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Sensitive determination of metal ions by liquid chromatography with tris(2,2'-bipyridine) ruthenium (II) complex electrogenerated chemiluminescence detection

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Abstract

Emetine dithiocarbamate metal complex, which is prepared from emetine, carbon disulfide, and metal (II), was found to indicate a large chemiluminescence intensity on the electrogenerated chemiluminescence of tris(2,2'-bipyridine)ruthenium(II). Liquid chromatography equipped with the chemiluminescence detection was developed for analyzing trace metal ions by use of the metal complex formation. The mixture of the Cu(II) and Co(II) complexes as a model sample was injected into the LC system. The two metal complexes and an excess emetine were successfully separated. The Cu(II) and Co(II) complexes were determined over the range 1–300 nM (the detection limit of 650 fg) and 30–5000 nM (the detection limit of 17 pg), respectively. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Recently, several studies have utilized tris(2,2'-bipyridine) ruthenium(II) ion ($\text{Ru}(\text{bpy})_3^{2+}$) as a chemiluminescence (CL) reagent. The oxidant ($\text{Ru}(\text{bpy})_3^{3+}$), which is obtained electrochemically, oxidizes various organic amines. In general, $\text{Ru}(\text{bpy})_3^{3+}$ reacts best with tertiary, next secondary, and primary alkyl amines [1,2]. The electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$ was used to determine antibiotic

compounds like erythromycin and clindomycin [3,4] as well as emetine as an alkaloid [5], which possess a reactive tertiary amine. Separation and detection of antihistamines, amino acids, and glyphosate compounds was also performed by liquid chromatography (LC) with the CL detection [6–8]. However, the electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$ has not been applied to the determination of metal ions.

Transition metal ions such as Cu(II), Ni(II), Zn(II), etc. react with diethyldithiocarbamate to form a stable complex. The diethyldithiocarbamate Cu(II) complex was, then, applied to determination of a small amount of Cu(II) up to 10 nM order by means of spectrophotometric method [9]. It is also known that such transition metal ions react with emetine as

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an alkaloid and carbon disulfide together to form stable dithiocarbamate complexes in a similar way [10]. It has been newly found by us that an emetine dithiocarbamate metal complex indicated a large CL intensity on electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$ [11]. 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 the analysis of metal ions.

In this study, on the basis of the above concept we proposed to develop LC with the CL detection of $\text{Ru}(\text{bpy})_3^{2+}$ for the sensitive analysis of metal ions. Detail examinations were made for the preparation of the dithiocarbamate metal complexes and the analytical condition of flow-injection analysis (FIA) system. The connection of the FIA system with a separation column, that is, the LC system was established in order to accomplish an on-line analysis. Cu(II) can be detected by atomic absorption spectrophotometry and inductively coupled plasma with the detection limits of 10 nM order, without any pretreatments such as preconcentration and column procedure [12,13]. The present LC for Cu(II) detection was found to be about 10 times more sensitive than the conventional detection methods. Luminol reagent has been applied to the determination of transition metal ions. Yan et al. detected Cu(II) with a detection limit of 1.25 nM by LC with luminol CL detection [14]. Recently, the CL flow-through sensor using luminol was reported for the trace detection of Cu(II); the sensor could detect up to about 2 nM Cu(II), but the method required electrochemically preconcentration [15]. The present method on the basis of the dithiocarbamate metal complex formation and their electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$ is clarified to become an effective analysis for trace amounts of metal ions.

2. Experimental

2.1. Reagents

All of the reagents used were of commercially available special grade. Ion-exchanged water was distilled for use. Tris(2,2'-bipyridine) ruthenium(II)

chloride and emetine were purchased from Nacalai Tesque, Inc.

2.2. Synthesis of emetine dithiocarbamate metal complexes and sample preparation

Emetine dithiocarbamate metal (Cu(II), Ni(II), and Co(II)) complexes were prepared according to the procedure reported previously [16]. The reaction scheme is shown in Fig. 1. Emetine and metal salt were dissolved in 100 ml citric acid at 20 and 10 mM, respectively. The pH of the solution was adjusted to be 10 with a small amount of 28% ammonia solution. After the addition of 0.5 ml of carbon disulfide, the solution was stirred for 3 h. The metal complexes produced thus in the aqueous solution were extracted with 100 ml of chloroform. In order to proceed with a thorough purification, the complexes were subjected to silica gel column using an eluent of ethyl acetate:methanol=1:1. The eluant was evaporated and the residue was dried. The solid compound was redissolved in the same solution as a carrier solution of FIA and LC to give desirable concentrations. Measurement for sample solutions prepared by the above procedure is called 'method A' in this study.

On the other hand, another procedure of sample preparation was performed as follows in order to explore the possibility of application for a practical sample. In this method 99 ml of metal ion solution at various low concentrations (≤ 5000 nM), 0.1 ml of 1 mM emetine, 0.5 ml of carbon disulfide, and 0.1 ml of 25 mM citric acid were added to 100 ml messflask in this order. The pH of the solution was adjusted to be 10 with 28% ammonia solution. Subsequently, 25

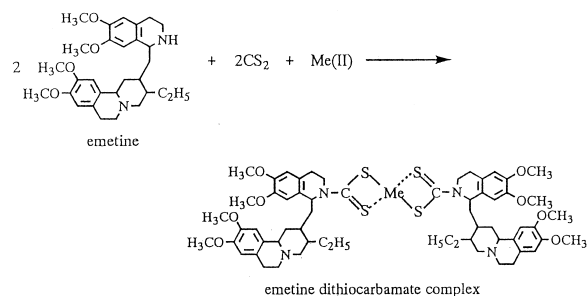


Fig. 1. Synthesis of emetine dithiocarbamate metal(II) complexes.

mM citric acid–ammonia solution (pH 10) was added up to 100 ml. After being stirred for 3 h, the solution as a sample was directly subjected to LC without any pretreatments such as extraction and column separation. It is called here ‘method B’.

2.3. Apparatus and procedures

A schematic diagram for LC with electrogenerated CL detector of $\text{Ru}(\text{bpy})_3^{2+}$ and electrochemical reactor is shown in Fig. 2. ODS column (L-column, Chemical Inspection and Testing Institute, Tokyo, Japan) was used in the LC. The FIA was performed by removing the column from the LC system. Carrier (100 mM phosphate buffer of pH 4.0 containing a definite amount of acetonitrile) and CL reagent ($0.3 \text{ mM Ru}(\text{bpy})_3^{2+}$ in $10 \text{ mM H}_2\text{SO}_4$) solutions were fed by pumps at flow-rates of 800 and 300 ml min^{-1} , respectively. The $\text{Ru}(\text{bpy})_3^{2+}$ was oxidized to $\text{Ru}(\text{bpy})_3^{3+}$ at electrolytic current 150 mA by carbon electrode in electrochemical reactor which was made referring to Uchikura’s report [5]. Sample solution of $10 \mu\text{l}$ was injected into the eluent or carrier solution. CL generated by mixing the carrier containing sample and $\text{Ru}(\text{bpy})_3^{3+}$ was measured by means of CL detector (ICA-3070, TOA electronics Ltd., Tokyo, Japan)

3. Results and discussion

3.1. Analysis of emetine dithiocarbamate $\text{Cu}(\text{II})$ complex with FIA and LC (method A)

Previously, three pieces of glass filter were used to pack glassy carbon in an electrochemical reactor [11]. However, it is laborious and time-consuming to cut the filters and fix them to the reactor. Furthermore, glassy carbon is easily loaded into porous small holes of the filter, so that it is difficult to keep a constant flow-rate of CL reagent. In this study, Polyflon filter which is made from pure polytetrafluoroethylene was selected after testing various materials. The filter provided an improvement in stability and repeatability of CL measurement.

The preliminary experiments for the FIA system were carried out with emetine sample as described below. The electrolytic current is one of the most important parameters in the electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$. The effect of the electrolytic current on the peak height and signal-to-noise ratio of emetine was examined for the FIA system. Fig. 3 shows that both increased with increasing current up to $150 \mu\text{A}$, while they gradually decreased above the current; $150 \mu\text{A}$ was chosen as an optimum value. 100 mM phosphate buffer–acetonitrile was used as a carrier

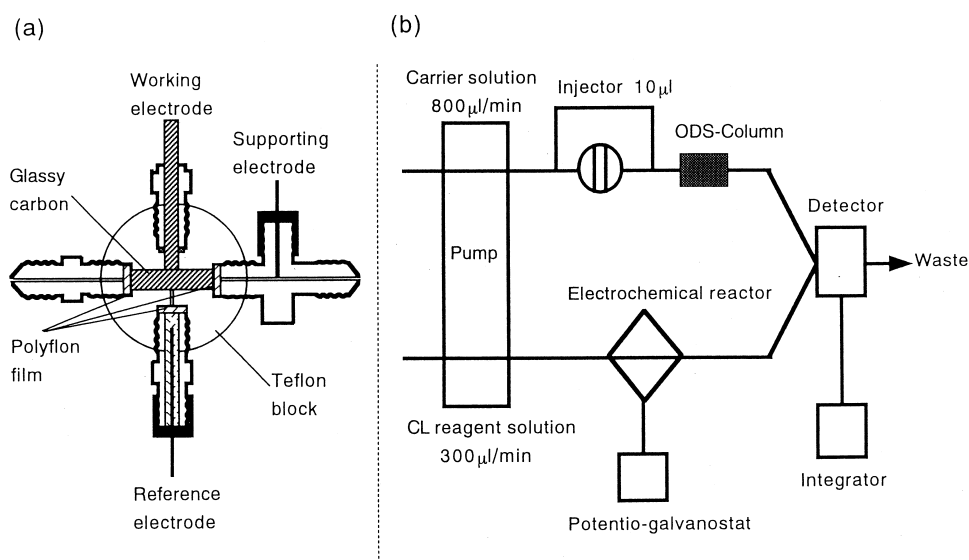


Fig. 2. Schematic diagrams of (a) electrochemical reactor and (b) apparatus for LC with the electrogenerated CL detection of $\text{Ru}(\text{bpy})_3^{2+}$.

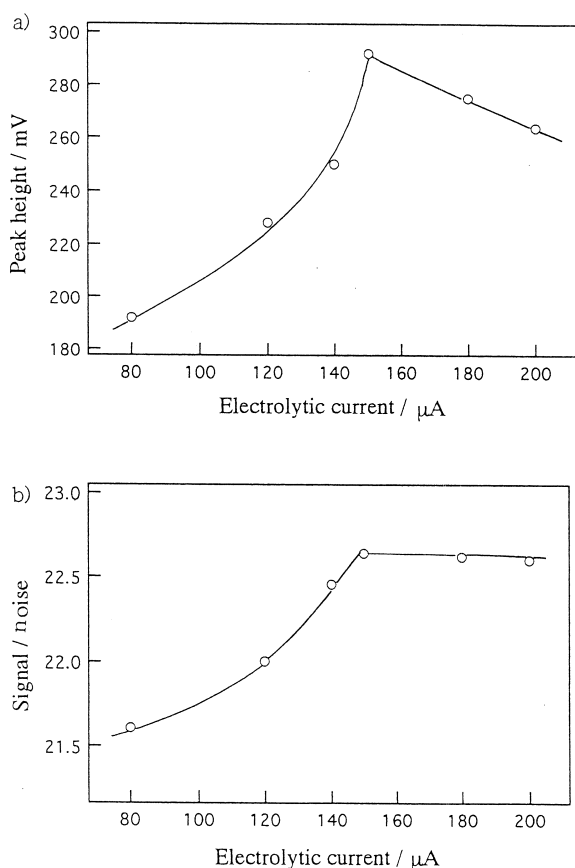


Fig. 3. Relationships between electrolytic current and peak height as well as between electrolytic current and signal-to-noise ratio. Conditions: CL reagent, 0.3 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM H_2SO_4 ; carrier solution, 100 mM phosphate buffer (pH 4)–acetonitrile (65:35, v/v); electrolytic current, 150 mA; and sample, 10 nM emetine.

solution in FIA. The peak height of emetine was found to increase up to pH 4 (Fig. 4). This pH value was used in all further experiments. Acetonitrile is often used as a solvent in the electrogenerated CL of $\text{Ru}(\text{bpy})_3^{2+}$ [17]. CL peak height of emetine was examined for carrier solutions which were the phosphate buffers of acetonitrile 30–80%. The amount of acetonitrile did not significantly influence the peak height over the range under the present conditions. However, poor repeatability and low CL intensity were observed if more than 80% of acetonitrile was used, probably due to the precipitation of phosphate salt.

The calibration curve of emetine was examined

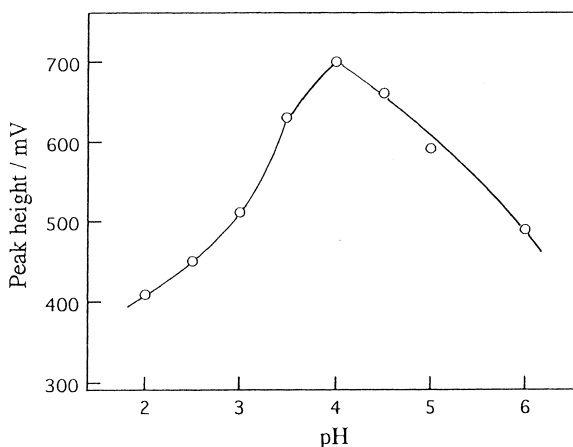


Fig. 4. Relationship between pH of phosphate buffer and peak height. Conditions: CL reagent, 30 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM H_2SO_4 ; carrier solution, 100 mM phosphate buffer–acetonitrile (65:35, v/v); electrolytic current, 150 mA; and sample, 10 nM emetine.

under the conditions recommended above. It responded over a range of 0.001–10 nM with the detection limit of 10 amol. The results supported that the analytical conditions and operating procedures for the FIA were appropriate for a sensitive determination of emetine and its analogs.

Transition metal ions react with diethyldithiocarbamate to form stable complexes [10]. The large stability constants are essential for a sensitive detection of metal ions. The diethyldithiocarbamate Cu(II) complex formation was, then, applied to the determination of a small amount of Cu(II) up to 10 nM order [9]. It is also known that such transition metal ions react with emetine and carbon disulfide together to form stable dithiocarbamate complexes [10]. We examined the response of an emetine dithiocarbamate Cu(II) complex to $\text{Ru}(\text{bpy})_3^{2+}$ electrogenerated CL by use of the HA system.

First, the conditions of the extraction and column procedure for purification were investigated. The pH value of an aqueous phase gives a large influence to extraction of dithiocarbamate metal complexes. Diethyldithiocarbamate Cu(II) and Ni(II) complexes are effectively extracted with chloroform at the pH range 4–11 [10]. However, as far as we know, a favorable pH for emetine dithiocarbamate metal complexes has not been examined in detail. Absorbance (440 nm) of emetine dithiocarbamate

Cu(II) complex in chloroform after the extraction was measured using the pH values (7–11) of aqueous phase. The pH over 10 was found to be appropriate to the extraction of the metal complex. The solvent of chloroform after the extraction must be subjected to the silica-gel column in order to separate the emetine dithiocarbamate metal complex from an excess emetine. Various solvents were preliminarily examined for the column separation by use of thin-layer chromatography (TLC). The mixture of methanol and ethylacetate (1:1) was adopted as an eluent for the separation.

Diethyldithiocarbamate metal complexes are known to be stable in an alkaline, but not in an acidic solution [10]. Since there is little information on the stability of emetine dithiocarbamate metal complexes, the stability of emetine dithiocarbamate Cu(II) was examined in phosphate buffer (pH 4)–acetonitrile (50:50, v/v). Fig. 5 shows that the peak height was constant for 3 h, after which it decreased gradually. Consequently, solutions of emetine dithiocarbamate metal complexes should be analyzed in 2–3 h after preparation.

The FIA system could determine the Cu(II) complex over the range 0.1–1000 nM with the detection

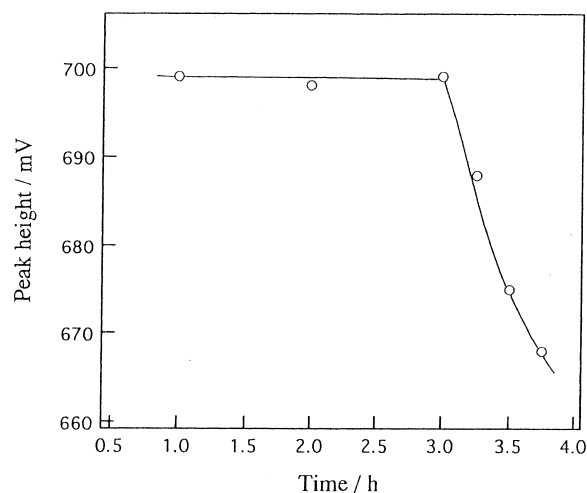


Fig. 5. Stability of emetine dithiocarbamate Cu(II) complex in the phosphate buffer (pH 4.0)–acetonitrile (50:50, v/v). Conditions: CL reagent, 30 mM Ru(bpy)₃²⁺ in 10 mM H₂SO₄; carrier solution, 100 mM phosphate buffer (pH 4.0)–acetonitrile (50:50, v/v); electrolytic current, 150 mA; and sample, 6 nM emetine dithiocarbamate Cu(II) complex.

limit of 0.1 nM Cu(II) (65 fg) ($S/N=2$). In general, Cu(II) can be detected by atomic absorption spectrophotometry and inductively coupled plasma with the detection limits of 10 nM order. The obtained result supports the possibility that the combination of the complex formation of Cu(II) and its CL detection using the electrogenerated CL can be applied to a sensitive detection method of Cu(II).

Next, we investigated the performance of LC with CL detection. Satisfactory separation of emetine and the emetine dithiocarbamate Cu(II) complex was found to require 50–60% acetonitrile. The emetine dithiocarbamate Cu(II) complex solution was diluted to suitable concentrations and analyzed with phosphate buffer–acetonitrile (40:60, v/v) as the eluent. The Cu(II) complex was determined over the range 0.1–1000 nM with the detection limit 65 fg ($S/N=2$). The determinable range and detection limit were similar to those obtained by the FIA. This result means that the sample dilution through the column and the effect of the eluent on CL must be negligible under the present conditions.

3.2. Analysis of emetine dithiocarbamate metal complexes with LC (method B)

In the above section of FIA and LC (method A), emetine dithiocarbamate Cu(II) complex solutions were prepared using a high concentration (10 mM) of Cu(II) and then diluted as needed. Here, the Cu(II) complex solutions were prepared from various low concentrations (≤ 5000 nM) of Cu(II) and then subjected to the LC system without any purifications (method B). The emetine and the Cu(II) complex indicated a base-line separation at 30 and 40% acetonitrile eluent.

The calibration curve of emetine dithiocarbamate Cu(II) complex was examined under the conditions of the phosphate buffer–acetonitrile (70:30, v/v) as an eluent. The plots of CL intensity vs. the Cu(II) concentration (1–300 nM) on log–log graph gave a good linearity (correlation coefficient, 0.999). The detection limit (650 fg ($S/N=2$)) was about 10 times as high as that obtained in the above section. The lowering of the sensitivity might be due to the poor complex formation at low Cu(II) concentration.

The mixture of emetine dithiocarbamate Cu(II) and Co(II) complexes which were 5 and 100 nM,

respectively, was subjected to LC. The chromatogram of Fig. 6 indicated good separation for emetine and the two metal complexes. The linear responding range of Co(II) was 30–5000 nM with a detection limit of 17 pg ($S/N=2$). The lower sensitivity compared to that for Cu(II) complex is probably due to the lower stability constant between Co(II) and emetine dithiocarbamate ligand. Tentatively, the mixture of emetine dithiocarbamate Cu(II), Ni(II) and Co(II) complexes was applied to LC (the data

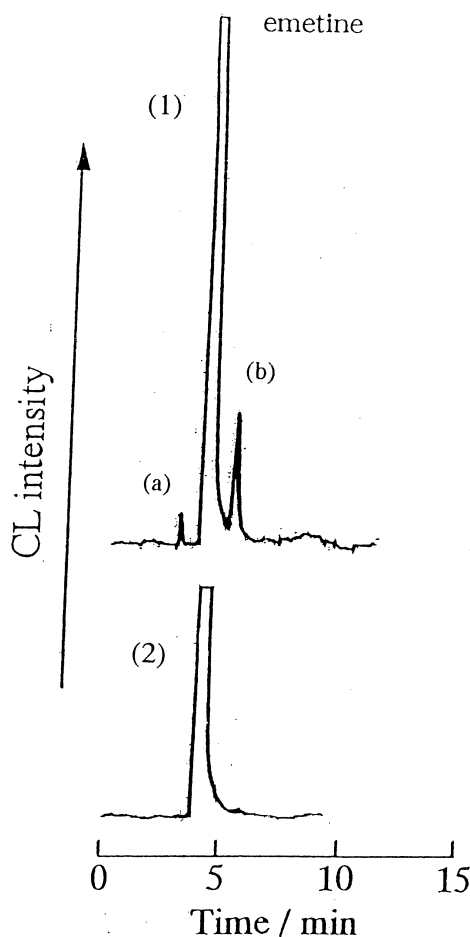


Fig. 6. (1) Chromatogram for mixture of emetine and emetine dithiocarbamate (a) Cu(II) and (b) Co(II) complexes. (2) Chromatogram for emetine solution not including metal ions (blank chromatogram). Conditions: CL reagent, 30 mM Ru(bpy)₃²⁺ in 10 mM H₂SO₄; eluent, 100 mM phosphate buffer (pH 4.0)–acetonitrile (70:30, v/v); electrolytic current, 150 mA; separation, ODS column; and sample, (a) Cu(II) complex 5 nM and (b) Co(II) complex 100 nM.

not shown). The peak of Ni(II) could be clearly distinguished from the peaks of Cu(II) and Co(II), but appeared overlapping the peak of emetine.

4. Conclusion

Emetine dithiocarbamate metal complexes were found to indicate sensitive responses on Ru(bpy)₃²⁺ electrogenerated CL system. The complexes were analyzed by use of FIA and LC with the electrogenerated CL detection. The Cu(II) and Co(II) complexes were determined over the range 1–300 nM (detection limit of 650 fg) and 30–5000 nM (detection limit of 17 pg), respectively. The present LC was about 10 times more sensitive than the conventional detection methods.

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