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 electognerated 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 $\sim 1.6$ with a change of $\sim 0.1$ could be detected.

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

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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 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 (~1.5 V vs. SCE) in the presence of 20 mM S2O82-. Concentration of Eu(dbm)3phen for ECL: 4 mM, and PL: 40 μM. Excitation wavelength of PL spectrum: 350 nm.

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...
close to that of the ECL maximum of Eu(dbm)₃phen (λₑcl = 612 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,

$$\text{Abs.} = -\log \frac{I_{\text{ECL}}}{I_{\text{ECL}}(c = 0)}$$  \hspace{1cm} (5)

where $I_{\text{ECL}}$ is the ECL intensity at different 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 μM 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 μM, which corresponds to 0.18 – 1.56 absorbance. At 8 μM, 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 PO₄³⁻ 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,

$$\text{H}_3\text{PO}_4 + 12\text{H}_2\text{MoO}_4 \rightarrow [\text{PO}_4(\text{MoO}_3)_{12}]^{3–} + 12\text{H}_2\text{O} + 3\text{H}^+$$  \hspace{1cm} (6)

$$[\text{PO}_4(\text{MoO}_3)_{12}]^{3–} + \text{HMG}^2+ + \text{H}^+ \rightarrow [\text{MG}^+]\text{[H}_2\text{PMo}_{12}\text{O}_{40}]$$  \hspace{1cm} (7)

The visible absorption spectra of the complex against the concentration of KH₂PO₄ 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 Eu(dbm)₃phen 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 ($\sigma$), 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 $3\sigma$ 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 PO₄³⁻, 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 Eu(dbm)₃phen could
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 Eu(dbm)₃phen showing a very sharp spectrum as a light source because of their broad spectra. Since the ECL source compared to ECLs of other luminophores that need an optical filter because of their broad spectra.

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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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