

Quantification of Cu^{2+} using 1,1'-oxalyldiimidazole chemiluminescence

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Abstract

Emission from two different peroxyoxalate chemiluminescence (PO-CL) reaction pathways (i.e., 1,1'-oxalyldiimidazole (ODI) and bis(2,4,6-trichlorophenyl)oxalate (TCPO) CL reactions) is significantly reduced in the presence of increasing Cu^{2+} concentrations. The CL intensity decrease in the ODI-CL reaction results from deactivation of the high-energy intermediate(s) by Cu^{2+} , whereas the apparent quenching in the TCPO-CL reaction results from both this effect and formation of Cu^{2+} -imidazole (ImH) complexes $[\text{Cu}(\text{ImH})_{n=1-4}]^{2+}$. Because the reaction between Cu^{2+} and ImH occurs competitively with the formation of a high-energy intermediate (X) in TCPO-CL reactions, accurately determining Cu^{2+} concentrations with a TCPO-CL-based system is more difficult versus an ODI-CL-based system. Using ODI-CL, the detection limit of Cu^{2+} dissolved in water was determined to be 5.0×10^{-8} M (signal:noise = 3) in a 1 cm fluorescence flow micro-cell. ODI-CL-based systems are expected to be useful for the rapid quantification of many quenchers in environmental and biological samples because the interactions between the high-energy intermediate(s) and quencher are very rapid relative to other competitive reactions.

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Keywords: Chemiluminescence; Copper; 1,1'-Oxalyldiimidazole (ODI); Peroxyoxalate; Quenching

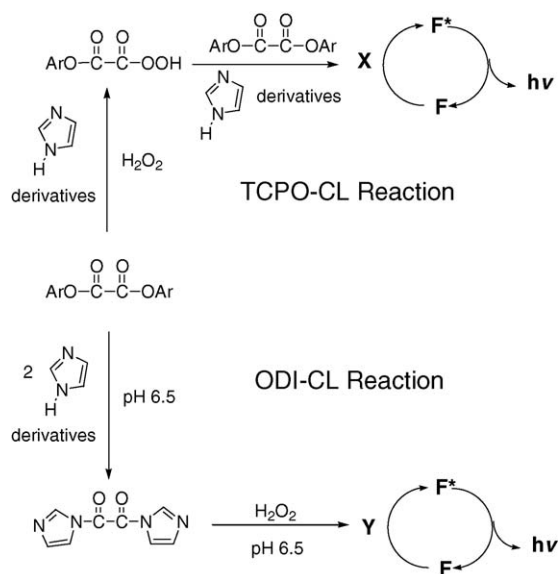
1. Introduction

Recently, we reported on the characteristics of two distinct peroxyoxalate chemiluminescence (PO-CL) reaction pathways in the presence of imidazole (ImH) derivatives (Scheme 1) [1–3]. When bis(2,4,6-trichlorophenyl)oxalate (TCPO) is added to a reaction cell containing ImH, H_2O_2 and fluorophore (TCPO-CL reaction), CL intensity versus time profiles having relatively long decay curves are observed. Conversely, 1,1-oxalyldiimidazole (ODI) is formed by the reaction between TCPO and ImH. When the remaining CL reagents (H_2O_2 and fluorophore) are then added to solutions containing ODI, a relatively fast decaying curve appears (ODI-CL reaction). These results imply that one (or more) of the critical PO-CL reagents could also react

with an analyte (e.g., fluorophore or quencher) to form unexpected products and/or unanticipated luminescent responses. If so, it will become more difficult to quantify analyte concentrations using PO-CL detection systems. For example, 2,4-dinitrophenol (DNP) is one of the products formed in DNP-catalyzed nucleophilic PO-CL reactions [4]. When a relatively low concentration of DNP is present in the reaction system, it acts as a nucleophilic catalyst to form one or more high-energy intermediates capable of transferring their energy to the fluorophore. When excess DNP molecules are added, however, they act as an apparent quencher of CL emission [4].

Here, we report for the first time that the interaction between an analyte (Cu^{2+}) and CL reagent (ImH) occurs competitively with the TCPO-CL reaction. In addition, we interpret the apparent CL quenching by Cu^{2+} in the two PO-CL reaction pathways (ODI-CL and TCPO-CL reactions) as resulting from the deactivation of high-energy intermediate(s) by Cu^{2+} .

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2. Experimental

2.1. Chemicals

Perylene, TCPO, ImH, H₂O₂ (30%) and Cu(ClO₄)₂ were purchased from Aldrich. Spectroscopic grade acetonitrile was purchased from Burdick & Jackson. Deionized water of resistivity greater than 17.8 MΩ cm (Super-Q™ Plus, Millipore) was used to prepare all aqueous solutions.

2.2. Methods

2.2.1. Measurements of 2,4,6-trichlorophenol (TCP), [Cu(ImH)_{n=1-4}]²⁺, Cu²⁺ and ImH absorbance

Rates of TCP formed from the reaction between TCPO and ImH in acetonitrile were followed by absorption at 290 nm using the kinetics function of an 8452A diode array spectrophotometer (Hewlett Packard). Rates of [Cu(ImH)_{n=1-4}]²⁺ complex formation from the reaction between Cu²⁺ and ImH in acetonitrile were determined by absorption at 325 nm using the same instrument.

Ultraviolet–visible (UV–vis) absorption spectra (resolution of 0.05 nm) of imidazole, Cu²⁺, and [Cu(ImH)_{n=1-4}]²⁺ were measured with a double-beam, double-monochromator spectrophotometer (Shimadzu 2501PC).

2.2.2. Fluorescence measurements

Steady-state fluorescence spectra of perylene at room temperature (23 ± 2 °C) in the absence and presence of Cu²⁺ were recorded with a PTI QuantaMaster™ spectrometer (Photon Technology International Inc.) equipped with a 75 W xenon arc lamp. The excitation wavelength used for perylene was 434 nm, and fluorescence emission was monitored over a wavelength range of 450–600 nm.

2.2.3. Chemiluminescence measurements

A typical CL reaction was conducted at room temperature (23 ± 2 °C) in 1 cm fluorescence cells for TCPO-CL reactions, and in a 1 cm fluorescence flow micro-cell for ODI-CL reactions, which were placed in the sample compartment of a spectrofluorometer (PTI Inc.). Fresh solutions were prepared daily and kept in the dark until used. The CL intensity versus time was monitored by the spectrofluorometer at the maximum emission wavelength (468 nm) of perylene without using an illuminating light source.

2.2.3.1. TCPO-CL reaction. A 0.4 ml H₂O₂ solution was added to the cell followed by addition of 0.4 ml each of a perylene solution, Cu²⁺ solution and an ImH solution. The reaction was initiated by injecting 0.4 ml of TCPO. Each experimental condition was replicated three times. A small magnetic stir bar in the fluorescence cell provided continuous mixing during each experiment.

2.2.3.2. ODI-CL reaction. Rapid ODI-CL decaying emission curves were recorded using the same spectrofluorometer equipped with a RX-2000 stopped-flow accessory (Applied Photophysics Ltd., UK). Equal volumes of perylene solution and H₂O₂ dissolved in acetonitrile were inserted in a drive syringe. To study the effects of Cu²⁺ on the reaction between TCPO and ImH in acetonitrile to form ODI, TCPO was reacted with ImH in the absence or presence of Cu²⁺ in the other drive syringe for 60 s. Then, both drive syringes were activated, simultaneously discharging their contents via a flow line (silica, glass and fluoro-carbon) into a silica micro-cell (80 μl) placed in the sample compartment of the spectrofluorometer. The mixing ratio between the two drive syringes was held constant at 1:1, and the stopped-flow system had a dead-time of no more than 6 ms. To investigate the quenching effects of Cu²⁺ in the ODI-CL reaction, Cu²⁺ dissolved in acetonitrile or water was inserted in the syringe containing perylene and H₂O₂. After TCPO reacted with ImH in the absence of Cu²⁺ in the other drive syringe for 60 s, both drive syringes were discharged into the silica micro-cell as described above.

3. Results and discussion

As shown in Table 1, strong and rapid CL emission was observed when ODI molecules (formed by reacting TCPO and ImH for 60 s) were subsequently reacted with H₂O₂ in the presence of perylene (i.e., the ODI-CL reaction). When ODI was formed by the same reaction in the presence of 0.1 mM Cu²⁺ under otherwise similar experimental conditions, we observed a much lower maximum emission intensity and slightly higher values for the times to reach maximum and half maximum emission (τ_{\max} and τ_{half} , respectively). When H₂O₂ and perylene were added to the otherwise same solution containing 0.5 mM Cu²⁺, however, only weak CL emission with much higher τ_{\max} and τ_{half} values was observed. These characteristics are not consistent with the ODI-CL reaction, but instead with the TCPO-CL reaction [1,2]. When a still higher Cu²⁺ concentration (1.0 mM) was used, no CL emission was observed (i.e., signal:noise < 3).

Table 1
Interference of Cu^{2+} in the reaction between TCPO and ImH to form ODI in acetonitrile at room temperature ($23.0 \pm 2.0^\circ\text{C}$)

| Cu^{2+} (mM) ^a | Relative I_{max} ^b | τ_{max} ^c (s) | τ_{half} ^d (s) |
|------------------------------------|--|--------------------------------------|---------------------------------------|
| 0 | 1000 | 0.1 | 1.1 |
| 0.1 | 113 | 0.3 | 3.7 |
| 0.5 | 0.6 ^e | 20.0 | 480 |
| 1.0 | nd ^f | nd | nd |

^a $\text{Cu}(\text{ClO}_4)_2$.

^b Maximum CL intensity.

^c Time to reach the maximum CL emission.

^d Time to reach the half maximum CL emission.

^e Relative I_{max} observed in the presence of Cu^{2+} was about 20 times higher than noise ($S/N \approx 20$).

^f nd, Not detected. Reaction conditions: $[\text{TCPO}] = 0.2 \text{ mM}$, $[\text{ImH}] = 2.0 \text{ mM}$, $[\text{perylene}] = 0.02 \text{ mM}$, $[\text{H}_2\text{O}_2] = 10.0 \text{ mM}$.

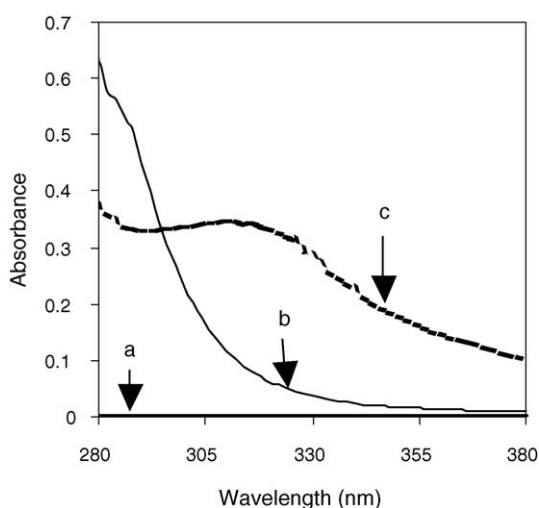
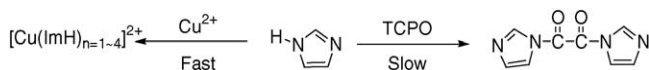


Fig. 1. Complexation between ImH and Cu^{2+} in acetonitrile at room temperature ($23.0 \pm 2.0^\circ\text{C}$). a: ImH; b: Cu^{2+} ; and c: $[\text{Cu}(\text{ImH})_{n=1-4}]^{2+}$.

Fig. 1 demonstrates the complexation between ImH (1.0 mM) and Cu^{2+} (0.2 mM), consistent with previous reports by other research groups [5–7]. We reported in a previous paper that 2,4,6-trichlorophenol (TCP) is formed when ImH reacts with TCPO [1]. In the present study, however, we determined that the pseudo first order rate of $[\text{Cu}(\text{ImH})_{n=1-4}]^{2+}$ complex formation ($5.29 \times 10^{-2} \text{ s}^{-1}$) resulting from the reaction between Cu^{2+} (0.1 mM) and ImH (1.0 mM) in acetonitrile is more than an order of magnitude and faster than the analogous rate of TCP formation ($1.94 \times 10^{-3} \text{ s}^{-1}$) resulting from the reaction between TCPO (0.1 mM) and ImH (1.0 mM) in acetonitrile (Scheme 2). When TCPO was added to ImH solutions containing Cu^{2+} , we also observed absorption spectra similar to c shown in Fig. 1. Therefore, the results of Table 1 can be explained as a disappearance of the relatively fast and intense ODI-CL emission due to the faster formation of $[\text{Cu}(\text{ImH})_{n=1-4}]^{2+}$ complexes in the presence of higher Cu^{2+} concentrations.



Scheme 2.

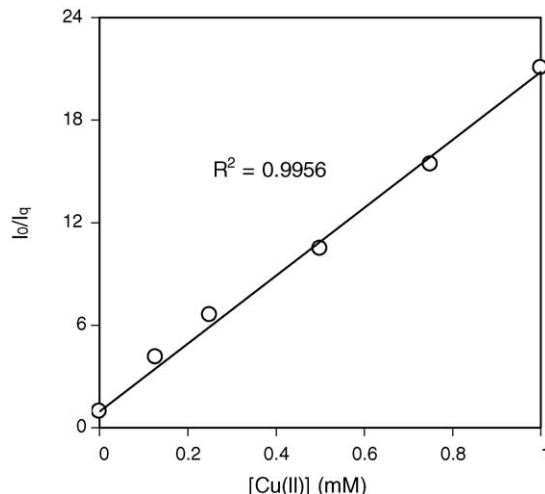
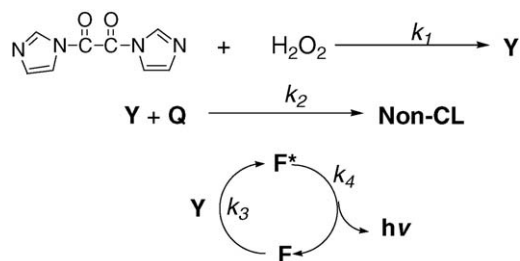


Fig. 2. I_0/I_q in ODI-CL reactions with increasing Cu^{2+} in acetonitrile. Reaction conditions: $[\text{TCPO}] = 0.2 \text{ mM}$, $[\text{ImH}] = 2.0 \text{ mM}$, $[\text{perylene}] = 0.02 \text{ mM}$, $[\text{H}_2\text{O}_2] = 10.0 \text{ mM}$. I_0 and I_q are the maximum CL intensities in the absence and presence of Cu^{2+} .

Fig. 2 shows true quenching of the emission generated by the ODI-CL reaction with increasing Cu^{2+} concentration. In other words, when Cu^{2+} , H_2O_2 and perylene were added to solutions containing ODI (formed by reacting TCPO and ImH for 60 s in the absence of Cu^{2+}), emission characteristics (i.e., rapid τ_{max} and τ_{half}) remained consistent with the ODI-CL reaction in the absence of Cu^{2+} . This result indicates that the emission quenching observed in Fig. 2 does not result from complexation of Cu^{2+} by ImH. Instead, the first-order dependence of I_0/I_q on Cu^{2+} concentration indicates that the major mechanism of ODI-CL emission quenching by Cu^{2+} is one of two possible alternative factors (Scheme 3): (a) competition between Cu^{2+} and perylene for the high-energy intermediate(s) (Y) formed in the ODI-CL reaction, or (b) collision between excited-state perylene (F^*) and Cu^{2+} analogous to the dynamic quenching of fluorescence. To test the likelihood of the latter factor, a dynamic quenching constant (k_d) was determined from perylene fluorescence intensities (excitation = 434 nm, emission = 468 nm) measured in acetonitrile solutions containing H_2O_2 and Cu^{2+} concentrations ranging from 0 to 1.0 mM [8]. The k_d value (0.68 mM^{-1}) determined from fluorescence quenching is much smaller than the slope (19.62 mM^{-1}) of the regression line shown in Fig. 2. This result thus indicates that alternative factor a is the most plausible explanation for the observations in Fig. 2. Based on



Scheme 3.

this reasoning, a plausible apparent quenching reaction pathway (Scheme 3) and corresponding modified Stern–Volmer equation for the apparent quenching of ODI-CL emission by Cu^{2+} is

$$I_0 = k_3[Y][F] \quad (1)$$

$$I_q = k_3 \left(\frac{k_3[F]}{k_3[F] + k_2[Q]} \right) [Y][F] \quad (2)$$

$$\frac{I_0}{I_q} = 1 + \frac{k_2}{k_3[F]}[Q] = 1 + K_q[Q]. \quad (3)$$

In Eqs. (1)–(3), I_0 and I_q are the maximum CL intensities in the absence and presence of Cu^{2+} , $[Q]$ the concentration of Cu^{2+} , and k_2 and k_3 are the rate constants. K_q (i.e., the slope of the regression line in Fig. 2) is the apparent quenching constant resulting from the interaction between Cu^{2+} and Y. As shown in Eq. (3), I_0/I_q is dependent on $k_2/(k_3[F])$. Therefore, to detect low concentrations of Cu^{2+} using the ODI-CL quenching system, $k_3[F]$ should be as small as possible. For example, using one of the complicated PO-CL reaction pathways in the presence and absence of ImH (but not the ODI-CL or TCPO-CL reaction pathway), van Zoonen et al. [9] was able to determine low detection limits for 21 quenchers (e.g., NO_3^- , SO_3^{2-} , aniline, benzylamine). Our previous papers [1–3] have reported that the ODI-CL reaction pathway results in much higher maximum emission intensities (I_{max}) and much lower τ_{max} and τ_{half} values. For example, we observed intense and rapid CL emission when 1-aminopyrene and H_2O_2 dissolved in water were mixed with an ODI derivative formed from the reaction between TCPO and an ImH derivative (4-methylimidazole) dissolved in an organic solvent mixture (acetonitrile and ethyl acetate) [3], because the ODI-CL reaction is much faster than ODI hydrolysis. Therefore, the ODI-CL reaction pathway can be used to measure the concentrations of quenchers dissolved in water without having to extract them first into organic solvents (e.g., acetonitrile, methanol). In other words, ODI-CL systems are expected to be useful for the rapid quantification of many quenchers (e.g., transition metals) present in environmental and biological samples. For example, using the ODI-CL spectra measured in the absence and presence of aqueous Cu^{2+} solutions (0–1.0 mM), we were able to determine a detection limit (signal:noise = 3) of 5.0×10^{-8} M for Cu^{2+} which is similar to or lower than the corresponding values determined with other methods reported recently [10–12].

Fig. 3 shows that the emission generated from the TCPO-CL reaction is also decreased upon addition of Cu^{2+} . In this case, however, I_0/I_q exhibits a second-order dependence on Cu^{2+} concentration. Based on the results shown in Figs. 1 and 3, it appears that the decreasing emission in the TCPO-CL system is influenced by two factors: (1) reduction in the concentration of ImH by complexation with Cu^{2+} , thus decreasing its ability to act as a nucleophilic catalyst in the TCPO-CL reaction and (2) competition between Cu^{2+} and perylene for the high-energy intermediate(s) formed in the TCPO-CL reaction. Therefore, determining K_q for factor 2 in the TCPO-CL system becomes more difficult versus the ODI-CL system. This is because the reaction between Cu^{2+} and ImH (factor 1) shown in Scheme 4

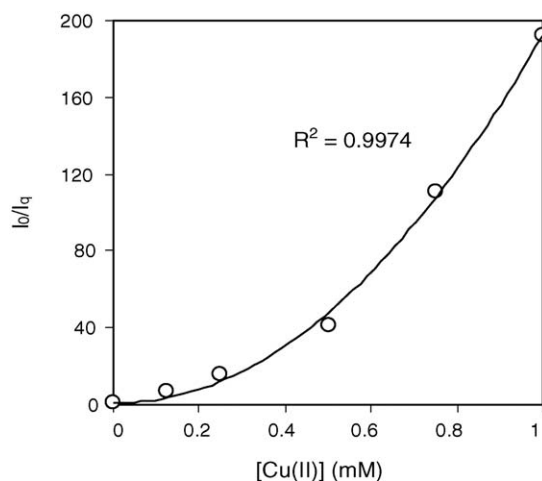
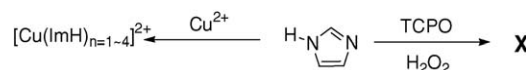


Fig. 3. I_0/I_q in TCPO-CL reactions with increasing Cu^{2+} in acetonitrile. Reaction conditions: $[\text{TCPO}] = 0.2$ mM, $[\text{ImH}] = 2.0$ mM, $[\text{perylene}] = 0.02$ mM, $[\text{H}_2\text{O}_2] = 10.0$ mM. I_0 and I_q are the maximum CL intensities in the absence and presence of Cu^{2+} .



Scheme 4.

occurs competitively with the formation of high-energy intermediate (X) in the TCPO-CL reaction.

4. Conclusions

Based on the rapid complexation of Cu^{2+} by ImH, we report for the first time in this paper that critical CL reagent(s) can react with analytes (fluorophore, quencher) and catalysts to form unpredicted byproduct(s). This implies that accurate quantification of certain analytes that can react quickly with one or more CL reagents may prove difficult. For example, the concentration of a fluorophore determined with the relatively slower PO-CL reaction pathway(s) (e.g., TCPO-CL reaction) will be underestimated if it reacts with a critical CL reagent. Conversely, the concentration of a quencher determined with the same PO-CL reaction pathway(s) will be overestimated if the reaction between quencher and a CL reagent (e.g., ImH) is much faster than the PO-CL reaction which forms the high-energy intermediate(s). Using an ODI-CL reaction system, however, we were able to circumvent these problems because the interactions between Cu^{2+} and high-energy intermediate(s) formed in the ODI-CL reaction were much faster than the complexation of Cu^{2+} by ImH. We expect that our quenching study using the ODI-CL reaction can be applied to develop new rapid and inexpensive analytical methods capable of quantifying analytes of interest to scientists in various research fields. For example, ongoing research is being conducted in our laboratories to develop portable analytical systems with microfluidic devices and ODI-CL detection to quantify toxic environmental pollutants on site.

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