Amino Acids (2009) 36:391–397
DOI 10.1007/s00726-008-0064-3

ORIGINAL ARTICLE

Kinetic determination of tryptophan by using the B-Z oscillating chemical system

Jinzhang Gao · Jie Qu · Wu Yang · Xiaoxia Wei · Hongxia Dai · Dongyu Lv · Jie Ren · Hua Chen

Received: 14 January 2008 / Accepted: 14 March 2008 / Published online: 20 May 2008

Abstract A simple and rapid method was devised for determination of tryptophan, based on the Belousov-Zhabotinskii (B-Z) oscillating chemical system. Changes in oscillating period and amplitude were linearly proportional to the negative logarithm of L-tryptophan concentration over the range of 6.44×10^{-7}–2.55×10^{-4} M, with the regression coefficients of near unity and a lower detection limit of 6.5×10^{-8} M. D-tryptophan was also examined although it is rarely found in most biological fluids, and perhaps not at all in natural proteins. The change of period against to negative logarithm of D-tryptophan concentration over the range of 4.9×10^{-5}–8.24×10^{-4} M is linear. Because the optimum conditions for determination of L- and D-tryptophan are not the same, a little amount of D-tryptophan does not affect the determination of L-tryptophan. Various influences were studied and a possible mechanism of perturbation to the B-Z oscillator by tryptophan was also discussed. Spectrophotometry and fluorescence spectrophotofluorimetry were used for comparision and confirmation of the results.

Keywords L-tryptophan · D-tryptophan · B-Z oscillating chemical reaction · Kinetic determination

J. Gao (✉) · J. Qu · W. Yang · X. Wei · H. Dai · D. Lv · J. Ren · H. Chen
College of Chemistry and Chemical Engineering, Northwest Normal University, 730070 Lanzhou, People's Republic of China
e-mail: jzgao@nwnu.edu.cn

Introduction

In the kinetic-catalytic analysis, the B-Z oscillating chemical reaction is one of the most popular approaches, and has been applied in the determining of organic and inorganic substances. The early FKN mechanism (Field and Schneider 1989) and the theoretical analysis by Taylor (2002) were highly important in the development of this field. The analytical application has also been summarized in two reviews (Jimenez-Prieto et al. 1998; Gao 2005). Compared to instrumental analysis, the oscillating chemical reaction as an analytical tool has many advantages such as a simple set-up, ease of operation, a largely linear range from approximately 10^{-7}–10^{-3} M, and, especially, a low detection limit (in the range of 10^{-6}–10^{-8} M). Due to these characteristics, many analysts are focusing on this approach (Strizhak et al. 2001; Gao et al. 2002, 2006a, b, c; Strizhak and Khavrus 2000; Raoof et al. 2005; Toledo et al. 2000).

Tryptophan is one of the protein amino acids that cannot be synthesized by humans and thus must be obtained from food or supplements. Tryptophan is essential for the production of several crucial substances in the body, including the neurotransmitter serotonin (5-hydroxytryptamine). Because serotonin plays a key role in mood and sleep patterns, tryptophan supplements have been used as antidepressants, sleep aids, and weight-loss aids.

In general, the determination of tryptophan is carried out by methods such as HPLC (Yust et al. 2004), fluorophotometry (Wu et al. 2005), and capillary electrophoresis (Yang et al. 2002), with detectable range from 10^{-4} to 10^{-7} M. In the present study, a new approach using the perturbation by tryptophan to the Belousov-Zhabotinskii (B-Z) oscillating profile has been examined in detail. Changes in oscillating period and amplitude were found to

be linearly proportional to the negative logarithm of tryptophan concentration, with a high sensitivity of detection.

Materials and methods

Instruments

The experimental assembly consisted of an oscillation reactor (ca. 50 mL) and a system for measuring potential. The reactor was coupled with a Model 501 thermostat and a Model ML-902 magnetic stirrer (Shanghai Pujing Analytical Instrument Factory, China) to keep the system at 308 ± 0.1 K. All electrodes were also from the above-mentioned factory. A CHI-832 (CHI, USA) analyzer was connected to the reactor through two Pt electrodes (Rex 213; one serving as the working electrode and another as the counter electrode), and to a K_2SO_4 reference calomel electrode (Rex 217 China) to record the potential changes. A Model 302 bromide selective electrode (Rex 302 China) was used to measure the change of bromide ion concentration.

Reagents

All chemicals used to establish a B-Z oscillating system ($KBrO_3$, malonic acid, H_2SO_4, $Ce(SO_4)_2$) were of analytical-reagent grade, and were used without further purification. Double-distilled-deionized water was used throughout. Solutions of $KBrO_3$, $Ce(SO_4)_2$ and $CH_2(COOH)_2$ were prepared in 0.7 M or 0.8 M sulfuric acid.

Stock solutions of 0.01 M L-tryptophan and D-tryptophan were prepared in double-distilled-deionized water. These solutions were stored in black polyethylene bottles and standardized before the use. Working dilutions of appropriate lower concentration were prepared with distilled water immediately before use.

Procedure

A mixture containing $KBrO_3$ (0.2 M), malonic acid (0.5 M) and $Ce(SO_4)_2$ (0.04 M) was placed in the reactor. The mixture was continuously magnetically stirred, and the system was kept at 308 ± 0.1 K. Then H_2SO_4 was added to a final volume of 20 mL. Meanwhile, the indicator, counter and reference electrodes were immersed into the reaction media and the data acquisition was started. When the amplitude and the period of oscillation stabilized, the workstock of tryptophan was added to the platinum electrode until the potential decreased to a minimum. The lowest position of the regular oscillating profile is the optimal injection point where the system will respond with the largest change in both period and amplitude.

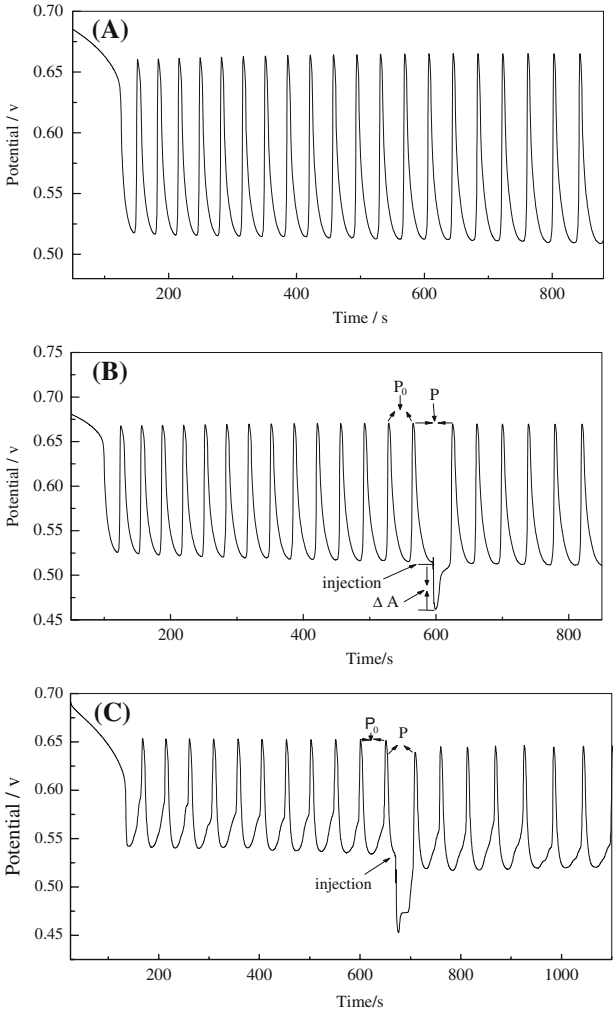


Fig. 1 **a** Without tryptophan, **b** at 8.35×10^{-6} M L-tryptophan. Common conditions: $KBrO_3$, 0.055 M, $CH_2(COOH)_2$, 0.168 M, Ce(IV), 0.0018 M, H_2SO_4, 0.7 M. **c** Profile of potential oscillation at 6.67×10^{-5} M D-tryptophan. Common conditions: $KBrO_3$ 0.065 M, $CH_2(COOH)_2$ 0.163 M, Ce(IV) 0.0020 M, H_2SO_4 0.8 M. One should note that the concentration of D-tryptophan in the above experiments was eight times higher than that of the L-isomer

Results and discussion

When tryptophan was injected into the reaction system, both period and amplitude of the oscillating system increased immediately. Figure 1 shows the typical potential oscillation profiles both with and without perturbation by tryptophan. The changes of period (ΔP) and amplitude (ΔA) were chosen as the analytical signals. These parameters can be described as follows:

$$\Delta P = P - P_0 \quad (1)$$

$$\Delta A = A - A_0 \quad (2)$$

In the above equations, P_o and P are the periods of oscillating profiles before and after adding tryptophan, respectively, while A_o and A represent the amplitudes of

the oscillating profiles before and after adding the tryptophan, respectively. It was found that the changes both in the period (ΔP) and the amplitude (ΔA) are directly proportional to the concentration of tryptophan needed to achieve the optimum response of the oscillator. Figure 1b, c represent the best perturbation profiles by L-tryptophan and D-tryptophan, respectively. One should note that the parameters of optimum responses of the oscillator to the tryptophan enantiomers are quite different.

Influences of experimental variables on tryptophan determination

In order to get the maximum sensitivity and accuracy possible in the determination of tryptophan, experimental conditions for constructing a suitable oscillating system must be established first. For L-tryptophan, the optimum conditions were defined as follows.

The B-Z oscillating system requires an acidic medium (Gao 2005). However, either a too high or a too low [H^+] can destabilize the oscillating system, making the oscillating profiles irregular. Regular oscillating profiles were observed in the sulfuric acid concentration range of 0.65–0.8 M (see Fig. 2a). Sulfuric acid at 0.7 M produced maximal oscillation period and amplitude, and this concentration was adopted for further study.

Effect of malonic acid concentration was studied over the range from 0.125 to 0.175 M. In this range, two maxima were obtained at 0.150 and 0.168 M (see Fig. 2b). The second peak, being larger and more symmetric, was used in this work.

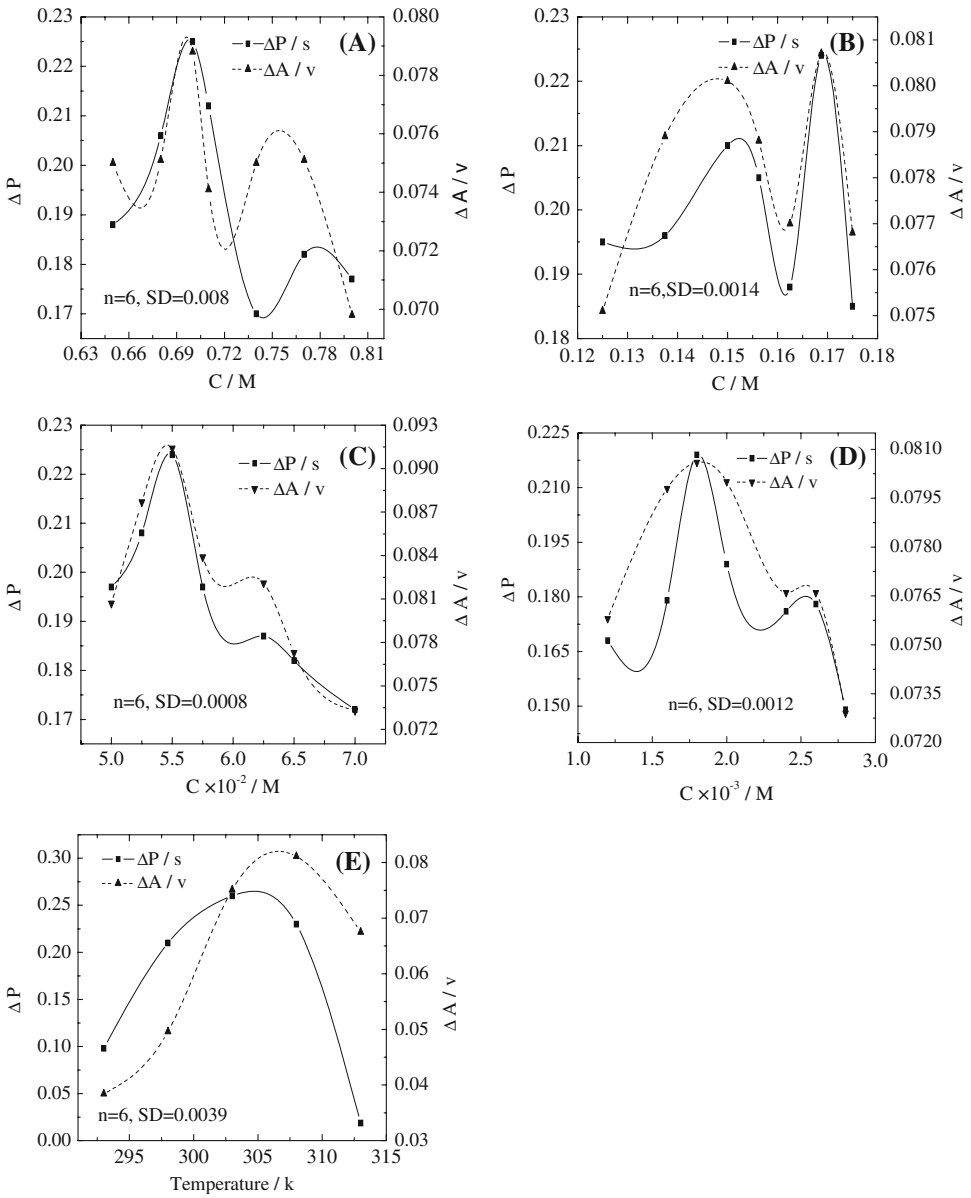


Fig. 2 **a** H_2SO_4, **b** $CH_2(COOH)_2$, **c** $KBrO_3$, **d** Ce(IV), **e** temperature. Common condition: [L-tryptophan] = 6.5×10^{-6} M, SD and *n* denote the standard error and the number of parallel experiments, respectively

Figure 2c shows the effect of $KBrO_3$ concentration in the range of 0.05–0.07 M. Below 0.05 M $KBrO_3$, the oscillation profiles were unstable. Above 0.065 M $KBrO_3$, both the oscillation period and amplitude decreased. In terms of stability and sensitivity, the optimal [$KBrO_3$] was close to 0.055 M.

For Ce(IV) ion that was used as a catalyst, the effect of the concentration change was examined over the range from 0.0012 to 0.0028 M. As seen in Fig. 2d, [Ce(IV)] of 0.0018 M was optimal for determination of L-tryptophan.

We also studied the effect of temperature in the range of 293 K to 313 K. In the presence of L-tryptophan, the maximal oscillation period appeared at 305 K, whereas the maximal amplitude was at 308 K (see Fig. 2e). Based on this, further work with L-tryptophan was carried out at 308 K.

As indicated by the above experiments, this method could be used for determination of L-trytophan even in the presence of a trace amount of D-trytophan. When using this oscillating system to detect D-trytophan, the boundary conditions of oscillating system should be chosen again. Table 1 provides the optimal concentrations of B-Z oscillator reactants for both enantiomers of tryptophan.

Determination of tryptophan

Under the optimal condition described above, the regular periods were found between the 10th and 20th period in the oscillating profile, indicating that the determination should be performed in this region. For this work, the 15th period was chosen as the time of sample addition. A plot of ΔP and ΔA against $-\lg C$ (where C is the concentration of L-tryptophan) over the range of 2.55×10^{-6}–2.55×10^{-4} M and 6.44×10^{-7}–$.55 \times 10^{-4}$ M was shown in Fig. 3a, b. When using this method to detect D-trytophan, the change in oscillating period (ΔP) should be chosen as a parameter due to its stability better than amplitude change (ΔA). A plot of ΔP against $-\lg C$ (where C is the concentration of D-tryptophan) over the range of 4.97×10^{-5}–8.24×10^{-4} M was shown in Fig. 3c.These linear relationships can be described by the following equations:

(A) $\Delta P(\mathrm{s}) = 0.973 - 0.170(-\lg C)$, $(N = 10,\ R = 0.9987)$

(B) $\Delta A(\mathrm{mV}) = 0.295 - 0.046(-\lg C)$, $(N = 12,\ R = 0.9980)$

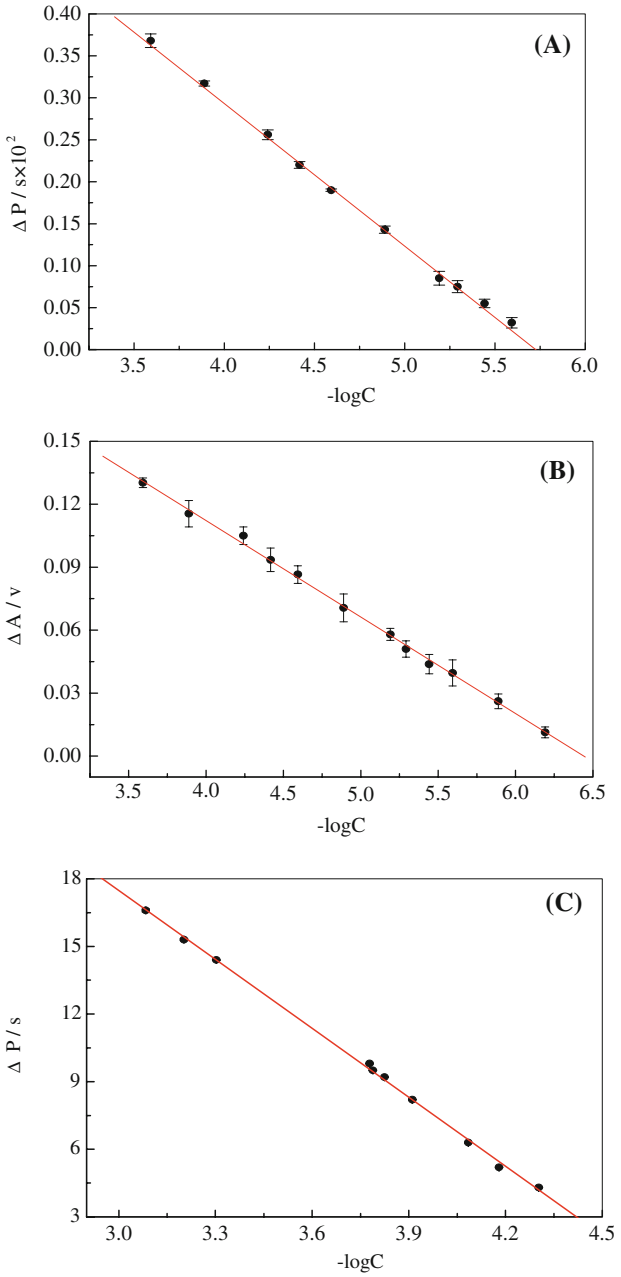


Fig. 3 Calibration curve of **a**: ΔP against −logC(C = [L-tryptophan]) in the range of 2.55×10^{-6}–2.55×10^{-4}M, **b**: ΔA against −logC(C = [L-tryptophan]) in the range of 6.44×10^{-7}–2.55×10^{-4}M, **c**: ΔP against −logC(C = [D-tryptophan]) in the range of 4.97×10^{-5}–8.24×10^{-4} M

(C) $\Delta P(\mathrm{s}) = 48.057 - 10.192(-\lg C)$, $(N = 10,\ R = 0.9994)$.

Table 1 Optimum conditions for determination of L-trytophan and D-trytophan

All component concentrations are in mole units

	H_2SO_4	$CH_2(COOH)_2$	$KBrO_3$	$Ce(SO_4)_2$	Temperature (K)
D-tryptophan	0.8	0.163	0.065	0.002	308.0
L-tryptophan	0.7	0.168	0.055	0.0018	308.0

Table 2 A comparison with other analytical methods used in the determination of L-tryptophan

Method	Linear range	Limit of detection	Reference
Chemiluminescent determination (catalyzer Ce^{4+})	7.8×10^{-6}–5.2×10^{-4} M	0.489 μM	Alwarthan (1995)
Chemiluminescent determination (catalyzer Cu^{2+})	5×10^{-6}–6×10^{-6} M	4.5 μM	Hanaoka et al. (2000)
High-performance liquid chromatographic	1.96×10^{-9}–7.84×10^{-7} M	10 pM	Yust et al. (2004)
Fluorescence	0–3.43×10^{-4} M	14.7 nM	Wu et al. (2005)
Capillary electrophoresis	2.5×10^{-6}–2.5×10^{-4} M	~1 μM	Yang et al. (2002)
Oscillating chemical reaction	6.44×10^{-7}–2.55×10^{-4} M	65 nM	Present work

Table 3 Effect of foreign species on the determination of 6.5×10^{-6} M L-tryptophan

Foreign species	Tolerance ratio (foreign/L-tryptophan)
Zn^{2+}, Mn^{2+}, Fe^{3+}, La^{3+}	2,000
NO_3^-	500
Methanol, ethanol, formic acid, acetic acid	100
Cl^-, I^-	100
Cu^{2+}, Hg^{2+}, Ag^+, Pb^{2+}	50
L-aspartic acid, DL-valine, L-glutamic acid	20
L-tyrosine, L-phenylalanine, L-lysine,	20
D-tryptophan	10

Comparison with other methods

To obtain a comparison of sensitivity, L-tryptophan was also measured using other techniques. As seen in Table 2, the proposed method is fairly sensitive, and should be useful for the routine analysis of L-tryptophan.

Interferences

It is known that oscillating chemical reactions could be highly sensitive to external ions and molecules (Gao et al. 2006). We therefore investigated effects of some common inorganic ions and organic compounds on the determination of L-tryptophan. Tolerance levels (defined as the maximum amount of foreign species causing an error lower than ± 5% (RSD) in the determination of 6.5×10^{-6} M L-tryptophan), are shown in Table 3. Generally, inorganic ions and small organic compounds had little influence on the determination. However, amino acids resembling L-tryptophan did perturb this oscillating system, producing a different potential. Amounts of D-tryptophan below 5% of L-tryptophan had no discernible effect on the measurement of the latter.

Possible mechanism for tryptophan activity in the oscillating system

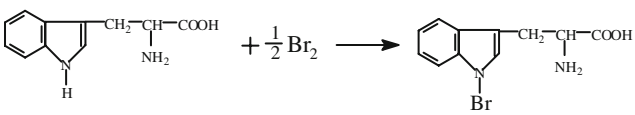
An oscillating chemical reaction consists of many kinetic steps involving several independent variables, in which the changes of some parameters could perturb the regular oscillating profile. Based on the FKN mechanism (Field and Schneider 1989; Taylor 2002), a typical B-Z oscillating system can be simplified as representing the three processes given below:

$$\text{Process A}: BrO_3^- + 2Br^- + 3CH_2(COOH)_2 + 3H^+ \rightleftharpoons 3BrCH(COOH)_2 + 3H_2O$$

$$\text{Process B}: BrO_3^- + 4Ce^{3+} + 5H^+ \rightleftharpoons HOBr + 4Ce^{4+} + 2H_2O$$

$$\text{Process C}: HOBr + 4Ce^{4+} + BrCH(COOH)_2 + H_2O \rightleftharpoons 2Br^- + 4Ce^{3+} + 3CO_2 \uparrow + 6H^+$$

All these processes produce circulation oscillations by means of positive and negative feedbacks during the chemical reactions. Due to the presence of the reaction between L-tryptophan and Br_2, the addition of L-tryptophan could perturb strongly Process C, and further perturb the entire oscillating profile. As has been already pointed out in FKN mechanism (Field and Schneider 1989), there is formation of Br_2 as higher concentrations of the Br^- ion, i.e., $5Br^- + BrO_3^- + 6H^+ \rightleftharpoons 3Br_2 + 3H_2O$. A substitution of L-tryptophan by bromine would occur as follows (Xu 1982):



The periodical changes of bromide concentration during the reaction process were recorded by a bromide-selective electrode. As seen in Fig. 4b, there is an apparent single oscillating cycle of [Br^-]. With prolonging the reaction, the highest value of [Br^-] (point A) decreases slightly down to point B and then decreases sharply to point C. After that, there is a slow increase to point D, and finally an abrupt increase back to the level of point A, starting another cycle. This implied that there would be critical values related to [Br^-] at the points of A, B, C and D. In order to understand that more clearly, the above positions were taken as the injection points. The maximum perturbation appeared at

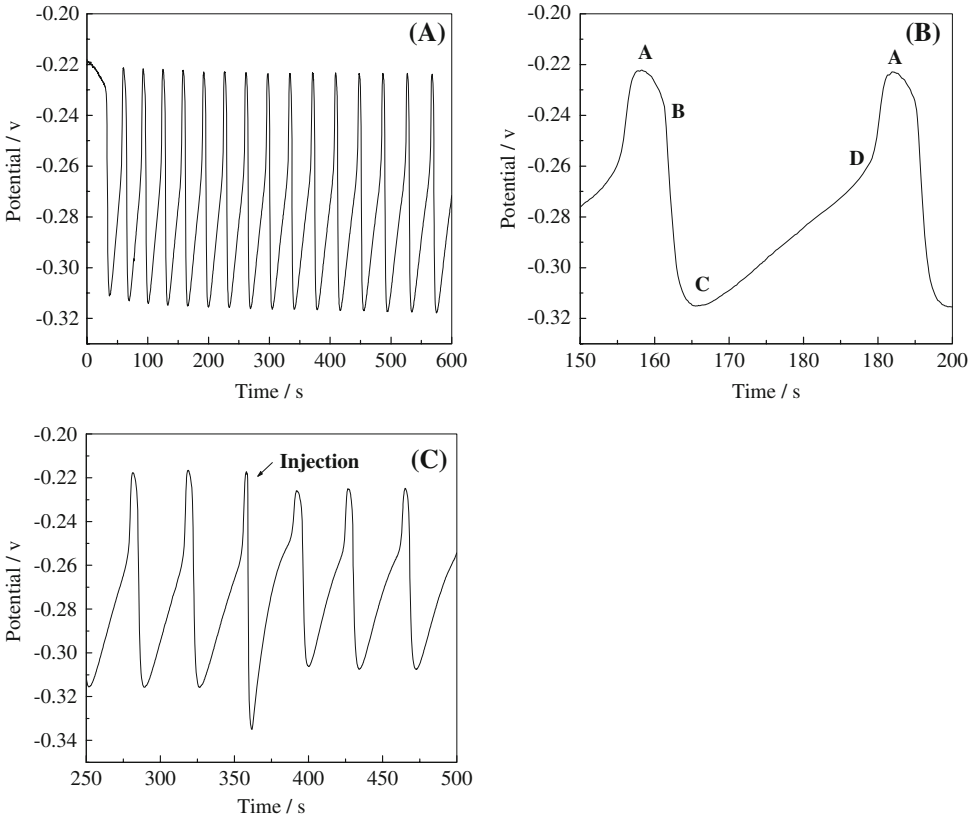


Fig. 4 **a** In the absence of L-tryptophan, **b** single oscillating cycle of [Br^-], **c** in the presence of 3.55×10^{-5} M L-tryptophan

the process from point A to B (see Fig. 4c), that is, [Br^-] decreased gradually due to formation of Br_2.

As we know, an oscillating chemical reaction is a system that is far from equilibrium, and includes multiple intermediary stages. Chelation of ceric ion by tryptophan (Masahiro et al. 2001) could be strongly influenced by the chiral form of tryptophan. Indeed, we observed a much higher reactivity for L-tryptophan (Table 1 and Fig. 1). The proposed method can thus be used for determination of L-tryptophan in the presence of trace amounts of D-tryptophan.

Sample analysis

For a quantitative comparison, we have assayed L-tryptophan (1.70×10^{-5}, 5.35×10^{-6}, and 1.70×10^{-6} M) using three different methods: fluorophotometry (Wu et al. 2005), spectrophotometry (Guo 1987), and the oscillator method. The results are listed in Table 4. As seen, all methods differed less than 3% of the common mean at any concentration tested.

Conclusion

A sensitive, simple and rapid method for tryptophan determination is needed for the routine analysis in many fields. Relative to the instrumental analysis, the equipment used in the proposed method is less expensive. Moreover, largely linear range (ca. 10^{-7}–10^{-4} M) and a low limit of detection (ca. 10^{-8} M) could meet the need of common determination. As an analytical approach, the oscillating chemical reaction is likely to be much investigated in the future.

Table 4 Determination of L-tryptophan using three different methods

Sample (M) method	1.70×10^{-5}	5.35×10^{-6}	1.70×10^{-6}
Fluorophotometry	1.75×10^{-5}	5.30×10^{-6}	1.69×10^{-6}
Spectrophotometry	1.68×10^{-5}	5.39×10^{-6}	1.67×10^{-6}
Present method	1.67×10^{-5}	5.33×10^{-6}	1.65×10^{-6}

Acknowledgments This work was supported in part by the Project of International Cooperation between China and Ukraine (043−05), the National Natural Science Foundation (20475044) and the project of KJCXGC-01, NWNU, China.

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