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Rapid hydrolysis and Electrochemical Detection of Cephalexin at a Heated Glassy Carbon Electrode

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The heated glassy carbon electrode (HGCE) was used to hydrolyze and detect the cephalexin without oil/water bath setup. The cephalexin could be rapid hydrolyzed in 15min by HGCE, and the good electro activity could be found in hydrolysate of cephalexin. Hence, the determination of cephaxlexin was developed by detecting degradation products using square wave voltammetry (SWV). The hydrolysis and detection were performed at the same HGCE. In addition, the HGCE could be renewed by mechanical polishing, which promotes the reproducibility of detection. Under the optimum conditions, the linear range for cephalexin from 6.0×10^{-7}~5.0×10^{-5} mol·L^{-1} and the detection limit (3σ/K) of 1.5×10^{-7} mol·L^{-1} were obtained. This method was applied to detect cephalexin in pharmaceutical formulations. The satisfactory reproducibility, relative errors and recoveries were gained. These results indicated that the application of HGCE provided a promising matrix in EC sensors for easy hydrolysis material, whose hydrolysis product had strong electroactive.

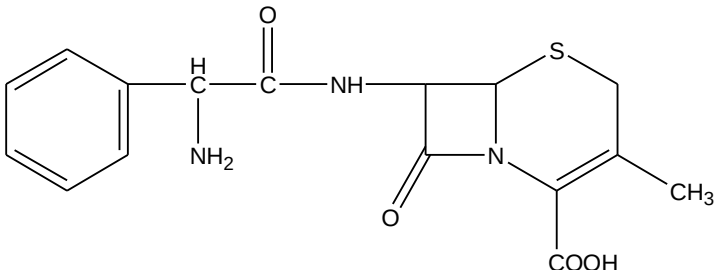
Keywords: heated electrode; cephalexin; electrochemistry; hydrolysis

1. INTRODUCTION

In recent years, heated electrodes technology [1, 2], in which the electrode temperature (*Te*) could be electrically heated, has been applied in many electrochemical systems. It is notable that the heating electrode can increase the temperatures in a thin hot solution layer near the surface of electrode, but keep the bulk solution at room temperature. The elevated *Te* can affect the thermodynamic and kinetic parameters of the electrode reaction, especially through thermal induction of the stirring effect, enhancing diffusion and accelerating the redox reaction rates.

Besides, heated electrode also was used to facilitate the hydrolysis process. Wei [3] designed a

heated screen-printed carbon electrode (HSPCE) and applied it in hydrolysis and electrochemical detection of trace carbofuran. This HSPCE was heated by high frequency alternating current in order to eliminate the disturbing effect of the heating current on the electrochemical signal [4]. In the present paper, the heated glassy carbon electrode (HGCE), which was indirectly heated by direct current (DC), was used in hydrolysis. The indirectly heated mode could avoid the interference between the heating current and electrochemical signal. And the using of DC could simplify the heating setup. In addition, differed from the HSPCE, the HGCE could be renewed by mechanical polishing, which promotes the reproducibility of detection. The hydrolysis and analytical performances of electrochemical detection systems based on HGCE were evaluated by determination of cephalexin.



Scheme 1. Structural formula of cephalexin

Cephalexin (Scheme 1), a kind of cephalosporin antibiotic, is an effective broad spectrum antibiotic that targets both Gram positive and Gram negative bacteria [5]. The widespread use of this compound requires fast and sensitive analytical methods. A great variety of methods to determine cephalexin have been reported, including chromatography [6-9], spectrophotometry [10-12], fluorescence method [13, 14], and immunoanalysis [15, 16]. Although the above methods are sensitive, they are time-consuming with complex and expensive instrumentation, and are not suitable for field routine operation[3, 17-19]. In recent years, several electrochemical determination methods [20-22] for cephalexin have been reported for both sensitivity and selectivity without the need of derivatization procedures. Cephalexin does not give peak current at a dropping mercury electrode, whereas its degradation product does, so on this basis adsorptive stripping voltammetry (ASV) was used for its determination; the peak current was directly proportional to its concentration [20]. For hydrolyzing, the oil or water bath was necessary in these reports, which was disadvantageous to field detection. The cephalexin was also determined based on the catalytic wave in presence of cobalt (II) [22], H_2O_2 [23] or β-lactamase [24], in which catalysts was necessary. The electrooxidation of cephalexin at boron-doped diamond electrodes (BDDE) and glass carbon electrode (GCE) were investigated by cyclic voltammetry (CV) [25]. The results showed that BDDE had better sensitivity than GCE, while the poor-defined response was obtained at GCE. However, the preparation of BDDE is very complex.

In this paper, the unmodified HGCE was used to the hydrolyze and detect the cephalexin. The *Te* could be rasied by electrically heating. Then, the cephalexin around the electrode could be hydrolyzed without oil or water bath apparatus. The hydrolysis product was electrochemically detected

at the same electrode. Parameters for hydrolysis and detection of cephalexin were investigated. The detection method was used for determination of cephalexin in pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Chemicals

Cephalexin was purchased from national institutes for food and drug control of China (Peking, China). Other reagents and chemicals were all commercially available and of analytical reagent grade. The stock standard solution was stored in the refrigerator, and the working standard solutions were freshly prepared before analysis. All the water used was de-ionized water prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Apparatus

Square wave voltammetry (SWV) measurements were performed with a CHI 660C electrochemical analyzer (CH Instruments, USA). An electrochemical cell with a three-electrode system was employed. The counter electrode was made of a platinum wire (99.99%). The reference electrode was Ag/AgCl in saturated KCl. The heated glassy carbon electrode (HGCE, 3 mm in diameter) was prepared as described earlier by Zhong [26], which was used as the working electrode. A glassy carbon stick that connected to a copper stick was coiled by a Cu lacquered wire (diameter 130 μm) through "twin-wire-wound coil method", which could cancel the magnetic field produced by the current passing through the Cu wire [27]. Before use, the HGCE was polished with shammy in suspension solution of alumina powder, rinsed with milli-Q water, and sonicated in ethanol and milli-Q water for 5 min, respectively. The electrochemical cell was covered with black cloth in order to reduce the light degradation of cephalexin. A DC power supply RXN-303A (Shengzhen Zhao Xin Electronic Equipments and Instruments Producer, Shengzhen, China) was connected to the Cu lacquered wire to provide a steady current for heating electrode.

2.3 Hydrolysis and electrochemical measurement of cephalexin

Briefly, cephalexin was dissolved in 6 mL of NaOH solution(0.1 $mol \cdot L^{-1}$). For wiping off the dissolved oxygen in the solution, the high pure nitrogen was bubbled into the solution for 10 min, and nitrogen flow was maintained during the experiment. The three-electrode system was immersed in the solution, and the electrode temperature (*Te*) was elevated to the desired temperature by DC heating for 15 min. Then, pH of hydrolyzate solution was adjusted to 11.73 by phosphate buffer solution (Na_2HPO_4+NaH_2PO_4, abbreviated to PBS, 0.2 $mol \cdot L^{-1}$, pH 10.0).

The EC measurement for hydrolyzate of cephalexin was performed by SWV. The SWV was recorded with potentials ranging from -0.2 to +1.0 V(vs. Ag/AgCl). The peak current values were plotted against the corresponding concentration of the cephalexin, and the calibration curve was

constructed by quintuplicate analysis. After each measurement, a preconditioning step was used to remove the previous deposits. In this step, the working electrode was cleaned by scanning the potential with -0.2~+1.0 V (vs. Ag/AgCl) in PBS(0.2 mol·L^{-1}, pH 7.0) at 75 ℃ for 20 successive CVs at 100 mV·s^{-1}. When the hydrolysis and detection were performed for five times, the HGCE was renewed by mechanical polishing and rinsing. In the experiment, the room temperature was stabilized at 22 ℃ by air-conditioning. It should be mentioned that the hydrolysis and detection were operated in a dark chamber to avoid the effect of light on the hydrolysis

2.4 Analysis of pharmaceutical samples

To prepare solutions of the cephalexin commercial samples, ten cephalexin capsules, whose shell were removed, were ground to homogeneous exiguous powder in a mortar. Then, an accurate mass of powder was dissolved in 1 L NaOH (0.2 mol·L^{-1}). Non-dissolved solids were filtered using a filter paper. The resulting solution was hydrolyzed at 70℃ by HGCE. The SWVs of hydrolyzate were obtained in PBS(0.2 mol·L^{-1}, pH 11.73). The cephalexin concentration in each sample solution was determined by calibration curve interpolation in an analytical curve previously obtained with standard solutions.

3. RESULTS AND DISCUSSION

3.1 Control and measurement of Te

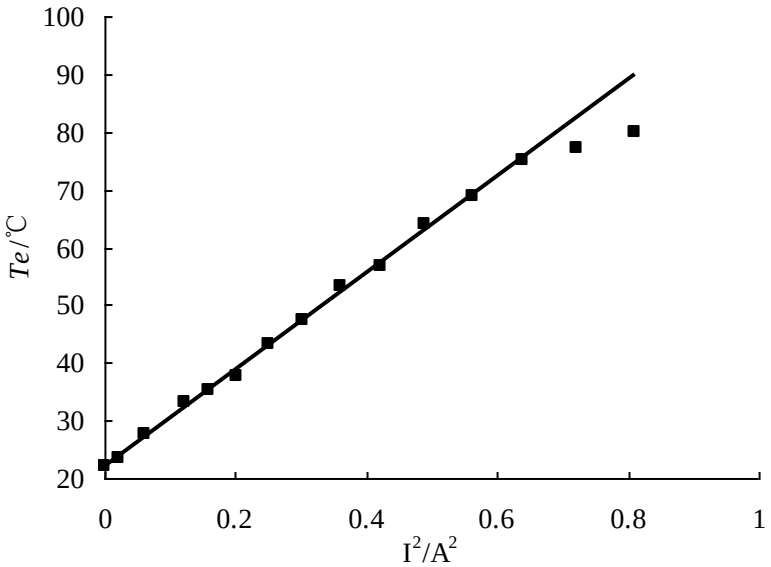


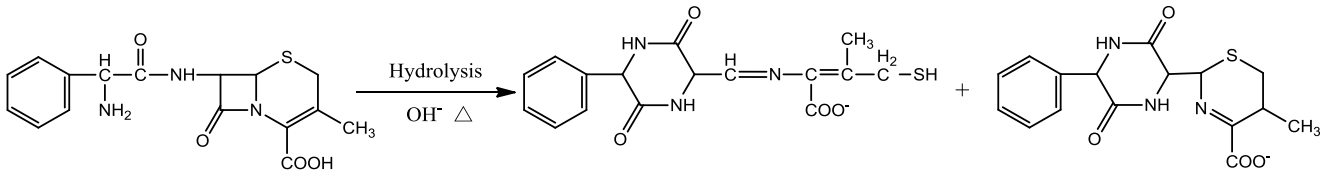
Figure 1. Relationship between the square of the heating current (I^2) and *Te*.

The prerequisite of using the heated electrode as an analytical tool is that the temperature at the surface of the electrode can be well controlled and easily measured [3]. The relationship between the heating current and *Te* should be examined before experiments. The procedure of electrode

temperature calibration was similar to the previous literatures [28, 29]. Fig. 1 displays the relationship between the heating current and *Te*. It shows that *Te* (22~75 ℃) nearly rises linearly with increase of the square of the heating current (I^2). Based on this relationship, *Te* could be changed easily by changing the heating current on the electrode. It should be mentioned that the thermal conductivity for different electrode was possibly inhomogeneous, so the temperature calibration should be performed for every HGCE.

3.2 SWV studies

According to earlier researches [20, 30], cephalexin with less electrochemical activity could be hydrolyzed in alkalescent aqueous solutions at high temperature (Scheme 2), which showed a good electrochemical response. Hence cephalexin can be indirectly determined by electrochemically detecting its hydrolysate product. The SWVs of cephalexin before(a) and after(b) its hydrolysis were shown in Fig.3.



Scheme 2 Hydrolysis of cephalexin

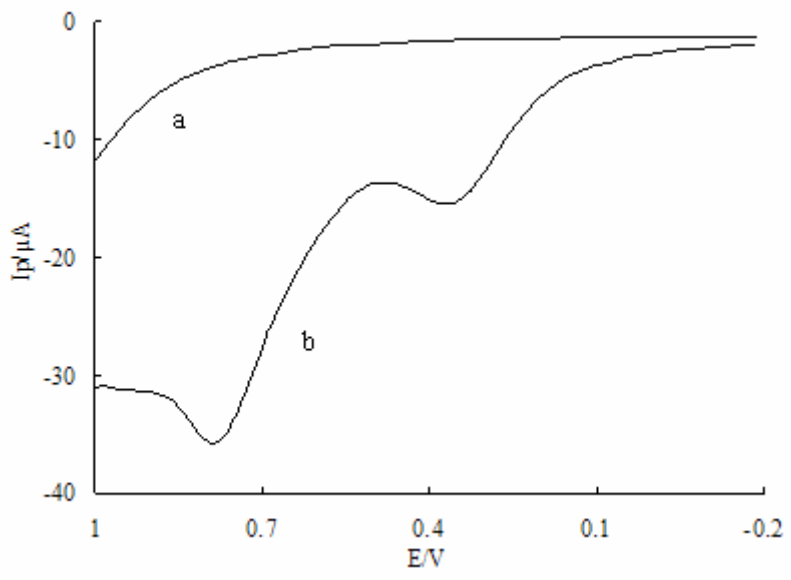


Figure 2. SWVs of cephalexin before(a) and after(b) its hydrolysis. [cephalexin]=10 μmol·L^{-1}; SW frequency, 50Hz; SW pulse amplitude, 45mV; detect buffer, PBS (pH=11.73, 0.2 mol·L^{-1}); hydrolysis time, 15 min; hydrolysis medium, NaOH(0.1 mol·L^{-1}).

As can be seen in Fig. 2, the direct oxidation of cephalexin was poorly defined wave. When the cephalexin was hydrolyzed at high temperature by HGCE, two visible current peaks were gained at 0.376V and 0.792V. The peak currents remained constant for 2 h at room temperature, which showed the stability of hydrolysis product. Considering the higher sensitivity, the peak current at 0.792V was used in quantitative analysis in our following experiments. The cephalexin was also hydrolyzed by oil bath at 100℃ for 30 min; the SWV coincided with the gained curve on HGCE. It showed that the hydrolysis behavior by HGCE was consistent with that by oil bath.

3.3 Optimization of factors affecting hydrolysis at HGCE

3.3.1 Effects of pH on hydrolysis

Effect of pH on peak currents of hydrolysis products was shown in Fig.3.

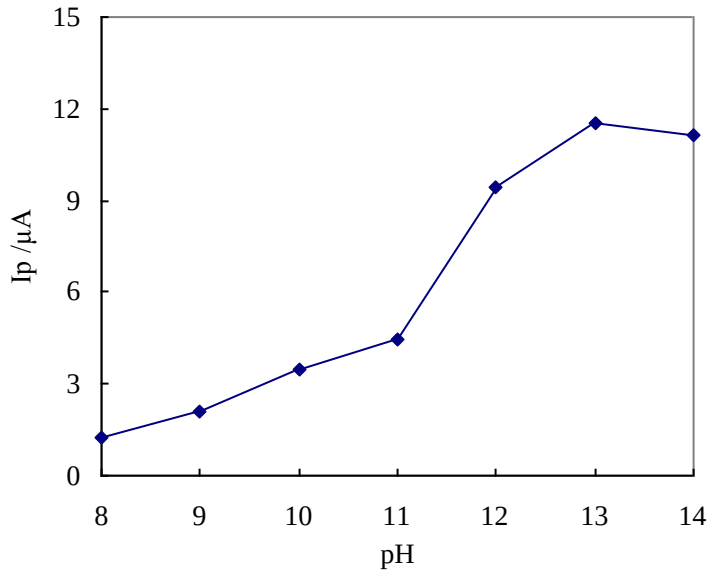


Figure 3. Plots of peak currents vs. pH of hydrolysis medium. Other conditions are the same as in Fig. 2.

The cephalexin was hydrolyzed in different pH (8.0~14.0), and the products were detected in PBS (pH 11.73). The obtained results showed that the peak current presented a maximum value at pH 13.0. Hence, the pH 13.0 was chosen as the acidity of hydrolysis medium.

3.3.2 Electrode temperature

The effect of the *Te* during hydrolyzation upon the SWV response of cephalexin at the HGCE was illustrated in Fig. 4. No obvious peak current was obtained when the *Te* was 22 ℃without heating. After heating the electrode, the cephalexin around the electrode surface was easily hydrolyzed. Then the significant peak currents were gained in the following electrochemical operations with SWV. It can

be seen that the SWV peak current is largely increased when raising the electrode temperature from 22 to 70 ℃ and slightly increased above 70 ℃. Hence, an electrode temperature of 70 ℃ was used in the subsequent experiments.

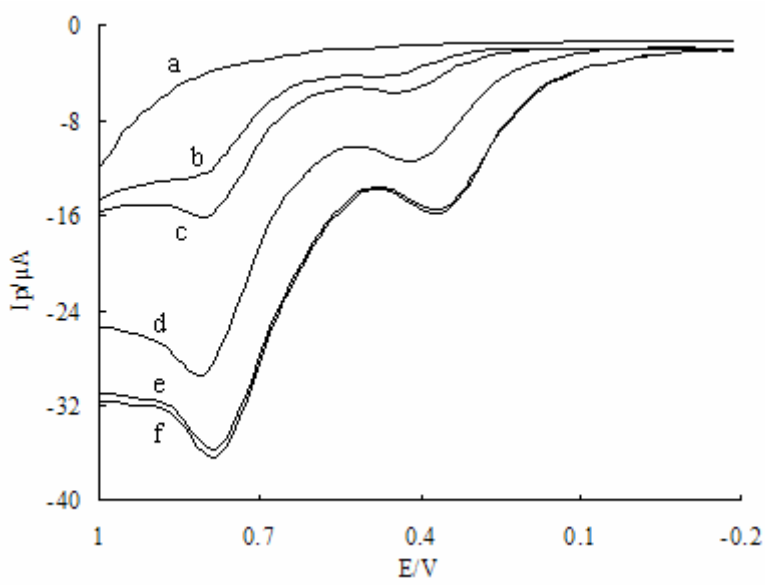


Figure 4. SWVs of hydrolyzing product of cephalexin at different electrode temperatures. a, 22 ℃; b, 40 ℃; c, 50 ℃; d, 60 ℃; e, 70 ℃; f, 75 ℃; Other conditions are the same as in Fig. 2.

3.3.3 Hydrolysis time

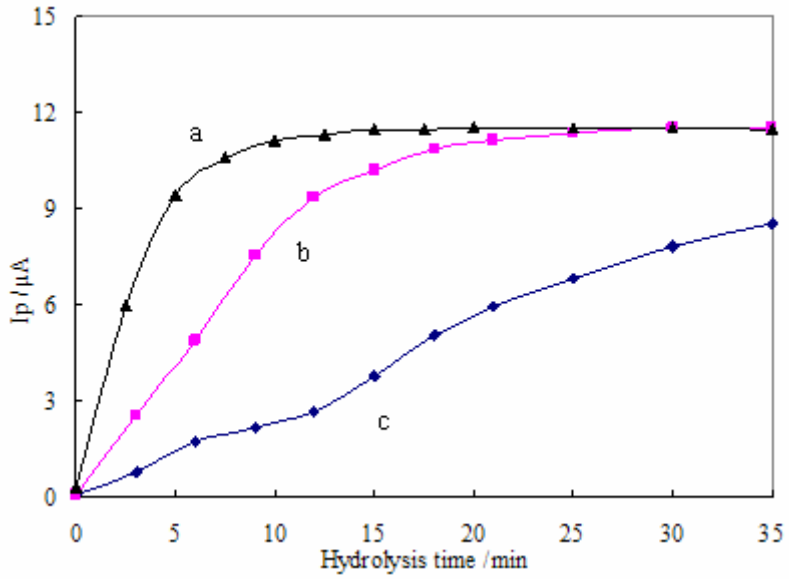


Figure 5. Plots of the peak current vs. heating time. a, 70 ℃ by HGCE; b, 100 ℃ by oil bath; c, 70 ℃ by oil bath; Other conditions are the same as in Fig. 2.

The effect of the hydrolysis time was also investigated. As shown in Fig.5, the response increased rapidly with hydrolysis time in 10 min, then changed to be steady and persisted with the time

(above 15 min). It indicated that the hydrolytic equilibrium was gained by heating electrode for 15 min. In the following experiment, 15 min was chosen as the hydrolysis time.

To compare the effect on hydrolysis by oil bath and HGCE, the cephalexin was also hydrolyzed by oil bath. The effect of hydrolysis time on the peak current by heating the entire solution with a thermostatic oil bath was displayed in Fig.5. When cephalexin was hydrolyzed at 100 ℃ by oil bath, the hydrolysis balance could de reached during about 30 min. And when the temperature of oil bath was 70 ℃, the hydrolysis would not be consummated in 35 min. Compared with the oil bath, the application of HGCE could shorten the heating time. The phenomena were possibly due to the characteristic of heating electrode technology. The temperature in a thin hot solution layer near the electrode surface could be significantly increased, while peak current was originated from the hydrolysis product on the electrode surface. Hence, the peak current could reach the maximum value in shorter time by HGCE.

3.4 Optimization of Analytical medium

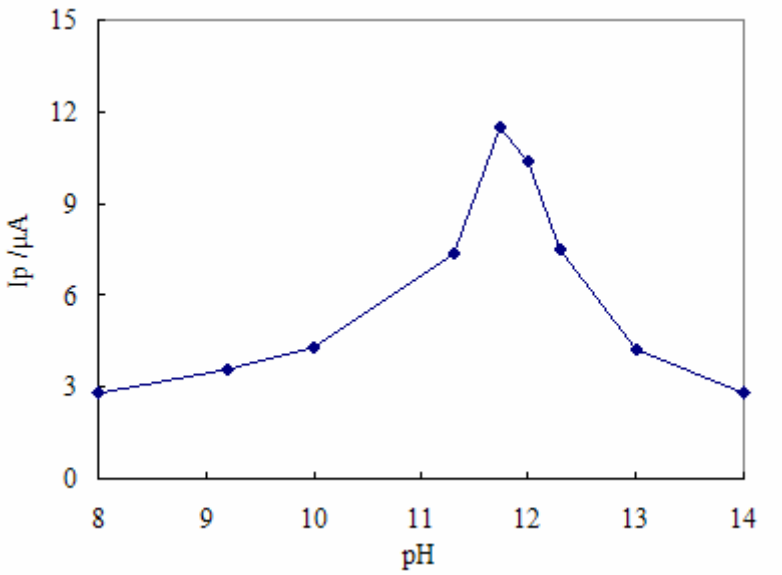


Figure 6. Relationship between peak currents and pH of Analytical medium. Conditions were the same as in Fig. 2.

The different supporting electrolytes, including PBS, Britton-Robinson buffer, borate buffer and NaOH-HCl were used as detection solution. The highest peak current was obtained in PBS. Then, pH of PBS was also optimized. Different with the pH of hydrolysis medium which affects the hydrolysis rate of cephalexin, the pH of analytical medium affects the detection sensitivity. Fig. 6 displayed the effect of pH on the peak current in PBS as detection solution. It was found that the maximum peak current was gained at pH 11.73. Thus, the subsequent cephalexin analytical determinations were carried out using PBS (0.2 $mol \cdot L^{-1}$, pH 11.73) as the analytical medium.

3.5 Analytical performance

After the cephalexin was hydrolyzed at 70 ℃ by HGCE, the oxidation peak current was linear with the concentration of cephalexin in the range of 6.0×10^{-7}~5.0×10^{-5} mol·L^{-1}. The corresponding regression equation (correlation coefficient of 0.9996) is -Ip(μA)=1.1798×C(μmol·L^{-1})-0.256, where the Ip is the peak current and the C is the concentration of cephalexin. The calculated value of the detection limit (3σ/K, three times the standard deviation of the blank solution/slope of the analytical curve) was 1.5×10^{-7} mol·L^{-1}.

As mentioned in 2.3, to remove the previous deposits, the working electrode was cleaned by scanning the potential with -0.2~1.0 V (vs. Ag/AgCl) in PBS (0.2 mol·L^{-1}, pH 7.0) at 75 ℃ for 20 successive cycles at 100 mV·s^{-1} after each measurement. The relative standard deviations (n=5) of peak current with 1.0×10^{-5} mol·L^{-1} cephalexin were 3.1 %. The electrode-electrode reproducibility (n=5) was determined for 1.0×10^{-5} mol·L^{-1} cephalexin with a freshly prepared HGCE as 4.5%.

3.6 The hydrolysis kinetics on surface of HGCE

Fig. 7 displayed a good linear relationship between the logarithm of cephalexin concentration (lnC) and the heating time, where the cephalexin concentration could be obtained by peak current through working curve (in section 3.5), which showed that hydrolysis of cephalexin followed first order kinetics.

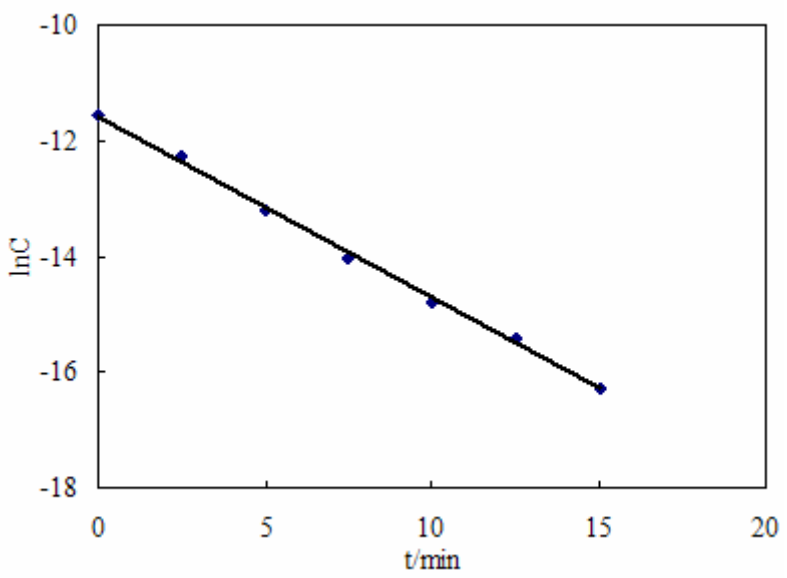


Figure 7. Relationship between degradation time and lnC. Conditions were the same as in Fig. 2.

3.7 Interferences

In order to determine cephalexin in samples by the proposed method, possible interferences from various inorganic cations, anions, and some organic substances were investigated by adding these

substances to the optimal supporting electrolyte containing 1.0×10^{-5} mol·L^{-1} cephalexin. The tolerable limit of foreign species was with a relative error not greater than 10%. No interference could be observed when 500 times of Na^+, K^+, Ca^{2+}, Cu^{2+}, Mg^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, CO_3^{2-}, SO_4^{2-}, NO_3^-, Cl^-, dextrin, amylum and glucose, 100 times of NH_4^+, Fe^{2+}, SO_3^{2-}, lactose, glucose and sucrose, and 10 times of citric acid, urea and uric acid, respectively, were added to 1.0×10^{-5} mol·L^{-1} cephalexin.

3.8 Sample analysis

Table 1. The determination results for cephalexin capsules.[a]

Sample	Label value (mg/capsule)	Reference method [b] (mg/capsule)	Proposed method [b] (mg/capsule)	Relative error (%)
1	125	124±2	124±1	-0.8 [c] 0 [d]
2	125	123±1	122±2	-1.6 [c] -2.4 [d]
3	125	125±3	126±4	0.8 [c] 0.8 [d]

[a] Conditions were the same as in Fig. 2.
[b] Average value of five measurements.
[c] Relative error between the measured values by proposed method and label value.
[d] Relative error between the measured values by proposed method and it by reference method.

Table 2. The addition and recovery of cephalexin capsules.[a]

Sample	Added (μmol·L^{-1})	Found (μmol·L^{-1})	Recovery (%)
1	1.00	0.95	95.0
1	5.00	5.19	103.8
1	10.00	9.29	92.9
2	1.00	1.02	102.0
2	5.00	5.44	108.8
2	10.0	9.90	99.0
3	1.0	0.93	93.0
3	5.0	4.95	99.0
3	10.0	10.32	103.2

[a] Conditions were the same as in Fig. 2.

The determination of cephalexin in commercial cephalexin capsules employing the proposed method and a standard method of the Chinese Pharmacopoeia [31] were performed. The amount of the cephalexin in each sample was determined by interpolation in an analytical curve previously obtained with reference (standard) solutions. Five determinations were done for each sample, and the standard deviations were calculated. As shown in Tab. 1, the found values by proposed method were consistent with the label value and the measured value by reference method.

An addition and recovery study was performed by adding known amount of standard solutions to a given sample followed by analysis using the proposed method. The results presented in Tab. 2 showed that the cephalexin recoveries were in the range from 92.9% to 108.8%, indicating absence of a matrix effect.

4. CONCLUSIONS

In this paper, an electrically heated glassy carbon electrode was successfully used in rapid hydrolysis and electrochemical detection of cephalexin. The application of proposed electrode in hydrolysis results in several advantages. Firstly, the hydrolysis and detection process could be carried out on the same electrode without oil/water bath, which simplified the device. Secondly, compared with heated by oil/water bath, the shorter hydrolysis time was obtained on HGCE. The cephalexin could be hydrolyzed in 15min with raised *Te*. Thirdly, the proposed method could be performed without the catalyzer and the modified electrode, which simplified the operation.

Under the optimum conditions, the linear range for cephalexin from 6.0×10^{-7}~5.0×10^{-5} mol·L^{-1} and the detection limit (3σ/K) of 1.5×10^{-7} mol·L^{-1} were obtained. This method was applied to detect cephalexin in pharmaceutical formulations. The satisfactory relative errors and recoveries were gained. These results indicated that the application of HGCE provided a promising matrix in EC sensors for easy hydrolysis materials, whose hydrolysis product had strong electroactive.

ACKNOWLEDGEMENT
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