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A study of chemiluminescence characteristics of a novel peroxyoxalate system using berberine as the fluorophore

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ABSTRACT

In the present work the chemiluminescence characteristics of a peroxyoxalate system using berberine as a novel fluorophore has been investigated. Berberine can accept energy via the formation of a dioxetane and emits intense yellow light. The characteristics of the chemiluminescence for this system under various concentrations of reagents including bis (trichlorophenyl)oxalate, sodium salicylate (as catalyst), hydrogen peroxide and the fluorophore were also studied. These parameters including the rise and fall rate constants for the chemiluminescence emission, theoretical and experimental maximum intensity, theoretical and experimental time to reach maximum intensity and total light yield emission were evaluated from intensity–time profiles using the pooled intermediate model by a non-linear least-squares curve fitting program, KINFIT.

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1. Introduction

Of the numerous chemiluminescence reactions known to date, peroxyoxalate chemiluminescence (PO-CL) is the most effective one (Quantum yield up to 50%). A typical PO-CL consists of the reaction of the active oxalates, such as bis (2,4,6-trichlorophenyl) oxalate (TCPO), hydrogen peroxide as oxidant and a catalyst, in which light emits from the excited states of various externally added fluorescent activators. In the peroxyoxalate system, 1,2-dioxetanones, including a 1,2-dioxetane and some cyclic peroxides have been suggested as the high energy key intermediates capable of producing an excited species by their thermal decomposition according to a chemically initiated electron exchange luminescence (CIEEL) process. These metastable intermediates form complexes with the fluorescent activator (fluorophore) so that one electron can be donated to the intermediate. This electron is then transferred back to the fluorophore raising it to an excited state and liberating light [1–6]. The CL spectra produced from these reactions could be used for different analytical applications, based on the determination of fluorophores, peroxides, catalysts or quenchers used in the PO-CL system. Such multi-scope character of the PO-CL systems enables them to be employed as powerful analytical tools for the detection of a wide variety of analytes such as fluorescent compounds [7], hydrogen peroxide catecholamines, carboxylic acids, carbonyl compounds, amines [8] and amino acids [9]. In recent years many fluorophores have been introduced for the PO-CL system. They emit light with different color regions as blue, green, yellow, orange and red [10–14]. Although, more attempts to introduce new types of fluorophores seem attractive and tempting.

Berberine, is an isoquinoline plant alkaloid, isolated from the Golden seal, *Hydrastis canadensis L.*, a member of the family *Ranunculaceae*. It is also found in many other plants including the *Berberineeris* species (Berberidaceae) and *Arcangelisia* flaw (Menispermaceae). (The molecular structure of berberine is shown in Fig. 1).

Berberine is an active ingredient used in traditional medicine for anti-bacterial [15], anti-inflammatory [16], anti-infectious and anti-depressant [17] and cholesterol level lowering activity [18]. It is now extensively studied and known to be an important lead compound in cancer therapy as berberine is demonstrated to possess anticancer activity [19] amongst a wide variety of other beneficial properties in vitro and in vivo [20]. This alkaloid also inhibits intestinal ion transport, reduces Cl^- secretion and can serve as a K^+ channel-blocking agent in cells [21]. For these reasons, utilization of berberine in medicine is rapidly on the increase.

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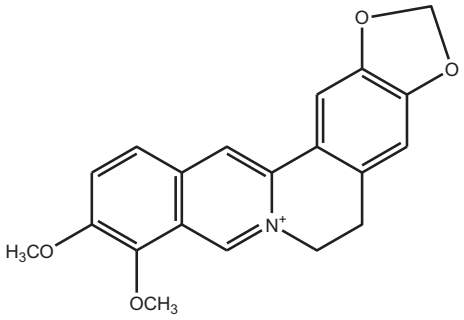


Fig. 1. Molecular structure of berberine.

It is also well known that berberine has high fluorescence activity [22–25]. Hence it is widely applied to study DNA, RNA, oligonucleotides and nanoparticles [26,27].

In present work, we have introduced berberine as a fluorophore for peroxyoxalate chemiluminescence system for the first time. Effects of the reagents on the CL parameters were also investigated.

2. Experimental section

2.1. Materials

Bis(2,4,6-trichlorophenyl) oxalate (TCPO), hydrogen peroxide (30% W/W) and sodium salicylate (SS) were purchased from Fluka and used without further purification. Berberine hydrochloride was obtained from Sigma Co (USA). All solvents (Analytical grade) were purchased from Merck.

2.2. Apparatus

Both of the steady state chemiluminescence and fluorescence studies were taken at spectrofluorimeter Shimadzu RF5301PC. In the fluorescence measurements the excitation and emission monochromators were set at 390 and 440 nm respectively. A spectral bandwidth of 5 nm was applied. In the chemiluminescence mode the Xenon lamp was turned off. Intensity as a function of time studies were made on a homemade luminometer equipped with photoelectric cell Hamamatsu RX80004 which was connected to personal computer with an appropriate interface. All measurements were done at ambient temperature 26 °C.

2.3. Procedure

The procedure was carried out in the following manner. The cuvette was filled with 400 μL sodium salicylate (in ethyl acetate), 850 μL TCPO (in ethyl acetate) and 400 μL berberine (in ethyl acetate). The samples were placed in luminometer and continuously mixed with a stirrer (450 rpm). Ten seconds after the luminometer began to record, reaction initiated by injecting 300 μL hydrogen peroxide (in acetonitrile) to the cuvette using a sampler. The fluorescence studies were carried out using freshly prepared 1.2×10^{-4} M berberine in methanol using a 3-cm quartz cuvette.

3. Result and discussion

Peroxyoxalate chemiluminescence is the most efficient nonenzymatic light producing system. The PO-CL reaction can be schematized in three basic steps. In the first step of reaction, an aryl oxalate ester like TCPO reacts with H_2O_2 to produce a key chemical intermediate, C_2O_4, containing the necessary excitation energy. The second step involves the chemiexcitation of a fluorophore, like berberine, to electronically excited states by the reactive intermediate via conversion of the chemical energy into electronic excitation energy. The final step is the emission of light energy by returning the excited fluorophore molecule to the ground state. In preliminary studies, it was found that by adding few drops of hydrogen peroxide to a cell containing TCPO, sodium salicylate and berberine, a very intense yellow light was observed at 519 nm. The emission wavelength maxima in both of the sensitized PO-CL spectrum of berberine and its fluorescence spectrum, recorded under comparable experimental conditions, was found to be similar (Fig. 2). This feature indicates that the singlet excited state of the fluorescent species is formed in the CL reaction and is the emitting species [3,4,7].

Due to the transient nature of the CL emissions, the optimization of PO-CL detection requires a thorough understanding of CL kinetics. Hence, to shed light on the kinetic parameters of the chemiluminescence process of H_2O_2–TCPO–Berberine–SS system from the corresponding CL intensity versus time profiles, a pooled intermediate simplified model was employed [28–30]:

$$R \rightarrow X \rightarrow P \tag{1}$$

In this model R, X and P represent pools of reactants, intermediates and products, respectively, and both the reaction steps are irreversible first-order reactions. The chemiluminescence signal is proportional to the concentration of intermediate X and the integrated rate equation of CL intensity versus time is given by:

$$I_{(t)} = \frac{Mk_r}{k_f - k_r}\left(e^{-k_r t} - e^{-k_f t}\right) \tag{2}$$

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 the first-order rate constants for the rise (faster step) and fall (slower step) of the burst of CL, respectively.

A further advantage of this model is that it not only allows the determination of M, k_r and k_f parameters, but also it permits an estimate of the intensity at maximum level (J), the time of maximum intensity (T_{max}) and the total yield (Y), as follows.

$$T_{max} = \frac{\ln\left(\frac{k_f}{k_r}\right)}{k_f - k_r} \tag{3}$$

$$J = M\left(\frac{k_f}{k_r}\right)\left(e^{-k_r t} - e^{-k_f t}\right) \tag{4}$$

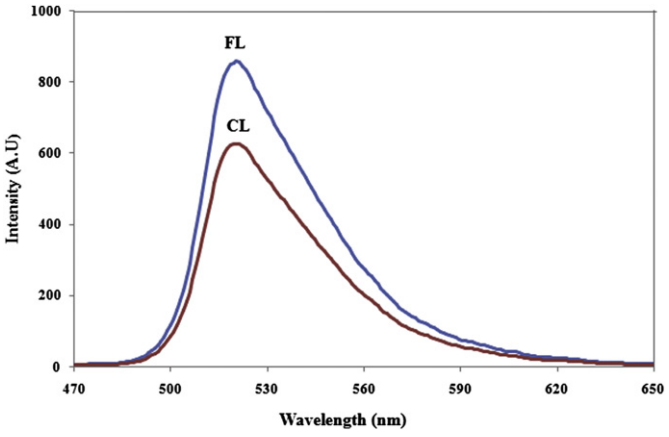


Fig. 2. Steady state fluorescence (FL) and chemiluminescence (CL) of berberine.

$$Y = \int_0^{\infty} I_{(t)}\mathrm{d}t = \frac{M}{k_f} \quad (5)$$

To evaluate M, k_r and k_f values from the corresponding CL intensity–time plots a non-linear least-squares curve fitting program KINFIT was used [31]. The other parameters J, T_{max} and Y were then obtained from Eqs. (3)–(5) using the k_r, k_f and M values.

3.1. Effect of TCPO concentration on PO-CL

The effect of concentration of TCPO on kinetic parameters was investigated and results were shown in Table 1. It was found that the increase of TCPO concentration until 2.5×10^{-3} M leads to the increase in CL intensity. This result is consistent with TCPO being the limiting reagent under this reaction condition [3,10,32]. It is pertinent to mention that fall and specially rise rate constants were almost independent from concentration of TCPO (within the experimental error). This observation confirms that the reaction is pseudo-first order in TCPO to a good approximation when H_2O_2 is in a large excess [30]. Fig. 3 shows intensity as a function of varying concentration of TCPO.

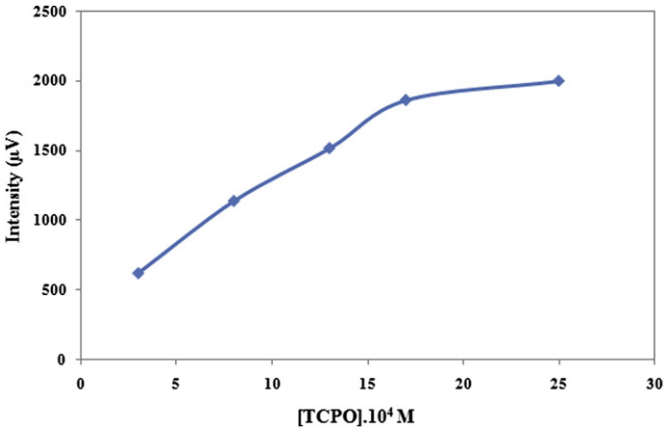


Fig. 3. Effect of TCPO concentration on CL intensity.

3.2. Effect of hydrogen peroxide concentration on PO-CL and possible mechanism

The kinetic parameters were obtained by varying the concentration of H_2O_2 in the range of 1.5×10^{-3}–1.6×10^{-2} M. According to Table 1 and Fig. 4 an increase in concentration of hydrogen peroxide leads to a linear increase in CL intensity. At above concentrations there was no significant variation observed in rate constants and CL intensity. Furthermore, it is pertinent to mention that both the pseudo-first order rise and fall rate constants show a linear increase with increasing concentration of peroxide. As seen in Fig. 5 and Table 1, rise rate constant (k_r) shows a linearity increase with concentration of peroxide, with a regression equation of 1.304 $[H_2O_2]$ + 15.23 and with a large intercept about 15.23 min^{-1} ($r^2 = 0.9811$). Meanwhile, fall rate constant (k_f) shows an increase with a regression equation of 0.0148$[H_2O_2]$ + 0.1387 and an intercept 0.1387 min^{-1} ($r^2 = 0.9722$). The possible reaction of TCPO, SS, berberine and hydrogen peroxide is depicted in Scheme 1.

On the basis of this mechanism, the nucleophilic substitution of H_2O_2 for 2,4,6-trichlorophenol (TCP) in TCPO results in an aryl-containing monoperoxy oxalic acid (**B**) analogous to the first reaction step proposed for peroxyoxalate chemiluminescence by other researchers [5,6]. This reaction results in the observed first-order dependence of k_r on H_2O_2 concentration. It is expected that this reaction is base catalyzed. Hence, it increases the dependence of k_r on sodium salicylate concentration.

Meanwhile, the direct formation of an intermediate leading to chemiluminescence results from the intramolecular cyclization of the aryl-containing monoperoxy oxalic acid (**F**) to form an aryl-containing hydroxyl dioxatanone, species (**D**). Subsequently, the species (**D**) can release a phenol group to produce1,2-dioxetanedione (**E**) and are structurally similar to the previous structures proposed for high-energy intermediates [4,29]. Dioxatanones similar to (**D**) and (**E**) have been shown to produce the singlet excited state of highly fluorescent acceptor molecules in

Table 1
CL parameters evaluated from computer fitting of the CL intensity–time plots for TCPO–SS–H_2O_2–Berberine system.

Parameter	Concentration (M)	k_r (min^{-1})	k_f (min^{-1})	M	J	J_{exp}	T_{max} (min)	T_{exp} (min)	Y
TCPO	3×10^{-4}	21.7	0.27	729	690	624	0.2	0.29	2701
	8×10^{-4}	23.2	0.24	1214	1157	1142	0.19	0.26	5058
	1.2×10^{-3}	24.2	0.26	1638	1560	1521	0.18	0.2	6302
	1.7×10^{3}	24.8	0.29	1944	1843	1866	0.18	0.19	6703
	2.5×10^{-3}	26.4	0.28	2107	2002	2003	0.17	0.2	7525
H_2O_2	1.5×10^{-3}	15.8	0.16	738	705	774	0.29	0.30	4618
	4×10^{-3}	22	0.19	1339	1285	1270	0.21	0.25	7052
	8×10^{-3}	25.7	0.26	1751	1671	1622	0.18	0.20	6736
	1.2×10^{-2}	31.2	0.34	2316	2203	2214	0.14	0.16	6813
	1.6×10^{-2}	35.6	0.36	2689	2566	2548	0.13	0.12	7471
SS	1×10^{-4}	15.2	0.17	978	930	884	0.29	0.31	5758
	2.5×10^{-4}	22.2	0.24	1269	1207	1180	0.20	0.24	5288
	5.5×10^{-4}	27	0.26	1707	1631	1670	0.17	0.18	6565
	7.5×10^{-4}	31.3	0.33	2211	2105	2087	0.14	0.16	6700
	1×10^{-3}	34.8	0.39	2706	2571	2598	0.13	0.14	6939
	1.5×10^{-3}	35.1	0.39	2570	2442	2483	0.12	0.12	6589
Berberine	2×10^{-6}	27.6	0.32	664	631	692	0.16	0.17	2076
	3.5×10^{-6}	28.2	0.30	906	863	878	0.16	0.16	3021
	5.5×10^{-6}	27.1	0.30	1183	1124	1082	0.16	0.17	3943
	8×10^{-6}	26.8	0.29	1517	1443	1395	0.17	0.16	5231
	2×10^{-5}	28	0.33	1705	1617	1624	0.16	0.15	5166
	4×10^{-5}	28.5	0.28	1774	1695	1702	0.16	0.16	6335
	6×10^{-5}	29.3	0.34	2049	1945	1932	0.15	0.15	6026
	8×10^{-5}	31.7	0.29	2286	2188	2227	0.15	0.14	7882
	1.2×10^{-4}	31	0.27	2529	2423	2460	0.15	0.14	9366

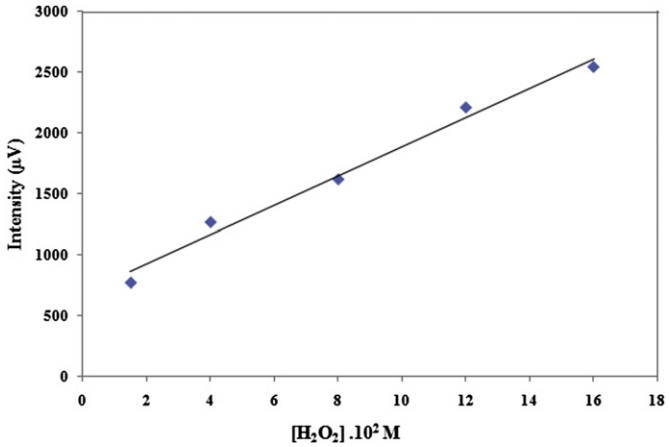


Fig. 4. Effect of hydrogen peroxide on CL intensity.

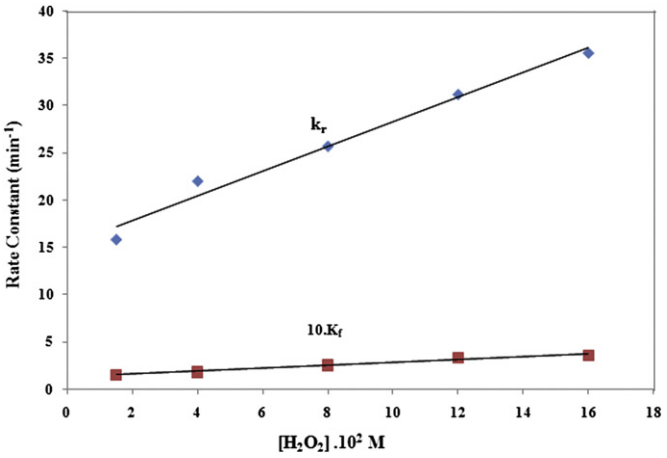
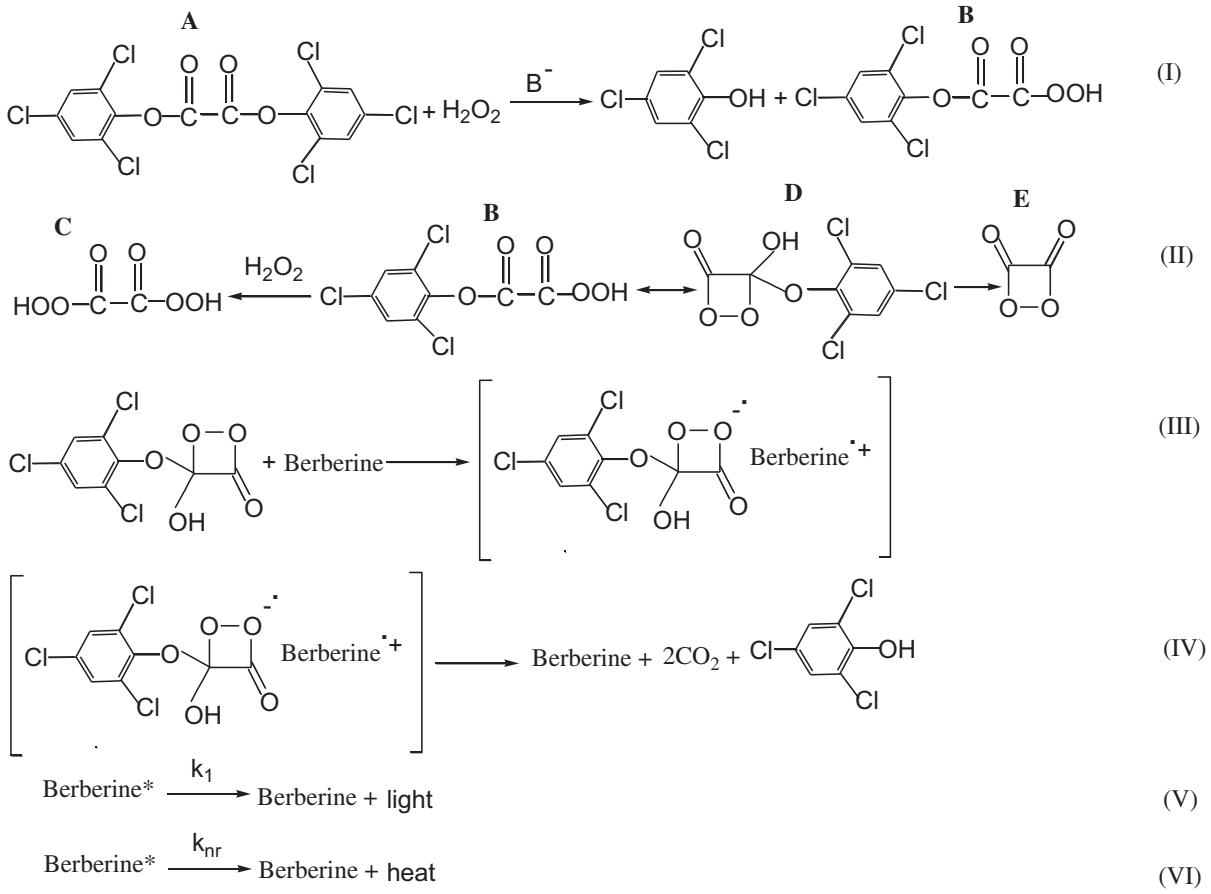


Fig. 5. Pseudo-first-order rise (k_r) and fall (k_f) rate constants as a function of hydrogen peroxide concentration.

a chemically initiated electron exchange luminescence (CIEEL) reaction [1,5,6]. The first-order dependence of k_f on H_2O_2 concentration is explained clearly by a second substitution of H_2O_2 for 2,4,6-trichlorophenol (TCP) to form the dihydroperoxyoxalate, species (**C**). It has been reported that species (**C**) does not lead to significant chemiluminescence via an analogous cyclization [30].

3.3. Effect of sodium salicylate concentration on PO-CL

The effect of the sodium salicylate (SS) concentration in the range from 1×10^{-4} to 1.5×10^{-3} M on the PO-CL kinetic parameters was investigated. Table 1 shows that as the SS concentration increased, the intensity and rate constants significantly increased while time to reach maximum intensity decreased which vividly verified the catalytic role of SS in the reaction. At higher concentration (1.5×10^{-3}) CL intensity and *Y* decreased. This is most probably due to the quenching effect of the base at higher concentrations, which begins to decompose the reactive intermediate dioxetane (**D** and **H**), hence, reduces the PO-CL light [10,11,28]. Effect of SS concentration on CL intensity was investigated and shown in Fig. 6.



Scheme 1. The possible mechanism of chemiluminescence of berberine generated from reaction of TCPO, sodium salicylate and hydrogen peroxide.

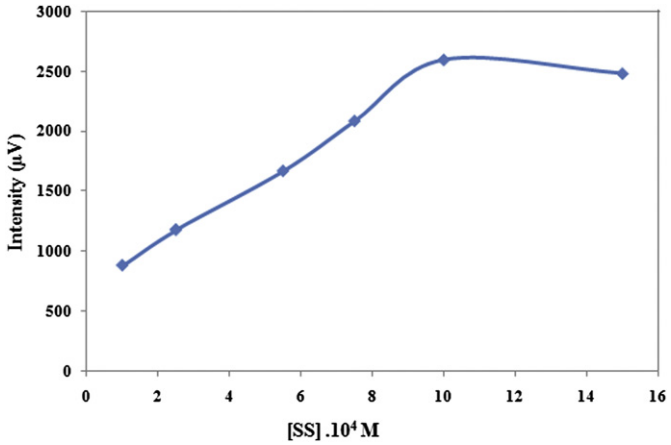


Fig. 6. CL intensity as a function of sodium salicylate concentration.

3.4. Effect of berberine concentration on PO-CL

As shown in Fig. 2 the PO-CL system in the presence of berberine emits intense yellow light at 519 nm. Fig. 7 shows intensity as a function of time profile in the presence of varying concentrations of berberine. As can be seen the peak intensity increases rapidly after mixing and reaches a maximum in a few seconds. Decay of the light-intensity from the maximum occurs at much longer period (i.e. 8 min). Effect of berberine concentration in the range of 2×10^{-6}–1.2×10^{-4} M on CL intensity, listed in Table 1 revealed an increase in concentration of berberine led to increase of CL intensity and *Y*, while it has no considerable effect on rate constants and T_{max}. In these reactions, the high-energy cyclic intermediates (dioxetane species) may transfer energy to a fluorophore via the chemically initiated electron exchange luminescence (CIEEL) mechanism [2–4]. Other researchers have clearly shown that the charge-transfer steps leading to chemiluminescence are much faster than the formation of an initial intermediate, so the final reactions leading to chemiluminescence (Eqs. (III)–(VI) in Scheme 1) are kinetically unobservable [3,33]. A typical computer fit of CL intensity–time plots was shown in Fig. 8. As seen from this figure, there is good agreement between the theoretical and experimental data that confirms the proposed model.

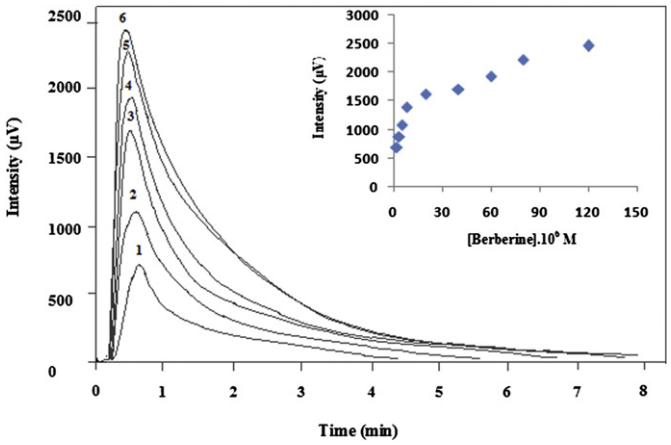


Fig. 7. CL intensity as a function of time for reaction of TCPO (2.5×10^{-3} M), SS (1×10^{-3} M) and hydrogen peroxide (1.6×10^{-2} M) in the presence of varying concentration of berberine (1) 2×10^{-6} M, (2) 5.5×10^{-6} M, (3) 2×10^{-5} M, (4) 6×10^{-5} M, (5) 8×10^{-5} M, (6) 1.2×10^{-4} M. (Inset) CL intensity as a function of berberine concentration.

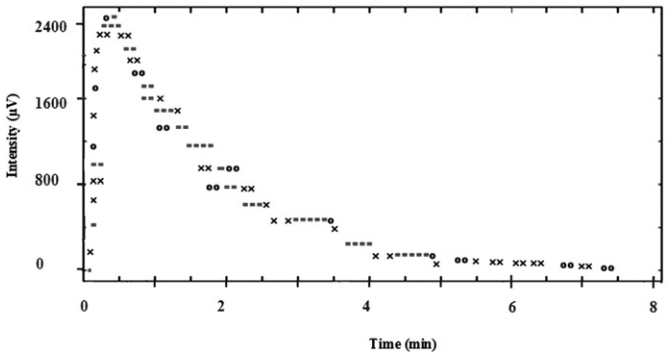


Fig. 8. Computer fit of the CL intensity–time plot for the TCPO–ImH–H_2O_2 system in the presence of 1.2×10^{-4} M berberine, (×) experimental point; (o) calculated point; (=) experimental and calculated points are the same within the resolution of the plot.

4. Conclusion

The present work deals with the first attempt to introduce berberine as fluorophore for peroxyoxalate chemiluminescence system. Berberine can accept energy from reaction of TCPO–H_2O_2–sodium salicylate probably through mechanism of chemically initiated electron exchange luminescence (CIEEL) and emits intense yellow light. Moreover, effects of concentration of reaction ingredients on kinetic chemiluminescence parameters were also investigated. These parameters including rise and fall rate constant for the chemiluminescence burst, theoretical and experimental maximum intensity, theoretical and experimental time to reach maximum intensity and total light yield emission were calculated from intensity–time profiles using KINFIT program.

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