Determination of Ascorbic Acid Using a New Oscillating Chemical System of Lactic Acid-Acetone-BrO_3^--Mn^{2+}-H_2SO_4

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Analyte pulse perturbation (APP) technique was applied to the study of the perturbation of ascorbic acid (AA) on the lactic acid-acetone-BrO_3^--Mn^{2+}-H_2SO_4 oscillating reaction, and AA was determined using this new oscillating chemical system. Influence of experimental variables was investigated. The linear range lies between 5.0×10^{-7}—5.5×10^{-5} mol/L, and the precision and throughput were quite good (2.43% as RSD, and 8—10 samples/h, respectively). This method was applied to the determination of real samples and the results were satisfactory. Some aspects of the potential mechanism of action of AA on the oscillating systems are discussed.

Keywords ascorbic acid, lactic acid, analyte pulse perturbation (APP) technique

Introduction

Oscillating reactions are complex dynamic systems that involve periodic changes in the concentration of some ingredients (whether a reactant, a product or an intermediate) with time. The similarities between life processes that exhibit oscillating behavior and oscillating chemical systems suggest that the biological and abiological phenomena conform to the same law. Therefore, oscillating chemical systems have been the focus of much research in the area of theoretical and experimental chemical dynamics.[1,2] Two of the famous oscillating chemical systems are the Belousov-Zhabotinskii (BZ)[3,4] and the Bray-Liebhafsky (BL) reactions.[5] As far as we know about these reactions, they follow that the systems must be far from thermodynamic equilibrium state, and one or more autocatalytic or cross-catalytic steps must take place between two steps of the reaction mechanisms.[6]

Before 1990's, studies on oscillating chemical reactions had focused on elucidating the intricate non-linear behaviors observed in the experiment systems from the physico-chemical standpoint, and few analytical applications were reported. The key to the analytical use of oscillating chemical reaction lies in the interaction of the analyte to be determined with an oscillating reaction and correlations between the changes in some characteristics of the oscillator (whether the induction period, oscillating period or amplitude) in the presence and absence of the analyte and its concentration.

Zhabontinskii was the first to apply oscillating reaction to analytical purpose.[7] He obtained a linear correlation between the reactant concentration and the oscillating period. Several other researchers have improved the idea by adding another inhibitor to the reaction system in order to affect the process of the reaction.[4,8-10]

Many analytical determinations were based on a closed system including the analyte involved labour-intensive procedures that entail re-starting the oscillating system before each new determination. This accounts for the little interest aroused so far by this type of reaction for analytical purpose.

The recent inception of an open system for application of the analyte pulse perturbation (APP) technique[11,12] by use of a CSTR (Continuous-flow Stirred

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Tank Reactor), which was developed by Dolores Pérez-Bendito, has opened up new avenues for oscillating reactions in routine analyses. As a result, there has been a gradual shift from theoretical to practical interest. The APP technique is used to derive quantitative analytical information in a straightforward and expeditious manner for some oscillating systems. It is based on the effect of a fast pulse of analyte on an oscillating system; for this purpose, a sample containing analyte is added to the oscillating system in a CSTR. The resulting perturbation causes a change in the oscillating amplitude and period proportional to the analyte concentration, then the system rapidly returns to the unperturbed state, and is ready for a new determination by use of another fast pulse of analyte. Sodium thiosulphate,[11] vanillin,[13] reduced glutathione,[14] vitamin B6,[15] gallic acid[16] and resorcinol acid[17] have been determined by APP technique.

Gao has determined ascorbic acid (AA) by the BZ oscillating chemical reaction in closed system.[18] In closed system, oscillating reaction will be damped in a short time and it is hard to analyze samples. In our experiment, APP technique was adopted to determine ascorbic acid using a new oscillating chemical system of lactic acid (LA)-acetone (Act)-BrO_3^--Mn^{2+}-H_2SO_4. CSTR was introduced into this oscillating system. It can ensure that the oscillating system will be permanently far from thermodynamic equilibrium state and the oscillating system can be used over long time (over 10 h) in successive analyte determination. In addition, this oscillating system was applied to determining real samples and gave satisfying results.

LA is an important intermediate product that is related to metabolic process of life. If the supply of oxygen is insufficient, glucose *in vivo* can be transformed to LA and give out energy to maintain normal function of a living organism. When oxygen is available again, LA can be oxidized to CO_2 and H_2O.[19] AA is one of the most common natural or artificially enriched ingredients in food, fruits and beverages. Our interest in AA results from the fact that AA has a major biological role as a natural antioxidant which can retard the progress of life senescence.[20] Clinical deficiency in AA leads to reduced drug metabolism and immuno-competence and thus affects social and work functions. A further study on the mechanism of its perturbation on the LA-Act-BrO_3^--Mn^{2+}-H_2SO_4 oscillating reaction will doubtlessly help to understand the mystique of life.

Experimental

Reagents

All chemicals used were of analytical grade without further purification and doubly distilled water was used to prepare solutions throughout.

Stock solutions of potassium bromate (0.040 mol/L), lactic acid (0.30 mol/L), acetone (0.30 mol/L) and Mn^{2+} (5.0×10^{-3} mol/L) were prepared separately with different concentrations of sulfuric acid. The concentration of sulfuric acid was chosen by the requirement of experiment to control the different acidity of oscillating reaction.

Stock solutions of AA (0.1 mol/L) were prepared with doubly distilled water. Solutions with lower concentrations were made freshly just prior to use.

Apparatus

The instrumental set-up used to implement the oscillating chemical reaction for determination of AA consisted of the following components: a glass CSTR of 20.0 mL capacity wrapped in a water recirculation jacket connected to a Model CS501 thermostat (Chongqing Yinhe Experimental Instrumental Factory), a MASTERFLEX LS peristaltic pump (Masterflax, Cole-Pammer Instrument) used to feed the CSTR with the reactant solutions and maintain the reaction volume to be constant in the CSTR, a Model 81-2 magnetic stirrer (Shanghai Sile Instrumental Factory), a Model 217 SCE (Shanghai Electroanalytical Instrumental Factory), a Pt electrode (self-made), a Model pH3-3C pH meter (Shanghai Leici Instrumental Factory) and a Type3056 recorder (Sichuan Instrumental Factory) used to indicate and record the oscillation potential changes, and a syringe used for injecting samples.

Procedure

The CSTR, thermostated at experimental temperature, was loaded with reactant solutions. Then, the electrodes were inserted and the peristaltic pump started to supply the reactant solutions (Fig. 1). The oscillating reaction began after an induction period. After the steady oscillating state had been set up, the system was perturbed by injecting different amounts of AA samples. Changes in the oscillating period following perturbation

were used as measurement to construct the calibration plot and dertermine AA.

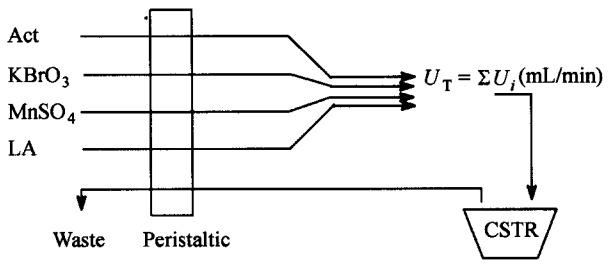


Fig. 1 Reactant and product flowing across the peristaltic pump. U_T indicates sum of every reactant's flow rate (mL/min).

Sample analysis

Twenty tablets were weighed and an average weight of a tablet was calculated before being ground into fine powder. A portion of the powder, equivalent to the average weight of a tablet was dissolved in water and filtered before making a volume of 50 mL. The final solution could be used for the determination. The results were obtained and are shown in Table 1.

Results and discussion

Basic phenomena of oscillating system

In the closed systems, when reactants were mixed in the reactor, oscillating reaction started immediately after an induction period.[21] However, because of reactant consumption by chemical reaction, the reactions exhibited damp oscillation for a limited time until thermodynamic equilibrium was achieved.[12] Thus, further study was hard to be carried out through. The application of CSTR can resolve this problem. A CSTR can be considered as a homogeneous, well-stirred system which exchanges mass and energy continuously with the surrounding environment. It can ensure that the oscillating system will be permanently far from thermodynamic equilibrium state and can be used over long time in successive analyte determination. In fact, the process of life is a non-equilibrated open system, and to some extent, CSTR is just the simulated reactor of this process.

Fig. 2 shows typical oscillation profiles obtained from the proposed oscillating chemical system in the absence and presence of an AA perturbation. In the process of reaction, the color of solution switched periodically between yellow and colorless.

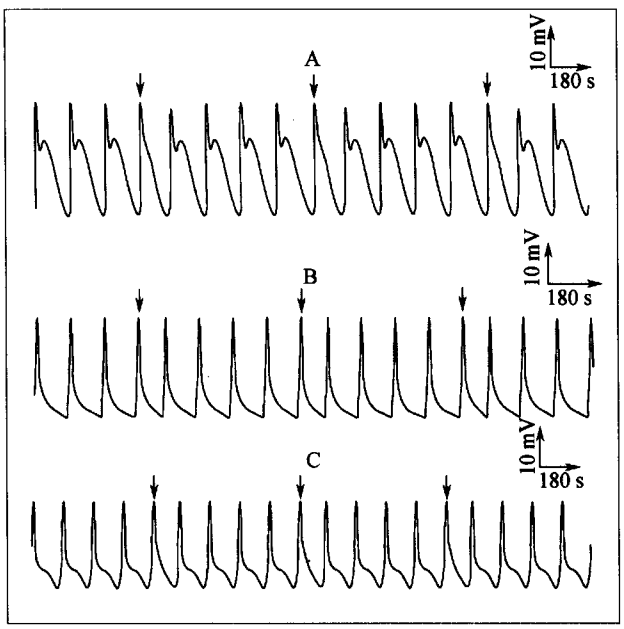


Fig. 2 Typical oscillation profiles of LA-Act-BrO_3^--Mn^{2+}-H_2SO_4 oscillating system in the CSTR. Arrowheads indicate the time at which oscillations were perturbed. Conditions: $c_{H_2SO_4} = 0.50$ mol/L, $c_{LA} = 0.15$ mol/L, $c_{Mn^{2+}} = 2.5 \times 10^{-3}$ mol/L. (A) $T = 299$ K, $c_{KBrO_3} = 0.025$ mol/L, $c_{Act} = 0.18$ mol/L, total flow rate = 0.49 mL/min; (B) $T = 305$ K, $c_{KBrO_3} = 0.020$ mol/L, $c_{Act} = 0.12$ mol/L, total flow rate = 1.42 mL/min; (C) $T = 301$ K, $c_{KBrO_3} = 0.025$ mol/L, $c_{Act} = 0.15$ mol/L, total flow rate = 1.02 mL/min.

Table 1 Determination of ascorbic acid in vitamin C tablet

Sample	Nominal content (mg per tablet)	Found (mg per tablet)	Average (mg per tablet)	Error (%)
Vitamin C tablet	100	95.3 97.5 99.3 98.7 96.6	97.5	2.5

In the CSTR three different kinds of oscillation profiles can be obtained under the different experimental conditions (*e. g.* temperature, total flow rate, concentration of each reactant, *etc.*). Whereas, only a kind of oscillation profile can be obtained in the closed system. Fig. 2A shows typical pseudo-biperiod oscillation profile; Fig. 2B shows typical single-period oscillation profile; Fig. 2C shows typical mixed mode oscillation profile of A and B.

Effect of reaction variables

As a rule, oscillating system is highly vulnerable to systematical conditions such as temperature, total flow rate and the variation of the reaction components in the medium. As for the proposed oscillating chemical system, under different conditions, not only different oscillation profiles could be obtained, but also the perturbation of AA on the oscillating system was different. The response to the AA perturbation could be 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 AA perturbation.

The effect of total flow rate was studied from 0.49 mL/min to 2.00 mL/min. As the total flow rate was increased, the oscillating period decreased roughly proportionally; the oscillation profile transferred from pseudo-biperiod to single-period; the effect of AA was increased slowly, then decreased after achieving maximum peak value (Fig. 3A).

The influence of potassium bromate concentration was studied over the range of 0.02—0.04 mol/L. As the concentration was increased, the oscillating period in-

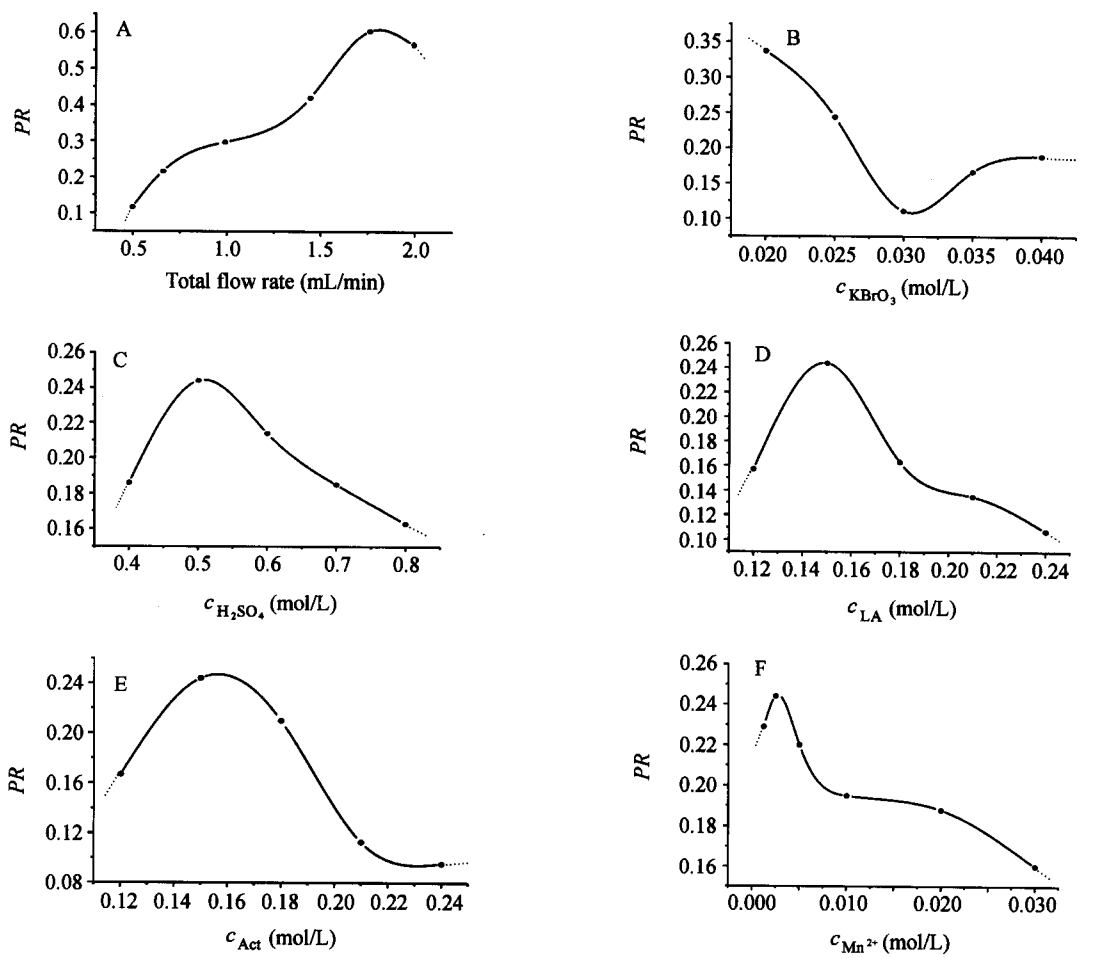


Fig. 3 Influence of the (A) total flow rate on the perturbation of AA and effect of the concentration of (B) $KBrO_3$, (C) H_2SO_4, (D) LA, (E) Act. and (F) $MnSO_4$ on the perturbation of AA. Dot line indicates that it is difficult to carry out oscillating reaction.

creased roughly proportionally; the oscillation profile transferred from single-period to pseudo-biperiod; the effect of AA was decreased sharply and then increased slowly (Fig. 3B).

Variations in tie sulfuric acid concentration had a marked effect on the oscillating system and the perturbation of AA. As sulfuric acid concentration was increased from 0.40 mol/L to 0.80 mol/L, the oscillating period increased roughly proportionally; the oscillation profile was not affected; the effect of AA was increased and then decreased (Fig. 3C). The influence of LA concentration was studied over the range of 0.12—0.24 mol/L, and a behavior similar to that of sulfuric acid was observed (Fig. 3D).

The effect of Act. concentration was investigated from 0.12 mol/L to 0.24 mol/L. As the Act. concentration was increased, the oscillating period decreased roughly proportionally; the oscillation profile transferred from single-period to pseudo-biperiod; the effect of AA was increased slowly, then decreased slowly and at last trended to be constant (Fig. 3E).

As the manganese sulfate concentration was increased from 1.25×10^{-3} mol/L to 3.00×10^{-2} mol/L, the oscillating period increased roughly proportionally; the oscillation profile transferred from single-period to pseudo-biperiod; the effect of AA was increased sharply, then decreased sharply and at last decreased slowly (Fig. 3F).

The temperature was found to affect oscillating system strongly and as the temperature increased from 297 K to 309 K, the oscillating period decreased roughly proportionally; the oscillation profile transferred from pseudo-biperiod to single-period. However, it did not affect the perturbation of AA.

Determination of AA

Based on the above discussion and analytical requirement including analytical sensitivity and analytical time, the following conditions were chosen to determine AA: $T = 303$ K, $c_{KBrO_3} = 0.020$ mol/L, $c_{H_2SO_4} = 0.50$ mol/L, $c_{LA} = 0.15$ mol/L, $c_{Act} = 0.15$ mol/L, $c_{Mn^{2+}} = 2.5 \times 10^{-3}$ mol/L, total flow rate = 1.76 mL/min.

Perturbing the oscillating system by injecting a sample containing a given amount of AA caused a change in the oscillation period (Fig. 4, Table 2) and it was found that square root of *PR* (*SPR*) was quantitatively related to the analyte concentration of the injected sample. Under the optimal conditions described above, the calibration plot (Fig. 5) over the concentration range of 5.0×10^{-7}—5.5×10^{-5} mol/L obeys the following linear regressive equation: $SPR = (0.1149 \pm 7.740 \times 10^{-3}) + (0.01399 \pm 2.755 \times 10^{-4}) c_{AA}$, $R = 0.9990$. And the precision and throughput were also quite good [2.43% ($n = 11$, $c_{AA} = 4.0 \times 10^{-5}$ mol/L) as RSD, and 8—10 samples/h, respectively].

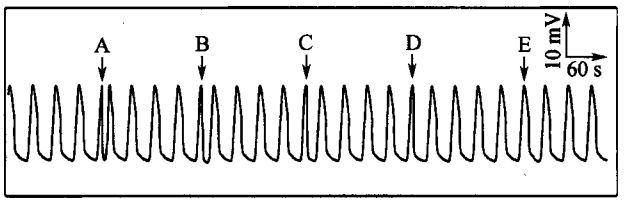


Fig. 4 Effect of AA on the oscillating system. A, B, C, D and E indicate injecting samples containing different concentrations of AA. Conditions: $T = 303$ K, $c_{KBrO_3} = 0.020$ mol/L, $c_{H_2SO_4} = 0.50$ mol/L, $c_{LA} = 0.15$ mol/L, $c_{Act} = 0.15$ mol/L, $c_{Mn^{2+}} = 2.5 \times 10^{-3}$ mol/L, total flow rate = 1.76 mL/min. AA concentrations (mol/L): (A) 5.0×10^{-5}; (B) 4.0×10^{-5}; (C) 3.0×10^{-5}; (D) 2.0×10^{-5}; (E) 1.0×10^{-5}.

Table 2 Effect of AA on the oscillating system

	Concentration of AA (μmol/L)	p_0 (s)	p (s)	*PR*
A	50	78	15	0.81
B	40	78	26	0.67
C	30	78	35	0.55
D	20	78	46	0.41
E	10	78	59	0.24

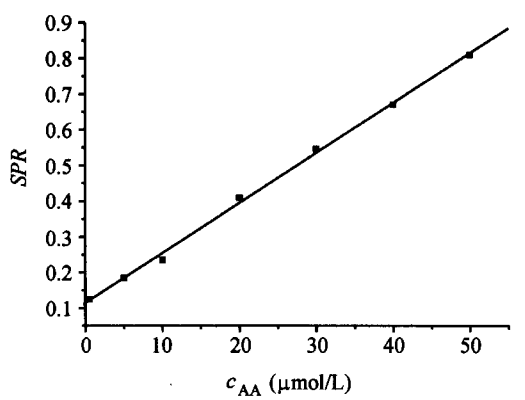


Fig. 5 Calibration plot for the determination of AA using APP technique.

Mechanism of action of AA *on the oscillating system*

Elucidating the nature of the interaction of the analyte with the oscillating reactions is very interesting and significant. Often, the oscillating system consists of many kinetic elementary steps involving several independent variables. In general, the well-known FKN mechanism can be applied to interpret the basic mechanism of bromide-driven chemical oscillator. Our group has ever put forward that this oscillating system might involve four overall reactions:[21,22]

A $BrO_3^- + 5Br^- + 6H^+ \rightleftharpoons 3Br_2 + 3H_2O$

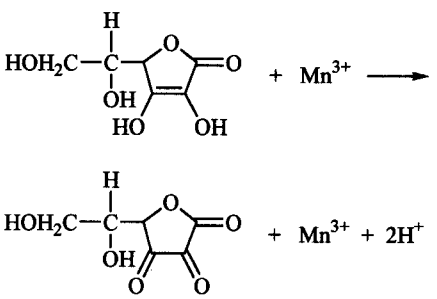
B $BrO_3^- + 4Mn^{2+} + 5H^+ \rightleftharpoons 4Mn^{3+} + HOBr + 2H_2O$

C $Mn^{3+} + CH_3CHOHCOOH \longrightarrow Mn^{2+}$ + oxidative product

D $Br_2 + CH_3COCH_3 \longrightarrow BrCH_2COCH_3 + Br^- + H^+$

The mechanism describes the two major competitive processes (A and B) that control the oscillating reaction and result in oscillations of the intermediate species. In process A, bromate ions and bromide react to form bromine. And in process B, manganese(II) is oxidized by bromate. Manganese(II) and bromide ions are reproduced in process C and process D, respectively. The oscillating period is dominated by the concentration of bromide ion and the oscillating amplitude is dominated by the ratio of Mn(III)/Mn(II). The reaction continues until the concentration of one of the reactants falls below the level necessary to sustain the cycle.

However, based on the above explanation, an approximation can be put forward. AA is a well-known antioxidant. Over a certain range of concentration, it may be assumed to be oxidized by manganese(III) owing to its reducing character. The process can be illustrated simply as follows:



Therefore, the AA reacted with manganese(III) and decreased the concentration of manganese(III), which resulted in the ascent of process B and inhibitory of process A. And the concentration of bromine, which is a product of process A, was lower than normal leading to the inhibitory of process D. The period was decreased as a result of a low generating rate of bromide ion, while the amplitude was decreased resulting from the decrease in the ratio of Mn(III)/Mn(II). Thus, the perturbation of AA leads to the decrease of period and amplitude.

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