

Determination of Cystein and Methionine by Oscillating Chemical Reaction using Conductometry Technique

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A new analytical method for determination of cystein and methionine is presented in this report. Sequential perturbation caused by different amounts of cystein and methionine on the oscillating chemical system of H_2O_2 -KSCN- CuSO_4 in an alkaline medium is proposed. The method relies on the linear relationship between the changes in the oscillation period of the chemical system and the concentration of cystein and methionine. Monitoring of oscillating frequency system was performed using a simple analytical method namely conductometry technique. Linear dynamic range were obtained in the ranges of $3.25 \times 10^{-5} \text{ M} - 2.35 \times 10^{-4} \text{ M}$ and $2.6 \times 10^{-4} \text{ M} - 1.1 \times 10^{-3} \text{ M}$ for cysteine and methionine respectively. Detection limits (2δ) of $2.1 \times 10^{-5} \text{ M}$ and $8.9 \times 10^{-5} \text{ M}$ were obtained for cysteine and methionine respectively. This is the first reported determination of cysteine and methionine based on an oscillating reaction by measuring the conductivity of the solution.

Keywords: Conductometry; Oscillating chemical system; cysteine; Methionine.

1. INTRODUCTION

Some far-from-equilibrium chemical systems exhibit an oscillating behavior as a result of their complex mechanisms including autocatalytic step [1], such systems are usually referred to as "oscillating reactions". Oscillating reactions can take place both in the liquid phase [2-4] and in the gaseous phase from heterogeneous catalytic systems [5]. Oscillating chemical reactions are always complex involving a large number of chemical species, which can be categorized as reactants, products, and intermediates that interact by unusual mechanisms [6, 7].

Basically, an oscillating chemical reaction is one where some species (usually a reaction intermediate) exhibits fluctuations of concentration; such fluctuations are periodic or non-periodic under specific reaction conditions. For such oscillating reactions, at least three requirements must be met [8]; (a) the system should be far from thermodynamic equilibrium, under such conditions,

spontaneous oscillations can be generated [9]; (b) there should be at least one autocatalytic step or, alternatively, cross-catalysis between two steps of their action mechanism; and (c) the system should possess at least two steady states under the initial conditions.

Several methods, such as pH-metry [10], potentiometry [11,12], luminometry [13,14], conductometry [15] and fluorimetry [16] have been applied to investigate of oscillating reaction

One of us (Samadi-Maybodi) for the first time reported that the oscillating system, H_2O_2 – KSCN – CuSO_4 – NaOH , could be easily monitored by measurement of the electric conductivity in solution [15]. Although this technique is very simple and very common, such studies have not been reported yet for determination of compounds by an oscillating reaction.

2. EXPERIMENTAL PART

Stock solutions were prepared from commercially available reagent-grade (Merck) potassium thiocyanate, 30% hydrogen peroxide, sodium hydroxide, copper sulfate pentahydrate; redistilled water was used throughout. The electric conductivity of the oscillating system was recorded with conductometer, JENWAY 4020. The apparatus was connected to a personal computer via an interface. The conductivity was calibrated using an aqueous solution of potassium chloride (1 M) of known conductivity. Stock solutions of cysteine and methionine were prepared with doubly distilled water. Solutions with lower concentrations were prepared freshly just prior to use.

2.1. Procedure

In a beaker solution contains 0.85 mol/L hydrogen peroxide, 0.122 mol/L sodium thiocyanate, 0.15 mol/L sodium hydroxide and 1.22×10^{-3} mol/L Cu(II) with final volume of 10 ml was prepared. Soon after mixing of the ingredients, the conductivities of the solution were recorded. Data were acquired at a rate of 1 point/s using software. All experiments were carried out at constant temperature (ca. 25 °C) using water jacket.

As soon as the signal and time increments for the oscillations leveled off, variable volumes (a few microlitres) of cysteine or methionine were sequentially injected. The oscillating periods was used as the measured parameter (i.e. analytical signal) to construct the calibration plot.

3. RESULTS AND DISCUSSION

Despite usual chemical reaction, during an oscillating chemical reaction the concentration of reactants decrease, the concentration of products increase, but the concentrations of intermediate or catalyst species execute oscillations as the conversion of reactants to products rushes toward equilibrium (Scheme 1).

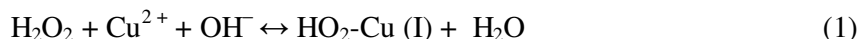


Scheme 1.

Mechanisms for oscillatory chemical systems have been pursued almost as soon as chemists finally recognized the possibility of oscillation in a homogeneous medium [17–21]. Understanding why and how such complicated behavior arises in terms of chemical species and the interaction among them is a fascinating subject in itself. In addition, this understanding facilitates the further design of new chemical oscillators. A successful mechanistic study also contributes to the knowledge of the general chemistry and kinetics of the chemical species that are involved.

In this study conductometry method was used to characterize the oscillating conductance in the system $\text{H}_2\text{O}_2\text{--KSCN--CuSO}_4\text{--NaOH}$. The mechanism for this oscillating system reaction was established from previous investigations by several authors [17-20]. The oscillation reaction of the $\text{H}_2\text{O}_2\text{--KSCN--CuSO}_4\text{--NaOH}$ system consists of 30 kinetic reactions using 26 independent variables. These reactions can be categorized into three groups, each containing reactions specific to one part of the overall reaction [21] that is briefly discussed below.

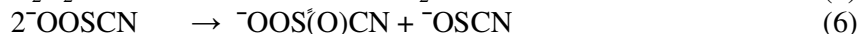
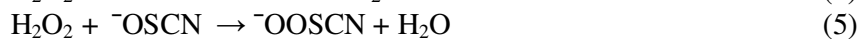
I. Reaction between H_2O_2 and Cu^{2+} ,



Where n may be 2, 3, or 4.

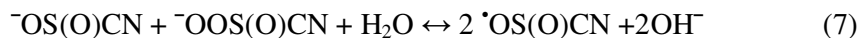
These reactions are chosen to represent the major effects of the copper-catalyzed alkaline H_2O_2 decomposition in the mixed reaction and to provide two essential intermediates, HO_2^- and $\text{Cu}^+ \{\text{SCN}\}_n$ for the copper part of the oscillation .

II. Reactions between H_2O_2 and KSCN,



This group of reactions describes a route for the oxidation of SCN. The mechanisms agrees with the experiments in the shape and frequencies of oscillation.

III. Reactions in a feedback loop:



These reactions explain a feedback loop that generates oscillatory behavior of this system. Ions have electrical conductance and variation in their concentration result in the variation of the

conductance. Therefore it can be proposed that the oscillating reaction causes fluctuation of the ionic species and consequently the conductance of the solution is oscillated.

3.1. Influence of experimental variables on the oscillating conductance

In order to ensure the maximum possible sensitivity and precision in the determination as well as the ability to perform large series of analyses, working conditions were optimized considering three factors, namely: (i) accomplishing the maximum oscillation amplitude to ensure higher sensitivity for determination of L-cysteine and methionine; (ii) obtaining oscillation period to allow the effect of the L-cysteine and methionine perturbation on the system to be accurately determined; and (ii) ensuring the maximum stability of the oscillating system to obtain reproducible results.

Series of experiments were performed, in each, the concentration of one of the species was changed but the others were held constant. The final concentration of the species was the same, except for the species that should be varied. Soon after mixing of the ingredients, the conductivity of the solution as a function of time was recorded. The concentration of ingredients can affect on the oscillating period (or oscillating frequency), this was proved by different techniques [10-16]. All experiments were carried out at room temperature (ca. 28 °C).

The influence of the sodium thiocyanate concentration was investigated over the range from 8.0×10^{-2} to 18.0×10^{-2} mol/L, and the results were shown in Figure 1. As can be seen, the oscillation period increased moderately with increasing in sodium thiocyanate concentration. However, at higher concentration 8.0×10^{-2} of KSCN the oscillating reaction is totally disturbed and no oscillating conductance can be observed at this circumstances.

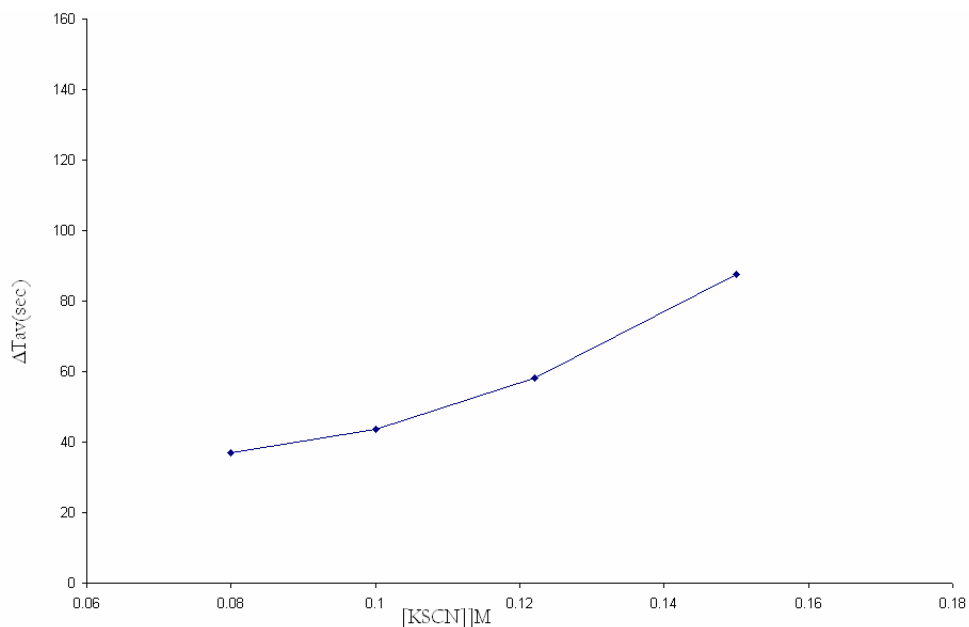


Figure 1. Influence of concentration of KSCN on period of oscillatory conductance at different KSCN concentrations [(a) 0.080, (b) 0.100, (c) 0.122 (d) 0.150 and (E) 0.170 M] with constant concentration of $[H_2O_2]$, 0.85 M; $[Cu(II)]$, 1.22×10^{-3} M and $[NaOH]$, 0.15 M.

The effect of the hydrogen peroxide concentration was studied over the range from 0.4 to 1.2 mol/L. Low hydrogen peroxide concentrations were found to prolong the induction period and adversely affect the oscillating system (Fig. 2). As the hydrogen peroxide concentration increased, the oscillation period decreased, and the induction period was shortened. By increasing hydrogen peroxide concentration more than 0.8 mol/L, the oscillation period is decreased slightly. A concentration of 0.85 mol/L was finally adopted as optimal.

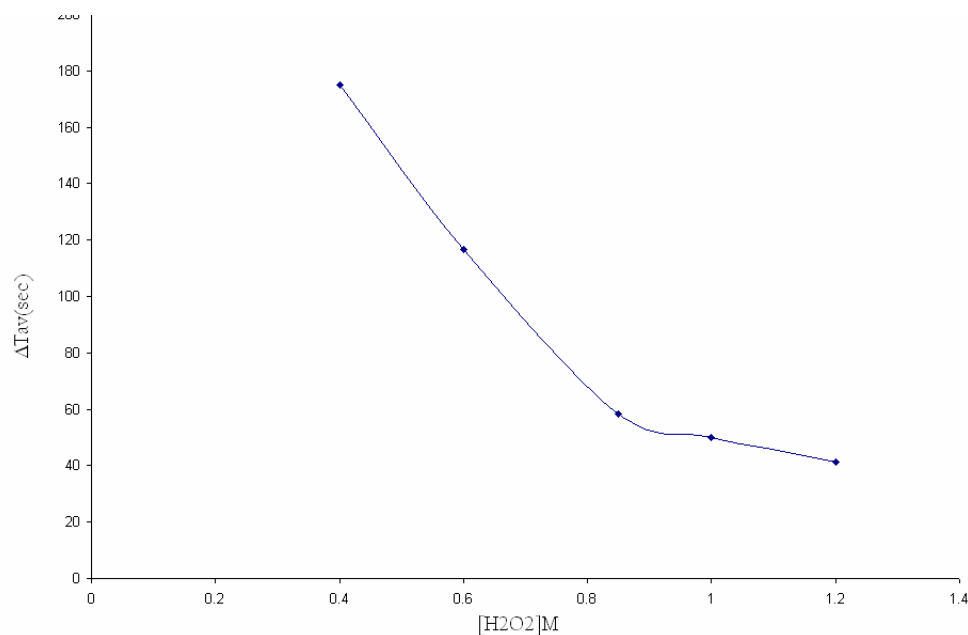


Figure 2. Influence of concentration of hydrogen peroxide on period of oscillating conductance at different concentrations of H₂O₂ [(a) 0.40, (b) 0.60, (c) 0.85 (d) 1 and (E) 1.20 M] with constant concentrations of [Cu(II)], 1.22×10^{-3} M; [NaOH], 0.15 M and [KSCN], 0.122 M.

The effect of the Cu(II) concentration on the behavior of the oscillating system over the range from 8.0×10^{-4} to 1.8×10^{-3} mol/L was studied. The oscillation period was found to decrease with increasing copper(II) concentration (Fig. 3). The oscillating period almost does not change at higher concentration of 1.8×10^{-3} mol/L of Cu(II) concentration. A 1.22×10^{-3} mol/L Cu(II) concentration was selected as optimal since it caused the system to oscillate highly uniformly prior to the perturbation and responded quite predictably.

Sodium hydroxide concentration was changed between 0.10 and 0.20 mol/L. As the concentration increased, the oscillation period decreased (Fig. 4). When sodium hydroxide concentration is higher than 0.14 mol/L, the oscillation period would maintain constant, a concentration of 0.15 mol/L was finally chosen as an optimized condition

Figure (5) shows the temporal evolution oscillation conductance in the system H₂O₂–KSCN–CuSO₄–NaOH with optimized concentration of H₂O₂, 0.85 M; KSCN, 0.122 M; CuSO₄, 1.22×10^{-3} M, and NaOH 0.15 M.

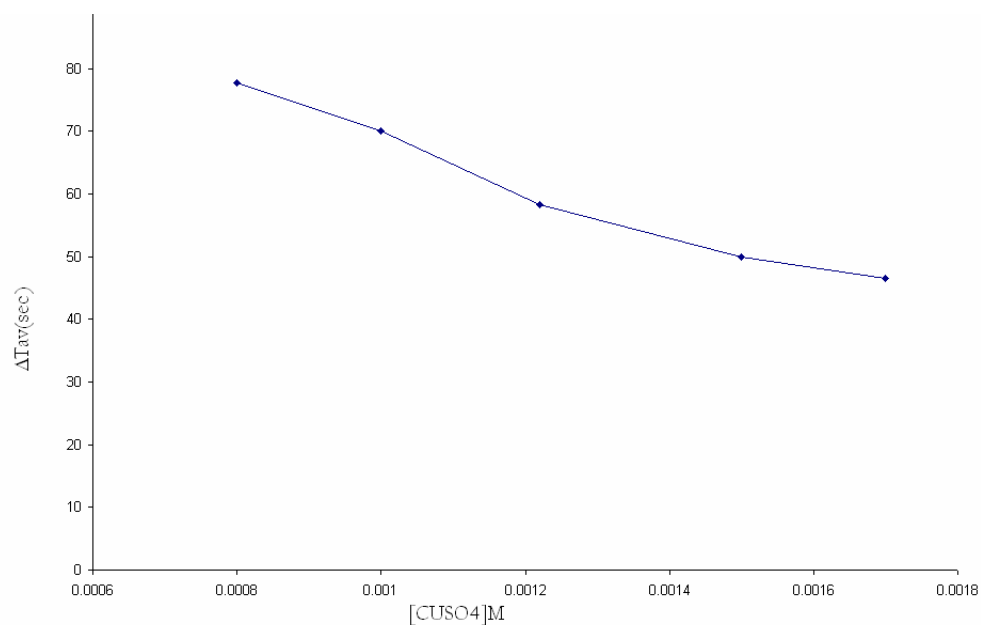


Figure 3. Influence of concentration of copper (II) on period of oscillating conductance for the H_2O_2 – KSCN – CuSO_4 – NaOH system with different copper concentrations [(a) 0.0008, (b) 0.0010, (c) 0.0012 (d) 0.0015 and 0.0017 M] with constant concentrations of $[\text{NaOH}]$, 0.15 M, $[\text{KSCN}]$, 0.122 M, and $[\text{H}_2\text{O}_2]$ 0.85 M.

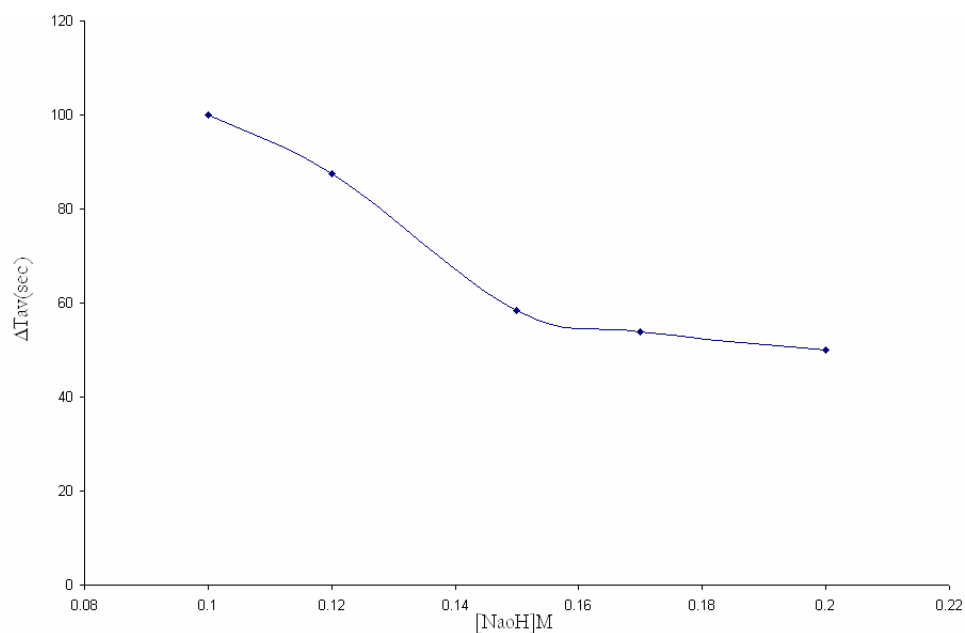


Figure 4. Influence of concentration of sodium hydroxide on period of oscillating conductance for the H_2O_2 – KSCN – CuSO_4 – NaOH system with different sodium hydroxide concentration [(a) 0.10, (b) 0.12, (c) 0.15 (d) 0.17 and (E) 0.2 M] with constant concentration of $[\text{KSCN}]$ 0.122 M, $[\text{H}_2\text{O}_2]$ 0.85 M and $[\text{Cu(II)}]$ 1.22×10^{-3} M

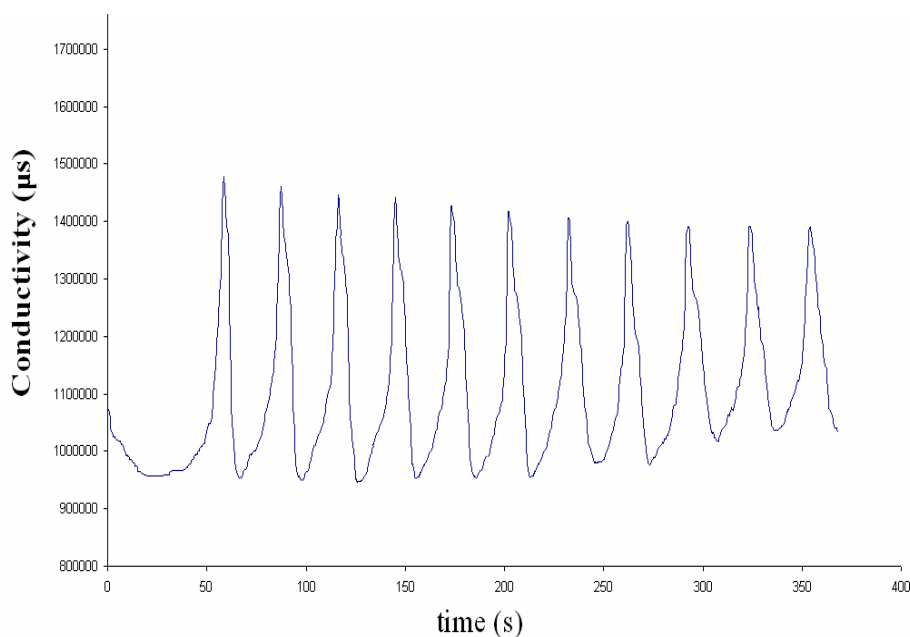


Figure 5. Temporal evolution of oscillatory conductance for the H_2O_2 –KSCN– CuSO_4 –NaOH system with final concentration of H_2O_2 , 0.85 M; KSCN, 0.122 M; CuSO_4 , 1.22×10^{-3} M, and NaOH 0.15 M.

3.2. Determination of Cysteine and Methionine

L-cysteine and methionine can interrupt the oscillating chemical system. In this study the period of oscillating ratio (P_r) is defined as an analytical signal which is referred to the ratio of the oscillating period after (P_a) and before (P_b) injection of the foreign species (i.e. L-cysteine or methionine).

$$P_r = P_a/P_b$$

Different concentrations of the L-cysteine and/or methionine was added to the reaction vessel containing H_2O_2 , KSCN, CuSO_4 and NaOH with final concentration of 0.122, 0.85, 1.22×10^{-3} , and 0.15 M respectively.

Calibration curves for determination of L-cysteine and methionine were constructed under the optimal conditions. The results, which were shown in Figs 6 & 7, exhibit the good relationship between the P_r and the concentrations of L-cysteine and methionine. The analytical parameters of this method were listed in Table 1. Calibration equations for L-cysteine and methionine were obtained as follows :

$$Y = 2268X + 1.0204$$

and

$$Y = 521.24 X + 0.9893$$

where Y is the conductivity in unit of micro siemens and X is the concentration in molar, it can be calculated that the detection limit (2σ) for L- cysteine and methionine are 2.55×10^{-5} and 8.9×10^{-5} mol/L respectively.

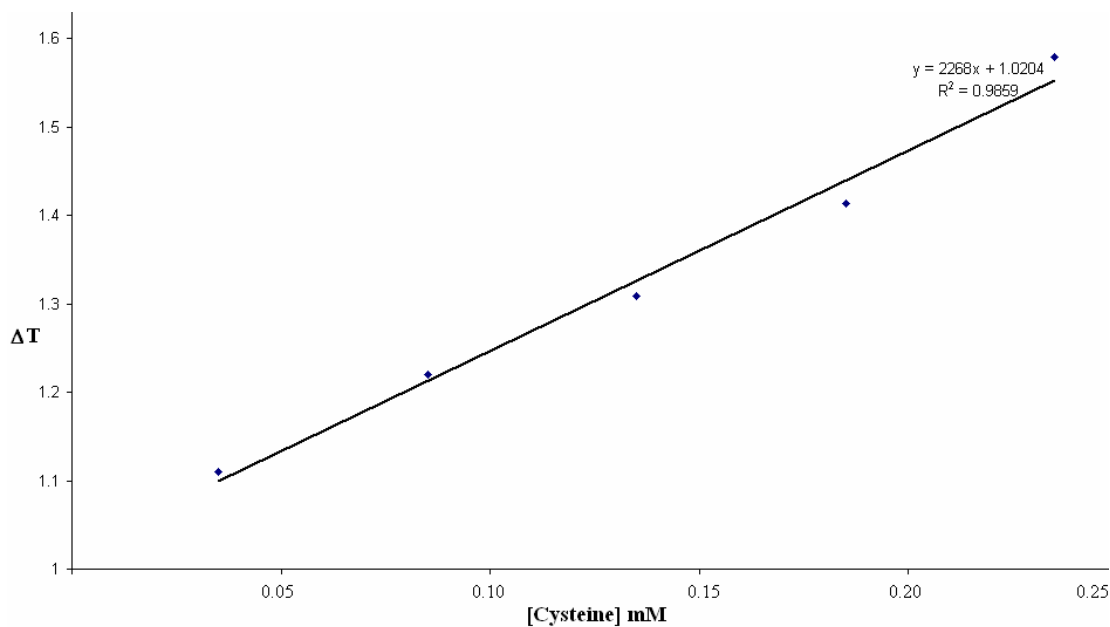


Figure 6. Calibration curve for the period with variable amounts of cysteine.

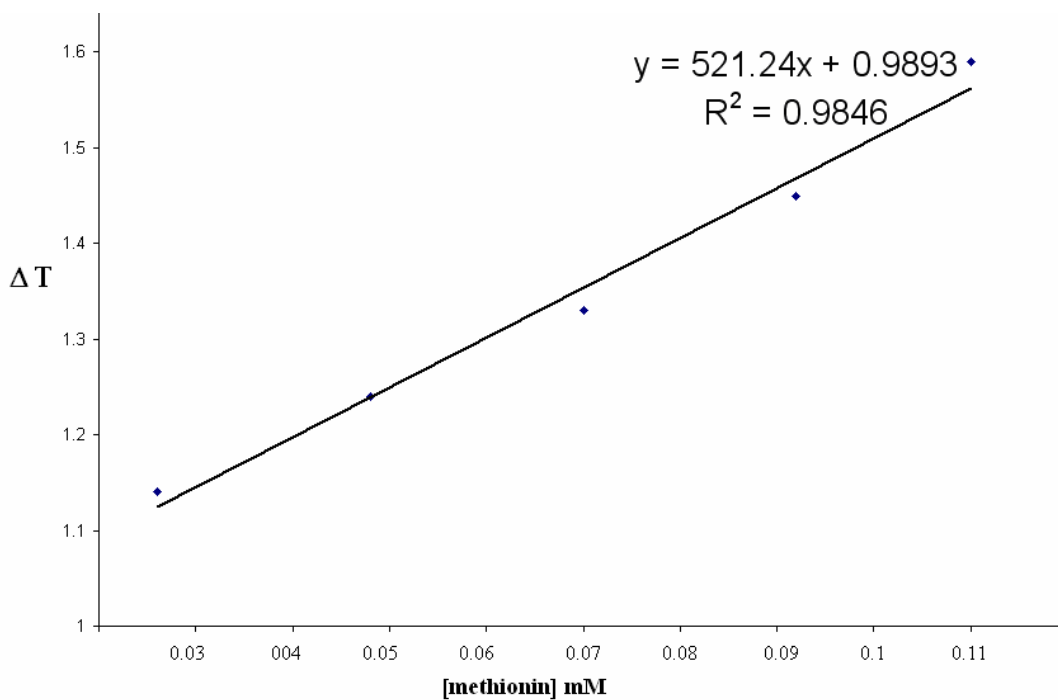


Figure 7. Calibration curve for the period with variable amounts of methionine

Table 1. Linear relationship for the determination of L-cysteine and methionin.

	Dynamic rang (M)	detection limit (M)	regression coefficient
L-cysteine	$3.25 \times 10^{-5} - 2.35 \times 10^{-4}$	2.55×10^{-5}	0.9929
Methionine	$2.6 \times 10^{-4} - 1.1 \times 10^{-3}$	8.9×10^{-5}	0.9922

3.3. Interferences

Cysteine and methionine are two sulfur-containing amino acids. As expected, methionine by sulfur atom in its structure has definite effect in the determination of L-cysteine (and vice versa), but the others have no effect in the determination of Cystein (or methionine) by this method that shows good selectivity of method. As an example some of amino acids such as glycine, alanine and leucine were examined results are shown in Table 2.

Table 2. Influence of some amino acids on the determination of L-cysteine

Ions and species	Tolerated ratio
Glycine	50
b-alanine	100
Leucine	200

Its supposed that the presence of sulfur atom in the structure of L-Cysteine result in a high capability of L-Cysteine in complexion with Cu (II), that has a catalyst role in the oscillating chemical reaction, remove the Cu (II) from the oscillating cycles can strongly affects on the oscillating reaction. Also methionin, with a sulfur atom in its structure like L-Cysteine, has a large unsuitable effect in the determination of L-Cysteine by this method.

Cysteine (or methionin) can react with Cu(II) through SH site and forms a stable complex. Concentration of Cu(II) is decreased to a varying degree in the presence of cysteine to form a stable complex with copper ion, i.e. it plays as a role of static quenching. As a result, by increasing the concentration of cysteine (or methionin), less amount of copper ions are available to be involved in the oscillating reaction and consequently the number of pulses are reduced.

4. CONCLUSIONS

The work here presents determination of cysteine and methionin by perturbation of oscillating conductance in the system of H_2O_2 -KSCN-CuSO₄-TMAOH using conductometry method. Results indicated that the period of the oscillating is changed by variation of cysteine or methionin concentration Linear dynamic range were obtained in the ranges of $3.25 \times 10^{-5} \text{ M} - 2.35 \times 10^{-4} \text{ M}$ and

2.6×10^{-4} M – 1.1×10^{-3} M of cysteine and methionine respectively. Detection limits (2δ) of 2.1×10^{-5} M and 8.9×10^{-5} M were obtained for cysteine and methionine respectively.

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