Electrogenerated Chemiluminescence of Tris(dibenzoylmethane)phenanthroline Europium(III) as a Light Source: An Application for the Detection of PO_4^{3-} Based on the Ion Associate Formation of Phosphomolybdic Acid and Malachite Green

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We demonstrate that electrogenerated chemiluminescence (ECL) of an organometallic Eu(III) complex, tris(dibenzoylmethane)phenanthroline europium(III), whose emission spectra is very sharp with the maximum wavelength of 612 nm, can be used as a light source for the detection of PO_4^{3-} based on changing the absorbance of the ion associate of malachite green (MG^+) and phosphomolybdic acid. The ECL was also applied to measure the absorbance of MG^+ . With the detection system we established, absorbance up to ~ 1.6 with a change of ~ 0.1 could be detected.

Keywords Electrochemiluminescence, molybdate, light absorption, tris(dibenzoylmethane)phenanthroline europium(III), malachite green

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Introduction

Eu(III) complexes are known to exhibit extremely sharp photoluminescence with the main maximum wavelength of around 610 nm.^{1,2} Generally, in Eu(III) complexes, the ligand absorbs light, and the energy transfers to the Eu(III) metal center, resulting in the formation of the excited state of the Eu metal center. Changing the electronic configuration of the 4f electrons is related to the formation of the excited states, and the luminescence is observed when the electronic configuration goes back to a ground state. The Stokes shift becomes very large because of the successive energy transfer from the ligand to the Eu metal center. In addition, because the 4f shell are effectively shielded by the outer 5s² or 5p⁶ shells, 4f electrons are hardly involved in chemical bonding.³ Therefore, the energy of the 4f orbitals are almost independent on the ligand, and very sharp emission spectra of Eu(III) complexes are observed. Such atomic-like light emissions have a high color purity and are applied in the field of organic light-emitting diodes (OLEDs).⁴ In analytical chemistry, the luminescence of Eu(III) is also attractive, and Eu(III) complexes with organic and inorganic materials have been recently applied for time-resolved luminescence assay^{5,6} and determination of tetracyclines based on energy transfer to Eu(III).⁷

Several studies on Eu(III) complexes have also been reported as materials for electrochemiluminescence (ECL).^{8,9} Although commonly, ECL intensities depending on the concentrations of analytes such as antigens (ECL immunoassay) and quenchers (ECL quenching based on energy or electron transfer) are utilized for quantitative analysis,^{10,11} we expect that the sharp

emissions of Eu(III) complexes are advantageous as a light source for light absorption analysis. OLEDs and ECL devices can be fabricated easily, and since these are compact and light, we also expect their applications as a portable light source. Several works have been published recently on the use of portable light emitting devices such as LEDs as a light source for the determination of analytes.¹²⁻¹⁴ We also applied organic photodiodes and LEDs for flow injection analysis,^{15,16} and recently, sharp emissions from OLEDs containing an Eu(III) or Tb(III) complex were utilized as a light source for the flow injection determination of phosphate¹⁷ and fluorescent flow injection immunoassay for alkylphenol ethoxylates.¹⁸

So far, we reported the photophysical and ECL properties of several light-emitting materials, whose emission spectra were broad,¹⁹⁻²³ and very recently, we also clarified the ECL behavior of several Eu(III) and Tb(III) complexes composed of N-donor ligands and enolate ions of β -diketonates.²⁴ It was shown that very sharp ECL spectra of the complex derived from the f-f transitions were able to be obtained. Among the complexes we explored, $\text{Eu}(\text{dbm})_3\text{phen}$ (where dbm represents the enolate form of dibenzoylmethanate, and phen means phenanthroline) presented moderately high ECL intensity.

In this paper, we apply the ECL of $\text{Eu}(\text{dbm})_3\text{phen}$ to measure the absorbance of malachite green (MG^+). Moreover, the ECL was applied for the detection of phosphate ion based on the method using phosphomolybdic acid and MG^+ .²⁵ Phosphate ion is a common analyte that presents in environmental water, and several methods such as potentiometric,²⁶ colorimetric,^{13,27} and luminescence quenching determinations^{28,29} have been proposed for the determination of phosphate ions. The ion associate of molybdophosphoric acid and MG^+ shows light absorption around the maximum wavelength of the ECL of $\text{Eu}(\text{dbm})_3\text{phen}$. Thus, detection of phosphate ion was chosen as an example of using the ECL as a light source.

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Fig. 1 An illustration of the experimental setup.

Experimental

Chemicals

Eu(dbm)₃phen was received from Minerva Light Lab. (Kyoto, Japan). Tetrabutylammonium hexafluorophosphate (TBAPF₆) was obtained from Sigma-Aldrich Co. (MO, USA). An HPLC grade of acetonitrile (MeCN) (Kanto Chemical Co., Inc., Tokyo, Japan) was used after dehydration with molecular sieves. Malachite green oxalate and ammonium molybdate were received from Wako Chemical Co., Ltd. (Osaka, Japan). (TBA)₂S₂O₈ was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Measurements

A Pt disk ($d = 3$ mm), Pt coil wire and Ag wire were used as working, counter and reference electrodes, respectively. The electrode potential was controlled by using an electrochemical analyzer (Model 715AN, BAS Inc., Tokyo Japan). An MeCN solution of 4 mM Eu(dbm)₃phen containing 0.1 M TBAPF₆ as a supporting electrolyte and 20 mM (TBA)₂S₂O₈ as a coreactant was used for ECL. The ECL was generated by applying a constant potential.

Aqueous solutions of the ion associate of phosphomolybdic acid and MG⁺ were prepared as follows. Ammonium molybdate (1.88 g) and malachite green oxalate (0.005 g) were added to a mixture of water (25 mL), methanol (12.5 mL), and conc. sulfuric acid (3.5 mL), and water was further added to prepare a solution with a total volume of 50 mL. Then, an aqueous solution of KH₂PO₄ was added to this solution to form the ion associate. The resultant concentrations of ammonia molybdate and MG⁺ of the solution were 0.19 M and 0.11 mM, respectively.

A quartz cell with a cap, whose optical path length of 1 cm, was filled with different concentrations of MG⁺ or ion associate solutions, and this was placed under the three-electrochemical cell. An optical fiber, which is connected to an electron multiplying charge coupled device (EMCCD) camera (proEM 512HS, Princeton Instruments, NJ, USA) equipped with a spectrometer (IsoPlane 160, Princeton Instruments, NJ, USA), was set under the quartz cell, and the ECL spectrum and intensity were recorded with the detection system in a dark room. For each measurement of the sample solutions, ECL

Fig. 2 ECL and PL spectra of Eu(dbm)₃phen in MeCN. ECL was generated by applying a constant potential (−1.5 V vs. SCE) in the presence of 20 mM S₂O₈^{2−}. Concentration of Eu(dbm)₃phen for ECL: 4 mM, and PL: 40 μM. Excitation wavelength of PL spectrum: 350 nm.

intensities through a blank solution (water) were also measured to calculate absorbance for each sample. A schematic diagram of the measurement is shown in Fig. 1. The visible absorption spectra were acquired using an UV-Vis spectrophotometer (V-560, JASCO Co., Tokyo, Japan).

Results and Discussion

ECL spectrum of Eu(dbm)₃phen

Figure 2 shows the ECL spectrum of Eu(dbm)₃phen with its photoluminescence (PL) spectrum. The ECL of Eu(dbm)₃phen was generated by applying a constant potential in the presence of S₂O₈^{2−} as a coreactant. A general ECL mechanism using S₂O₈^{2−} can be written as,^{30,31}

where R is a light-emitting molecule. A sharp ECL spectrum with the main maximum wavelength (λ_{ECL}) of 612 nm derived from the f-f transition was observed. The full width at half maximum of the main emission was 6 nm. The ECL spectrum agrees with the PL spectrum well. The small deviation at 630–690 nm is because the ECL originated from the ligands.²⁴ As reported elsewhere, in the system of Eu(dbm)₃phen and S₂O₈^{2−}, the ECL intensity derived from the f-f transition reached the maximum around the first reduction peak potential of Eu(dbm)₃phen (−1.53 V vs. SCE²⁴). Hence, by applying a constant potential around the first reduction peak potential, the ECL can be used as a light source for the detection of both MG⁺ and the ion associate of MG⁺ and phosphomolybdic acid, as described in the following section. The cyclic voltammogram of an MeCN solution of Eu(dbm)₃phen containing S₂O₈^{2−} is displayed in Fig. S1 (Supporting Information).

Absorbance of MG⁺ using ECL as a light source

We applied the ECL for MG⁺ detection in a wide absorbance range. The visible absorption spectra at different concentrations of MG⁺ are shown in Fig. 3(a). The absorption maximum of MG⁺ appeared at 620 nm, and the maximum wavelength is very

Fig. 3 (a) Visible absorption spectra of MG^+ solutions. (b) ECL responses after passing through different concentrations of MG^+ . (c) Absorbance calculated from the curves in panel (b). (d) Absorbance estimated with ECL as a light source as a function of the concentration of MG^+ . Each value is an average between 500 to 1000 ms in panel (c). For comparison, absorbance at 612 nm estimated from panel (a) is also displayed.

close to that of the ECL maximum of $\text{Eu}(\text{dbm})_3\text{phen}$ ($\lambda_{\text{ECL}} = 612 \text{ nm}$).

In the experimental setup as illustrated in Fig. 1, the ECL intensities after passing through different concentrations of MG^+ are shown in Fig. 3(b). The ECL was generated when the potential was stepped to -1.5 V vs. SCE, and in 100 ms, the ECL intensities reached maximum, then these decreased (Fig. 3(b)). The ECL response is related to the current response (Fig. S2, Supporting Information); after the potential step,

the current monotonically decreased because of the development of the diffusion layer in the vicinity of the electrode surface. The ECL intensities depending on the MG^+ concentration were converted into the absorbance (Fig. 3(c)) with the Lambert-Beer equation as,

$$\text{Abs.} = -\log \frac{I_{\text{ECL}}}{I_{\text{ECL}}(c=0)} \quad (5)$$

where I_{ECL} is the ECL intensity at difference concentrations of MG^+ , and $I_{\text{ECL}}(c=0)$ indicates the ECL intensity when the concentration of MG^+ is 0. Although the ECL intensities were changed while the potential was set to -1.5 V vs. SCE (Fig. 3(b)), it can be seen that the absorbance values are moderately stable. At levels over $8 \mu\text{M}$ of MG^+ , the ECL signals are comparable to the background noise. The absorbance as a function of the MG^+ concentrations measured with the ECL system is presented in Fig. 3(d). For comparison, the absorbance values estimated with the UV-Vis absorption spectrometer are also shown. A good linear relationship was obtained when the concentration of MG^+ was in the range of 1 to $8 \mu\text{M}$, which corresponds to 0.18 – 1.56 absorbance. At $8 \mu\text{M}$, the absorbance estimated with the ECL system was slightly lower than that with the UV-Vis absorption spectrometer. This is probably because of stray light; the scattered ECL on the glass surface, which reaches to the optical fiber, is not negligible when the transmitted ECL intensity is very weak.

Determination of PO_4^{3-} using ECL as a light source

In an acidic solution, the phosphate ion reacts with molybdic ion to form phosphomolybdic acid (Mo-P). Furthermore, phosphomolybdic acid forms the ion associate with MG^+ (Mo-P-MG), which shows a green color. The reactions can be written as,²⁵

The visible absorption spectra of the complex against the concentration of KH_2PO_4 based on the method is shown in Fig. 4(a). As the concentration of phosphate increased, the absorbance at 550 to 700 nm increased. Changing absorption spectra due to the formation of the ion associate was detected using the ECL of $\text{Eu}(\text{dbm})_3\text{phen}$ as a light source. The change in the absorbance with respect to the concentration of phosphate is displayed in Fig. 4(b). Although the estimated absorbance with ECL is comparable to that with the UV-Vis spectrometer, the standard deviations (σ), which are originated from the stability of the ECL, are relatively large; these were estimated to be in the range of 0.015 – 0.033 absorbance from the three measurements. It is suggested that 3σ of 0.05 – 0.1 absorbance may be a detection limit with this system. The ECL intensities in three measurements for a blank solution (water) are shown in Fig. S3 (Supporting Information) to display the reproducibility. The slope of the absorbance change from 0 to 0.2 ppm is smaller than that from 0.2 to 0.6 ppm. This is because of a larger amount of free MG^+ compared to that of the ion associate. At low concentrations of PO_4^{3-} , the light absorption by free MG^+ is not negligible. To minimize the background absorption by free MG^+ , solvent extraction could be useful.

Conclusions

In this paper, we showed that the ECL of $\text{Eu}(\text{dbm})_3\text{phen}$ could

Fig. 4 (a) Visible absorption spectra of Mo-P-MG solutions. (b) Absorbance estimated with ECL as a light source as a function of the concentration of KH_2PO_4 . The standard deviations were calculated from three experimental results. For the comparison, absorbance at 612 nm estimated from panel (a) is also displayed.

be applied to the light absorption measurements of MG^+ and the ion associate of molybdophosphoric acid and MG^+ . Although the obtained standard deviations are relatively large, absorbance up to ~ 1.6 could be detected. Overall, it is advantageous to use ECL of $\text{Eu}(\text{dbm})_3\text{phen}$ showing a very sharp spectrum as a light source compared to ECLs of other luminophores that need an optical filter because of their broad spectra. Since the ECL emission system is very simple, the experimental setup can be easily assembled. We expect that, in addition to the molybdophosphoric acid method, the ECL of $\text{Eu}(\text{dbm})_3\text{phen}$ can be applied to the detection of several analytes having absorption around 610 nm. At the present point, as a light source, commercial red LEDs are less expensive and more reliable compared to the proposed ECL method. As a feature of ECLs, the emission area and pattern can be controlled easily with the electrode size and pattern. It is possible to realize a large emission area of ECL and array ECL emission by using large electrodes and patterned electrodes. We believe that this feature is useful for multi-sample measurements.

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Supporting Information

This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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