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### **A STUDY OF CHEMILUMINESCENCE FROM REACTIONS OF PEROXYOXALATE ESTERS, HYDROGEN PEROXIDE, AND 7-AMINO-4-TRIFLUOROMETHYLCUMARIN**

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**A STUDY OF CHEMILUMINESCENCE  
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**ABSTRACT**

The chemiluminescence arising from the reaction of bis(2,4,6-trichlorophenyl)oxalate (TCPO) and bis(2,4-dinitrophenyl)oxalate (DNPO) with hydrogen peroxide in the presence of 7-amino-4-trifluoromethylcumarin (ATFMC) have been studied. The influences of stirring solution, nature of peroxyoxalate and solvent properties on the resulting chemiluminescence were investigated. The relationship between the chemiluminescence intensity and concentrations of TCPO and ATFMC are reported.

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\*Corresponding author.

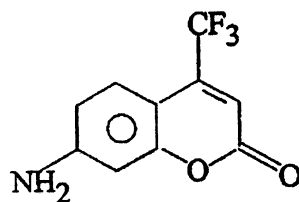
*Key Words:* Chemiluminescence; 7-Amino-4-trifluoromethyl-cumarin; Peroxyoxalate;  $\text{H}_2\text{O}_2$

## INTRODUCTION

The phenomenon of chemiluminescence is one of the most fascinating demonstrations of chemical energy<sup>1</sup>. The brilliant emission resulting from the reaction of some oxalic acid derivatives is one of the most outstanding examples of chemiluminescence<sup>2,3</sup>. This group of chemiluminescent reactions has been classified as the peroxyoxalate chemiluminescence (PO-CL) reactions. The PO-CL reactions have been reported as one of the most sensitive analytical tools to detect a wide range of fluorescent compounds<sup>4,5</sup>. These are based on the reaction of hydrogen peroxide with an activated oxalate which result in the formation of one or more energy-rich intermediate(s). These intermediates are capable of exciting a large number of fluorophores<sup>1,4-7</sup> through the chemically initiated electron exchange luminescence mechanism<sup>8</sup>.

The choice of activated oxalate for the PO-CL reactions has been the subject of numerous efforts<sup>9-14</sup>. On the basis of the results obtained, there are at least two characteristics necessary in the design of useful reagents for the PO-CL. First, the existence of electron-withdrawing groups around the central peroxyoxalate moiety which facilitate the generation of reactive intermediates responsible for the transfer of the excitation energy to a fluorophore compound<sup>13,14</sup>. Second, the sufficient solubility of the leaving groups in the solvent for which the reagent was designed. Detailed discussion of the possible mechanisms of the PO-CL reactions are frequently reported and reviewed in the literature<sup>15-19</sup>. The results have led to a preferential use of bis(2,4,6-trichlorophenyl) oxalate (TCPO), bis(2,4-dinitrophenyl) oxalate (DNPO) and bis[2-(3,6,9-trioxadecyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO) in analytical detection systems.

Cumarin derivatives are widely distributed in the plant kingdom, some of them being physiologically active<sup>20</sup>. Antimicrobial properties of some coumarins have been investigated<sup>21,22</sup>. Many coumarin derivatives are of great practical interest<sup>20</sup>. They are widely used as optical brighteners and dyes for natural and synthetic materials as well as in lasers<sup>23</sup>. 7-Amino-4-trifluoromethylcoumarin (ATFMC) is used in the synthesis of a substrate for the fluorometric assay of proteolytic enzymes in biological fluids<sup>24</sup> and for use as a laser dye<sup>25</sup> and as a marker for proteinase<sup>26</sup>. In this paper we report the first study of chemiluminescence from reactions of peroxyoxalate esters, hydrogen peroxide and ATFMC.



ATFMC

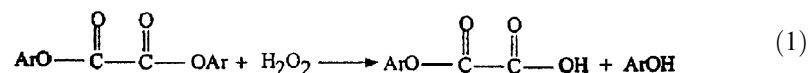
### EXPERIMENTAL

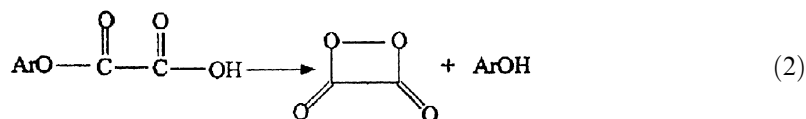
All chemicals were of the reagent-grade from Fluka chemical company and used as received. TCPO and DNPO were prepared from the reaction of 2,4,6-trichlorophenol and 2,4-dinitrophenol, respectively, with oxalyl chloride in the presence of triethylamine as described previously<sup>3</sup>. Hydrogen peroxide (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 shaken well on an electrical shaker. After 10 h, the organic phase was separated, dried on anhydrous Na<sub>2</sub>SO<sub>4</sub> and the H<sub>2</sub>O<sub>2</sub> 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  $5.0 \times 10^{-3}$  M sodium salicylate) was prepared from this solution. The stock solutions of TCPO, DNPO and ATFMC (0.01 M) were also prepared in ethyl acetate and methanol solutions.

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 in flattened bottom glass cells of 15 mm diameter at room temperature.

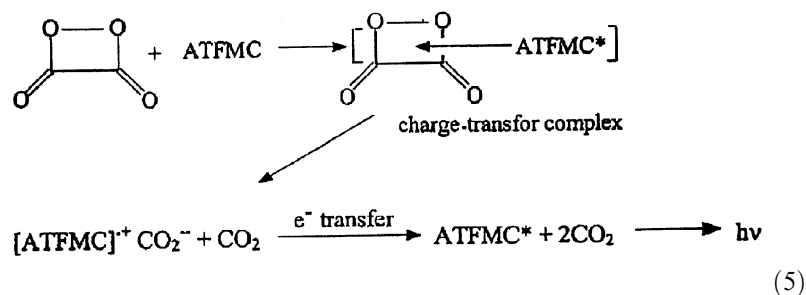
### RESULTS AND DISCUSSION

Peroxyoxalate chemiluminescence (PO-CL) is one of the most efficient non-biological light producing systems. The most frequently proposed mechanism for the PO-CL process involves the following steps<sup>1,3,16,27</sup>:





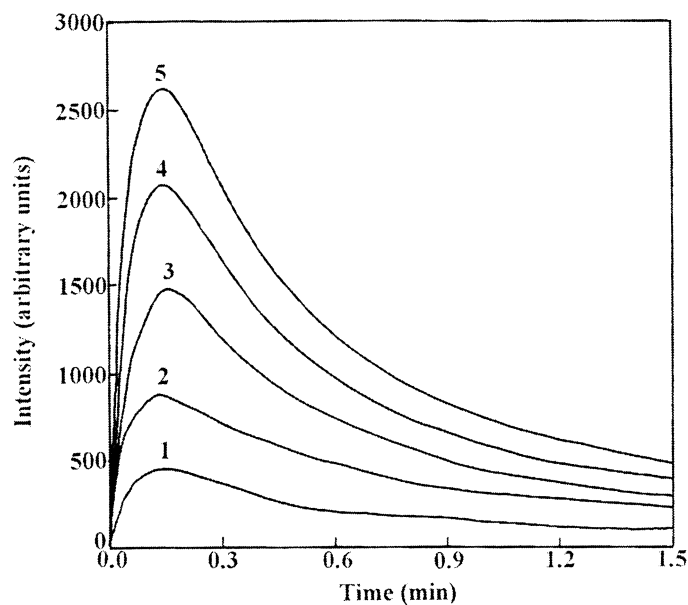
Excitation of the fluorescer ATFMC, step (3), most probably occurs via the chemically initiated electron exchange luminescence pathway (CLEEL) as follows<sup>27,28</sup>:



In preliminary experiments, it was found that the addition of a few ml of the stock solution of hydrogen peroxide to a nearly colorless ethyl acetate solution containing  $1.0 \times 10^{-3}$  M ATFMC and 0.01 M TCPO results in a very intense blue light.

Upon the use of alcohol, instead of ethyl acetate, the light intensity was greatly reduced and the color shifted to green. It is worthy to note that, in the absence of sodium salicylate as a base, the emission of light was relatively long-lived but the time taken to reach maximum emission and the duration of the emission were not reproducible. However, in the presence of the base, reproducible emission intensity decay curves were obtained.

Typical intensity decay curves for the chemiluminescence of varying concentrations of ATFMC in the presence of fixed amounts of TCPO and  $\text{H}_2\text{O}_2$  are shown in Fig. 1. As seen, the peak intensity increases sharply with increasing concentration of ATFMC as a suitable fluorescer. In all cases, the light intensity increases rapidly after mixing and reaches a maximum in  $t_m = 8\text{--}10$  s. Whereas, the decay of light intensity from the maximum occurs at longer periods of time (up to 90 s) via an exponential process. The arbitrary



**Figure 1.** Emission intensity as a function of time for reaction of TCPO ( $5.0 \times 10^{-3}$  M) with  $\text{H}_2\text{O}_2$  (0.14 M) in ethyl acetate at various concentrations of ATFMC: (1)  $5.0 \times 10^{-4}$  M, (2)  $1.0 \times 10^{-3}$  M, (3)  $2.0 \times 10^{-3}$  M, (4)  $5.0 \times 10^{-3}$  M, (5)  $1.0 \times 10^{-2}$  M.

quantity  $t_{1/2}$  (during which the luminescence light intensity decays to  $1/2$  of its maximum value) was chosen to permit the comparison of emission lifetimes (see Tables 1 and 2). It is interesting to note that the spectral distribution of chemiluminescence was found to essentially resemble the

**Table 1.** Effect of Stirring Sample Solution on  $t_m$  and  $I$  of Chemiluminescence of TCPO- $\text{H}_2\text{O}_2$ -ATFMC System in Ethyl Acetate

ATFMC (M)	Stirred		Unstirred	
	$t_m$ (s)	$I$	$t_m$ (s)	$I$
$5.0 \times 10^{-4}$	8	493	29	275
$1.0 \times 10^{-3}$	8	893	20	633
$2.0 \times 10^{-3}$	9	1499	18	1223
$5.0 \times 10^{-3}$	8	2084	17	1359
$1.0 \times 10^{-2}$	9	2636	13	1791

**Table 2.** Effect of the Nature of Oxalate Ester on  $t_m$ , I and  $t_{1/2}$  of Chemiluminescence of Various Concentrations of ATFCM

ATFCM Concentration (M)	TCPO			DNPO		
	$t_m$ (s)	I	$t_{1/2}$ (s)	$t_m$ (s)	I	$t_{1/2}$ (s)
$5.0 \times 10^{-4}$	8	493	30	< 5	90	< 3
$1.0 \times 10^{-3}$	8	893	38	< 5	93	5
$2.0 \times 10^{-3}$	9	1499	36	< 5	118	6
$5.0 \times 10^{-3}$	8	2084	31	< 5	120	< 5
$1.0 \times 10^{-2}$	9	2636	32	< 5	123	< 5

**Table 3.** Effect of Solvent on I and  $k_L$  of TCPO- $H_2O_2$ -ATFCM System at Various Concentrations of TCPO

TCPO (M)	Ethyl Acetate		Methanol	
	I	$k_L$ (min)	I	$k_L$ (min)
$3.0 \times 10^{-4}$	38	0	—	—
$5.0 \times 10^{-4}$	76	0.33	17	—
$1.0 \times 10^{-3}$	257	0.86	21	—
$3.0 \times 10^{-3}$	765	1.22	45	—
$5.0 \times 10^{-3}$	1554	1.48	116	0.15
$8.0 \times 10^{-3}$	2515	1.64	251	0.17

fluorescence spectrum of ATFCM, emphasizing that the first singlet excited state of the fluorescer is the chemiluminescent emitter.

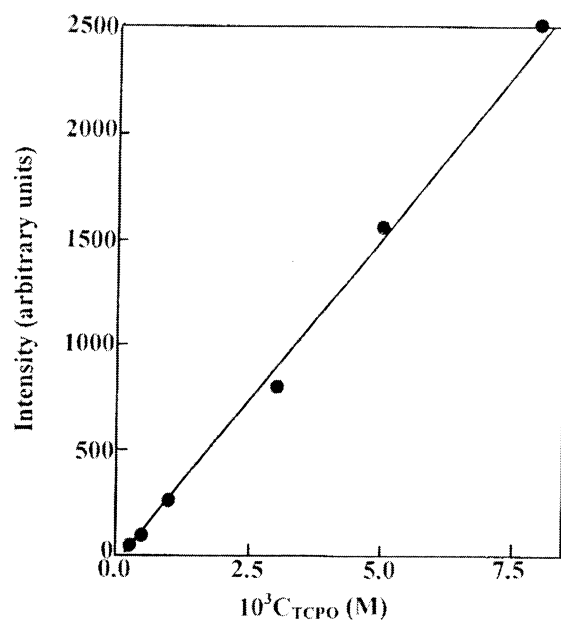
The influence of stirring sample solution (at a rate of 500 rpm) on the time required to reach maximum intensity,  $t_m$ , and the chemiluminescence light intensity, I, for TCPO- $H_2O_2$ -ATFCM system was investigated and the results are given in Table 1. As is obvious, stirring the sample solution resulted in a pronounced decrease in  $t_m$  value and, at the same time, a considerable enhancement in the chemiluminescence light intensity, at entire concentration range of the fluorescer studied.

In Table 2, the chemiluminescence characteristics of TCPO- $H_2O_2$ -ATFCM and DNPO- $H_2O_2$ -ATFCM systems in ethyl acetate solution are compared. It is well known that the nature of the leaving groups of the oxalic ester has a profound effect on the efficiency of the PO-CL systems. Dieryl esters of oxalic acid are reported to be among the most efficient substrates<sup>1-19</sup>.

However, the nature of the substituents on the phenyl ring greatly affects the overall efficiency of the chemiluminescent systems<sup>1,2,6,10,12,29</sup>.

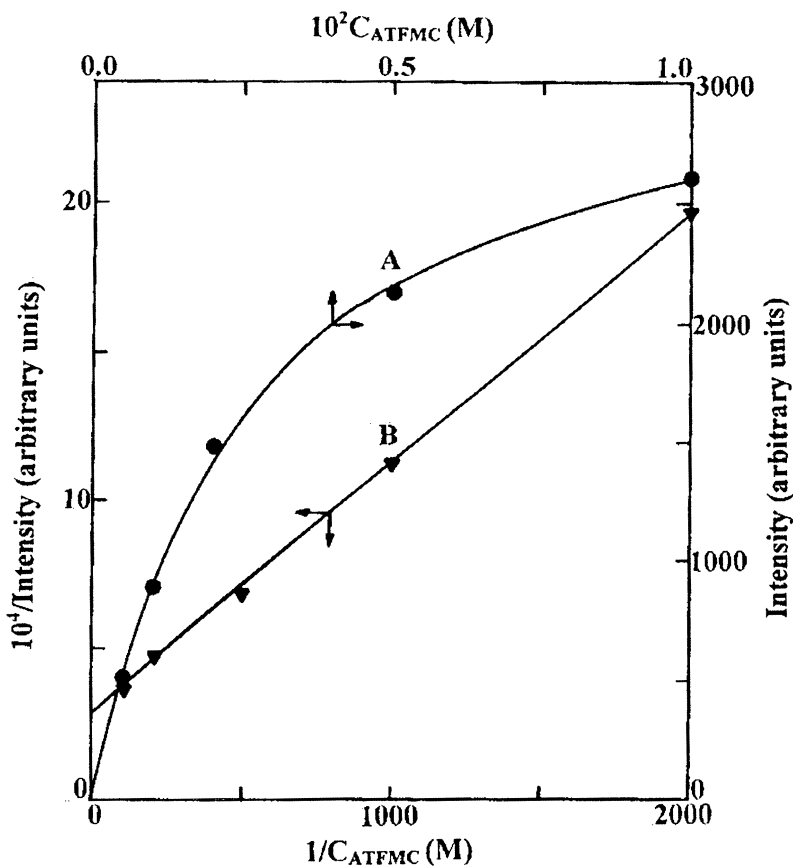
As it is obvious from Table 2, the use of TCPO resulted in a great enhancement in chemiluminescence of the cumarin derivative used over that of DNPO. The observed trend could be due not only to the different electron-withdrawing effects of the chloro and nitro groups on TCPO and DNPO, respectively, but also to the influence of the amino group of ATFMF on the PO-CL system. It is noteworthy that such influence of the chemical structure of the fluorophores<sup>6</sup> and the catalytic effects of nitrogen containing heterocyclic compounds<sup>30,31</sup> on the peroxyoxalate chemiluminescence reactions are fully investigated.

The influence of solvent properties on the chemiluminescence of TCPO-H<sub>2</sub>O<sub>2</sub>-ATFMF system was investigated by using ethyl acetate and methanol as solvent and the results are summarized in Table 3. It should be noted that, in Table 3,  $k_L$  is the rate constant for exponential decay of the chemiluminescence intensity of the system. Obviously, the efficiency of the PO-CL system studied in ethyl acetate is much higher than that in methanol solution. It is well known that, in the CIEEL mechanism, the influence of



**Figure 2.** Effect of TCPO concentration on the chemiluminescence intensity of TCPO-H<sub>2</sub>O<sub>2</sub>-ATFMF system in ethyl acetate solution.





**Figure 3.** Effect of ATFMC concentration on the chemiluminescence intensity of TCPO- $\text{H}_2\text{O}_2$ -ATFMC system in ethyl acetate solution: (A)  $I$  vs.  $C_{\text{ATFMC}}$ , (B)  $I^{-1}$  vs.  $C_{\text{ATFMC}}^{-1}$ .

different solvents on the chemiluminescence intensity depends on their ability to stabilize the intermediate charge-transfer complex, in equation (5), within a solvent cage<sup>27,32</sup>. The viscous non-polar solvents such as ethyl acetate (as compared with methanol) are usually the best solvents for this purpose.

The influence of varying TCPO concentration in the presence of excess amounts of hydrogen peroxide was investigated and the results are given in Table 3 and Fig. 2. As it is seen from 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 detail in the literature<sup>15</sup>.

The effect of ATFMF concentration, at constant amount of TCPO, was studied and the results are shown in Table 2 and Fig. 3(A) and 3(B). As it has been clearly shown before<sup>33</sup>, there is an exponential increase in chemiluminescence of the TCPO-H<sub>2</sub>O<sub>2</sub>-ATFMF system with increasing concentration of the fluorescer ATFMF (Fig. 3A). However, the reciprocal plots of chemiluminescence intensity against fluorescer concentration was found to result in a linear calibration plot, the slope and intercept of which were both dependent upon the initial TCPO concentration<sup>33</sup>.

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