Scientific paper

A Study of Chemiluminescence from Reaction of Bis(2,4,6-trichlorophenyl)oxalate, Hydrogen Peroxide and Dithranol as Antipsoriatic Drug and its Analytical Application

Morteza Hosseini,[a,*] Shiva Dehghan Abkenar,[a] Mohammad Javad Chaichi[b] and Mojtaba Shamsipur[c]

[a] *Department of Chemistry, Islamic Azad University, Savad Kooh Branch & Young Researcher Club, Savad Kooh, Iran*

[b] *Department of Chemistry, Mazandaran University, Babolsar, Iran*

[c] *Department of Chemistry, Razi University, Kermanshah, Iran*

** Corresponding author: E-mail: hossiny54@yahoo.com; Tel.: +98-123-2236707; Fax: +98-124-5221208*

Received: 28-06-2007

Abstract

The chemiluminescence arising from the reaction of bis(2,4,6-trichlorophenyl)oxalate with hydrogen peroxide in the presence of dithranol has been studied. The influence of concentration of peroxyoxalate, hydrogen peroxide, dithranol, catalyst and temperature on the resulting chemiluminescence was investigated. The kinetic parameters for the peroxyoxalate-chemiluminescence of dithranol were evaluated from computer fitting of the resulting intensity-time plots. The activation parameters E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ were evaluated from temperature dependence of the fall rate constants. The chemiluminescence intensity is proportional to the concentration of dithranol. Based on these findings, a simple and rapid flow-injection chemiluminescence method has been developed for the determination of dithranol, which has been satisfactory applied in different pharmaceutical preparations.

Keywords: Peroxyoxalate-chemiluminescence; dithranol; bis(2,4,6-trichlorophenyl)oxalate (TCPO); activation parameters

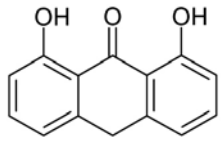
1. Introduction

Dithranol (1,8-dihydroxy-9-anthrone) is an extremely effective and economical antipsoriatic agent. It was safely applied for many years for the treatment of psoriasis and its uses are still developing.[1] Psoriasis occurs in all age groups and is approximately equally distributed in men and women. People with psoriasis may suffer discomfort, restricted motion of joints, and emotional distress. Although dithranol is considered to be an irritant, its principal therapeutic action is the reduction of epidermal DNA synthesis and mitotic activity. Dithranol is used in the treatment of alopecia aerate, eczema and other chronic dermatomes.[2]

There are various analytical methods for the assay of dithranol, such as reversed phase liquid chromatography,[3] laser microprobe mass spectrometry,[4] biochemical techniques,[5] spectrophotometric method[6] and differential pulse polarographic method.[7] However, these methods are often costly, tedious, time consuming and/or need a prior separation procedure, or suffer from the disadvantages of low sensitivity and narrow linear range. An alternative analytical method needs to be simple, rapid and sensitive.

Chemiluminescence (CL) methods provide some advantages for pharmaceutical determinations such as high sensivity, high selectivity, small amount of chemical consumption, cost effectiveness, simple sample preparation and instrumentation.[8–14] An efficient CL reaction requires a chemical pathway that results in the excited state of the product or intermediate, and this excited molecule has to be capable of emitting energy as light (direct CL) or transferring its energy to another molecule of fluorophore (4)

(sensitized CL). Peroxyoxalate-chemiluminescence (POCL) is a type of sensitized CL in which the decomposition of the postulated intermediate dioxetanedione may provide the chemical energy required for excitation. In principal, POCL reaction involves hydrogen peroxide oxidation of an peroxyoxalate ester that transfers energy to a variety of fluorescent molecules named fluorophores which in turn emit light during relaxation from the first singlet exited state in the presence of a suitable week base as a catalyst.[15,16] Therefore, it can be used for the detection of a fluorophore or hydrogen peroxide. Since the advent of its use with combination of high-performance liquid chromatography (HPLC), flow injection analysis (FIA), capillary electrophoresis (CE).[17]



Dithranol

In this paper, the feasibility of using the POCL reaction for the sensitive and selective determination of dithranol is demonstrated. Dithranol acted as a fluorophore and accepted energy from peroxyoxalate ester degradation intermediates and emitted yellow light. The CL intensity is proportional to the concentration of dithranol. Based on these findings, a simple and rapid FIA-CL method has been developed for the determination of dithranol, which has been satisfactory applied for analysis of various pharmaceutical preparations.

2. Results and Discussion

Peroxyoxalate chemiluminescence (POCL) reaction is one of the most efficient non-biological light producing systems. The mechanism of POCL process has been postulated to involve at least one highly energetic intermediate (possibly a dioxetane species) capable of exciting a fluorescent receptor molecule,[17–21] as shown in the following scheme (dithranol: Di).

In preliminary experiments it was found that the addition of few drops of the stock solution of hydrogen

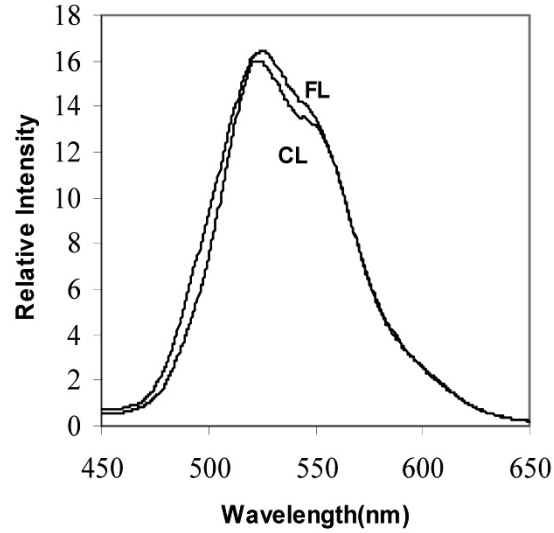
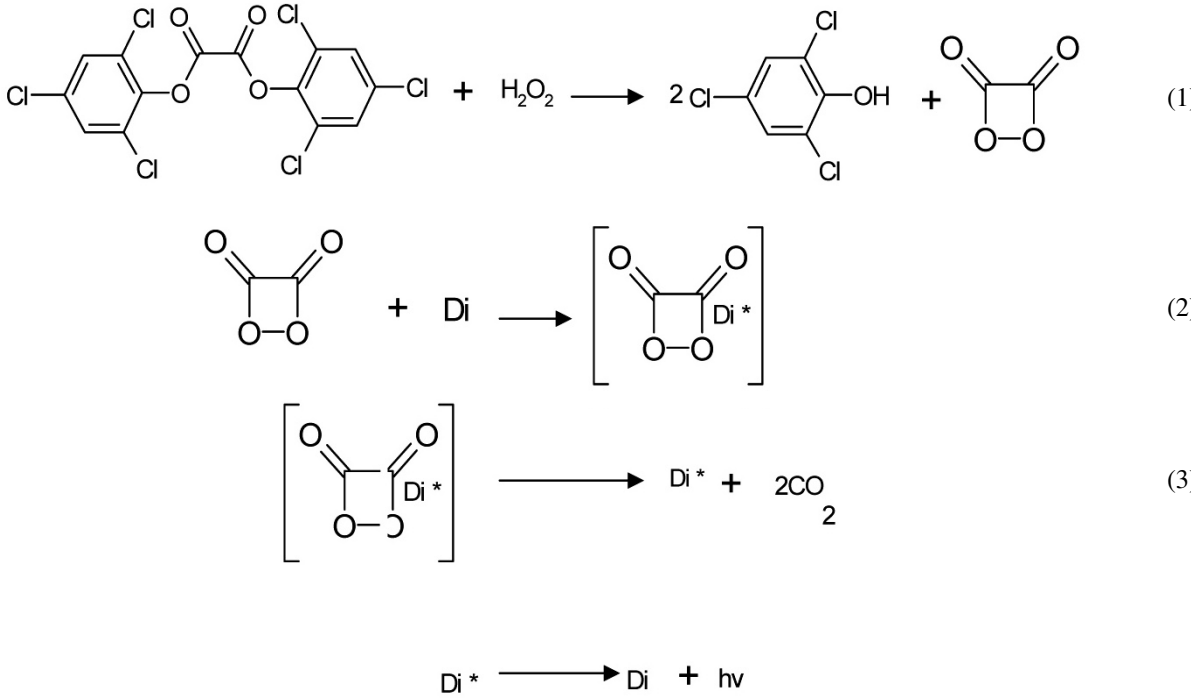


Figure 1. The fluorescence (FL) and chemiluminescence (CL) spectra of dithranol (4×10^{-4} M), TCPO (0.01 M), sodium salicylate (2.2×10^{-4} M) and H_2O_2 (0.2 M) in ethyl acetate.



peroxide to an ethyl acetate solution containing 4×10^{-4} M of dithranol and 0.01 M bis(2,4,6-trichlorophenyl)oxalate (TCPO) results in a very intense yellow light. The sensitized POCL spectrum of dithranol together with its fluorescence spectrum is shown in Fig. 1. Since the light emission steps for both chemiluminescence and fluorescence are essentially analogous it can be seen that the emission wavelength maxima in both processes are similar (i.e., 526 nm).[22,23]

As expected, the intensity of the POCL emission was found to be affected by the initial concentration of the reactants.[8–14] Thus, in the next step, the influence of concentrations of dithranol, TCPO, H_2O_2, and catalyst, as well as the effect of temperature on the POCL system was studied and some of the resulting CL intensity-time plots are shown in Figs. 2–5.

2. 1. Effect of TCPO Concentration

The influence of varying TCPO concentration in the presence of excess amounts of H_2O_2 on CL signal was investigated and the results are given in Table 1 and Fig. 2. As seen from Fig. 2, the peak intensity increases rapidly after mixing and reaches a maximum in a few seconds. Whereas, the decay of light-intensity from the maximum occurs at much longer periods (e.g. >300 s at a TCPO concentration of 2.2×10^{-3}M). As it is obvious from the Fig. 2, there is a nice linear correlation between the chemiluminescence intensity and the TCPO concentration. The basis for such linear correlation has already been discussed in the literature.[19]

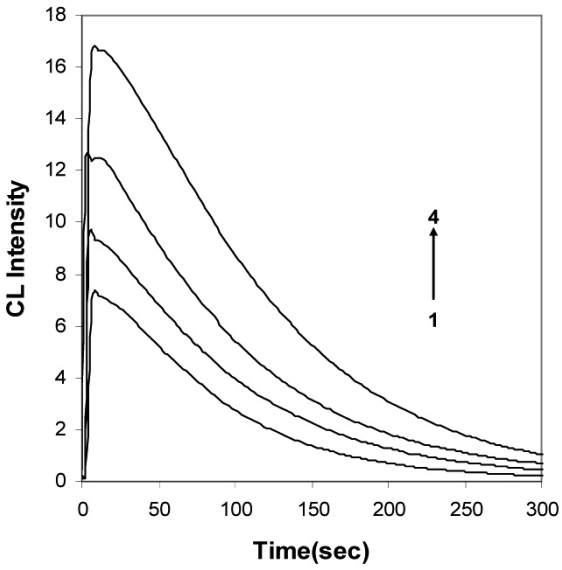


Figure 2. CL intensity as a function of time for reaction of H_2O_2 (0.2 M), sodium salicylate (4.4×10^{-4} M) and dithranol (5.5×10^{-6} M) in ethyl acetate in the presence of varying concentration of TCPO: (1) 8.8×10^{-4} M, (2) 1.3×10^{-3} M, (3) 1.7×10^{-3} M, (4) 2.2×10^{-3} M.

2. 2. Effect of H_2O_2 Concentration

The influence of H_2O_2 concentration on the POCL of dithranol was studied. It was found (Fig. 3) that the signal reached a minimum value when hydrogen peroxide concentration was about 0.7 M. The reduction of response might be due to the increase of reaction rate (Table 1) and hence excitation and de-excitation of dithranol prior to entering the spiral coil for CL measurement. Thus, the optimum concentration of hydrogen peroxide (0.2 M) was chosen in our research work.

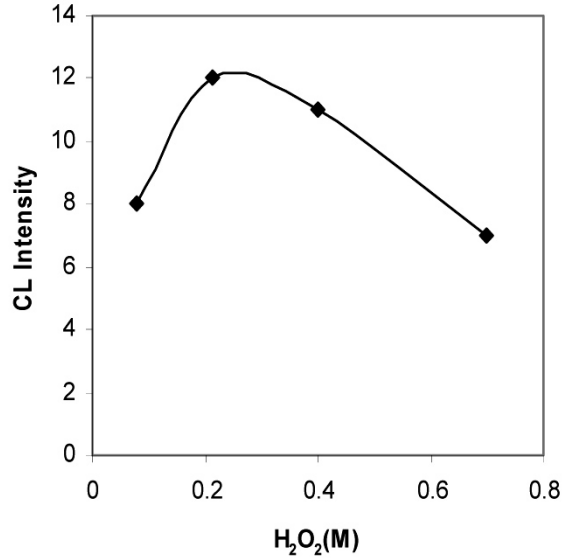


Figure 3. Effect of H_2O_2 concentration on the CL intensity of TCPO-H_2O_2-dithranol system

2. 3. Effect of Sodium Salicylate Concentration

The POCL intensity of a 5.5×10^{-6} M solution of dithranol, under the optimal constant concentration of TCPO and H_2O_2 was found to increase significantly in the presence of sodium salicylate, a behavior that is clearly indicative of the catalytic effect of the salt on the POCL system studied.[9,23]

In order to investigate the optimal concentration of sodium salicylate, the CL response of the H_2O_2-TCPO-dithranol system was measured against the varying concentrations of the base and the resulting plot is shown in Fig. 4. The POCL intensity sharply increased with increasing concentration of sodium salicylate until a concentration of 6.6×10^{-4} M is reached, the observed intensity enhancement being indicative of the catalytic effect of the base. However, further addition of sodium salicylate revealed a gradual decrease in the CL intensity. This is most probably due to the quenching effect of the base at higher concentration, which begins to decompose the reactive intermediate, dioxetanedione, and hence reduces the CL

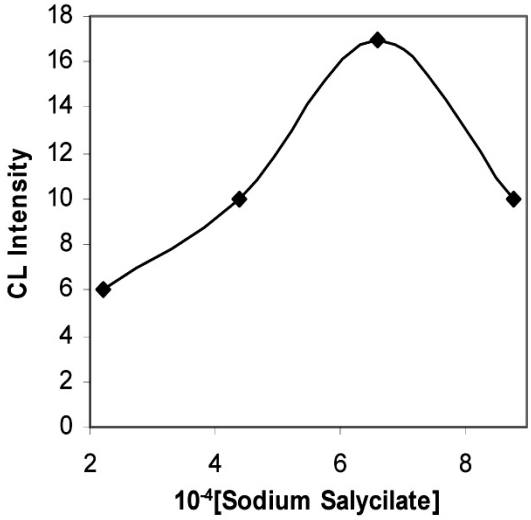


Figure 4. Effect of sodium salicylate concentration on the CL-intensity of TCPO-H_2O_2-dithranol system

light.[23] It should be noted that maximum rise and fall constants is also observed at the sodium salicylate concentration of 8.8×10^{-4} M.

2. 4. Effect of Dithranol Concentration

The effect of dithranol concentration, at a constant amount of TCPO, was studied and the results are shown in Fig. 5 and Table 1. As it is seen from Fig. 5, the peak intensity decreases with increasing dithranol concentrations. This is most probably due to the self-absorption effect and to the formation of dimer structure in high concentration.[24]

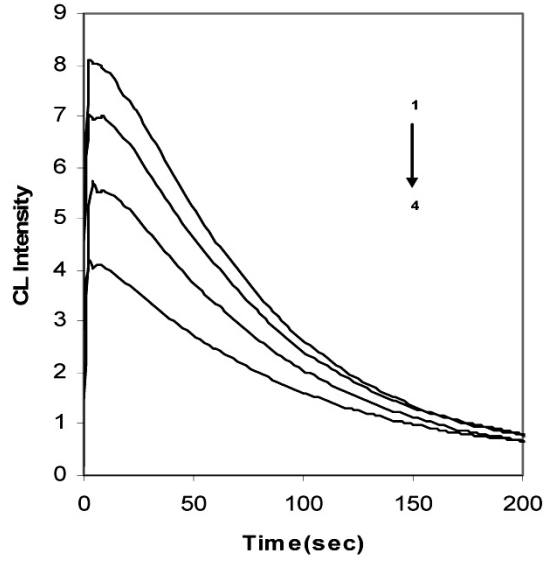


Figure 5. CL intensity as a function of time for reaction of varying concentrations of dithranol with H_2O_2 (0.2 M) in the presence of sodium salicylate (4.4×10^{-4} M) and TCPO (1.3×10^{-3} M), in ethyl acetate: (1) 5.5×10^{-6} M, (2) 1.1×10^{-5} M, (3) 2.2×10^{-5} M, (4) 3.3×10^{-5} M

2. 5. Effect of Flow Rate

The effect of flow rate on the CL emission intensity was studied over the range of 0.3–2.8 ml min^{-1} in each stream.

An increase in the emission intensity was obtained with increase in the flow rate in the range of 0.3–1.2 ml min^{-1} for CL system, but higher flow rates caused a decrease in emission intensity. Thus, a flow rate of 1.2 ml min^{-1} was chosen as the optimum flow rate.

2. 6. Kinetic Data

In order to evaluate the kinetic data for the POCL system studied from the resulting CL intensity-time plots, a pooled-intermediate model was used.[25–26] According to this model the CL reaction is simplified as:

$$A \xrightarrow{k_r} B \xrightarrow{k_f} C \tag{5}$$

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 linear dependence of log I_f on the time of chemiluminescence ($R = 0.9991$) proved this model is true in this experiments.[12] The integrated rate equation for the CL intensity versus time is:

$$I_t = \frac{M}{k_f - k_r}\left[e^{-k_r t} - e^{-k_f t}\right] \tag{6}$$

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 burst of CL. 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 estimation of the intensity at maximum level (J), the time of maximum intensity (τ_{max}) and the total light yield (Y), as follows:

$$J = M\frac{k_f}{k_r}\left[\frac{k_f}{(k_r - k_f)}\right] \tag{7}$$

$$\tau_{max} = \frac{\ln\left(\frac{k_f}{k_r}\right)}{k_f - k_r} \tag{8}$$

$$Y = \int_0^\infty I_t dt = \frac{M}{k_f} \tag{9}$$

In this work, a non-linear least-squares curve fitting program KINFIT[27] was used to evaluate the M, k_r and k_f values from the corresponding CL intensity-time plots.

A typical computer fit of the CL intensity time plots is shown in Fig. 6. The other parameters J, τ_{max} and Y were then evaluated from equations 7–9 using the k_r, k_f and M values. The kinetic parameters thus obtained for all experiments carried out are summarized in Tables 1 and 2. The data given in Table 1 also indicate that in all cases there is a satisfactory agreement between the calculated (J) and experiment (J_{exp}) value of the intensity at the maximum CL.

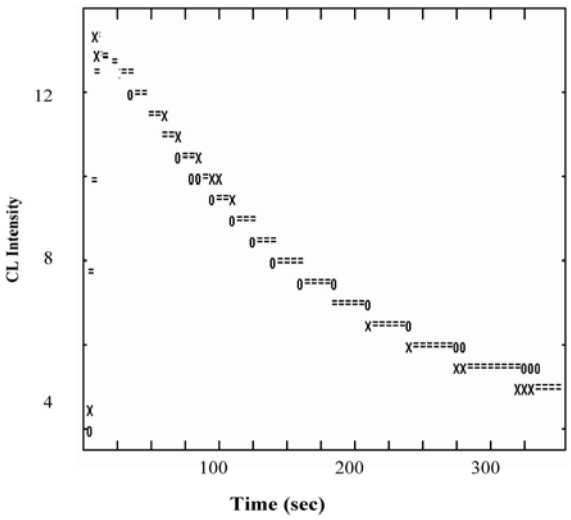


Figure 6. Computer fit of the CL intensity time plot for the H_2O_2-TCPO-dithranol system (see Fig. 2): (×) experimental points; (o) calculated point; (=) experimental and calculated points are the same within the resolution of the plot.

50 °C are shown in Fig.7. As it is illustrated in Fig. 7, the POCL intensity increased with increasing temperature, due to the enhanced population of the activated dithranol* molecules at higher temperatures. Whereas the decay of light-intensity from the maximum occurs at shorter times as the solution temperature increases.

The activation energy for the fall step of the POCL process was obtained from the slope of the corresponding

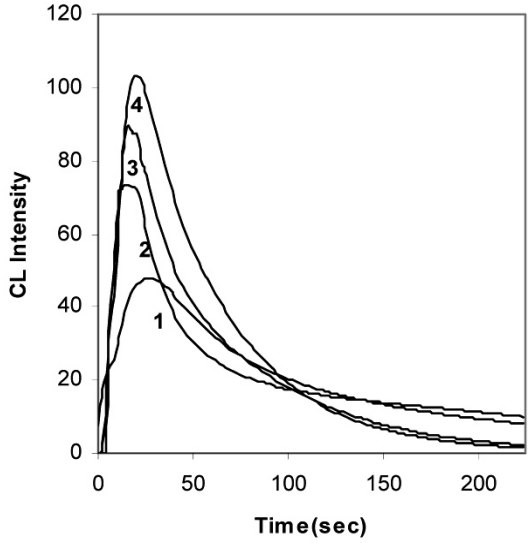


Figure 7. The CL intensity as a function of time for TCPO-H_2O_2-dithranol system at 293 K (1), 303 K (2), 313 K (3), 323 K (4). Experimental conditions: dithranol 5.5×10^{-6} M; TCPO 1.3×10^{-3} M; H_2O_2 0.2 M; sodium salicylate 4.4×10^{-4} M

Table 1. The CL parameters evaluated from computer fitting of the CL intensity-time plots for H_2O_2-TCPO-dithranol-sodium salicylate system

Parameter	Concentration (M)	k_r (10^{-3} s^{-1})	k_f (10^{-3} s^{-1})	M (μV)	J (μV)	τ_{max} (s)	Y (μV/ s)	J_{exp}
H_2O_2	0.08	77.5 ± 0.03	4.05 ± 0.03	10	6	5	2469	8
	0.2	86.01 ± 0.02	7.4 ± 0.02	12.3	12	4	1662	12
	0.3	73.2 ± 0.03	8.11 ± 0.01	11.5	10	4	1356	11
	0.7	69.72 ± 0.02	9.32 ± 0.02	7	6	4	751	7
TCPO	8.8×10^{-4}	157.1 ± 0. 2	12.8 ± 0.1	9.02	7	4	702	7
	1.3×10^{-3}	250.2 ± 0. 2	10.7 ± 0.1	10.97	10	4	1024	10
	1.7×10^{-3}	170.1 ± 0.1	9.85 ± 0.2	14.32	14	4	1454	13
	2.2×10^{-3}	190.1 ± 0.1	9.1 ± 0.2	20.08	17	4	2206	17
Sodium salicylate	2.2×10^{-4}	80.2 ± 0.2	6.58 ± 0.2	16.07	5	4	2345	6
	4.4×10^{-4}	100.1 ± 0.1	10.4 ± 0.1	18.32	11	4	1832	10
	6.6×10^{-4}	130.2 ± 0.2	18.1 ± 0.1	25.24	18	4	1400	17
	8.8×10^{-4}	180.5 ± 0.1	25.6 ± 0.4	24.77	11	4	991	10
Dithranol	5.5×10^{-6}	99.3 ± 0.1	12.3 ± 0.2	9.17	9	2	743	8
	1.1×10^{-5}	98.2 ± 0.1	11.7 ± 0.3	8	7.9	2	678	7
	2.2×10^{-5}	72.3 ± 0.2	11.3 ± 0.2	6.36	6	2	456	5
	3.3×10^{-5}	145.5 ± 0.2	10.2 ± 0.2	4.46	4	4	444	4

2. 7. Effect of Solution Temperature

The influence of solution temperature on the chemiluminescence of the H_2O_2-TCPO-dithranol system, at constant concentrations of all reagents involved, was studied and the resulting response curves at 20, 30, 40, and

Arrhenius plot of ln k_f versus $1/T$ and the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated by using Eyring transition-state theory,[28] from the slope and intercept of the linear plot of ln (k_f/T) versus $1/T$. The resulting activation parameters thus determined for the fall of the POCL burst

Table 2. CL parameters evaluated from computer fitting of the intensity-time plots for H_2O_2-TCPO-dithranol-base system at various temperatures; H_2O_2 0.2 M, TCPO 1.3×10^{-3} M, sodium salicylate 4.4×10^{-4} M, dithranol 5.5×10^{-6} M.

T (K)	k_r (10^{-3} s^{-1})	k_f ($10^{-3}s^{-1}$)	M (μV)	J (μV)	τ_{max} (s^{-1})	Y (μV s^{-1})	J_{exp}
293	105.2 ± 0.3	10.6 ± 0.3	57.69	45	24	5395	47
303	192.2 ± 0.3	13.7 ± 0.3	71.155	70	14	5192	73
313	110.4 ± 0.4	21.1 ± 0.1	109	83	16	5181	89
323	73.3 ± 0.2	26.4 ± 0.2	160	98	20	6066	103

are follows: E_a = 24.57 kJ mol^{-1}, $\Delta H^{\ddagger}$ = 21.93 kJ mol^{-1}, $\Delta S^{\ddagger}$ = –207.85 J $mol^{-1}K^{-1}$ and $\Delta G^{\ddagger}$ = 41.04 kJ mol^{-1}.

2. 8. Analytical Application

As a result of the optimization procedure, the increase of CL intensity was proportional with the concentration of dithranol over the range of 0.1–5.0 μg/mL. In order to improve the precision of detection, the calibration curve was drawn at different concentration ranges. The regression equation of calibration curve for dithranol is:

$$I = 7.723C + 6.19 \ (r = 0.9989, n = 4).$$

where I is the analytical signal in mV and C is the concentration expressed in μg/mL. The limit of detection, calculated at three times the standard deviation of the baseline was determined to be 0.03 μg/mL.

A typical recording output of the proposed CL system for the measurements of different concentrations of dithranol is shown in Fig. 8.

This method shows a good reproducibility for the POCL system of dithranol. The relative standard deviation for 6 parallel measurements of 2.0 μg/mL dithranol is 1.62.

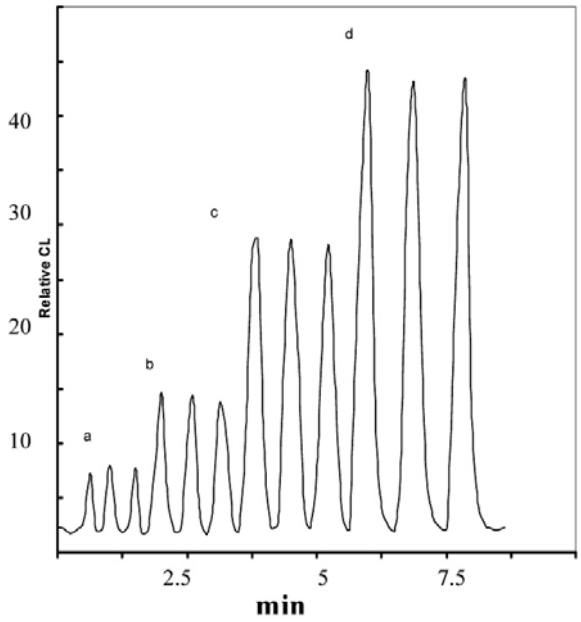


Figure 8. A typical recording output of the proposed CL system for the measurements of different concentrations of dithranol (a: 0.10 μg/mL; b: 1.5 μg/mL; c: 3.0 μg/mL; d: 5.0 μg/mL)

The proposed method was applied for the determination of dithranol in pharmaceutical creams and commercial drug formulations and the results are showed in Table 3. The results agreed well with those obtained by official methods.[5]

Table 3. The comparison of the analysis of dithranol containing solutions of pharmaceutical preparations

Sample	Claimed (%)	Proposed method (CL)	Official method (UV)
Cream	0.25	0.23 ± 0.03	0.24 ± 0.02
	0.5	0.47 ± 0.02	0.48 ± 0.03
Commercial	0.25	0.24 ± 0.04	0.24 ± 0.04
Solution	0.5	0.49 ± 0.03	0.48 ± 0.02

3. Experimental

3. 1. Reagents

All chemicals were of the reagent grade from Fluka and Merck chemical companies. TCPO was synthesized by the reaction of 2,4,6-trichlorophenol with oxalyl chloride in the presence of triethylamine as described before.[29]

TCPO and sodium salicylate solutions (0.01 M) were prepared daily by dissolving 0.4489 g of TCPO and 0.16 g of sodium salicylate (Merck) in ethyl acetate into 100 mL volumetric flask. Hydrogen peroxide (Merck, Super Pure, 30% in water) solutions were assayed via potassium permanganate titration.

Stock solutions of dithranol were freshly prepared by dissolving 0.0561 g standard sample in ethyl acetate and diluting with ethyl acetate to 25 mL, stored in the refrigerator (4 C) and protected from light. Working standard solutions were prepared from the stock solution by appropriate dilution with ethyl acetate before use and protected from light and heat. All other chemicals were of the best grade available and were used as received.

3. 2. Procedure for Dithranol Solution from Commercial Drugs

Two different amounts of dithranol are found in commercially pharmaceutical creams, 0.25% (w/w) and

0.5% (w/w) and commercial solutions 0.25% (w/v) and 0.5% (w/v). These creams also contain 0.2% salicylic acid, 12% glycerol monostearate, 5% spermaceti, 11.5% liquid vaseline, 0.15% niposal, 5% nipagine M and 65.7% distilled water. A certain amount of cream samples were taken into ethyl acetate, shaken for an hour and stored at 0°C overnight. By this way, waxy ingredients of dissolved cream samples were precipitated. The supernatant was decanted and diluted with ethyl acetate to a desired concentration. Dithranol standard solutions (0.5%, 0.25% in methanol) were freshly prepared from reagent grade of this compound.[7]

3. 3. Apparatus

A peristaltic pump was used to deliver flow streams in the FIA system. The PTFE tubing (1 mm i.d.) was used as a connection part in the flow system. The sample was injected with a Rheodyne sample injector, model 7125. Two solutions merged in a spiral flow cell in front of a photomultiplier tube (PMT). The flow cell was a glass spiral (2 mm i.d, 500 L internal volume) positioned in front of a mirror in a sealed housing. CL spectra of the proposed system were investigated using a model LS-50B luminescence spectrometer (Perkin-Elmer).

3. 4. Procedures

The flow lines A and B were connected to TCPO and sodium salicylate solution, hydrogen peroxide and ethyl acetate solution reservoir vessels, respectively. A 500 L standard or sample solution was injected into the carrier stream. This stream was combined with hydrogen peroxide and TCPO solution, and then reached the flow cell. The concentration of the sample was quantified by the recorded relative CL intensity.

3. 5. Conclusion

The proposed method is simple, sensitive, rapid, suitable for automatic and continuous analysis, and can be applied for the determination of dithranol in pharmaceutical preparations with satisfactory results. The proposed FIA system might be coupled with some separation techniques, such as HPLC and CE, and used as a post-column detection system for determination of dithranol after separation from the other contaminants in the commercial drugs.

4. Acknowledgements

This work was supported by grants from Islamic Azad University Branch of Savadkooh.

5. References

1. W. Wiegrebe, E. Plumier, K. K. Mayer, U. Runne, W. Schultz-Amling, J. Rosmarinowski, G. J. Safar, K. D. Kubka, *Arch. Dermatol. Res*. **1985**, *277*, 153–155.
2. Remington's Pharmaceutica Science 18th Edition **1990**, 763–765.
3. D. E. Wurster, S. M. Upadrashta, *J. Chromatogr*. **1986**, *362*, 71–78.
4. The International Pharamcopoeia, **2001**, *4*, 86–89.
5. U. Runne, J. Rosmarinowski, G. J. Safar, K. D. Kupka, W. Schultz-Amling, E. Plumier, W. Wiegrebe, *Arch. Dermatol. Res*. **1983**, *275*, 269–270.
6. British Pharmaceutical Codex, The Pharmaceutical Press, London, **1973**, 767–769.
7. A. Temizer, D. Özer, M. T. Orbey, *Anal. Lett*. **1994**, *27*, 717–722.
8. K. W. Sigvardson, J. M. Kennish, J. W. Birks, *Anal. Chem*. **1984**, *56*, 1096–1102.
9. A. G. Mohan, in: J. G. Burr (Ed.), Chemi and Bioluminescence, Marcel Dekker, New York, **1985**, pp. 245–258.
10. K. Robards, P. J. Worsfold, *Anal. Chim. Acta* **1992**, *266*, 147–173.
11. A. G. Hadd, J. W. Birks, in: R. E. Sievers (Ed.), Selective Detectors, Chemical Analysis, vol. 131, Wiley, New York, **1995**, pp. 209–215.
12. M. Shamsipur, M. J. Chaichi, *Spectrochim. Acta A*. **2001**, *57*, 2355–2358.
13. M. Shamsipur, M. J. Chaichi, *J. Photochem. Photobiol. A*. **2003**, *155*, 69–72.
14. M. Shamsipur, M. J.Chaichi, *Spectrochim. Acta A* **2005**, *61*, 1227–1231.
15. O. A. K. Campbell in: Chemiluminescence: Principles and Applications in Biology and Medicine, VCH, Weinheim, **1988**, pp.17–25.
16. A. G. Hadd, A. Seeber, J. W. Birks, *J. Org. Chem*. **2000**, *65*, 2675–2683.
17. S. M. Silva, F. Casallanovo, K. H. Oyamaguchi, L. F. L. M Ciscato, C. V. Stevani, W. J. Baader, *Chemiluminescence* **2002**, *17*, 313–320.
18. G. B. Schuster, *Acc. Chem. Res*. **1979**, *12*, 366–373.
19. C. L. R. Catherall, T. F. Palmer, R. B. Cundall, *J. Chem. Soc., Faraday. Trans. 2*. **1984**, *80*, 823–836.
20. G. Orosz, *Tetrahedron* **1989**, *45*, 3493–3506.
21. C. V. Stevani, S. M. Silva, W. J. Baader, *Eur. J. Org. Chem*. **2000**, 4037–4046.
22. C. L. R. Catherall, T. F. Palmer, R. B. Cundall, *J. Chem. Soc., Faraday Trans. 2*. **1984**, *80*, 837–849.
23. M. Sigbrand, T. Jonsson, E. Ponten, K. Irgum, R. Bos, in: A. M. La Campana, W. J. Baeyens (Eds.), Chemiluminescence in Analytical Chemistry, Marcel Dekker, New York, **2000**, pp. 135–150.
24. I. Delneuville, J. P. Dechesne, L. Delattre, *Int. J. Pharm*. **1998**, *168*, 109–118.

25. R. S. Givens, R. L. Schowen, in: J. W. Birks (Ed.) Chemiluminescence and Photochemical Reaction Detection in Chromatography (Chapter 5), VCH, New York, **1989**.
26. M. Orlović, R. L. Schowen, R. S. Givens, F. Alvarez, B. Matuszewski, N. Parekh, *J. Org. Chem.* **1989**, *54*, 3606–3610.
27. J. L. Dye, V. A. Nicely, *J. Chem. Educ*. **1971**, *48*, 443–448.
28. S. H. Lin, K. P. Li, H. Eyring, in: H. Eyring, D. Handerson, W. Yost (Eds.), Physical Chemistry, an Advanced Treatise, Academic Press, New York, **1977**, Vol. II, pp. 5–10.
29. A. G. Mohan, N. J. Turro, *J. Chem. Educ*. **1974**, *51*, 528–535.

Povzetek

Raziskali smo kemoluminiscenco, ki se pojavi pri reakciji med bis(2,4,6-triklorofenil)oksalatom in vodikovim peroksidom v prisotnosti ditranola. Ugotavljali smo vpliv koncentracije peroksioksalata, vodikovega peroksida, ditranola, katalizatorja in temperature na nastalo kemoluminiscenco. S pomočjo računskega ujemanja dobljenih odvisnosti intenzivnosti od časa smo določili kinetične parameter za peroksioksalatno kemoluminiscenco ditranola. Aktivacijske parametre E_a, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ in $\Delta G^{\ddagger}$ smo določili iz temperaturne odvisnosti konstant upadanja. Intenziteta kemoluminiscence je bila sorazmerna koncentraciji ditranola. Na osnovi teh ugotovitev smo razvili enostavno in hitro pretočno kemoluminiscenčno metodo za določanje ditranola, ki smo jo zadovoljivo dobro uporabili na različnih farmacevtskih pripravkih.