The Study of Chemiluminescence of Acridinium Ester in Presence of Rhodamin B as a Fluorescer

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ABSTRACT: *The reaction of hydrogen peroxide and pheny -9-acridinecarboxylate in the presence of rhodamin B as a fluorophore leads to the emission of either bright blue light or bright red light, depending on the structure of fluorescer and reaction conditions. The light emitter responsible for the Chemi Luminescence (CL) reaction is acridone and that the blue light produced with out the fluorescers is a result of direct CL. The intensity and kinetic parameters for the CL systems were evaluated from fitting of the resulting intensity-time plots. The CL systems were investigated as concentration dependent of fluorescer, hydrogen peroxide, acridinium ester and sodium hydroxide as a catalyst.*

KEY WORDS: *Chemiluminescence, Acridinium ester, Rhodamin B, Lucigenin, Hydrogen peroxide.*

INTRODUCTION

Chemi- and bioluminescence reaction that efficiently produce singlet states are generally considered to require dioxetane or dioxetanone intermediates as keys to the excitation process [1,2]. The synthesis and investigation of the properties of acridine-based compound are not new. They are used in a wide variety of applications from dyestuffs to antibacterial agents and chemiluminescence [3,4]. The other well known CL reagents are lucigenin, a bis acridinium, and a hydrazid, luminol [5,6].The chemiluminescence efficiency of acridinium ester are often better than that of lucigenin and luminol. The mechanism of light emission of the acridinium salts is very well understood, Fig. 1.

Particular advantages of these compounds are the very low background light level and the extreme simplicity of the light yielding reaction conditions, no catalyst other than base being required [7,8].

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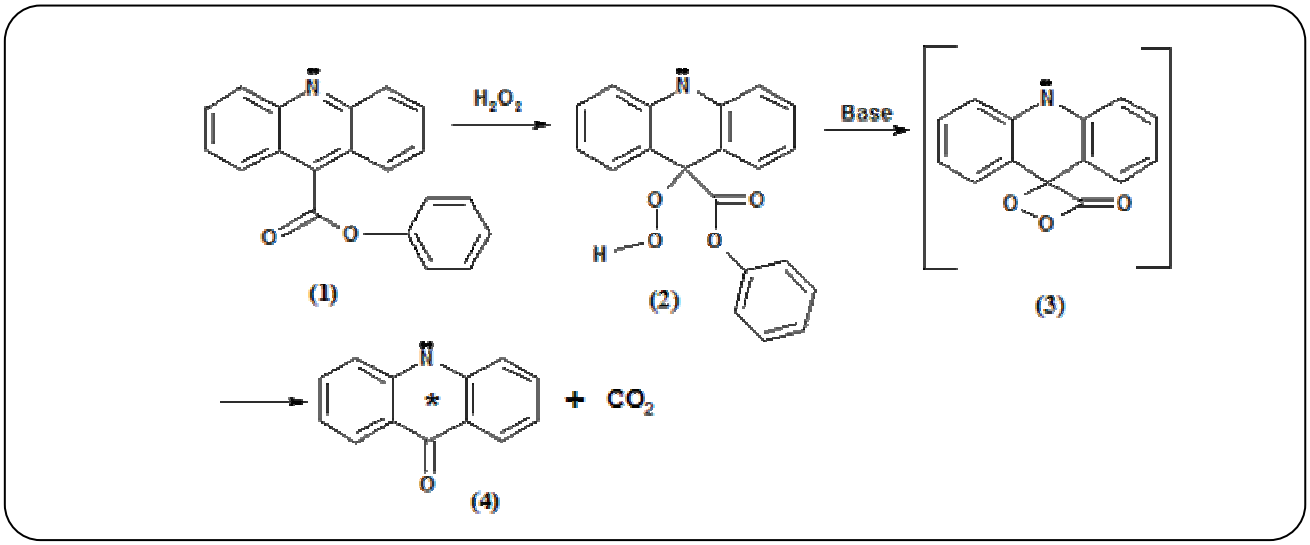

Fig. 1: Mechanism of the CL of acridinium esters.

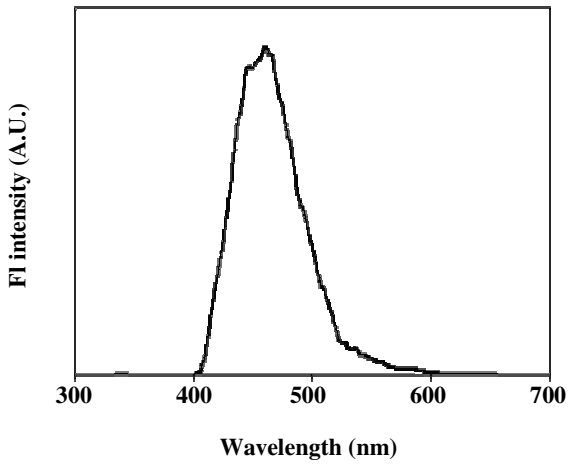

Fig. 2: The fluorescence of axcited acridone.

Hydrogen peroxide as an oxidizing agent in the CL system is more nucleophilic than hydroxyl ion and the Peroxide (2) is formed in about 90% yield. The dioxetanone (3) is an intermediate and decomposes in a fast step to the excited acridone(4), the fluorescence of which is blue (442 nm), Fig. 2.

The similarity between CL of peroxy oxalate and acridinium ester is the formation of dioxetane intermediate and the role of acidic phenol as a leaving group [8,9]. The reaction between phenyl-9-acridine carboxylate (acridinium ester) with hydrogen peroxide in the presence of fluorophores lead to the emission of either bright blue light or bright red light depending on the structure of fluorescer and reaction conditions. Excitation energy transfer between acridinium ester (as donor) and rhodamin B (5) (as acceptor) is reported in this paper, based on the mechanism that shown in Fig. 3.

EXPERIMENTAL SECTION

Reagents

All the chemicals used were reagent-grade, purchased from Fluka and used as received. Acridinium ester was synthesized by the method of *Akhavan-Tafti* [10]. The least expensive route starts with acridine.

Synthesis of 9-cyanoacridine

9-cyanoacridine was synthesized in 65% yield from acridine by the method of *Lehmstedt & Wirth* [11]. Acridine (2.5 g) is dissolved in ethanol (11 mL) and the solution is added to glacial acetic acid (0.8 mL). A solution of KCN (1.2 g) in water (2 mL) is added and the mixture is stirred under reflux for 1h. After cooling, the precipitate is filtered off and washed with 2 mol L^{-1} NaOH solution and water, dissolved in chloroform and dried over anhydrous $MgSO_4$. Evaporation of the filtrate to dryness andrecrystallization from n-propanol yields 9-cyanoacridine (1.12 g, 50%) mp. 184-185 ˚C.

Synthesis of acridine-9-carboxylic acid

The acridine 9-cyanid 1.5 g is added to concentrated sulfuric acid (12mL) and heated on a steam bath for 2.5 h. It is cooled to 0 ˚C in an iced water bath and

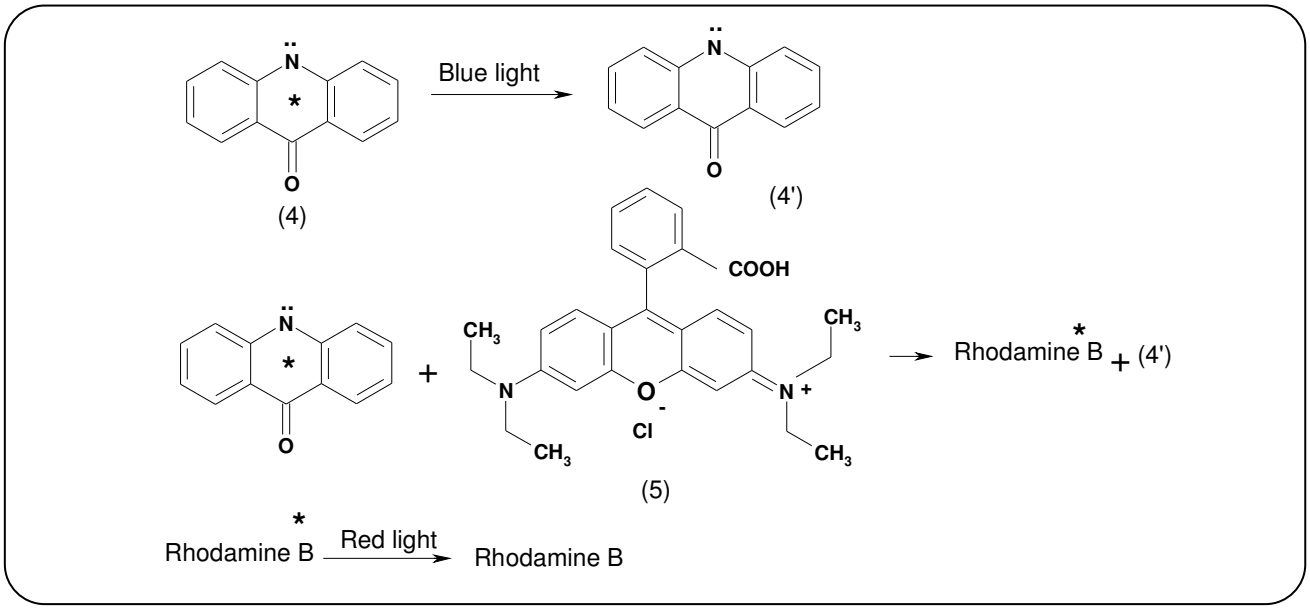

Fig. 3: The CL mechanism of acridinium ester in the presence of rhodamin B.

sodium nitrite 5.5 g is added slowly. It is important to allow time for each portion of sodium nitrite to react. Brown fumes are produced. The mixture is then heated carefully until no more gas is given off. It is then stirred at 100 °C for 2 h, cooled, and poured slowly into iced water to precipitate the yellow product. This is filtered off, washed with water, and sucked dry. The product is dissolved in the minimum of 2 mol L^{-1} NaOH solution and filtered through a sintered funnel. The deep red filtrate solution is treated with concentrated HCl until the yellow precipitate reformed. This is filtered off, washed with water, and dried. It is dried further at 55 °C under reduced pressure for 24 h (yield 1.1 g, 85.75%).

Synthesis of phenyl-acridine-9-carboxylate

phenyl-acridine-9-carboxylate was synthesized by the method of *Akhavan-Tafti* [10]. Acridine-9-carboxylic acid (1 g) was suspended in thionyl chloride (5mL) and the reaction mixture refluxed for 3 h. The solvent was removed under reduced pressure leaving a yellow solid which was dissolved in methylen chloride and small amount of pyridine (about 350 µL) under argon. This solutin was cooled in an ice bath and a solution mixture was stirred overnight at room temperature. After evaporation of solvent, the residue was redissolved in ethyl acetate and washed with water.The organic layer was dried over $MgSO_4$ and concentrated to obtain a crude material. Recrystallization gives the pure product as a yellow solid in 70% yield. Recrystallization from toluene gave needles, mp 180-182 °C, nmr δ7.3-8.42 complex aromatic pattern, ir 1750 cm^{-1}.

Instrumentations and measurements:

CL detection was carried out with a home-made apparatus equipped with a model BPY47 photocell (Leybold, Huerth, Germany). The apparatus was connected to a personal computer via a suitable interface (Micropars, Tehran, Iran). The FL and CL emission spectra were recorded on a model LS-50B Perkin-Elmer instruments. Experimental were carried out with magnetic stirring in a spectrofluorimeter light-tight flattened bottem quartz cell.

Chemiluminescence of Acridinium ester

The fluorimeter cell containing 100 µL Rhodamin B (1.0×10^{-4} mol L^{-1} in MeOH), 300 µL Acridinium ester (0.005 mol L^{-1} in MeOH) and 50 µL Sodium hydroxide (0.4 mol L^{-1} in acetone/water 70/30). The CL signals were obtained in 35 min intervals by injection of 100 µL Hydrogen peroxide (35%) into the cell. The CL intensity-time curves were obtained.

Chemiluminescence of Lucigenin

To a fluorometric cell containing 250 µL lucigenin (0.002 mol L^{-1} in MeOH), was added 100 µL triethyl amine (0.1 mol L^{-1} in acetone/water 70/30) and 100 µL rhodamin B (0.0001mol L^{-1} in MeOH). The CL signal

produced by injection of 100 μL hydrogen peroxide (35%) into the cell, were obtained in about 15 min intervals. The experiment was also carried out in the home made luminometer.

The CL intensity was recorded while the solution was stirred magnetically during measurement. For investigation of concentration of all reagents on the intensity of CL light produced, the variable amounts of reagents were injected to a fluorometric or luminometric cells.

RESULTS AND DISCUSSION

The CL oxidation of aliphatic and aromatic esters of acridinium ester by H_2O_2 in alkaline solution is a well-known reaction. In the CL reaction of acridinium ester, a number of factors play role. The CL reaction is catalyzed by a base but at high pH values there is a competing non-CL reaction leading to a diminishing of the light output [12]. The effect of the NaOH concentration of the starting reagent on the CL kinetics is shown in Fig. 4.

Triethyl amine instead of NaOH was used for studying the CL of lucigenin. Sodium hydroxide reacts rapidly with lucigenin and change it to a dark green compound (6) [13]. The formula of proposed green compound is shown in Fig. 5.

The effect of hydrogen peroxide concentration on CL intensity is shown in Fig. 6, a very small amount of hydrogen peroxide is needed to start the CL.

Certain acridinium species are among the most highly CL synthetic molecules currently known. The light emitting reaction is rather simpler than the luminol and isoluminol systems. First, it requires none of the many and varied catalysts of these phethalhydrazide molecules and second, it does not proceed with as many "active oxygen" species as do these systems [14]. Like lucigenin and its derivatives, acridine CL has no catalytic requirement for the production of high quantum yields. The oxidation and CL mechanism of lucigenin has been reported previously [15-19].

The effects of acridinium ester and lucigenin concentration are compared and some of the resulting CL intensity-time plots are shown in Figs. 7 and 8.

The increasing concentration of acridinium ester and lucigenin increase the emitted light intensity. But according to the experimental condition, the chemiluminescence intensity of lucigenin is higher than that of the acridinium ester. Decomposition of lucigenin

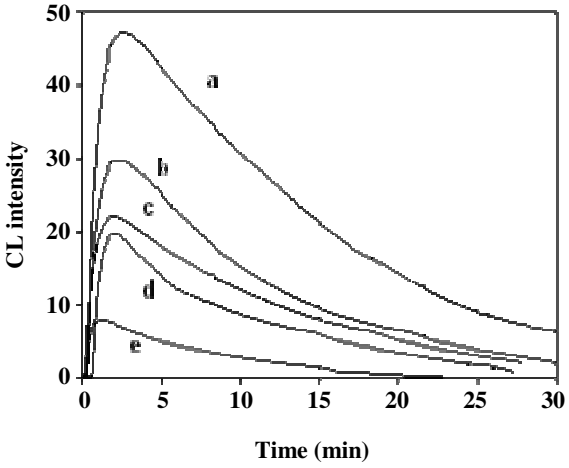

Fig. 4: CL intensity of time for reaction of acridinium ester (5×10^{-3} mol L^{-1}) with H_2O_2 (35%) in the presence of rhodamin B (1×10^{-4}mol L^{-1}) and varying concentration of sodium hydroxide in acetone/water 70/30 (v/v) solutions: (a) 10×10^{-6}, (b) 20×10^{-6}, (c) 40×10^{-6}, (d) 60×10^{-6},(e) 80×10^{-6} mol L^{-1}.

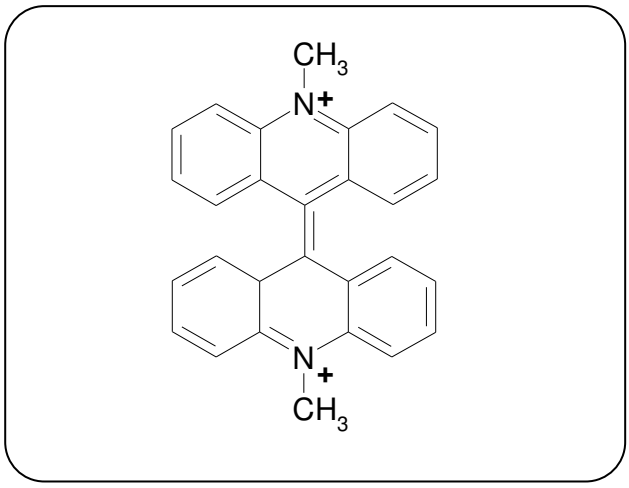

Fig. 5: The proposed formula for the dark green product of lucigenin CL in presence of triethyl amine.

molecule produces two acridone products, but the acridinium ester produces one molecule. Therefore the probability of production of the excited state in lucigenin reaction is more than that of acridinium ester. The acridone product of lucigenin also has an electron donating methyl group on the nitrogen atom and led to the more intense fluorescence and chemiluminescence intensity rather than the acridinium ester as shown in Figs. 7 and 8.

The effect of rhodamin B (as fluorophore) concentration, at a constant amount of acridinium ester and lucigenin was studied and the results are shown in Figs. 9 and 10.

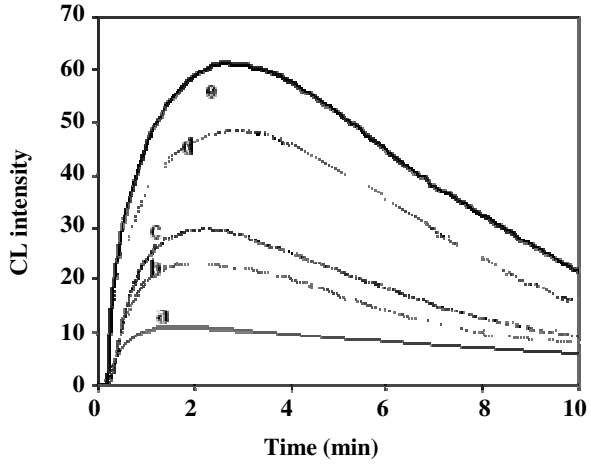

Fig. 6 : CL intensity as a function of time for reaction of acridinium ester (5×10^{-3} mol L^{-1}) with varing concentration of H_2O_2 in the presence of rhodamin B (1×10^{-4} mol L^{-1}) in methanol: (a) 0.58×10^{-3}, (b)1.16×10^{-3} (c) 1.17×10^{-3}, (d) 2.32×10^{-3}, (e) 2.91×10^{-3} mol L^{-1}.

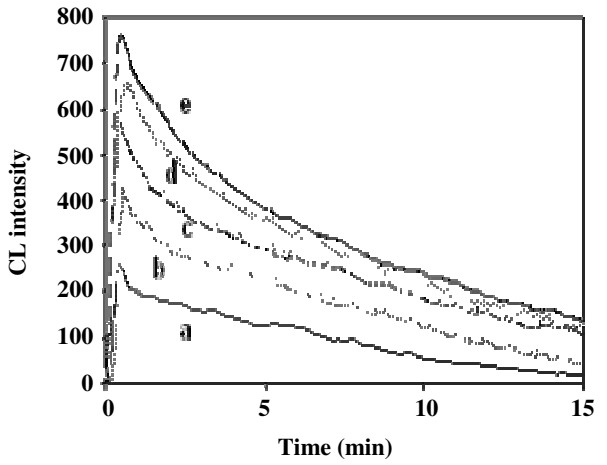

Fig. 8: The CL intensity as a function of time for reaction of varying concentration of lucigenin with H_2O_2 (35%) in the presence of triethyl amine (0.1 mol L^{-1}) in acetone/water (70/30 v/v) as a solvent by a home-made luminometer: (a) 1.82×10^{-7}, (b) 3.64×10^{-7}, (c) 5.45×10^{-7}, (d) 7.27×10^{-7}, (e) 9.1×10^{-7} mol L^{-1}.

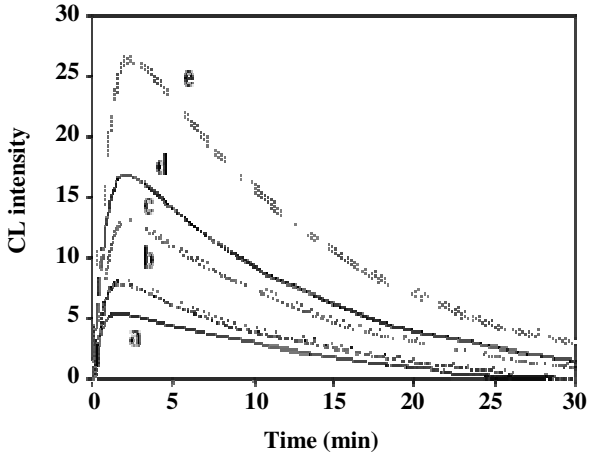

Fig. 7: CL intensity as a function of time for reaction of varying concentration of acridinium ester with H_2O_2 (35%) in the presence of sodium hydroxide (0.4 mol L^{-1}) in acetone/water (70/30 v/v) as a solvent by a home-made luminometer: (a) 0.5×10^{-6}, (b)1×10^{-6}, (c) 1.5×10^{-6}, (d) 2×10^{-6}, (e) 2.5×10^{-6} mol L^{-1}.

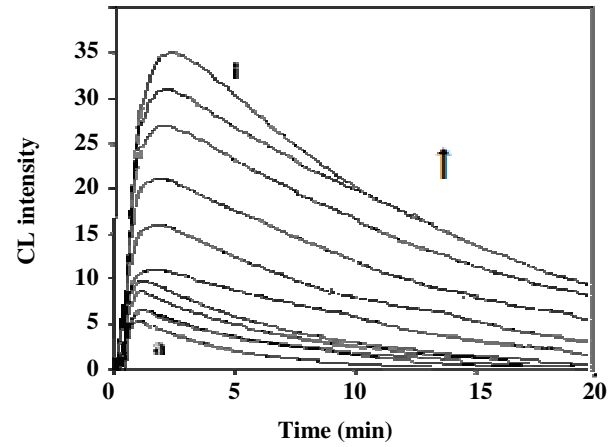

Fig. 9: The CL intensity as a function of time for reaction of acridinium ester (5×10^{-3} mol L^{-1}) with H_2O_2 (35%) in the presence of varying concentration (1×10^{-9}, 2×10^{-9}, 3×10^{-9}, 4×10^{-9} , 5×10^{-9}, 6×10^{-9}, 7×10^{-9}, 8×10^{-9} , 9×10^{-9}, 10×10^{-9} mol L^{-1} ; a-i) of rhodamin B in methanol for 10-100 µL injection.

As it has been shown, there is an exponential increase in CL of H_2O_2-acridinium ester-rhodamin B system with increasing concentration from (10-100 µL) and decreasing from (100-300µL)of the fluorescer rhodamin B.

Peroxy oxalate Chemiluminescence (PO-CL) is well-known as a powerful means of detecting various fluorophores [20-23]. To evaluate the kinetic data for the PO-CL systems studied, a simplified pooled intermediate model (1) was used [24-28].

$$A \xrightarrow{k_r} B \xrightarrow{k_f} C \qquad (1)$$

Where A, B and C represent pools of reactants, intermediates and products, respectively, and both reaction steps designated by the rate constants k_r and k_f are irreversible first order reactions. The integrated rate equation for the CL intensity versus time is:

$$I_t = [M/k_f - k_r][\exp(-k_r t) - \exp(-k_f t)] \qquad (2)$$

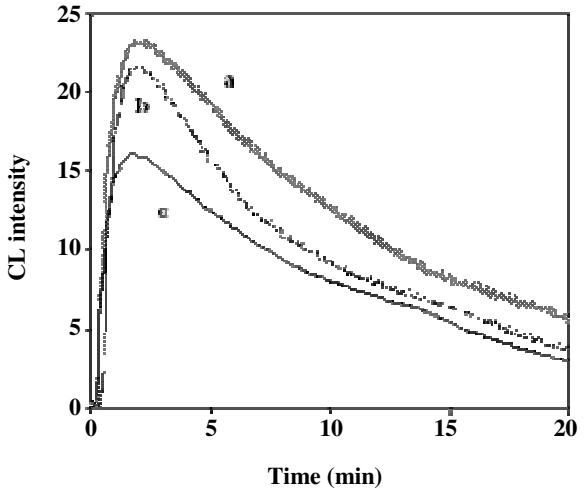

Fig. 10 : The CL intensity as a function of time for reaction of varying concentration of rhodamin B (a-c: 0.1×10^{-7}-0.3×10^{-7} mol L^{-1}) in methanol for 100-300 µL injection.

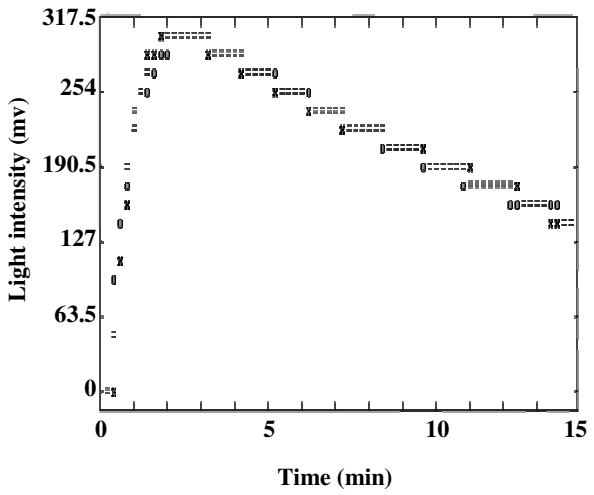

Fig. 11: A typical computer fit of the CL intensity time plot for the acridinium ester -H_2O_2 –Rhodamin B system according to the data of luminometric measurements. The acridinium ester concentration is 2×10^{-6} mol L^{-1}. (×) experimental point; (o) calculated point; (=) experimental and calculated points are the same within the resolution of plot.

Where, I_t is the CL intensity at time t, M is a theoretical maximum level of intensity if the reactants were entirely converted to a CL-generating material and k_r and k_f are, respectively, the first order rate constants for the rise and fall of the CL burst. A further advantage of this model is that it not only allows the determination of parameters M, k_r and k_f , but also it permits an estimate of the intensity at maximum level (J), the time of maximum intensity (T_{max}) and the total light yield (Y), as follows:

$$J = M\left(\frac{k_f}{k_r}\right)\left[\frac{k_f}{k_r - k_f}\right] \quad (3)$$

$$T_{max} = \frac{\ln\left(k_f/k_r\right)}{k_f - k_r} \quad (4)$$

$$Y = \int_0^{\infty} I_t dt = \frac{M}{k_f} \quad (5)$$

In this work, a non-linear least-squares curve fitting program KINFIT [29] was used to evaluate the M, k_r and k_f values from the corresponding CL intensity-time plots. A typical fit of the CL intensity time plots is shown in Fig. 11.

The other parameters J, T_{max} and Y were evaluated by equations (3)-(5) using the k_r , k_f and M values [20]. The time required for half and three-quarters of total light emission, $t_{½}$ and $t_{¾}$ respectively, are obtained directly from the light intensity-time curves. The kinetic parameters thus obtained for all experiments carried out are summarized in Table 1.

The influence of H_2O_2 concentration on the kinetics of the CL of acridinium ester was studied in the presence of sodium hydroxide and the results are given in Fig. 4 and Table 1. There is a direct linear relationship between the concentration of hydrogen peroxide and CL intensity of the system, Fig.12.

In the presence of sodium hydroxide, the light intensity observed is clearly indicative of the catalytic effect of sodium hydroxide on the PO-CL system studied. In order to investigate the optimal concentration of sodium hydroxide, the CL response of the acridinium ester-H_2O_2-Rhodamin B system measured against the concentration of base, Fig. 13.

As it is obvious from Fig. 13, further addition of sodium hydroxide revealed a gradual decrease of CL intensity. This is most probably due to the quenching effect of base at higher concentration, which begins to decompose the reactive intermediate, dioxetadione and hence reduces the CL light [30,31,32].

The influence of varying acridinium ester concentration in the presence of H_2O_2 was investigated and the results are given in Table 1 and Figs. 7 and 14. Fig. 14 shows a linear correlation between the CL intensity and the acridinium ester concentration.

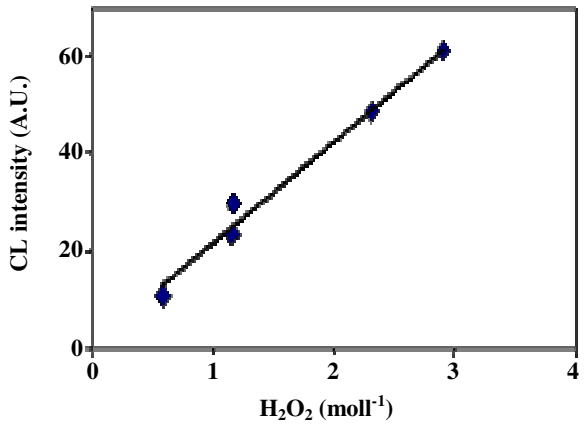

Fig. 12: Effect of H_2O_2 concentration (10^{-3} mol L^{-1}) on the CL intensity of acridinium ester -H_2O_2 –Rhodamin B system in the presence of (0.4 mol L^{-1}) sodium hydroxide. Y= 20.837x+0.8283, R^2 = 0.9821.

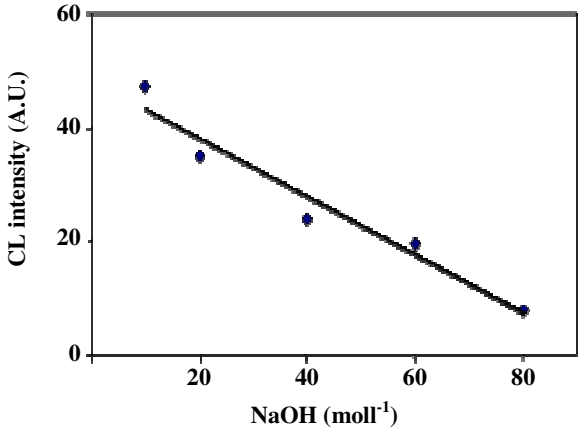

Fig. 13: The Effect of sodium hydroxide concentration (10^{-6} mol L^{-1}) on the CL intensity of acridinium ester -H_2O_2 - Rhodamin B system Y=-0.5085x+48.159, R^2=0.9492.

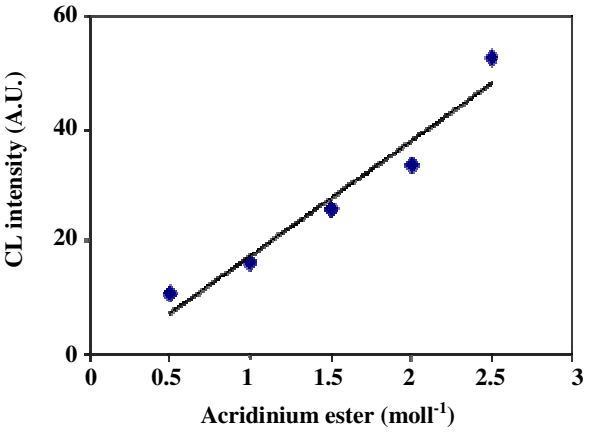

Fig. 14: The Effect of acridinium ester concentration (10^{-6} mol L^{-1}) on the CL intensity of acridinium ester -H_2O_2- Rhodamin B system.Y= 20.2x-2.52, R^2= 0.9471.

CONCLUSIONS

Acridinium esters CL in presence of hydrogen peroxide in basic media are relatively intense and fast light emitting. Rhodamine B was found to act as a red fluorescer and effect on the light emission intensity. The kinetic parameters for the CL reaction were evaluated from the fitting of the corresponding CL intensity-time plots.

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