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Separation and determination of emetine dithiocarbamate metal complexes by capillary electrophoresis with chemiluminescence detection of the tris(2,2'-bipyridine)–ruthenium(II) complex

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Abstract

Emetine dithiocarbamate metal complexes, which were prepared from emetine, carbon disulfide, and metal(II), indicated large chemiluminescence intensities on tris(2,2'-bipyridine)–ruthenium(II) chemiluminescence. Capillary electrophoresis with chemiluminescence detection was developed for analyzing emetine and the metal complexes. After the optimization of various analytical conditions, the mixture of the Cu(II), Ni(II), and Co(II) complexes as a model sample was injected into the capillary electrophoresis system with chemiluminescence detection. They were successfully separated and detected with a detection limit of 50 nM. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Tris(2,2'-bipyridine)–ruthenium(II) ion $[\text{Ru}(\text{bpy})_3^{2+}]$ as a chemiluminescence (CL) reagent has received much attention in the field of analytical chemistry. The oxidant $[\text{Ru}(\text{bpy})_3^{3+}]$, which is obtained chemically or electrochemically, oxidizes various organic amines. $\text{Ru}(\text{bpy})_3^{3+}$ reacts best with tertiary, then secondary, and primary alkyl amines [1,2]. The CL of $\text{Ru}(\text{bpy})_3^{2+}$ was used to determine

antibiotic compounds like erythromycin and clindamycin [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 were also performed by liquid chromatography with CL detection [6–8].

On the other hand, transition metal ions such as Cu(II), Ni(II), Zn(II), etc., react with diethyldithiocarbamate to form stable complexes. The diethyldithiocarbamate Cu(II) complex was, then, applied to the determination of a small amount of Cu(II) down to the 10 nM order by means of a spectrophotometric method [9]. It is also known that such transition metal ions react with emetine as an alkaloid and carbon disulfide together to form stable dithiocarbamate complexes in a similar way [10].

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Uchikura reported that the CL of the $\text{Ru}(\text{bpy})_3^{2+}$ system could detect a 10^{-12} M level of emetine [5]. We have studied the possibility to develop a highly sensitive detection method for metal ions taking advantage of the complex formation of emetine and metal ions, and found that the emetine dithiocarbamate Cu(II) complex indicated large CL intensity on the CL of $\text{Ru}(\text{bpy})_3^{2+}$; $\text{Ru}(\text{bpy})_3^{3+} + \text{emetine-metal ion complex} \rightarrow [\text{Ru}(\text{bpy})_3^{2+}]^* \rightarrow \text{Ru}(\text{bpy})_3^{2+} + h\nu$ [11]. A combination of the dithiocarbamate complex formation of transition metal ions and their CL response to $\text{Ru}(\text{bpy})_3^{2+}$ must be useful for the analysis of metal ions.

Capillary electrophoresis (CE) with CL detection has been focused on since the early 1990s [12,13]. Several groups have reported CE equipped with CL detection of $\text{Ru}(\text{bpy})_3^{2+}$ [11,14]. In this study, CE with CL detection was developed for the analysis of metal ions, which took advantage of dithiocarbamate complex formation. We briefly described the preliminary results of this investigation in a previous communication [11]. The mixture of emetine dithiocarbamate metal complexes were separated and detected with detection limits in the nanomolar-order levels. The present CE with CL detection method must become a promising means of separation and determination of small 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.

A $\text{Ru}(\text{bpy})_3^{3+}$ solution was prepared as follows; a $\text{Ru}(\text{bpy})_3^{2+}$ solution (0.3 mM) was prepared by dissolving tris(2,2'-bipyridine)-ruthenium(II) chloride with 10 mM H_2SO_4 solution. Lead(IV) oxide was added to the solution and the solution was stirred for 10 min. In order to give a homogeneous $\text{Ru}(\text{bpy})_3^{3+}$ solution which does not influence the CL performance, PbO_2 particles were removed from the solution by filtering.

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 [15]. The reaction scheme is shown in Fig. 1. A citric acid solution (99 ml of 25 mM), an emetine solution (0.1 ml of 100 mM), a metal ion [Cu(II), Ni(II), or Co(II)] solution (0.1 ml of 10 mM), and carbon disulfide (0.3 ml) were added to a 100-ml volumetric flask in this order. The pH of the solution was adjusted to 10 with 28% ammonia solution. A citric acid-ammonia buffer solution (25 mM, pH 10) was subsequently added up to 100 ml. 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 eluent was evaporated and the residue was dried. The solid compound was redissolved in the same solution as a migration buffer of CE, giving desirable concentrations. It is called here "method A".

On the other hand, another sample preparation procedure was performed as follows in order to explore the possibility of application for a practical sample. A metal ion solution (99 ml) at various low concentrations (≤ 5000 nM), an emetine solution (0.1 ml of 1 mM), carbon disulfide (0.5 ml), and a citric acid solution (0.1 ml of 25 mM) were added to a 100-ml volumetric flask in this order. The pH of the solution was adjusted to be 10 with 28% ammonia solution. A citric acid-ammonia solution (25 mM, pH 10) was subsequently added up to 100 ml. After stirring for 3 h, the solution as a sample

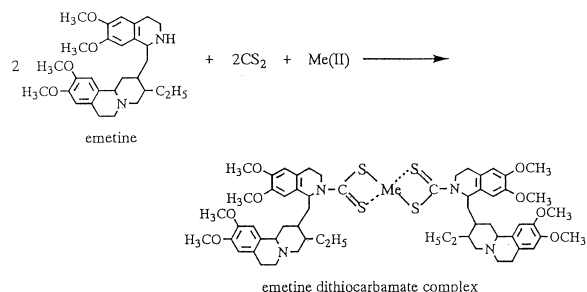


Fig. 1. Synthesis of emetine dithiocarbamate metal(II) complexes.

was directly subjected to CE without any pretreatments such as extraction and column separation. It is called here “method B”.

The data in Sections 3.1–3.3 were obtained by method A and the data in Section 3.4 were obtained by method B.

2.3. Apparatus and analytical procedure

An outline of the CE apparatus with CL detection and an enlargement figure of the CL detection are shown in Fig. 2.

A fused-silica capillary (70 cm × 75 μm I.D. × 150 μm O.D.) was inserted into a 0.5 mm I.D. PTFE tube. As shown in Fig. 2B, the PTFE tube was put in just front of a photomultiplier tube (Hamamatsu, R-464) as a detection cell.

A fresh capillary was treated with 1 M NaOH for 30 min and washed with distilled water. A migration buffer solution (100 mM phosphate buffer containing

acetonitrile) was treated through a filter and degassed for use. The capillary was filled with the buffer solution in advance. An Ru(bpy)₃³⁺ solution (0.3 mM in 10 mM H₂SO₄) was fed by siphoning from a 15 cm height (80 μl min⁻¹). Then, it was mixed with the eluate at the tip of the capillary. A sample solution (ca. 60 nl) was introduced into the capillary for 15 s from 35 cm height by siphoning. After introducing the sample solution, a voltage of 0–15 kV was gradually applied for 60 s. Monitoring started just after the voltage reached 15 kV. The resulting CL at the tip of capillary was detected by the photomultiplier tube connected to a photon counter (Hamamatsu C1230).

3. Results and discussion

3.1. Preliminary experiments using an emetine sample

The Ru(bpy)₃³⁺ solution (green) was prepared by the oxidation of the Ru(bpy)₃²⁺ solution (bitter orange) as described in the Experimental section. However, the color of Ru(bpy)₃³⁺ solution gradually changed from green to yellow green after ca. 2 h in the preparation. The reduction would proceed in the Ru(bpy)₃³⁺ solution. We also examined the relationship between the standing time of Ru(bpy)₃³⁺ solution and the CL intensity of emetine. The CL intensity indicated constant values up to ca. 110 min, whereas the intensity gradually decreased after that. The Ru(bpy)₃³⁺ solution was used for the experiments within 110 min after the oxidation.

It was reported that acetonitrile acts as a sensitizer to the CL of Ru(bpy)₃²⁺ [16] so that 100 mM phosphate buffer containing acetonitrile was used as a migration buffer in the present CE system. The CL intensity of emetine was examined for the migration buffers containing acetonitrile (5–50%, v/v) (Fig. 3). In more than 60% (v/v) acetonitrile, poor reproducibility and low CL intensity were observed, probably due to the precipitation of phosphate salt. As shown in Fig. 3, the CL intensity increased up to 30%, but above that the intensity did not change. Consequently, the phosphate buffer containing ace-

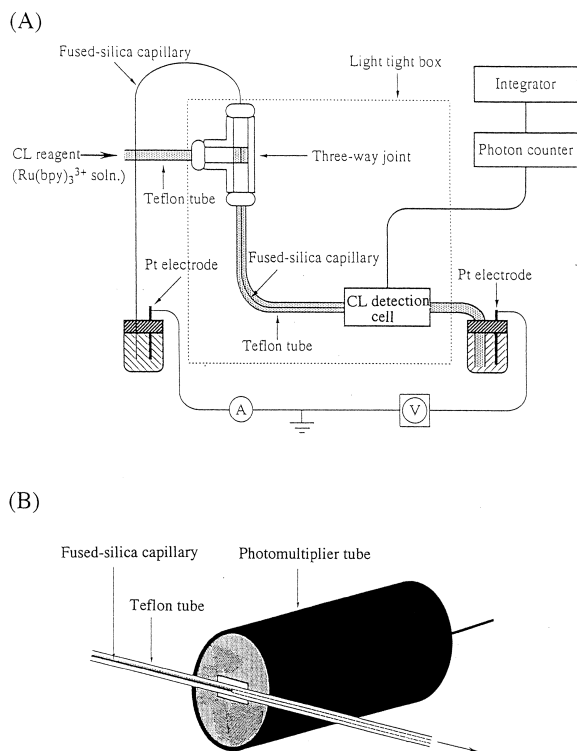


Fig. 2. Schematic diagrams of (A) apparatus for CE with CL detection of Ru(bpy)₃²⁺ and (B) enlargement figure of the CL detection cell.

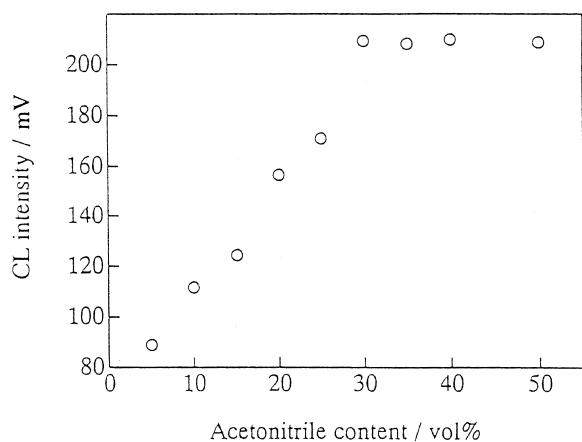


Fig. 3. Relationship between volume percentage of acetonitrile and CL intensity of emetine. Conditions: CL reagent, 0.3 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM; migration buffer, 100 mM phosphate buffer (pH 4)–acetonitrile; and sample, 0.1 mM emetine.

tonitrile (30–50%, v/v) was recommended for this study.

The $\text{Ru}(\text{bpy})_3^{3+}$ solution was delivered by siphoning as described above. The flow-rate of the solution was regulated with the height of the vessel of $\text{Ru}(\text{bpy})_3^{3+}$ solution. The heights of 5, 10, 15, and 20 cm corresponded to the flow-rates of 40, 55, 80, and 100 $\mu\text{l min}^{-1}$, respectively. The effect of the flow-rate of $\text{Ru}(\text{bpy})_3^{3+}$ solution on the CL intensity and half-peak width was examined (Fig. 4). The CL

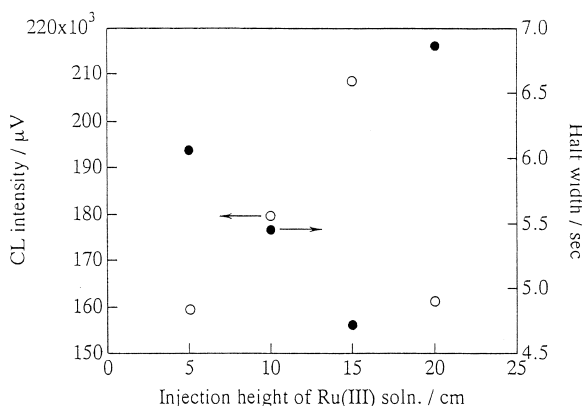


Fig. 4. Relationships between an injection height of $\text{Ru}(\text{bpy})_3^{3+}$ solution and CL intensity as well as a half-peak width. Conditions: CL reagent, 0.3 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM H_2SO_4 ; migration buffer, 100 mM phosphate buffer (pH 4)–acetonitrile (65:35, v/v); and sample, 0.1 mM emetine.

intensity became maximum and the width became minimum around 15 cm height or 80 $\mu\text{l min}^{-1}$. The height of 15 cm was used for the experiments.

The calibration curve of emetine was examined under the conditions recommended above. It responded over a range of 0.1 nM–0.5 mM with a detection limit of 8 amol ($S/N=3$). The results supported that the analytical conditions and operating procedures for CE were appropriate for a sensitive determination of emetine and its analogs.

3.2. Detection of emetine dithiocarbamate metal complexes

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

First, the stability of the CL intensity of emetine dithiocarbamate Cu(II) complex was examined in phosphate buffer (pH 4)–acetonitrile (50:50, v/v). The CL intensity was constant for 160 min, after which it decreased gradually; the reason has not been well known. Consequently, solutions of the metal complexes should be measured within 160 min after preparation.

The mixture of emetine and emetine dithiocarbamate metal complex [Cu(II), Ni(II), or Co(II)] was analyzed by the present system. Each metal complex was successfully separated from emetine. The peak of emetine which has smaller molecular mass than the complex appeared earlier than that of the complex; the migration times of emetine and the metal complex are 9.4 and 10.3 s, respectively.

The plots of CL intensity vs. the metal concentration on log–log graph gave a good linearity

(correlation coefficient, 0.998–0.999). The Cu(II), Ni(II), and Co(II) complexes were determined over a range of 3–10 000 nM (detection limit of 190 amol; $S/N=3$), 20–100 000 nM (detection limit of 1.3 fmol; $S/N=3$), and 50–100 000 nM (detection limit of 3.2 fmol; $S/N=3$), respectively. Their sensitivities seem to be equal to or higher than those reported by CE with absorption detection [17–19]. However, as these metal complexes have very similar characteristics regarding mass and charge, all of them indicated

the same migration time. The complexes could not be separated from each other under the conditions.

3.3. Separation of emetine dithiocarbamate metal complexes

It was difficult to separate the Cu(II), Ni(II), and Co(II) complexes by CE as described above. However, there are some differences between these metal ions or their complexes in an ionic radius and

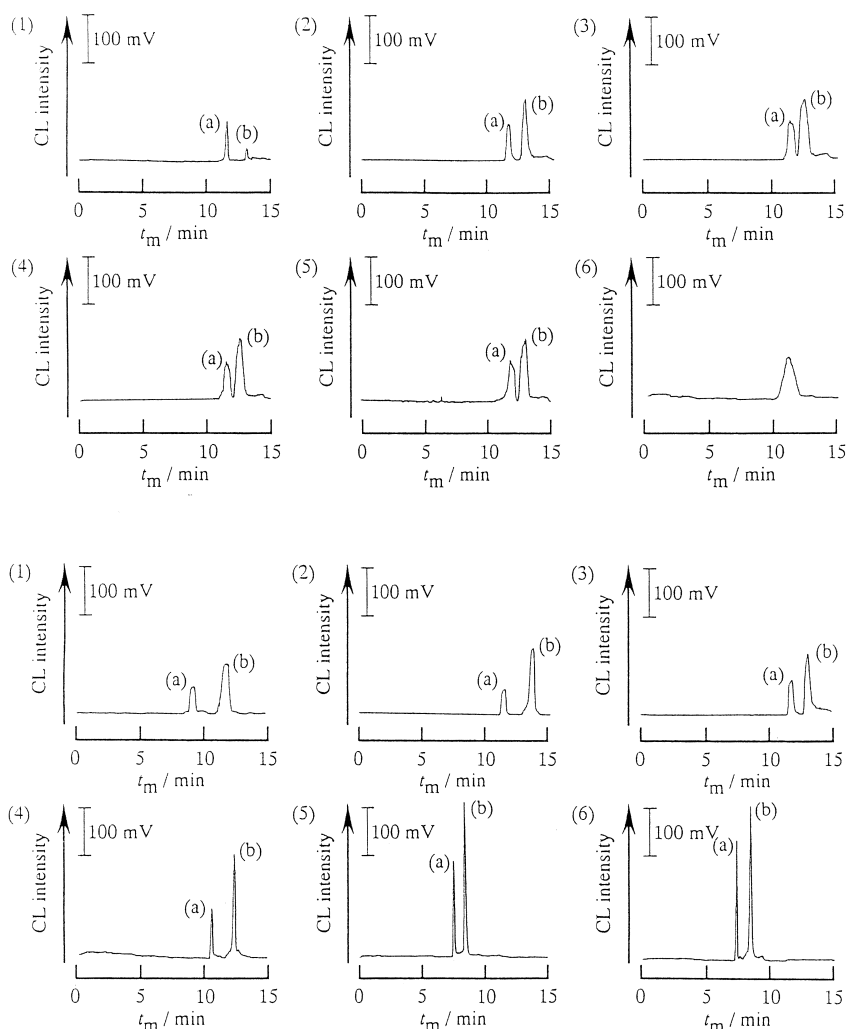


Fig. 5. Effect of (A) volume percentage of acetonitrile and (B) pH of migration buffer on electropherogram. (A) (1), 10%, (2), 20%, (3), 30%, (4), 40%, (5), 50%, (5), 60% and; (B) (1) pH 2, (2) pH 3, (3) pH 4, (4) pH 5, (5) pH 6, (6) pH 7. Conditions: CL reagent, 0.3 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM H_2SO_4 ; migration buffer, 100 mM phosphate buffer–acetonitrile; and sample, emetine dithiocarbamate (a) Cu(II) ($1 \cdot 10^{-7}$ M) and (b) Co(II) ($1 \cdot 10^{-6}$ M) complex.

configuration (square planar, octahedral, tetrahedral, etc.). We examined the effect of pH and acetonitrile percentage of a migration buffer on the separation. They must influence the coordination behavior of anion species to the complexes and the solvation, leading to change of migration behavior of the metal complexes in CE.

The effect of volume percentages (10–60%) of acetonitrile in the 100 mM phosphate migration buffer on separation was examined. The mixture of the dithiocarbamate Cu(II) and Co(II) complexes was used as a model sample. The obtained electropherograms are shown in Fig. 5A. By considering the resolution and the peak height, 20% (v/v) acetonitrile was recommended for the separation. The effect of pH (2.0–7.0) of the 100 mM phosphate migration buffer was also examined (Fig. 5B); pH 6–7 was recommended.

A model sample containing emetine and three kinds of emetine dithiocarbamate metal complexes [Cu(II), Ni(II), and Co(II)] was analyzed by use of the above-recommended migration buffer [100 mM phosphate buffer (pH 7.0)–acetonitrile (80:20, v/v)]. They were successfully separated and detected within 10 min (Fig. 6).

3.4. Application to a metal ion sample at low concentration

In the above section, emetine dithiocarbamate metal complex solutions were prepared using a high concentration (10 mM) of the metal ion and then diluted as needed (method A). Here, the complex solutions were prepared from various low concentrations (≤ 5000 nM) of metal ion and then subjected to CE without any purifications (method B). The emetine and the Cu(II), Ni(II), and Co(II) complexes indicated a baseline separation similarly to Fig. 6. However, the detection limits of these metal ions were 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 metal concentration.

4. Conclusion

We developed, for the first time, CE with CL

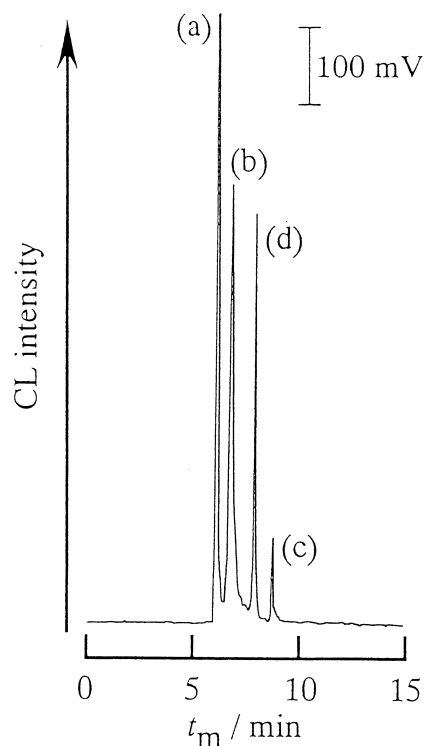


Fig. 6. Electropherogram of mixture of emetine and emetine dithiocarbamate Cu(II), Ni(II), and Co(II) complexes. Conditions: CL reagent, 0.3 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 10 mM H_2SO_4 ; migration buffer, 100 mM phosphate buffer (pH 7)–acetonitrile (80:20, v/v); and sample, (a) emetine ($1 \cdot 10^{-7}$ M), emetine dithiocarbamate (b) Cu(II), (c) Ni(II), and (d) Co(II) complex ($1 \cdot 10^{-6}$ M).

detection of $\text{Ru}(\text{bpy})_3^{2+}$ for the analysis of metal ions. We took advantage of the phenomenon that emetine dithiocarbamate metal complexes, which were prepared from emetine, carbon disulfide, and metal(II), indicated large CL intensities on the $\text{Ru}(\text{bpy})_3^{2+}$ CL system. The mixture of the Cu(II), Ni(II), and Co(II) complexes was successfully separated and detected with detection limits in the nanomolar-order levels. The present CE with CL detection method must become a promising method for the separation and determination of small amounts of metal ions.

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