) and N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]urea (PCZ-U), which are then degraded to 2,4,6-TCP, present as free and conjugated metabolites, together with traces of 2,4,6-trichlorophenoxyacetic acid. In rat, the major metabolites result from the opening of the imidazole ring to form PCZ-U, which is then further metabolized to 2,4,6-trichlorophenoxyethanol and the corresponding acid [19].

Here, a novel disposable electrode doped with nano-aperture medical stone was fabricated, which was more stable, simple, convenient and inexpensive than screen-printed electrode. To the best of our knowledge, a sub-nanomole level electrochemical method was firstly proposed for detecting PCZ and its metabolites as 2,4,6-TCP based on the nano-aperture medical stone doped disposable electrode (MSDDE).

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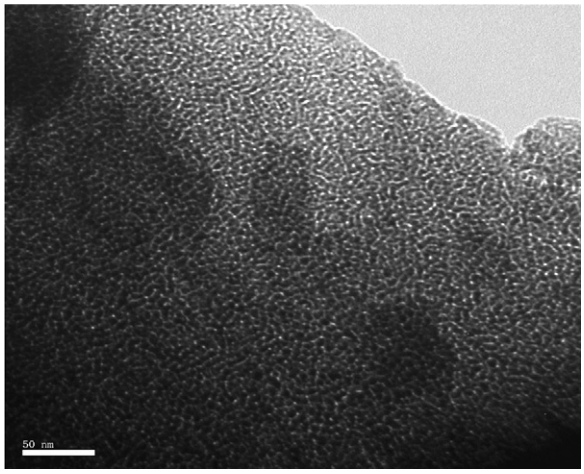

Fig. 1. Transmission electron micrograph of nano-aperture medical stone.

2. Experimental

2.1. Reagents and solutions

PCZ and 2,4,6-TCP were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Oranges were bought from local supermarket. The standard solutions of 1.0 mmol L^{-1} PCZ or 2,4,6-TCP was prepared by dissolving them in ethanol and then stored in the dark. Medical stone was provided by cooperator in Japan. Crystalline flake graphite and pyridine hydrochloride were from Shanghai Reagent Corporation (China). Phosphate buffered saline (PBS, 0.1 mol L^{-1}) solutions with different pH were prepared by mixing three stock solutions of 0.1 mol L^{-1} H_3PO_4, NaH_2PO_4 and Na_2HPO_4. All reagents were of analytical grade and used without any further purification. Any of electrochemical experiments were carried out at room temperature.

2.2. Apparatus

CHI760D electrochemical workstation (Shanghai CH Instruments, China) was used for all electrochemical measurements. A conventional three-electrode system was used throughout the experiments, including a doped disposable working electrode, a platinum wire counter electrode and a saturated calomel electrode used as the reference. All potentials mentioned in this work refer to the reference electrode. All cyclic voltammetry (CV) experiments were carried out with a scan rate of 100 mV s^{-1}. The pH measurements were carried out with a pHS-25 pH-meter (Shang-hai Leici Instrument Plant, China) at room temperature. The extracts were obtained by KQ118 ultrasonicator (Kunshan Ultrasonic Instrument Co. Ltd, China). Structural observation for medical stone was performed in Philips Tecnai 12 transmission electron microscope (Netherlands). The gas chromatographic analysis was carried out using a Varian 3300 gas chromatograph (GC) equipped with electron-capture detector (USA).

2.3. Fabrication of doped disposable electrode

Here, a new method for making disposable electrode was proposed. The disposable electrode was composed of prepreg substrate and copper foil, which were pressed by automatic vacuum lanminating machine. Prior to pressing, prepreg substrate (Fig. 3A-b), copper foil (Fig. 3A-c) and heat-resistant plastic film as an auxiliary material (Fig. 3A-a) were lapped together into a panel (Fig. 3A). They were put into a pressing instrument after many panels were accomplished (Fig. 3B). When the pressing process finished, the heat-resistant plastic film was removed and the panel was cut into many pieces. Each piece was drilled with a 1.0 mm hole in diameter at one end and a 5.0 mm hole at the other end (Fig. 3C). Crystalline flake graphite, medical stone and paraffin liquid were mixed according to a certain proportion and grinded uniformly by pestle in mortar. Then a proper amount of the mixture was stuffed in the 5.0 mm hole and smoothed with spatula, and the other hole was connected with conducting wire (Fig. 3D). The fabrication process in detail was shown in Fig. 3(A–D). The fabrication process of

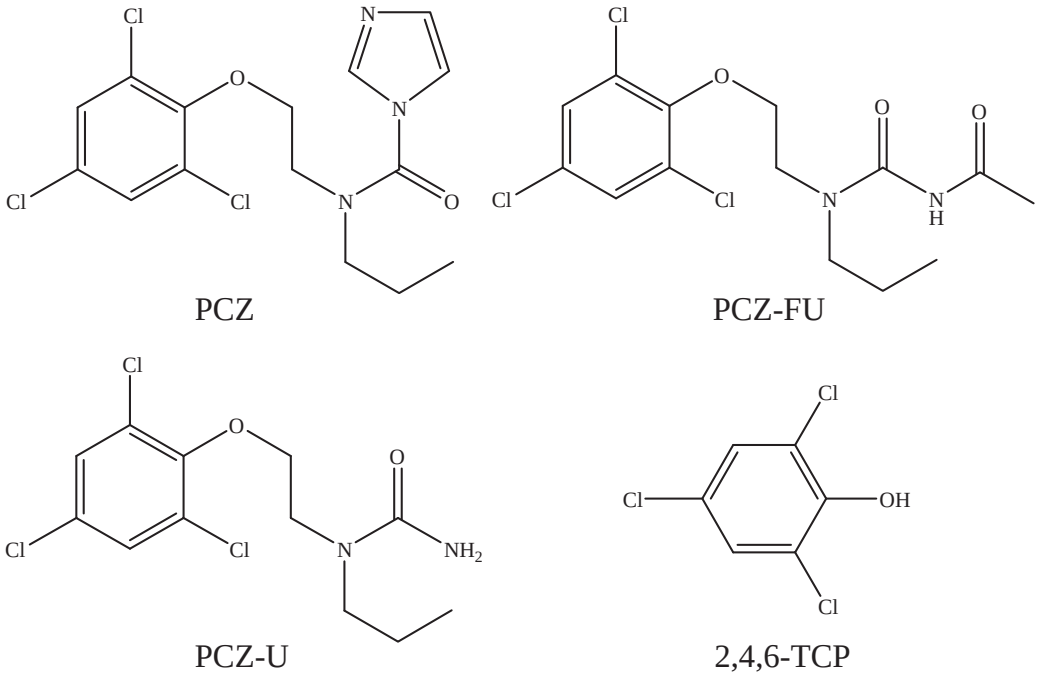

Fig. 2. Chemical structures of PCZ and its metabolites.

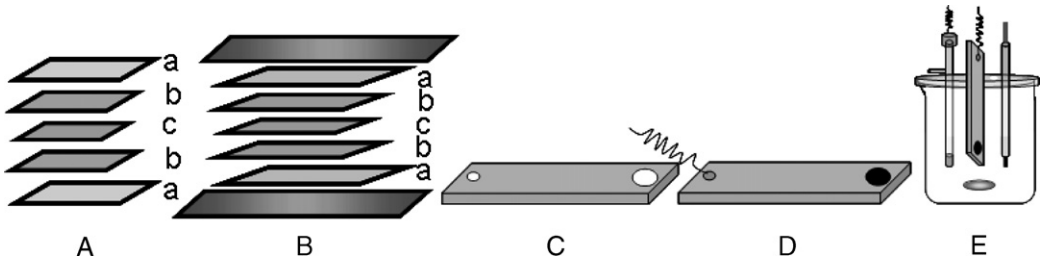

Fig. 3. Detailed fabrication process of medical stone doped disposable electrode and three-electrode system. The a, b, c in A means heat-resistant plastic film, prepreg substrate and copper foil, respectively.

undoped disposable electrode (UDE) was as the same as the above only without medical stone. Finally, the doped disposable working electrode was used to determine PCZ and its metabolites in orange rind, which can be seen in Fig. 3(E).

2.4. Sample preparation

Fresh orange rinds were cut into small pieces with scissors, chopped well in an electro-coffee mill for 2 min, and stored at −18 °C if not used immediately. Chopped orange rind (10.0 g) was extracted with 50.0 mL acetone followed by reextraction with 50.0 mL ethyl acetate for two times. The 2.0 mL extract was transferred into a glass ampoule and then pyridine hydrochloride (0.1 g) was added. And the ampoule was sealed and heated at 200 °C for 3 h. PCZ and its metabolites were converted into 2,4,6-TCP, dissolved in 0.1 mol L^{-1} PBS for electrochemical experiment and extracted with hexane for GC test. The similar method was adopted for the hydrolysis of standard PCZ. The above extraction method was obtained by referring to the literature [20]. The results were expressed as PCZ equivalents by correcting the measured 2,4,6-TCP concentration for the molecular weight factor of 1.9.

2.5. Analytical procedure

The required volume of 2,4,6-TCP standard solution or sample solution was added to 10 mL PBS and underwent a pre-adsorption process on optimized conditions. Then CV or differential pulse stripping voltammetry (DPSV) was recorded, respectively. The DPSVs were recorded with amplitude of 0.05 V, pulse width of 0.05 s and pulse period of 0.2 s.

The GC analysis was carried out using a Varian 3300 GC equipped with electron-capture detector (ECD), an on-column injector and a connecting Varian 4290 reporting integrator. The megabore column was a DB-5 fused-silica column (30 m × 0.53 mm i.d., 0.83 μm; J&W Scientific, Folsom, CA, USA). The injector and detector were operated at 240 °C and 300 °C, respectively. The oven temperature was programmed as follows: 140 °C for 1 min, increasing to 240 °C (10 °C min^{-1}) to 260 °C (20 °C min^{-1}) and holding for 10 min. Nitrogen (99.999% pure) was for the carrier (2 mL min^{-1}) and makeup (28 mL min^{-1}) gas.

3. Results and discussion

3.1. Electrochemical behavior of PCZ and its hydrolysate

The electrochemical behavior of PCZ and its hydrolysate (2,4,6-TCP) were studied, respectively. As can be seen in Fig. 4, there is no oxidation peak at curve a in PBS (pH = 4.0), which indicates that the oxidation reaction for PBS has not taken place at the potential window from 0.0 to 1.2 V. The weak oxidation peak which appears at curve b is due to the trace raw material of 2,4,6-TCP untreated completely, which is confirmed with curve b of Fig. 5. However, a markedly oxidation peak appears at curve c for the 1.0 μmol L^{-1}

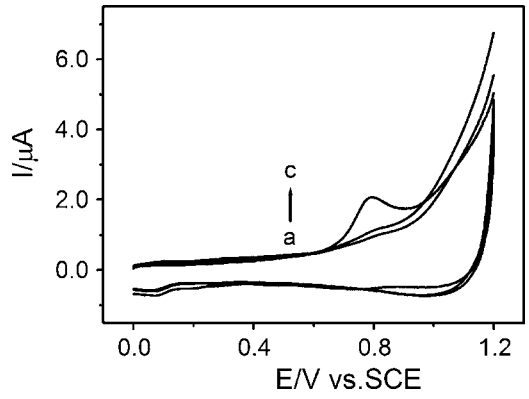

Fig. 4. CV curves of 1.0 μmol L^{-1} PCZ and its hydrolysate in PBS (pH 4.0) at a MSDDE: (a) blank buffer; (b) in the presence of PCZ; (c) in the presence of PCZ hydrolysate.

hydrolysate of PCZ demonstrating that the hydrolysate of PCZ has good electrochemical activity, which can be used to detect PCZ and its metabolites.

3.2. Electrochemical behavior at different electrodes

The electrochemical responses to 2,4,6-TCP in PBS (pH = 4.0) were examined using CV at UDE and MSDDE, which were shown in Fig. 5. An oxidation peak for 1.0 μmol L^{-1} 2,4,6-TCP is observed at the UDE (curve a). The oxidation peak potential is at 0.83 V and the peak current is very low. However, the oxidation peak of 2,4,6-TCP shifts negatively to 0.79 V at the MSDDE (curve b). Moreover, a highly sensitive oxidation peak is observed for 1.0 μmol L^{-1} 2,4,6-TCP. Furthermore, compared with that at the UDE, the oxidation peak current of 2,4,6-TCP remarkably increases. The considerable peak current enhancement of 2,4,6-TCP clearly suggests that medical stone exhibits excellent accumulation efficiency to 2,4,6-TCP

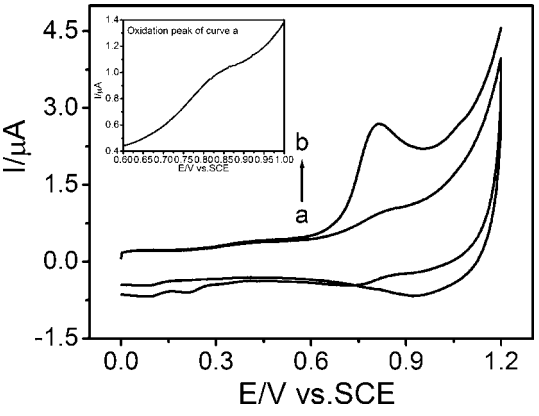

Fig. 5. CV curves of 1.0 μmol L^{-1} 2,4,6-TCP in PBS (pH 4.0): (a) UDE; (b) MSDDE. Inset, enlarged drawing of linear curve a.

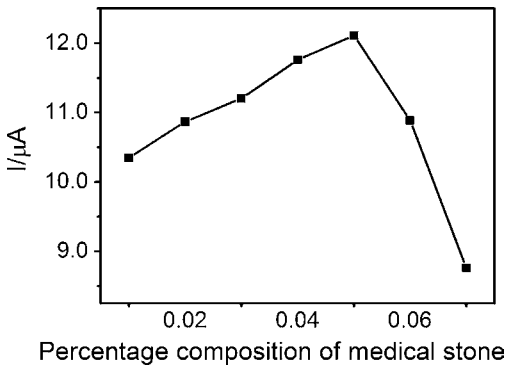

Fig. 6. Effect of percentage composition for medical stone on peak current.

due to its nano-aperture. In brief, conclusion can be made that MSDDE is more active to 2,4,6-TCP and can greatly improve the sensitivity of determining PCZ and its metabolites.

3.3. Optimization of medical stone

It is very clear that the MSDDE can remarkably improve the oxidation peak current of 2,4,6-TCP. However, further studies show that the amount of medical stone contained in the doped disposable electrode also affects the electrochemical response to 2,4,6-TCP. Fig. 6 shows the influence of medical stone amount on the oxidation peak current of 2,4,6-TCP. As the content of medical stone gradually improving, the oxidation peak current firstly increases gradually until reaching maximum at 5% of the compound electrode material and then sharply decreases. As the amount of medical stone gradually rising, the active site for 2,4,6-TCP correspondingly increases and so the oxidation peak current greatly increases. However, the conductivity of MSDDE lowers as the content of medical stone furtherly improving, which blocks the electron transfer of 2,4,6-TCP and increases the background current. Thus, the oxidation peak current of 2,4,6-TCP contrarily decreases when the content of medical stone is too high. In this work, the best mass content of medical stone was found at 5%.

3.4. Influence of pH

The effect of solution pH value on the electrochemical response to 2,4,6-TCP was also studied. Variation of the peak potential and current as a function of solution pH in the range of 3.0–8.0 is shown in Fig. 7. The oxidation peak potential shifted negatively with the increase of pH, which can be explained that pH value affects the concentration of hydrogen ion and therefore influences the peak potential. Due to the acidic hydroxyl groups of 2,4,6-TCP, the pK_a value of it is 6.23 [21], slight less than 7. Therefore, a maximum CV peak current of 2,4,6-TCP less than pH 6.23 is expected. In fact, it is clear that the CV peak current of 2,4,6-TCP increases gradually with the increase of solution pH till reaches maximum at pH 4.0 and then decreases rapidly. Therefore, pH 4.0 was chosen in the subsequent measurements.

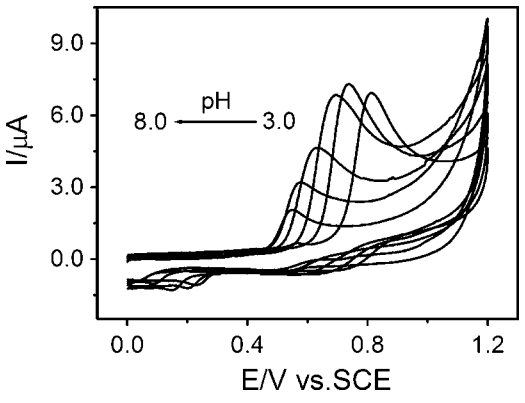

Fig. 7. Effect of solution pH on the peak current of 1.0 μmol L^{-1} 2,4,6-TCP by CV.

3.5. Effects of accumulation potential and time on peak current

The effects of the accumulation potential and time on peak currents were also studied, respectively. As shown in Fig. 8A, when the accumulation time was firstly fixed at 60 s and the accumulation potential changed from −0.4 to 0.4 V, the peak current for 10.0 μmol L^{-1} 2,4,6-TCP in PBS (pH 4.0) firstly increased gradually until reaching maximum at 0 V and then sharply decreased. So 0 V was favorable to the accumulation of 2,4,6-TCP at the MSDDE. With the increase of the accumulation time, the peak height dramatically increased until reaching maximum at 300 s (see Fig. 8B), which indicated that the accumulation of 2,4,6-TCP on the doped electrode surface nearly reached a saturation state at 300 s. In this work, 0 V and 300 s were selected as the optimal accumulation potential and time, respectively.

3.6. Calibration curve

On the optimal conditions, the peak current of 2,4,6-TCP was measured by DPSV at the MSDDE. From the electrochemical responses in Fig. 9, the oxidation peak current increased linearly with the concentrations for 2,4,6-TCP over the range from 6.0×10^{-9} to 8.0×10^{-5} mol L^{-1}. The linear regression equation is IPa (μA) = 0.3634 + 0.1928 C (μmol L^{-1}) (r = 0.9974). The detection limit for prochloraz and its metabolites was calculated to be 8.4×10^{-10} mol L^{-1} (0.3 ng g^{-1}) at 3σ, which was lower three orders of magnitude than that of reports [2]. The reproducibility was evaluated by measuring nine standard solutions containing 8.0 μmol L^{-1} 2,4,6-TCP. The relative standard deviation is 3.5%, showing good reproducibility of the proposed method.

3.7. Stability and reproducibility

The stability and reproducibility of the MSDDE were studied under optimal conditions. The results indicated that the peak heights of the same batch MSDDE changed less than 5.0%. In addition, the reproducibility was evaluated by measuring nine standard solutions containing 6.0 μmol L^{-1} 2,4,6-TCP with nine MSDDEs of the same batch, respectively. The relative standard deviation is 3.4%, showing good reproducibility of the proposed method.

3.8. Interference of foreign species

The interference of the possible species was examined. The influences of 200-fold common metal ions were studied, such as Fe^{3+}, Al^{3+}, Na^{+}, Zn^{2+}, K^{+}, Cu^{2+}, Pb^{2+}, Ca^{2+}, Ag^{+} and Mg^{2+}. It was found that the above metal ions could be negligible for quantitative detection of 2,4,6-TCP on the selected experiment conditions, which might be ascribed to the used positive potential window, in which many metal ions do not produce oxidation peaks. With the individual addition of 100-fold glucose, fructose, citric acid, vitamin A and vitamin B1 into the standard solution containing 2,4,6-TCP, no interference (signal change >5%) was observed for the determination of 2,4,6-TCP.

3.9. Samples analysis

Total residues of PCZ and its metabolites in orange rind were determined as 2,4,6-TCP by DPSV described here. The analytical

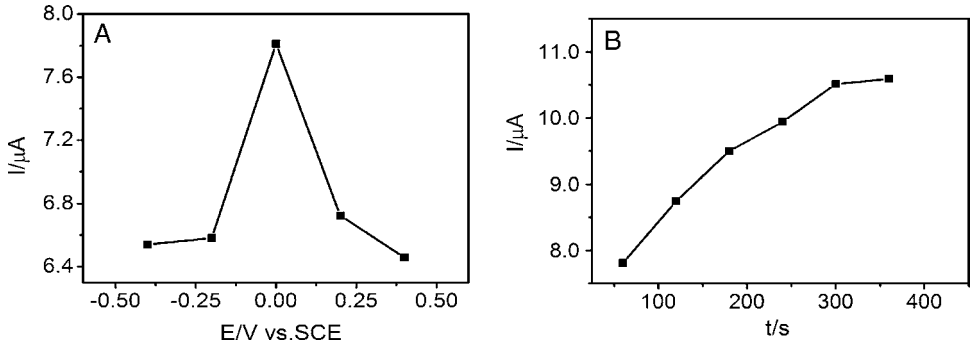

Fig. 8. Effects of accumulation potential and time on peak current for 10.0 μmol L^{-1} 2,4,6-TCP in PBS (pH 4.0) at a MSDDE.

Table 1
Measurement results for total residues of PCZ and its metabolites in orange rind ($n = 3$).[a]

Analyte	GCM (nmol L^{-1})	ECM (nmol L^{-1})	Added (nmol L^{-1})	Found (nmol L^{-1})	R.S.D. (%)	Recovery (%)
Orange rind	9.21	9.15	0	9.13	3.6	99.8
	9.18	9.12	1.0	10.25	3.4	113.0
	9.15	9.19	2.0	11.16	3.1	98.5
	9.33	9.27	3.0	12.38	3.9	103.7

[a] n is the repetitive measurements number; GCM and ECM are the abbreviations of gas chromatography and electrochemical method, respectively.

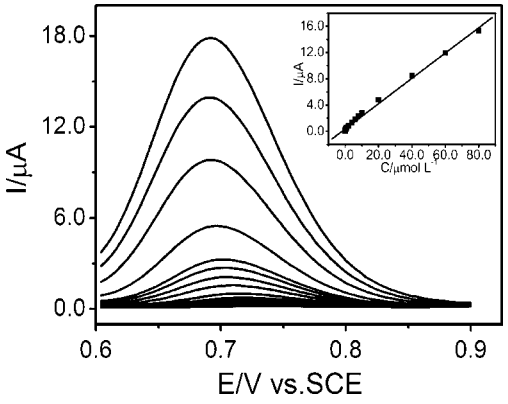

Fig. 9. DPSVs for different concentration of 2,4,6-TCP at MSDDE. 2,4,6-TCP concentration (from bottom to top, μmol L^{-1}) is 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 40.0, 60.0 and 80.0, respectively. Inset, the relationship between 2,4,6-TCP concentration and peak current.

result was shown in Table 1. The result of the electrochemical experimental accorded very well with those obtained by GC, which confirmed the accuracy and reliability of the approach, and it has a great potential for the practical analysis of PCZ and its metabolites in orange rind.

4. Conclusion

A sub-nanomole level electrochemical method for determining total residues of prochloraz and its metabolites as 2,4,6-TCP based on a nano-aperture MSDDE was firstly proposed. The electrochemical responses to 2,4,6-TCP were examined in details. It was found that the oxidation peak current of 2,4,6-TCP remarkably increases at the MSDDE. The significant peak current enhancement strongly suggests that nano-aperture medical stone exhibits excellent adsorption ability to 2,4,6-TCP, compared with undoped electrode. Compared with the reported chromatography, this new method has characteristics of ultrasensitivity, rapid response and extreme simplicity.

Acknowledgements

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