

Interaction of Luminol with the Oscillating System  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$ Simeen Sattar<sup>†</sup> and Irving R. Epstein\*

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Two types of oscillating chemiluminescence are observed when luminol is added to the oscillating system  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$ . Narrow bursts of light, with intensity proportional to the concentration of luminol, occur at the same time as the sharp increase in the concentration of a copper peroxide complex that is a key species in the oscillation of the luminol-free system. A second set of broad, low-intensity oscillations is apparent at luminol concentrations below  $10^{-4}$  M. These are ascribed to the slower reaction of luminol with superoxide. A skeleton mechanism for the action of luminol is proposed, but testing it will require refinement of the mechanism of the  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$  oscillator.

## Introduction

Oscillating chemiluminescence, produced by the addition of luminol (3-aminophthalhydrazide) to the oscillating system  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$ ,<sup>1</sup> is an intriguing phenomenon, because of the sharp bursts of light which punctuate the course of the reaction. In the absence of luminol, the  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$  system shows oscillations in color (between yellow and colorless), in platinum electrode potential, and in dissolved oxygen concentration, under both closed (batch) and open (continuous flow) conditions.<sup>2</sup> A recently published mechanism<sup>3</sup> accounts for the oscillatory behavior of the luminol-free system by linking the alkaline  $\text{H}_2\text{O}_2\text{-CuSO}_4$  and  $\text{H}_2\text{O}_2\text{-KSCN}$  subsystems via a feedback network. The sharp pulses of blue light produced by the addition of luminol are unlike other oscillations characteristic of this system.

We describe here further experiments with the luminol- $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$  system and suggest pathways to account for the interaction of luminol with the oscillating reaction.

The light-generating reactions of luminol have been the subject of much research.<sup>4-6</sup> In basic aqueous solution, chemiluminescence results from the oxidation of luminol by hydrogen peroxide catalyzed by a transition-metal ion. Light is emitted by the excited oxidation product, 3-aminophthalate. Chemiluminescence can also be initiated by irradiation of luminol solutions. We shall draw upon results of these studies.

## Experimental Section

**Chemicals.** All chemicals were analytical grade reagents. Hydrogen peroxide solutions were freshly prepared by diluting 30% solution and were then stored in polyethylene bottles.

**Apparatus and Methods.** In both batch and flow experiments, the initial concentrations of  $\text{H}_2\text{O}_2$ , KSCN,  $\text{CuSO}_4$ , and NaOH were fixed at 0.25, 0.025,  $7.5 \times 10^{-5}$ , and 0.025 M, respectively. Luminol solutions were made up in NaOH. Solutions were stirred magnetically or by bubbling  $\text{N}_2$  gas through the reaction vessel. The continuous flow stirred tank reactor (CSTR), which was fitted with quartz windows, had a volume of 42 mL and a path length of 4.2 cm. The reactor was fed by a peristaltic pump (Sage 375A). A typical flow rate (reciprocal residence time) was  $5.0 \times 10^{-3} \text{ s}^{-1}$ . All experiments were done at ambient temperatures, about 20 °C.

Two kinds of optical observations were made. The chemiluminescence of luminol, which peaks at 425 nm, was detected by a fluorescence spectrophotometer (Perkin-Elmer MPF-4). Absorbance was measured with a UV-visible spectrophotometer (Perkin Elmer 552A). Platinum electrode potential (versus a calomel reference electrode) and optical measurements could be recorded simultaneously.

**Chemiluminescence Standard.** The experimental procedures for using the hemin-catalyzed oxidation of luminol by hydrogen peroxide as a standard for total light emission and absolute in-

tensity have been detailed by Lee et al.<sup>7</sup> Bovine hemin was purchased from Aldrich.

## Results

We performed a series of experiments in a CSTR, in which the luminol concentration was varied. At luminol concentrations above  $1 \times 10^{-3}$  M, the sharp chemiluminescent pulses, about 3 s wide at half-height, have a slight shoulder. The light intensity then drops to a low plateau, visible in the dark, for 2 min before the next pulse. As the luminol concentration is reduced to  $1 \times 10^{-5}$  M, these features are resolved into two distinct events, illustrated in Figure 1. Between the sharp pulses a second set of oscillations, smoother and lower in intensity, is now apparent. In the following discussion, each type of oscillation is considered separately.

The magnitude and period of oscillations in platinum electrode potential are unchanged by luminol, suggesting that it does not perturb the course of the oscillating system.

**Sharp Pulses.** In this study, luminol displays simple kinetic behavior. The height of the narrow light pulses is found to be linearly proportional to the luminol concentration over a 200-fold concentration range (Figure 2). For a fixed composition of the oscillatory subsystem, the measured light intensity is given by

$$I = k[\text{luminol}] \quad (1)$$

In a closed system with an initial luminol concentration of  $3.5 \times 10^{-4}$  M, the absolute intensity at the peak of the light pulses was  $1 \times 10^{-8}$  mol photons  $\text{L}^{-1} \text{ s}^{-1}$ . From the same experiment, we estimate that  $10^{10}$  photons  $\text{L}^{-1}$  are emitted per oscillation, which indicates that the luminescence is generated by only a small fraction of the luminol.

As stated earlier, the  $\text{H}_2\text{O}_2\text{-KSCN-CuSO}_4\text{-NaOH}$  system oscillates between yellow and colorless states. The yellow color arises from a copper-peroxide complex,  $\text{HO}_2\text{-Cu(I)}$ , a key intermediate in the Cu(II)-catalyzed decomposition of hydrogen peroxide.<sup>8</sup> The very sharp rise in absorbance at 390 nm, shown in Figure 3, coincides in time with the chemiluminescent pulses. The rise time accounts for 5 s of a period that varies from 1 to 6 min in a range of initial  $[\text{CuSO}_4]$  between  $1 \times 10^{-4}$  and  $4 \times 10^{-5}$  M. The intensity of the chemiluminescent pulses increases over this range. At lower initial copper sulfate concentrations, the peak height decreases, and the oscillations cease below  $3 \times 10^{-5}$  M.

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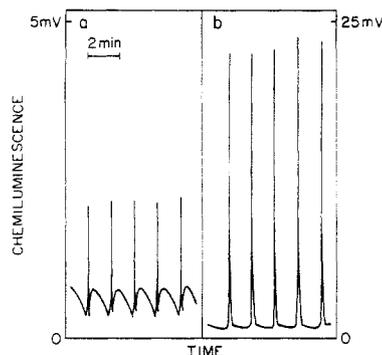
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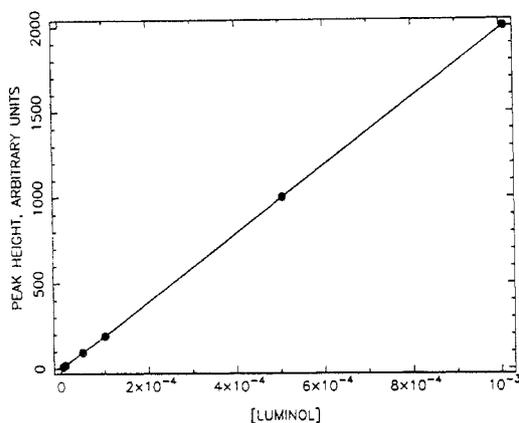
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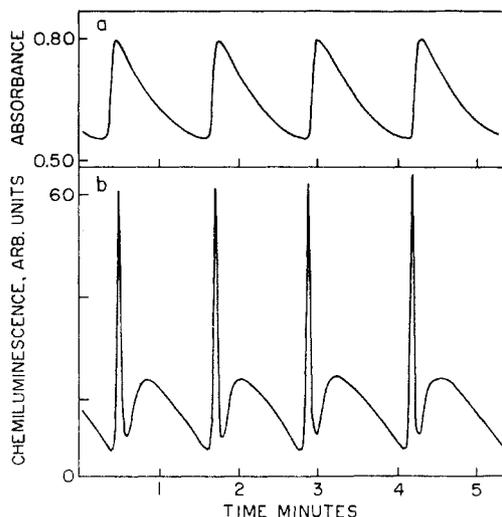
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**Figure 1.** Oscillatory chemiluminescence at different luminol concentrations (M): (a)  $1.0 \times 10^{-5}$ ; (b)  $1.0 \times 10^{-3}$ . Input concentrations:  $[\text{H}_2\text{O}_2]_0$ , 0.25 M;  $[\text{KSCN}]_0$ , 0.025 M;  $[\text{CuSO}_4]_0$ ,  $7.5 \times 10^{-5}$  M;  $[\text{NaOH}]_0$ , 0.025 M. Flow rate =  $5.0 \times 10^{-3} \text{ s}^{-1}$ . Gain in (a) is 10 $\times$  gain in (b).



**Figure 2.** Peak height vs luminol concentration for sharp pulses. Input concentrations and flow rate same as in Figure 1.



**Figure 3.** Oscillatory behavior as a function of time. Input concentrations and flow rate same as in Figure 1.  $[\text{Luminol}]_0 = 7.2 \times 10^{-5} \text{ M}$ . (a) Absorbance at 390 nm; (b) chemiluminescence at 425 nm.

**Secondary Oscillations.** Between  $5 \times 10^{-6}$  and  $1 \times 10^{-4} \text{ M}$  luminol, when the weak oscillations are distinguishable from the narrow pulses (Figure 1), the total light emitted increases linearly with luminol concentration, falling off slightly at the upper end of the range. Examination of Figure 1 shows that these oscillations are offset from the center of the interval between the narrow light pulses, even at the lowest luminol levels.

At  $5 \times 10^{-6} \text{ M}$  luminol, the peak-height ratio of the two types of oscillations is 2. Thus the intensity of the sharp luminescence peaks varies much more strongly with the luminol concentration than does that of the broader, secondary peaks.

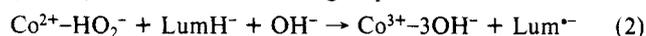
**TABLE I: Rate Constants for Luminol Reactions**

reaction	$k$	ref
5	$(2.3 \pm 0.3) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$	13
6	$2.5 \times 10^5 \text{ s}^{-1}$	a
-6	$10^9 \text{ M}^{-1} \text{ s}^{-1}$	a
7	$(2 \bullet 0.5) \times 10^3 \text{ s}^{-1}$	11
8	$(1.8 \pm 0.3) \times 10^5 \text{ s}^{-1}$	11

<sup>a</sup> Estimated from the equilibrium constant in ref 12.

### Discussion

In a study of the cobalt(II)-catalyzed reaction of luminol with hydrogen peroxide, Burdo and Seitz<sup>9</sup> proposed that a cobalt-peroxide complex is the critical intermediate required for chemiluminescence. Its reaction with luminol produces a luminol radical ( $\text{Lum}^{\bullet}$ ) in the rate-determining step.



Under the prevailing alkaline conditions, luminol is present as a monoanion,  $\text{LumH}^-$  ( $\text{p}K_{a1} = 6$ ).<sup>10</sup>

Although an analogous equation can be written in the present instance



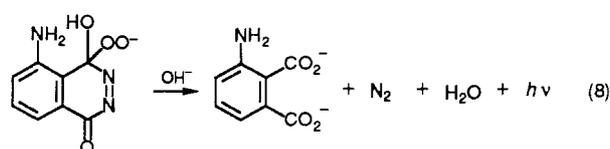
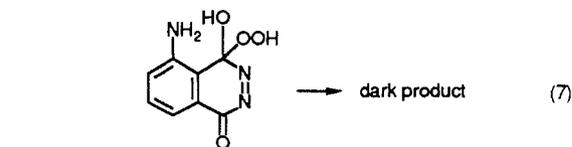
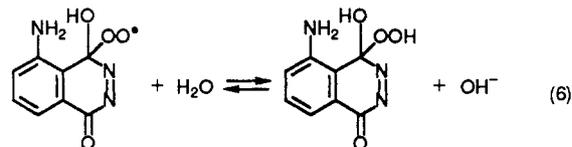
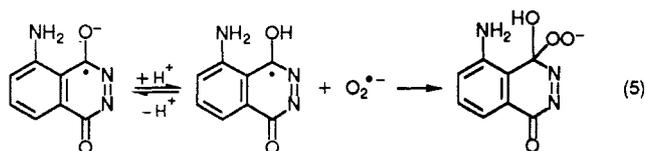
it alone cannot account for the luminescence, because the light pulse decays while the copper-peroxide concentration is still high.

Inclusion of a reversible reaction that binds a large fraction of the luminol can remove this difficulty. A reaction of the form of eq 4 would also be consistent with the observation that only a small portion of the luminol molecules actually emit light.

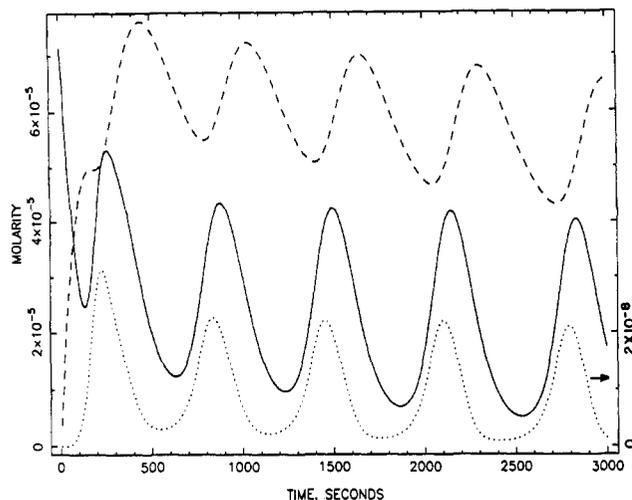


We observe that a dark brown solid with  $[\text{luminol}]:[\text{Cu}] = 1:1$  precipitates upon addition of copper sulfate to luminol. Also, the luminescence increases as the initial  $[\text{CuSO}_4]$  is lowered. However, we note that, at the highest luminol concentrations employed in this study, the input concentration of copper sulfate is insufficient to bind a large fraction of the luminol in a 1:1 complex.

An overall scheme for the reaction of luminol with basic aqueous hydrogen peroxide has been developed from pulse radiolysis experiments.<sup>11-13</sup> After the one-electron oxidation of luminol by hydroxyl radicals, the following sequence occurs:



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**Figure 4.** Simulations showing [HO<sub>2</sub>-Cu(I)] (solid line), [O<sub>2</sub><sup>•-</sup>] (dashed line), and [SO<sub>3</sub><sup>•-</sup>] (dotted line). Input concentrations and flow rate same as in Figure 1.

Rate constants at pH 11.0 are summarized in Table I. Superoxide (~10<sup>-5</sup> M) is supplied by the H<sub>2</sub>O<sub>2</sub>-CuSO<sub>4</sub> subsystem<sup>8</sup> and inhibits recombination of luminol radicals. The hydroperoxide adduct is a key intermediate with a pK<sub>a</sub> of 10.4 ± 0.1.<sup>12</sup> Its basic form (peroxide adduct) is the precursor for the light-emitting species. The dark reaction (7) is significant only in batch when the pH drifts to lower values toward the end of the oscillations.

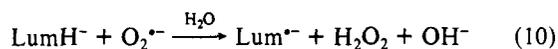
The weak oscillations reflect a slow step in the generation of aminophthalate, the light-emitting species. We identified two species in the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH mechanism as likely oxidants: superoxide and the sulfite radical ion from SCN<sup>-</sup>. In the latter case, SO<sub>3</sub><sup>•-</sup> might abstract a hydrogen atom:



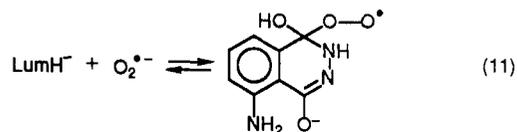
An analogue of this reaction involving CO<sub>3</sub><sup>•-</sup> has a rate constant of (9 ± 3) × 10<sup>8</sup> M<sup>-1</sup> s<sup>-1</sup>.<sup>13</sup> Simulations of the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH system,<sup>3</sup> however, show that the sulfite radical ion oscillates nearly in phase with the copper peroxide complex (Figure 4). Thus, reaction of luminol with SO<sub>3</sub><sup>•-</sup> cannot account for the weak chemiluminescent oscillations.

Superoxide, on the other hand, is one-quarter cycle out of phase with the copper-peroxide complex (Figure 4). This is consistent with the phase difference between the pulses and the secondary oscillations observed at low luminol concentrations.

Superoxide is weakly reactive toward luminol between pH 7 and 11.<sup>14</sup> Total light emission was found to level off around 10<sup>-4</sup> M luminol at fixed superoxide concentration and pH 9.3. Merényi et al.<sup>14</sup> conclude that luminescence is not initiated by a direct one-electron oxidation of the form



They propose instead formation of an adduct



followed by reactions that generate the chemiluminescence.

The proposed skeleton mechanism provides a plausible explanation for the role of luminol in the system and gives qualitative agreement with the observed behavior. However, efforts to simulate the complete five-component system failed to yield fully satisfactory agreement with the experimentally observed oscillations. We believe that the major source of discrepancy lies not in the description of the luminol reactions, the remaining details of which should have relatively little influence on the observed oscillations, but in the incomplete treatment of the copper-catalyzed H<sub>2</sub>O<sub>2</sub> decomposition included in the mechanism<sup>3</sup> of the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH oscillator. In order to keep the number of elementary steps and independent species (30 and 26, respectively) to manageable levels, Luo et al.<sup>3</sup> deleted a number of reactions contained in the earlier description<sup>8</sup> of the peroxide decomposition. They also limited the number of copper-containing species to three. The omitted reactions and species were not necessary to generate the overall dynamics (oscillation period and amplitude, bistability, etc.) of the oscillator, but do affect the waveform of the oscillation. For example, the simulated oscillations in [HO<sub>2</sub>-Cu(I)] shown in Figure 4 are nearly symmetric, in contrast to the marked asymmetry of the experimental absorbance and chemiluminescence traces in Figure 3. If, as seems likely, the sharp peak in the chemiluminescence is related to a rapid rise in the concentration of a copper-containing species, the mechanism of the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH system must reflect this behavior before any derived mechanism for the luminol-containing reaction can be expected to properly describe the chemiluminescent spikes. In particular, it will probably be necessary to treat the different copper complexes in more detail.

In this study, we have focused on how luminol interacts with the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH system. Amrehn et al.,<sup>1</sup> in contrast, were interested in the overall dynamical behavior of the luminol-containing system. They suggested that the very sharp peaks in the luminescence required highly nonlinear kinetics to account for the behavior of luminol. The present study suggests that, if the luminol-free system is described in sufficient detail, it may well be possible to account for the observed behavior without invoking any higher order nonlinearities.

Further elucidation of the mechanism will require both refinement of the mechanism<sup>3</sup> of the H<sub>2</sub>O<sub>2</sub>-KSCN-CuSO<sub>4</sub>-NaOH system and detailed studies of the alkaline H<sub>2</sub>O<sub>2</sub>-CuSO<sub>4</sub>-luminol system.

**Acknowledgment.** We thank Kenneth Kustin, Yin Luo, Bob Olsen, and Miklós Orbán for many helpful discussions, and Colin Steel for assistance with the luminescence measurements. This work was supported by the National Science Foundation (Grant No. CHE-8800169) and by the donors of the Petroleum Research Fund, administered by the American Chemical Society.

**Registry No.** H<sub>2</sub>O<sub>2</sub>, 7722-84-1; SCN<sup>-</sup>, 302-04-5; Cu, 7440-50-8; luminol, 521-31-3.

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