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Original scientific paper

Determination of epinephrine by a Briggs–Rauscher oscillating system using a non-equilibrium stationary state

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Abstract: A highly sensitive method for the determination of epinephrine is proposed, which is based on the perturbation of epinephrine to a Briggs–Rauscher oscillating system involving malonic acid, Mn^{2+}, H^+, IO_3^- and H_2O_2 at a non-equilibrium stationary state. The concentration of KIO_3 was chosen as a control parameter to find the bifurcation point in this study. The results showed that a good linear relationship between the difference in the potential and the negative logarithm of the concentrations of epinephrine existed in the range 1.1×10^{-7}–5.2×10^{-9} mol L^{-1} with a lower detection limit of 6.8×10^{-10} mol L^{-1} and a correlation coefficient of 0.9974. Compared to the classical oscillating reaction, this method has a lower detection limit and a wider linear range. The effects of some foreign species, which may possibly exist with epinephrine, on determination were also investigated. The proposed method was successfully used to determine epinephrine both in serum and adrenaline hydrochloride injection.

Keywords: Briggs–Rauscher oscillating system; epinephrine; determination; non-equilibrium stationary state.

INTRODUCTION

The application of classical oscillating chemical reactions in analytical chemistry has made significant progress since a continuously stirred tank reactor (CSTR)[1] was combined with the analyte pulse perturbation technique (APP).[2] The simple equipment used, the large linear range (*ca.* 10^{-7}–10^{-4} mol L^{-1}) and low detection limit (*ca.* 10^{-6}–10^{-8} mol L^{-1}) are its unique advantages, in general, which could satisfy the need of common determinations in many fields. In recent years, for improving further the sensitivity, the Perez-Bendito group[3,4] and the Strizhak group[5,6] investigated theoretically the largest Lyapunov exponent in the transient chaotic regime with the BZ (Belousov–Zhabotinsky) oscillating system and developed a new analytical method with a very high sensitivity (detection li-

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mit ≤ 10^{-12} mol L^{-1}). Gao *et al.*[7,8] reported that a sulfide-modified BZ oscillating chemical system is very sensitive to trace amounts of some metal ions. Vukojevic and Pejic *et al.*[9–11] studied the characteristics of a non-equilibrium stationary state close to the bifurcation point between the non-oscillatory and oscillatory state, and proposed successfully a novel kinetic method for the determination of organic compounds and inorganic ions. The above methods are basically a BZ oscillating chemical system, *i.e.*, a cerium ion catalyzed oxidation reaction of malonic acid by BrO_3^- in sulfuric acid. In addition to the BZ oscillating reaction, the BR (Briggs–Rauscher) oscillating reaction is also very interesting in analytical chemistry.

The classical BR reaction[12] is the Mn^{2+} catalyzed oxidation of malonic acid by iodate and hydrogen peroxide in sulfuric or perchloric acid medium, which was reported by Briggs and Rauscher in 1973. Although the sustained oscillating time of the BR is shorter than that of the BZ oscillating reaction, it has been successfully used to analyze some antioxidant-type substances with one or more phenolic hydroxyl groups, such as polyphenolic compounds,[13] virgin olive oil[14] and red wine.[15] It is meaningful to determine food and drugs using the BR oscillating system because the pH employed is similar to the acidity of the fluids in the stomach[13] and the stomach is part of the digestive system. In the process of disease treatments, drugs are involved in some non-linear oscillation such as the human blood circulation and metabolism; hence exploring therapeutic mechanisms through studying oscillating reactions could more truly reflect the nature of the drugs.

As a natural catecholamine in the human body, epinephrine (the chemical structure of which is shown in Fig. 1) is an important compound for message transfer in the mammalian central nervous system, and it can also excite the heart, contract blood vessels and relax bronchial smooth muscle contraction. Many life phenomena are related to its concentration, thus, it is meaningful to develop an efficient determination method to study its physiological function and diagnosis in some diseases in clinical medicine. In this paper, a new method for the determination of epinephrine is proposed and compared with other methods, such as fluorimetry,[16] chemiluminescence,[17] voltammetry,[18] and molecular imprinting.[19] The results indicated that the sensitivity of the proposed method is better than those of the others.

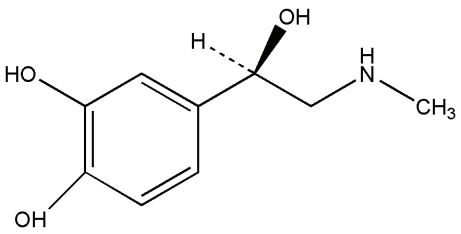

Fig. 1. Chemical structure of epinephrine.

EXPERIMENTAL

Apparatus

A SY-601 thermostat (Tianjin Ounuo Instrument Ltd. Co., China) with an accuracy±0.1 K and a Model ML-902 magnetic stirrer (Shanghai Pujiang Analytical Instrumental Factory, China) were used to maintain the temperature constant. A CHI-832 electrochemistry analyzer (Shanghai Chenhua Instrument Co., China) was directly connected to the reactor through two Pt-electrodes (Rex, 213, China), whereby, one served as the working electrode and the other as auxiliary electrode, and a Hg_2SO_4 reference electrode to record the potential changes. An injector was used to add the sample solutions.

Reagents

All the employed chemicals were of analytical-reagent grade and doubly distilled water was used throughout to prepare the working solutions, *i.e.*, malonic acid (0.25 mol L^{-1}), $MnSO_4$ (0.025 mol L^{-1}), H_2SO_4 (0.16 mol L^{-1}), H_2O_2 (4.8 mol L^{-1}) and KIO_3 (0.25 mol L^{-1}). The H_2O_2 solution was standardized by $KMnO_4$ solution before the use and preserved in a brown reagent-bottle. A stock solution of epinephrine was prepared with distilled water and stored in a refrigerator. Working solutions with lower concentrations were prepared by dilution immediately prior to use.

Procedure

The experiments were performed in a glass reactor (*ca*. 50 mL) coupled with a SY-601 thermostat and a Model ML-902 magnetic stirrer. A mixture containing malonic acid, $MnSO_4$, H_2SO_4, H_2O_2 and KIO_3 was placed in the reactor at 295±0.1 K. Then doubly distilled water was added to a final volume of 20 mL. Finally, the three electrodes were immersed into the reaction media under stirring, and the time–potential curve was recorded immediately.

RESULTS AND DISCUSSION

Finding bifurcation point

Generally, the initial concentration of reactants and the specific flow rate, as well as temperature, can be chosen as the control parameter to study a non-equilibrium stationary state. In this study, the initial concentration of KIO_3, which was varied from 5.75×10^{-2} to 4.0×10^{-3}mol L^{-1}, was chosen as the control parameter to define the bifurcation profile. With decreasing initial concentration of KIO_3, the amplitude of the system gradually decreased and eventually disappeared at a KIO_3 concentration 5.0×10^{-3} mol L^{-1} (Fig. 2A), *i.e.*, the system transformed from a steady oscillation state to a non-equilibrium stationary state. Then, the bifurcation profile (Fig. 2B) using the initial concentration of KIO_3 as the control parameter was made following Fig. 2A.

The theoretical bifurcation point of the system was also found at a concentration of 4.1×10^{-3} mol L^{-1} by linear extrapolation,[9] *i.e.*, a plot of the square of the amplitude of the oscillations *versus* the initial concentration of KIO_3 (Fig. 3).

In order to investigate the determination sensitivity in different non-equilibrium stationary states, the same amount of epinephrine was added into this system at different concentrations of KIO_3. Fig. 4 indicates that the closer to the

concentration of bifurcation point, the higher sensitivity is. Hence, a KIO_3 concentration of 5.0×10^{-3} mol L^{-1} was used in this study.

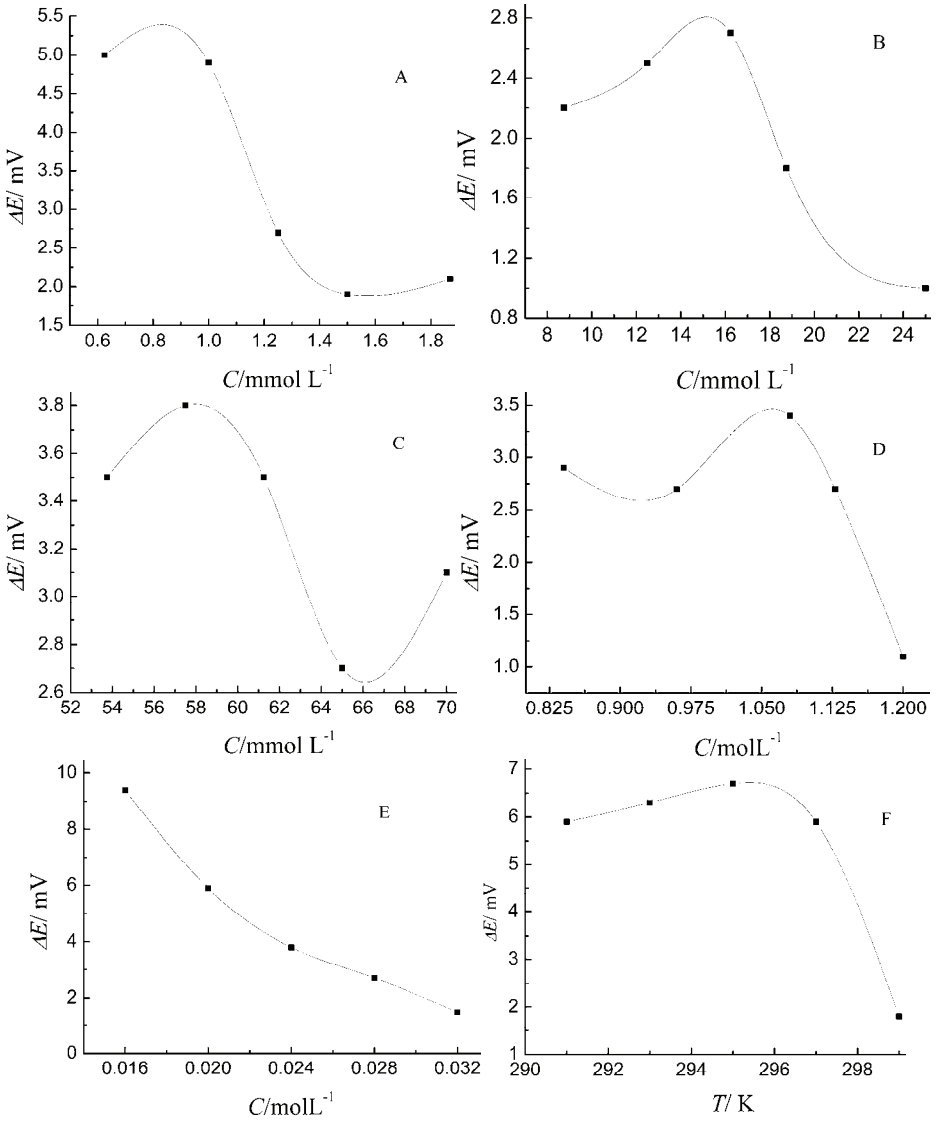

Fig. 2. A. Time series profiles at different initial concentration of KIO_3; A) 7.0×10^{-3}, B) 6.0×10^{-3}, C) 5.8×10^{-3}, D) 5.6×10^{-3} and E) 5.0×10^{-3} mol L^{-1}; F) bifurcation profile using the initial concentration of KIO_3 as the control parameter. Common conditions: [malonic acid] = 1.625×10^{-2} mol L^{-1}, [$MnSO_4$] = 1.0×10^{-3} mol L^{-1}, [H_2SO_4] = 0.02 mol L^{-1}, [H_2O_2] = 1.08 mol L^{-1}, T = 295 K.

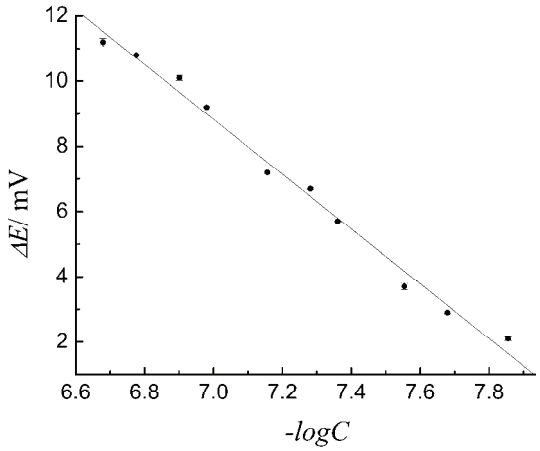

Fig. 3. The plot of the square of the amplitude *versus* the initial concentration of KIO_3 in mol m^{-3}.

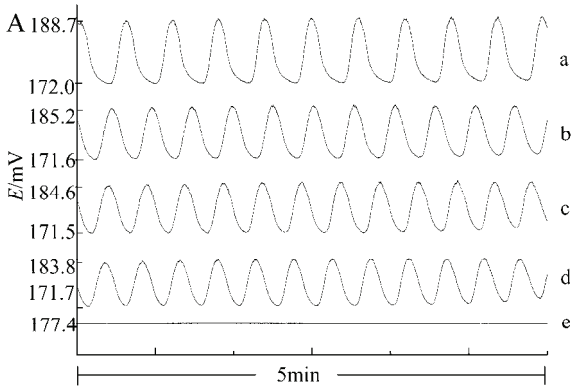

Fig. 4. The profiles of adding 2.1×10^{-8} mol L^{-1} epinephrine near to the point of bifurcation. [KIO_3]: a) 5.0×10^{-3}, b) 4.7×10^{-3}, c) 4.4×10^{-3}, d) 4.1×10^{-3} and e) 3.8×10^{-3} mol L^{-1}. The other conditions were the same as those given in the caption to Fig. 2.

Determination of epinephrine in non-equilibrium stationary state

In the vicinity of the bifurcation point, the system is extremely sensitive to surrounding change. When the epinephrine was injected into the system, the potential of system changed. In the range of 1.1×10^{-7} to 5.2×10^{-9} mol L^{-1}, the potential difference ΔE ($\Delta E = E - E_p$, where E and E_p are the potential of the system before and after addition of epinephrine, respectively) was linearly proportional to the negative logarithm of the epinephrine concentration (–log c), and the detection limit was 6.8×10^{-10} mol L^{-1} (Fig. 5). The linear relationship can be expressed by the following regression equation:

$$\Delta E \text{ (mV)} = 41.33–3.82 \text{ (–log } c \text{ / mol L}^{-1}\text{)} \quad (r = 0.9974, N = 11)$$

Moreover, a comparative study between the classical oscillating profile and the bifurcation point was performed for the same system.

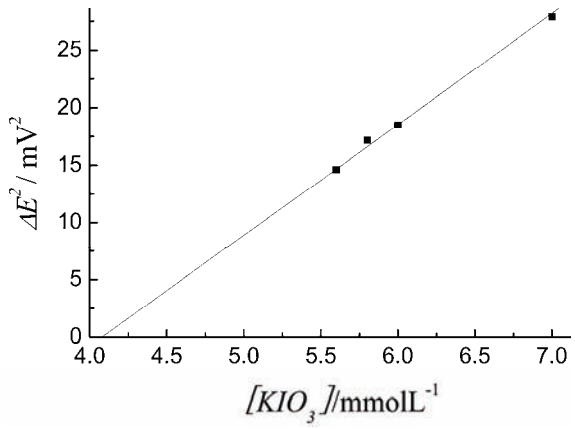

Fig. 5. Calibration curve for the determination of epinephrine using the non-equilibrium stationary state.

Determination of epinephrine in a regular oscillation system

In order to gain higher sensitivity, the effects of experimental variables on the determination were investigated. As shown in Fig. 6, 1.625×10^{-2} mol L^{-1} malonic acid, 5.75×10^{-2} mol L^{-1} KIO_3, 1.08 mol L^{-1} H_2O_2 and 295 K were chosen as the optimal conditions. In addition, the effects of the $MnSO_4$ concentration and solution acidity were studied over the range from 6.25×10^{-4} to 1.87×10^{-3} mol L^{-1} and from 0.016 to 0.032 mol L^{-1}, respectively. As the $MnSO_4$ and H_2SO_4 concentrations decreased, the perturbations of epinephrine increased. When the $MnSO_4$ concentration was lower than 6.25×10^{-4} mol L^{-1} and the H_2SO_4 concentration was lower than 0.016 mol L^{-1}, the oscillating profiles became irregular. In terms of stability and sensitivity, 1.0×10^{-3} mol L^{-1} $MnSO_4$ and 0.02 mol L^{-1} H_2SO_4 were finally adopted for further study.

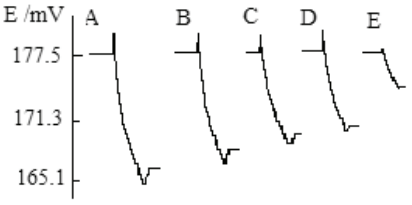

Fig. 6. Influence of the concentration of A) $MnSO_4$, B) malonic acid, C) KIO_3, D) H_2O_2, E) sulfuric acid and F) temperature on the determination of 5.24×10^{-8} mol L^{-1} epinephrine.

Under the optimal conditions mentioned above, a regular oscillating profile (*i.e.*, constantly oscillating amplitude and period) was obtained and then, the determination of was performed. The results showed that the difference in the oscillating amplitude ΔE ($\Delta E = E - E_0$, where E_0 and E are the amplitudes before and after injection of epinephrine, respectively) was linearly proportional to the negative logarithm of epinephrine concentration over the range of 1.4×10^{-8}–-2.1×10^{-7} mol L^{-1} (Fig. 7), with a detection limit of 1.0×10^{-8} mol L^{-1}. The linear relationship can be expressed by the following regression equation:

ΔE (mV) =67.65–8.40 (–log c / mol L^{-1}) (r = 0.9950, N = 10)

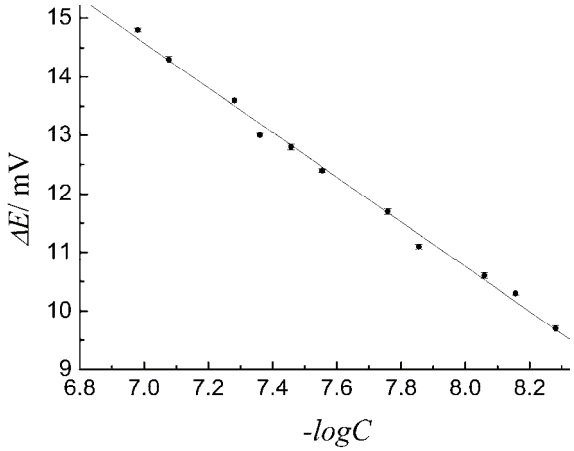

Fig. 7. Calibration curve for determination of epinephrine using the regular oscillating reaction.

Interferences

It is well known that a non-equilibrium stationary state is highly vulnerable to foreign species; hence, some potentially interfering species that may possibly be present in adrenaline hydrochloride injection or serum were investigated. The tolerable ratio (defined as the maximum amount of foreign species causing an error of less than ±5 % in the determination of 5.24×10^{-8} mol L^{-1} epinephrine) are listed in Table I. It can be seen that common inorganic ions, glucose and EDTA have no influence on the determination of epinephrine, while adenine affects the determination slightly.

TABLE I. Effects of foreign species on the determination of 5.24×10^{-8} mol L^{-1} epinephrine

Foreign species	Tolerable ratio (foreign/epinephrine)
K^+, Mg^{2+}, Na^+, Fe^{2+}	3000
Glucose, $EDTA^-$	2000
HPO_4^{2-}, HCO_3^-, Cl^-	500
Uric acid	100
Adenine	40

Comparison with other methods

In order to confirm the applicability and sensitivity of the non-equilibrium stationary state, the method developed in this study was compared with other methods employed for the determination of epinephrine, including regular oscillating reactions. From Table II, it can be seen that the detection limit of the proposed method was the lowest.

TABLE II. Comparison of the proposed method with others employed for the determination of epinephrine

Method	Linear range, mol L^{-1}	Detection limit, mol L^{-1}	Reference
Fluorescence	6.0×10^{-8}–1.0×10^{-5}	1.5×10^{-8}	16
Chemiluminescence	5.0×10^{-9}–1.0×10^{-7}	3.0×10^{-9}	17
Voltammetry	5.0×10^{-8}–5.5×10^{-4}	9.4×10^{-9}	18
Molecular imprinting	5.0×10^{-8}–2.0×10^{-5}	2.0×10^{-8}	19
Regular oscillating system	1.4×10^{-8}–2.1×10^{-7}	1.0×10^{-8}	This paper
Non-equilibrium, stationary state	5.2×10^{-9}–1.1×10^{-7}	6.8×10^{-10}	This paper

Sample analysis

Epinephrine in adrenaline hydrochloride injection (Tianjin Pharmaceutical Group, Xinzheng Ltd. Co., China) and serum were determined by the regular oscillator and bifurcation profile. Adrenaline hydrochloride injection and serum were directly used after dilution. The recovery was also examined by standard addition method using the non-equilibrium stationary state of the BR oscillating chemical reaction. Results in Tables III and IV indicated that this method could be used in routine analysis of epinephrine, benefiting from its reproducibility and accuracy.

TABLE III. The results of the determination of epinephrine in adrenaline hydrochloride injection

Sample No.	Original$\times10^8$, mol L^{-1}	Added$\times10^8$, mol L^{-1}	Found$\times10^8$, mol L^{-1}	Recovery, %
1	1.019	0	1.045	102.5
2	1.019	2.094	3.076	98.8
3	1.019	3.490	4.597	101.9
4	1.019	0.698	1.738	101.2

TABLE IV. The results of the determination and recovery analysis of epinephrine in serum

Sample No.	Original$\times10^9$, mol L^{-1}	Added$\times10^9$, mol L^{-1}	Found$\times10^9$, mol L^{-1}	Recovery, %
1	8.725	0	8.885	101.8
2	8.725	6.980	15.65	99.6
3	8.725	13.96	22.47	99.1
4	8.725	20.94	29.64	99.9

CONCLUSIONS

In this paper, epinephrine was successfully determined using a non-equilibrium stationary state in a BR oscillating chemical system. Moreover, larger linear range (*ca.* 10^{-9}–10^{-7} mol L^{-1}) and lower detection limit (*ca.* 10^{-10} mol L^{-1}) could satisfy the needs of routine determinations. It could be used for real sample due to its advantages compared with other instrumental analysis, such as ease of operation, inexpensive set-up, *etc*. In addition, the BR oscillating system is of

more interest for understanding the oscillators in biological system because the employed pH of about 1.7 is similar to the acidity of the fluids in the stomach.

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ИЗВОД

ОДРЕЂИВАЊЕ ЕПИНЕФРИНА ОСЦИЛАТОРНИМ СИСТЕМОМ BRIGGS–RAUSCHER ПРИ НЕРАВНОТЕЖНИМ СТАЦИОНАРНИМ СТАЊИМА

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Развијен је високо селективан метод за одредјивање епинефрина, базиран на пертурбацији Briggs–Rauscher осцилаторног система који укључује малонску киселину, Mn^{2+}, H^+, IO_3^- и H_2O_2 при неравнотежним стационарним стањима. У раду је изабрана концентрација KIO_3 као контролни параметер за утврђивање бифуркационе тачке Резултати су показали добро линерно слагање (корелациони коефицијент 0,9974) између разлике потенцијала и негативног логаритма концентрације епинефрина у опсегу $1{,}1\times10^{-7}$–$5{,}2\times10^{-9}$ mol L^{-1}, и детекциони лимит $6{,}8\times10^{-10}$ mol L^{-1}. У поређењу са класичном осцилаторном реакцијом, овај метод има нижи детекциони лимит и шири опсег линеарности. Утицаји других врста, које могу постојати поред епинефрина су такође испитани. Развијен метод се може користити за одређивање епинефрина у серуму и адреналинским инекцијама.

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