

## Solvent Effects on Chemiluminescence from the Hydrogen Peroxide-Lucigenin Reaction: Kinetics of Light Emission in Mixed Polar Solvents

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**Summary.** The effect of reaction media composition on reaction kinetics was studied for the reaction of lucigenin (10,10'-dimethyl-9,9'-biacridinium nitrate) with hydrogen peroxide and alkali. Chemiluminescent emission as well as lucigenin disappearance were recorded in mixtures of water with the co-solvents methanol, ethanol, 1-propanol, dimethylsulfoxide, and dimethylformamide. The kinetic results (base and peroxide concentration influence on the reaction rate and the relative chemiluminescence yield) are very similar in all the reaction media, suggesting that the fundamental step in the disappearance of lucigenin and in light emission decay is  $\text{HO}_2^-$  addition to lucigenin. Lucigenin can also disappear through dark reactions with  $\text{OH}^-$  or  $\text{H}_2\text{O}_2$ . The co-solvent acts as a catalyst for the reaction with  $\text{HO}_2^-$  and increases both the initial chemiluminescence intensity and the decay rate constant.

**Keywords.** Chemiluminescence; Lucigenin; Solvent effects.

### Lösungsmittelleffekte bei der Chemilumineszenz der Wasserstoffperoxid-Lucigenin Reaktion. Kinetik der Lichtemission in gemischten polaren Lösungsmitteln

**Zusammenfassung.** Es wurde der Einfluß der Zusammensetzung des Reaktionsmediums auf die Kinetik der Reaktion von Lucigenin (10,10'-dimethyl-9,9'-biacridiniumnitrat) mit Wasserstoffperoxid und Alkali untersucht. Die Emission der Chemilumineszenz und das Verschwinden von Lucigenin wurde in Mischungen von Wasser mit den Kosolventien Methanol, Ethanol, 1-Propanol, Dimethylsulfoxid und Dimethylformamid gemessen. Die kinetischen Resultate (Einfluß der Basen- und Peroxid-Konzentration auf die Reaktionsgeschwindigkeit und die relative Chemilumineszenzausbeute) sind für alle Reaktionsmedien sehr ähnlich; das legt den Schluß nahe, daß der grundlegende Schritt im Verbrauch des Lucigenin unter Lichtemission die Addition von  $\text{HO}_2^-$  an Lucigenin ist. Lucigenin kann auch über Dunkelreaktionen mit  $\text{OH}^-$  oder  $\text{H}_2\text{O}_2$  verschwinden. Das Kosolvens agiert als Katalysator für die Reaktion mit  $\text{HO}_2^-$  und erhöht sowohl die anfängliche Chemilumineszenzintensität als auch die Zerfallsgeschwindigkeitskonstante.

### Introduction

Chemiluminescence (CL) from 10,10'-dimethyl-9,9'-biacridinium nitrate (lucigenin, *L*), first reported in 1935 [1], has been studied in reactions with a wide variety of substances [2], mainly in aqueous solutions.

The reaction of lucigenin with aqueous hydrogen peroxide has been extensively studied [3–5]. N-Methylacridone (*NMA*) is the major fluorescent product but the recorded CL spectrum does not match the fluorescence spectrum of *NMA*. The CL reaction with excess hydrogen peroxide is first order and follows the same rate law as the disappearance of lucigenin from solution [3].

This reaction has also been characterized in aqueous solutions of different types of membrane mimetic agents, such as anionic surfactants and cyclodextrins [6–10]. While cyclodextrins increase CL intensity [7, 8], the presence of anionic surfactants causes a decrease [6]. Some surfactants also change the recorded CL spectrum [9]. However, in spite of these examples of the important role played in this reaction by the physical and chemical nature of the reaction media, few papers have been devoted to the subject.

The main purpose of this work was to determine the effects of co-solvents such as aliphatic alcohols, dimethylsulfoxide (DMSO), and dimethylformamide (*DMF*) on the CL of lucigenin. Moreover, the analysis of these effects gave an indication of the nature of the reaction.

This paper presents the kinetic results for CL generation in the hydrogen peroxide-lucigenin reaction in mixtures of water with co-solvents.

## Experimental

Lucigenin (Ega-Chemie) was recrystallized once from ethanol and dried at 110°C. UV-Vis absorption and fluorescence spectra were in good agreement with those reported in the literature [3, 7]. Doubly distilled (in glass) water was employed. The other reagents and co-solvents were of analytical or spectroscopic grade and were used without further purification.

CL intensity-time curves were obtained using a Laser Power Meter 820 (Newport Research Corporation) photometer attached to an Omniscrite D/5000 (Houston Instruments) strip chart recorder. Correction of such curves for spectral response of photometer was unnecessary because of the small differences in the CL spectra recorded in the various reaction media used.

UV-Vis absorption measurements were performed with a Cary-17 or a Pye-Unicam SP-1800 Spectrophotometer. Fluorescence and CL spectra were obtained using a Perkin-Elmer LS-5 Luminescence Spectrometer.

3 ml of the solvent mixture containing the desired concentrations of hydrogen peroxide and lucigenin were added to a special glass cuvette. 1 ml of aqueous sodium hydroxide of the appropriate concentration was added manually and the intensity-time curves and absorbance-time curves were immediately recorded. The injection of sodium hydroxide simultaneously initiated the CL reaction and the recording. For recording intensity-time curves the cuvette was placed in front of the photometer in a specially constructed dark chamber.

Recording times (with detectable emission) were longer than 5 min in all cases. The first 10 seconds (on the average) of the intensity-time curves were not taken into account for rate constant determinations because during this time the reactions were strongly influenced by mixing effects. No active mixer was used.

## Results and Discussion

In studying reaction kinetics we employed NaOH and H<sub>2</sub>O<sub>2</sub> at higher concentrations than lucigenin to obtain pseudo-first order conditions for lucigenin. This ensured that both the disappearance of lucigenin, measured as the decrease in UV absorbance at  $\lambda_{\max}$  ( $\lambda_{\max} = 368$  nm in 80:20, *v/v*, water:ethanol), and the decay of

light emission, followed the first order rate law to at least 3 half-lives. Moreover, both rate constants were about the same.

Profiles obtained by plotting the logarithm of the pseudo-first order rate constant ( $k$ ) vs. the initial concentration of hydrogen peroxide  $[\text{H}_2\text{O}_2]_0$  are shown in Fig. 1, for the reaction in mixtures of 80% *v/v* water with ethanol or *DMSO*. The two curves are very similar; the rate constant grows linearly with  $[\text{H}_2\text{O}_2]_0$  until  $[\text{H}_2\text{O}_2]_0$  becomes higher than  $[\text{NaOH}]_0$  (initial hydroxide concentration).

The  $\log k$  vs.  $[\text{NaOH}]_0$  curves are shown in Fig. 2 for two quite different reaction mixtures. For the *DMSO*:water mixture (35:65 *v/v*), the rate constant grows linearly with  $[\text{NaOH}]_0$  until  $[\text{NaOH}]_0$  becomes higher than  $[\text{H}_2\text{O}_2]_0$ .

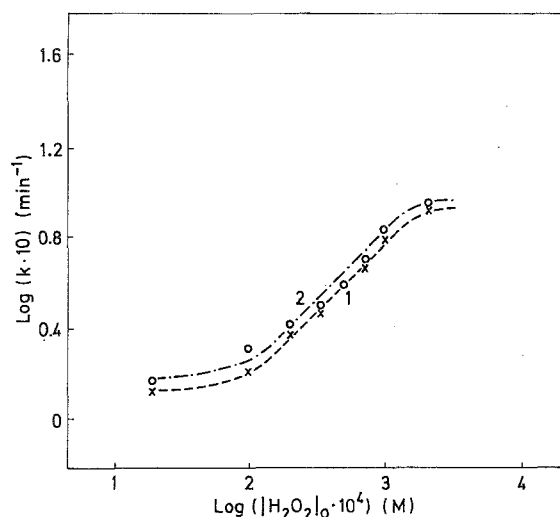


Fig. 1. Change in rate constant with peroxide concentration for the hydrogen peroxide-lucigenin reaction in: (1) water:ethanol, 80:20 (*v/v*), and (2) water:*DMSO*, 80:20 (*v/v*).  $[L]_0 = 6.25 \cdot 10^{-6} M$ ,  $[\text{NaOH}]_0 = 0.1 M$

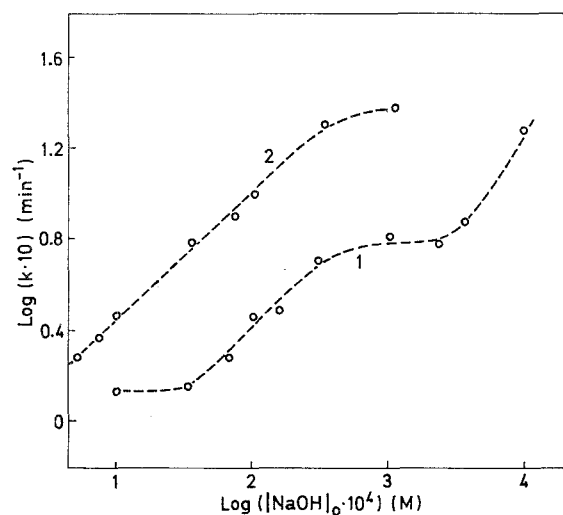
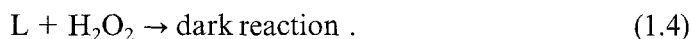
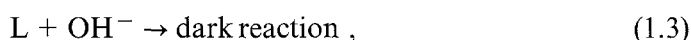
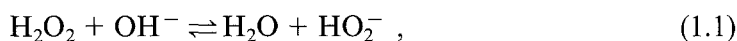


Fig. 2. Change in rate constant with base concentration in: (1) water:ethanol, 80:20 (*v/v*),  $[\text{H}_2\text{O}_2]_0 = 0.1 M$ , and (2) water:*DMSO*, 65:35 (*v/v*),  $[\text{H}_2\text{O}_2]_0 = 0.05 M$ ,  $[L]_0 = 6.25 \cdot 10^{-6} M$

The ethanol : water mixture (20 : 80) behaves similarly, but in this case a second interval of linear growth is observed at  $[\text{NaOH}]_0$  values higher than  $[\text{H}_2\text{O}_2]_0$ .

These results, in view of reaction (1.1) (set 1), appear to indicate that the fundamental step for lucigenin disappearance and light emission decay is the nucleophilic addition of the hydroperoxide anion ( $\text{HO}_2^-$ ) to lucigenin (reaction (1.2) in set 1). The last interval of linear growth in Fig. 2 [2] reflects the existence of a second process which causes the disappearance of lucigenin through its reaction with  $\text{OH}^-$  (reaction (1.3))

Set 1



Relative chemiluminescence yields ( $R$ ) were measured in different reaction mixtures. The results are shown in Figs. 3 and 4. The values of  $R$  were calculated from the area defined by the intensity-time curves. They can also be calculated from the initial CL intensities ( $I_m$ ) and the decay rate constants. If the decay follows the pseudo-first order rate law, then  $R$  is given by the following equation:

$$\text{Area} = R = I_m/k . \quad (1)$$

The two values of  $R$  are in good agreement ( $\pm 5\%$ ).

Once the relative CL yields are known, Eq. (1) can be used to obtain information on the reaction mechanism. Assuming the rate-controlling step in the CL process is the nucleophilic addition of  $\text{HO}_2^-$  to lucigenin, and working under pseudo-first

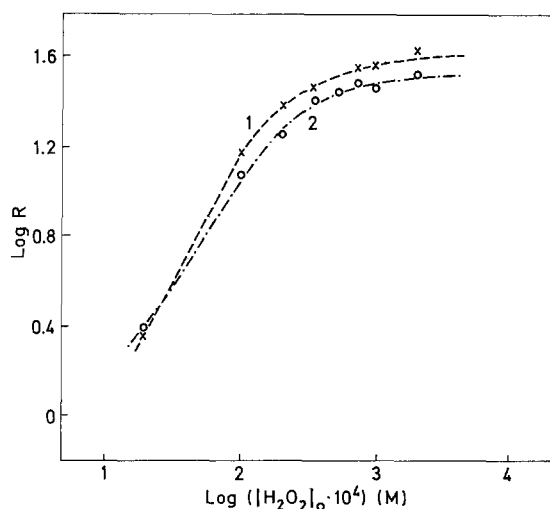


Fig. 3. Change in relative chemiluminescence yield ( $R$ ) with peroxide concentration in: (1) water : ethanol, 80 : 20 (v/v), and (2) water : *DMSO*, 80 : 20 (v/v),  $[L]_0 = 6.25 \cdot 10^{-6} M$ ,  $[\text{NaOH}]_0 = 0.1 M$

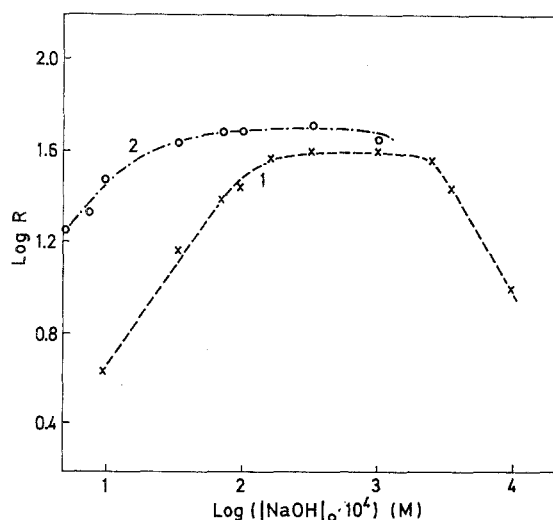


Fig. 4. Change in  $R$  with base concentration in: (1) water : ethanol, 80 : 20 (v/v),  $[\text{H}_2\text{O}_2]_0 = 0.1 \text{ M}$ , and (2) water : *DMSO*, 65 : 35 (v/v),  $[\text{H}_2\text{O}_2]_0 = 0.05 \text{ M}$ ,  $[L]_0 = 6.25 \cdot 10^{-6} \text{ M}$

order conditions for lucigenin ( $[\text{HO}_2^-] \gg [L]$ ), then

$$Im = Re Rq k_1 [\text{HO}_2^-] [L]_0, \quad (2)$$

where  $Re$  = emission efficiency and  $Rq$  = excitation efficiency (relative yield in the generation of excited state *NMA* from lucigenin).

The positive slope interval observed in the  $\log R$  vs.  $[\text{H}_2\text{O}_2]_0$  curves (Fig. 3) at low peroxide concentrations, combined with the negative slope interval observed in the  $\log R$  vs.  $\log [\text{NaOH}]_0$  curves at high base concentrations, indicates that the reaction of lucigenin with base (reaction (1.3) in set 1) does not contribute to the generation of excited states. Unlike Reaction 1.2, this process contributes only significantly to the disappearance of lucigenin when  $[\text{NaOH}]_0$  is much higher than  $[\text{H}_2\text{O}_2]_0$ .

The zero slope intervals in Figs. 3 and 4 indicate that reaction (1.2) is the most important in the disappearance of lucigenin when base and peroxide concentrations are similar.

Finally, the positive slope intervals observed in the  $\log R$  vs.  $[\text{NaOH}]_0$  plots at low values of  $[\text{NaOH}]_0$  (Fig. 4) reveal the existence of another dark process (reaction (1.4) in set 1). Hydrogen peroxide participates in this process, which becomes significant in the disappearance of lucigenin when  $[\text{H}_2\text{O}_2]_0$  is much higher than  $[\text{NaOH}]_0$ . Reaction 1.4 cannot be detected in the  $\log R$  vs.  $\log [\text{H}_2\text{O}_2]_0$  or the  $\log R$  vs.  $\log [\text{H}_2\text{O}_2]_0$  plots because oxygen bubble formation prevents kinetic measurements at very high peroxide concentrations.

Thus, the pseudo-first order rate constant for the disappearance of lucigenin and the decay of light emission is given by the following equation:

$$k = k_1 [\text{HO}_2^-] + k_2 [\text{HO}^-] + k_3 [\text{H}_2\text{O}_2]. \quad (3)$$

Inspection of Figs. 1–4 reveals that the kinetic results are very similar for all the mixtures used as reaction media. Also, inspection of the CL spectra recorded in these mixtures reveals that *NMA* is the primary emitter in all cases [11]. This

**Table 1.** Initial chemiluminescence intensity (arbitrary units), in alcohol-water mixtures, as a function of alcohol species and concentration.  $[L]_0 = 2 \cdot 10^{-5} M$ ;  $[NaOH]_0 = 0.065 M$ 

Alcohol concentration % (v)	Methanol	Ethanol	1-Propanol
0	3.5	3.5	3.5
4.25	3.3	5.4	5.3
10.6	6.9	9.5	11.5
21.3	13.3	19.5	51.5
42.5	93.0	380	315

**Table 2.** Rate constants ( $\text{min}^{-1}$ ) in alcohol-water mixtures as a function of alcohol species and concentration.  $[L]_0 = 2 \cdot 10^{-5} M$ ,  $[NaOH]_0 = 0.065 M$ ,  $[H_2O_2]_0 = 0.05 m$ 

Alcohol concentration % (v)	Methanol	Ethanol	1-Propanol
0	0.113	0.113	0.113
4.25	0.11	0.125	0.113
10.6	0.146	0.163	0.175
21.3	0.21	0.27	0.46
42.5	0.73	3.7	4.2

includes those reaction media, such as aqueous solutions, in which lucigenin modifies the recorded spectrum, making it different from the *NMA* fluorescent emission. These results appear to indicate that the reaction mechanism is independent of the co-solvent used.

However, addition of co-solvent increases both the initial CL intensity and the decay rate constant in comparison with the values measured in aqueous solution. Both such increases are proportional to co-solvent concentration over the range studied (Tables 1 and 2).

The increases depend also on the nature of the co-solvent. Thus, in the series of aliphatic alcohols, both increases are proportional to the reciprocal of the dielectric constant, as can be seen in Tables 1 and 2. Likewise, the increases are higher in  $H_2O$ -*DMF* mixtures than in  $H_2O$ -*DMSO* mixtures of the same composition.

According to Eq. (2) and (3), the increases observed in both *Im* and *k* suggest that the co-solvent acts a catalyst for nucleophilic addition of  $HO_2^-$  to lucigenin. This result agrees with the usual effect of these co-solvents on reactions between ions in solution [12], and confirms the fundamental role of this process in the reaction of lucigenin with basic hydrogen peroxide.

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