

Determination of Cu²⁺ by Lophine Chemiluminescence

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The chemiluminescence reaction of lophine with H₂O₂ in alkaline solution has been investigated for use in determination of Cu²⁺ ions. The observed chemiluminescence intensity is found to be a function of the concentration of Cu²⁺. Under the optimum reagent concentrations such as 4×10⁻⁴ M lophine, 0.8 M KOH, 0.2M H₂O₂, λ_{em}, 533 nm, the linear range and the detection limit were found to be 0.048 μg/ml-48.32 μg/ml (R=0.99897) and 0.005 μg/ml respectively. Relative standard deviation for five determinations of 24.16 μg/ml Cu²⁺ is 2.35%. The interference from other species was investigated. The proposed method was applied to the determination of Cu²⁺ in different water samples.

key words: Chemiluminescence, Lophine, Copper, Mixing mode, Water sample

INTRODUCTION

Copper is an essential but toxic element in the environment. Copper ions are usually introduced into water body by industrial dumping and they have effected toxic for human life. Therefore, their maximum tolerated concentrations have been fixed by legislation: 50 mg l⁻¹ for Cu²⁺ (Real Decreto 928/88 legislación española). In order to assess its accumulation or deficiency in biological and environmental samples, a sensitive, reproducible and accurate analytical method is required. Chemiluminescence (CL) reaction has appeared of great importance for the determination of trace amount of chemical species. The method involves inhibition or catalysis of the redox reaction of CL reagents such as luminol, lucigenin, lophine and gallic acid. Applications of CL method are gaining interest in analytical chemistry because it shares a number of advantages [1], including (1) low detection limits (in the nanogram-or even subnanogram-per-milliliter region, (2) wide dynamic ranges (up to six orders of magnitude), (3) high signal to noise ratios resulting from the absence of a light source and the consequent absence of noise, (4) absence of Rayleigh and Raman scattering, (5) instrumental simplicity and affordability and (6) absence of toxic effects from the usual CL reagents.

Cu²⁺ was determined by a number of chemiluminescence methods: Luminol- KCN [2], Luminol-H₂O₂ [3], Indigo carmine-H₂O₂ [4], o-Phenanthroline-hydrogen peroxide-CTMAB-copper (II) chemiluminescence system [5]. Nakano and co-workers

[6] have reviewed the CL methods for the determination of Cu²⁺ with continuous and batch type procedures. Analysis of labile Cu²⁺ in fresh waters using the Cu²⁺-catalyzed oxidation of 1,10 phenanthroline by superoxide anion radical has been reported by Sangi et al. [7]. A flow injection method for the determination of Cu²⁺ based on coproporphyrin I-Cu²⁺/TCPO/H₂O₂ chemiluminescence reaction for the screening of waters has been studied by Meseguer-Lloret et al. [8]. Thiosemicarbazide (TSC)-H₂O₂-CTMAB chemiluminescence reaction used for the determination of Cu²⁺ by Sorouraddin et al [9] was based on the catalytic effect of Cu²⁺ on the oxidation of TSC with H₂O₂ to produce light emission.

Lophine (2,4,5-triphenylimidazole) emits yellow CL. The mechanism is thought to involve attack by H₂O₂ to form a hydroperoxide, conversion to a dioxetane intermediate, and cleavage of the peroxide bond resulting in light emission [10]. Metal ions catalyze the chemiluminescence reaction between lophine and hydrogen peroxide [11] and their catalytic phenomena can be utilized for their determinations. However, thus far no method of Cu²⁺ determination using lophine CL detection has been reported. Our present study indicates that under proper conditions, Cu²⁺ concentrations enhanced the CL of lophine in basic H₂O₂ solutions. Thus reaction ingredient concentrations were optimized for a Cu²⁺ detection limit and the interference from other species was also studied.

EXPERIMENTAL

Reagents and solutions

Lophine (2,4,5-triphenylimidazole, 98%, Aldrich) has limited solubility in water; all lophine solutions were prepared in 95 % methanol (Duksan pure chemical Co., Ltd, Korea). Methanol was degassed by boiling prior to dissolution of the

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lophine in the hot methanol in order to obtain best results. Lophine solutions were preserved in refrigerator when not in use. The stock standard solution of Cu^{2+} (1×10^{-2} M) was prepared from copper(II)nitrate (Shinyo pure chemicals). A 0.8 M standard solution of KOH (Samchun pure chemical industries ltd) and 0.2 M H_2O_2 (Junsei, Japan) solution were prepared in water.

Apparatus

The light was measured by photomultiplier tube (Hamamatsu, Model R928) operated at 950 V. Spex (Edison, NJ, USA) Model FL111 spectrofluorimeter was used for CL measurements and spectra data were collected by Spex DM3000 spectroscopy computer. During the measurements, the light source of the spectrofluorimeter was switched off. Peak height was measured for each signal and expressed as current output of the PMT.

Basic procedure

The basic analysis procedure consisted of addition of the following quantities of the equilibrated solutions into the reaction cell: 1.0 ml lophine or blank, 0.5 ml Cu^{2+} solution and 0.5 ml KOH solution. The contents of the reaction cell were allowed to mix for 10 second prior to injection of 0.5 ml of H_2O_2 solution with an Ismatec 404 peristaltic pump (Tygon tubing, 2 mm i.d). The analytical signal was taken as the difference in the CL peak height between a blank and analyte run. The cell was then evacuated and rinsed twice with 0.1M HNO_3 .

RESULTS AND DISCUSSION

Basic studies

Kinetic aspect

Emission chemiluminescence spectrum was obtained for the (a) blank and (b) Lophine- Cu^{2+} -KOH- H_2O_2 system following static conditions [4×10^{-4} M lophine, 2×10^{-4} M Cu^{2+} (48.32 $\mu\text{g}/\text{ml}$), 0.8 M KOH and 0.2 M H_2O_2]. In Figure 1 it can be seen that there is an emission maximum at 533 nm. Reaction kinetic curve at 533 nm was also obtained. From Figure 2 it can be seen that the maximum CL signal is observed after 38 second (Residence time) of mixing of the reagents and then the signal is decayed slowly. The Fig. 2 also indicates that the reaction is fast.

Selection of solvent

As lophine is sparingly soluble in water, a water miscible organic solvent may be used to make the lophine solution. The use of organic solvents has some advantages [12]: (1) higher concentrations of the CL ingredient in the reaction mixture may provide better detection limits, (2) water insoluble reaction products may be kept in solution and increase precision and prevent memory effect. In our study, lophine stock and standard solutions were prepared in both 95

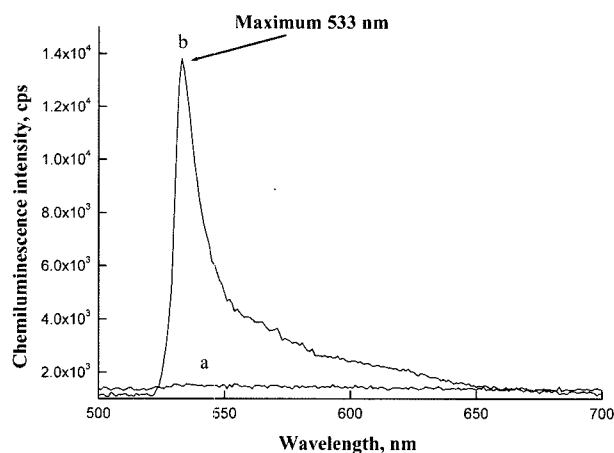


Figure 1. Emission chemiluminescence spectra for (a) blank, (b) Lophine- Cu^{2+} - KOH- H_2O_2 system. Conditions: [lophine] = 4×10^{-4} M, [Cu^{2+}] = 2×10^{-4} M (48.32 $\mu\text{g}/\text{ml}$), [KOH] = 0.8 M, [H_2O_2] = 0.2 M.

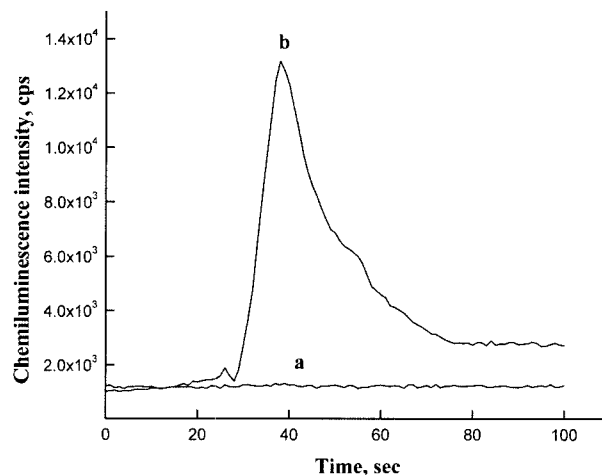


Figure 2. Kinetic curves for lophine Chemiluminescence signals using optimized reagents concentrations. (a) Blank, (b) Lophine- Cu^{2+} - KOH- H_2O_2 system; Conditions: [lophine] = 4×10^{-4} M, [Cu^{2+}] = 2×10^{-4} M (48.32 $\mu\text{g}/\text{ml}$), [KOH] = 0.8M, [H_2O_2] = 0.2M, [λ_{em}] = 533 nm.

% methanol and 95% ethanol. As can be shown in Figure 3, under optimum experimental conditions methanol was found to be the optimum solvent because the light intensity was 3.27 times greater than with 95% ethanol and because the stock solution of lophine showed reasonable storage stability. Therefore, methanol was selected as a solvent of lophine for determination of Cu^{2+} .

Mixing mode of the analyte with CL reagents

Chemiluminescence is usually generated by fast reactions and hence the phenomenon can be followed only when the chemical reaction is initiated in front of the light detector. Since the CL detection requires chemiluminescent reagent, the mixing mode of the analyte with CL reagent influences the light intensity significantly. Different mixing modes might rise to light quench even under the same conditions [13, 14].

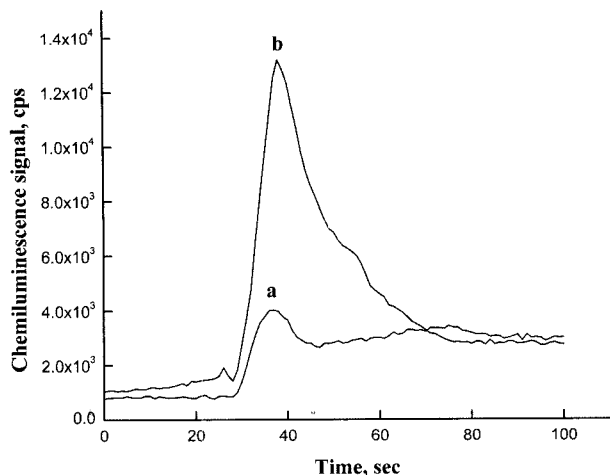


Figure 3. Solvent effect on the determination of Cu^{2+} . Peak (a) for the determination of Cu^{2+} using lophine prepared in 95% ethanol. Peak (b) for the determination of Cu^{2+} using lophine prepared in 95% methanol. Conditions: $[\text{lophine}] = 4 \times 10^{-4} \text{ M}$, $[\text{Cu}^{2+}] = 2 \times 10^{-4} \text{ M}$ (48.32 $\mu\text{g/ml}$), $[\text{KOH}] = 0.8 \text{ M}$, $[\text{H}_2\text{O}_2] = 0.2 \text{ M}$, $[\lambda_{\text{em}}] = 533 \text{ nm}$.

We investigated several mixing modes of the analyte with CL reagents. (1) Analyte and CL reagents such as lophine, H_2O_2 mixed together as cell components and KOH as injection component, (2) CL reagents such as lophine, KOH and H_2O_2 mixed together as cell components and analyte as injection component, (3) Analyte and CL reagents such as lophine and KOH mixed together as cell components and H_2O_2 as injection component. We found that mode (1) and (2) in which H_2O_2 was added and mixed in advance and KOH/ Cu^{2+} were injected later (10 second interval) lead to a significant decrease in light intensity. It was proposed that light loss resulting from mixing of lophine and hydrogen peroxide in advance has a great effect on the sensitivity of the detection. In order to establish a sensitive and reproducible system of CL detection we proposed mode (3) as described in the basic procedure (experimental section). We observed the strongest light signal due to the fast kinetic catalyst reaction.

Optimization studies

KOH concentration optimization

Lophine reacts with hydrogen peroxide to produce light emission in basic solution. The concentration of potassium hydroxide versus signal intensity was studied at different concentrations from 0.0002M to 2M (cell concentrations). It is found that the signal intensity increases with the concentration of potassium hydroxide; however, the blank signal also increases with increasing the concentration of potassium hydroxide. So the S/N (signal/noise) ratio was used to evaluate the potassium hydroxide effect. As can be seen from Figure 4, the CL intensity increases with increasing the concentration of potassium hydroxide up to 0.8 M, thereafter falls off to 2M potassium hydroxide. Higher concentration of KOH over 0.8 M could induce the decomposing of H_2O_2 . As

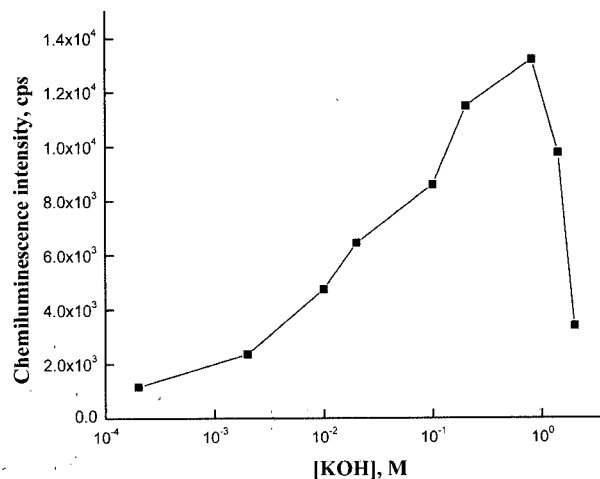


Figure 4. KOH optimization. Conditions: $[\text{lophine}] = 4 \times 10^{-4} \text{ M}$, $[\text{Cu}^{2+}] = 2 \times 10^{-4} \text{ M}$ (48.32 $\mu\text{g/ml}$), $[\text{H}_2\text{O}_2] = 0.2 \text{ M}$, $[\lambda_{\text{em}}] = 533 \text{ nm}$.

a compromise between the sensitivity and the background level, an optimum point was selected at 0.8 M KOH (this represents a precell concentration of 4M) for the present work.

H_2O_2 concentration optimization

Concentration of H_2O_2 in the reagent solution is an important factor influencing signal magnitude. There was no CL signal observed in the absence of hydrogen peroxide in the CL reaction. The effect of concentration of H_2O_2 on the CL intensity was examined in the range of 0.0002M-2.2M H_2O_2 (cell concentrations). Figure 5 shows the CL signal as a function of hydrogen peroxide concentration. As can be seen, the CL intensity is increased with increasing the concentration of H_2O_2 up to 0.2 M (this represents a precell concentration of 1M). The raising of hydrogen peroxide concentration over 0.2 M caused the decrease of relative CL intensity but the blank

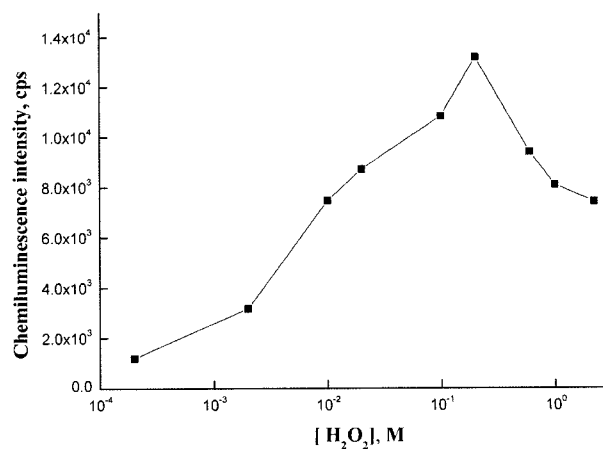


Figure 5. H_2O_2 optimization. Conditions: $[\text{lophine}] = 4 \times 10^{-4} \text{ M}$, $[\text{Cu}^{2+}] = 2 \times 10^{-4} \text{ M}$ (48.32 $\mu\text{g/ml}$), $[\text{KOH}] = 0.8 \text{ M}$, $[\lambda_{\text{em}}] = 533 \text{ nm}$.

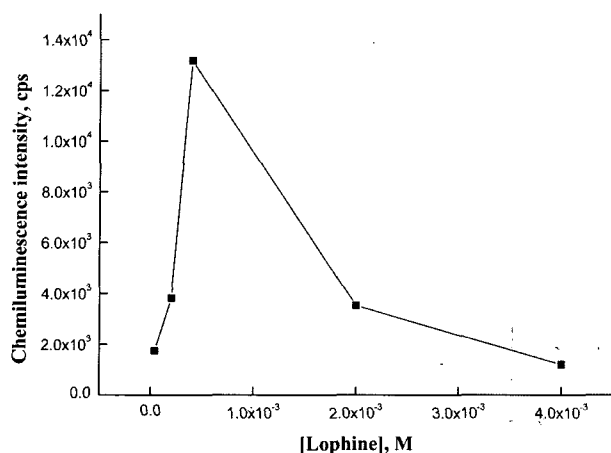


Figure 6. Lophine optimization. Conditions: $[\text{Cu}^{2+}] = 2 \times 10^{-4} \text{M}$ (48.32 $\mu\text{g/ml}$), $[\text{KOH}] = 0.8 \text{M}$, $[\text{H}_2\text{O}_2] = 0.2 \text{M}$, $[\lambda_{\text{em}}] = 533 \text{nm}$.

signal was independent of the H_2O_2 concentration. Therefore, 0.2M H_2O_2 (this represents a precell concentration of 1M) was used for further experiment.

Lophine concentration optimization

Figure 6 illustrates the results of the lophine concentration optimization study. For lophine, the maximum chemiluminescence signal and the best detection limit were obtained at a lophine concentration of $4 \times 10^{-4} \text{M}$ (this represents a precell concentration of $1 \times 10^{-3} \text{M}$). The CL signal starts to fall off rapidly at concentration higher than $4 \times 10^{-4} \text{M}$, but this is not due to the self-absorption, as lophine absorbs at 367 nm, fluoresces at 438 nm. The CL signal occurs at a maximum of 533 nm. A concentration higher than $4 \times 10^{-4} \text{M}$ could not be used due to the bubble formation from the breakdown of the peroxide in the presence of KOH. Thus, $4 \times 10^{-4} \text{M}$ was selected for application of the proposed method to the chemiluminometric determination of Cu^{2+} .

Analytical parameters

A series of 10 standard solutions (from 0.05 $\mu\text{g/ml}$ to 80 $\mu\text{g/ml}$) were used to study the linear range of the calibration graph under the optimized conditions such as $[\text{lophine}] = 4 \times 10^{-4} \text{M}$, $[\text{KOH}] = 0.8 \text{M}$, $[\text{H}_2\text{O}_2] = 0.2 \text{M}$, $[\lambda_{\text{em}}] = 533 \text{nm}$. According to previous reports and many similar chemiluminescence methods [15, 16, 17], the calibration line was not linear over the entire concentration range. Figure 7 shows a calibration curve for Cu^{2+} obtained using the above procedure. The chemiluminescence intensity linearly increases with an increase in the concentration ranges below 50 $\mu\text{g/ml}$ and was saturated at concentrations higher than 50 $\mu\text{g/ml}$. A concave calibration curve was obtained between Cu^{2+} concentration and chemiluminescence intensity when various concentrations of standard Cu^{2+} samples (0.05 $\mu\text{g/ml}$ to 80 $\mu\text{g/ml}$) were applied to the CL system under optimum conditions. However calibration approximates linearity in the limited range of 0.05

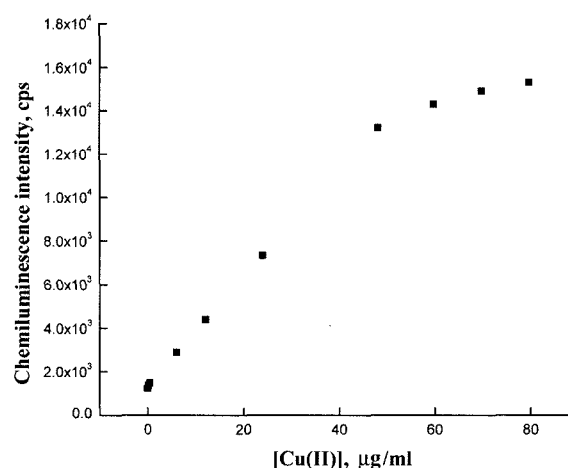


Figure 7. Cu^{2+} calibration curve. Conditions: $[\text{lophine}] = 4 \times 10^{-4} \text{M}$, $[\text{KOH}] = 0.8 \text{M}$, $[\text{H}_2\text{O}_2] = 0.2 \text{M}$.

$\mu\text{g/ml}$ -48.32 $\mu\text{g/ml}$. The equation for calibration graph is $x = 0.004061y - 5.425$ ($R = 0.99897$), where x is the concentration of Cu^{2+} expressed in $\mu\text{g/ml}$ and y is the chemiluminescence intensity (cps unit). The limit of detection as defined by IUPAC, $C_{\text{LOD}} = 3S_b/m$ (where S_b is the standard deviation of the blank signals and m is the slope of the calibration graph) was found to be 0.005 $\mu\text{g/ml}$. The relative standard deviation (R.S.D) for 5 repeated measurements of 24.16 $\mu\text{g/ml}$ Cu^{2+} was 2.35%.

Interference studies

In a real sample, the analyte under investigation will be in the presence of interferences. They may suppress or enhance the CL, although they have no significant effect on the CL reaction of lophine. The influence of several metal ions on the determination of 0.0001M (24.16 $\mu\text{g/ml}$) Cu^{2+} by the proposed method was studied (Table 1). A foreign ion was considered to interfere seriously when it showed a determination error of more than 5%. From the table it is obvious that Mn^{2+} , Ni^{2+} , Cd^{2+} interfered to a lesser extent. The most important interferences occurred from Co^{2+} and Cr(VI) , although their concentrations are low relative to Cu in natural water. The rest of metal ions mentioned in the table have high tolerable concentration ratios. Therefore we can propose the method to be selective for the determination of Cu^{2+} .

Table 1. Tolerable concentration ratios with respect to Cu(II) for some interfering metal ions

Interferents	Tolerable concentration ratio
Zn^{2+} , Hg^{2+}	2500
Na^+ , K^+	1500
Mg^{2+} , Al^{3+} , As^{3+}	1000
Mn^{2+} , Ni^{2+} , Cd^{2+}	2.5
Ba^{2+} , Fe^{2+} , Fe^{3+}	250
Co^{2+} , Cr(VI)	1

Table 2. Determination of Cu²⁺ in water samples

Samples	Cu ²⁺ found (µg/L)	R.S.D (%)	Cu ²⁺ added (µg/L)	Cu ²⁺ recovered (µg/L)	Recovery (%)
^a River water	27.32	1.43	10	35.81	95.50
^b Sea water	78.26	0.60	10	83.250	94.30
^c Mineral water	33.06	1.54	10	42.75	99.5
^d Tap water	22.35	1.87	10	31.48	98.4
^e Deionized water	Not detected		10	9.50	95.0

^aNaktong River, in the Yongnam area of the provinces of North Kyongsang and South Kyongsang, southeastern South Korea.

^bSeaport, Pusan, South Korea

^cBonsagoan (Dormitory), Kyungpook National University, South Korea

^dKyungpook National University area, South Korea

^eSpectrochemical Analysis Laboratory, Kyungpook National University, South Korea

Application of proposed method

The method was applied to determine Cu²⁺ in different water samples and the results are shown in the Table 2. Tests of recovery efficiency for known amounts of Cu²⁺ in water samples were also made. The results shown in the Table 2 indicate that quantitative recoveries 94.3-99.5% were obtained by the proposed method. The results are seen to be satisfactory.

CONCLUSION

The Cu²⁺-lophine system gives a detection limit of 0.005 µg/ml and linearity of three orders of magnitude and the lophine CL method for Cu²⁺ speciation described herein is rapid, sensitive, chemically and instrumentally less complex and specific for real samples.

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