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Determination of L-aspartic Acid by using the Cu(II)-Catalyzed Oscillating Reaction

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Foi proposto um procedimento conveniente por meio de um sistema químico oscilante como uma ferramenta analítica para a determinação de ácido L-aspártico (L-Asp). O sistema envolve a reação entre peróxido de hidrogênio e tiocianato de sódio, catalizada por íons Cu(II) em meio alcalino. O método baseia-se na relação linear entre as mudanças no período de oscilação e o logarítmo da concentração de ácido L-aspártico. Condições otimizadas foram posteriormente validadas para garantir a confiabilidade do método. Sob condições otimizadas, o gráfico de calibração é linear e proporcional à concentração do ácido L-aspártico no intervalo de $1,17 \times 10^{-5}$ mol L^{-1} a $7,10 \times 10^{-8}$ mol L^{-1} com um coeficiente de regressão linear de 0,9990 e RSD de 2,75%. O limite de determinação (LOD) foi de $5,58 \times 10^{-8}$ mol L^{-1}. O método proposto foi empregado na determinação do ácido L-aspártico em amostras de medicamentos. Além disto, a técnica HPLC foi empregada para confirmar os resultados.

A convenient procedure by means of oscillating chemical system as an analytical tool to determine the L-aspartic acid (L-Asp) is proposed. The system involves the Cu(II)-catalyzed reaction between hydrogen peroxide and sodium thiocyanate in an alkaline medium. The method relies on the linear relationship between the changes in the oscillation period and the logarithm of concentration of L-aspartic acid. Optimized conditions were further validated to ensure the reliability of the method. Under optimum conditions, the calibration plot is linearly proportional to the concentration of L-aspartic acid over the range from 1.17×10^{-5} mol L^{-1} to 7.10×10^{-8} mol L^{-1} with a corresponding regression coefficient of 0.9990 (RSD 2.75%). A lower limit of determination (LOD) was 5.58×10^{-8} mol L^{-1}. The proposed method has been used to determine L-aspartic acid in drug sample. Additionally, the HPLC technique was also used to confirm the determination results.

Keywords: oscillating chemical reaction, analyte pulse perturbation technique, L-aspartic acid, drug sample

Introduction

In the biochemical process the well-known Krebs cycle illustrated the conversion of various compounds and the transformation of energy, for example, L-aspartic acid (L-Asp) can be converted into α-ketoglutaric acid to enter this cycle. In clinic, L-Asp is necessary for the treatment of heart disease, hepatopathy, and hypertension; it can also help people providing and recovering from fatigue. Thereby, a rapid and convenient determination method is required in many fields. At present, some methods have been reported for the determination of L-Asp such as ion exchange chromatography,[1] high-performance liquid chromatography (HPLC)[2] and capillary electrophoresis (CE) coupled with flow injection (FI).[3] Although these methods have higher sensitivity and good selectivity, generally, the pre-treatment of column is not easy to be done.

Compared to these methods, the application of oscillating chemical reaction for the determination of trace amounts of substances has gained interest from many analysts[4-5] because of its simplicity, largely linear range (*ca.* 10^{-7} - 10^{-3} mol L^{-1}), and lower detection limit (*ca.* 10^{-8} - 10^{-6} mol L^{-1}). The use of oscillating chemical reactions for analytical monitoring has concentrated on

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two oscillators. One is the most widely studied and used B-Z oscillating reaction, particularly, the Ce(IV)-catalyzed reaction between malonic acid and potassium bromate in acidic medium. The early FKN mechanism[6] and a recently theoretical analysis by Taylor[7] impel the study to the deep progress. Another is the Cu(II)-catalyzed reaction between hydrogen peroxide and sodium thiocyanate in the alkaline medium, which has developed by Orban *et al*.[8-9] A lot of papers concerning the determination of organic[10-14] and inorganic substances[15-22] have also been published.

In general, the response of a regular oscillating system (such as its induction period, oscillating amplitude or oscillating period) to the perturbation from the surroundings relies on the interaction between the sample and the oscillating-system substrates. The stronger the response is, the higher sensitivity. For the purpose of analytical determination, the response of the system without analyte (*i.e.*, a stable regular oscillating profile) is defined as a baseline just like common instrumental analysis; and then, the sample was added into the stable regular oscillating system to perturb it. The response-change relative to baseline (*i.e.*, the stable regular oscillating profile) was recorded to calculate the quantity of analyte. This is the basic principle of analytical determination in oscillating reaction.

In this work, adding a few of L-Asp into the Cu(II)-catalyzed oscillating reaction between hydrogen peroxide and sodium thiocyanate in alkaline medium which was implemented in a closed system, causes a change in oscillation period. Such a response implied that the L-Asp has reacted with some of components in this system. The change of oscillation period is correlated with the amount of adding L-Asp. The purpose of this work is to broaden the scope of application in the Cu(II)-catalyzed oscillating chemical reaction. The most salient advantage of this proposed method is very rapid and convenient for determination. Moreover, its good reproducibility, sensitivity and precision have also been accepted.

Experimental

Reagents

All chemicals used were of analytical-reagent grade and doubly distilled-deionized water was used throughout. Stock solution of 1.40 mol L^{-1} H_2O_2 was made freshly just prior to use, and was standardized with the standard $KMnO_4$ solution. The stock solution was kept in a black polyethylene bottles and stored in refrigerator at 5 °C to avoid decomposition. Solution of 0.0750 mol L^{-1} sodium thiocyanate was prepared by dissolving the appropriate amount of NaSCN in 0.310 mol L^{-1} sodium hydroxide solution. The 0.310 mol L^{-1} NaOH solution was checked by the acid-base titration method. Copper sulfate stock solution (1.80×10^{-3} mol L^{-1}) in 1.20 mol L^{-1} NaCl solution was also made. Solution of 0.01 mol L^{-1} L-aspartic acid was prepared using biochemical reagent dissolved in doubly distilled-deionized water and stored in refrigerator.

Apparatus

The oscillating assembly consists of a 50 mL glass reaction vessel fitted with a thermostated jacket connected to a Model CS 501 thermostat (Shanghai Experimental Instrumental Factory, China), with an accuracy ± 0.1 K. A Model ML-902 magnetic stirrer was used for homogenization (Shanghai Pujiang Analytical Instrumental Factory, China). The oscillation was monitored by means of two platinum electrodes (Rex, 213, China), and an Hg | Hg_2SO_4 | K_2SO_4 reference (Rex, 217, China), which were directly connected to an electrochemical instrument (Shanghai Chenhua CHI 832, China) with a potential measurement error ± 0.1 mV. Signals were recorded as a function of time at intervals of 0.1 s. A micro-injector was used for adding different amounts of sample solution.

Chromatograms were recorded on a SHIMADZU LC-16A high-performance liquid chromatographic instrument (Shimadzu, Japan) equipped with a SPD-16A UV spectrophotometric detector and a CR-3A data processor.

Procedure

The following procedure was used in all experiments for determination of L-Asp: A 23.5 mL of Cu(II) catalyzed reaction mixture solution was consisted of 2.50 mL of 1.80×10^{-3} mol L^{-1} $CuSO_4$ (prepared by using 1.20 mol L^{-1} NaCl), 7.00 mL of 7.50×10^{-2} mol L^{-1} NaSCN (prepared by using 0.310 mol L^{-1} NaOH), and 3.00 mL of 1.40 mol L^{-1} H_2O_2, along with 11.00 mL of distilled-deionized water. After immersing three electrodes into the mixture solution, the mixture was stirred magnetically at 600 rpm and kept constant at 309 ± 0.1 K, and then, the data acquisition was started. Signals of the potential of Pt electrode were recorded as a function of time with a time step 0.1 s. Once a regular oscillating profile was appeared, various amounts of L-aspartic acid were added to perturb the system. Because the highest point (or the lowest point) of cyclic profile is being at the critical value of potential, that is, very sensitive to the surroundings, the sample must be injected in this position as shown in Figure 1.

Results and Discussion

The copper sulfate-catalyzed oscillating reaction of hydrogen peroxide with sodium thiocyanate is the most representative example of an oscillating system involving no halogen compounds. This oscillating system has a high potential for analytical determination, owing to the different responses to sample perturbation exhibited by this oscillation.[4] In this work, adding a trace amount of L-Asp can perturb the oscillating profile, implying that the L-Asp has reacted with some of components in oscillating system. Figure 1 shows typical oscillation profiles for the proposed system in the absence (I) and presence (II) of L-aspartic acid. As mentioned above, the position of adding sample was chosen at the highest point of oscillating profile, and repeated again to ensure the reproducibility. The result perturbed is that the period is increased and its change is relative to the amount of L-Asp injected.

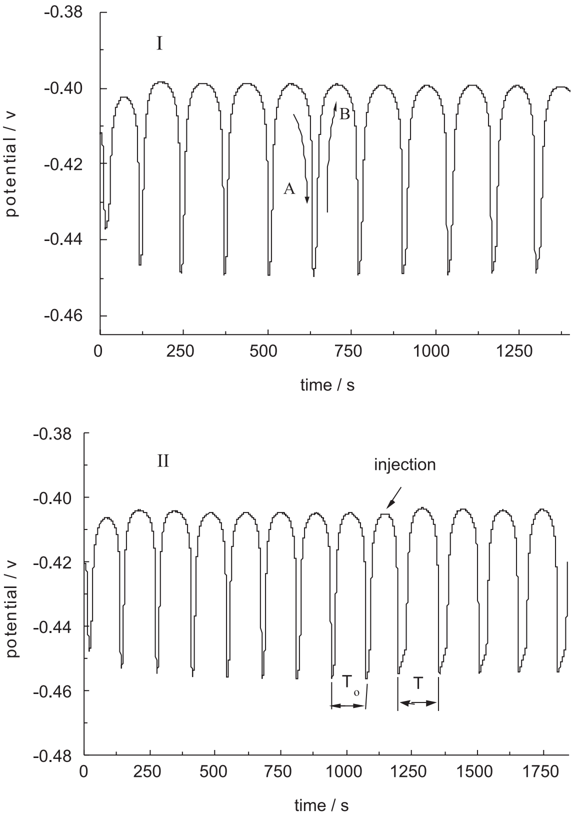

Figure 1. Typical oscillation profiles obtained in the absence (I) and presence (II) of 8.50×10^{-7} mol L^{-1} of L-aspartic acid under the optimum conditions.

Firstly, we defined T_o and T as the periods before and after perturbing, respectively. Then, the values of ΔT can be expressed as follow:

$$\Delta T = T - T_o$$

Influence of experimental variables

To obtain a stable regular oscillating system for determining L-Asp with higher sensitivity and accurate, the effects of various variables on the behavior of the oscillating system were tested thoroughly. The effect of hydrogen peroxide concentration was studied in the range of 0.75-3.0 mol L^{-1}. With increasing the concentration, the period would be decreased, the induction time become shorter and eventually disappeared. When the concentration of hydrogen peroxide increased from 2.0 mol L^{-1} to 3.0 mol L^{-1}, the oscillation profile was drifted up obviously. If the sodium thiocyanate concentrations were changed from 0.01 to 0.12 mol L^{-1}, the period would be lengthened. The NaOH concentration and pH have strong effects on the oscillating reaction, which were studied over the range of 0.1 - 0.5 mol L^{-1}, in which the oscillation amplitude and period increased with the increase of NaOH concentration. The influence of the Cu(II) concentration was also examined, it can be seen that an increase of Cu(II) in the range of 1.0×10^{-3} mol L^{-1} - 4.0×10^{-3} mol L^{-1} would cause a decrease in the oscillation period while the amplitude has not changed.

Adding L-Asp to the regular oscillating system to ensure that the highest sensitivity and precision were obtained. For ease of representation, we use the change of oscillation period (ΔT) as a reference standard to analyze the variables on the determination of L-Asp. Results are given in Figure 2 and Table 1.

The influence of temperature was also investigated over the range of 303-313 K. It is clear that with increasing the temperature the frequency becomes quicker and eventually the profile drifts up (see Figure 3). For the purpose of determination, the optimal temperature of 309 K was chosen.

Sodium chloride was used to keep a moderate ionic strength of oscillating system and to avoid passivation of the Pt electrode[23]. Thus, 1.20 mol L^{-1} of NaCl was adopted in this work.

Determination of L-aspartic acid

Under the optimum conditions, a stable regular oscillating profile was obtained and could be lasting for 2000 s. That is to say, the determination of L-Asp would begin. All data were recorded by personal computer. A plot of ΔT against the amounts of L-Asp in the range of 7.10×10^{-8} - 1.17×10^{-5} mol L^{-1} was made (in Figure 4) that was fitted to the following linear regression equation very well:

$$\Delta T\ (s) = (2.94 \pm 0.04)\ \log C_{L\text{-}Asp} + (27.6 \pm 0.2)$$
(r = 0.9995; n = 14; RSD = 2.75%)

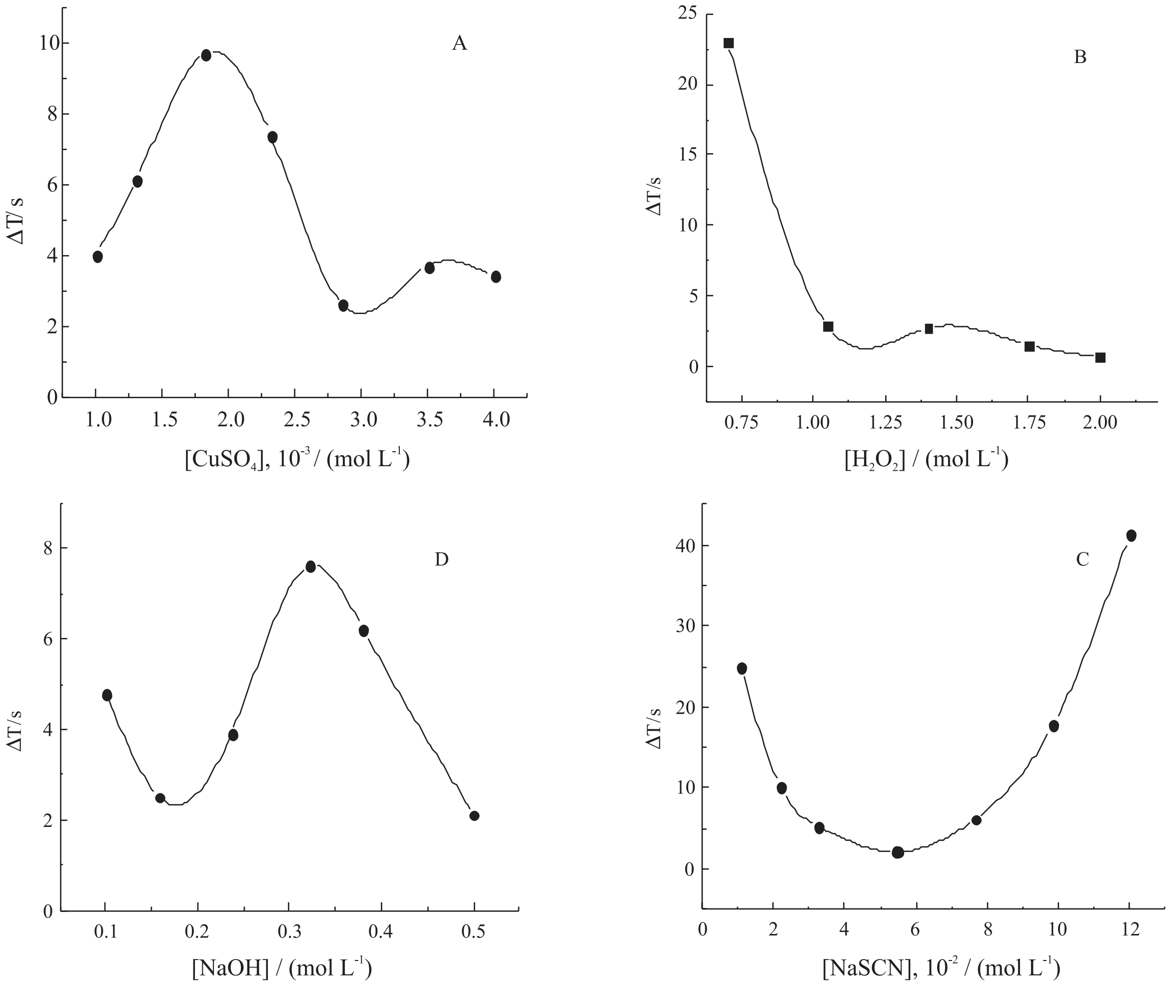

Figure 2. Influence of the concentrations of $CuSO_4$ (A), H_2O_2 (B), NaSCN (C) and NaOH (D) on the L-aspartic acid pertubed oscillating period change. Experimental conditions: T = 309K, [L-Asp] = 5.60×10^{-6} mol L^{-1}; (A) 1.40 mol L^{-1} H_2O_2, 7.65×10^{-2} mol L^{-1} NaSCN, 1.20 mol L^{-1} NaCl, 0.310 mol L^{-1} NaOH; (B) 1.80×10^{-3} mol L^{-1} $CuSO_4$, 7.65×10^{-2} mol L^{-1} NaSCN, 1.20 mol L^{-1} NaCl, 0.310 mol L^{-1} NaOH; (C) 1.80×10^{-3} mol L^{-1} $CuSO_4$, 1.40 mol L^{-1} H_2O_2, 1.20 mol L^{-1} NaCl, 0.310 mol L^{-1} NaOH; (D) 1.80×10^{-3} mol L^{-1} $CuSO_4$, 1.40 mol L^{-1} H_2O_2, 7.65×10^{-2} mol L^{-1} NaSCN, 1.20 mol L^{-1} NaCl.

Table 1. Optimum conditions of the L-aspartic acid determination

Variables	$CuSO_4$ / mol L^{-1}	H_2O_2 / mol L^{-1}	NaSCN / mol L^{-1}	NaOH / mol L^{-1}	T/K
Selected value	1.80×10^{-3}	1.40	0.0750	0.310	309

It is known that oscillating chemical reactions are very sensitive to the foreign species both ion and molecule. We therefore investigated effects of some common inorganic ions and organic compounds on the determination of L-Asp. Tolerance levels (defined as the maximum amount of foreign species causing an error lower than ± 5% (RSD) in the determination of 8.50×10^{-7} mol L^{-1} L-Asp) are shown in Table 2. Generally, inorganic ions and some organic compounds with small molecular weight have little influence on the determination.

Table 2. Influence of foreign species on the determination of L-aspartic acid

Foreign species	Tolerated ratio (foreign/ L-aspartic acid)
Zn^{2+}, Mn^{2+}, Ca^{2+}, Ni^{2+}	2000
NO_3^-, Cl^-, Br^-, I^-	800
Threonine	100
Lysine, Cysteine	20
L-Glutemic acid, Glycin	10

Perturbation of L-aspartic to the oscillating system

The copper(II)-catalyzed oscillating reaction between hydrogen peroxide and sodium thiocyanate has been widely studied by Orban[8] in 1986 and Luo[24] in 1988 proposing that the mechanism for this oscillating reaction consists of 30 kinetic steps involving 26 independent variables.[4] The centerpiece of the mechanism is the positive and negative feedback loops

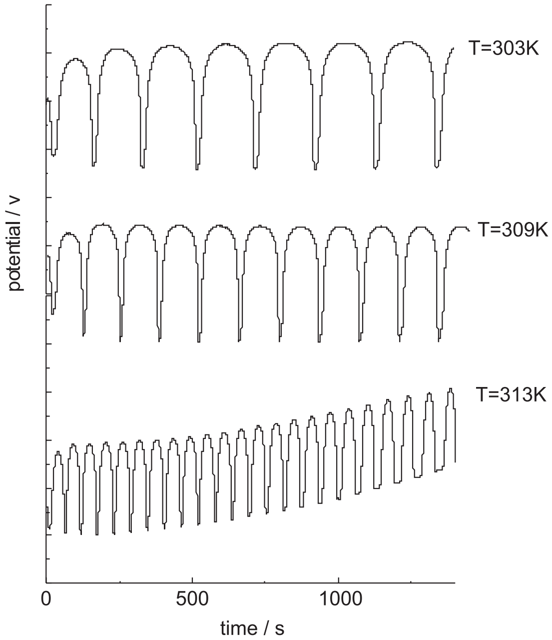

Figure 3. Influence of temperature on the oscillating profile. Other Experimental conditions are as same as the Figure 2 used.

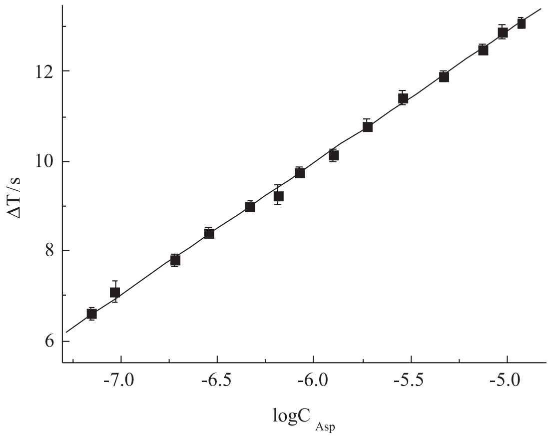

Figure 4. Calibration curve of the ΔT in period versus the logarithm of L-Asp concentration.

on which the autocatalytic process relies. Thus, the positive feedback loop produces the yellow superoxide-copper(I) complex $[HO_2\text{-Cu(I)}]$ that governs the rate of the process:

$$OS(O)CN^- + OOS(O)CN^- + H_2O \Leftrightarrow 2OS(O)CN\bullet + 2OH^-$$
$$OS(O)CN\bullet + Cu^+[SCN]_n \rightarrow OS(O)CN^- + Cu^{2+} + nSCN^-$$
$$H_2O_2 + Cu^{2+} + OH^- \Leftrightarrow HO_2\text{-Cu(I)} + H_2O$$

which disappears in the negative feedback loop:

$$HO_2\text{-Cu(I)} + nSCN^- \rightarrow Cu^+[SCN^-]_n + HO_2\bullet$$
$$OS(O)CN^- + HO_2\bullet \rightarrow SO_3\bullet^- + HOCN$$
$$SO_3\bullet^- + Cu^+[SCN^-]_n \rightarrow SO_3^{2-} + Cu^{2+} + nSCN^-$$

where n is 2, 3, or 4.

Intermediate $OS(O)CN^-$ is produced very slowly in the following reaction:

$$2OOS(O)CN^- + OH^- \rightarrow OS(O)CN^- + SO_4^{2-} + HOCN$$

and it initially has a very low concentration. It is generated auto-catalytically through the formation and reduction of the radical $OS(O)CN\bullet$, when the concentration of $OS(O)CN^-$ and $Cu^+[SCN^-]_n$ become high enough to make this pathway significant. The Cu^+ is simultaneously oxidized to Cu^{2+}, and the yellow superoxy complex is produced almost immediately. The sharp increase in color stops when most of the Cu^+ has been consumed. The amino in the L-aspartic acid molecule may be oxidized by peroxohypothiocyanate ion ($OS(O)CN^-$), which may delay both the positive and negative feedback loop, this may prolong the survival of the yellow superoxide-complex and increase the oscillating period as observed in practice.

Determination of L-aspartic acid in pharmaceutical

We assessed the potential of the proposed method for determining L-Asp in three samples that produced by different pharmaceutical company. 100 μL of sample solution was diluted to 100 mL with distilled-deionized water. To test the accuracy of the proposed procedure, we employed HPLC method to determine the content of L-Asp in the same pharmaceutical samples. Each real sample was analyzed for 8 times in parallel and the results are listed in Table 3.

Table 3. Determination of L-aspartic acid by using different methods for pharmaceutical samples

Method	Sample		Average found/ mg cm^{-3}	% RSD
	labelled	amount /mg cm^{-3}		
Present method	1	79~91	81.9	2.52
	2	85.0	83.9	2.41
	3	45.1	46.1	2.59
HPLC	1	79~91	82.0	1.49
	2	85.0	84.5	1.51
	3	45.1	45.7	1.55

Notes: 1-Potassium aspartate and magnesium aspartate injection (Shandong Chenxin Pharmaceutical Co. Ltd, China). 2-Potassium aspartate and magnesium aspartate injection, (Chengdu Tiantaishan Pharmaceutical Co. Ltd, China). 3-Potassium magnesium aspartate oral solution, (Suzhou Changzheng-Xinkai Pharmaceutical Co. Ltd, China).

Conclusions

A simple and convenient method for the determination of L-Asp without pretreatment was developed. Compared with other analytical instruments, the set-up used in the proposed method is rather cheap in price. However, the sensitivity and reproducibility are very well. It can offer a linear range of 7.10×10^{-8} - 1.17×10^{-5} mol L^{-1} (RSD 2.75%), with a lower limit of detection (LOD) of 5.58×10^{-8} mol L^{-1}. Simple set-up, convenient operation would be easy received by clinic analysis.

Acknowledgements

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