

# Polarographic determination of berberine in the presence of $\text{H}_2\text{O}_2$ in medicinal plants

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## Abstract

The mechanism of the parallel catalytic wave of berberine in the presence of  $\text{H}_2\text{O}_2$  was studied. The results showed that the reduction process of the C=N bond of berberine was two successive one-electron transfers, in which an intermediate free radical was involved. When  $\text{H}_2\text{O}_2$  was present, it oxidized the free radical to the original C=N bond, producing the parallel catalytic wave of berberine. In  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M)– $\text{H}_2\text{O}_2$  (4 mM) supporting electrolyte, the peak current of the catalytic wave was linear to the berberine concentration in the range of  $1.0 \times 10^{-8}$ – $3.0 \times 10^{-7}$  M. The limit of detection was  $7.0 \times 10^{-9}$  M. The catalytic wave can be applied to direct determination of berberine in medicinal plant *Coptis chinensis* Franch, after sufficiently diluting the water extraction without preliminary separation. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Berberine; Hydrogen peroxide; Polarographic catalytic wave; Free radical; Medicinal plant; *Coptis chinensis* Franch

## 1. Introduction

Berberine was a natural green drug, which was mainly obtained from medicinal plants such as *Coptis chinensis* Franch and *Phellodendron amurense* Rupr by water extraction. Berberine and some medicinal plants in which berberine was major component had been used as bacterium resistant in clinical practice for long time due to a good curative effect. As compared with high-performance liquid chromatographic [1,2] and extraction spectrophotometric methods [3,4] for the

determination of berberine, polarographic method is a simple and quick. Some polarographic methods for the determination of berberine in crude drug and pharmaceutical preparation were reported. Although the most sensitive polarographic technology, differential pulse (DPP) and square-wave polarography (SWP) were employed, the lowest detectable concentration was  $5.0 \times 10^{-7}$  M using DPP [5],  $2.0 \times 10^{-7}$  M using SWP [6] on basis of its reduction wave in NaCl–HCl (pH 2, 0.1 M) solution, and sub-ppm level by DPP [7] on basis of its catalytic hydrogen wave in Britton–Robbinson buffer (pH 10). In addition, it is well known that the polarographic catalytic wave was an important aspect in polarography. Recently,

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studies and applications on polarographic catalytic wave were extended to organic compound field. Polarographic catalytic wave of such classes of organic compounds that contain thiol [8,9], disulfide linkage (protein) [10,11], ethylenic bond conjugated to carbonyl bond (steroid [12,13], cinnamic acid [14] and glycyrrhetic acid [15]) and others had been reported. They can be obtained with the help of convenient chemical matter, and were used not only to attain high analytical sensitivities and but also to study reaction kinetics of the electrogenerated free radical of organic compounds by conventional polarographic equipment. We observed a polarographic catalytic wave of berberine in  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M)– $\text{H}_2\text{O}_2$  (4 mM) buffer, and used it to determine berberine in berberine tablet [16].

As berberine is a typical one of the nitrogen-containing heterocyclic alkaloid, it is necessary to study the production mechanism of the catalytic wave for interpreting the redox process of berberine in the electrochemical system and for developing quick and sensitive method for determining the class of the alkaloids. The aim of this work was, firstly, to study both the reduction process of the reduction wave and the mechanism of the catalytic wave of berberine and, secondly, to further use the polarographic catalytic wave to directly determine berberine in medicinal plant.

## 2. Experiment

### 2.1. Materials

Berberine sulfate was of biochemical-reagent grade and was purchased from the Medicine Factory attached to the Second Military Medical Institute (Shanghai, PR China). A stock solution of berberine (1 mM) was prepared. Other standard working solutions of berberine were obtained by diluting the stock solution with water. All chemicals used were of analytical reagent grade. A  $\text{H}_2\text{O}_2$  solution (0.1 M) was standardized by  $\text{KMnO}_4$  standard solution. Buffers of  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) and  $\text{Na}_2\text{B}_4\text{O}_7\text{--NaH}_2\text{PO}_4$  (pH 8.6, 0.06 M) were prepared. A *N,N*-dimethyl formamide (DMF) mix-

ture containing supporting electrolyte tetraethyl ammonium bromide,  $(\text{C}_2\text{H}_5)_4\text{NBr}$ , (0.1 M) was prepared. *o*-Nitrophenyl octyl ether was used without further purification. Twice-distilled water was used through the experiments.

### 2.2. Apparatus

A model CH660 electrochemical workstation (CH Instrument, USA), with a three-electrode system consisting of a model 303A stationary mercury drop working electrode (EG&GARA Corp, USA) (for cyclic voltammetry) or a mercury pool cathodic electrode (for coulometry), a platinum wire counter electrode and a saturated calomel reference electrode (SCE). A model JP-2 linear-potential scan polarograph (Chengdu Instrumental Factory, China), with a three-electrode setup consisting of a dropping mercury working electrode (DME), a platinum wire counter electrode and a SCE. A model UV-2101PC UV-Vis spectrophotometry (Shimadzu, Japan).

### 2.3. Experimental procedure

Cyclic voltammetry of berberine was respectively performed in  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) buffer and in DMF– $\text{H}_2\text{O}$  (10:0.01, v/v) mixture with and without  $\text{H}_2\text{O}_2$  after removing dissolved oxygen by passage of oxygen-free nitrogen.

Controlled-potential coulometry was carried out in  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) buffer containing berberine (1  $\mu\text{M}$ ). During the electrolysis, the solution was purged with oxygen-free nitrogen. Firstly, the cathodic potential was controlled at  $-1.20$  V, pre-electrolysis was 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  $-1.45$  V. The consumed electricity  $q$  of berberine in electrolysis process was automatically recorded.

### 2.4. Sample analysis

#### 2.4.1. Construction of the calibration graph

The varied amounts of berberine standard working solution, 20 ml of  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$

(pH 9.4, 0.08 M) buffer and 1 ml of  $\text{H}_2\text{O}_2$  (0.1 M) solution were successively added into 25 ml volumetric flask, and was then diluted to mark with water. The prepared solution was transferred to polarographic cell without deaeration. Linear-potential scan polarogram was recorded by scanning cathodically from  $-1.10$  to  $-1.60$  V. First-order derivative peak current of the catalytic wave at  $-1.30$  V was measured. The calibration graph was constructed by plotting the peak current of the catalytic wave against berberine concentration.

#### 2.4.2. Preparation and analysis of sample

About 0.1792 g of smashed powder of medicine plant *C. chinensis* Franch was weighed, and dipped in water overnight. It was then transferred into a 100 ml volumetric flask. The supernatant liquid was diluted 100 times with water again. Other measurements were completed as the procedure mentioned above. The berberine content in sample was calculated according to the calibration graph.

### 3. Results and discussions

#### 3.1. Reduction process of berberine

Berberine was a quaternary isoquinolinium salt.

Only a reduction wave was generally observed in aqueous solution. The reduction wave was ascribed to the reduction of the C=N bond in isoquinolinium ring prior to the reduction of the conjugated C=C bond [5,6,17].

The electrode process of the reduction wave was examined in detail. Cyclic voltammetry in  $\text{Na}_2\text{B}_4\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) buffer (Fig. 1a) after deaeration showed a single symmetrical reduction wave of berberine with peak potential  $E_p = -1.30$  V at cathodic branch and no oxidation wave at anodic branch. With potential scan rate  $v$  increasing, the peak current  $i_p$  of the cathodic wave increased. The  $i_p$  was linear to the  $v$  rather than to the one-two order  $v^{1/2}$  of the  $v$  for the diffusion-controlled wave. The relationship of the  $i_p$  with the  $v$  was  $i_p = 0.049 + 0.458v$  ( $r = 0.998$ ,  $n = 8$ ). Moreover, the peak potential  $E_p$  linearly shifted into more negative direction with increasing the  $v$  from 0.5 to 20 V/s. The relationship of the  $E_p$  with  $\log v$  was  $-E_p = 1.327 + 0.048 \log v$  ( $r = 0.986$ ,  $n = 8$ ). Therefore, the reduction wave of berberine was an irreversible one with adsorptive characteristic [18].

The electron transfer number  $n$  for the single reduction wave was determined by potential-controlled coulometry and by means of the relationship of the  $E_p$  with  $\log v$  that obtained using linear-scan voltammetry. The  $n$  was 2.1 on the basis of the data obtained by potential-controlled

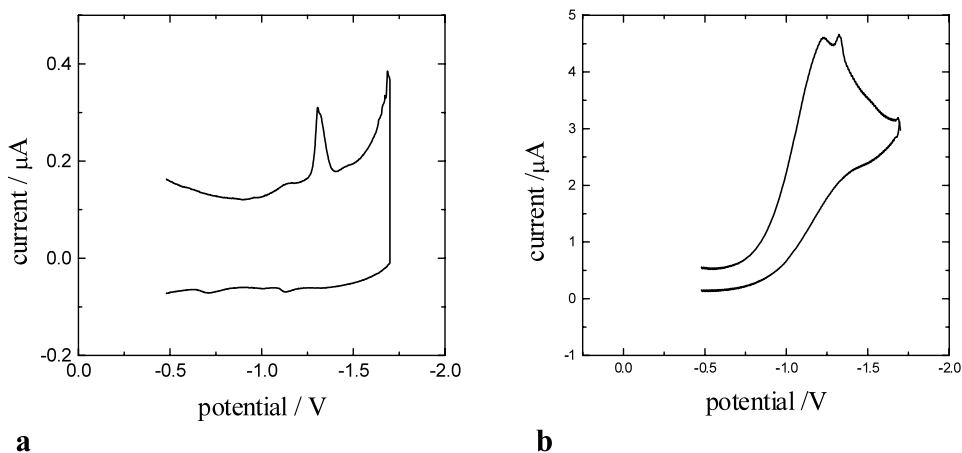


Fig. 1. Cyclic voltammograms of berberine (0.1  $\mu\text{M}$ ) in  $\text{Na}_4\text{B}_2\text{O}_7\text{--Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) buffer (a) in the absence; and (b) in the presence of  $\text{H}_2\text{O}_2$  (1 mM). Scan rate, 0.25 V/s.

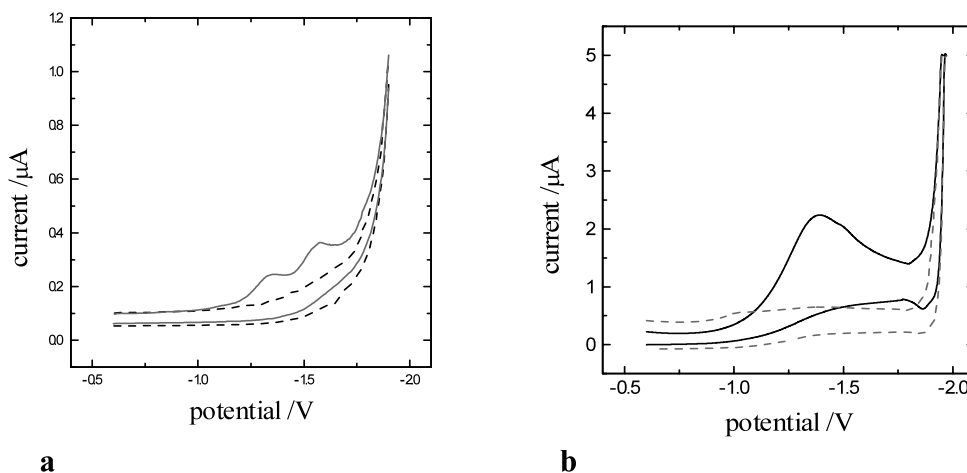


Fig. 2. Cyclic voltammograms of berberine (0.1  $\mu\text{M}$ ) in DMF–H<sub>2</sub>O(10:0.01, v/v)–(C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>NBr (0.1 M) mixture (a) in the absence; and (b) in the presence of H<sub>2</sub>O<sub>2</sub> (1 mM). Scan rate, 0.25 V/s, dash curve, base solution; solid curve, base solution + berberine.

Table 1

Effect of volume ratio of H<sub>2</sub>O to DMF (v/v) in to DMF–H<sub>2</sub>O mixture on both peak current and peak potential of berberine

H <sub>2</sub> O/DMF ratio (v/v)	0.01:10	0.04:10	0.08:10	0.12:10	0.16:10	0.20:10
$i_{p1}$ ( $\mu\text{A}$ )	0.113	0.135	0.177	0.192	0.203	0.221
$-E_{p1}$ (V)	1.35	1.35	1.35	1.35	1.34	1.34
$i_{p2}$ ( $\mu\text{A}$ )	0.110	0.086	0.045	0.031	0.022	0.000
$-E_{p2}$ (V)	1.57	1.54	1.51	1.47	1.42	1.34

coulometry. Furthermore, according to linear-scan voltammetric relationship of  $E_p$  with  $\log v$  for irreversible process with adsorption of reactant on electrode surface [19]:

$$E_p = E^0 + \frac{2.303 RT}{\alpha n F} \left( \log \frac{RT}{\alpha n F} k^0 - \log v \right)$$

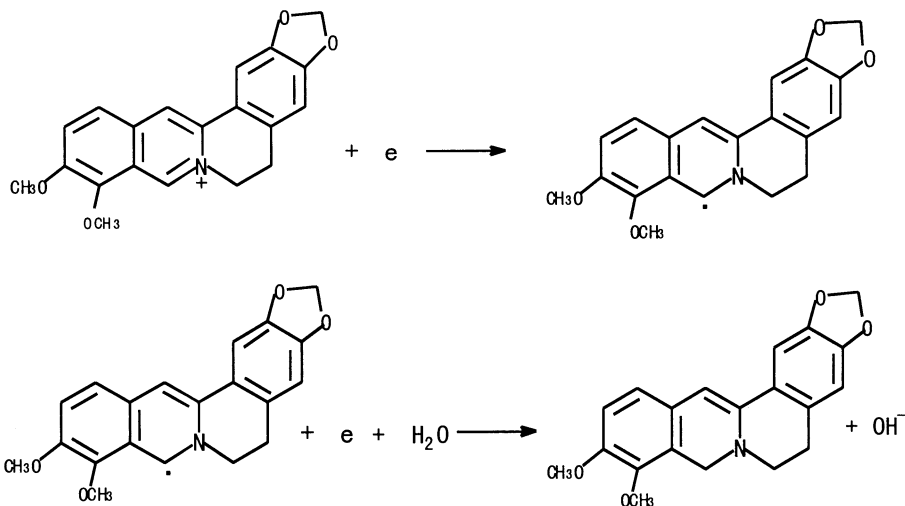
where the symbols have general meanings. The  $n$  obtained was 2.3 from the slope value 0.048 of the  $E_p \sim \log v$  relationship (assuming electron transfer coefficient  $\alpha = 0.5$ ). These results in this work were in agreement with that obtained in acidic aqueous solution [5,6,17], and demonstrated that the single reduction wave was a two-electron transfer of the C=N bond of berberine in aqueous solution.

Due to the fact that the proton condition of medium largely influenced the reduction process

of organic compound [20], organic aprotic medium had been used to obtain details about reduction process owing to prolonging the lifetime of the intermediate free radical [14,21]. For this reason, voltammetric behavior of berberine in DMF–H<sub>2</sub>O mixture was further examined. In the DMF–H<sub>2</sub>O (10:0.01, v/v)–(C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>NBr (0.1 M) mixture (H<sub>2</sub>O had to be added for the introduction of berberine standard solution or H<sub>2</sub>O<sub>2</sub> solution), cyclic voltammetry (Fig. 2a) displayed that berberine yielded two reduction waves,  $P_1$  and  $P_2$ , at cathodic branch, and no oxidation wave at anodic branch. The peak potential  $E_{p1}$  of the wave  $P_1$  was located at  $-1.35$  V and the  $E_{p2}$  of the wave  $P_2$  at  $-1.57$  V. Table 1 listed the data about the effect of the volume ratio of H<sub>2</sub>O added to DMF in the DMF–H<sub>2</sub>O mixture on these two reduction waves. With the ratio increasing, both

the  $E_{P1}$  and the  $E_{P2}$  shifted to positive direction. However, the positive shift of the  $E_{P2}$  was more than that of the  $E_{P1}$ . At this time, the increase of the peak current  $i_{p1}$  of the wave  $P_1$  was simultaneous with the decrease of the peak current  $i_{p2}$  of the wave  $P_2$ , while the sum  $i_{p1} + i_{p2}$  of peak currents of these two waves remained constant. When the volume ratio of  $H_2O$  to DMF achieved to 10:0.2 (v/v), the two waves emerged to a single one with the peak potential  $-1.34$  V, and the peak current of the single wave was approximately equal to the sum  $i_{p1} + i_{p2}$ .

Above experiments demonstrated that the two reduction waves in DMF– $H_2O$  mixture corresponded to the single reduction wave in aqueous solution. The reduction processes of the C=N bond of berberine was two successive one-electron reduction one in nature. The C=N bond was first reduced in one-electron addition to an intermediate free radical, producing wave  $P_1$ ; the free radical was further reduced in the same matter, producing the wave  $P_2$ . Therefore, the reduction process of the C=N bond of berberine was described as follows:



### 3.2. Polarographic catalytic wave of berberine

When a certain amount of  $H_2O_2$  solution was added into the  $Na_2B_4O_7$ – $Na_2CO_3$  (pH 9.4, 0.08 M) buffer, the peak potential of the reduction wave of berberine remained unchanged and the peak current increased sharply. Cyclic voltammet-

ric experiment in this media showed that a small reduction wave appeared on the reverse scan (Fig. 1b), besides the peak potential of the reduction wave remained unchanged and the peak current increased sharply. At this time, the reduction wave became a catalytic one. As the reduction wave of  $H_2O_2$  itself was located at  $-1.10$  V, that was closed to the catalytic wave of berberine, the first-order derivative approach was used to good resolve the latter from the former and to accurately measure the peak current of the catalytic wave of berberine. The first-order peak current  $i'_{p,1}$  of the catalytic wave increased with  $H_2O_2$  concentration increasing (Fig. 3) and the ratio  $i'_{p,1}/i'_p$  of the  $i'_{p,1}$  to the first-order peak current  $i'_p$  of the corresponding reduction wave was linearly proportional to the square root of  $H_2O_2$  concentration. The relation equation was  $i'_{p,1}/i'_p = -21.0 + 761.5 C_{H_2O_2}^{1/2}$  ( $r = 0.9995$ ,  $n = 5$ ). These characters mentioned above demonstrated that the catalytic wave of berberine in the presence of  $H_2O_2$  was a parallel one.

It is well-known that  $H_2O_2$  was a active oxidant, and it can cause the polarographic catalytic

waves of transfer metal ions, such as Fe(III) and Ti(IV), and of organic compounds, such as cinnamic acid [14] and glycyrrhetic acid [15], which resulted from that  $H_2O_2$  oxidizing the reduction intermediate product of transfer metal ion with low-value state, such as Fe(II) and Ti(III), and the free radicals of the C=C bond of both glycyrrhetic

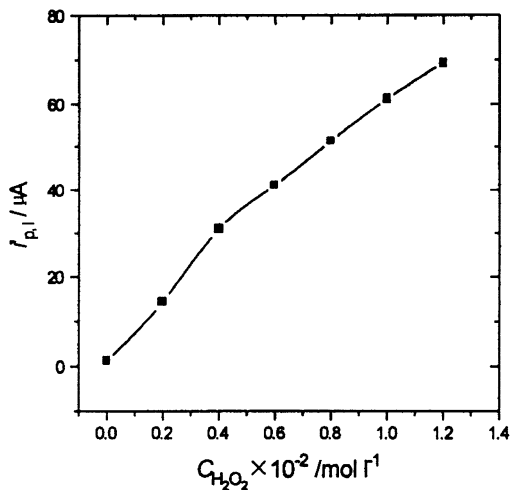
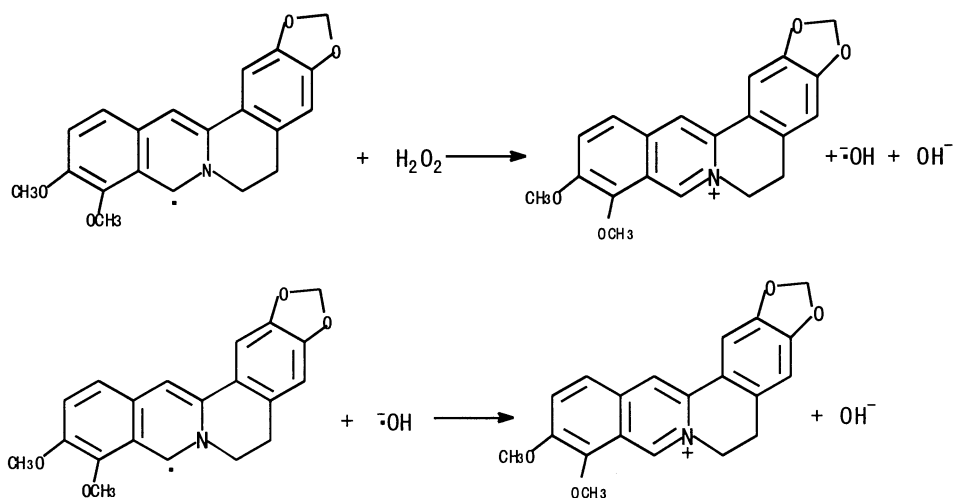


Fig. 3. Effect of  $H_2O_2$  concentration on the first-order derivative peak current  $i'_{p,1}$  of the catalytic wave of berberine, other conditions as in Fig. 1a.

acid and cinnamic acid. Therefore, it can be concluded that  $H_2O_2$  oxidized the free radical produced in the reduction process of the C=N bond of berberine to regenerate the original that can continue to proceed the reduction on the electrode surface. The cycle of the electroreduction and chemical regeneration of the C=N bond resulted into the production of the parallel catalytic wave. The conclusion was further proved by cyclic voltammetric behavior of berberine in DMF– $H_2O$  (10:0.1, v/v)– $(C_2H_5)_4NBr$  (0.1 M) mixture containing  $H_2O_2$  (1 mM). As showed in Fig. 2b, the wave  $P_1$  increased sharply and the wave  $P_2$  disappeared

on cathodic scan. And a small reduction wave on anodic scan was observed too. The reduction wave of  $H_2O_2$  itself was located at  $-1.8$  V, and not influenced the catalytic wave of berberine. In addition, the effect of pH on peak potential  $E_p$  of the catalytic wave in  $Na_2B_4O_7$ – $Na_2CO_3$  (0.08 M) buffer in the presence of  $H_2O_2$  (1 mM) was explored. The results indicated that the  $E_p$  was no longer dependent on the pH value of the medium in the range of 6.0–11.2, locating at  $-1.30$  V. This difference of the catalytic wave from the reduction wave of berberine [5,6] in the dependence of the  $E_p$  on the pH value indicated that no proton took part in the reduction process of berberine in the presence of  $H_2O_2$ . That is, the electrode reaction of the C=N bond of berberine in this case was only a one-electron reduction process of the C=N bond to its free radical. No reduction of the free radical was probable due to that the oxidation rate of the free radical by  $H_2O_2$  was larger than the reduction rate of the free radical.

On the other hand, the reduction process of oxidant  $H_2O_2$  was in two successive one-electron uptakes through a free radical  $\cdot OH$  as intermediate product of  $H_2O_2$  reduction either chemically or electrochemically, and the free radical  $\cdot OH$  is more active than  $H_2O_2$  in reaction ability. Undoubtedly, the  $\cdot OH$  also took part in the oxidation reaction of the free radical of berberine. Thus, the oxidation reaction of the free radical of berberine by both  $H_2O_2$  and  $\cdot OH$  was described as follows:



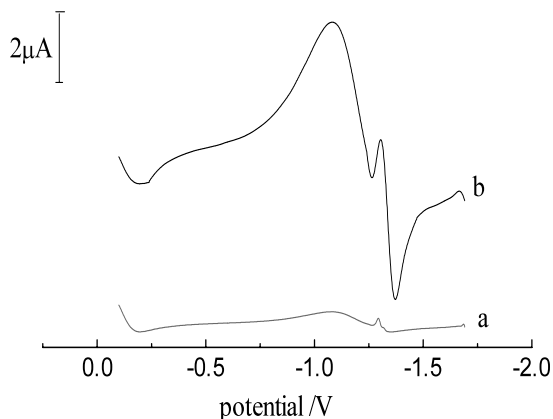
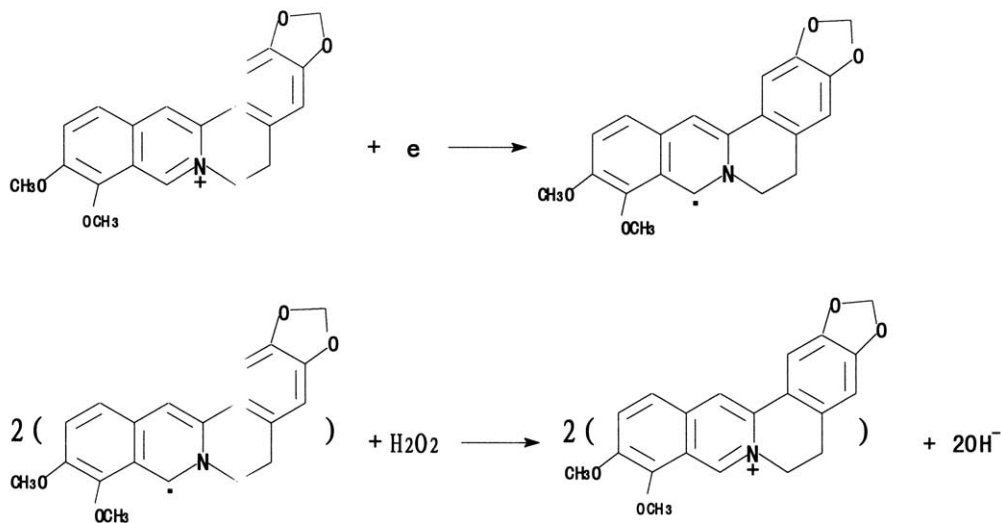


Fig. 4. First-order derivative linear-potential scan polarograms of berberine ( $0.1 \mu\text{M}$ ) in  $\text{Na}_2\text{B}_4\text{O}_7\text{-Na}_2\text{CO}_3$  (pH 9.4, 0.08 M) buffer in the absence (curve a) and the presence (curve b) of  $\text{H}_2\text{O}_2$  (4 mM).

In summary, the production of the polarographic catalytic wave was ascribed to that the one-electron reduction of the C=N bond of berberine was simultaneous with chemical regeneration through the oxidation of the free radical of the C=N bond by both  $\text{H}_2\text{O}_2$  and  $\cdot\text{OH}$ . The mechanism of the catalytic wave was represented as follows:



#### 4. Analytical application

The medium condition of the catalytic wave for analytical purpose was optimized [16]. The

$\text{Na}_2\text{B}_4\text{O}_7\text{-Na}_2\text{CO}_3$  (pH 9.4, 0.08 M)– $\text{H}_2\text{O}_2$  (4 mM) buffer was used as the optimal supporting electrolyte. A conventional linear-potential scan polarograph was employed. As shown in Fig. 4, the first-order derivative peak of the catalytic wave of berberine was perfectly resolved from neighboring  $\text{H}_2\text{O}_2$  reduction wave. The first-order derivative peak current  $i'_{p,1}$  of the catalytic wave was rectilinear to berberine concentration in the range of  $1.0 \times 10^{-8}$ – $3.0 \times 10^{-7}$  M. The linear-regression equation was  $i'_{p,1}/\mu\text{A} = -0.3 + 4.0 \times 10^7 C_{\text{berberine}}$  per M.

The correlation coefficient  $r$  was 0.9996 ( $n = 7$ ). The R.S.D. values for the slope and the intercept were 2.1 and 0.29%, respectively. The limit of detection was  $7.0 \times 10^{-9}$  M.

The catalytic wave was very stable. Both the peak current and the peak potential remained unchanged within at least 8 h. The relative standard deviation was found to be less than 1.8% in 10 replicate determinations of berberine ( $0.1 \mu\text{M}$ ).

With using the catalytic wave was used to determine berberine in natural medical plants, the interference mainly comes from these coexistent alkaloids similar to berberine in chemical structure, polmatine and coptisine. It was found that,

however, the content of berberine in *C. chinensis* Franch were generally between 5 and 8%, even 10% in weight, and far large than that of both polmatine and coptisine. On the other hand, as

Table 2  
Berberine content in *C. chinensis* Franch

Diluted sample, solution taken (ml)	Berberine content, by proposed method (%)			Average (%)	R.S.D. (%)	Berberine content, by extraction spectrophotometry (%)
0.25	6.79	6.90	6.60	6.65	0.15	6.55
0.40	6.62	6.87	6.50			
0.50	6.53	6.71	6.30			

Table 3  
Recovery results in sample

Added $\times 10^8$ (M)	Found $\times 10^8$ (M)		Recovery (%)	Average recovery (%)
1.20	1.21	1.21	100.8	99.3
2.00	1.98	1.99	99.0	
3.20	3.14	3.12	98.2	

the catalytic wave has a very high sensitivity with the limit of detection  $7.0 \times 10^{-9}$  M, it allows to take only several micrograms of the sample for the determination of berberine. Moreover, water extraction of berberine eliminated the extraction of both polmatine and coptisine. Hence, berberine content in *C. chinensis* Franch was determined after the water extraction was sufficiently diluted without any pre-separation. Under these conditions of this work, only the catalytic wave of berberine and no interference of these coexistent alkaloids in *C. chinensis* Franch were observed in practical analysis of sample. The berberine contents in *C. chinensis* Franch were determined by both the catalytic wave method and the extraction spectrophotometric method by Arai [4]. The results obtained by two methods were listed in Table 2, and were in good agreement. The recovery test was done in a sample solution to which added a certain amounts (0.01  $\mu$ M) of berberine standard solution to validate the reliability of the catalytic wave method. The results were showed in Table 3. The recoveries were in the range of 97.5–100.8%, with average recovery of 99.3%.

## 5. Conclusion

In this work, the existence of an intermediate free radical of the C=N bond of berberine in its

two successive one-electron reduction process was proved. And the production of the parallel catalytic wave of berberine in the presence of  $H_2O_2$  was ascribed to a redox cycle that consisted of both electroreduction of the C=N bond and chemical oxidation of its free radical by both  $H_2O_2$  and  $\bullet OH$ .

As compared with those known polarographic methods, the parallel catalytic wave method was more convenient and sensitive. It permits direct determination of berberine in medicinal plants after sufficiently diluting the water extraction. If it were integrated with adsorption stripping technique, the analytical sensitivity for berberine would be further improved.

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