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# Enhanced chemiluminescence of the luminol-hydrogen peroxide system by colloidal cupric oxide nanoparticles as peroxidase mimic

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## ABSTRACT

As a peroxidase mimic, cupric oxide nanoparticles were found to enhance the chemiluminescence (CL) of luminol-H<sub>2</sub>O<sub>2</sub> system up to 400 folds. The CL spectra and radical scavengers were conducted to investigate the possible CL enhancement mechanism. It was suggested that the enhanced CL could be attributed to the peroxidase-like activity of CuO nanoparticles, which effectively catalyzed the decomposition of hydrogen peroxide into hydroxyl radicals. The effects of the reactant concentrations and some organic compounds were also investigated. The proposed method could be used as a sensitive detection tool for hydrogen peroxide and glucose.

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## 1. Introduction

Owing to its attracting features such as high sensitivity, simple and inexpensive instrumentation, rapid analysis and low background signals, chemiluminescence (CL) has attracted extensive interest since the chemiluminescence phenomenon of luminol was first reported by Albrecht [1]. Chemiluminescence related analytical techniques have been developed as important and powerful tools in diverse fields, such as biotechnology, food analysis, environmental analysis, pharmaceutical analysis and clinical assay [2–7].

In contrast to bioluminescence systems, the CL emission generated during oxidation of organic molecules is of relatively low intensity due to low quantum yield. Enhancement of CL emission for the purpose of higher sensitivity is therefore necessary for application in trace analysis. Catalysts, such as transition metal ions and peroxidase, have normally been used as signal enhancers for that purpose [8–11]. In recent years, nanomaterials with unique redox catalytic properties have gained increasing attention as a novel alternative to catalyze redox CL reactions, providing amplified CL emission. Many investigations have indicated that the use of metal or semiconductor nanoparticles in CL reactions could provide new approaches to enhance the inherent

sensitivity and expand new applications of this mode of detection. For example, Cui et al. have reported many prominent works about noble metal nanoparticles-catalyzed CL systems. It has been demonstrated that gold, silver and platinum nanoparticles could greatly enhance a series of CL reactions including luminol-H<sub>2</sub>O<sub>2</sub> [12], luminol-K<sub>3</sub>Fe(CN)<sub>6</sub> [13], luminol-AgNO<sub>3</sub> [14], luminol-hydrazine [15], rhodamine 6G-cerium(IV) [16], Ce(IV)-Na<sub>2</sub>SO<sub>3</sub> [17], KIO<sub>4</sub>-NaOH/Na<sub>2</sub>CO<sub>3</sub> [18], lucigenin-KI [19], and tris(2,2'-bipyridyl)ruthenium-cerium (IV) [20]. In other cases, metal oxide nanoparticles, such as Fe<sub>2</sub>O<sub>3</sub> [21], ZnO [22], Co<sub>3</sub>O<sub>4</sub> [23], CoFe<sub>2</sub>O<sub>4</sub> [24], and CeO<sub>2</sub> [25], have also been applied to CL reactions.

Cupric oxide (CuO), a p-type semiconductor with a narrow band gap (1.2 eV), has been studied intensely because of its interesting properties and widespread applications in heterogeneous catalysis [26], high-temperature superconductor [27], battery [28], gas sensor [29], biosensor [30,31], field-emission emitter [32], and so forth. In recent study, we have found that cupric oxide nanoparticles exhibited significant peroxidase-like activity [33]. Furthermore, these cupric oxide nanoparticles are considerably more stable and possess an almost unchanged catalytic activity over a wide range of pH and temperatures. However, to the best of our knowledge, there are no reports exploring the catalytic property of cupric oxide nanoparticles in liquid-phase CL reactions.

Luminol-H<sub>2</sub>O<sub>2</sub> CL reaction, a popular CL reaction, has been widely applied for the detection of various substances [25,34–38]. In this work, we chose the luminol-H<sub>2</sub>O<sub>2</sub> CL reaction as a model

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system and explored the effect of colloidal solutions of cupric oxide nanoparticles on the CL for the first time. It was found that CuO nanoparticles could greatly enhance the CL signal of luminol-H<sub>2</sub>O<sub>2</sub> system. A possible enhancement mechanism was discussed. Based on the effect of cupric oxide nanoparticles on the luminol-H<sub>2</sub>O<sub>2</sub> CL system, the feasibility of using the proposed method for H<sub>2</sub>O<sub>2</sub> and glucose detection was studied. Under optimum conditions, the CL intensity was linear with H<sub>2</sub>O<sub>2</sub> concentration. When the catalytic reaction was coupled with the glucose catalytic oxidation reaction by glucose oxidase (GOx), a simple, low-cost, and sensitive CL glucose biosensing system was constructed.

## 2. Experimental

### 2.1. Reagents and materials

All chemicals and reagents were of analytical grade and used without further purification, and ultra pure water was used throughout. Cupric acetate, sodium hydroxide, glucose and 30% (v/v) H<sub>2</sub>O<sub>2</sub> were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glucose oxidase (GOx) was purchased from Aladdin Reagent Company (Shanghai, China). A  $1.0 \times 10^{-2}$  M stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving luminol (Sigma) in 0.1 M sodium hydroxide solution. Working solutions of luminol were prepared by diluting the stock solution. Working solutions of H<sub>2</sub>O<sub>2</sub> were prepared fresh daily by dilution of 30% (v/v) H<sub>2</sub>O<sub>2</sub>. A 20 mM stock solution of glucose was allowed to mutarotate at 4 °C overnight before use. The standard glucose solutions were prepared by proper dilution of the stock. Clinical serum samples were provided by the Second Hospital of Fuzhou.

### 2.2. Synthesis of cupric oxide nanoparticles

The cupric oxide nanoparticles were prepared via a previously reported quick-precipitation method [39]. Briefly, 150 mL of 0.02 M copper acetate aqueous solution was mixed with 0.5 ml glacial acetic acid in a round-bottomed flask equipped with a refluxing device. The solution was heated to boiling with vigorous stirring. Then 10 mL of 0.04 g mL<sup>-1</sup> NaOH aqueous solution was rapidly added into the above boiling solution, where a large amount of black precipitate was simultaneously produced. The precipitate was centrifuged, washed three times with absolute ethanol, and dried in air at room temperature.

The morphology and size distribution of the synthesized CuO nanoparticles were characterized by using a transmission electron microscope (TEM) of Tacnai-12 (Philips, Netherlands). Statistical analysis of TEM data revealed that the product consists of spherical particles with a regular morphology and significantly narrow size distribution. The average particle diameter is found to be approximately 6.0 nm.

The as-prepared CuO nanoparticles, even without any surface modification, can well disperse in distilled water to form a transparent brown solution. Both the appearance and the UV-vis absorption spectrum of the solution remains unchanged even after 6 months, which plays an important role in our later flow injection chemiluminescence system.

### 2.3. General procedure for CL detection

The chemiluminescence detection was conducted on a model IFFM-E flow injection CL system (Xi'an Remax Company, Xi'an, China). Two peristaltic pumps with three channels were used to deliver the reactants to the flow cell. One peristaltic pump was used to deliver CuO NPs and H<sub>2</sub>O<sub>2</sub> (or samples) solution with two

channels and the other pump was used to carry luminol solution at 1.6 mL/min. The CL signals were monitored by a PMT placed close to the flow cell and the output signals were obtained by a computer automatically. Data acquisition and treatment were performed with REMAX software running under Windows XP. All the studies, including selectivity, inhibition compounds and analytical application, were realized in the flow system. When the CL system was used to investigate the effect of organic compounds and free radical scavengers, one peristaltic pump was used to deliver CuO NPs and the mixture of H<sub>2</sub>O<sub>2</sub> and luminol with two channels and the other pump was used to carry organic compound or free radical scavenger at 1.6 mL/min, respectively.

### 2.4. Glucose determination in real serum samples

The serum samples were pretreated by ultrafiltration to remove protein before glucose assay. Glucose determination was carried out by adding 250 μL pretreated human serum sample, 50 μL of 2 mg mL<sup>-1</sup> GOx, and 200 μL of 10 mM phosphate buffer solution (pH 5.5) into an EP tube. The mixture was then incubated at 37 °C for 30 min to yield the testing sample solution. Before CL testing, the sample solutions were diluted 100 times by water. The results were compared with that of the glucose oxidase endpoint method by using automatic biochemical analyzer, a widely used clinical method in hospitals for glucose assay. Standard addition experiments were further conducted in the real serum samples, in which different amounts of external glucose were added and the recoveries were calculated.

## 3. Results and discussion

### 3.1. Enhancement of luminol CL

The effects of cupric oxide nanoparticles on the luminol-H<sub>2</sub>O<sub>2</sub> chemiluminescent system were investigated. As shown in Fig. 1, the kinetic curve shows that the oxidation of luminol by H<sub>2</sub>O<sub>2</sub> generates weak CL in alkaline media. In presence of cupric oxide nanoparticles, the CL signal intensity could be greatly enhanced up to about 400 folds. As compared with other nano-catalysts reported in the literatures, the cupric oxide nanoparticles exhibit the highest catalytic activity on the luminol-H<sub>2</sub>O<sub>2</sub> CL system to the best of our knowledge (Table 1).

In order to explore the CL-enhancing phenomena, the CL spectra for cupric oxide colloids mixed with luminol-H<sub>2</sub>O<sub>2</sub> was acquired. It was clearly indicated in Fig. 2 that the maximum emission of the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system was ~425 nm, revealing that the luminophor was still the excited-state 3-aminophthalate

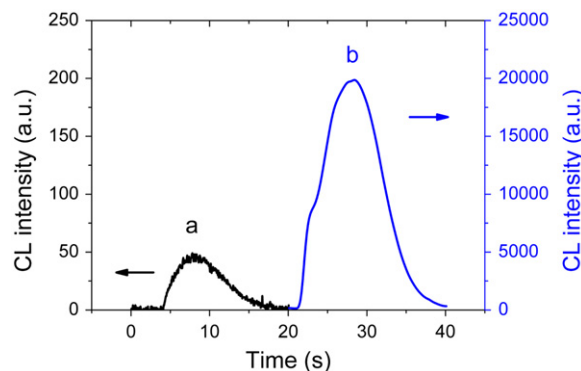
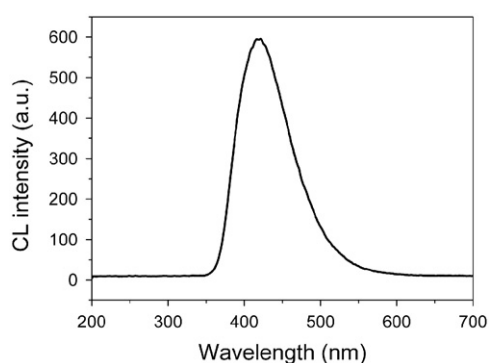


Fig. 1. Kinetic characteristics of (a) the luminol-H<sub>2</sub>O<sub>2</sub> CL system and (b) the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system. Luminol solution:  $5.0 \times 10^{-4}$  M, pH 11.5. H<sub>2</sub>O<sub>2</sub> solution:  $3.0 \times 10^{-6}$  M. CuO colloids: 2.0 mg L<sup>-1</sup>.

anions (3-APA\*) [43,44]. Therefore, the addition of cupric oxide nanoparticles did not lead to the generation of a new luminophor for this CL system. The enhanced CL signals were thus ascribed to the possible catalysis from cupric oxide nanoparticles.

**Table 1**  
Enhancement factor of various nanoparticles on luminol-H<sub>2</sub>O<sub>2</sub> CL system.

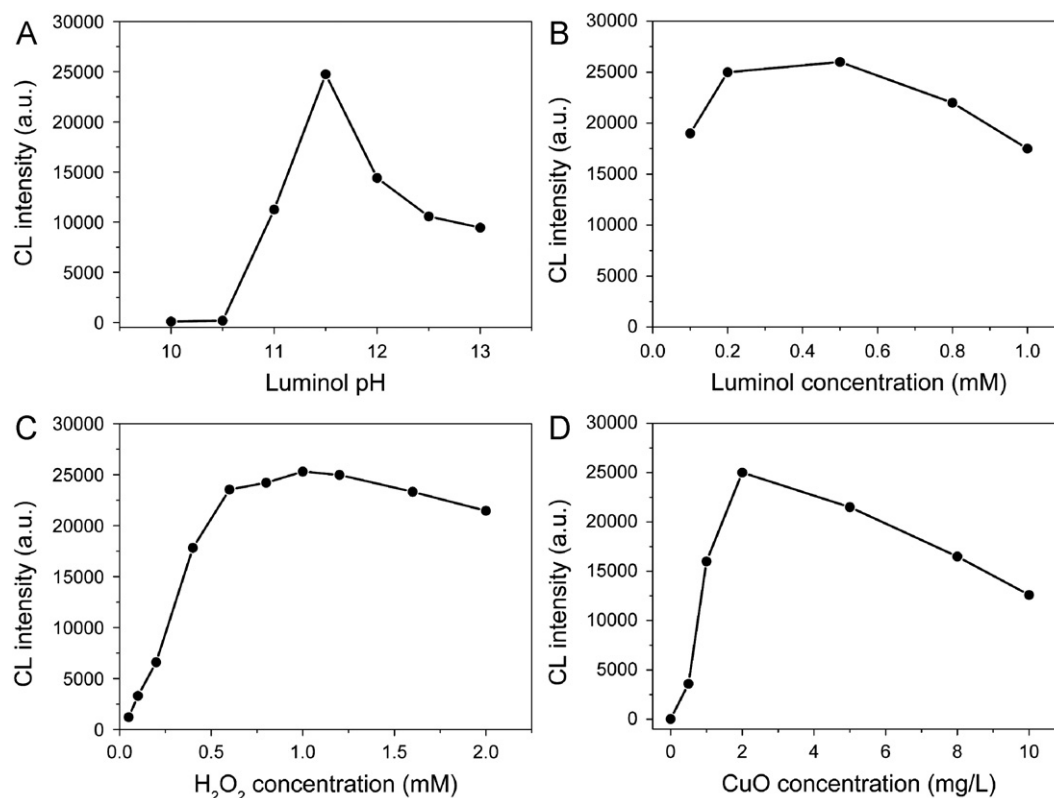
Nanoparticle catalyst	Enhancement factor	Literature
Au NPs	100	[12]
Pt NPs	120	[40]
Ag NPs	3–10	[41]
Au–Ag alloy NPs	5	[42]
Fe <sub>2</sub> O <sub>3</sub> NPs	18	[21]
ZnO NPs	6	[22]
CoFe <sub>2</sub> O <sub>4</sub> MNPs	50	[24]
CeO <sub>2</sub> NPs	22.5	[25]
CuO NPs	400	This work



**Fig. 2.** Chemiluminescence spectra for luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids system.

### 3.2. Optimization of reaction condition

The reaction conditions were optimized for the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system as shown in Fig. 3. The pH of luminol solution played an important role in the CL reaction, so the effect of pH on the CL was firstly studied in the pH range from 10.0 to 13.0. As shown in Fig. 3A, the maximum CL intensity was obtained at pH 11.5 in sodium hydroxide solution. When the pH of luminol solution was higher than 11.5, the CL intensity decreased dramatically. The effect of luminol concentration was investigated from  $1.0 \times 10^{-4}$  to  $1.0 \times 10^{-3}$  M. It was found that the CL intensity increased with the luminol concentration from  $1.0 \times 10^{-4}$  to  $5 \times 10^{-4}$  M. A higher concentration of luminol produced self-absorption of the emitted radiation and decreased the CL intensity (Fig. 3B). The effect of H<sub>2</sub>O<sub>2</sub> concentration on the CL was studied in the range of 0.05–2.0 mM (Fig. 3C). The CL intensity increased with increasing H<sub>2</sub>O<sub>2</sub> concentration in the range of 0.05–1.0 mM and slightly decreased when the concentration of H<sub>2</sub>O<sub>2</sub> was larger than 1.0 mM. The effect of concentration of cupric oxide nanoparticles was also investigated. As shown in Fig. 3D, the CL intensity increased with the concentration of cupric oxide nanoparticles and reached a maximum CL emission by increasing concentration of cupric oxide nanoparticles up to  $2.0 \text{ mg L}^{-1}$ . With higher concentration of cupric oxide nanoparticles, the CL intensity decreased dramatically. Considering the CL intensity and the consumption of the reagents, the optimized conditions for the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system were as follows:  $5 \times 10^{-4}$  M luminol in NaOH solution (pH 11.5), 1.0 mM H<sub>2</sub>O<sub>2</sub>, and  $2.0 \text{ mg L}^{-1}$  cupric oxide colloids. By carefully controlling the experimental conditions, the between-run relative standard deviation of the newly developed method is less than 4%, which demonstrates high robustness of the method.



**Fig. 3.** Effects of the (A) luminol pH, (B) luminol concentration, (C) H<sub>2</sub>O<sub>2</sub> concentration, and (D) CuO concentration on the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system.

### 3.3. Mechanism discussion

The CL-generation mechanism for luminol oxidation in aqueous solution has been extensively studied. Some important oxygen-related radicals such as superoxide radical anion  $O_2^-$  and the hydroxyl radical  $OH\cdot$ , have been reported to be important intermediates leading to luminescence [45,46]. As for the luminol- $H_2O_2$  system, such oxygen-related radicals were supposed to be generated from  $H_2O_2$ . In the absence of a catalyst, the reaction of luminol with hydrogen peroxide in alkaline solution is relatively slow, leading to relatively weak CL. Therefore, it is assumed that the enhanced CL by cupric oxide nanoparticles may be ascribed to their interaction with the reactants or the intermediates of the reaction of luminol with hydrogen peroxide. As a peroxidase mimetic, cupric oxide nanoparticle might break up the O–O bond of  $H_2O_2$  into two hydroxyl radicals. Then the resulting hydroxyl radicals reacted with luminol anion and  $HO_2^-$  to facilitate the formation of luminol radicals and superoxide radical anions, which further reacted with each other to form the excited-state 3-aminophthalate anions. It was also possible that oxygen dissolved in the solution reacted with luminol radicals to generate superoxide radical anions [46]. Deaeration experiments supported that the dissolved oxygen was involved in the CL reaction because significant changes in CL intensity were observed when  $N_2$  was bubbled into the reactant solutions for 20 min before the reaction.

To obtain further insight into the mechanism of the CL system, the effects of various active oxygen radical scavengers were investigated. Ascorbic acid is a well-known common free radical scavenger. As a classical reducing agent, ascorbic acid can terminate active oxygen radicals by electron transfer. Quenching of the CL was observed in the presence of ascorbic acid. It confirmed the radical reaction mechanism of the proposed CL system, in which the generation of free radicals appeared to be the critical controlling factors.

In order to further confirm the generation of  $O_2^-$  and  $OH\cdot$  in the CL reaction, superoxide dismutase (SOD) and thiourea [47] were introduced into the luminol- $H_2O_2$ -CuO NPs system. SOD catalyzes

the dismutation reaction of superoxide anion radicals to give ground state molecular oxygen and  $H_2O_2$ . Therefore, CL signals from the reaction involving superoxide anion radicals can be halted by the addition of SOD to the CL systems. The experimental results show the inhibition effect of SOD on the luminol- $H_2O_2$ -CuO nanoparticles system, which confirmed that superoxide anion radicals were involved in the CL process. Hydroxyl radical is always considered to be one of the most potent oxidizers among the oxygen-centered free radicals. Thiourea is an effective radical scavenger for hydroxyl radical. The inhibited CL signals were measured, respectively, after the addition of thiourea at different concentrations to the CL system. The results showed that percentage inhibition for thiourea increased with its concentration, confirming the role of hydroxyl radical in the CL process.

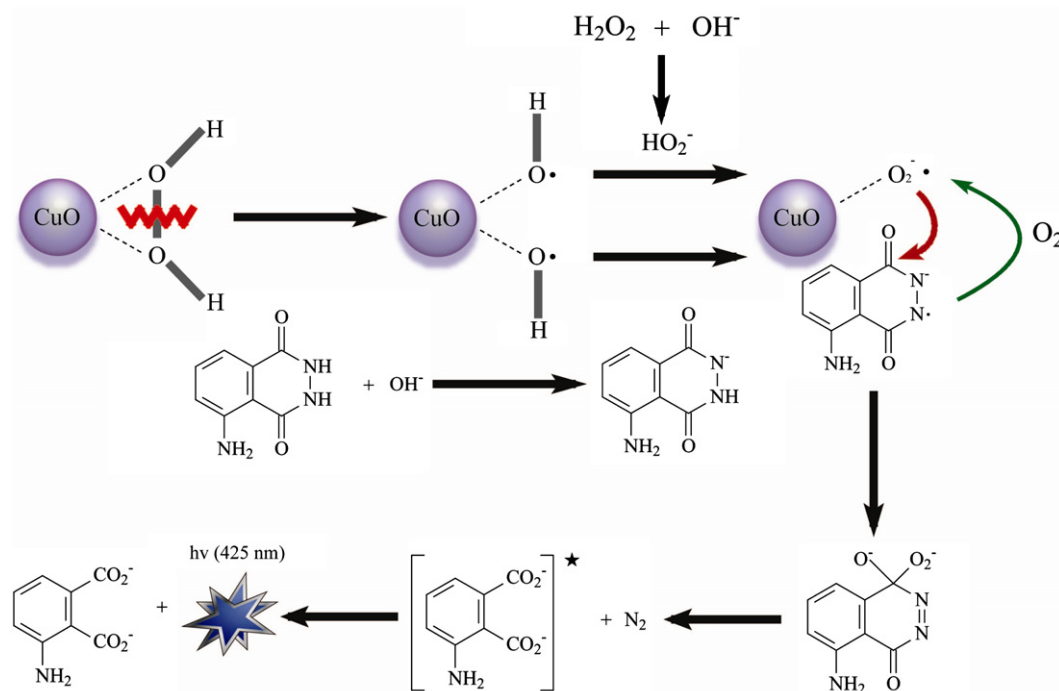
On the basis of the above results, the whole enhance mechanism is summarized in Scheme 1.

### 3.4. Inhibition effects of organic compounds

It has been reported that the reducing groups of OH,  $NH_2$ , or SH are likely to compete with luminol for the oxygen-containing intermediate radicals, leading to a decrease in CL intensity [22]. On the other hand, these compounds may interact with cupric oxide nanoparticles to reduce the active surface area, interrupting the formation of luminol radicals and hydroxyl radicals taking place on the surface of nanoparticles. Herein, the effects of some organic compounds containing groups of OH,  $NH_2$ , or SH on the luminol- $H_2O_2$ -CuO colloids CL system were investigated. The results were listed in Table 2. As expected, all the tested compounds inhibited the CL signal of luminol- $H_2O_2$ -CuO colloids system. In addition, the inhibition percentage varied with the species and concentration of the compounds.

### 3.5. Analytical applications

The analytical determination of hydrogen peroxide is of considerable importance for medical diagnosis since hydrogen peroxide is formed as an intermediate product in the case of a large



Scheme 1. Possible mechanism for the luminol- $H_2O_2$ -CuO colloids CL system.

number of important detection processes. The possibility of using the proposed method for the determination of hydrogen peroxide was investigated. Under the optimum condition as described above, the log–log calibration graph of relative CL emission intensity versus hydrogen peroxide concentration was linear in the  $1.0 \times 10^{-7}$ – $5.0 \times 10^{-6}$  M range. The regression equation was  $\log \Delta I = 13.71 + 1.722 \log C$  (M),  $r = 0.9984$  ( $n = 10$ ). The limit of detection (LOD) for hydrogen peroxide was  $1.1 \times 10^{-8}$  M. The RSD was 3.3% for  $1.0 \times 10^{-6}$  M hydrogen peroxide ( $n = 6$ ). As compared with the analytical methods previously published in literatures using luminol-H<sub>2</sub>O<sub>2</sub> CL system with or without nanoparticles in terms of LODs (Table 3), the proposed method shows high sensitivity for H<sub>2</sub>O<sub>2</sub> analysis.

Reliable and fast determination of glucose is of considerable importance in biotechnology, clinical diagnostics and food industry. Various techniques such as optical sensor [54], fluorescence [55], phosphorescence [56], chemiluminescence [57], electrochemiluminescence [58], and electrochemistry [59] have been applied in glucose determination. Since hydrogen peroxide is one of the products of the glucose oxidase (GOx)-catalyzed reaction, CL detection of glucose could therefore be realized by coupling the

luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids system with the glucose catalytic reaction by GOx. Glucose determination was carried out by adding 250  $\mu$ L of glucose of different concentrations, 50  $\mu$ L of 2 mg mL<sup>-1</sup> GOx, and 200  $\mu$ L of 10 mM phosphate buffer solution (pH 5.5) into an EP tube. The mixture was then incubated at 37 °C for 30 min to yield the testing sample solution. Before CL testing, the sample solutions were diluted 100-times by water. The results of measurements are shown in Fig. 4. The linear range for glucose is from  $5 \times 10^{-6}$  to  $6 \times 10^{-5}$  M, and the limit of detection is  $2.9 \times 10^{-6}$  M. The regression equation is  $\Delta I = 296.87C - 855.28$  (where C is the glucose concentration,  $\times 10^{-6}$  M) with a correlation coefficient of 0.9981 ( $n = 7$ ). The RSD was 2.8% for  $3 \times 10^{-5}$  M glucose ( $n = 6$ ).

The selectivity experiments were carried out by using a series of solutions containing  $3 \times 10^{-5}$  mol/L glucose plus various amounts of maltose, lactose, fructose or sucrose. The tolerance limit was estimated as less than 5% of the error. No clear interference could be found when 5 folds maltose was added into the CL reaction. More than 2 folds lactose or fructose could decrease the CL signal intensity, possibly because lactose and fructose can reduce H<sub>2</sub>O<sub>2</sub> produced from the enzymatic reaction. However, equal amount of sucrose could increase the CL signal due to the excess glucose produced by sucrose hydrolysis in acidic environment.

To evaluate the feasibility of the sensing system for analysis of glucose in biological samples, the developed method was applied to the determination of glucose in human serum samples. The results are listed in Table 4. From Table 4, it can be seen that the results obtained by the proposed method were in good agreement with those measured by glucose oxidase endpoint method. The practical applicability of the proposed method was further

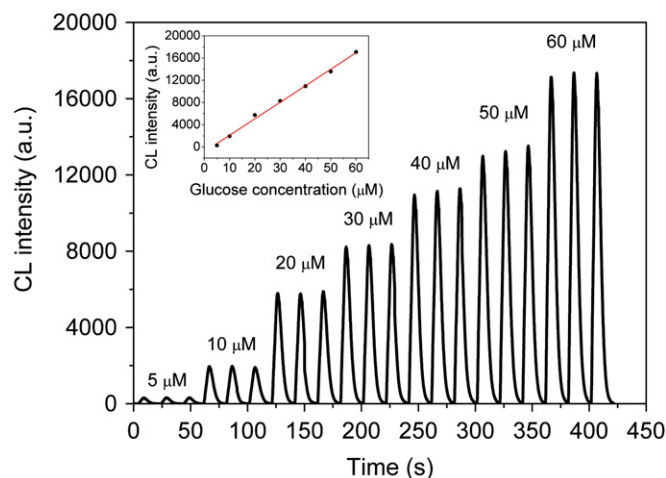
**Table 2**  
Inhibition effects of organic compounds ( $1.0 \times 10^{-4}$  and  $1.0 \times 10^{-5}$  M) on luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system<sup>a</sup>.

Organic compounds	Quenching (%)	
	$1.0 \times 10^{-4}$ M	$1.0 \times 10^{-5}$ M
Catechol	0	1.1
Resorcinol	4.7	87.9
Hydroquinone	17.2	77.9
Pyrogallol	0.1	0.7
Ascorbic acid	0.1	16.8
Dopamine	0	12.8
4-aminophenol	21.8	81.9
L-alanine	7.3	93.9
L-glutamic acid	5.1	53.9
L-glycine	7.5	90.0
L-leucine	7.5	98.7
L-isoleucine	3.0	85.9
L-serine	4.1	92.2
L-arginine	13.9	80.0
L-phenylalanine	13.4	100
L-threonine	0.6	47.5
L-tryptophan	3.8	83.8
L-histidine	1.2	29.9
L-methionine	10.5	77.5
L-valine	5.3	89.9
L-proline	0.6	78.8
L-aspartic acid	1.4	74.6
tannin	0	0

<sup>a</sup> The percentage of quenching was calculated as  $I/I_0$ . The blank CL signal  $I_0$  was obtained by luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids CL system without the tested organic compounds.

**Table 3**  
Comparison of this work with some established methods using chemiluminescence for hydrogen peroxide.

System	Detection limit ( $\mu$ M)	Linear range (M)	Reference
Hexacyanoferrate(III)-luminol-H <sub>2</sub> O <sub>2</sub>	1.8	$2.2 \times 10^{-6}$ – $2.1 \times 10^{-4}$	[48]
Immobilized Co <sup>2+</sup> and sodium lauryl sulfate-luminol-H <sub>2</sub> O <sub>2</sub>	0.26	$5.8 \times 10^{-7}$ – $4.7 \times 10^{-5}$	[49]
KIO <sub>4</sub> -luminol-H <sub>2</sub> O <sub>2</sub>	0.03	$2.0 \times 10^{-7}$ – $6.0 \times 10^{-4}$	[50]
Immobilized HRP-luminol-H <sub>2</sub> O <sub>2</sub>	670	$1.0 \times 10^{-4}$ – $3.0 \times 10^{-3}$	[51]
Ferric oxide nanoparticles-luminol-H <sub>2</sub> O <sub>2</sub>	1250	$2.0 \times 10^{-3}$ – $1.0 \times 10^{-1}$	[21]
Au nanoparticles-Hb/PMMA-luminol-H <sub>2</sub> O <sub>2</sub>	0.2	$1.0 \times 10^{-6}$ – $1.0 \times 10^{-4}$	[52]
Au nanoflower-luminol-H <sub>2</sub> O <sub>2</sub>	10	$3.0 \times 10^{-5}$ – $3.0 \times 10^{-3}$	[53]
$\beta$ -CD/CoFe <sub>2</sub> O <sub>4</sub> MNPs-luminol-H <sub>2</sub> O <sub>2</sub>	0.02	$1.0 \times 10^{-7}$ – $4.0 \times 10^{-6}$	[24]
CuO nanoparticles-luminol-H <sub>2</sub> O <sub>2</sub>	0.01	$1.0 \times 10^{-7}$ – $5.0 \times 10^{-6}$	This work



**Fig. 4.** CL intensity of the luminol-Glucose/GOx-CuO colloids system in presence of different concentration of glucose under the optimized conditions. Inset: calibration curve for glucose.

**Table 4**  
Analytical results of glucose in human serum.

Serum samples	Proposed method (mM)	Glucose oxidase endpoint method (mM)
Sample 1	4.37 ± 0.02	4.32 ± 0.13
Sample 2	5.65 ± 0.22	5.74 ± 0.17
Sample 3	11.14 ± 0.17	11.08 ± 0.33
Sample 4	14.91 ± 0.35	15.23 ± 0.46

verified through standard addition experiments, with the recoveries of glucose in three serum samples ranging from 94.9% to 99.3%. The results demonstrate that this new method is suitable and satisfactory for glucose analysis of real samples.

#### 4. Conclusions

In summary, we have found that cupric oxide nanoparticles could enhance the luminol-H<sub>2</sub>O<sub>2</sub> CL signals greatly up to 400 folds. The enhancement of CL was suggested to attribute to the peroxidase-like activity of CuO nanoparticles, which effectively catalyzed the decomposition of hydrogen peroxide into hydroxyl radicals. Some organic compounds containing hydroxyl, amino, or mercapto groups interacting with oxygen-containing intermediate radicals and/or cupric oxide nanoparticles were observed to inhibit the CL signals of the luminol-H<sub>2</sub>O<sub>2</sub>-CuO colloids system at the experimental conditions, which could be potentially applied in the analysis of these compounds. In addition, the proposed novel CuO-based enzyme nano-mimic CL method was used successfully for H<sub>2</sub>O<sub>2</sub> and glucose detection. This work is believed to be beneficial for the insight of the enzyme nano-mimics and their potential applications in CL and bioanalysis.

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