Luminol Chemiluminescence-Delay Method for Determination of Copper(II)

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The copper(II)-catalyzed oxidation of cysteamine with oxygen was carried out in a basic medium involving luminol. After preferential oxidation of cysteamine to disulfide, a Cu(II)-catalyzed luminol chemiluminescence (CL) reaction was subsequently commenced with hydrogen peroxide accumulated during the oxidation of cysteamine. Thus, a delay time from reaction initiation to a sharp flash of CL was observed. This delayed CL of luminol was applied to the determination of copper(II) by measuring the delay time or CL intensities. The delay time and the CL intensity at maximum light emission were linearly correlated with the Cu(II) concentration over the range from the detection limit of 3.0×10^{-6} to 2.0×10^{-5} M. The relative standard deviation of the CL intensity and the delay time in five successive experiments was 3.0% at 1.0×10^{-5} M of Cu(II). The tolerance limits of Fe(III), Ni(II), and Zn(II) in the present method were 20- to 100-times higher than those in the conventional luminol CL method.

The conventional chemiluminescence (CL) reaction of luminol with hydrogen peroxide (H_2O_2) catalyzed by metal ions proceeds quickly; CL emission begins immediately after the initiation of the CL reaction. On the other hand, a few studies have been conducted on the delayed CL reaction of luminol in which a CL flash suddenly appears after a certain period from reaction initiation. Frew et al. reported the CL-delay in the ferrihaem-catalyzed oxidation of luminol with H_2O_2 in the presence of ascorbic acid and uric acid.[1,2] The delay time was linearly dependent upon the concentration of ascorbic acid and uric acid, thus making it possible to determine these reductants.[1] In addition, the CL-delay was observed in the Cu(II)-catalyzed oxidation of cysteine with oxygen in the presence of luminol.[3] A mechanism for the formation of H_2O_2 during the Cu(II)-catalyzed oxidation of cysteine has been proposed.[3] However, no reports have been found concerning the application of this reaction to the determination of Cu(II).

We previously showed that the rate of the Cu(II)-catalyzed oxidation of cysteine with oxygen is dependent on the concentration of Cu(II) as a catalyst.[4] Additionally, the CL intensity in the Cu(II)-catalyzed luminol CL reaction with H_2O_2 was dependent on the concentration of Cu(II).[5] Therefore, Cu(II) could be determined by measuring the delay time or CL intensities in the CL-delay reaction. For this purpose we surveyed the possibilities of the CL-delay as a new principle for a trace Cu(II) determination.

Experimental

Reagents. Guaranteed-grade reagents were used as received with no further purification. All of the solutions used were prepared with water from a Millipore Milli-Q water purification system. A 1.0×10^{-3} M (1 M=1 mol dm^{-3}) stock solution of Cu(II), prepared from copper(II) nitrate in 0.1 M hydrochloric acid, was standardized by EDTA. Working solutions of Cu(II) were obtained by serial dilution of the stock solution with water. A luminol solution was prepared by dissolving the compound with a 0.1 M NaOH solution. Standard solutions of such thiols as L-cysteine {$HSCH_2CH(NH_2)COOH$} and cysteamine ($H_2NCH_2CH_2SH$) were made daily.

Apparatus. All CL measurements were made using a luminometer constructed in this laboratory. A glass cuvette (22 mm i.d. ×20 mm) was placed on a magnetic stirrer in a dark box. The light output was detected with a Hamamatsu Photonics R453 photomultiplier (PMT). The resultant photocurrent was measured with a TOA Electronics PM-18 type current meter and displayed on a chart recorder. A luminol solution and a thiol solution were injected through Teflon tubing into the cuvette by using an injector (Iatron Laboratories, Inc., dualpette type).

The absorption spectra were measured with a Hitachi U-2000-type spectrophotometer equipped with 1-cm quartz cells. A Toa Model HM-60S pH meter was employed for the pH measurements.

Recommended Procedure. A 0.5 cm^3 portion of an aqueous solution containing Cu(II) was added with an Eppendorf pipet into the cuvette. The solution was saturated with oxygen by bubbling. Next, a 1 cm^3 portion of a 2.5×10^{-3} M luminol solution and a 1 cm^3 portion of a 1.0×10^{-3} M cysteamine solution were simultaneously injected into the cuvette. The entire solution, thus prepared, was referred to a final solution. The CL reaction was initiated, and the CL emission was detected by the PMT. Bubbling of oxygen at a rate of 50 cm^3 min^{-1} and vigorous agitation by a magnetic stirrer were continued during the reaction. All measurements were made at room temperature. The maximum light emission was referred to as the CL intensity. The time period from the reaction initiation to a CL flash was defined as the delay time.

Analytical Procedure for Thiols and H_2O_2. The concentration of thiol consumed and H_2O_2 formed during the Cu(II)-catalyzed oxidation of thiol was determined as follows: A 5 cm^3 portion of 6.0×10^{-5} M Cu(II) solution was taken in a 50-cm^3 volumetric flask. The solution was saturated with oxygen. The catalytic reaction was then started by adding 10 cm^3

of a 6.0×10^{-4} M thiol solution and 10 cm^3 of a 0.1 M NaOH solution. Oxygen bubbling and vigorous agitaion by a magnetic stirrer were continued during the reaction. A 1 cm^3 portion of the reaction mixture was arbitrary pipetted into the cell, in which a 0.1 cm^3 portion of 1.0×10^{-3} M EDTA solution was added to stop the catalytic reaction. Determinations of thiol and H_2O_2 were carried out spectrophotometrically. Thiol was determined at 428 nm with 2,2'-dithiobis(5-nitropyridine),[6] and H_2O_2 at 408 nm with a $TiCl_4$ reagent.[7]

Results and Discussion

Effect of Thiol on a Delayed CL of Luminol. The CL measurements were carried out according to the recommended procedure in which a 2.5×10^{-3} M solution of luminol, a 6.0×10^{-4} M solution of the thiol and a 6.0×10^{-5} M solution of Cu(II) were used. Typical CL response curves are shown in Fig. 1. A CL flash suddenly appeared after a dark period of 1—4 min from the start of the reaction. Cysteamine gave a shorter delay time than did cysteine, whereas cysteamine showed a greater CL intensity than did cysteine. On the other hand, no light emission was detected in the blank, which contained no Cu(II).

The appearance of the delay time could be explained on the basis of a mechanism proposed by Cavallini.[3] The Cu(II)-catalyzed oxidation of the thiols with oxygen proceeds preferentially, resulting in the formation of H_2O_2 and disulfide. During thiol oxidation, Cu(II) is strongly complexed with thiol, thereby being ineffective for the luminol CL reaction. When thiol is almost completely oxidized to disulfide, Cu(II) is again free.

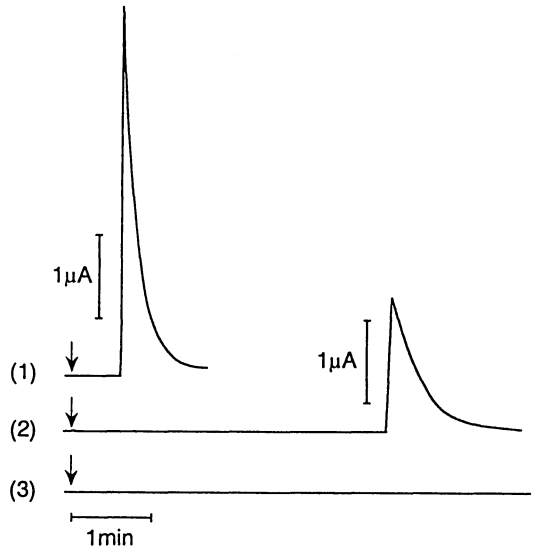

Fig. 1. Typical chemiluminescence response curves for solutions containing cysteamine and cysteine. (1): cysteamine, (2): cysteine. Conditions: 2.5×10^{-3} M luminol, 6.0×10^{-4} M thiol, 6.0×10^{-5} M Cu(II). (3): blank. Conditions: 2.5×10^{-3} M luminol, 6.0×10^{-4} M cysteamine. At the arrow luminol and thiol were added.

As a result, the Cu(II)-catalyzed luminol CL reaction is subsequently commenced using H_2O_2 accumulated as an oxidizing agent, and a CL emission suddenly appears after a dark period.

We then examined the effects of thiols on the delay time and the CL intensity. The time course for thiol consumption and H_2O_2 formation is shown in Fig. 2. The concentration of H_2O_2 increased with a decrease in the thiol concentration. When thiol was almost completely consumed, the decomposition of H_2O_2 was initiated. The time at the beginning of H_2O_2 decomposition was in accordance with the delay time, as shown in Fig. 1, for the respective thiols. The difference in the delay times between cysteine and cysteamine is mainly ascribable to the difference in the oxidation rates of cysteine and cysteamine, as shown in Fig. 2. On the other hand, the magnitude of CL intensities were dependent on the thiols used. As is evident in Fig. 2, cysteamine gives a higher H_2O_2 concentration than does cysteine. Consequently, the difference in the CL intensities between cysteamine and cysteine is probably due to the extent of H_2O_2 formation during thiol oxidation, since the CL intensity in general increases with increasing H_2O_2 concentration. When thiols are converted quantitatively to H_2O_2, the reaction yield (mol%) of H_2O_2 should be 50%,[4] where the reaction yield is defined as the ratio of the amount of H_2O_2 formed to that of thiols used. However, Fig. 2 gives the reaction yield as 43% for cysteamine and 29% for cysteine, respectively, thus suggesting the oxidation of thiol, in part, with the formed H_2O_2.

Optimum Conditions for the Delayed CL Reaction. When the delayed CL of luminol is to be used for the determination of Cu(II), cysteamine is a good choice with respect to the sensitivity and analysis speed. In subsequent studies, the conditions were determined by measuring the CL intensities, so as to be maximal under optimum conditions.

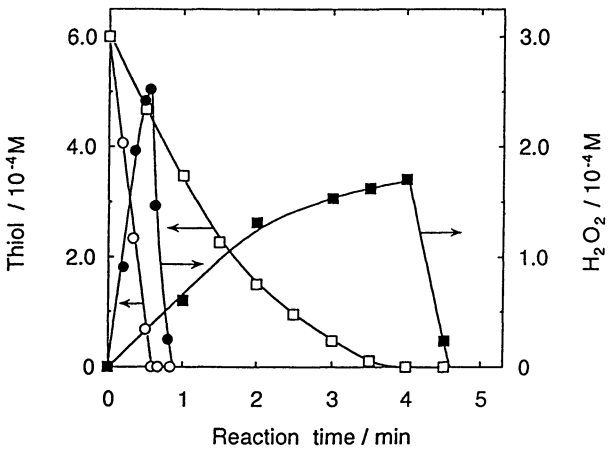

Fig. 2. Time course of the thiol consumption and the formation of hydrogen peroxide. ○, ●: cysteamine, □, ■: cysteine. Conditions: 6.0×10^{-4} M thiol, 6.0×10^{-5} M Cu(II).

The influence of the pH in the final solution was examined in the 11.9—13.3 pH range. The pH values were adjusted by adding sodium hydroxide to the luminol solution. Figure 3 shows the effect of pH on the CL intensity and the delay time. The CL intensity was maximal at pH 12.5, when the luminol solution was prepared with a 0.1 M NaOH solution. The delay time was constant between pH 11.9 and 12.5, and increased above 12.5. Thus, a pH of 12.5 was chosen for the recommended procedure.

The effect of the luminol concentration was tested in the 2.0×10^{-4}—5.0×10^{-3} M range. The optimization curves for luminol concentrations are shown in Fig. 4. The CL intensity increased to a maximal value at 2.5×10^{-3} M of luminol, after which the CL intensity gradually decreased with increasing luminol concentration. The delay time was constant in the range tested. The optimum luminol concentration was thus determined to be 2.5×10^{-3} M.

The results concerning the cysteamine optimization study are shown in Fig. 5. The CL intensity exhibits a maximum at 1.0×10^{-3} M, while the delay time was increased with an increase in the cysteamine concentration. Thus, the optimum cysteamine concentration was chosen to be 1.0×10^{-3} M.

Analytical Results and Parameters. Analytical calibration curves were prepared under optimized conditions. In Fig. 6 two logarithmic calibration curves are presented. One is based on the CL intensity, which increased with an increase in the Cu(II) concentration; the other is based on the delay time, which decreased

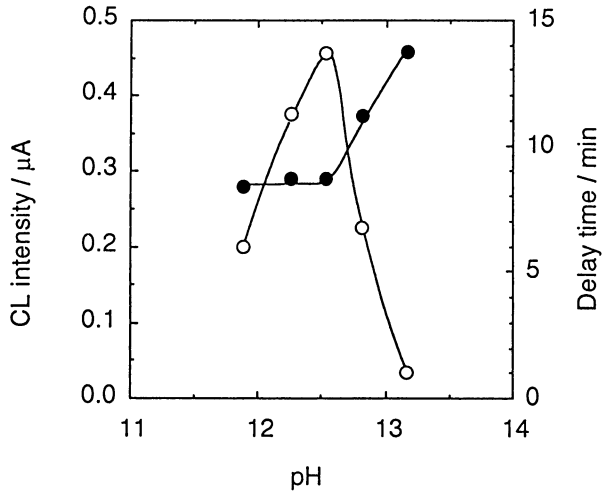

Fig. 3. Effect of pH on chemiluminescence intensity and delay time. ○: CL intensity, ●: delay time. Conditions: 2.5×10^{-3} M luminol, 1.0×10^{-3} M cysteamine, 1.0×10^{-5} M Cu(II).

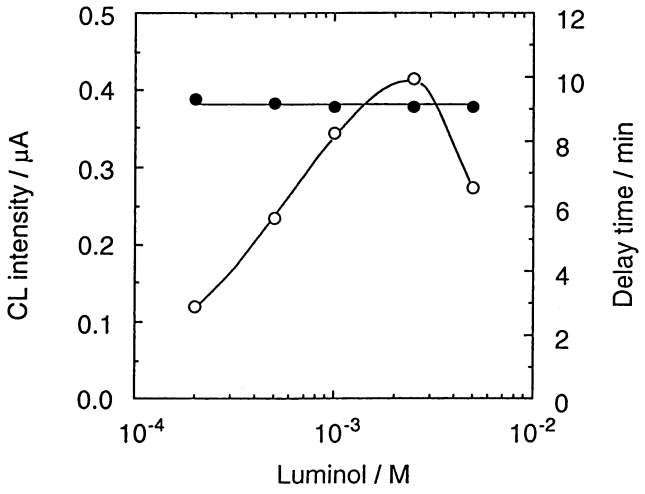

Fig. 4. Variation of chemiluminescence intensity and delay time with luminol concentration. ○: CL intensity, ●: delay time. Conditions: 1.0×10^{-3} M cysteamine, 1.0×10^{-5} M Cu(II).

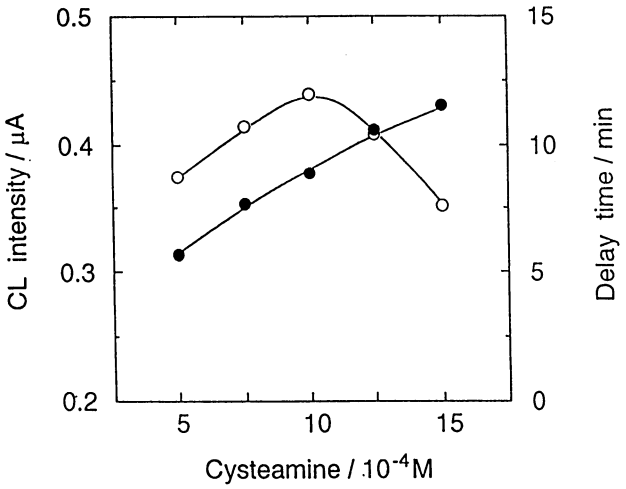

Fig. 5. Variation of chemiluminescence intensity and delay time with cysteamine concentration. ○: CL intensity, ●: delay time. Conditions: 2.5×10^{-3} M luminol, 1.0×10^{-5} M Cu(II).

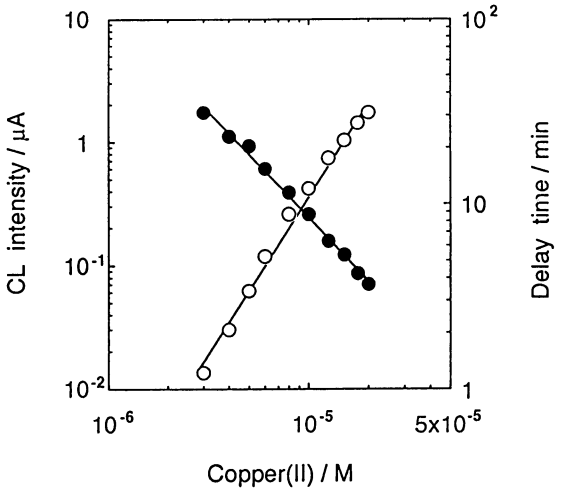

Fig. 6. Calibration curve for copper(II). ○: CL intensity, ●: delay time. Conditions: 2.5×10^{-3} M luminol, 1.0×10^{-3} M cysteamine.

with an increase in the Cu(II) concentration. The two calibration curves are linear over the range from the detection limits of 3.0×10^{-6} to 2.0×10^{-5} M Cu(II). The detection limit was defined as being the concentration of Cu(II) that produced a CL intensity equal to three-times the noise signal in the blank solution. The relative standard deviation of the CL intensity and the delay time in five successive experiments was 3.0% at 1.0×10^{-5} M of Cu(II).

We next compared the detection limit of the present method with that of the conventional luminol CL method. The latter was made according to the recommended procedure, except that a H_2O_2 solution was employed in place of a thiol solution. The calibration curve of the conventional luminol CL method was obtained under optimized conditions for H_2O_2. The detection limit of the luminol method is a factor of about 20-times better than that of the delayed CL method. This is mainly ascribable to a difference in the concentration of H_2O_2 between the two methods. That is, the optimum H_2O_2 cnoncentration for the conventional luminol CL method was 2.0×10^{-2} M, while the concentration of H_2O_2 formed in the present method was 2.6×10^{-4} M, as shown in Fig. 2.

The effect of several metal ions on the determination of Cu(II) at a level of 1.0×10^{-5} M was examined according to the recommended procedure in which such metal ions as Cr(III), Mn(II), Fe(III), Co(II), Ni(II), and Zn(II) were added to the Cu(II) solution. These metal ions are accepted to be an effective catalyst for the luminol CL reaction. In addition, the same experiments as above were carried out for the conventional luminol CL method, in order to compare the selectivities of the two methods. The tolerance limit ratio of each metal ion was taken as being the largest amount yielding an error of less than 3% in the CL intensity and delay time.

The results from interference studies are summarized in Table 1. Serious interferences from all of the metal ions were observed in the conventional luminol CL method, even at a level equal to that of Cu(II). This is probably due to the effect of the metal ions on the decomposition of H_2O_2 with Cu(II). On the other hand, in the CL-delay method based on measuring the CL intensities, Fe(III) and Zn(II) were tolerable in greater amounts than those in the conventional luminol CL method. As can be seen in Table 1, the effect of foreign ions on the delay time is not so serious as that on the CL intensity. These results indicate that the foreign ions give rise to significant effects on the catalytic activity of Cu(II) for H_2O_2 decomposition, rather than for the oxidation rate of cysteamine.

Table 1. Tolerance Limit Ratio

Tolerated ratio	Ions tested		
	Luminol CL	Delayed-luminol CL	
	CL intensity	CL intensity	Delay time
<1	Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}	Cr^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}	Cr^{3+}, Mn^{2+}, Co^{2+}
10		Fe^{3+}	
20			Ni^{2+}
50			Fe^{3+}, Zn^{2+}
100		Zn^{2+}	

In conclusion, the delayed CL reaction of luminol has been successfully applied to the determinaiton of Cu(II) by measuring the delay time or CL intensities. The delayed CL reaction is promising to overcome mutual metal interference in the conventional luminol CL reaction, which does not differentiate between different metal ions. In the present method, Fe(III) and some metal ions were tolerable in greater amounts than those in the conventional luminol CL method. Furthermore, the CL-delay method is potentially useful for obviating background signals observed in the conventional luminol CL reaction. This is due to the fact that the present method is based on measurements of the CL intensities or delay times after a dark period from reaction initiation.

References

1) J. E. Frew and P. Jones, *Anal. Lett.*, **18**, 1579 (1985).
2) T. E. G. Candy, M. Hodgson, and P. Jones, *J. Chem. Soc., Perkin Trans.*, **1990**, 1385.
3) D. Cavallini, C. D. Marco, and S. Dupre., *Arch. Biochem. Biophys.*, **124**, 18 (1968).
4) T. Kamidate, K. Itoh, and H. Watanabe, *Anal. Sci.*, **6**, 769 (1990).
5) I. E. Kalinichenko, T. M. Tkachuk, and A. T. Pilipenko, *Zh. Anal. Khim.*, **40**. 1237 (1985).
6) A. Swaitditat and C. C. Tseu, *Anal. Biochem.*, **45**, 349 (1972).
7) H. Pobiner, *Anal. Chem.*, **33**, 1423 (1961).