

Carbon Dioxide Effects on Luminol and 1,10-Phenanthroline Chemiluminescence

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Luminol and 1,10-phenanthroline are widely used chemiluminescent (CL) reagents for the analysis of a wide range of metals and inorganic and organic complexes. While the fundamental mechanism for luminol and 1,10-phenanthroline chemiluminescence is understood, the analytical application of these reagents is largely empirical and often poorly described mechanistically. For example, CL signals observed from metal–luminol systems are strongly dependent on the pH of the sample, even though the final pH of the reaction mixture is controlled to a narrow range by a buffer. Other investigators report significant changes in CL signal due to freshness and the acidity of reagents. Our work shows that many of these effects are due to dissolved CO₂ present or formed in the analytical system. The hypothesis that carbon dioxide plays a pivotal role in enhancing luminol CL is supported by direct manipulation of CO₂(aq) concentrations by the addition of CO₂(g) or carbonic anhydrase. In contrast, Cu(II) analysis using the CL reagent 1,10-phenanthroline is completely quenched in the presence of CO₂(aq). A plausible mechanism for these observations involves the reaction between superoxide, produced in these analytical systems, and CO₂(aq) to form the peroxycarbonate radical, [•]CO₄[−]. The formation of [•]CO₄[−] has very important analytical implications since this species appears to enhance or quench the CL signal from luminol and 1,10-phenanthroline, respectively.

The luminol-based chemiluminescence method has been widely used in the quantitative determination of many metal, organic, and inorganic compounds at very low concentrations. Developments in these analytical methods were described in several recent reviews.^{1–6} The major advantages of these methods are easy setup, inexpensive instrumentation, and very low detec-

tion limits. Unfortunately, the methods can also suffer from poor selectivity and, sometimes, capricious sensitivity. In all cases, the analyte of interest is a rate-determining species in the oxidation of luminol to 3-aminophthalate. The analytical application of chemiluminescence (CL) for trace determination is based on the premise that the CL signal is proportional to the concentration of analyte and is often linear over several decades of analyte concentration. Analysis of different species is achieved by modifying the analytical matrix to make specific species more or less reactive with luminol or its intermediate products.

The lack of complete mechanistic information for this system makes the analysis process highly empirical. This is reflected in many puzzling observations of the CL system performance. For example, no definite explanation has been given as to why the CL sensitivity is dependent on the length of time that the luminol solution is allowed to age.⁷ Furthermore, a number of reagents were found to enhance or quench the CL signal. Chang and Patterson showed that the CL intensity generated by the metal/luminol/H₂O₂ reaction is enhanced 6-fold in the presence of 0.3 M bromide ion.⁸ We recently reported that CO₃^{2−} greatly enhances the CL signal.⁹ Utilizing this enhancement effect, we were able to determine Cr(III) at the 1 part per trillion (ppt) level in basic media. Lan and Mottola found that passing carbon dioxide gas through a luminol solution (pH ~11) remarkably enhanced CL light emission.⁷ In addition, several studies found that the CL produced from well-buffered luminol reaction mixtures are still strongly dependent on sample pH.⁹ We now attribute many of these observations to the presence or transient formation of CO₂(aq) in the analytical system.

Before Lan and Mottola's observation of the CO₂ effect on CL,⁷ several similar observations had been reported. Bersis and Nikokavouras observed luminescence emission when a rapid stream of CO₂(g) was passed through a basic solution of luminol containing NaCl, H₂O₂, and MnCl₂.¹⁰ A striking burst of light was observed when CO₂ was bubbled through a Co(II) dosimeter containing 0.1 mM Co(II), 0.44 M H₂O₂, 0.57 mM luminol, and 30 mM NaOH.¹¹ It was also observed that carbon dioxide affects NO determination using luminol/H₂O₂-based chemiluminescence.¹² The formation of the carbonate radical via the decomposi-

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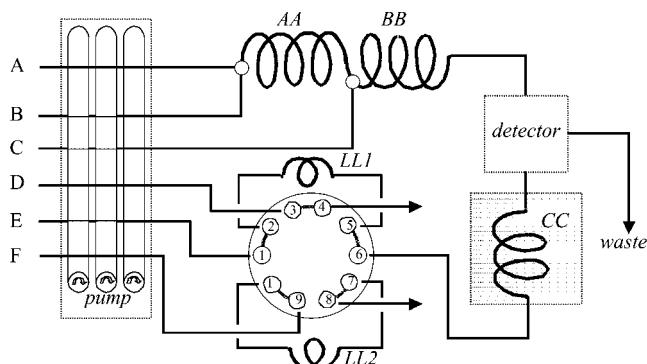


Figure 1. Flow injection analysis system used in this work. Reagent composition, flow rates, and tubing lengths are provided in Table 1. Loop LL₁ provides the optional capability of injecting a sample with each valve cycle.

tion of peroxybicarbonate (HOOCO_2^-) was proposed to explain the carbon dioxide effect,⁷ but no kinetic or spectroscopic methods were applied to establish the suggested mechanism. Moreover, the variations in CL caused by differences in the concentration of ubiquitous carbon dioxide have not been addressed. In this work, we show that luminol CL in aqueous solutions is not only enhanced by CO_2 but CO_2 is fundamental to the CL reactions for most common analytical applications. A plausible mechanism involving the reaction between superoxide ion and $\text{CO}_2(\text{aq})$ to form the peroxy carbonate radical, $\cdot\text{CO}_4^-$, is proposed. Utilizing this mechanistic understanding, we have been able to consistently enhance the CL signal by several orders of magnitude. As a practical matter, understanding the CO_2 effect on luminol CL explains many of the empirical variations in CL signal described above. We demonstrate that such understanding has very important analytical implications for current and future CL-based detectors.

EXPERIMENTAL SECTION

Apparatus. The flow system is shown schematically in Figure 1. All solutions are delivered with an eight-channel peristaltic pump (Dynamax) using Tygon tubing. All other tubing is either (1.57-mm o.d., 0.76-mm i.d.) PEEK or Teflon tubing. The length of the tubing is relevant in this work and is provided in Table 1 along with reagent compositions and flow rates. The loop labeled CC in Figure 1 is made of highly gas-permeable Teflon AF (1.0-mm o.d., 0.7-mm i.d.) and is contained in a 250-mL chamber containing air, Ar, or CO_2 at a total pressure of 0.1 MPa. A Waterville Analytical FIA instrument with CL detection is used in this work. The instrument incorporates a Valco Inc. automated 10-port injection valve, a CL flow cell and detector, and control electronics and software.^{9,13} The voltage supplied to the PMT in this work is 700 V, except where otherwise stated.

Reagents. A 20.9% (w/w) environmental grade plus NH_3 solution was obtained from Alfa, with detected transition metals in the range of 1–20 ng L⁻¹. Ultrapure 68% (w/w) HNO_3 was obtained from J. T. Baker. A 30% (w/w) H_2O_2 solution was purchased from Aldrich (Milwaukee, WI). Luminol (97%, Sigma)

was dissolved by adding a minimal amount of NaOH to prepare the 0.01 M stock solution. Other reagents used in this work include the following: anhydrous sodium carbonate (Baker analyzed reagents, 0.0001% heavy metal as Pb, and 0.0001% Fe), sodium bicarbonate (Fisher Scientific Co., 2.5 ppm heavy metal and 0.0004% Fe), 1,10-phenanthroline (99%, Aldrich), tetraethylenepentamine (technical grade, Aldrich), cetyltrimethylammonium bromide (85% Aldrich), and 99% sodium EDTA (Aldrich). Carbonic anhydrase (from bovine erythrocytes, 91% protein, 5710 W-A units/mg of protein) was purchased from Sigma. A 3 mM superoxide stock solution was prepared by saturating KO_2 (Sigma) in dried DMSO (Sigma). All solutions were prepared using water purified in a Barnstead Nanopure ion exchange system. In several experiments, it was necessary to exclude CO_2 from the reagents and samples. In these cases, pure water was sparged with 99.999% Ar(g) overnight. All subsequent solutions were prepared using the CO_2 -free water. The solution bottle tops were fitted with ascarite columns (1.5-cm i.d., 10-cm length) to prevent recontamination with atmospheric CO_2 while allowing reequilibration with atmospheric oxygen.

Cr(III), Co(II), and Cu(II) standards were prepared by diluting a 1000 mg L⁻¹ SpecPure standard stock solution (Alfa) with 0.02 M HNO_3 . Cr(III) standards (1–50 ng L⁻¹) were freshly prepared by diluting a 100 ng L⁻¹ Cr(III) stock solution in 0.015 M HNO_3 with 5 mM NH_3 solution. Fe(II) solutions were prepared from ferrous ammonium sulfate dissolved in 10 mM HCl to prevent oxidation. All bottles for the storage of Cr(III) standards, NH_3 , HNO_3 , and other high-purity reagent solutions were soaked in 1 M HNO_3 for more than 10 h and then in pure water for another 2–10 h.

RESULTS AND DISCUSSION

(i) Sample pH Effect. Figure 2 shows the pH dependence of method A for cobalt analysis by CL. Similar pH dependence was also observed when reagent A was 10 mM luminol in a 0.1 N NaOH solution. A strong pH dependence of the CL signal was also found for the luminol/ H_2O_2 method in Cr(III) analyses.⁹ For pH below 3.5, the CL signal is so sensitive to the sample acidity that it is difficult to determine Co(II) or Cr(III) in the 1–100 ppt range. It was found that the presence of millimolar ammonia in the sample matrix reduces the Co(II) CL signal. However, the pH and ammonia effects on the CL signal were eliminated if the samples were mixed with the luminol solution before reaching the flow cell and the hydrogen peroxide solution was introduced into the flow cell via the sample loop (Co(II) method B). A satisfactory calibration curve could be obtained from standards with different pH values (1.2–4.0) and different ammonia concentrations (0–5 mM).

(ii) Carrier Effect on Co(II) CL. Figure 3 shows the CL signals of a 12.8 ppt Co(II) standard obtained using different carriers (Co(II) method B). Note that the CL signals observed when fresh ultrapure water was used as the carrier were much weaker than those observed when the carrier was aged in a polyethylene bottle for 1 week. The significance of this observation is obvious. We were often frustrated by the fact that the CL signal kept increasing with time during CL analyses so that it was impossible to perform a calibration. As also shown in Figure 3, the CL signals become much weaker if a 0.1 N NaOH solution was used as the carrier.

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Table 1. Experimental Parameters for Flow Configurations Described in Figure 1^a

analyte (ref)	reagent/flow rate (mL/min)					LL ₂ /mL	AA/cm	BB/cm	CC/cm
	F	E	A	B	C				
Fe(II) ^b (13)	sample/1.0	H ₂ O/12	0.5 mM L ₁ /4			0.5			100
•O ₂ ^{-b}	sample/1.0	H ₂ O/12	0.5 mM L ₁ /4						100
H ₂ O ₂ ^b	sample/1.0	H ₂ O/12	0.5 mM L ₁ /4						100
Co(II) A (8)	sample/1.0	H ₂ O/3.0	0.5 mM L ₂ /3	60 mM H ₂ O ₂ /1.2		0.10			
Co(II) B	60 mM H ₂ O ₂ /1.0	H ₂ O/3.0	0.5 mM L ₂ /3	sample/1.0		0.10			
Cr(III) (9)	sample/1.0	H ₂ O/2.0	0.1M Na ₂ CO ₃ /0.5	0.12 M H ₂ O ₂ /0.5	1 mM L ₃ /1.2	0.72	400	70	
Cu(II) (15)	sample/1.0	H ₂ O/1.2	0.06 mM L ₄ /1.2	1.5 M H ₂ O ₂ /1.2		0.10	120		

^aL₁, luminol in 1.0 M NH₃ (pH 11); L₂, luminol in 0.2 M Na₂CO₃ (pH 11.2); L₃, luminol in 30 mM NH₃ (pH 10.8); L₄, 1,10-phenanthroline in 75 mM NaOH containing 400 nM tetraethylenepentamine and 20 mM cetyldimethylammonium bromide. ^bAll solutions for these analyses were prepared with CO₂-free, ultrapure water.

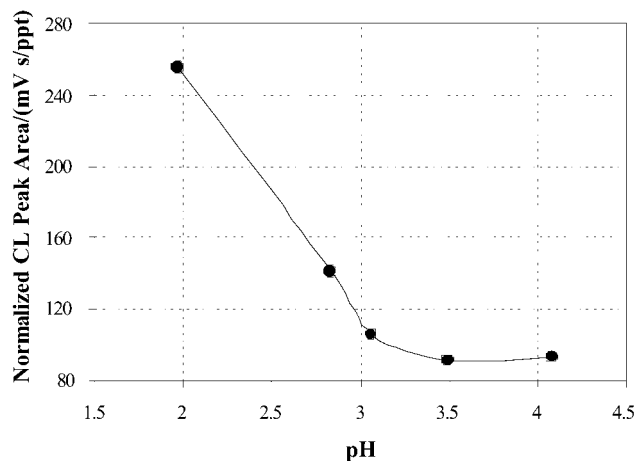


Figure 2. Normalized CL peak areas obtained from Co(II) standards (25–35 ppt) as a function of the sample pH. Conditions are given in Table 1 (Co(II) A). The pH of the standard solutions was adjusted with an ultrapure HNO₃ solution. The line was drawn through the points as a visual aid.

(iii) **CO₂ Effect on Co(II) CL.** Why is the CL signal for Co(II) and Cr(III) so sensitive to the sample pH when the pH of the final, mixed solution in the flow cell is controlled effectively (within 0.1 pH unit) by either a buffer or a large excess of base (0.1 N NaOH)? Why is the CL signal affected by the freshness of the carrier water when the role of the carrier is only to push the hydrogen peroxide solution in the injection loop into the flow cell? We propose that variable carbon dioxide concentrations in FIA system explain our observations. An alternative explanation is that the carrier may affect the background (baseline) signal. However, as shown in Figure 3, the baselines observed with fresh water and the aged water were not significantly different.

Similar to the light burst observed when CO₂(g) is passed through luminol solutions,^{10,11} we found that a large CL signal is always observed when air bubbles are accidentally introduced into the reagent streams or the sample loop when switching the sample, whereas the presence of He(g) and O₂(g) bubbles in the sample loop reduces the CL signal. A normal PMT voltage of 700 V was used in the experiment presented in Figure 3, and if a few CO₂(g) bubbles were injected into the loop, a huge CL signal was observed (>10⁶ mV or ~1000 times higher than the first peak in Figure 3). As shown in Figure 3, the CL signal for a 12.8 ppt Co(II) solution was enhanced significantly by the carrier water that was briefly contacted with CO₂(g) (passing CO₂(g) over the carrier water at 100 mL min⁻¹ for 10 s). On the basis of these

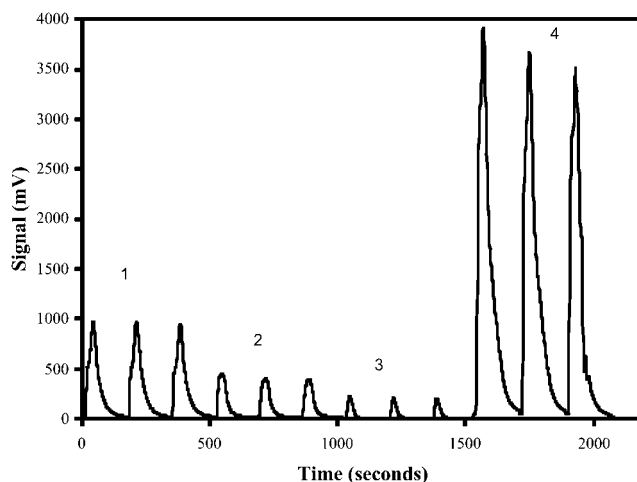


Figure 3. CL signals of a 12.8 ppt Co(II) standard obtained using different carriers (Co(II) B in Table 1): 1, aged ultrapure water; 2, fresh ultrapure water (18 MΩ); 3, 0.1 M NaOH; 4, CO₂-contaminated ultrapure water.

observations, we believe that the unusual sample pH effect and the effect of the freshness of water on the CL signal resulted from the presence of aqueous CO₂. When the acidic sample solution first met the basic luminol solution, which inevitably contained small amounts of carbonate, aqueous carbon dioxide was produced at the mixing boundary and promoted CL emission. This also explains why light emission during the neutralization of a basic luminol solution was observed many years ago by Behrens et al.¹⁶ Light pulses were observed following injection of 0.5 mL of 0.2 M HCl into 5 mL of 0.2 M NaOH containing 1.5×10^{-7} mol of luminol. They found that the type of acid did not seem to be important and the spectrum of light emitted during the partial neutralization of the basic luminol solution was the same as the CL spectrum obtained from the K₂S₂O₄/luminol reaction and the fluorescence spectrum of luminol under similar pH conditions.

Figure 4 also shows the CL signals obtained when carbonic anhydrase was added to the carrier water. The significant increase in the peak size is due to the catalytic activity of the enzyme producing CO₂(aq).¹⁷ Even in the system without carbon dioxide added intentionally, the enzyme greatly increases the dehydra-

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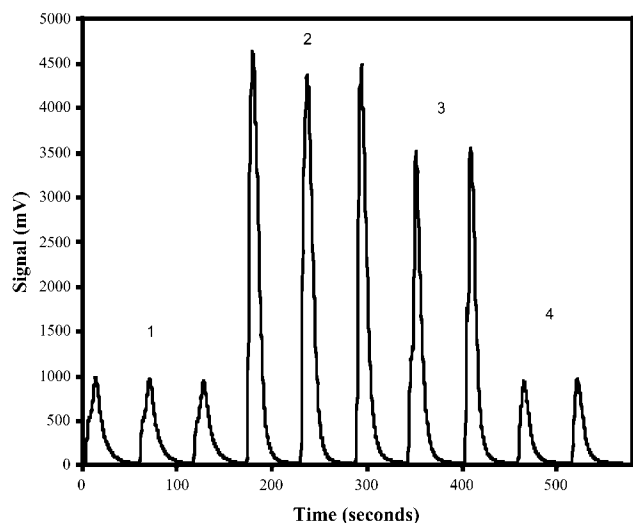


Figure 4. CL signals of a 27.1 ppt Co(II) standard solution obtained using a 3 ppm carbonic anhydrase solution as the carrier (Co(II) B in Table 1): 1, aged ultrapure water; 2, fresh ultrapure water containing 3 ppm carbonic anhydrase; 3, the same experimental condition as 2 except a 20- μ L air bubble was introduced in the injection loop containing the hydrogen peroxide solution; 4, thermally deactivated carbonic anhydrase (heated in a closed polyethylene bottle in a 90 $^{\circ}$ C water bath for 3 h).

tion rate of carbonate upon mixing with an acidic solution and thereby enhances the CL light emission by producing transient, local increases in $\text{CO}_2(\text{aq})$ concentration. Note that hydration of $\text{CO}_2(\text{aq})$ and dehydration of HCO_3^- are relatively slow processes in the absence of catalysts.¹⁷ Thermally deactivated enzyme served as a control and had a much smaller effect on the CL signal. Note that no further enhancement was observed when air bubbles were present in the sample loop in the presence of carbonic anhydrase. These observations confirm our assumption that the significant increase in the CL signal with decreasing sample pH is caused by the formation of small amounts of $\text{CO}_2(\text{aq})$ upon mixing of the acidic sample with the basic luminol solution.

(iv) Other Metals. The effect of carbon dioxide in other luminol-based CL systems was also examined. If the carrier water was contaminated with carbon dioxide during the CL analysis of Cr(III), using the reagent flow configuration described in Table 1 and Figure 1,⁹ a very large baseline signal was observed. When our CL method for Cr(III) was published, we were not aware that fresh pure water was essential to the determination of Cr(III) below 10 ppt. We now realize that the Cr(III) method could be modified to detect the presence of carbon dioxide in pure water if other system solutions are free of carbon dioxide.

To investigate directly the effect of CO_2 , we modified our flow system to allow quantitative addition of CO_2 . The tubing coil CC in Figure 1 is constructed of highly gas-permeable Teflon AF. By placing the tubing coil in a chamber containing CO_2 , Ar, or air the gas content of the sample stream could be regulated.

Figure 5 shows the effect of CO_2 exclusion on the chemiluminescence signal of Fe(II). Almost no signal was observed when $\text{CO}_2(\text{g})$ was absent. Ar(g) was used in the gas exchanger to prevent any uptake of $\text{CO}_2(\text{g})$ by the highly permeable tubing. When $\text{CO}_2(\text{g})$ was added in place of the Ar(g), an immediate increase in signal was observed, reaching an equilibrium signal after several minutes. The effect was reversible. Replacing

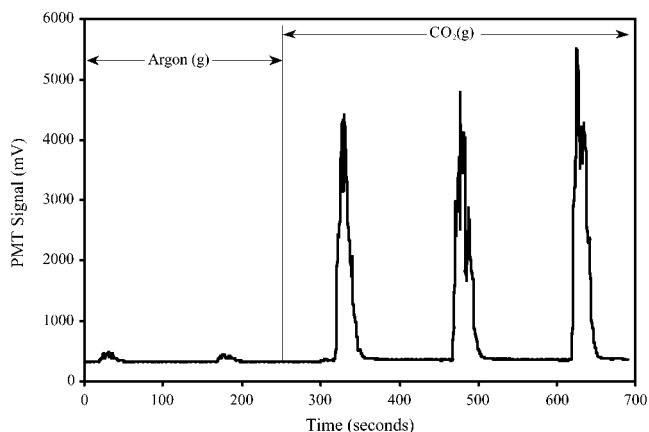


Figure 5. Argon/ $\text{CO}_2(\text{g})$ test demonstrating the signal enhancement of Fe(II) CL by $\text{CO}_2(\text{g})$. Concentrations of the Fe(II) (20 nM) and luminol reagent, pump speed, and PMT voltage (900 V) were held constant.

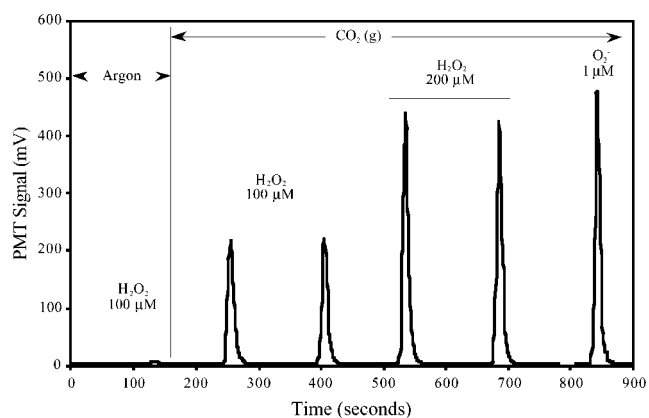
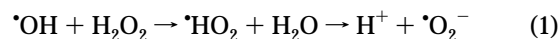


Figure 6. Signal due to H_2O_2 and O_2^- in the presence and absence of $\text{CO}_2(\text{g})$. Concentration of each species is labeled above each peak. Analytical conditions were the same as for Fe(II) shown in Figure 5.

$\text{CO}_2(\text{g})$ with Ar(g) produced a rapid drop in the signal, and the signal continued to decrease as the $\text{CO}_2(\text{g})$ was replaced with Ar(g). These results imply that $\text{CO}_2(\text{aq})$ not only enhances the signal but is also fundamental to the Fe/luminol system.

The mechanism of Fe(II) chemiluminescence involves a superoxide intermediate produced by the oxidation of Fe(II) by O_2 .¹³ It is logical to assume that the Lewis base, O_2^- , reacts with $\text{CO}_2(\text{aq})$ added intentionally or inadvertently to the CL systems described above. Figure 6 shows the effect of adding CO_2 to CO_2 -free systems with O_2^- or H_2O_2 as samples (see Table 1 for system configuration). In the absence of CO_2 , essentially no CL was observed for either sample. When the samples were exposed to 0.1 MPa of $\text{CO}_2(\text{g})$ in the gas exchanger (loop CC), a dramatic increase in signal was observed. The O_2^- signal is over 200 times more intense, suggesting that the H_2O_2 signal is a result of the slow production of O_2^- .¹⁸



It is also interesting to note that the CL signal for Fe(II) shown

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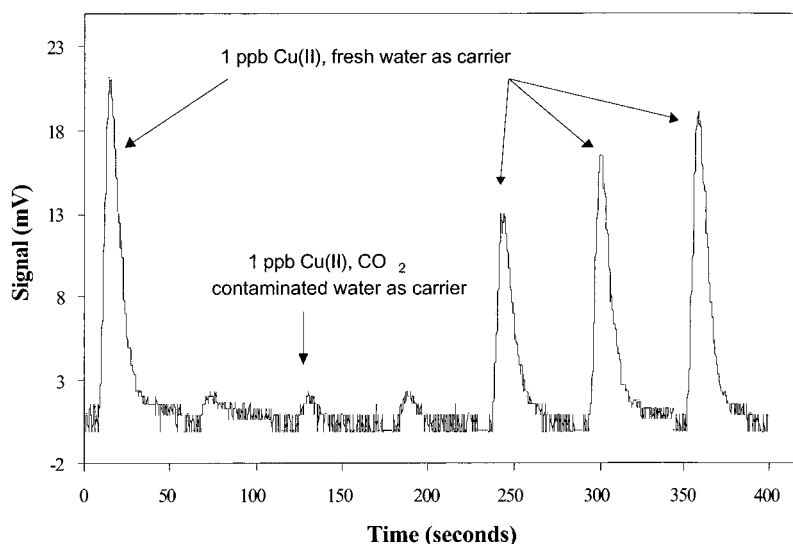


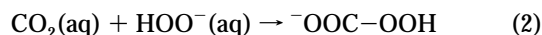
Figure 7. Cu(II) CL signals obtained using the method reported by Coale et al.¹⁵ Note that the signal of a 1 ppb Cu(II) standard gradually reached the previous level after the carrier line was switched back to the normal fresh ultrapure water.

in Figure 5 is 500 times greater than the signal for $\cdot\text{O}_2^-$. This will be significant in subsequent mechanistic discussions.

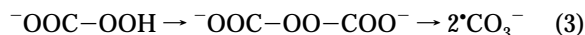
In all cases involving luminol as a CL reagent, we found that $\text{CO}_2(\text{aq})$ is essential for chemiluminescence. For comparison, we also investigated the effect of $\text{CO}_2(\text{aq})$ on the detection of Cu(II) using 1,10-phenanthroline,^{15,19} a very different CL system. Figure 7 shows that the CL signal of a 1 ppb Cu(II) standard was almost entirely quenched if the carrier water was in brief contact with $\text{CO}_2(\text{g})$ by passing $\text{CO}_2(\text{g})$ over it at 100 mL min^{-1} for 10 s (reaction pH in the flow cell does not change).

Implications to CL Analyses and a Possible Mechanism.

Lan and Mottola⁷ speculated that aqueous carbon dioxide interacts with the CL light-emitting processes via an intermediate species peroxycarbonate, which is formed by the reaction of CO_2 with the peroxide ion in the strong basic medium:



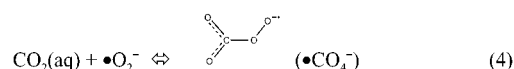
The decomposition of peroxycarbonate results in the formation of carbonate radicals:



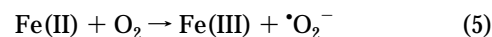
In the explanation of the carbonate enhancement effect of the Cr(III) CL, we argued that carbonate enhances the CL signal, because it converts the very reactive hydroxy radical to a less reactive carbonate radical.⁹ The carbonate radical reacts selectively with luminol to produce a luminol radical, which leads to formation of the light-emitting intermediate 3-aminophthalate, whereas the hydroxy radical attacks more than one carbon site on the aromatic ring of luminol, producing various species, which do not lead to the light-emitting intermediate.^{18,20} We observed that metal impurities initially present in the hydrogen peroxide solution, or in the carbonate buffer, not only increase the baseline but also enhance

the CL signal. Note that the carbonate radical can be produced by the reactions 2 and 3, without the need for initiation of the radical decomposition reaction of hydrogen peroxide. Thus, the presence of $\text{CO}_2(\text{aq})$ may enhance the luminol/ H_2O_2 chemiluminescence by promoting the formation of carbonate radicals.

However, the above mechanism does not explain the CO_2 enhancement in the Fe(II) and $\cdot\text{O}_2^-$ experiments. Furthermore, the mechanism is inconsistent with the $\cdot\text{O}_2^-$ and H_2O_2 experiments. These data support a direct reaction between $\text{CO}_2(\text{aq})$ and $\cdot\text{O}_2^-$. This reaction is well documented in aprotic media,^{21,26} but has not been observed in aqueous solutions.



We propose that the peroxycarbonate radical, $\cdot\text{CO}_4^-$, is an effective oxidant of luminol **1** to the luminol radical **2** as depicted in Figure 8. The CL precursor, hydroperoxide **3**, is then formed by the rapid reaction between **2** and $\cdot\text{O}_2^-$ or the disproportionation of **2** to **1** and **4**. This mechanism does not preclude the formation of the carbonate radical as an additional source of **2**, but the carbonate radical is not required. The enhancement of Fe(II) CL relative to $\cdot\text{O}_2^-$ (Figures 5 and 6) is consistent with the relatively slow production of $\cdot\text{O}_2^-$ in the flow cell when Fe(II) reacts with the alkaline luminol reagent.¹³



Superoxide produced by reaction 5 will react with $\text{CO}_2(\text{aq})$ and **1** to produce **2**, which will increase to a steady-state concentration.

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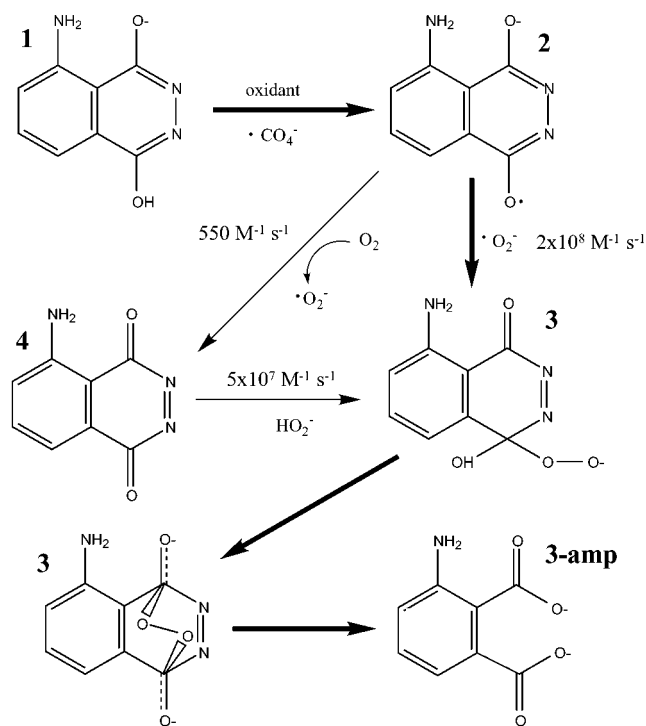


Figure 8. Proposed mechanism of luminol CL including the peroxycarbonate radical. Reaction rates are from refs 20 and 24.

Subsequent production of $\cdot\text{O}_2^-$ may react with $\text{CO}_2(\text{aq})$ or **2** to produce **3** and light. In contrast, the rapid reaction between $\text{CO}_2(\text{aq})$ and $\cdot\text{O}_2^-$ in luminol/ H_2O_2 and luminol/ $\cdot\text{O}_2^-$ systems precludes significant reaction with **2**. This is because all the $\cdot\text{O}_2^-$, either injected as sample or produced via reactions 1 and 2, is consumed by reaction 4 before any significant accumulation of **2** can occur. As a consequence, the CL signal for 20 nM Fe(II) shown in Figure 5 is 500 times greater than the signal for 1 μM $\cdot\text{O}_2^-$ on a concentration-normalized scale. The fast reaction between $\cdot\text{O}_2^-$ and $\text{CO}_2(\text{aq})$ is also evidenced in the Cu(II)/1,10-phenanthroline CL system.

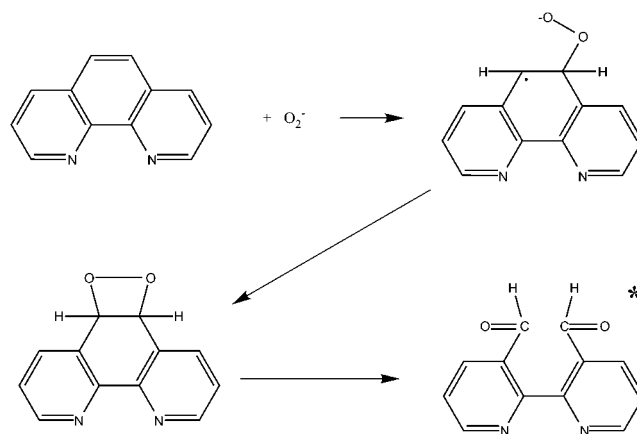


Figure 9. Proposed mechanism of 1,10-phenanthroline CL. Based on ref 22.

In the method used by Coale et al.^{15,19} for Cu(II) determination, 1,10-phenanthroline rather than luminol was used as the chemiluminescence reagent. This reaction is completely quenched by elevated $\text{CO}_2(\text{aq})$ in the carrier or reagents. Fedorova et al. proposed a CL mechanism involving direct reaction of 1,10-phenanthroline with $\cdot\text{O}_2^-$ (Figure 9).²² Enhancement of CL by added cationic micellar solutions is thought to involve more efficient reaction of 1,10-phenanthroline with $\cdot\text{O}_2^-$ in the Stern region of the micelles.¹⁹ Formation of the peroxycarbonate radical in the presence of $\text{CO}_2(\text{aq})$ blocks the coupling reaction between 1,10-phenanthroline and $\cdot\text{O}_2^-$ and quenches the reaction. Similar effects are observed when other $\cdot\text{O}_2^-$ scavengers such as NBT are added to the 1,10-phenanthroline system.²² The important implication of this quenching effect is that moderate concentrations of $\text{CO}_2(\text{aq})$ (500 μM) are capable of complexing a significant fraction of $\cdot\text{O}_2^-$ in solution.

The luminol CL system is clearly complicated. The final CL emission is a result of competing fast radical reactions that are affected by trace amounts of dissolved gases (i.e., O_2 and CO_2) and metal ion contaminants. This makes it very difficult to study

Table 2. Summary of Luminol CL Mechanism^a

system	reactions/rate or equilibrium constants	comments	key refs
luminol/ H_2O_2 luminol/ $\cdot\text{O}_2^-$	$\text{H}_2\text{O}_2 \rightarrow 2\cdot\text{OH}/\text{dependent of light and catalyst}$ $\text{H}_2\text{O}_2 \rightarrow \text{HOO}^- + \text{H}^+ / 1.5 \times 10^{-12}$ $\text{HOO}^- + \cdot\text{OH} \rightarrow \text{OH}^- + \cdot\text{O}_2\text{H} / 7.5 \times 10^9$ $\cdot\text{O}_2\text{H} \rightleftharpoons \cdot\text{O}_2^- + \text{H}^+ / 10^{-4.7}$ $\text{CO}_2(\text{aq}) + \cdot\text{O}_2^- \rightarrow \cdot\text{CO}_4^- / \text{unknown}$ $\cdot\text{OH} + \mathbf{1} \rightarrow \mathbf{2} + \text{others} / 8.8 \times 10^9$ $\cdot\text{CO}_4^- + \mathbf{1} \rightarrow \mathbf{2} / \text{unknown}$ $\mathbf{2} + \cdot\text{O}_2^- \rightarrow \mathbf{3} / 2.3 \times 10^8$	CO_2 is fundamental for all the luminol CL systems. CO_2 may be present in the reagent stream or a pH mixing boundary between the basic luminol solution and acidic sample	b, 16, 18, 24, 27, 31
luminol/ M^{n+}/O_2	$\text{M}^{n+} + \text{O}_2 \rightarrow \cdot\text{O}_2^- + \text{M}^{n+1} / 10^{-13.77 + 2.0 \text{ pH}}(\text{Fe})$	Fe(II) oxidation rates at pH > 9 favors this reaction relative to other metal ions	7, 14, 28, 34
luminol/ $\text{H}_2\text{O}_2/\text{M}^{n+}$	$\text{H}_2\text{O}_2 + \text{M}^{n+} \rightarrow 2\cdot\text{OH} + \text{M}^{n+}$	transition metal ions that have two available oxidation states usually catalyze the radical decomposition of H_2O_2 , so they can be detected by CL	14, 27, 29, 30, 32, 33
luminol/ $\text{H}_2\text{O}_2/\text{M}^{n+}/\text{CO}_3^{2-}$ luminol/ $\text{H}_2\text{O}_2/\text{M}^{n+}/\text{carbonic anhydrase}$	$\cdot\text{OH} + \text{CO}_3^{2-} \rightarrow \cdot\text{CO}_3^- + \text{OH}^- / 3.65 \times 10^8$ $\cdot\text{CO}_3^- + \mathbf{1} \rightarrow \mathbf{2} / 9 \times 10^8$ acidic pH + basic pH + carbonic anhydrase $\rightarrow \text{CO}_2(\text{aq})$	carbonate enhancement an example of how luminol CL can be used to analyze any analyte that can affect one or more reactions listed above	8, 9, 24 b

^a Luminol systems for bioanalysis is reviewed in ref 1; unit for rate constant is $\text{L mol}^{-1} \text{s}^{-1}$. More reactions involving luminol intermediates are shown in Figure 8. ^b This work.

its mechanism by means of traditional spectroscopic or kinetic means. Nevertheless, much insight has been gained on luminol reactivity from the careful work of Merenyl and co-workers using radiolysis.^{20,23–25} Many empirical observations have further refined the analytical application of luminol and, with care, may also help to understand the mechanistic aspect of CL reactions. Table 2 details significant mechanistic information on luminol CL that may assist the development of new analytical applications using this CL system.

CONCLUSIONS

We have demonstrated that CO₂(aq) is responsible for a number of unusual observations in luminol- and 1,10-phenanthroline-based chemiluminescence. These observations include the sample pH effect and the effect of the acidity and freshness of the carrier water. In all cases involving luminol as a CL reagent, we found that CO₂(aq) is essential for chemiluminescence. Since it is difficult to exclude dissolved CO₂ or carbonate entirely from the reagents used in CL experiment, the presence of CO₂(aq) in the CL system could have affected many other systems described in the CL literature. In a recent paper on luminol CL in unbuffered solutions with a cobalt(II)–ethanolamine complex,³⁴ it would be worthwhile examining the possible role of CO₂ in addition to O₂ and N₂ gases. The role of CO₂ demonstrated in this work could explain the very low detection limits achieved with a batch apparatus,³⁵ where the reagents and the sample are mixed in an open container enhancing gas exchange.

The CO₂ enhancement effect certainly can be employed to develop other CL methods, including methods for the determination of the CO₂ content in both liquid and gaseous samples, as demonstrated by Lan and Mottola.³⁶ To our knowledge, our work is the first time that a chemiluminescence phenomenon associated with the direct production of CO₂ in the presence of carbonic anhydrase has been reported. We believe that luminol/H₂O₂-based CL can be developed to study the activity of this enzyme and its interactions with inhibitors.

The possible interferences caused by carbon dioxide should be explored during the method development and troubleshooting in luminol analyses. The degree of the carbon dioxide effects on the CL signal is dependent on the reagent flow/mixing pattern. For example, in a recently reported Co(II)/luminol method for the determination of H₂O₂ in seawater,³⁷ no carrier was used. The alkaline CL reagent was injected directly into an alkaline seawater sample. In this unsegmented flow injection configuration, the problems that may be caused by using a carrier were minimized unintentionally.

The observations presented here demonstrate clearly that CO₂ plays an important role in the radical reactions involving $\cdot\text{O}_2^-$. More definitive studies of this system by means of kinetic and spectroscopic methods are needed since CO₂/ $\cdot\text{O}_2^-$ is involved in many biological, industrial, and natural systems.

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