

Letters to the Editor

High-Sensitivity Determination of Emetine Dithiocarbamate Copper(II) Complex Using the Electrogenerated Chemiluminescence Detection of Tris(2,2'-bipyridine)ruthenium(II)

Kazuhiko TSUKAGOSHI[†], Kazuhide MIYAMOTO*, Eiji SAIKO*, Riichiro NAKAJIMA*, Tadashi HARA* and Kaoru FUJINAGA**

*Department of Chemical Engineering and Materials Sciences, Faculty of Engineering, Doshisha University, Tanabe, Kyoto 610-03, Japan

**Interdisciplinary Faculty of Science and Engineering, Department of Material Sciences, Shimane University, Nishikawatsu-cho 1060, Matsue 690, Japan

Keywords Electrogenerated chemiluminescence, tris(2,2'-bipyridine)ruthenium(II), dithiocarbamate complex, copper(II), emetine, flow injection analysis, capillary electrophoresis

Chemiluminescence (CL) has been shown to be a highly sensitive detection method in both flow injection analysis (FIA) and high-performance liquid chromatography (HPLC). Recently, several studies have utilized tris(2,2'-bipyridine)ruthenium(II) ion ($\text{Ru}(\text{bpy})_3^{2+}$) as a CL reagent, where $\text{Ru}(\text{bpy})_3^{3+}$, which is obtained electrochemically, oxidizes various organic amines. The oxidant, $\text{Ru}(\text{bpy})_3^{3+}$, generally reacts best with tertiary, next secondary, and primary alkyl amines.^{1,2} The CL reaction was, for example, utilized to determine antibiotic compounds, such as erythromycin and clindomycin, which both have a reactive tertiary amine.^{3,4} Uchikura *et al.*⁵ reported on a sensitive FIA for the determination of various alkaloid using the electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$.

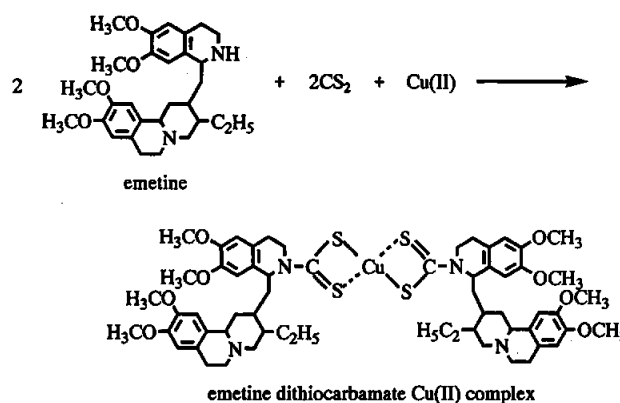
In this study, it was newly found that the emetine dithiocarbamate Cu(II) complex, which was prepared from emetine as an alkaloid, carbon disulfide, and Cu(II), indicated a large CL intensity on the electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$. When the Cu(II) complex was subjected to FIA equipped with electrogenerated CL detection, the complex could be determined over a wide range of 1×10^{-10} – 1×10^{-6} mol dm⁻³. Furthermore, capillary electrophoresis (CE) with electrogenerated CL detection was for the first time established, by which a mixture sample of emetine and emetine dithiocarbamate Cu(II) complex was successfully separated and determined with a nl order sample volume. The information provided here, though initial data, must be very interesting and useful for analyzing such transition elements as Cu(II).

Experimental

Reagents

All of the reagents used were of commercially available special grade. Ion-exchanged water was distilled before use. Tris(2,2'-bipyridine)ruthenium(II) chloride and emetine were purchased from Nacalai Tesque, Inc.

Emetine dithiocarbamate Cu(II) complex was prepared from emetine, carbon disulfide, and Cu(II) according to a previously reported procedure.⁶ The reaction scheme is shown in Scheme 1. After the Cu(II) complex had been extracted with chloroform, in order to proceed with a thorough purification, the complex was subjected to a silica-gel column using an eluent of ethyl acetate:methanol=1:1.



Scheme 1 Synthesis reaction of emetine dithiocarbamate Cu(II) complex.

[†] To whom correspondence should be addressed.

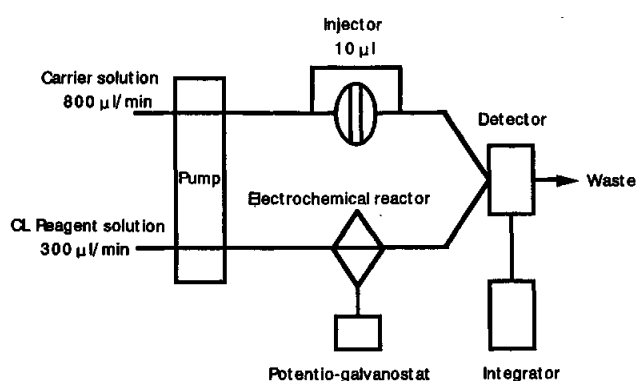


Fig. 1 Schematic diagram of the apparatus for FIA using the electrogenerated CL detection of $\text{Ru}(\text{bpy})_3^{2+}$.

Apparatus and procedures

A schematic diagram of an FIA⁷ equipped with an electrogenerated CL detector of $\text{Ru}(\text{bpy})_3^{2+}$ is shown in Fig. 1. Carrier (10 mol dm^{-3} phosphate buffer (pH 4.0) containing 35% acetonitrile) and CL reagent ($3 \times 10^{-4} \text{ mol dm}^{-3}$ $\text{Ru}(\text{bpy})_3^{2+}$ in $1.0 \times 10^{-2} \text{ mol dm}^{-3}$ H_2SO_4) solutions were fed by pumps at flow rates of 800 and $300 \mu\text{l min}^{-1}$, respectively. The $\text{Ru}(\text{bpy})_3^{2+}$ was oxidized to $\text{Ru}(\text{bpy})_3^{3+}$ at an electrolytic current of $150 \mu\text{A}$ at a carbon electrode in an electrochemical reactor. A sample solution of $10 \mu\text{l}$ was injected into the carrier solution.

An outline CE apparatus with an electrogenerated CL detector is also shown in Fig. 2. All of the tubes used were made of Teflon. A $500 \mu\text{m}$ i.d. Teflon tube, which was covered with black Tygon-tape and had an 11 mm detection length in front of a photomultiplier tube

(Hamamatsu Photonics Co., Ltd., R-464), was used as a detection cell. A fresh fused-silica capillary tube (50 mm i.d., 50 cm length) was treated with 1 mol dm^{-3} sodium hydroxide for 30 min and washed with distilled water. A migration buffer solution (25 mol dm^{-3} phosphate (pH 3.5) containing 35% acetonitrile) was treated through a filter and degassed before use. A capillary tube was filled with the buffer solution in advance. A $\text{Ru}(\text{bpy})_3^{2+}$ solution (1.2 mol dm^{-3} in 10 mol dm^{-3} H_2SO_4) was fed at a rate of 40 ml min^{-1} by a pump, which was oxidized using an electrolytic current of 100 mA in an electrochemical reactor. It was then mixed with the eluate at the tip of the capillary tube. A sample solution was introduced into a capillary tube having a positive-electrode side for 20 s from 15 cm height by siphoning. After introducing the sample solution, a voltage of 0–20 kV was gradually applied for 60 s. Monitoring was started just after the voltage reached 20 kV. The resulting CL at the tip of capillary was measured by a photon counter (Hamamatsu Photonics Co., Ltd., C1230).

Results and Discussion

Determination of an emetine dithiocarbamate $\text{Cu}(\text{II})$ complex with FIA

FIA with the $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL detection was made while referring to Uchikuras' report.⁷ First of all, the optimum electrolytic current was determined to be $150 \mu\text{A}$ by examining the noise level and the CL intensity of emetine as a sample. Under these conditions, emetine responded over a range of 1×10^{-12} – $1 \times 10^{-8} \text{ mol dm}^{-3}$.

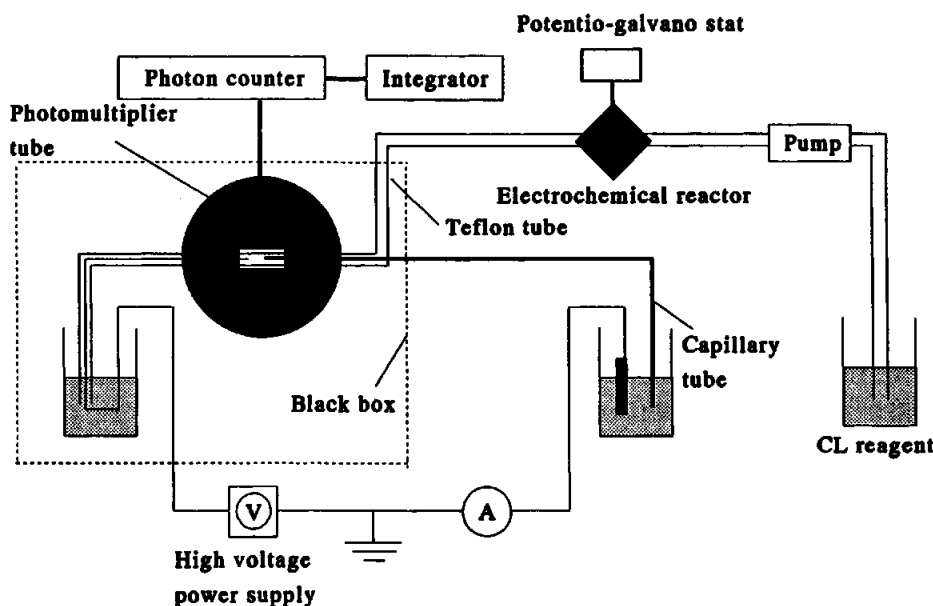


Fig. 2 Schematic diagram of the apparatus for CE using the electrogenerated CL detection of $\text{Ru}(\text{bpy})_3^{2+}$.

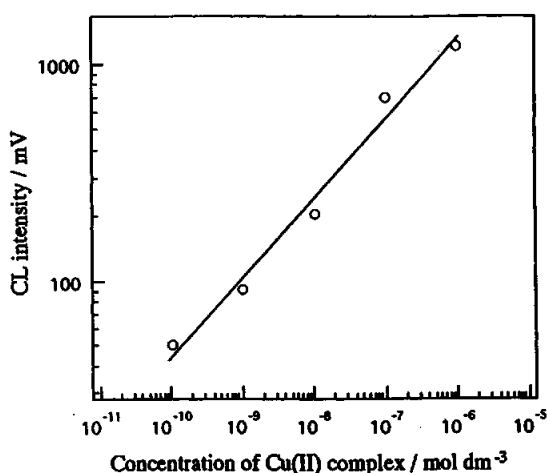


Fig. 3 Relationship between the concentration of emetine dithiocarbamate Cu(II) complex and the CL intensity by FIA using the electrogenerated CL detection of $\text{Ru}(\text{bpy})_3^{2+}$. Conditions: carrier solution, 10 mmol dm^{-3} phosphate buffer (pH 4.0) containing 35% acetonitrile; CL reagent, $3 \times 10^{-4} \text{ mol dm}^{-3}$ $\text{Ru}(\text{bpy})_3^{2+}$ in $1.0 \times 10^{-2} \text{ mol dm}^{-3}$ H_2SO_4 ; and electrolytic current, $150 \mu\text{A}$.

Transition metal ions such as Cu(II), Ni(II) and Zn(II), react with diethyldithiocarbamate to form a stable complex; for example, the stability constants of the diethyldithiocarbamate complexes of Cu(II), Ni(II), and Zn(II) were reported as $10^{28.8}$, $10^{12.9}$, and $10^{11.4}$, respectively.⁸ The diethyldithiocarbamate Cu(II) complex was then applied to the determination of a small amount of Cu(II) up to $10^{-8} \text{ mol dm}^{-3}$ order.⁹ It is also known that such transition metal ions react with emetine and carbon disulfide together to form stable dithiocarbamate complexes in a similar way.⁶ We examined the response of the emetine dithiocarbamate Cu(II) complex to $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL by using the FIA system. As shown in Fig. 3, the Cu(II) complex responded well over the range of 1×10^{-10} – $1 \times 10^{-6} \text{ mol dm}^{-3}$ with a detection limit of $1 \times 10^{-10} \text{ mol dm}^{-3}$ Cu(II) (65 fg) ($S/N=2$). In general, Cu(II) can be detected by atomic absorption spectrophotometry and inductivity coupled plasma with detection limits of 10^{-8} – $10^{-7} \text{ mol dm}^{-3}$ order. The obtained result supports the possibility that the combination of the complex formation of Cu(II) and its CL detection using electrogenerated CL could be applied to a sensitive detection method of Cu(II). In order to accomplish an on-line analysis for Cu(II), the connection of the FIA system with a separation column is now under investigation.

Development of a CE equipped with a $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL detector

We have reported on CE-CL detection methods using TCPO– H_2O_2 and luminol– H_2O_2 systems.^{10–12} Very small amounts of metal ions, proteins, dyestuffs, and so on were separated and determined by using these CE-CL detection methods. This time, we newly developed a

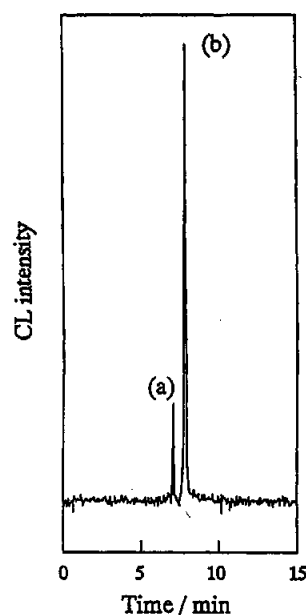


Fig. 4 Electropherogram of a mixed-sample solution of (a) emetine and (b) emetine dithiocarbamate Cu(II) complex obtained by CE using the electrogenerated CL detection of $\text{Ru}(\text{bpy})_3^{2+}$. Conditions: capillary, 50 cm of 50 μm i.d. fused silica; applied voltage, 20 kV; migration buffer, 25 mmol dm^{-3} phosphate (pH 3.5) containing 35% acetonitrile; CL reagent, 1.2 mmol dm^{-3} in 10 mmol dm^{-3} H_2SO_4 ; electrolytic current, $100 \mu\text{A}$; and sample, reactant of $5 \times 10^{-5} \text{ mol dm}^{-3}$ and $1.1 \times 10^{-4} \text{ mol dm}^{-3}$ emetine.

CE-CL detection method using $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL. When a mixed sample of emetine and emetine dithiocarbamate Cu(II) complex was subjected to the present CE-CL detection method, an electropherogram as shown in Fig. 4 was observed; they were successfully separated and detected. Emetine and the Cu(II) complex were determined over a range of 3×10^{-8} – $1 \times 10^{-5} \text{ mol dm}^{-3}$ and 3×10^{-7} – $5 \times 10^{-5} \text{ mol dm}^{-3}$, respectively. Although the detection limits might not be satisfied when the result obtained by FIA was taken into consideration, the analysis could be easily and rapidly carried out with a very small injection volume, on the nl order. The main cause of the low sensitivity would be due to dilution of the sample at the tip of the end capillary by being mixed with a CL reagent. An improvement of the sensitivity is now being undertaken by modifying the detection-cell geometry, flow rate of the CL reagent, and so on.

In conclusion, emetine dithiocarbamate Cu(II) complex was found to indicate a sensitive response on a $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL system. The Cu(II) complex was analyzed using FIA and CE with electrogenerated CL detections. A combination of the dithiocarbamate complex formation of transition metal ions and their CL response to $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL is expected to be useful for analyzing transition metal

ions. Recently, various analytical methods using $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL have been reported.^{3,4,13,14} The CE-CL detection method with the $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL detector, which was for the first time developed by the present authors, must become a promising way for the separation and determination of various substances which are related to the electrogenerated CL reaction.

The authors are grateful to Professor Sorin Kihara at Kyoto Institute of Technology for the gift of a carbon electrode.

References

1. J. B. Noffsinger and N. D. Danielson, *Anal. Chem.*, **59**, 865 (1987).
2. J. B. Noffsinger and N. D. Danielson, *J. Chromatogr.*, **387**, 520 (1987).
3. N. D. Danielson, L. He, J. B. Noffsinger and L. Trelly, *J. Pharm. Biomed. Anal.*, **7**, 1281 (1989).
4. M. A. Targove and N. D. Danielson, *Anal. Chim. Acta*, **277**, 55 (1993).
5. K. Uchikura and M. Kirisawa, Abstract of the 111th Annual Meeting of Pharmacia Society of Japan, 1991, No. 28LC.
6. F. Feigel, "*Spot Test in Organic Analysis*", p. 462, Elsevier Publishing Company and Maruzen Comany Ltd., 1961.
7. A. Momose, K. Uchikura and Y. Kabasawa, *Bunseki Kagaku*, **32**, 142 (1983).
8. K. Ueno, T. Imamura and K. L. Cheng, "*Handbook of Organic Analytical Reagents*", 2nd ed., p. 457, CRC Press, Boca Raton, 1992.
9. H. Onishi and I. Tsukahara, "*Kyukobunko-ho (Spectrophotometric Analysis, in Japanese)*", p. 89, Kyoritsusyuppan, Tokyo, 1983.
10. K. Tsukagoshi, A. Tanaka, R. Nakajima and T. Hara, *Anal. Sci.*, **12**, 525 (1996).
11. K. Tsukagoshi, H. Akasaka, R. Nakajima and T. Hara, *Chem. Lett.*, **1996**, 467.
12. K. Tsukagoshi, S. Fujimura and R. Nakajima, *Anal. Sci.*, **13**, 279 (1997).
13. D. R. Deaver, *Nature [London]*, **377**, 758 (1995).
14. M. Maeda, *Bunseki*, **1996**, 452.

(Received April 28, 1997)
(Accepted June 19, 1997)