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Quenching effect of DL(±)α-methylbenzylamine on peroxyoxalate chemiluminescence of 7-amino-4-trifluoromethylcumarin

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Abstract

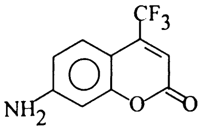
The quenching effect of DL(±)α-methylbenzylamine on strong chemiluminescence of bis(2,4,6-trichlorophenyl)oxalate-hydrogen peroxide system in the presence of 7-amino-4-trifluoromethylcumarin was investigated. The chemiluminescence parameters were evaluated from computer fitting of the resulting intensity–time plots. The system resulted in a Stern–Volmer plot with a K_Q value of 569 M^{-1}, in the quencher concentration range of 2.8×10^{-4} to 2.8×10^{-3} M.

Keywords: Peroxyoxalate chemiluminescence; 7-Amino-4-trifluoromethylcumarin; DL(±)α-methylbenzylamine; Quenching

1. Introduction

Peroxyoxalate chemiluminescence (PO-CL) is a well known and powerful means of detecting hydrogen peroxide [1–3] and a variety of fluorophores [4–7]. Thus, the kinetics and mechanism of PO-CL have been extensively investigated [8–17]. It has been shown that the quenched chemiluminescence of peroxyoxalate systems has a potential for the detection of easily oxidizable compounds such as anilines, organosulfur compounds and certain inorganic anions such as nitrite, sulfite, iodide and bromide [18–20]. This phenomenon has been of increasing, interest because (1) it gives an insight in the role of possible interferences on analytical procedures using the PO-LC reactions and (2) the analytical potential of PO-CL can be extended to the analyses of quenchers under favorable experimental conditions.

Cumarin derivatives are widely distributed in plants, some of them being physiologically active [21] and some possessing antimicrobial properties [22,23]. Meanwhile, cumarin derivatives are of fundamental practical interest [21]. Many of them are widely used as optical dyes and brighter for natural and synthetic materials, as well as in lasers [24]. The 7-amino-4-trifluoromethylcumarin (I) is an interesting cumarin derivative which has been successfully used in the synthesis of a substrate for the fluorometric assay of proteslytic enzymes in biological fluids [25] and for use as a laser dye and as a marker for proteins [26]. We have recently investigated the chemiluminescence reactions of some peroxyoxalate esters, hydrogen peroxide and cumarin (I) [27]. In this paper, we report the quenching effect of DL(±)α-methylbenzylamine (MBA) on the peroxyoxalate chemiluminescence in the presence of cumarin (I). It is noteworthy that, MBA is a well known chiral handle which has been widely used in different important processes such as resolution and determination of the enantiomeric purity of acids [28], stereocontrolled synthesis of prostaglandins [29], asymmetric synthesis of stereochemically homogeneous α-methyl-α-aminonitriles [30], enantioselective synthesis of ring-C aromatic steroids [31] and stereoselective reduction of chiral iminium ions [32]. The results of this study revealed that cumarin (I) with an inherently high PO-CL intensity is very useful for the MBA detection by the quenched chemiluminescence of peroxyoxalate–H_2O_2–cumarin (I) system.



(I)

2. Experimental

All chemicals were of the reagent-grade from Fluka chemical company and used as received. Bis(2,4,6-trichloro-

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phenyl)oxalate (TCPO) was prepared from the reaction of 2,4,6-trichlorophenol with oxalyl chloride in the presence of triethylamine, as described elsewhere [33]. Hydrogen peroxide (30%) was concentrated via freeze drying (using a model FD-1 Fyela freeze dryer) up to 60% mixed with dimethyl phthalate in a 1:1 (v/v) portions, and shook well on an electrical shaker. After 10 h, the organic phase was separated, dried on anhydrous Na_2SO_4 and the H_2O_2 concentration was determined by a standard potassium permanganate solution. Then a standard stock solution of hydrogen peroxide (1.5 M in 80:20 (v/v) dimethyl phthalate *tert*-butyl alcohol containing 5.0×10^{-3} M sodium salycilate) was prepared from this solution. The stock solutions of TCPO and cumarin (I) (0.01 M) were also prepared in ethyl acetate.

Chemiluminescence detection was performed with a homemade 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). Experiments were carried out with magnetic stirring (500 rpm) in a light-tight flattened bottom glass cell of 15 mm diameter at room temperature. The fluorescence spectra were recorded on a Model LS-50B Perkin-Elmer instrument.

3. Results and discussion

The PO-CL is one of the most efficient non-biological light producing systems that can be quenched by some easily oxidizable compounds and certain inorganic anions [18–20]. The most frequently proposed mechanistic pathways that a quencher (Q) can follow in the reaction with TCPO and H_2O_2 are [5,9,20,33–36]:

$$\mathrm{TCPO} + H_2O_2 \rightarrow \text{intermediate } (C_2O_4) + 2\mathrm{TCP}\ (2,4,6\text{-trichlorophenol}) \quad (1)$$

$$C_2O_4 + \mathrm{I} \rightarrow [C_2O_4{}^{-}\mathrm{I}^{+}] \quad (2)$$

$$C_2O_4 + Q \rightarrow \text{non-CL} \quad (2a)$$

$$[C_2O_4{}^{-}\mathrm{I}^{+}] \rightarrow \mathrm{I}^{*} + 2CO_2 \quad (3)$$

$$\mathrm{I}^{*} \rightarrow \mathrm{I} + h\nu \quad (4)$$

$$\mathrm{I}^{*} + Q \rightarrow \mathrm{I} \quad (4a)$$

$$\mathrm{I}^{*} \rightarrow \mathrm{I} + \text{heat} \quad (5)$$

The asterisk signifies the excited state of the fluorophore (I).

Our preliminary experiments revealed that the addition of excess amount of hydrogen peroxide to a colorless ethyl acetate solution containing 0.01 M TCPO and 1.0×10^{-3} M cumarin (I) results in a very intense blue light. In Fig. 1, the CL spectrum for TCPO–H_2O_2 reaction in the presence of I is compared with the fluorescence spectrum of the fluorescer (I), obtained under comparable conditions. As is obvious, a good correspondence was obtained for the CL and fluorescence spectral distributions of the fluorescer, indicating that the singlet excited state of the fluorescent additive is formed in the reaction and is the emitting species [37,38].

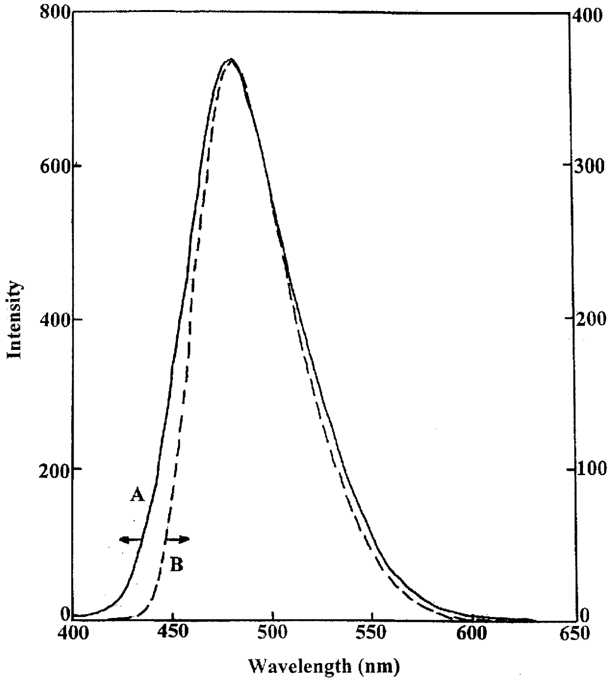

Fig. 1. Fluorescence (A) and chemiluminecence (B) spectra of cumarin (I).

The resulting CL intensity decay curves for the TCPO–H_2O_2–I chemiluminescent system in the absence and presence of MBA are shown in Fig. 2. As seen, in the absence of quencher, the peak intensity increases rapidly after mixing and reaches a maximum at a T_m of about 5 s. Whereas the decay of light intensity from the maximum

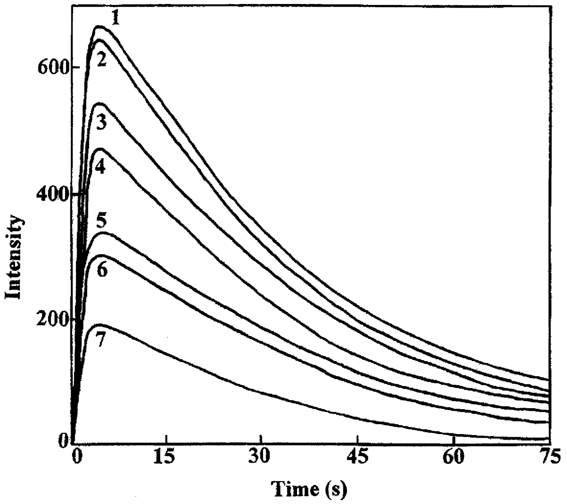

Fig. 2. CL-intensity as a function of time for the reaction of TCPO (5.0×10^{-3} M), H_2O_2 (0.15 M) and cumarin (I) (5.0×10^{-4} M) in ethyl acetate in the presence of varying concentrations of MBA as quencher. The MBA concentration (M) is: (1) 0, (2) 2.8×10^{-4}, (3) 5.6×10^{-4}, (4) 1.1×10^{-3}, (5) 1.9×10^{-3}, (6) 2.2×10^{-3}, (7) 2.8×10^{-3}.

occurs at longer periods of time (up to 90 s) via an exponential process. However, the presence of MBA was found to quench the chemiluminscence system significantly. The resulting intensity decay curves in the presence of increasing MBA concentration are also included in Fig. 2 (2–2 to 2–7).

In order to investigate the kinetics of the chemiluminescence process of TCPO–H_2O_2–Cumarin (I) system in the absence and presence of increasing concentration of MBA from the corresponding CL intensity versus time profiles, a previously reported simplified model was employed [39,40]:

$$R \xrightarrow{k_r} X \xrightarrow{k_r} P \tag{6}$$

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

$$I_t = \left[\frac{Mk_f}{k_f - k_r}\right][\exp(-k_r t) - \exp(-k_f t)] \tag{7}$$

where I_t is the CL intensity at time t, M the theoretical maximum level of intensity if the reactions were entirely converted to a CL-generating material and k_r and k_f are the pseudo-first-order rate constants for the rise and fall of the burst of CL, respectively. In addition, this pooled-intermediate model permits an estimate of intensity at the maximum CL, J, time of maximum intensity, T_{max}, and the total light yield, Y, as follows:

$$J = M\left(\frac{k_f}{k_r}\right)\left[\frac{k_f}{k_r - k_f}\right] \tag{8}$$

$$T_{max} = \frac{\ln(k_f/k_r)}{k_f - k_r} \tag{9}$$

$$Y = \int_0^{\infty} I_E\, dt = \frac{M}{k_f} \tag{10}$$

The k_r, k_f and M values were evaluated by computer fitting of the CL intensity–time profiles to Eq. (7), using a non-linear least-squares curve fitting program KINFIT [41]. A sample computer fit of the CL intensity–time plots is shown in Fig. 3.

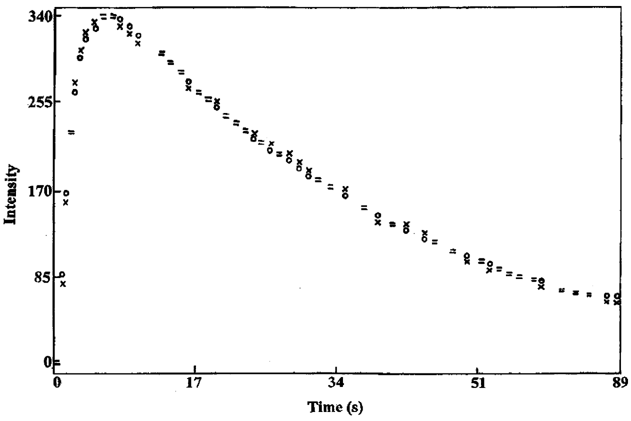

Fig. 3. Computer fit of the CL intensity–time plot for TCPO–H_2O_2–cumarin (I) system in the presence of 1.9×10^{-3} M of MBA in ethyl acetate solution: (x) experimental point; (o) calculated point, (=) experimental and calculated points are the same within the resolution of the plot.

The other parameters J, T_{max} and Y were then evaluated from Eqs. (8)–(10), using the k_r, k_f and M values. All the resulting parameters are summarized in Table 1. As expected, the maximum CL and total eight yield sharply decreased with increasing concentration of MBA. The data given in Table 1 also indicates that there is a satisfactory agreement between the calculated (J) and experimental (J_{exp}) values of the intensity at the maximum CL. The data are clearly in support of a strong quenching effect of MBA on the TCPO–H_2O_2–cumarin (I) chemiluminescence system.

The chemiluminescence quenching terms have been described by Fletcher and Heller in details [42]. In the presence of a quencher, the CL intensity is reduced from I_o to I_Q, the ratio which being directly proportional to the quencher concentration, $[Q]$, as expressed by the Stern–Volmer equation as [43]:

$$\frac{I_o}{I_Q} = 1 + K_Q[Q] \tag{11}$$

where K_Q is the Stern–Volmer quenching constant. According to Eq. (11), a plot of I_o/I_Q versus $[Q]$ will result in a linear graph with an intercept of 1 and a slope of K_Q.

Table 1
The CL parameters evaluated from computer fitting of the CL intensity–time plots for TCPO–H_2O_2–cumarin (I) system

MBA concentration (M)	k_r (s^{-1})	k_f (s^{-1})	M	J	J_{exp}	T_{max}	Y
0	39.4 ± 0.7	1.67 ± 0.01	777 ± 3	661	674	6	464
2.8×10^{-4}	33.5 ± 0.5	1.74 ± 0.01	747 ± 3	646	643	5	429
5.6×10^{-4}	33.3 ± 0.8	1.62 ± 0.01	625 ± 3	532	540	6	387
8.3×10^{-4}	32.2 ± 0.5	1.66 ± 0.01	594 ± 2	508	518	6	358
1.1×10^{-3}	31.4 ± 0.5	1.90 ± 0.01	570 ± 3	462	473	7	300
1.4×10^{-3}	30.2 ± 0.6	1.78 ± 0.01	533 ± 3	446	451	6	298
1.7×10^{-3}	30.1 ± 0.5	1.80 ± 0.01	446 ± 2	373	371	6	247
1.9×10^{-3}	28.6 ± 0.4	1.72 ± 0.01	406 ± 2	339	339	6	238
2.2×10^{-3}	28.3 ± 0.5	1.82 ± 0.01	368 ± 2	305	300	6	202
2.5×10^{-3}	27.4 ± 0.7	2.09 ± 0.02	339 ± 2	282	287	5	162
2.8×10^{-3}	25.4 ± 0.5	2.38 ± 0.03	246 ± 2	195	193	6	103

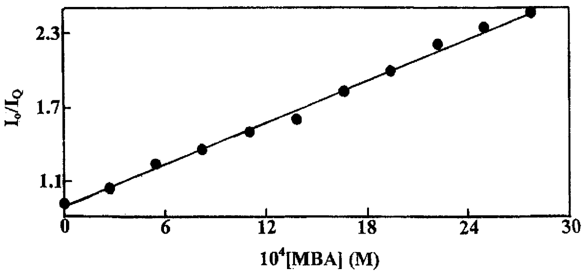

Fig. 4. Stern–Volmer plot of I_o/I_Q vs. [MBA].

For a measurement system based on quenching, K_Q should be as large as possible. A plot of I_o/I_Q versus [MBA] is shown in Fig. 4. The resulting regression equation in the concentration range 2.8×10^{-4} to 2.8×10^{-3} M is $I_o/I_Q = 0.879 + 569\,[Q]$ ($r = 0.9881$), with $K_Q = 569\,M^{-1}$. Given the excited state lifetime of cumarin derivative (I) as approximately 10 ns [44], the actual quenching rate constant, k_q, can be estimated as $5.7 \times 10^{10}\,M^{-1}\,s^{-1}$. This value is in the same order of the k_qs' reported for several quenched luminescence systems, including O_2 quenching of probes bound to DNA (Hoechst no. 33258) [44].

The most likely mechanism for the quenching of TCPO–H_2O_2–cumarin (I) chemiluminescence by MBA, as an easily oxidizable compound [32], could be imagined as an electron transfer quenching pathway [7,18–20,39,44]. According to this mechanism, after the reduction of cumarin, the resulting cumarin radical anion may react with C_2O_4 to produce $C_2O_4^-$ and free cumarin. The net result would be the diminished amount of the reactive intermediate $[C_2O_4^- I^+]$ in solution (see Eq. (3)) and, consequently, the reduced chemiluminscence intensity of the TCPO–H_2O_2–cumarin (I) system. The quencher MBA may also undergo a reduction reaction with the excited state cumarin I^* (see Eq. (4a)) to further decrease the chemiluminescence of the system.

It should be noted that the MBA detection by the quenched chemiluminescence of TCPO–H_2O_2–cumarin (I) system is based on a reduction in the CL signal. Hence, the noise of thus signal should be reduced as much as possible to achieve a favorable signal-to-noise ratio for the quenched chemiluminescence. This can be obtained most easily at a high I_o value, since a greater dynamic range in then obtainable. From these considerations, it can be concluded that cumarin (I) with an inherently high PO-CL intensity is very appropriate for the quenched chemiluminenscence mode.

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