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Determination of copper(II) based on its catalytic effect on thiosemicarbazide–H_2O_2–CTMAB chemiluminescence reaction

M.H. Sorouraddin*, J.L. Manzoori, M. Iranifam

Analytical Chemistry Department, Faculty of Chemistry, Tabriz University, Tabriz, Iran

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Abstract

A novel chemiluminescence (CL) reaction, thiosemicarbazide (TSC)–H_2O_2, for the determination of copper at nanogram per milliliter level in batch type is described. The method is based on the catalytic effect of copper(II) on the oxidation of TSC with hydrogen peroxide to produce light emission. The emitted light was observed by using a conventional fluorescence detector. In the optimum conditions, calibration graph was linear in the range of 0.1–1.3 ppm. The limit of detection was 10 ppb. The relative standard deviation for five determinations of 0.5 ppm copper(II) was 1.93%. The proposed method permitted the selective and sensitive determination of Cu(II) in human hair and wheat flour with sufficient precision. The possible mechanism for the new chemiluminescence reaction was also discussed.

Keywords: Copper(II); Chemiluminescence; Thiosemicarbazide; Human hair; Wheat flour

1. Introduction

In the past two decades, the utilization of the kinetic-based methods including chemiluminescence (CL) reactions have markedly increased for the determination of trace amount of chemical species [1–6]. Most of these methods involve inhibition or catalysis of the redox reaction of CL reagents such as luminol, lucigenin, lophine and gallic acid. CL analysis is becoming increasingly important in various fields because of its simplicity of operation, low cost of instrumentation, high selectivity, wide dynamic range, reproducibility and rapidity [7].

Nakano and co-workers [2] have reviewed the CL methods for determination of Cu(II) with continuous and batch type procedures. Analysis of labile Cu(II) in fresh waters using the Cu(II)-catalyzed oxidation of 1,10 phenanthroline by superoxide anion radical has been investigated by Sangi et al. [4]. A flow injection method for the determination of Cu(II) based on coproporphyrin I-Cu(II)/TCPO/H_2O_2 chemiluminescence reaction for the screening of waters has been studied by Meseguer-Lloret et al. [3].

In spite of the above-mentioned merits, the application of the CL methods for the analysis of real samples is limited due to the lack of selectivity [8–11]. Therefore, new CL methods with better selectivity are to be developed. This paper reports a novel method, utilizing a new CL reagent, thiosemicarbazide, for determination of nanogram per milliliter amounts of copper, based on its catalytic activity on thiosemicarbazide (TSC)–H_2O_2 CL system in the presence of a surfactant.

TSCs could be considered as derivatives of thiourea and it is known that TSCs and their complexes are effective as bactericidal compounds [12]. As an analytical demonstration, trace amounts of copper(II) were conveniently detected and quantified in samples with complex matrix such as human hair and wheat flour. In this method, the reagents are stable, non-toxic and inexpensive. The analysis process is simple and can easily be automated. In addition, the possible mechanism was discussed for the new chemiluminescence reaction.

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* Corresponding author. Tel.: +98 912 1307417; fax: +98 411 3340191.
E-mail address: soruraddin@tabrizu.ac.ir (M.H. Sorouraddin).

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2. Experimental

2.1. Apparatus

The chemiluminescence measurements were made on a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) with closed excitation slit and using emission wavelength of 440 nm. Slit width of emission monochromator was set at 40 nm. All pH measurements were made with a digital pH meter (Metrohm 744).

2.2. Reagents and solutions

All the reagents used were of analytical-reagent grade. Doubly distilled water was used throughout. The stock standard solution of copper(II) (1 mg ml^{-1}) was prepared from copper(II) nitrate (Aldrich). A 0.1 M stock solution of TSC (Hopkin and Williams) was prepared in water. A 5% hydrogen peroxide solution was prepared by diluting 16.66 ml of the 30% H_2O_2 (Merck) to 100 ml. The buffer used for analytical determinations was 0.1 M $Na_2HPO_4{}^{-}$–NaOH (pH 12). Cetyltrimethylamonium bromide (CTMAB), sodium dodecyl sulfate (SDS) and Triton-X-100 were purchased from Merck (Darmstadt, Germany). A 2% (m/v) stock solution of CTMAB was prepared in doubly distilled water. Strong base anion exchanger (anion exchanger III, Merck) packed in Teflon column (70 mm length × 10 mm i.d.) was used for eliminating the anionic interferences.

2.3. General procedure

Light producing reactions were carried out in a 1.0 cm quartz cell placed in the spectrofluorimeter cell holder. A 2.0 ml portion of the sample solution containing 0.1–1.3 μg ml^{-1} Cu(II), 0.005 M TSC and 0.35% CTMAB in 0.1 M phosphate buffer (pH 12) (reagent 1) was added into the cell. Then 1.0 ml of 5% H_2O_2 solution (reagent 2) was injected to initiate CL reaction. The cover was closed and the progress of the CL was continuously monitored on a recorder connected to the spectrofluorimeter.

2.4. Sample treatment

2.4.1. Wet ashing of human hair

The procedure of wet ashing of human hair is described by Pournaghi-Azar and Dastangoo [13]. The sample was rinsed with acetone, chloroform and doubly distilled water and then dried at 60 °C. About 1 g of dried sample was accurately weighed and placed in a 50 ml beaker, and 12 ml concentrated HNO_3 and 2 ml concentrated $HClO_4$ were added. The contents in the beaker were heated on a hot plate (initially at 100 °C for 45 min and then at 150 °C for 45 min). After dissolution, the solution was cooled to 70 °C and 5 ml of 30% hydrogen peroxide was added. The mixture was heated to dryness at 200 °C to yield a whitish residue. About 10 ml of 0.1 M HNO_3 was added to the beaker and the contents were heated at 100 °C for 1 h and the volume was made in a 50 ml conical flask. This solution was passed through the ion exchanger III packed column with flow rate of 4 ml/min. The amount of Cu(II) in the collected solution was determined according to the general procedure and using standard addition method.

2.4.2. Wet ashing of wheat flour

The procedure for wet ashing of wheat flour is as published elsewhere [14,15]. Wheat flour was dried at 90 °C for 24 h and a portion of 5.0 g was digested with nitric acid and percloric acid, and heated gently on a hot plate to dryness. The ash was dissolved in water; the mixture was filtered and then diluted to 10 ml with distilled water. This solution was passed through the ion exchanger column with flow rate of 4 ml/min. The amount of Cu(II) in the collected solution was determined according to general procedure and using standard addition method.

3. Results and discussion

Preliminary studies indicated that upon injection of H_2O_2 into the mixed solution of Cu(II)–TSC-surfactant in basic media, CL occurred and emission intensity was dependent on Cu(II) concentration. The presence of surfactant was critical for obtaining CL emission, so that in the absence of surfactant, no detectable signal was observed. The effect of anionic (SDS), cationic (CTMAB) and non-ionic (Triton-X-100) surfactants on the CL emission was studied. It was observed that the CL intensity is highest in the presence of CTMAB; therefore, this surfactant was chosen further studies. The maximum emission wavelength of Cu(II)–TSC–CTMAB system was 440 nm.

3.1. Kinetic aspect

One of the most important characteristics of CL reaction is its kinetic profile. Fig. 1 shows the CL intensity–time profile for the new reaction used in this study. The height of profile was used as analytical signal for the rest of the study. This profile has been obtained for 1 μg ml^{-1} Cu(II), 0.35% CTMAB, 0.005 M TSC and 5% H_2O_2 at pH 13. As it can be seen from this figure, the maximum CL signal is observed after about 1 min of mixing of the reagents, and then the signal is decayed slowly.

3.2. Optimization of the CL reaction conditions

Several variables affecting the CL reaction were optimized to establish the most suitable reaction conditions. Fig. 2 shows the effect of pH on the CL intensity. As it can be seen, the CL reaction is critically dependant on pH of the solution and only occurs at pH values above 10. According to this figure, pH 12.0 gives the highest CL intensity and hence, was chosen as the optimum value. This pH was adjusted

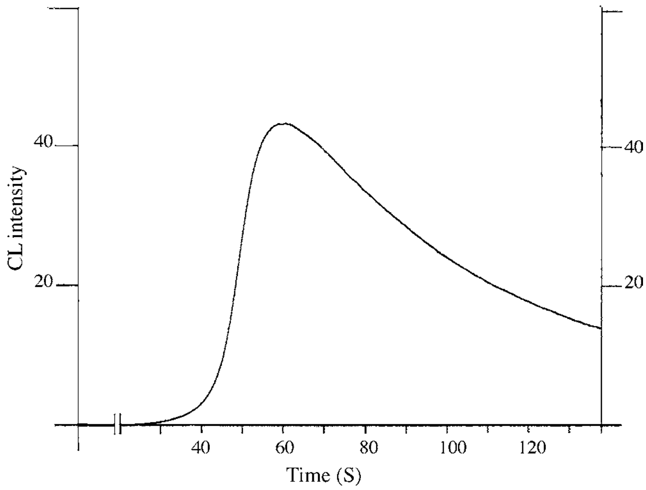


Fig. 1. Chemiluminescence (CL) emission intensity vs. time profile after mixing reagent 1 with reagent 2. Reagent 1: 1 ppm Cu(II), 0.35% CTMAB, 0.005 M TSC (pH 13); reagent 2: 5% H_2O_2.

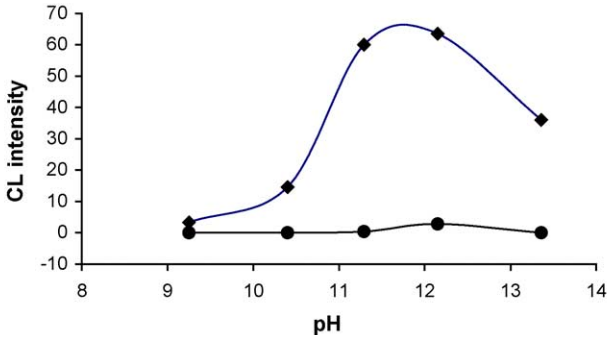


Fig. 2. The effect of pH (reagent1) on CL emission intensity in the presence (♦) or in the absence (●) of 1 ppm Cu(II). Reagent 1: 1 ppm Cu(II), 0.35% CTMAB, 0.005 M TSC; reagent 2: 5% H_2O_2.

with phosphate buffer. The concentration of this buffer did not affect the CL intensity up to 0.2 M. The effect of TSC concentration on the CL intensity is illustrated in Fig. 3. Maximum CL signal was attained at 0.005 M that was chosen for subsequent works. Fig. 4 shows the influence of hydrogen peroxide concentration on the CL reaction. As it can be seen, the CL intensity is increased with increasing H_2O_2 concentration up to 5% and reached a plateau at higher concentrations. Therefore, a 5% hydrogen peroxide was taken as optimum value.

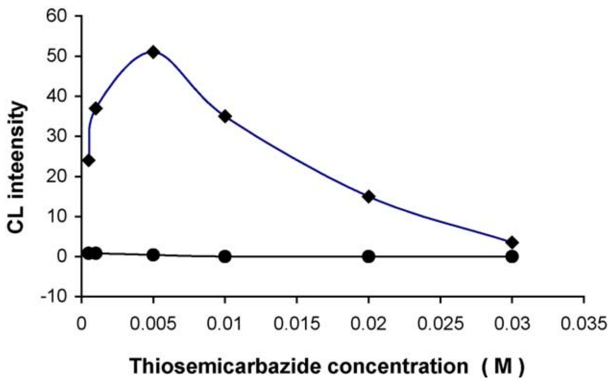


Fig. 3. The effect of TSC concentration (reagent1) on CL emission intensity in the presence (♦) or in the absence (●) of 1 ppm Cu(II). Reagent 1: pH 12, 1 ppm Cu(II), 0.35% CTMAB, TSC; reagent 2: 5% H_2O_2.

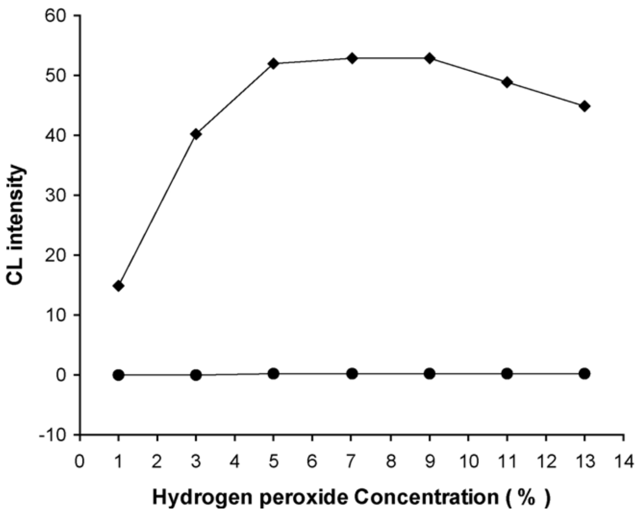


Fig. 4. The effect of H_2O_2 concentration (reagent2) on CL emission intensity in the presence (♦) or in the absence (●) of 1 ppm Cu(II). Reagent 1: pH 12, 1 ppm Cu(II), 0.35% CTMAB, 0.005 M TSC; reagent 2: H_2O_2.

The effect of concentration of CTMAB on the CL intensity was also investigated. The results are shown graphically in Fig. 5. According to this figure, 0.5% (m/v) was chosen as the most suitable concentration. The enhancement of CL in the presence of CTMAB micelles is probably due to more efficient reaction of TSC with $^{\bullet}O^{2-}$ radicals in the Stern layer of the micelles. This may arise because $^{\bullet}O^{2-}$ is attracted to the positively charged CTMAB micellar surface, forming a dioxetane intermediate, which initiates the reaction with TSC [4,16].

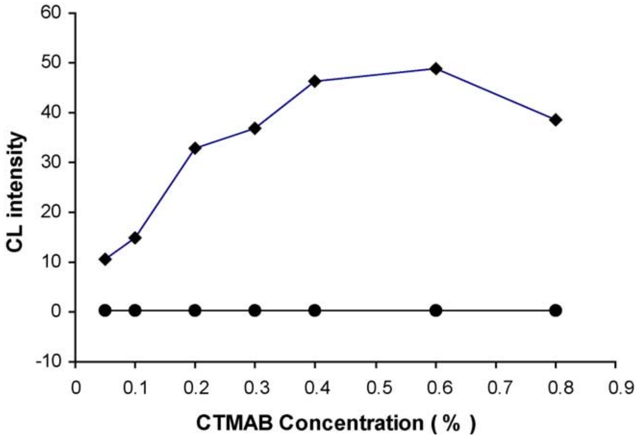


Fig. 5. The effect of CTMAB concentration (reagent 1) on CL emission intensity in the presence (♦) or in the absence (●) of 1 ppm Cu(II). Reagent 1: pH 12, 1 ppm Cu(II), 0.005 M TSC, CTMAB; reagent 2: 5% H_2O_2.

Table 1
Tolerable Concentration ratios with respect to Cu(II) for some interfering species

Substance	Tolerable concentration ratio
K^+	1000
Ba^{2+}, NH_4^+	500
Ca^{2+}, Mg^{2+}, Pb^{2+}	250
Al^{3+}, Sr^{3+}	100
Zn^{2+}	50
Fe^{3+}	25
Co^{2+}	5
Ni^{2+}, Mn^{2+}	1

3.3. Analytical parameters

Under the optimum conditions, the calibration graph was obtained for Cu(II) determination by using a series of seven standard solutions. The calibration-graph was found to be linear in the range of 0.1–1.3 $\mu g\,ml^{-1}$. The equation for calibration graph is $I = 0.025 + 50.62X$ ($r = 0.997$), where I is the CL intensity (arbitrary units) and X is the concentration of Cu(II) expressed in $\mu g\,ml^{-1}$. The limit of detection as defined by IUPAC, $C_{LOD} = 3S_b/m$ (where S_b is the standard deviation of blank and m is the slope of the calibration graph) was found to be 0.01 $\mu g\,ml^{-1}$. The relative standard deviation (R.S.D.) for 0.5 $\mu g\,ml^{-1}$ Cu(II) was 1.9% based on five replicated measurements.

3.4. Interferences

The influence of foreign ions on the determination of 0.3 $\mu g\,ml^{-1}$ Cu(II) by the proposed method was examined (Table 1). A foreign ion was considered to interfere seriously when it gave a determination error of more than 5%. From the results, it was shown that the most important interferences were from Mn(II) and Ni(II), while Fe(III), Co(II) and Zn(II) interfere to a lesser extent. On the other hand, the high concentrations of some anions, especially NO_3^-, interfere strongly with the CL reaction and cause precipitation. Passing the sample solution through an anion exchanger column can solve this problem.

3.5. Applications

We have examined the applicability of the proposed CL method for the selective determination of trace amount of copper in human hair and wheat flour as a typical example of application. Based on the results for interference described above and the reported concentration of metal ions in human hair and wheat flour [17,18], we concluded that selective determination of Cu(II) in human hair and wheat flour free from any interference was achieved by our novel CL system (after passing the sample through an anion exchanger column). The results obtained by the proposed method were compared with FAAS standard method (Table 2) by two-sided *t*-test [18]. Statistical comparison at the 95% confidence level showed no significant difference between the results obtained with the proposed procedure and those of the FAAS standard method.

Table 2
Results of the determination of Cu(II) in samples of human hair and wheat flour

Sample	Amount found[a] ($\mu g/g$)	
	Proposed method	FAAS
Human hair	24.7 ± 0.5	23.9 ± 0.2
Wheat flour	0.57 ± 0.02	0.55 ± 0.01

[a] Mean of three determinations.

3.6. Summery of possible CL reaction mechanism

Based on the mechanisms present in the literature for the similar compounds [4,20,21], the following mechanism is proposed as possible route for the new CL reaction. Transition metal ions that have two available oxidation states usually catalyze the radical decomposition of H_2O_2, so they can be detected by CL reaction [20]. The superoxide ion (anion radical) is produced by the radical decomposition of H_2O_2 [19,7]. We propose that the superoxide ion, $^{\bullet}O^{2-}$, reacts with TSC(I, II) to form an intermediate product (III), which emits light by fragmenting into two parts (IV, V). Enhancement of CL by added cationic micellular solutions (CTMAB) is thought to involve more efficient reaction of TSC with the $^{\bullet}O^{2-}$ in the Stern region of the micelles [16].

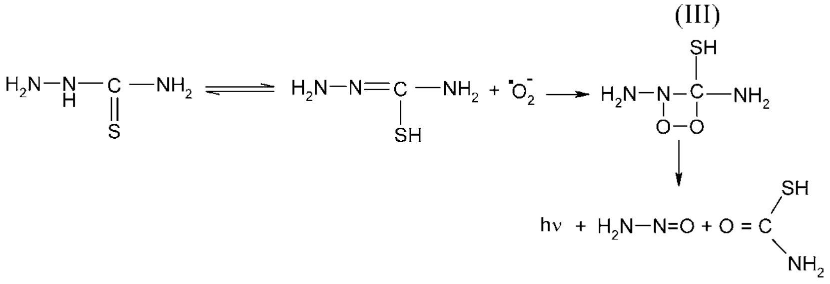
$$H_2O_2 \rightarrow\ 2^{\bullet}OH \tag{1}$$

$$H_2O_2 + Cu^{2+} \rightarrow\ 2^{\bullet}OH\ +\ Cu^{2+} \tag{2}$$

$$H_2O_2 \rightarrow\ HOO^- + H^+ \tag{3}$$

$$HOO\ +\ ^{\bullet}OH\ \rightarrow\ OH + ^{\bullet}O_2H \tag{4}$$

$$^{\bullet}O_2H\ \leftrightarrow\ ^{\bullet}O^{2-} + H^+ \tag{5}$$



(I) (II) (V) (IV)

4. Conclusions

The CL method proposed here is relatively simple and showed improved selectivity. Furthermore, it gives sufficient sensitivity without using an expensive CL reagent. The results indicated that the proposed CL reaction system is not only appropriate for automated flow systems but is also suitable for batch type systems due to the relatively long emission lifetime. Utilizing the proposed method, the copper content of human hair or wheat flour can be directly determined with reasonable selectivity and sensitivity, without any need for a preconcentration step. The sensitivity and detection limit can be further improved if an ultra sensitive Luminometer is employed.

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