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Determination of caffeine using oscillating chemical reaction in a CSTR

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Abstract

A new analytical method for the determination of caffeine by the sequential perturbation caused by different amounts of caffeine on the oscillating chemical system involving the manganese(II)-catalyzed reaction between potassium bromate and tyrosine in acidic medium in a CSTR was proposed. The method exposed for the first time in this work. It relies on the relationship between the changes in the oscillation amplitude of the chemical system and the concentration of caffeine. The calibration curve fits a second-order polynomial equation very well when the concentration of caffeine over the range 4.0×10^{-6}–1.2×10^{-4} M ($r = 0.9968$). The effect of influential variables, such as the concentration of reaction components, injection point, temperature, flow rate and stirring rate were studied. Some aspects of the potential mechanism of action of caffeine on the chemical oscillating system were also discussed. A real sample was determined and the result was satisfactory.

Keywords: Determination; Caffeine; Oscillating chemical reaction; Continuous-flow stirred tank reactor (CSTR)

1. Introduction

Kinetic methods of analysis are useful for the determination of chemical compounds in solution [1–3]. These methods are based on an ability of a substance to change kinetic parameters of chemical reactions. The signal obtained in experiment usually reflects reaction rate which depends on the concentration of a compound to be determined. Chemical reactions may exhibit various dynamic regimes, for example, monotonic, oscillatory, quasi-periodic, chaotic, etc. [4]. However, only monotonic regimes have been widely applied in kinetic methods for trace analysis. The first paper concerning application of regular chemical oscillations for determination of trace amounts of ruthenium(III) was published in 1978 [5]. In the later years several studies have been performed involving of analytical application of regular chemical oscillation [6–8]. There are also several references to naturally occurring oscillating reactions such as those resulting in periodic changes in the calcium concentration in a variety of cell types, which are highly significant to protein phosphorylation [9].

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The most widely known and studied chemical oscillating systems are the Belousov-Zhabotinskii [10–12] and the copper sulfate-catalyzed reaction of hydrogen peroxide with sodium thiocyanate system [13–15]. More recently, oscillating chemical systems involving amino acids have aroused increasing attention for they have close relevance to human metabolism [16–18].

For chemical oscillations to last long enough, the reagents inflow and products outflow must be carefully maintained constant in order to ensure that the reactor is kept under steady concentration conditions; this is usually accomplished by using a continuous-flow stirred tank reactor (CSTR) [19,20]. The CSTR provides several advantages over batch systems for the study of oscillating phenomena. First, a stable oscillatory state can be maintained and studied as long as desired, whereas in batch any oscillatory state must of necessity be a transient one. Second, by supplying reactants or removing products at an appropriate rate, the flow configuration increases the number of systems capable of undergoing oscillation and broadens the range of oscillatory conditions for those systems that can oscillate in batch. Third, maintenance of a far from equilibrium environment by use of the CSTR allows for the possibility of new dynamic phenomena more complex than simple periodic oscillation. Finally, form the analytical point of view, it allows the steady state to be rapidly regained after each perturbation by adding microvolume of the analyte, which affords many determinations on the same oscillating and rapid manner.

The analyte pulse perturbation (APP) technique [19] was developed fairly recently has set up a new field for the use of oscillating chemical reactions in routine quantitative analytical determination. It uses a CSTR to maintain oscillations for a long time (several hours), thereby providing an inexhaustible indicator system for successively added analyte pulse.

The proposed method widens the scope of oscillating chemical reactions in quantitatively analytical determinations. It relies on the analytical potential of the manganese(II)-catalyzed reaction between potassium bromate and tyrosine in acidic medium to determine caffeine with the aid of a CSTR. The oscillating chemical system is perturbed with variable amounts of caffeine, which results substantial changes in the oscillation amplitude that are relevant to the concentration of caffeine.

Some other methods have been reported for the determination of caffeine. Karin Carlesson and his coworker determined caffeine by using a microvolume liquid–liquid flow-extraction system with one-capillary spectroscopic [21], the calibration graph for the extraction of caffeine is linear over the range 1–8 mg l^{-1}, and the limit determination was determined to amount to 0.6 mg l^{-1}. In contrast, the new method has wider linear range and it has great potential in analytical determination.

2. Experimental

2.1. Reagents

All reagents were analytical grade and were used without further purification. Stock solutions of 0.2 M potassium bromate, 0.02 M tyrosine and 0.2 M manganese sulfate were prepared in 0.8 M sulfuric acid, respectively. Solution of 0.2 M acetone was made in 0.8 M sulfuric acid daily. Because the solubility of caffeine in water is very small, the stock solution of caffeine was prepared in 0.05 M sulfuric acid. Doubly distilled disionized water was used throughout.

2.2. Apparatus

All experiments were conducted using a set-up which is shown schematically in Fig. 1. The oscillating assembly comprised a 50 ml CSTR fitted with a thermostated jacked connected to a Model CS-501 thermostat (Shanghai Pujiang Analytical Instrumental Factory) and a magnetic stirrer Model ML-902 (Shanghai Pujiang Analytical Instrumental Factory) for homogenization, a Model DDB-600 6-channel peristaltic pump (Zhejiang Xiangshang Haitian Instrumental Factory). Three of the channels were used to deliver the reactants and the others to remove the waste and keep the volume of reaction mixture in the CSTR

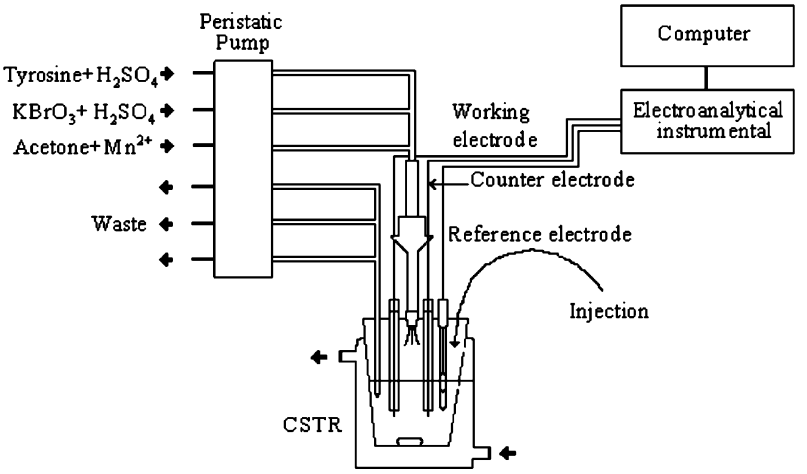


Fig. 1. Experimental set-up for the determination of implementation of oscillation reactions in a CSTR.

constant. State of the system was monitored by measuring. A Type 213 platinum electrode was used as the working electrode, another Type 213 platinum electrode as the counter electrode and a Type 217 saturated calomel electrode as the reference electrode against which all potential were recorded. A Type CHI832 electrochemical analytical instrumental (Shanghai Chenhua Instrumental Company) connected with a personal computer was used to record the potential change. Signals were recorded as a function of time with time step 0.1 s.

2.3. Procedure

The CSTR thermostated at 35 ± 0.05 °C was fed as the following: 1.5 ml of 8 M sulfuric acid, 1 ml of 0.2 M manganese sulfate, 7 ml of 0.02 M tyrosine, 7 ml 0.2 M potassium bromate and 7 ml 0.2 M acetone. Then the electrodes were inserted and the peristaltic pump was started to supply the reactants—the overall feed stream was 1.26 M in sulfuric acid, 0.06 M in potassium bromate, 6×10^{-3} M in tyrosine, 8.7×10^{-3} M in manganese sulfate and 0.06 M in acetone, at a constant flow rate of 0.3 ml min^{-1} of each channel. After attaining the steady state (constant oscillation amplitude and period), the system was perturbed by injection variable amounts of caffeine. Perturbations decreased the amplitude of the oscillation cycle. The change in amplitude ΔA ($\Delta A = A_0 - A$, where A_0 and A are the amplitudes before and after the injection, respectively) against the concentration of caffeine fits a second-order polynomial equation. After the responses were recorded, the flow rate was raised to 5 ml/min for 2 min to flush most reaction products out of the system, which ensured the system return to the steady state quickly, and then the initial working flow rate was reset. When the steady state was regained, the system was ready for a new determination.

3. Results and discussion

3.1. Results

Perturbing the oscillating system by injection a microvolume of sample containing a given amount of caffeine caused a change in the oscillation amplitude that was quantitatively related to the analyte concentration in the injected sample. Fig. 2 and Fig. 3 show the typical oscillation profiles obtained for the proposed oscillating chemical system in the absence and presence of caffeine perturbation under the above-described experimental conditions. When the concentration of caffeine is over the range 4.0×10^{-6}–1.2×10^{-4} M, the changes of oscillation amplitude (ΔA) against the caffeine concentrations provided a

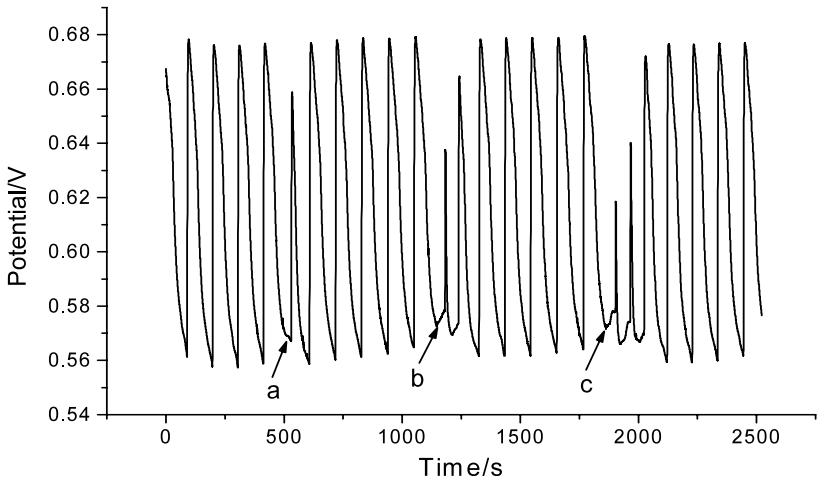


Fig. 2. The typical oscillation profile for the proposed oscillating system obtained in the absence and in the presence of variable amounts of caffeine perturbation. The arrows indicate the times at which oscillations were perturbed. (a) 250 μl 0.02 M caffeine; (b) 500 μl 0.02 M caffeine; (c) 750 μl 0.02 M caffeine.

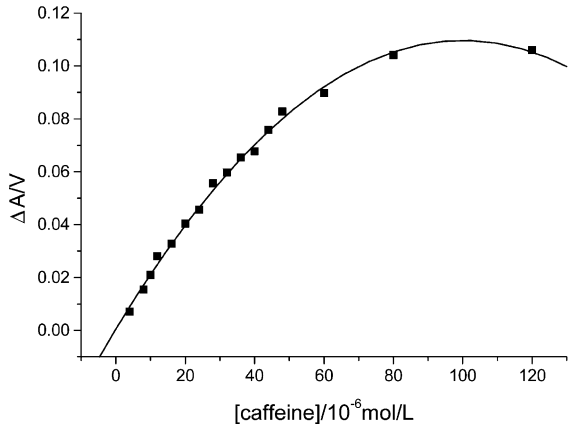


Fig. 3. Calibration curve of the decrease in amplitude vs. the concentration of caffeine in the range 40×10^{-6}–1.2×10^{-4} M (other conditions are the same as that described in the text).

calibration graph that is fitted to the following second-order polynomial equation (Fig. 4):

$$\Delta A = 4.9864E-4 + 0.00875[\text{caffeine}] - 1.7446E-4[\text{caffeine}]^2 \quad (N = 16,\ r = 0.9968)$$

Oscillating systems are reportedly very easily altered by the presence of foreign species in the reaction medium. Consequently, we investigated the effect of some potential interference. The results obtained are shown in Table 1. As can be seen, large amounts of amino acids, phenols and some drugs have no effect on the determination.

3.2. Effect of experimental variables on the determination

Changing each variable in turn while keeping all the other constant optimized the working system. The influence of these variables was carried out with two primary objectives in mind, namely: (a) to achieve the maximum possible stability in the oscillating system over time—essential requirement to obtain reproducible results; and (b) to ensure that the oscillation amplitude allowed the effect of caffeine on it to be accurately determined.

3.2.1. Effect of reaction components

The effect of potassium bromate in the system was investigated over the range from 0.04 to 0.08 M. With the increasing of the concentration of potassium bromate, the responses of caffeine decrease as shown in Fig. 4A. However, the oscillating system is not very stable with low concentration of potassium bromate. So an initial concentration of 0.06 M was adopted. The influence of sulfuric acid was studied in the range from 0.9 to 1.4 M, and a similar behavior was observed (Fig. 4E). Just as in our previous work, when the concentration of sulfuric acid was lower than 1.15

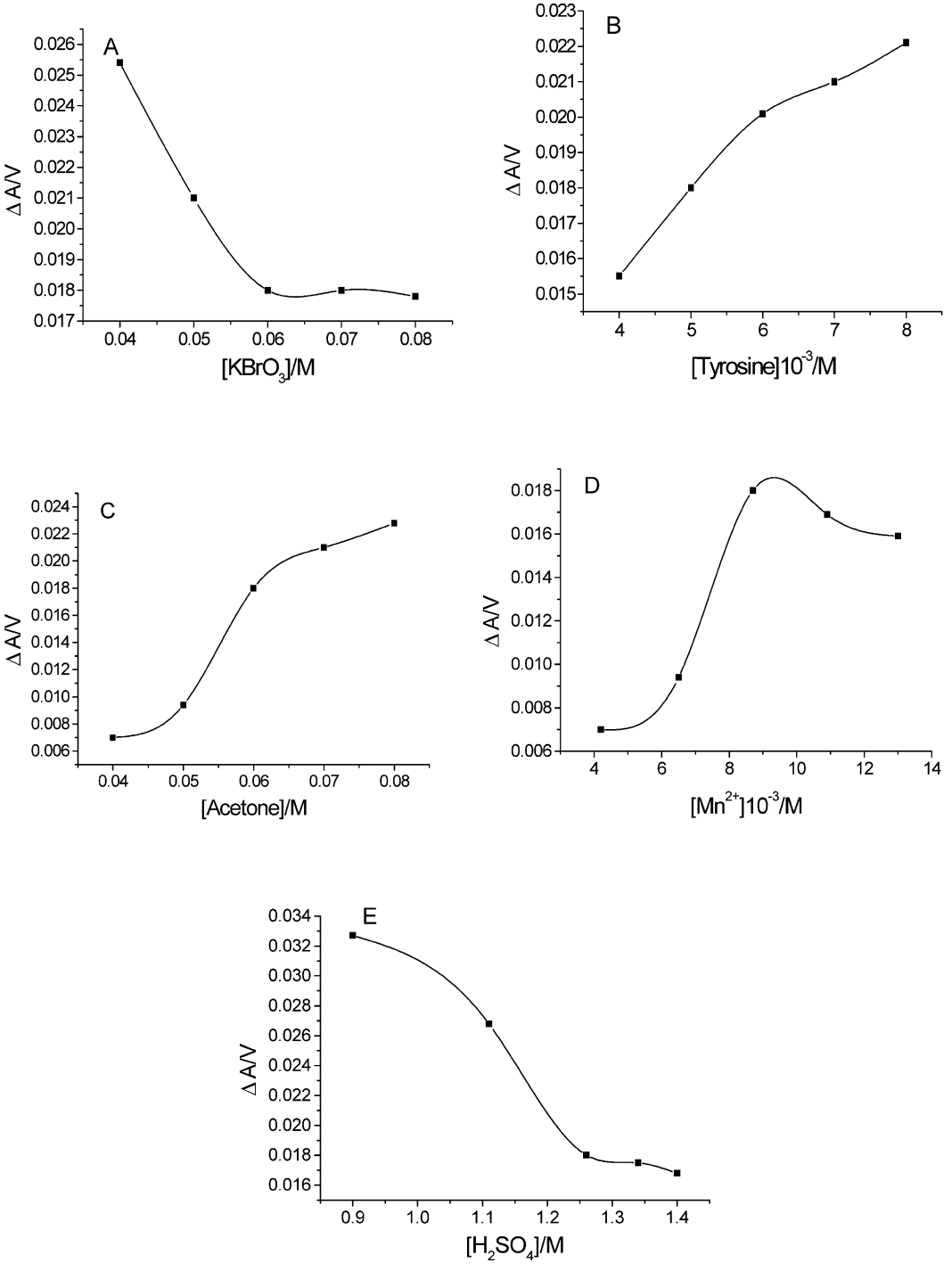

A
ΔA/V
[KBrO3]/M
B
ΔA/V
[Tyrosine]10-3/M
C
ΔA/V
[Acetone]/M
D
ΔA/V
[Mn2+]10-3/M
E
ΔA/V
[H2SO4]/M

Fig. 4

Table 1
Influence of chemically related species on the determination of caffeine

Foreign species	Tolerated ratio (error ±5%)
Glucose, clysine, glutamic acid, glycine, phenylalanine, cystein, alanine	100
Serine, asparagine, valine, ephedrine	64
Phenol, hydroquinone, diacetyl morphine	10
Ascorbic acid	2

M, the potential decreased at the minimum of the cycle. Finally, in the proposed system, the concentration of sulfuric acid was chosen as 1.26 M.

The tyrosine in the CSTR was changed between 4×10^{-3} and 8×10^{-3} M. As the concentration was increased, the value of ΔA increased (Fig. 4B). However, a too high concentration of tyrosine made the duration of oscillating short, we thus chose to employ 6×10^{-3} M tyrosine in the CSTR. The influence of acetone was similar to that of tyrosine (Fig. 4C). Nevertheless, the more acetone was fed into the oscillating system, the larger background noise was obtained. So 0.06 M acetone was employed as optimal.

In contrast, the effect of manganese(II) was different from that of potassium bromate, tyrosine, acetone and sulfuric acid. The result of the manganese(II) in the presence of caffeine is illustrated in Fig. 4D. A manganese(II) concentration of 8.7×10^{-3} M was finally chosen in order to maximize the system response to the caffeine perturbation. Under the optimized conditions, the duration of the system is about 6 h, it is long enough for determination sixty samples.

3.2.2. Effect of injection point

In order to ensure accurate and reproducible results, where the injection should be performed was two crucial variables, and therefore, required careful study. Several injection points have been tested throughout the oscillation cycle, and it can be seen in Fig. 5. The oscillation cycle can be divided into three zones. In Zone A, the potential drops rapidly while in Zone B, it drops slowly and then increases to the starting state in Zone C. Two injection points were chosen in each zone and the results show that the analyte should be injected in Zone B.

3.2.3. Effect of temperature

The effect of temperature was investigated over the range from 20 to 50 °C. In the absence of caffeine, the oscillation period decreases with increasing in the temperature while the oscillation period remained virtually constant. When the temperature is too low, it would take a long time for each determination. However, the temperature is too high, it is difficult to inject the samples at the same point of the oscillation cycle. Because of a compromise between these, we finally chose to employ 35 °C in the CSTR as the optimal.

3.2.4. Effect of flow rate

The overall reactant flow rate fed to the CSTR significantly influenced the behavior of the oscil-

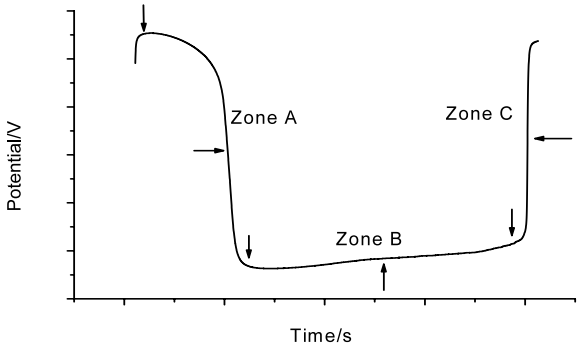


Fig. 5. Influence of injection points on the determination of caffeine. The arrows indicate the points at which oscillations were perturbed.

Fig. 4. Influence of the concentration of (A) potassium bromate, (B) Tyrosine, (C) Acetone, (D) Mn(II) and (E) sulfuric acid on the caffeine perturbed oscillating reaction. Conditions: (A) 6.0×10^{-3} M Tyrosine, 0.06 M Acetone, 8.7×10^{-3} M Mn(II) and 1.26 M H_2SO_4; (B) 0.06 M $KBrO_3$, 0.06 M Acetone, 8.7×10^{-3} M Mn(II) and 1.26 M H_2SO_4; (C) 0.06 M $KBrO_3$, 6.0×10^{-3} M Tyrosine, 8.7×10^{-3} M Mn(II) and 1.26 M H_2SO_4; (D) 0.06 M $KBrO_3$, 6.0×10^{-3} M Tyrosine, 0.06 M Acetone and 1.26 M H_2SO_4; (E) 0.06 M $KBrO_3$, 6.0×10^{-3} M Tyrosine, 0.06 M Acetone and 8.7×10^{-3} M Mn(II). Common condition: 250 μl 0.02 M caffeine.

lating system [22–24]. Increasing flow rate led to an increasingly shorter oscillation period. The influence of flow rate was carried out with two criteria in mind, namely: (a) a long enough time for the analyte effect the oscillating system and (b) the time needed for the steady state to be regained after each perturbation possible short. Because too high a flow rate might sweep part of the analyte before it could exert its effect on the oscillating system and too low a flow rate lengthened the restoration time of the steady state. So the flow rate of each channel of 0.3 ml min^{-1} was adopted to perform perturbations and record responses.

3.2.5. *Effect of stirring rate*

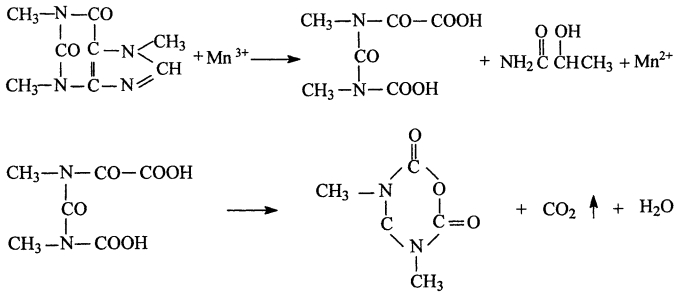
The previous work [25] indicated that the dependence on the stirring rate is due to oxygen: the rate of transport of oxygen into the reaction mixture depends on the stirring rate. For the reaction mixture did not expose in the air during our experiments, the stirring rate hardly effected the determination.

3.3. *Mechanism of action of caffeine on the oscillating system*

The mechanisms of oscillation reaction are rather complex. Elucidating the nature of the interactions of the analytes with the oscillation reactions was no easy task. The oscillating system in this work was proposed recently by us. The accurate mechanism of it is unknown up to now. For the proposed oscillating system is a bromate ion-driven oscillating chemical reaction, the well-known FKN mechanism [26] can be applied to elucidate the action of caffeine on the oscillating system.

The mechanism of caffeine on the oscillating system made the oscillation amplitude changed while the period kept almost constant. The change of oscillation amplitude means the change of $\ln([Mn^{3+}]/[Mn^{2+}])$. When caffeine was injected into the oscillating system, the concentration of Mn^{3+} decreased while that of Mn^{2+} increased for the total amount of manganese is constant. In addition, there had a lot of bubbles produced when caffeine was injected into the system.

The possible mechanism is that when caffeine is introduced into the system, it can be oxidized to binary carboxylic acid and oxyammonia by Mn^{3+} in strong acidic medium, that led to the concentration of Mn^{3+} in the system decreased while that of Mn^{2+} increased, so the potential decreased apparently. The binary carboxylic acid can easily happen decarboxylation and form stable inner ester with a hexatomic ring and liberate carbon dioxide at the same time. This is consistent with the phenomena observed in the experiment. The reaction can be expressed as following:



With the feeding of reactants and the products flowing out, the system regained the steady state and a new determination can be started.

3.4. Practical application

The proposed method was applied to the determination of caffeine in tablets. Commercial caffeine tablets were dissolved in water and diluted to a concentration of 8.0×10^{-5} M. The average caffeine content was found to be 7.8×10^{-5} M.

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