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Polarographic behavior of cephalexin and its determination in pharmaceuticals and human serum

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Abstract

Cephalexin gives a reduction wave in 0.03 mol/l HCl medium at ca. −1.24 V. With cephalexin concentration higher than 2.5×10^{-5} mol/l, another reduction wave is observed at ca. −0.90 V. These reduction waves are attributed to the reduction of ethylenic bond of a six-membered dihydrothiazine ring. When H_2O_2 is present, the reduction wave at ca. −0.90 V is catalyzed by H_2O_2 and its reduction intermediate hydroxyl radical $^{\bullet}OH$, producing a catalytic wave. However, the reduction wave at ca. −1.24 V remains nearly unchanged. A sensitive polarographic method for the determination of cephalexin is proposed based on the reduction wave of cephalexin. The second-order derivative peak current of the wave at ca. −1.24 V is rectilinear to the cephalexin concentration in the range 1.0×10^{-7} to 2.5×10^{-5} mol/l, and the detection limit is 5.0×10^{-8} mol/l. The proposed method is applied to the individual tablet dosage form and human serum.

Keywords: Cephalexin; Polarographic catalytic wave; H_2O_2; Pharmaceutical analysis

1. Introduction

Cephalexin, 7-(D-amino-2-phenylacetamido)-3-methyl-8-oxo-5-thia-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (Scheme 1), is a cephalosporin antibiotic. It is closely related to the penicillin family and is used for patients allergic to penicillin.

The widespread use of this compound and clinical, and pharmacological study requires fast and sensitive analytical methods to determine the drug in pharmaceutical formulations and serum samples. A great variety of methods to determine cephalexin have been reported over these years, including chromatography [1–3], spectrophotometry [4–7], a fluorescence method [8], a flow-through immunoanalysis [9] and electroanalytical methods [10–22].

Several polarographic methods for the determination of cephalexin have been reported. However, because cephalexin was thought that it contains an unsubstituted 3-methyl group and no other reducible group, and does not give a peak at a mercury electrode, some authors [15–20] used its degradation products acting as depolarizers. Fogg et al. [18] devised a method for the determination of cephalexin, based

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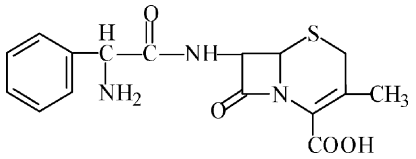


Scheme 1. Structural formula of cephalexin.

on polarographic measurement of one of its degradation products after intensive hydrolysis in pH 7.4 buffer at 100 °C for 1 h. Li and Chen [19] studied the electrochemical behavior and the mechanism of its degradation in 0.1 mol/l NaOH at 100 °C for 20 min. Yet Benner [21] reported that cephalexin gives a d.c. polarographic wave at −1.25 V (versus SCE) in 0.125 mol/l sulphuric acid which provides linear relationships between the current and concentration in the range 1–70 μg/ml. Hall et al. [22] also reported that Δ^3-deacetoxycephalothin, which has an unsubstituted 3-methyl group, produced a wave at low pH value but the wave merged into the electrolyte discharge at pH values above 3.2. To achieve a proper and most efficient use of determination methods, a review on the electroanalytical chemistry of cephalosporins and cefamycins was recently presented [23].

The aim of this study is to investigate the electrochemical behavior of cephalexin in the absence and the presence of H_2O_2 and to optimize the conditions for the determination of this compound in pharmaceutical dosage forms and human serum using linear-potential scan polarographic techniques. In this work, the information about polarographic properties and the reaction of cephalexin with H_2O_2 in 0.03 mol/l HCl medium are described. This method is potentially useful for the determination of cephalexin, because of its simple procedure in obtaining measurement results without any particular hydrolysis procedure.

2. Experimental

2.1. Apparatus

Linear-potential scan polarograms were recorded by model JP-303 polarographic analyzer (Chengdu Instrument Factory, Chengdu, China). A three-electrode set-up was equipped with a dropping mercury working electrode, a platinum-wire auxiliary electrode and a saturated calomel reference electrode. Cyclic voltammograms were recorded on a model CH660 electrochemical workstation (CH Instruments, USA) coupled with a model 303 A static mercury drop electrode system (EG&G PARC, USA), including a hanging mercury drop working electrode, a platinum-wire auxiliary electrode and a saturated calomel reference electrode. The workstation was controlled by CH660 software and operated under Windows 98 environment.

Unless otherwise stated, potentials were referred to the potential of the saturated calomel electrode.

2.2. Reagents

A stock standard solution of cephalexin (Sigma, Germany) (1.0×10^{-3} mol/l) in water was prepared by dissolving 0.0365 g cephalexin of 99.0% purity in twice distilled water, transferring the solution into a 100 ml volumetric flask, diluting to volume and mixing. The solution was stored in dark under refrigeration. Working standard solutions (1.0×10^{-4} and 1.0×10^{-6} mol/l) were prepared by dilution with water. A H_2O_2 solution (0.1 mol/l) was standardized by $KMnO_4$ standard solution. All chemicals were of analytical-reagent grade or better, and were used without further purification. Twice distilled water was used throughout the experiments.

2.3. Procedure

Cyclic voltammograms of cephalexin in 0.03 mol/l HCl medium in the absence and the presence of H_2O_2 were recorded on a model CH660 electrochemical workstation after deaeration with high-purity nitrogen for 10 min.

Determination of cephalexin was carried out on a model JP-303 polarographic analyzer. A certain amount of standard or sample solution of cephalexin and 0.6 ml HCl (0.5 mol/l) solution were successively pipetted into 10 ml volumetric flask and diluted to the mark with water. The prepared solution was transferred into a polarographic cell without deaeration. Linear-potential scan was cathodically performed from –0.80 to −1.40 V at a scan rate of 0.25 V/s. The

second-order derivative peak current of the reduction wave at ca. −1.24 V was recorded versus the concentration of cephalexin. The cephalexin content in the samples was obtained by using the standard curve method.

3. Results and discussion

3.1. The polarographic catalytic wave of cephalexin

Polarographic behavior of cephalexin was examined in such media as H_2SO_4, HCl, $HClO_4$, HAc–NaAc, KH_2PO_4–$Na_2B_4O_7$, KH_2PO_4–Na_2HPO_4, $NH_3{\cdot}H_2O$–NH_4Cl, and KCl solutions. Only in acidic media (H_2SO_4, HCl and $HClO_4$) cephalexin produced the polarographic response, and the best-defined reduction wave was obtained in HCl medium. From the voltammograms of cephalexin in 0.03 mol/l HCl solution, as shown in Fig. 1, a single reduction wave appeared at ca. −1.24 V on the cathodic scan when cephalexin concentration was lower than 2.5×10^{-5} mol/l. The absence of an oxidation wave on the reverse scan showed that the reduction is totally irreversible.

The experiments showed that the peak current of the reduction wave was a linear function of cephalexin concentration from 1.0×10^{-7} to 2.5×10^{-5} mol/l. And the peak current decreased with increasing pH in the range 0–3. On the other hand, the peak potential shifted negatively with increasing pH in the range 0–3. The relationship equation is $-E_p(\text{mV}) = 1154.6 + 68\,\text{pH}$ ($r = 0.9932$, $n = 7$). In order to validate the electrode process, constant potential coulometric analysis was used to identify the number of electrons transferred in the reduction process. Three 25.0 ml portions of 0.03 mol/l HCl and 2.0×10^{-5} mol/l cephalexin solution were electrolyzed on a model CH660 electrochemical workstation coupled to a model 303 A electrode system, including a mercury pool electrode serving as a working electrode, a platinum-wire auxiliary electrode and a saturated calomel reference electrode. During the electrolysis, the solution was stirred under purging with high-purity nitrogen. The pre-electrolysis at the potential −1.10 V was first run until the current fell to a minimum value. Then, the electrolysis proceeded until the current fell to a minimum value again at the controlled potential −1.30 V. The coulometric measurements were performed three times. The number of transferred electrons obtained was 2.12, 1.98 and 2.08, respectively, thus $n = 2$. From the E_p–pH relationship $-E_p(\text{mV}) = 1154.6 + 68\text{pH}$ mentioned above, the number of proton H^+ transferred was two, too. Therefore, the electrode reduction of cephalexin was a $2e^-$, $2H^+$ process. From the chemical structure of cephalexin, it belongs to α, β-unsaturated acid. Because the ethylenic bond was activated by the conjugated carboxyl group, it can be reduced at more positive potential than that of carboxyl group. Among these α, β-unsaturated acids, maleic acid can be used as a model compound. In comparison, the relationship of peak current of reduction wave of maleic acid with pH is similar to that of cephalexin, and the peak potential shifted negatively with increasing pH in the range 0–3 and the relationship equation is $-E_p(\text{mV}) = 584.4 + 73.3\text{pH}$ ($r = 0.9944$, $n = 7$). From their similarities in polarographic behavior and chemical structure, it can be deduced that the reduction of cephalexin in acidic medium on mercury electrodes is due to the reduction of ethylenic bond. Moreover, $KMnO_4$ is the known reagent to clarify ethylenic bond. Experiments showed that after appropriate $KMnO_4$ solution was added into the 0.03 mol/l HCl solution, the reduction peak current of cephalexin disappeared. Therefore, the electrode process of the reduction wave of cephalexin can be described

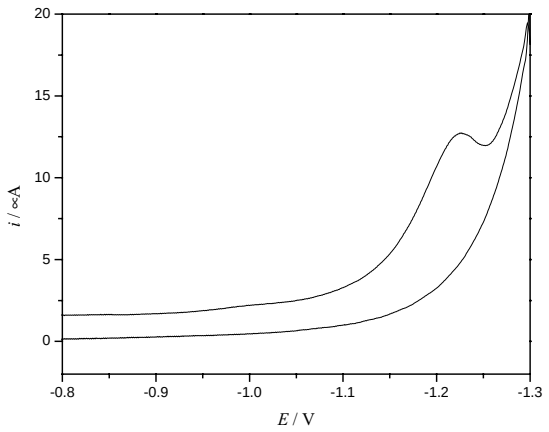
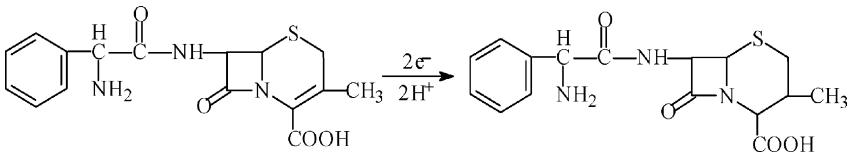


Fig. 1. Cyclic voltammogram: 0.03 mol/l HCl; 1.0×10^{-5} mol/l cephalexin. Initial potential −0.80 V; potential of reverse −1.30 V; scan rate 0.1 V/s.

as follows:



With cephalexin concentration increasing higher than 2.5×10^{-5} mol/l another reduction wave was observed in 0.03 mol/l HCl medium at ca. −0.90 V (Fig. 2, curve c). The peak potential shifted negatively from −0.864 to −1.068 V with increasing cephalexin concentration from 2.5×10^{-5} to 1.1×10^{-4} mol/l. The peak current increased with increasing pH in the range 0–2, and decreased with increasing pH in the range 2–3. On the other hand, the peak potential shifted negatively from −0.544 to −1.210 V with increasing pH in the range 0–3.

The reduction wave at ca. −0.9 V could be catalyzed by oxidant H_2O_2. As shown in Fig. 3, the peak current observed for 6.0×10^{-5} mol/l cephalexin increased gradually with increasing H_2O_2 concentration in the range 1.0×10^{-3} to 6.0×10^{-3} mol/l and the maximum current was obtained at 6.0×10^{-3} mol/l H_2O_2. However, the peak potential remained unchanged on the cathode scan and as expected, a reduction wave at ca. −0.92 V appeared on the reverse scan (Fig. 2, curve d), producing a catalytic wave. The peak current of the catalytic wave in the presence of 6.0×10^{-3} mol/l H_2O_2 was ca. 20 times higher than that of the corresponding reduction wave. When H_2O_2 concentration was in the range 1.0×10^{-3} to 6.0×10^{-3} mol/l, the ratio $i_{p,l}/i_{p,c}$ of the peak current $i_{p,l}$ of the catalytic wave to the peak current $i_{p,c}$ of the corresponding reduction wave was linearly proportional to the square root of H_2O_2 concentration. The relationship equation obtained was $i_{p,l}/i_{p,c} = 88.7 \times (C_{H_2O_2}(\text{mol/l}))^{1/2}$ ($r = 0.9987$, $n = 6$). Further study showed that the current function $i_p/v^{1/2}$ of the catalytic wave decreased sharply with increasing scan rate v in the range 0.025–0.20 V/s, and then leveled off in the range 0.20–0.50 V/s (Fig. 4, curve b). These characteristics indicated that the catalytic wave at ca. −0.9 V was a parallel one.

From the above results, it can be concluded that the formation of the reduction wave was caused by the reduction of ethylenic bond of cephalexin and the reduction wave at ca. −0.9 V could be catalyzed by H_2O_2. According to literature [24,25], the reduction of the conjugated ethylenic bond undergoes an intermediate radical. Therefore, when H_2O_2 was present, H_2O_2 ox-

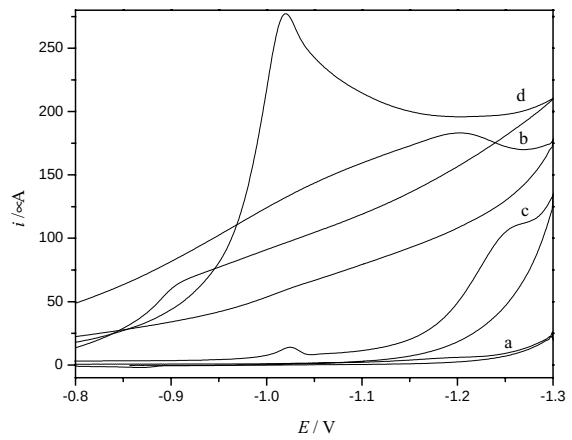


Fig. 2. Cyclic voltammograms: (a) 0.03 mol/l HCl; (b) $a + 6.0 \times 10^{-3}$ mol/l H_2O_2; (c) $a + 6.0 \times 10^{-5}$ mol/l cephalexin; (d) $a + 6.0 \times 10^{-5}$ mol/l cephalexin + 6.0×10^{-3} mol/l H_2O_2. Initial potential −0.80 V; potential of reverse −1.30 V; scan rate 0.1 V/s.

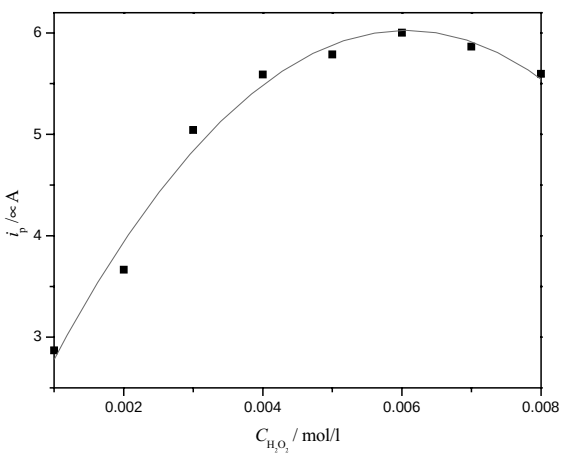


Fig. 3. Effect of H_2O_2 concentration on the reduction peak current at −0.9 V: 0.03 mol/l HCl; 6.0×10^{-5} mol/l cephalexin. Scan rate 0.25 V/s.

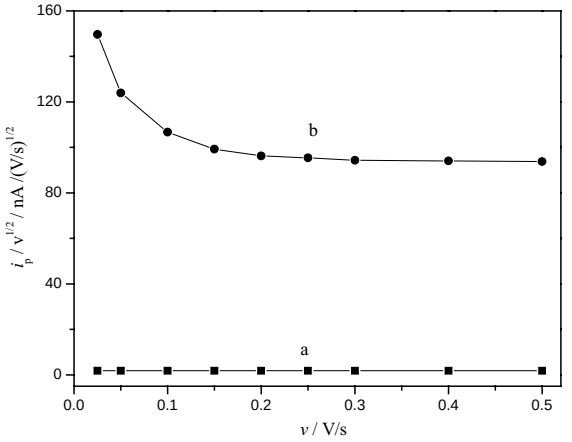


Fig. 4. Current function $i_p/v^{1/2}$: (a) 0.03 mol/l HCl + 6.0×10^{-5} mol/l cephalexin; (b) $a + 6.0 \times 10^{-3}$ mol/l H_2O_2.

idized the intermediate radical of the ethylenic bond of cephalexin at mercury electrodes, to reproduce the original, resulting in a large catalytic current. In addition, it was well known that the reduction of H_2O_2 involved two successive one-electron processes via the hydroxyl radical •OH. The •OH was a stronger oxidizing agent and was more active than H_2O_2. Because H_2O_2 chemically oxidized the intermediate radical of cephalexin to yield •OH, when the intermediate radical of cephalexin was produced, it was immediately oxidized by H_2O_2 and •OH to the original, forming the catalyzed reduction cycle.

However, the reduction wave at −1.24 V was not catalyzed by H_2O_2 possibly because the chemosorption was produced on mercury electrodes, resulting in the intermediate radical of cephalexin reacting with mercury.

3.2. Condition optimization

As mentioned above, this paper chose HCl solution as the supporting electrolyte. The effect of HCl concentration on the reduction wave at ca. −1.24 V showed that increasing HCl concentration from 0.001 to 1.0 mol/l led to a gradual increase of the peak current of the wave, while E_p shifted positively from −1.382 to −1.164 V. In HCl concentration range 0.025–0.040 mol/l the peak current of the reduction wave at ca. −1.24 V showed good sensitivity and stability, and therefore 0.03 mol/l HCl was selected as the supporting electrolyte.

3.3. Calibration curve, detection limit and precision

It was known that the derivative technique had a good resolving power. Therefore, the second-order derivative was used to determine the peak current of cephalexin. In the optimal supporting electrolyte chosen in this work, the second-order derivative peak current i_p'' of the reduction wave at ca. −1.24 V was linearly proportional to cephalexin concentration in the range 1.0×10^{-7} to 2.5×10^{-5} mol/l. The linear regression equation was i_p'' (nA/s^2)$=-1.9 + 3.5 \times 10^7 C$ (mol/l) with a regression coefficient $r = 0.9993$ ($n = 7$). The detection limit of 5.0×10^{-8} mol/l was calculated from the calibration curves as $3s_1/m$, where s_1 is the standard deviation of the intercept and m is the slope. A relative standard deviation (R.S.D.) of 1.4% was obtained for 11 independent determinations of 1.0×10^{-6} mol/l cephalexin. The precision was evaluated by repeating six independent determinations of 1.0×10^{-6} mol/l cephalexin. The peak current remained nearly unchanged over 8 h and relative standard deviation was calculated to be 1.6%.

3.4. Interferences

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^{-6} mol/l cephalexin. The experiments showed that a 500-fold excess of Na^+, K^+, Cu^{2+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+}, Cl^-, Br^-, NO_3^-, HPO_4^{2-}, CO_3^{2-}, amylum, glucose, carbamide, ascorbic acid, oxalic acid, and phenylformic acid; a 200-fold excess of Mn^{2+}, Fe^{3+}, $S_2O_3^{2-}$, cystine, serine, glutamic acid, and valine; a 100-fold excess of Fe^{2+}, Ni^{2+}, Co^{2+}, and arginine, and; a 10-fold excess of tyrosine did not interfere with the determination of cephalexin.

3.5. Sample analysis

3.5.1. Determination of cephalexin in tablets

Ten tablets were powdered and mixed further. A portion of the powder obtained was accurately weighed, transferred into a 50 ml volumetric flask, and dissolved in water. The contents of the flask were sonicated for 15 min to ensure complete dissolution.

Table 1
Analytical results of tablets

Samples	Label value (mg)	Determined value (mg)	Mean value (mg)	R.S.D. (%)
A	125	123.8, 124.2, 123.9, 124.5, 123.2, 124.9	124.1	0.5
B	250	248.9, 247.9, 248.2, 247.6, 248.5, 247.2	248.1	0.3

Table 2
Analytical results of the samples ($n = 6$)

Samples	Added (mol/l)	Found (mol/l)	Recovery (%)	R.S.D. (%)
Tablet	8.00×10^{-7}	7.99×10^{-7}	99.9	1.9
	1.00×10^{-6}	1.01×10^{-6}	101.0	2.3
	4.00×10^{-6}	3.96×10^{-6}	99.0	2.2
Serum	8.00×10^{-7}	7.94×10^{-7}	99.3	3.6
	1.00×10^{-6}	9.64×10^{-5}	96.4	4.1
	4.00×10^{-6}	3.92×10^{-6}	98.0	4.5

Appropriate solutions were prepared by taking suitable aliquots of the supernatant liquor. The sample solution obtained above was used for the polarographic determination of cephalexin. The results are summarized in Table 1.

To study the accuracy of the proposed methods, and to check interference from excipients used in the dosage forms, recovery experiments were carried out by standard addition method. Each recovery was calculated by comparing the results obtained before and after addition. The results are shown in Table 2.

3.5.2. Determination of cephalexin in spiked serum samples

Serum samples obtained from healthy individuals (from the Hospital of Northwest University, Xi'an) were stored frozen until assay. After gentle thawing, an aliquot of sample was fortified with cephalexin dissolved in twice distilled water to achieve the appropriate concentration. The supernatant was taken carefully. An appropriate volume of supernatant liquor was transferred to a 10 ml volumetric flask, diluted to volume, and the sample solution obtained above was used for polarographic determination of cephalexin. The results are summarized in Table 2.

4. Conclusion

The polarographic behaviors of cephalexin in the absence and the presence of H_2O_2 at mercury electrodes have been described in this paper. When cephalexin concentration is lower than 2.5×10^{-5} mol/l, a sensitive reduction wave at -1.24 V is produced in 0.03 mol/l HCl medium. Furthermore, with cephalexin concentration higher than 2.5×10^{-5} mol/l, another reduction wave is observed at ca. -0.90 V. These reduction waves are attributed to the reduction of ethylenic bond of cephalexin. With respect to the reduction wave at -1.24 V, an application to pharmaceuticals is possible after a simple dilution step. Developed procedures have also been used for serum samples, with good recoveries obtained at the levels tested. When H_2O_2 is present, the reduction wave at ca. -0.90 V is catalyzed by H_2O_2 and its reduction intermediate hydroxyl radical, producing a parallel catalytic wave. Using the parallel catalytic wave, it is helpful to study the intermediate radical of cephalexin.

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