行政院國家科學委員會專題研究計畫 成果報告

界面活性劑增強 luminol 之化學發光及其應用之研究
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2113-M-002-033-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學化學系暨研究所

計 畫 主 持 人：林萬寅

計畫參與人員：博士班研究生-兼任助理：陳育錚、陳異仁
碩士班研究生-兼任助理：林郁珊、方巍儒、郭家銘、柯廷璋、郭奕生

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 11 月 01 日

行政院國家科學委員會補助專題研究計畫 ☑ 成 果 報 告
□期中進度報告

界面活性劑增強 luminol 之化學發光及其應用之研究

計畫類別：☑ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 94－M－2113－002 －033－
執行期間：　95 年 8 月 1 日至 96 年 7 月 31 日

計畫主持人：林萬寅
共同主持人：
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立台灣大學

中　華　民　國　96　年　10　月　30　日

中文摘要：

陽離子型界面活性劑 CTAC 可以使 luminol、pyrogallol 及 lophine 之氧化產生化學發光。界面活性劑將發光試劑、氧化劑、催化劑及增強劑聚集在微胞內部或表面，並讓反應中間體或放光物質遠離自由基或螢光消滅劑，而顯著增強化學發光。催化劑、氧化劑、增強劑濃度對化學發光都有很大的影響。這些系統可用於檢驗參與或影響化學發光反應之物質，包括化學發光試劑、增強劑、抑制劑(如抗氧化劑、尿酸、多巴胺等)。

關鍵詞：化學發光、界面活性劑、luminol

Abstract

The presence of cationic surfactant (e.g., CTAC) induced the chemiluminescence (CL) from the oxidation of luminol, pyrogallol and lophine. CL enhancement was achieved by gathering the CL reagent, oxidant, catalyst and enhancer in the interior or interface of the micelle and shielding the CL intermediate and emitting species from external scavengers or quenchers. The concentrations of CL reagent, oxidant, and catalyst dramatically affect the CL emission. The enhanced CL systems were employed to determine the substances that participate or influence the CL reactions, such as CL reagents, enhancers, and inhibitors (e.g., antioxidants, uric acids, dopamine etc.).

Keywords: Chemiluminescence, surfactant, luminol

計劃緣由與目的：

Methods based on Chemiluminescence (CL) have the advantages of being simple, fast, sensitive, cheap, and versatile. In the past few years, we have been interested in the enhancement of CL emission from the oxidation of luminol. Intense CL emission was observed for the oxidation of luminol with H_2O_2 or m-chloroperoxybenzoic acid catalyzed by Fe- or Mn-microperoxidase.[1] Moreover some enhancers, such as Na_2CO_3, Tris, guanidine hydrochloride, are capable of increasing the CL emission by 1-2 orders of magnitude, depending on the catalysts and oxidants used.[2-5]

In this report, we have found that the use of a cationic surfactant (e.g., CTAC) caused a dramatic enhancement of the CL emission from the oxidation of luminol, pyrogallol, and lophine. We also investigated the factors that affect the CL emission and the potential applications of these enhanced CL systems (especially the

luminol CL) in chemical analysis.

結果與討論：

Effect of CTAC on CL emission

Fig. 1 demonstrated the effect of CTAC (cetyltrimethylammonium chloride) on the CL emission from the oxidation of luminol with chloramine T/iodide, pyrogallol with KIO_4 and lophine with H_2O_2 or NaOCl measured by the technique of flow injection analysis (FIA). The presence of 20 mM CTAC resulted in an intense CL emission for all three systems, whereas essentially no CL emission was observed without CTAC.

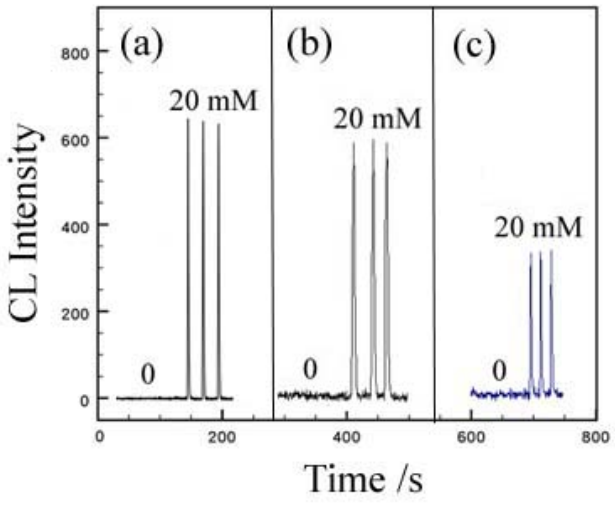

Fig. 1. FIA CL emission for the oxidation of (a) luminol (b) pyrogallo, and (c) lophine with and without the presence of 20 mM CTAC.

The use of anionic (e.g., SDS) or neutral (e.g., triton-X) surfactants or cyclodextrins did not have any enhancing effect on the CL emission. These results suggest that the cationic micellear intersurface may play a significant role in CL enhancement. It is proposed that the attraction of negatively charged reagents (I^-, IO_4^-, OCl^-, luminol anion, pyrogallol anion) onto the micellear interface and the hydrophobic reagents (chloramine T, lophine, CL intermediates) to the micellar interior will bring all the reactants to close vicinity for an efficient reaction to occur. Meanwhile, the incorporation of the CL intermediates and the emitting species in the micelle will protect them from potential radical scavengers or fluorescence quenchers in the solution, thereby increasing the CL efficiency and the fluorescence quantum yield. All these factors will contribute to the CL enhancement.

CL from the oxidation of luminol by chloramine T and iodide was found to be strongly dependent on the concentrations of chloramine T, iodide, CTAC and pH. Fig. 2 showed the effect of the concentrations of chloramine T on the CL emission. No CL emission was observed in the absence of the oxidant chloramine T. The CL intensity increased rapidly when 0.5-10 μM of chloramine T was added, indicating that chloramine T was essential for CL emission.

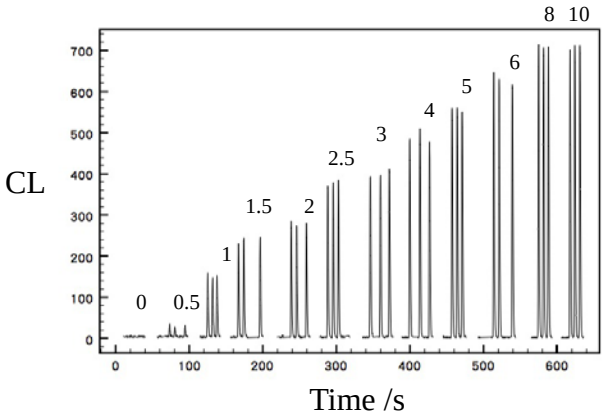

Fig. 2. FIA-CL emission for the oxidation of luminol (50 μM) with chloramine T (0-10 μM) at pH 10.10 in the presence of I^- (5 μM) and CTAC (20 mM).

The effect of the concentration of iodide on the CL emission was illustrated in Fig. 2. No CL emission was observed in the absence of iodide. The CL intensity increased rapidly when 0.3-10 μM of iodide was added, indicating that iodide was also essential for CL emission.

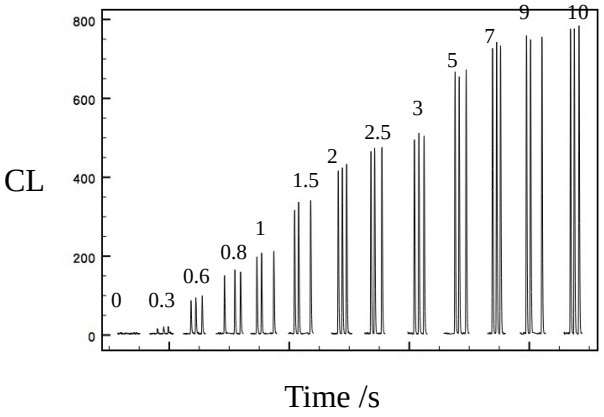

Fig. 3. FIA-CL emission for the oxidation of luminol (50 μM) with chloramine T (5 μM) at pH 10.10 in the presence of I^- (0-10 μM) and CTAC (20 mM).

In the chloramine T/I^-/luminol system, the actual oxidant for the CL reaction is iodine. Oxidation of iodide by chloramine T produced iodine, which is more soluble in the micelle than in the solution. The iodine in the micelle reacts with luminol anion in the interface, initiating the follow-up CL reactions.

The effect of the concentration of CTAC on the CL emission was demonstrated in Fig. 4. No CL emission was observed in the absence of CTAC. The CL intensity increased linearly when 0-40 mM of CTAC was added, indicating that CTAC was essential for CL emission.

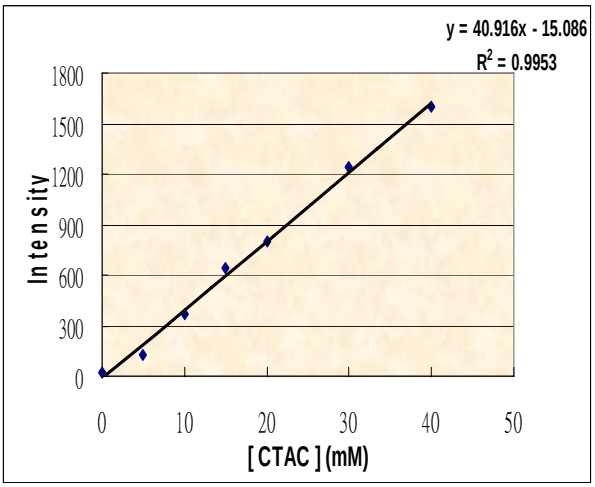

Fig. 4. FIA-CL emission for the oxidation of luminol (50 μM) with chloramine T (5 μM) at pH 10.10 in the presence of I^- (5 μM) and CTAC (0-40 mM).

Detection of iodide and doapmine

The enhanced CL systems can be used to determine a variety of substances. For example, the chloramine T/I^-/luminol/CTAC system can be used to determine chloramine T (0-10 μM) and iodide (0-10 μM). The detection limit (3σ) is 0.1 μM. This CL system is selective for iodide. The interference

studies showed that anions (F^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-) and cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, alkali metal ions) at 100 folds will not interfere with the CL emission. The CL method was successfully employed to determine the iodide content in iodized salt and in seawater (data not shown).

The chloramine T/I^-/luminol/CTAC system can also be used to determine the concentration of dopamine as illustrated in. Fig. 5.

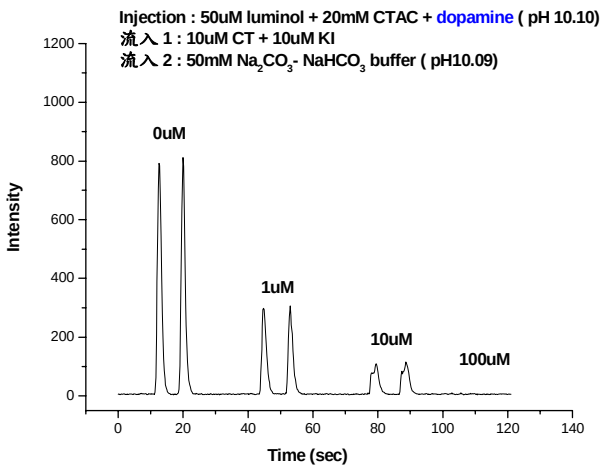

Fig. 5. FIA-CL emission for the oxidation of luminol (50 μM) with chloramine T (5 μM), I^- (5 μM) and CTAC (20 mM) at pH 10.10 in the presence of. dopamine (0-100 μM)

Significant inhibition of the CL emission was observed upon addition of dopamine (1-10 μM), allowing the determination of dopamine at sub-μM level.

The chloramine T/I^-/luminol/CTAC CL system can also be used to determine polyphenols other than dopamine (e.g., catechol, adrenaline, some flavonoids). The CL detection in conjunction with HPLC separation will provide a sensitive and powerful means to determine mixtures of polyphenols. We also found some antioxidants (e.g., vitamin C, trolox, 3-hydroxyflavone) that are capable of scavenging radicals can also be determined by this CL system.

CL emission from pyrogallol

Pyrogallol is another CL reagent that requires CTAC for detecting CL emission (Fig. 1). We found that CL from the oxidation of pyrogallol with periodate was enhanced significantly by the presence of acetaldehyde as illustrated in Fig. 6.

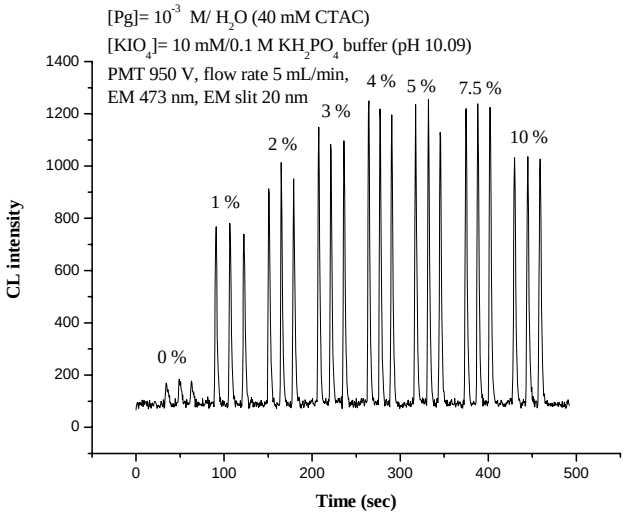

Fig. 6. FIA-CL emission for the oxidation of pyrogallol (1 mM) with KIO_4 (10 mM) and CTAC (40 mM) in the presence of. CH_3CHO (0-10%).

The presence of 4% CH_3CHO enhanced the CL emission by one order of magnitude.

The reason for the CL enhancement caused by CH_3CHO is still not clear and requires further investigation.

The CL emission from pyrogallol was found to be dependent on the concentrations of pyrogallol (0-1 mM), KIO_4 (0-10 mM), and CTAC (0-40 mM). The CL intensity of this system is much weaker than that of luminol. However, it is more easily affected than luminol by other substances, allowing the detection of those substances.

Detection of uric acid

The pyrogallol system can be used to determine the concentration of uric acid as shown in Fig. 7. Addition of uric acid (0-80 μM) caused a significant inhibition of the CL emission from pyrogallol, allowing its quantification.

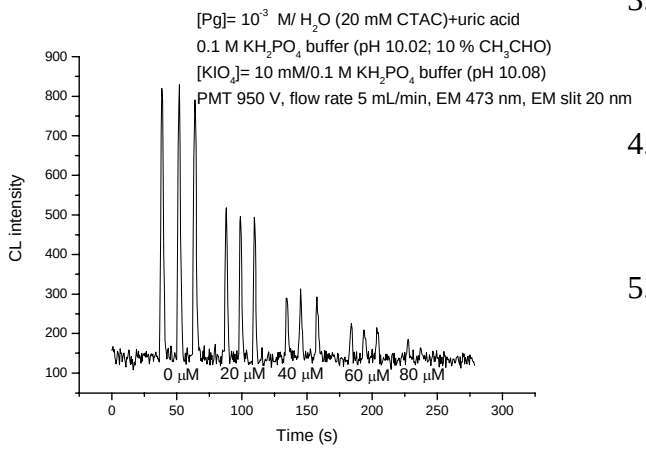

Fig. 7. FIA-CL emission for the oxidation of pyrogallol (1 mM) with KIO_4 (10 mM), CTAC (40 mM) and CH_3CHO (4%) in the presence of uric acid (0-80 μM).

Conclusion

We have found that CTAC is capable of enhancing the CL emission from the oxidation of luminol, pyrogallol, and lophine with appropriate oxidants. The enhancement in CL caused by surfactant was attributed to the attraction of the relevant CL reagents to the interior or interface of the micelle for efficient reaction. The concentrations of CL reagent, oxidant, and catalyst dramatically affect the CL emission. The enhanced CL systems were employed to determine iodide, dopamine, uric acid, and antioxidants.

References:

1. Yeh, H. –C.; Lin, W. –Y. *Anal. Chim. Acta*, **2001**, *442*, 71-77.
2. Yeh, H. –C.; Lin, W. –Y. *Anal. Bioanal. Chem.* **2002**, *372*, 525-531.
3. Yeh, H. –C.; Lin, W. –Y. *Talanta,* **2003,** *59*, 1029-1038.
4. Yeh, H. –C.; Lin, W. –Y. *J. Chin. Chem. Soc.* **2003**, *50*, 81-88.
5. Yeh, H. –C.; Hsu, W. –T.; Lin. W. –Y. *J. Chin. Chem. Soc.* **2005**, *52*, 657-664.