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A new method for determination of uric acid by the lactic acid–acetone-BrO_3^-–Mn^{2+}–H_2SO_4 oscillating reaction using the analyte pulse perturbation technique

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Abstract

A new analytical method for the determination of uric acid (UA) by the perturbation of UA on the Belousov–Zhabotinsky oscillating reaction is proposed. The method is based on the linear relationship between the changes in the oscillating period and the concentration of UA. The calibration curve is linear over the range of 2.0×10^{-5} to 5.0×10^{-4} M, with a detecting limit of 3.28×10^{-6} M. The method features good precision (R.S.D.: 3.59%) and excellent throughput (10 samples h^{-1}). The possible mechanism of the perturbation of UA on the oscillating reaction is discussed.

Keywords: Oscillating chemical reaction; APP; Uric acid; CSTR

1. Introduction

Oscillating chemical systems have been the focus of many researchers for the past two decades because of the wealth of dynamic behavior in these far-from-equilibrium complex systems. Not only single period, but also a wide variety of non-linear behaviors have been observed in experimental systems: quasi-double-period, complex oscillations, chaos and so on [1–4]. A number of approaches have been taken to investigate on the non-linear behaviors in order to elucidate the very complex reaction mechanisms. The most widely studied chemical system is the Belousov–Zhabotinsky (BZ) reaction [5]. In general, the BZ reaction accomplishes the oxidation of an organic substrate by bromate under a catalyst in an acidic environment.

However, most biological systems are commonly placed under far-from-equilibrium state with dissipative structure. There has been an explosion of interest in the BZ reactions, as a simple chemical model of biological systems, because of the similarities between chemical oscillating systems and life process that exhibit oscillatory behavior. Lactic acid (LA) is an important intermediate product that is related to metabolic process of life. Under anaerobic conditions, glucose in vivo can be transferred to LA and produce energy to maintain normal functions of living organism. Under aerobic conditions, LA can be oxidized to CO_2 and H_2O [6]. Therefore, we consider the LA–acetone–BrO_3^-–Mn^{2+}–H_2SO_4 oscillating reaction as a better model for studying on antioxidation. Mn(II)-catalyzed Belousov–Zhabotinsky oscillator with LA was extensively studied by Peter Sevcik and co-workers [7,8]. They found the system oscillates only if the bromine formed during the reaction is removed by bubbling inert gas [9]. It was firstly reported by our group in 1997 that acetone can be used instead of inert gas, which has active methylene radical by the action of enolization in acid medium. In this paper, the modified reaction was investigated in open system for the first time in order to get sustained stable oscillations.

Continuous-flow stirred-tank reactor (CSTR) is widely used to ensure the oscillating reaction be permanently far from thermodynamic equilibrium. Under the optimum

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conditions by CSTR, the state of the oscillation would be stable at least 10 h, so that we have enough time for the quantitative analysis. The analyte pulse perturbation technique, developed by Pérez-Bendito and co-workers [10–13] have been considered as a powerful analysis tool for CSTR systems. The literatures have been gradually accumulated for analytical determination using this technique in the last few years [14–17].

Free radicals have been postulated to be a major factor contributing to many diseases for its oxidative damage to cellular components, such as cancer, cardiovascular disease [18,19] and sickle cell anemia [20]. However, antioxidants can inhibit the levels of active oxygen. Uric acid, the end product of purine metabolism, is a natural antioxidant that exists in plasma in relatively high concentration (in men, 320 $\pm$ 60 μM; in women, 234 $\pm$ 52 μM) [21]. UA can prevent lipid from peroxidating and clear up ozone and hypochlorous acid in body. UA reactions with hydroxy radical produce peroxy radical, which is more inactive and harmfulness than hydroxyl. But excess UA peroxy radical can make alcohol dehydrogenase and α-antiprotease inactive [22,23]. Therefore, determination of uric acid would be often recommended in many clinical situations. Uric acid is often determined by chromatographic [24,25] and electrophoretic [26,27] methods. Up to now, there have been few literatures on the determination of UA by kinetic analytical method, which will help us better understand antioxidation of UA. Moreover, our new analytical method is more rapid and simple after the oscillations become stable.

A new method for the determination of uric acid based on the perturbation on the LA–acetone–BrO_3^-–Mn^{2+}–H_2SO_4 oscillating system by CSTR is proposed in this paper. After regular single period displayed in the system, a few microliters solution of uric acid was injected into the oscillation system. Changes in oscillating period were recorded and calculated for quantitative analysis of uric acid. We also found that the ratio of the oscillating period changes (PR) was not simply increased with the concentration of uric acid. But PR was gradually decreased as the increase of uric acid concentration over 5.0×10^{-4} M.

2. Experimental

2.1. Reagents

All chemicals used were of analytical grade and doubly distilled, de-ionized water was used to prepare solutions throughout. Solutions of LA (0.15 M), Act (0.12 M), $MnSO_4$ (5.0×10^{-3} M), and $KBrO_3$ (0.035 M) were prepared separately with different concentrations of H_2SO_4. The concentration of H_2SO_4 was chosen by the requirement of experiment to control the different acidity of oscillating reaction. Stock solution of uric acid (0.01 M) was prepared with 0.1 M sodium hydroxide.

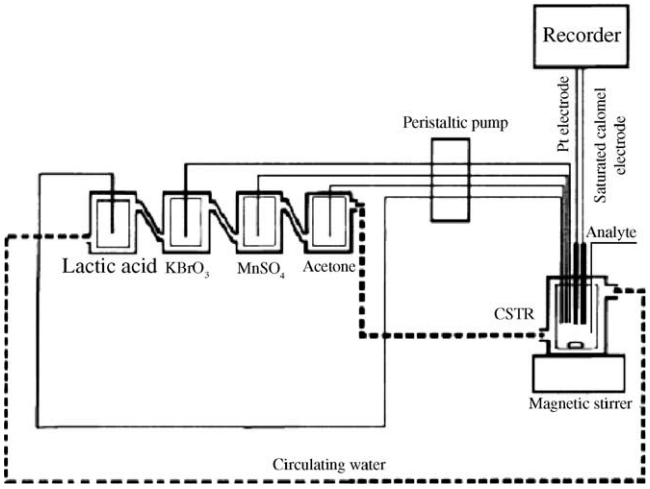


Fig. 1. Schematic diagram of the set-up used to study the oscillating chemical oscillation in a CSTR.

2.2. Apparatus

The experimental instrument (in Fig. 1) consists of a 20 mL glass vessel connected to a Model CS501 thermostat (Chongqing Experimental Instrumental Factory), Pt electrode and $Hg/Hg_2SO_4/K_2SO_4$ reference electrode (Rex, 217) which were used to monitor the signals of oscillations, a magnetic stirrer (Shanghai Experimental Instrumental Factory, 81-2). The reactants were fed by a peristaltic pump (Masterflax, Cole-Pammer Instrument). A syringe was used for injecting samples.

2.3. Procedures

All the solutions of reactants and the CSTR were thermostated at 299 K. Then, the electrodes were inserted and peristaltic pump started to supple the reactant streams at a constant flow rate of 1.13 mL min^{-1}(Fig. 1). Oscillations immediately start when the reactants mixed. After the stable oscillatory state had been set-up, different amounts of UA samples or water were injected. It would spend a few periods for the system to regain its stable oscillatory state and then a new determination could be taken. Voltage signals of the reaction were acquired by our data-collecting system. Changes in the oscillating period following perturbation were used as measurement to construct the calibration plot and determine UA.

3. Results and discussion

In the process of Mn(II)-catalyzed oxidation of lactic acid by BrO_3^- in strong acid medium, the color of the solution switched periodically between yellow and colorless. Fig. 2 shows three kinds of different oscillation profiles we obtained by CSTR under different experimental conditions, whereas only one kind of oscillation profile was obtained in closed sys-

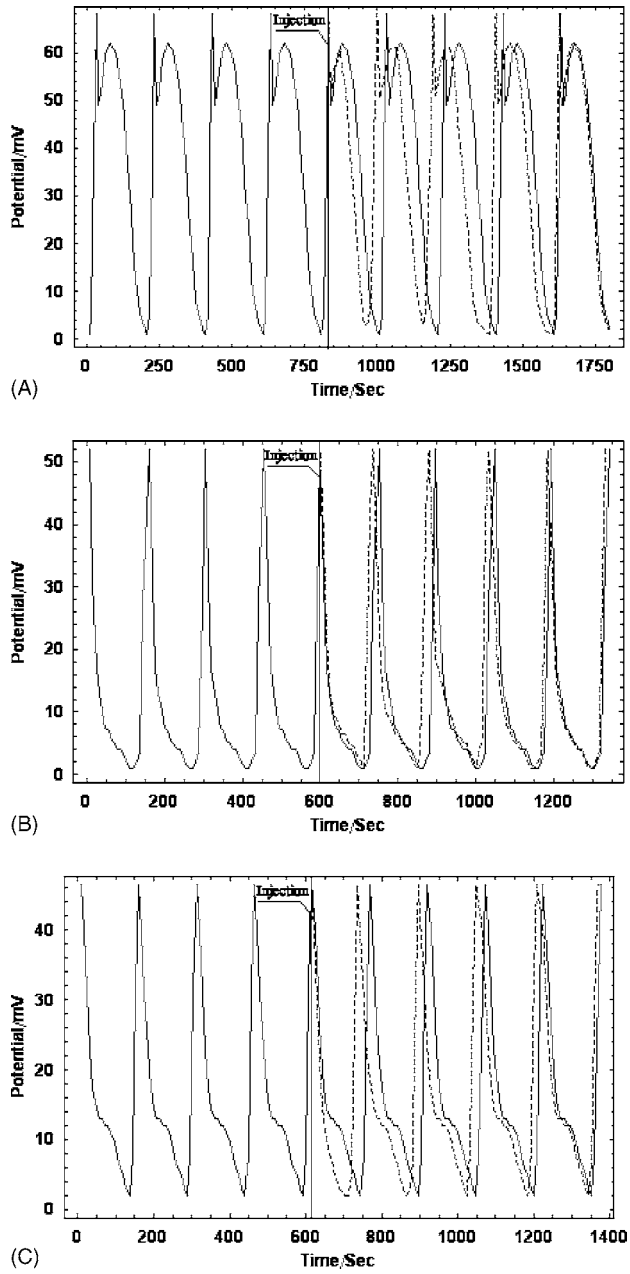


Fig. 2. Typical oscillation profiles obtained in the absence (—) and presence (- - -) of 4×10^{-5} M uric acid. Uric acid was injected into the system after the oscillating state had been stable: (A) quasi-double-period; (B) single period; (C) mix-mode period. Experimental conditions: $C_{H_2SO_4} = 0.50\,mol\,L^{-1}$, $C_{LA} = 0.15\,mol\,L^{-1}$, $C_{Act} = 0.12\,mol\,L^{-1}$, $C_{Mn^{2+}} = 3.3 \times 10^{-3}\,mol\,L^{-1}$, total flow rate = 0.52 mL min^{-1}. (A) $T = 301$ K, $C_{KBrO_3} = 0.040\,mol\,L^{-1}$; (B) $T = 303$ K, $C_{KBrO_3} = 0.020\,mol\,L^{-1}$; (C) $T = 299$ K, $C_{KBrO_3} = 0.020\,mol\,L^{-1}$.

tem. After a uric acid solution of 4.0×10^{-5} M was injected into the system, the effects of the perturbation on the three oscillatory states would fade away after about three oscillating periods. So this method can give us excellent throughput (10 samples h^{-1}). There are some differences among the effects of perturbation: under single period and quasi-double-period conditions, the oscillating periods shortened; under the mix-mode oscillation, not only the oscillating periods shortened but also the state converted to single period. All the effects can be used for our determination.

The point of the cycle in which the analyte is injected, is an important factor in analytical determination oscillating system. The optimum injection point, for maximum response, should be at the point, which the potential is at top of the cycle as shown in Fig. 2. In order to get good reproducibility, the injection point of every determination should be uniform.

3.1. Influence of experimental variables

The effects of experimental variables on the oscillating reaction were studied in order to optimize the working condition for determining uric acid. Different behaviors of the oscillation were observed under different conditions, and the effects of uric acid on the oscillation were also different. Changing each variable in turn while keeping other variables constant, we optimized the system in the absence and presence of UA for obtaining the maximum possible sensitivity and precision.

3.1.1. Influence of acidity

The influence of H_2SO_4 concentration was studied over the range 0.5–1.2 M. Sulfuric acid was almost of no effect on the oscillating amplitude, while the induction period gradually shortened. As can be seen in Fig. 3A, PR increases firstly and then decreases with increase of acidity. 0.9 M of H_2SO_4 was chosen as our working condition.

3.1.2. Influence of temperature

Temperature was an important variable for the proposed system. The oscillating period was highly vulnerable to any changes of the system temperature. Raising the system temperature, induction period and oscillating period both shortened for the reaction rate improved. The system response to UA perturbation was almost not altered by the temperature changes below 300 K, while PR rapidly decreased above 300 K with raising the temperature of system. We chose 299 K as our working condition for the best sensitivity and reproducible oscillations.

3.1.3. Influence of overall reactant flow rate

The overall reactant flow rate fed to CSTR, the other key parameter of the oscillation, was also investigated. The parameter influenced the behaviors of the oscillation and the system resistance to the perturbations. Increasing it from 0.39 to 2.04 mL min^{-1}, the state of oscillation converted quasi-double-period into single period. The maximum of PR was obtained at 1.13 mL min^{-1} which we chose in the single period of the oscillation.

3.1.4. Influence of the concentrations of reactants

The effect of the LA concentration by CSTR was studied over the range 0.12–0.24 M. With the concentration increased, the induction period shortened while the oscillating period and amplitude kept constant. The effect of the UA

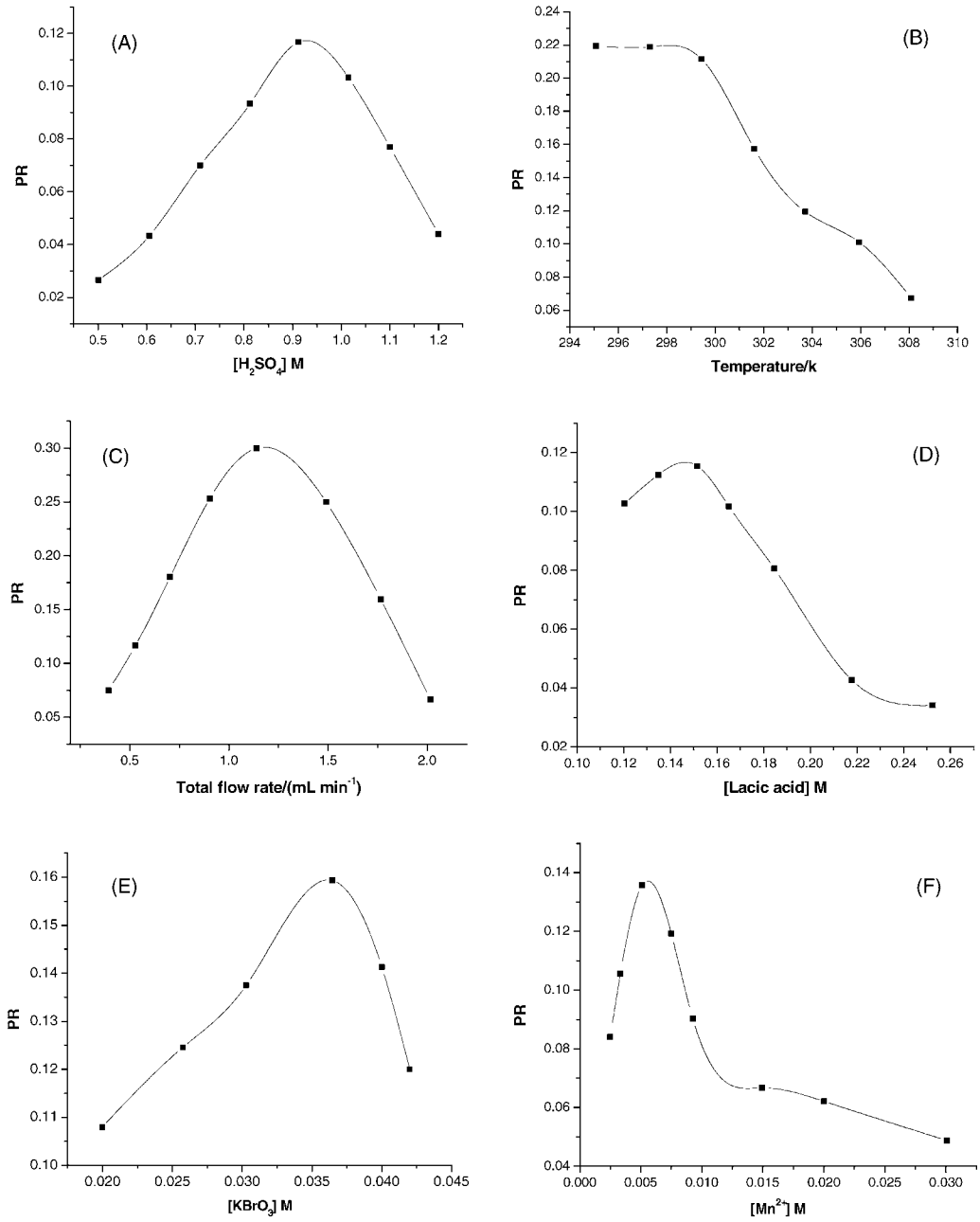


Fig. 3. Influence of: (A) acidity of the solution, (B) temperature, (C) total flow rate on the perturbation of uric acid and the effect of concentration of (D) lactic acid, (E) $KBrO_3$, (F) Mn^{2+} on the uric acid-perturbed oscillating system.

perturbation was such that it increased firstly and then decreased rapidly. A LA concentration of 0.15 M was chosen for the optimum condition.

The effect of the $KBrO_3$ concentration in the reactor was studied over the range 0.020–0.040 M and was similar to the effect of LA on the oscillation. Increasing the $KBrO_3$ concentration, we found that the oscillation state was converted from single period to quasi-double-period. A $KBrO_3$ concentration of 0.035 M was chosen for the highest sensitivity.

The Mn^{2+} concentration in the reactor, which Mn^{2+} was considered as a catalyst, was changed between 0.003 and 0.030 M. As the Mn^{2+} concentration was increased, the induction period shortened and the system transformed single period into quasi-double-period. As a result of increasing the Mn^{2+} concentration, PR was increased firstly and then decreased as shown in Fig. 3F. The Mn^{2+} concentration of 0.0033 M was adapted in order to get high sensitivity.

Finally, the effect of the acetone concentration was studied over the range 0.10–0.25 M. As the concentration was increased, the induction period lengthened and the system converted from single period to quasi-double-period. But there were almost no changes about PR. We chose an acetone concentration of 0.12 M as our working condition.

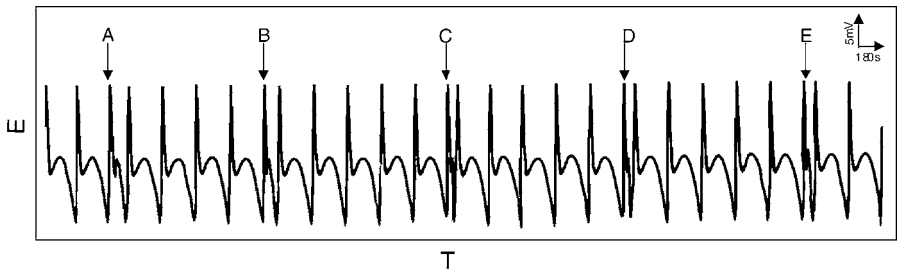


Fig. 4. Effects of different concentrations of uric acid on the oscillating system. A–E indicate injecting samples containing different concentrations of uric acid. Experimental conditions: $T = 299$ K, $C_{KBrO_3} = 0.035$ mol L^{-1}, $C_{H_2SO_4} = 0.90$ mol L^{-1}, $C_{LA} = 0.15$ mol L^{-1}, $C_{Act} = 0.12$ mol L^{-1}, $C_{Mn^{2+}} = 3.3 \times 10^{-3}$ mol L^{-1}, total flow rate = 1.13 mL min^{-1} (A), 3.0×10^{-4} M, (B) 4.0×10^{-4} M, (C) 5.0×10^{-4} M, (D) 6.0×10^{-4} M, (E) 7.0×10^{-4} M.

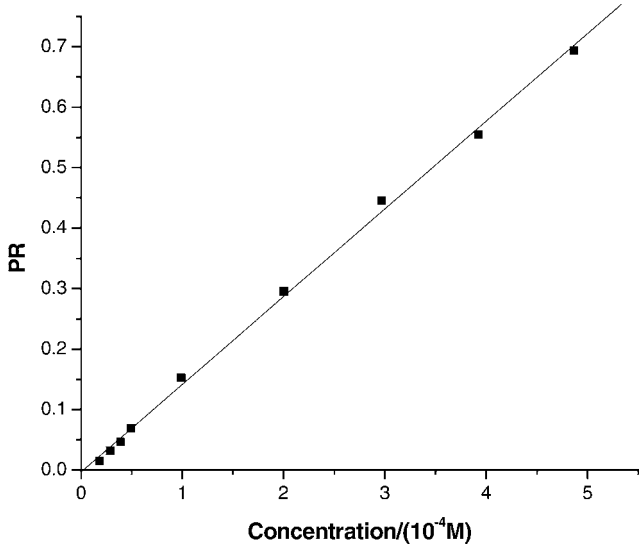


Fig. 5. Calibration plot of the PR versus concentrations of uric acid.

3.2. Determination of uric acid

Different amounts of uric acid were injected into the system after the oscillation state was stable under the optimum condition. The response to the UA perturbation was evaluated by variational ratio of period (PR): PR = $(P_0 - P)/P_0$, where P_0 is the period of the cycle immediately before the perturbation was applied, and P is the one corresponding to the UA perturbation (Fig. 4).

As can be seen in Fig. 5, there was good linear relationship between PR and the injected analyte concentration. It may be considered acceptable that applied this method for determination of uric acid. Table 1 summarizes the figures of merit of the calibration graphs. The precision (R.S.D.: 3.59%) and the sensitivity (detection limit: 3.28×10^{-6} M) of the method were quite good while the UA concentration was in the range of 2.0×10^{-5} to 50×10^{-5} M. Under the working conditions, the chemical oscillating reaction would regain the stable oscillatory state after each perturbation, and the throughput was estimated to be about 10 samples h^{-1}.

Table 1
Analytical figures of merit for the determination of uric acid

Parameter	Value
Linear range (M)	2.0×10^{-5} to 50×10^{-5}
Detecting limit (M)	3.28×10^{-6}
Slope	0.14293 ± 0.0021
Correlation coefficient	0.9992
Precision (%, R.S.D., $n = 11$)	3.59

3.3. Interferences

It was reported that oscillating system is vulnerable to some foreign species. We investigated the effects of some foreign ions and potential interferences from substances with chemical structures or characteristics resembling that of uric acid. The tolerant level was defined as the maximum amount of foreign species causing an error of less than 5% in the determination of 4×10^{-5} M uric acid. The results obtained are shown in Table 2.

3.4. Mechanism of uric acid on the oscillating system and numerical simulations

Since the FKN model for the Belousov–Zhabotinsky reaction was first reported by Field et al. in 1972 [28], which consists of over 20 elementary reactions, the literatures have gradually accumulated on the investigation of the mechanism. The model considered here, which has been studied deeply by An (our group) in 1997 [6], is a simplification of the FKN model. The basic idea of the mechanism is that there are four important processes in every oscillating period which are listed as follows:

- Process A:

$$Br^- + BrO_3^- + 2H^+ \rightleftharpoons HBrO_2 + HOBr \quad (1)$$

$$HBrO_2 + Br^- + H^+ \rightleftharpoons 2HOBr \quad (2)$$

Table 2
Effect of foreign species on the determination of 4×10^{-5} M uric acid

Foreign species	Tolerated ratio
Ca^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, Cu^{2+}	1000
Phenol	500
Thiamine, pyridoxamine	10
Ascorbic acid, tocopherol	3

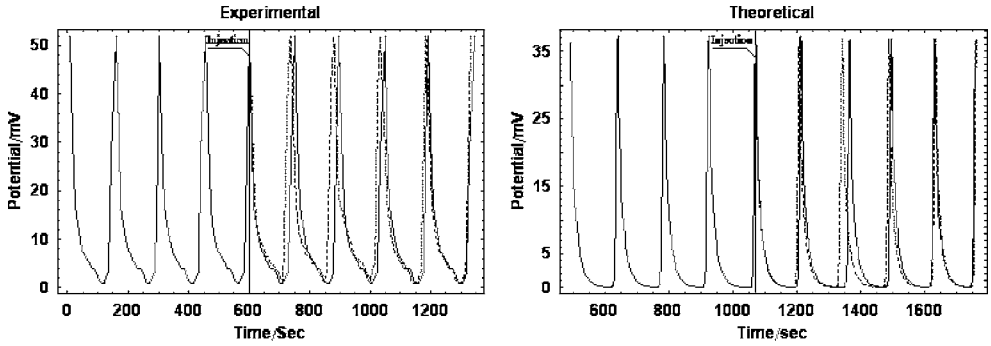


Fig. 6. Comparison of the BZ oscillations (—) and the perturbations (- - -) of uric acid between experimental data and numerical simulations. A uric acid solution of 4×10^{-5} M was injected into the oscillation.

$$Br^- + HOBr + H^+ \rightleftharpoons Br_2 + H_2O \quad (3)$$

- Process B:

$$2HBrO_2 \rightleftharpoons HOBr + BrO_3^- + H^+ \quad (4)$$

$$HBrO_2 + BrO_3^- + H^+ \rightleftharpoons 2BrO_2^\bullet + H_2O \quad (5)$$

$$BrO_2^\bullet + Mn^{2+} + H^+ \rightleftharpoons Mn^{3+} + HBrO_2 \quad (6)$$

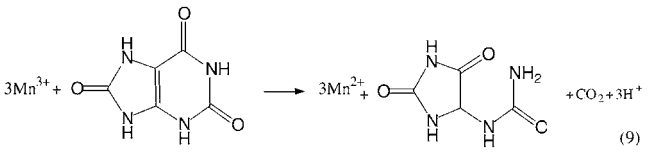
- Process C:

$$Mn^{3+} + CH_3CHOHCOOH + H_2O \rightarrow Mn^{2+} + CH_3COOH + CO_2 + 4H^+ \quad (7)$$

- Process D:

$$Br_2 + CH_3COCH_3 \rightarrow BrCH_2COCH_3 + Br^- + H^+ \quad (8)$$

The key of the oscillation contain the autocatalytic reaction of $BrO_2^\bullet$ that is mainly depended on the concentration of Br^-. With the increase of [Br^-], the production of $BrO_2^\bullet$ becomes more and more slowly because of the restriction of Process B. When the concentration of Br^- decreased, on the contrary, Process A is restricted and Process B becomes the main reaction of the system, with the formation of Mn^{3+}.



(9)

When uric acid was injected into the oscillation, the analyte can react with Mn^{3+} which was reduced to Mn^{2+} by lactic acid. As a result of the increase of [Mn^{2+}], the reaction (9) cooperates with Process C. Therefore, Process B is dominated in the whole reactions causing the decrease of Br^- which controls the oscillating period.

All our numerical simulations are based on the modified FKN model mentioned above. Simulations were performed using the LSODE implementation of the Gear's algorithm for stiff ordinary differential equations (LSODE, Livermore solver for ordinary differential equations). The integrator works in double precision, while the output file is single precision. Data were sampled once a second.

Fig. 6 illustrates the comparison between our experimental data and numerical simulations. Oscillations and the perturbations on this system were both simulated under the condition of single period. Numerical simulations are in agreement with the data of actual experiments very well.

4. Conclusions

Our results demonstrate a suitable use of LA–acetone–BrO_3^-–Mn^{2+}–H_2SO_4 oscillating reaction for the determination of uric acid, which has an effect on the Mn^{2+} concentration. The calibration curve is linear over the range of 2.0×10^{-5} to 5.0×10^{-4} M, with a detecting limit of 3.28×10^{-6} M. The method features good precision (R.S.D.: 3.59%) and excellent throughput (10 samples h^{-1}). It will help us better understand the antioxidation of UA on the biological systems.

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