The Oxidation of Luminol an Experiment to Maximize the Efficiency of Chemiluminescence from Luminol

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Abstract: In this work, seven inorganic salts, KCl, Na_2SO_4, $MgSO_4·7H_2O$, $ZnCl_2$, Na_2CrO_4, $CuSO_4·5H_2O$, and $K_3[Fe(CN)_6]$, were used as catalysts to induce chemiluminescent luminol oxidation in alkaline aqueous media. It was observed that simple salts containing either Mg^{2+}, Zn^{2+}, Na^+ and K^+ cations or SO_4^{2-} and Cl^- anions, are not active as catalysts. On the other hand, the relative order of activity detected for the active chemiluminescent salts containing Fe(III), Cu(II) and Cr(VI) cations is $K_3[Fe(CN)_6] > CuSO_4•5H_2O > Na_2CrO_4$. The intensity of the emitted light agrees with the standard reduction potentials of the corresponding redox couple and with the presence of paramagnetic species in the aqueous solutions. The inhibition effect of mannitol was also studied.

Introduction

There are many fascinating examples of chemical reactions that are chemiluminescent, that is, they emit light without an appreciable quantity of heat. In nature, there are the examples of the firefly, glowworm, and microorganisms involved in the decay of vegetable matter. The basic reason for the chemiluminescence emission is the formation of one intermediate in an electronically excited state, which decays as soon as it is formed, emitting its characteristic excitation energy in the visible region. Since many of these reactions take place with a small activation energy, the excited state of the emitting species cannot lie very much higher in energy than the reagents [1–4].

The chemiluminescence reaction of luminol from **I** to **IV** is an oxidation process, represented in Scheme 1. Different oxidants are required, and slightly different chemiluminescent spectra are observed in different media. In aprotic solvents, only molecular oxygen and a strong base are needed to produce chemiluminescence from luminol, and emission occurs at 485 nm [5]**.** In protic solvents, the reaction requires a strong base, either molecular oxygen or peroxide, and an auxiliary oxidant such as hypochlorite, ferricyanide, or persulfate ions. The emission in protic solvents occurs at 425 nm [6, 7]. Although the reaction mechanism varies with the solvent, in both protic and aprotic solvents, the emitting species has been identified in an aprotic medium, such as DMSO, as the aminophthalate ion, **III**, or its tautomeric quinoidal form **II** [6].

In both types of solvents, considerable data have been accumulated on the mechanism of the oxidation of luminol. Mechanisms in protic and aprotic media differ in their early steps. In protic solvents, a free-radical species is involved, whereas in aprotic media, a luminol dianion intervenes [8]. Because the conditions required for the reaction are milder in the aprotic solvents, the mechanism in such solvents is better understood. No definitive luminol mechanism is known, but much information about the intermediate species in the oxidation has been gathered [6].

In aqueous systems, an activator is always required [9]. These activators, which are frequently transition-metal complexes such as the hexacyanoferrate(III) ion [9], may act either as catalysts, (Scheme 2) or co-oxidants (Scheme 1). Frequently, the transition metal requires complexing agents to prevent the precipitation of the metal hydroxide in the strongly alkaline environment required for chemiluminescent oxidation [9]. Here, the species required to initiate the oxidation in aqueous media is the superoxide radical anion, $O_2^{\bullet-}$ formed in aqueous media by the decomposition of hydrogen peroxide [10]. The transition-metal complex may be also involved in an electron-transfer step such as illustrated in Scheme 2 [11]. The chemiluminescence of luminol in aqueous alkaline media is inhibited by superoxide dismutase or by other radical scavengers, for example, mannitol, diphenylfuran, and histidine [12, 13].

In this work we present a simple and qualitative experiment for undergraduate students beginning courses in inorganic chemistry. This experiment may be used as an appropriate introduction to the phenomenon of chemiluminescence and related catalysis, providing the student with an example of the role of cations in the efficiency of luminol oxidation.

Seven inorganic salts KCl (**1**), Na_2SO_4 (**2**), $MgSO_4•7H_2O$ (**3**), $ZnCl_2$ (**4**), Na_2CrO_4 (**5**), $CuSO_4•5H_2O$ (**6**), and $K_3[Fe(CN)_6]$ (**7**), containing different cations and anions, are used to analyze the chemiluminescence of luminol in aqueous alkaline media. In order to understand the inhibition phenomenon, mannitol is used as a quencher of free radicals in all those solutions containing the active metals.

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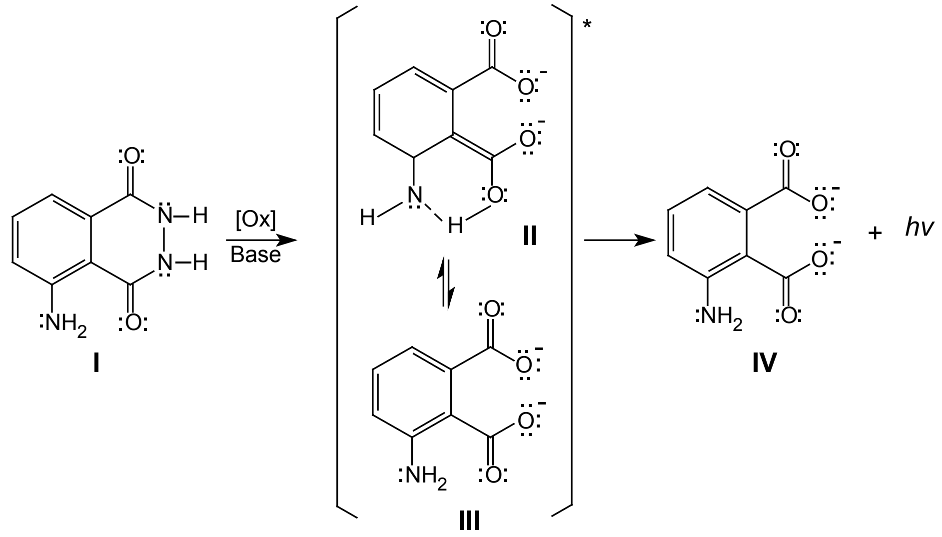
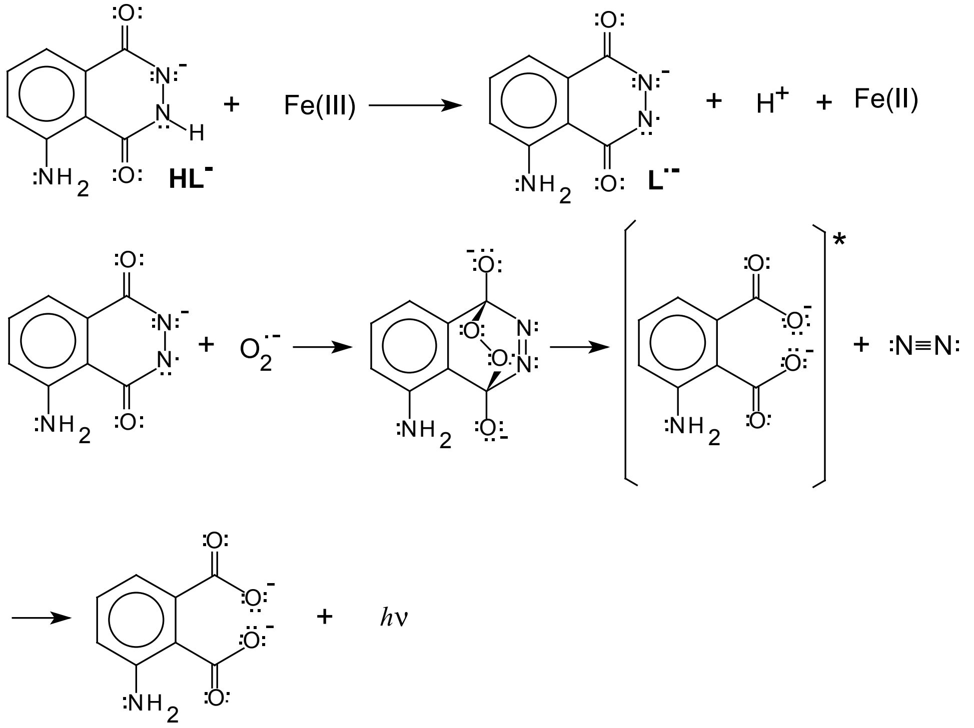


Scheme 1. The luminol oxidation in presence of co-oxidant, [Ox].



Scheme 2. Reaction mechanism of luminol oxidation using [Fe(CN)$_6^{3-}$] as catalyst.

Experimental

Chemicals. Luminol, H_2O_2, NaOH, salts, and the inhibitor were obtained from commercial sources and used without further purification. The following salts were used as catalysts: KCl (**1**), Na_2SO_4 (**2**), $MgSO_4•7H_2O$ (**3**), $ZnCl_2$ (**4**), Na_2CrO_4 (**5**), $CuSO_4•5H_2O$ (**6**), and $K_3[Fe(CN)_6]$ (**7**). Mannitol was used in the inhibition experiments.

Procedure

Catalytic Effect. The experiment is performed by seven groups. Each group prepares two types of solutions. Solution **A**: identical solutions, labeled 1–7, are prepared containing 0.05 g of luminol, 5 mL of NaOH (5%), and 450 mL of H_2O in a 500-mL Erlenmeyer flask. Solution **B**: identical solutions, labeled 1–7, are prepared containing 5 mL of NaOH (5%), 10 mL of H_2O_2 (3%) and ~1.0×10^{-4} mole of the given salts in 450 mL of H_2O in a 500-mL Erlenmeyer flask. KCl (**1**), 0.0075 g; Na_2SO_4 (**2**), 0.0142 g; $MgSO_4•7H_2O$ (**3**), 0.0246 g; $ZnCl_2$ (**4**), 0.0136 g; $K_2Cr_2O_7$ (**5**), 0.0162 g; $CuSO_4•5H_2O$ (**6**), 0.0250 g; and $K_3[Fe(CN)_6]$ (**7**), 0.0329 g. Solution **B** is then added to solution **A** in a 1-L Erlenmeyer flask in a dark room.

Questions. The students should answer the following questions at the end of the experiment.

What do you observe?

How do you assess the catalytic effect of each aqueous cation: Cu^{2+}, Na^+, K^+, Mg^{2+}, and Zn^{2+}?

Table 1. Intensity and duration of chemiluminescence of luminol in the presence of different salts in alkaline aqueous solution

Salts	Species in solution	Light intensity	Light duration
1 and 7	$K^+_{(aq)}$	---	---
2 and 5	$Na^+_{(aq)}$	---	---
3	$Mg^{2+}_{(aq)}$	---	---
4	$ZnO_2^{2-}{}_{(aq)}$	---	---
6	$[Cu(OH_2)_6]^{2+}_{(aq)}$	Medium	Long
1 and 4	$Cl^-_{(aq)}$	---	---
5	CrO_4^{2-}	Weak	Short
3 and 6	$SO_4^{2-}{}_{(aq)}$	---	---
7	$[Fe(CN)_6]^{3-}_{(aq)}$	Strong	Medium

Table 2: Standard reduction potentials of the seven salts used in the luminol oxidation

Redox couple	Reduction Reaction	E° (Volts)
K^+/K^o	$K^+_{(ac)} + e^- \rightleftharpoons K^o$	–2.924
Na^+/Na^o	$Na^+_{(ac)} + e^- \rightleftharpoons Na^o$	–2.7129
Mg^{2+}/Mg^o	$Mg^{2+}_{(ac)} + 2e^- \rightleftharpoons Mg^o$	–2.375
ZnO_2^{2-}/Zn^o	$ZnO_2^{2-}{}_{(ac)} + 2e^- \rightleftharpoons Zn^o + 4OH^-_{(ac)}$	–1.216
$CrO_4^{2-}/Cr(OH)_3$	$CrO_4^{2-}{}_{(ac)} + 4H_2O + 3e^- \rightleftharpoons Cr(OH)_3 + 5OH^-_{(ac)}$	–0.12
Cu^{2+}/Cu^+	$Cu^{2+}_{(ac)} + e^- \rightleftharpoons Cu^+_{(ac)}$	0.158
$[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$	$[Fe(CN)_6]^{3-}_{(ac)} + e^- \rightleftharpoons [Fe(CN)_6]^{4-}_{(ac)}$	0.46
ClO^-/Cl^-	$ClO^-_{(ac)} + 2H_2O + 2e^- \rightleftharpoons Cl^-_{(ac)} + 2OH^-_{(ac)}$	0.90
$S_2O_8^{2-}/SO_4^{2-}$	$S_2O_8^{2-}{}_{(ac)} + 2e^- \rightleftharpoons 2SO_4^{2-}{}_{(ac)}$	2.00

How do you assess the catalytic effect of each aqueous anion: SO_4^{2-}, Cl^-, $Cr_2O_7^{2-}$, and $Fe(CN)_6^{3-}$?

Which of these compounds enhance the chemiluminescent reaction?

What would you expect for other paramagnetic metal and other oxidant agents?

Why do solutions **B** contain the same concentration in mol•L^{-1} of the added salt?

Inhibition effect

Two new sets of solutions should be prepared by the students, solutions **A,** containing all seven salts, as described above, and solutions **B**, containing only the active salts **5, 6,** and **7**. To solutions **A,** each containing one of the seven salts, mannitol (0.0364 g , 2.0 × 10^{-4} mole) is then added as a radical scavenger. Solutions **B** are then added to the resulting mixtures **A** in 1-L Erlenmeyer flasks, as described above.

Questions. The following questions may then be put to the students, What do you observe in the experiments that contain mannitol as compared to the previous experiment?

Suggest an explanation for the observed inhibition.

Results and Discussion

The students observe that simple salts containing either Mg^{2+}, Zn^{2+}, Na^+, and K^+ cations, or SO_4^{2-} and Cl^- anions, are not active as catalysts or co-oxidants. The blue light emission is observed only when the resulting solution contained $Cu^{2+}_{(aq)}$, $CrO_4^{2-}{}_{(aq)}$, or $[Fe(CN)_6]^{3-}_{(aq)}$ ions. The relative order detected for the active chemiluminescent aqueous ions is $[Fe(CN)_6]^{3-} > [Cu(OH_2)_6]^{2+} > CrO_4^{2-}$. The relative intensity and duration of luminescence with each ion are shown in Table 1.

The redox couples in alkaline media for each cation are given in Table 2. The students should conclude that luminol, which acts as a reducing agent, reacts only with the most strongly oxidizing species $[Fe(CN)_6]^{3-}$, $[Cu(OH_2)_6]^{2+}$, and CrO_4^{2-} ions, discarding all other metallic cations. The relative order of detected activity, $[Fe(CN)_6]^{3-} > Cu(OH_2)_6^{2+} > CrO_4^{2-}$, is also in agreement with the oxidizing power of the corresponding redox couple ($E^o = 0.46$ V > 0.158 V > –0.12 V, respectively).

Additionally, the students understand that those salts containing paramagnetic transition metals, $Cu^{2+}(3d^94s^0)$ and $Fe^{3+}(3d^54s^0)$, act as free radicals, and are more effective catalysts than the CrO_4^{2-} anion. Although the $Zn^{2+}(3d^{10}4s^0)$ cation is a transition metal, it is not active, as a consequence of its diamagnetic character, due to the five completely filled 3d orbitals. According to Scheme 2, the active paramagnetic metal complexes must have the ability to remove one electron from the **HL**$^-$, in order to generate the luminol radical, **L•**$^-$. By contrast, the role of the CrO_4^{2-} anion is best explained as that of a co-oxidant, Scheme 1.

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