Fluorimetry Studies of Oscillating Chemilumnescence in the Luminol−H₂O₂−KSCN−CuSO₄−TMAOH System

Abdolraouf Samadi-Maybodi,[1,2] Niloofar Naseri,[1] and M. J. Chaichi[1]

Oscillating chemical reactions are complex systems, involving a large number of chemical species. In oscillating chemical reaction, some species, usually a reaction intermediate, exhibit fluctuation in its concentration. In this report, visible oscillating chemiluminescence produced by the addition of luminol (3-aminophthalhydrazide) to the oscillating system of H_2O_2—KSCN—$CuSO_4$—TMAOH was investigated using spectrofluorimetry method. The effects of ingredient concentration of the oscillating system and complexing agents like citric acid and cysteine on the behavior of the oscillating system were investigated. Moreover, the influence of nonaqueous solvents such as ethanol and ethylene glycol has been studied.

KEY WORDS: Oscillating chemiluminescence; luminol; fluorimetry; complexing agent; non-aqueous solvents.

INTRODUCTION

Oscillating chemical reactions are always complex systems involving a large number of chemical species, which can be categorized as reactants, products and intermediates that show unusual mechanisms [1,2]. Basically, an oscillating chemical reaction is the one where some species (usually a reaction intermediate) exhibit fluctuations in their concentration; such fluctuations are periodicity or non-periodicity under specific reaction conditions. For such oscillating reaction, at least three requirements must be met [3]. (a) The system should be far from thermodynamic equilibrium, under such conditions, spontaneous oscillations can be generated [4]; (b) there should be at least one autocatalytic step or, alternatively, cross catalysis between the two steps of the reaction mechanism; and (c) the system should possess at least two steady states under the initial conditions.

The fluctuations are reflected in color changes (if the oscillating species exhibit different colors), pH changes (variations in the H^+ or OH^- concentration in the medium), redox potential changes, *etc*. Several methods, such as pH metery, potentiometry [5–8] and chemiluminescence [9–11], have been applied in the investigation of oscillating reactions. One of the oscillating reactions reported recently has an initial composition of H_2O_2—KSCN—$CuSO_4$—TMAOH, determined using conductometry method [12].

The sharp pulses of blue light ($\lambda_{max} = 424$ nm) produced by the addition of luminol to the oscillating system H_2O_2—KSCN—$CuSO_4$—TMAOH are unlike the oscillation characteristic to this system. In basic aqueous solution, chemiluminescence results from the oxidation of luminol by hydrogen peroxide catalyzed by a transition-metal ion such as copper or cerium. The light-generating reaction of luminol has been the subject of many researches.

Typical oscillation attributes such as the oscillation period and amplitude were recently used to evaluate their use in chemical analysis [7,8]. In regular oscillations, the oscillating period and amplitude depend strongly on the concentrations of the reaction ingredient [11].

[1] Department of Chemistry, Faculty of Basic Science, Mazandaran University, Babolsar, Iran.
[2] To whom correspondence should be addressed. E-mail: samadi@umz. ac.ir

In this study, using spectrofluorimetry technique, both factors were studied with luminol—H$_2$O$_2$—KSCN—CuSO$_4$—TMAOH system. We have performed a series of experiments in which for each series, the concentrations of one of the species was varied and the other was held constant. The effects of complexing agent such as citric acid and L-cysteine also have been examined on the behavior of oscillating reaction. Furthermore, the influences of alcohols such as ethanol and ethylene glycol have been studied.

EXPERIMENTAL

Reagents

Stock solutions were prepared from commercially available reagent-grade (Merck); potassium thiocyanate, 30% hydrogen peroxide, tetramethyl ammonium hydroxide (TMAOH), copper sulphate pentahydrate, luminol, citric acid and cysteine without further purification. Bi-distilled water was used throughout.

Apparatus

The oscillating reaction was monitored by a spectrofluorimeter (Filter Fluorimetry, LS-2B Perkin-Elmer). To record the light emitted from the oscillating chemiluminescence reaction, no excitation source was applied to the sample cell. The sharp pulses of blue light ($\lambda = 424$ nm) produced by the addition of luminol have been recorded.

Data were collected and excel program was used for plotting graphs (intensity *vs.* time). Experiments were carried out at ambient temperature *ca.* 24°C and chemiluminescence intensity is given in terms of relative units.

RESULTS AND DISCUSSIONS

Despite the usual chemical reaction, during chemiluminescence 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).

$$A + B + \cdots \leftrightarrow X,Y,\cdots \leftrightarrow P + Q + \cdots$$

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 [13–15].

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, spectrofluorimetry technique has been used to characterize the oscillating chemiluminescence in the system of luminol—H$_2$O$_2$—KSCN—CuSO$_4$—TMAOH. The mechanism of the oscillating reaction was established from previous investigation by several authors [1–10]. The oscillation reactions of such a system consist of 30 kinetic reactions using 26 independent variables. The reaction between hydrogen peroxide and sodium thiocyanate in alkaline medium is catalyzed by copper(II) and is first-order in each reactant [4,6,16].

$$4H_2O_2 + SCN^- \rightarrow HSO_4^- + NH_4^+ + HCO_3^- + H_2O$$

$$(1)$$
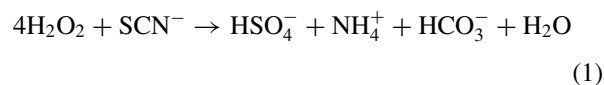
The rate-determining step is

$$4H_2O_2 + SCN^- \rightarrow HOSCN + OH^- \qquad (2)$$

It is proposed that the SCN$^-$ acts to separate in time the steps in which copper(I) forms and the latter is temporarily stabilized by SCN$^-$. Copper(I) is then reoxidized to the bivalent state with the simultaneous oxidation of the SCN$^-$ [6].

However, recent studies [16] have revealed the occurrence of other intermediates such as cyanosulfite, $^-$OS(O)CN, peroxocyanosulfite, $^-$OOS(O)CN, hypothiocyanite, $^-$OSCN, and peroxohypothiocyanate ions, $^-$OOSCN.

In order to be able to carry out large number of determinations with the maximum possible sensitivity, the influence of selected experimental variable was studied to achieve the maximum possible stability in the oscillating system over time.

Figure 1 shows the temporal evolution oscillation chemiluminescence in the system of luminol—H$_2$O$_2$—KSCN—CuSO$_4$—TMAOH with optimized final concentration of luminal (0.003 M), H$_2$O$_2$ (0.48 M), KSCN (0.069 M), CuSO$_4$ (5.5×10^{-4} M). The oscillating behavior is the same as reported with the other methods such as potentiometery [5–8] and luminometry [9–11]. The behavior of the oscillations can be influenced by changing the solvent or by employing foreign species.

Figure 2 shows the graph of oscillating light intensity with time for the system of luminol–H$_2$O$_2$–KSCN-CuSO$_4$–TMAOH with final concentration of luminal (0.003 M), H$_2$O$_2$ (0.48 M), KSCN (0.069 M), CuSO$_4$
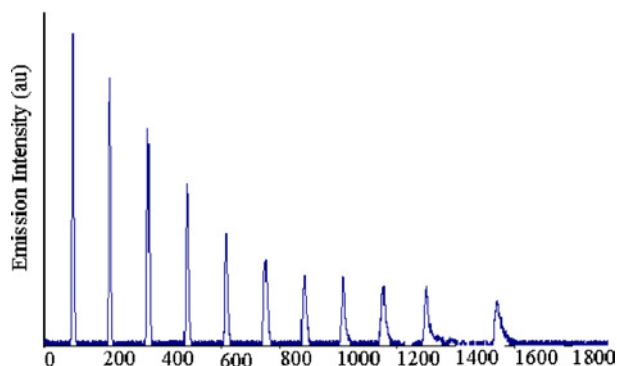
Fig. 1. A typical fluorescence spectrum of the luminol—H_2O_2—KSCN—$CuSO_4$—TMAOH oscillating system with final concentration of [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [$CuSO_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.

(5.5×10^{-4} M) and TMAOH (0.015 M). As can be seen, the oscillating light intensity decreased exponentially with time, which reveals the characteristic of pseudo-first-order reaction. The curve fitting was applied by solver program and satisfactory result have been obtained.

Figure 3 illustrates the fluorescence spectra of the oscillating chemiluminescence at constant concentrations of luminol (0.003 M), H_2O_2 (0.48 M), KSCN (0.069 M) and $CuSO_4$ (5.5×10^{-4} M), but different concentration of TMAOH, i.e., 9.0×10^{-3}, 1.2×10^{-2}, 1.5×10^{-2} and 1.8×10^{-2} M, respectively. As can be seen, increasing the concentration of TMAOH causes the number of oscillations to increase significantly, i.e. for concentration of 0.009 M of TMAOH six pulses can be observed; whilst for 0.018 M of TMAOH 11 pulses are observed. Results obtained from these experiments notify that the overall time of oscillating reaction greatly increases with increase in the concentration of TMAOH, while the period of oscillation does not change considerably with the variation in the TMAOH concentration.
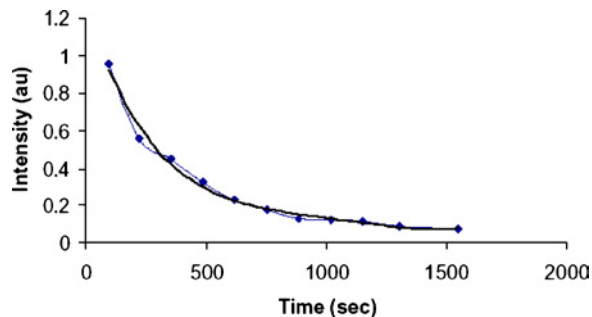

Fig. 2. Graph of oscillating light intensity *vs.* time for the luminol—H_2O_2—KSCN—$CuSO_4$—TMAOH oscillating system. Final concentration: [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [$CuSO_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.
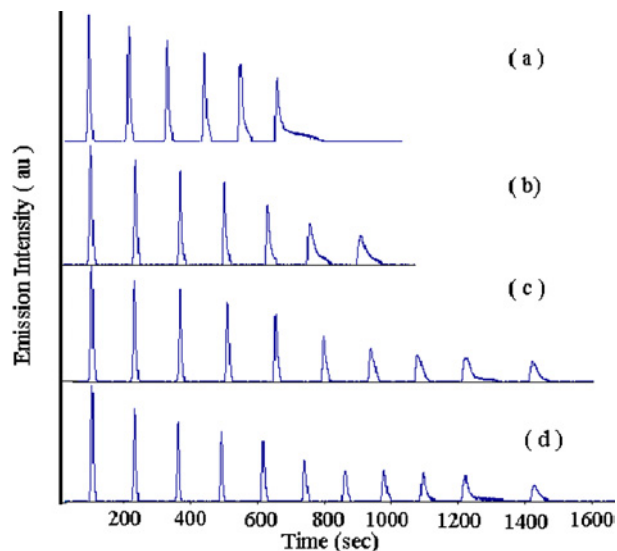

Fig. 3. Fluorescence spectra of luminol—H_2O_2—KSCN—$CuSO_4$—TMAOH oscillating system at different TMAOH concentrations [(a) 9.0×10^{-3}, (b) 1.2×10^{-2}, (c) 1.5×10^{-2} and (d) 1.8×10^{-2} M] with constant concentration of [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M and [$CuSO_4$], 5.5×10^{-4} M.

We also have performed a series of experiments in which for each series, the concentration of one of the species was varied but the other was held constant. Results obtained from these experiments were the same as previous work's, which studied this system using luminometry and conductometry methods [11,12].

Solvent Effect

To investigate the effect of non-aqueous solvents on the behavior of the oscillating chemilumnescence, we have examined ethanol and ethylene glycol as non-aqueous solvents. In these studies, during the oscillating reaction, the emitted light was monitored using the fluorimetry technique. Factors such as the number of pulses, period of oscillating, total time of oscillating chemiluminescence and intensity of emitted light have been studied.

The effect of ethanol on luminol–H_2O_2–KSCN–$CuSO_4$–TMAOH oscillating system was studied in a series of experiments at different ethanol concentration but constant concentrations of TMAOH, KSCN, Cu SO_4, H_2O_2 and luminol. This experiment has been performed at different ethanol concentrations of 0.0, 10, 30 and 60% v/v, and final concentration of luminal, H_2O_2, KSCN, Cu(II) and TMAOH of 0.003, 0.48, 0.069, 5.5×10^{-4} and 0.015 M, respectively.

The fluorescence spectra of the oscillating chemiluminescence in the presence of ethanol have been shown in Figs. 4a–4d. Results obtained from this experiment reveal
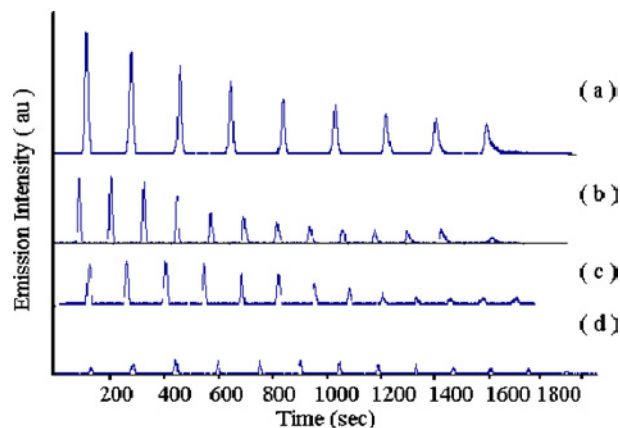
Fig. 4. Fluorescence spectra of luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH oscillating system in the presence of ethanol, (a) without ethanol, (b) 10% v/v, (c) 30% v/v and (d) 60% v/v ethanol. Final concentration: [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [CuSO$_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.

that by increasing the ethanol concentration, a significant reduction of light intensity occurs. Since solubility of luminol in alcohol is more than that of water, therefore by increasing ethanol, more amount of luminals interact with ethanol and accordingly less amount of luminol is involved in the oscillating reaction. Consequently, the emission intensity is decreased. However, it should be noted that no appreciable changes have occurred in the oscillating system, which means the other factors such as pulse duration (i.e. half width of light pulses), number of pulses and period of oscillation are moderately constant at different concentrations of ethanol. This can be expected, since ethanol has no direct effect on the oscillating reaction.

Figure 5 illustrates the fluorescence spectra of luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH oscillating system in the presence of ethylene glycol. This experiment has been performed at different ethylene glycol concentrations of 0.0, 10, 30 and 60% v/v, and final concentration of luminal, H_2O_2, KSCN, Cu(II) and TMAOH of 0.003, 0.48, 0.069, 5.5×10^{-4} and 0.015 M, respectively.

In spite of the earlier results, here no considerable changes can be seen by the addition of ethylene glycol and a regular oscillation is observed in all conditions. Therefore, it can be realized that ethylene glycol has no interaction with the ingredients of the oscillating system, i.e. no solute–solvent interaction is carried out by ethylene glycol. Consequently, ethylene glycol exerts no interruption in the oscillating reaction.

Effect of Copper Complexing Agents

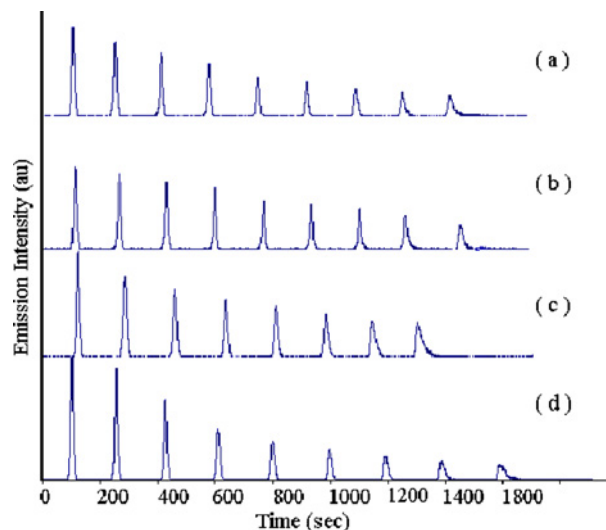Copper plays the role of a catalyst in the oscillating system. It was shown that the number of oscillations as

Fig. 5. Fluorescence spectra of luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH oscillating system in the presence of ethylene glycol, (a) without ethylene glycol, (b) 10% v/v, (c) 30% v/v and (d) 60% v/v ethylene glycol. Final concentration: [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [CuSO$_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.

well as the overall time of reaction decreases with decrease in the concentration of the copper [11,12]. Therefore, it is worthwhile to examine the effect of complexing agents in this system. Addition of complexing agent causes copper ion to react with those reagents and consequently affects the behavior of the oscillating system. In this study, citric acid and cysteine were used as complexing agents to be added in the oscillation system of luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH.

The fluorescence spectra of the luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH oscillating system were investigated at different concentrations of cysteine, 1.0×10^{-3}, 7.5×10^{-4}, 5.0×10^{-4} and 2.0×10^{-4} M, with constant concentration of luminal, H_2O_2, KSCN, Cu(II), and TMAOH with concentration of 0.003, 0.48, 0.069, 5.5×10^{-4} and 1.5×10^{-2} M, respectively. Figure 6 represents fluorescence spectra of the luminol–H_2O_2–KSCN–CuSO$_4$–TMAOH oscillating system in the presence of cysteine. Results obtained from this experiment reveal that by increasing the concentration of the cysteine, the number of oscillations is decreased. As can be seen, spectrum obtained from the highest concentration of cysteine (i.e. 0.001 M) shows only three signals (Fig. 6a), whilst spectrum obtained from the oscillating system without cysteine exhibits up to 11 signals.

Copper ion tends to react with sulfide ion to form an insoluble compound of CuS ($K_{sp} = 4 \times 10^{-38}$) [17]. Cu(II) can reacts with enzymes containing –SH group. Therefore, –SH groups of cysteine and glycine can react
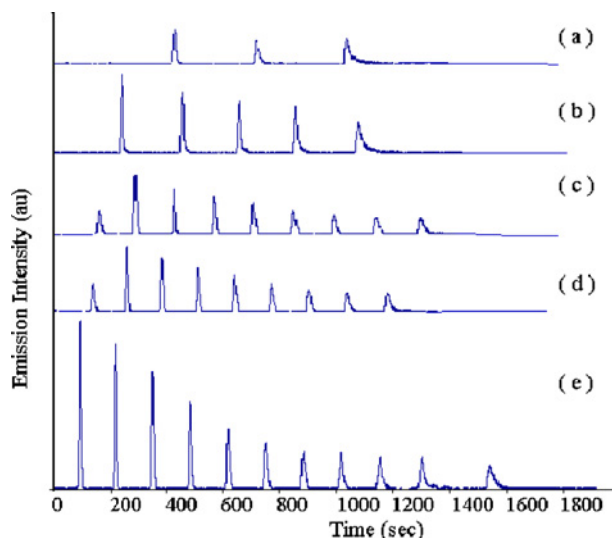
Fig. 6. Effect of cysteine on the fluorescence spectra of luminol–H_2O_2–KSCN–$CuSO_4$–TMAOH oscillating system, at different cysteine concentrations [(a) 1.0×10^{-3}, (b) 7.5×10^{-4}, (c) 5.0×10^{-4}, (d) 2.0×10^{-4} M and (e) without cysteine] with final concentration of [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [$CuSO_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.

with Cu(II) to form a stable complex. The formation constant of such complexes (K_f) is large, for example, the formation constant of the complex of Cu–glysine is 1.86×10^{16} (log $K_f = 16.27$) [18].

Cysteine 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, less amount of copper ions are available to be involved in the oscillating reaction and consequently the number of pulses are reduced. It is worthwhile to point out that the first pulse of light is delayed, as concentration of the cysteine is increased, i.e. the first signal appears at 66, 106, 135, 200 and 386 s for the solution containing, 0.0, 7.5×10^{-4}, 5.0×10^{-4}, 2.0×10^{-4} and 1.0×10^{-3} M, respectively (Fig. 6). This phenomenon also can be attributed to the formation of Cu–cysteine complex, which in terms it influences on the oscillating reaction.

The effect of citric acid was performed with final concentrations of cysteine 1.5×10^{-3}, 1.25×10^{-3}, 1.0×10^{-3} and 5.0×10^{-4} M, but constant concentrations of luminal, H_2O_2, KSCN, Cu(II), and TMAOH with concentration of 0.003, 0.48, 0.069, 5.5×10^{-4} and 1.5×10^{-2} M, respectively.

Figure 7 illustrates that citric acid significantly influences the behavior of the oscillating reaction. As can be

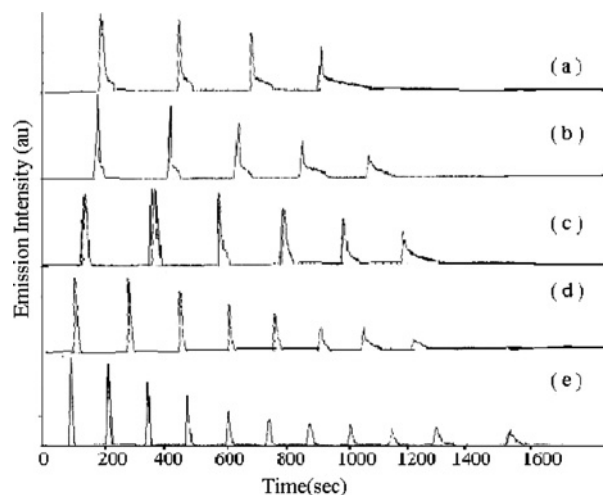**Fig. 7.** Effect of citric acid on the fluorescence spectra of luminol–H_2O_2–KSCN–$CuSO_4$–TMAOH oscillating system, at different citric acid concentrations of [(a) 1.5×10^{-3}, (b) 1.25×10^{-3}, (c) 1.0×10^{-3}, (d) 5.0×10^{-4} M and (e) without citric acid] with final concentration of [luminal], 0.003 M; [H_2O_2], 0.48 M; [KSCN], 0.069 M; [$CuSO_4$], 5.5×10^{-4} M and [TMAOH], 0.015 M.

seen, increasing concentration of the citric acid causes the period of the oscillating reaction to decrease; however, the pulse number of lights is increased. Copper ion tends to react with citric acid to form a strong complex; the formation constant of the complex is significant (log $K_f = 14.2$, where K_f is the overall formation constant) [18]. As a result, it can be deduced that by increasing the concentration of citric acid, the amount of Cu(II) involved in the reaction of oscillating system is reduced and consequently the oscillating frequency is decreased. Similar behavior has been reported when the copper concentration was decreased in luminol–H_2O_2–KSCN–$CuSO_4$–TMAOH oscillating system [11,12].

Results obtained from this experiment specifies that the citric acid can also affects the peak shape of signals (Fig. 7). Citric acid is a polyprotic acid ($HOC(CH_2COOH)_3$), which undergoes stepwise dissociation (p$K_1 = 3.128$, p$K_2 = 4.761$, p$K_3 = 6.396$) [17]. The pH of solution varies during the reaction of the luminol–H_2O_2–KSCN–$CuSO_4$–TMAOH oscillating system. Hence, it is expected that ionic species of LH_2^-, LH^{2-} and L^{3-}; where L: $HOC[(CH_2COO)_3]^{3-}$, can also present in the solution. This fact influences the oscillating reaction and consequently has an effect on the peak shape of the fluorescence spectra.

CONCLUSIONS

This work presents fluorimetry studies of oscillating chemiluminesecence in the system of

H_2O_2–KSCN–CuSO$_4$–TMAOH. Results have shown that although ethanol has no effect on the oscillating behavior, the light intensity is strongly influenced by changing the ethanol concentration. The copper complexing agents such as cysteine and citric acid are considerably affected by the behavior of the oscillating reaction.

REFERENCES

1. R. M. Noyes (1989). Some models of chemical oscillators. *J. Chem. Educ.* **66** (3), 190–191.
2. R. J. Field and F. W. Schneider (1989). Oscillating chemical reactions and nonlinear dynamic. *J. Chem. Educ.* **66**(3), 195–204.
3. I. R. Epstein (1989). The role of flow systems in far-from-equilibrium dynamics. *J. Chem. Educ.* **66**(3), 191–195.
4. R. J. Field, E. Koros, and R. M. Noyes (1972). Oscillating chemical system. II. Through analysis of temporal oscillation in the bromate–cerium–malonic acid system. *J. Am. Chem. Soc.* **45**(25), 8649–8664.
5. I. R. Wilson and G. M. Harris, (1960). Oxidation of thiocyanate, a pH-independent reaction. *J. Am. Chem. Soc.* **82**(82), 5415–4517.
6. M. Orbán (1986). Oscillation and bistability in the Cu(II)-catalysed reaction between H_2O_2 and KSCN. *J. Am. Chem. Soc.* **108**(22), 6893–6898.
7. R. Jiménes-Prieto, M. Silva, and D. P. Péres-Bendito (1995). Analytical pulse perturbation technique: A tool for analytical determinations in far-from equilibrium dynamic systems. *Anal. Chem.* **67**(4), 729–734.
8. R. Jiménes-Prieto, M. Silva, and D. P. Péres-Bendito (1998). Approching the use of oscillating reaction for analytical monitoring. *Analyst* **123**, 1R–8R.
9. S. Sattar and I. R. Epstein (1990). Interaction of luminol with the oscillating system H_2O_2–KSCN–CuSO$_4$–NaOH. *J. Phys. Chem.* **94**(1), 275–277.
10. J. Amrehn, A. Resch, and F. W. Schneider (1988). Oscillating chemiluminescence with luminol in the continuous flow stirred tank reactor. *J. Phys. Chem.* **92**(12), 3318–3320.
11. A. Samadi-Maybodi and S. M. Ourad (2003). Studies of visible oscillating chemiluminescence with a luminol–H_2O_2–KSCN–CuSO$_4$–NaOH system in batch reactor. *Luminescence* **18**(1), 42–48.
12. A. Samadi-Maybodi and M. K. Tavakoli-Amiri (2004). Studies of oscillating chemical in the H_2O_2–KSCN–CuSO$_4$–NaOH system using a conductometry method. *Transition Metal Chem.* **29**(7).
13. W. C. Bary (1921). A periodic reaction in homogeneous solution and its reaction to catalysis. *J. Am. Chem. Soc.* **43**, 1262–1266.
14. R. J. Field (1972). A reaction periodic time and space. *J. Chem. Educ.* **49**(5), 308–311.
15. B. L. Clarke (1992). Stoichiometric network analysis of the oxalate-persulfate-silver oscillator. *J. Chem. Phys.* **97**(4), 2459–2472.
16. Y. Luo, M. Orbàn, K. Kustin, and I. R. Epstein (1989). Mechanistic study of oscillations and bistability in the Cu(II)-catalyzed reaction between H_2O_2 and KSCN. *J. Am. Chem. Soc.* **111**(13), 4542–4548.
17. R. C. Weast (1978). *Handbook of Chemistry and Physics*, 1st student ed., CRC Press, Boca Raton, p. B-106.
18. J. A. Dean (1995). *Analytical Chemistry Handbook*, McGraw-Hill, New York.