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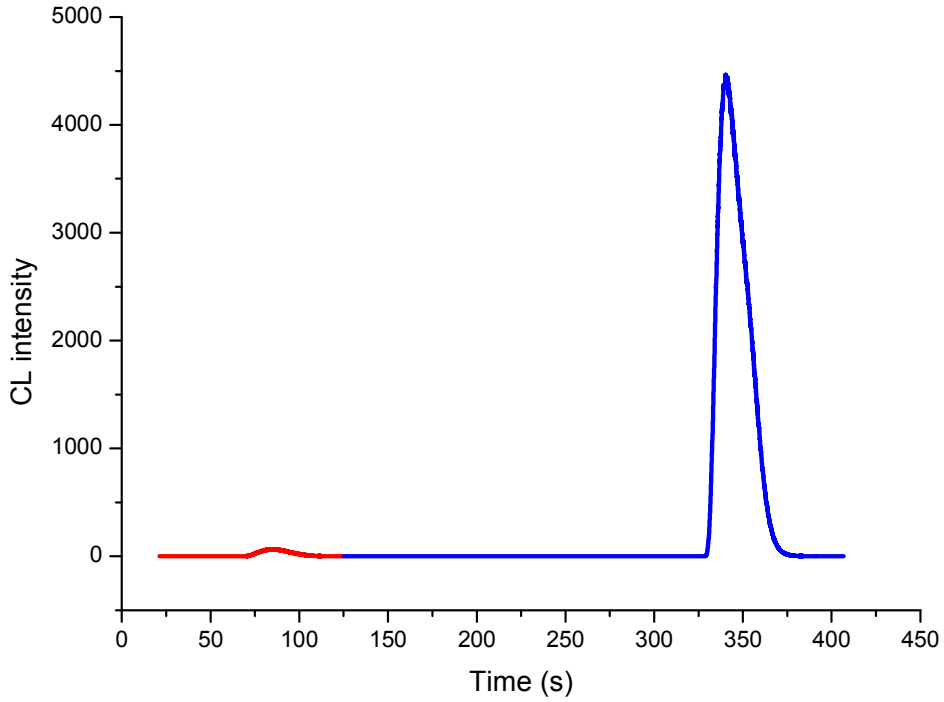
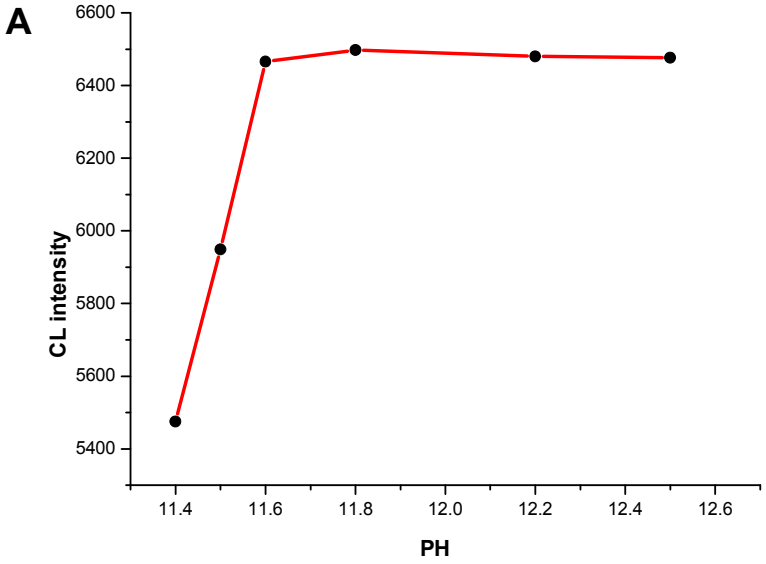


Figure 1. Kinetic curves of chemiluminescence systems: The red line: luminol-H_2O_2; The blue line: luminol-H_2O_2-Cu NCs. Luminol: 5×10^{-5} M in pH 11.8 (sodium hydroxide solution); H_2O_2: 0.15 M; Cu NCs: 12.8 mg L^{-1}.



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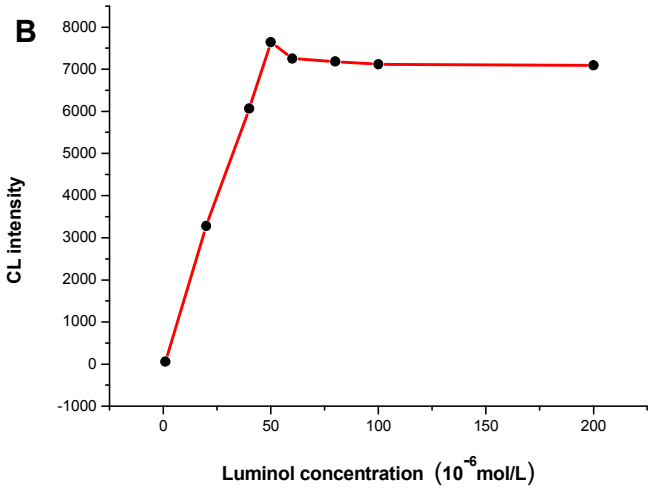

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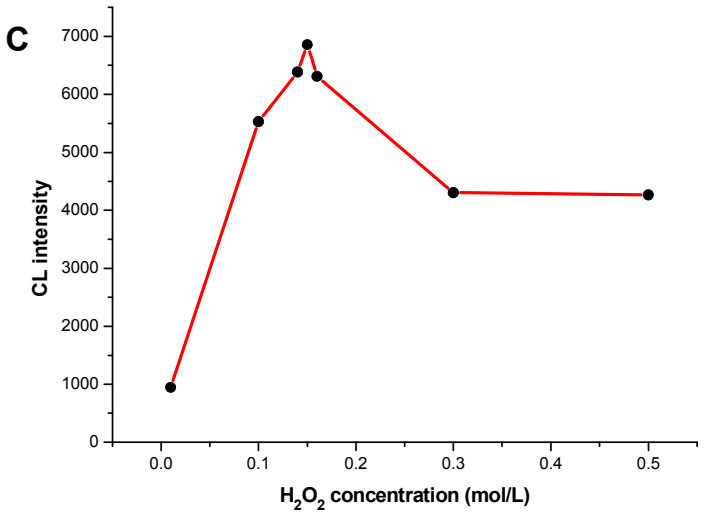

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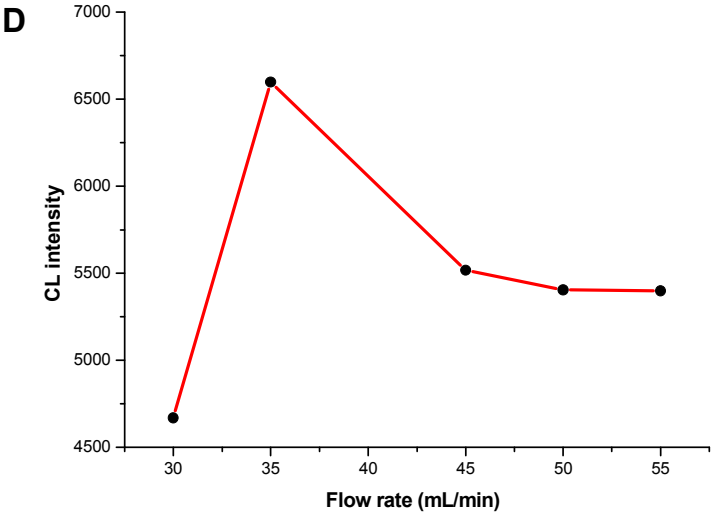

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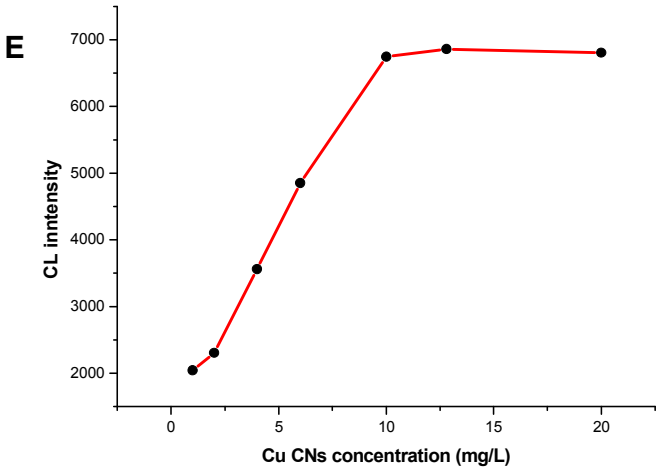


Figure 2. Effects of the reaction conditions on the luminol-H2O2-Cu NCs CL system. (A) Effects of pH :Luminol: 1×10^{-5} M; H_2O_2: 0.15 M; Cu NCs: 12.8 mg L^{-1} (B) Effect of luminol concentration: pH: 11.8; H_2O_2: 0.15 M; Cu NCs: 12.8 mg L^{-1} (C) Effect of H_2O_2 concentration : PH:11.8; Luminol: 5×10^{-5} M; Cu NCs: 12.8mg L^{-1} (D) Effect of flow rate: Luminol: 5×10^{-5} M; H_2O_2: 0.15 M; pH: 11.8. (E)Effect of Cu NCs: Luminol: 5×10^{-5} M; H_2O_2: 0.15 M; pH: 11.8.

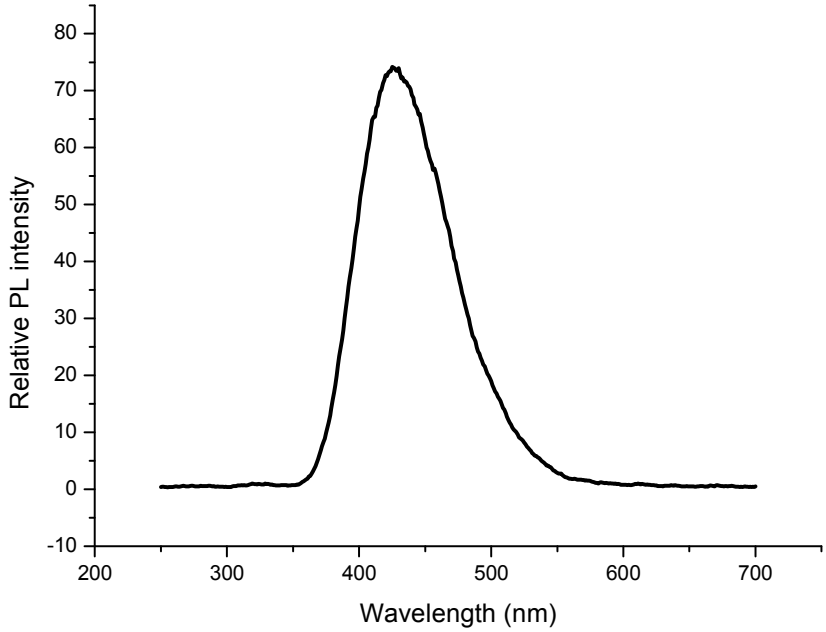


Figure 3. Chemiluminescence spectra for luminol-H_2O_2-Au NCs system. Luminol: 5×10^{-5} M in pH 11.8 (sodium hydroxide solution); H_2O_2: 0.15 M; Cu NCs: 12.8 mg L^{-1}.

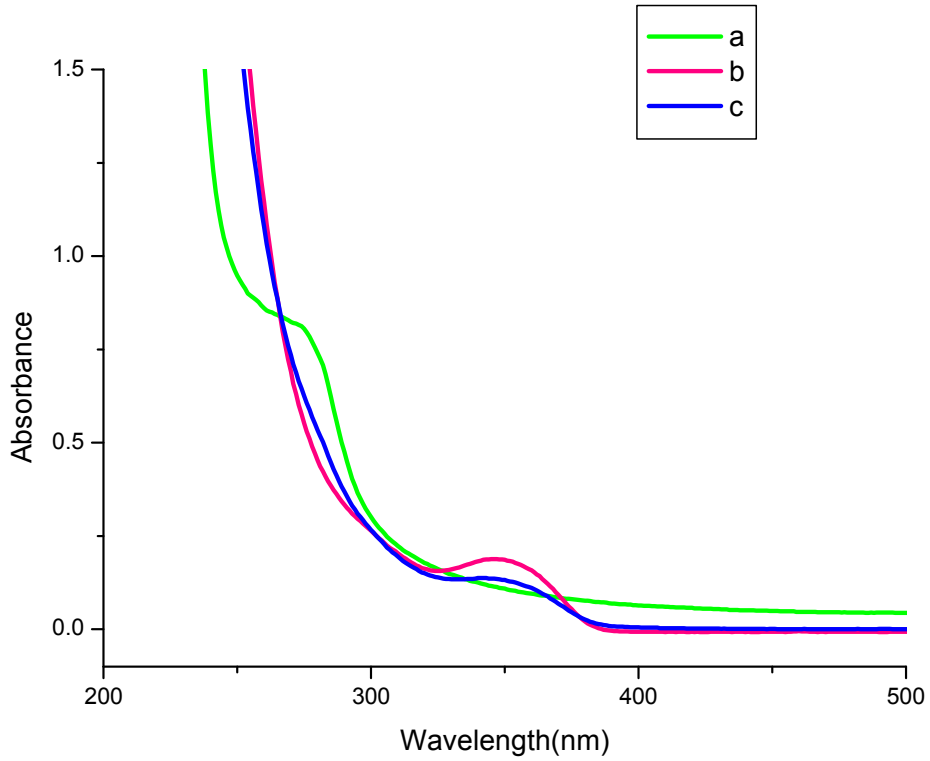


Figure 4. UV-visible absorption spectra of (a) Cu NCs; (b) luminol-H_2O_2-Cu NCs; (c) luminol-H_2O_2- H_2O.

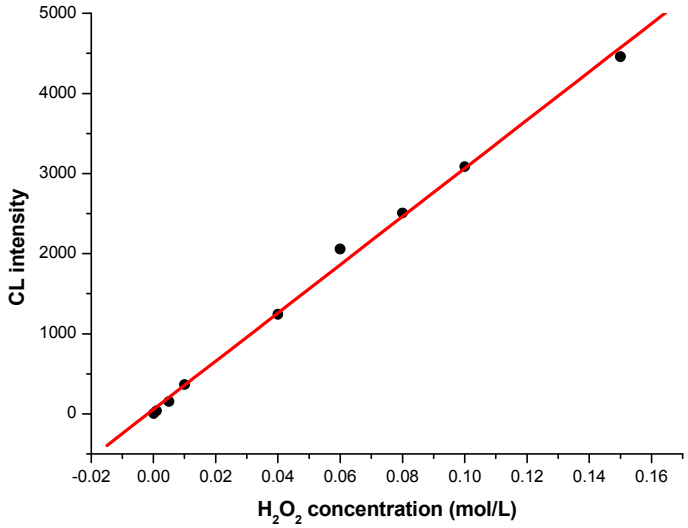


Figure 5. Standard calibration curve for H_2O_2 assay.

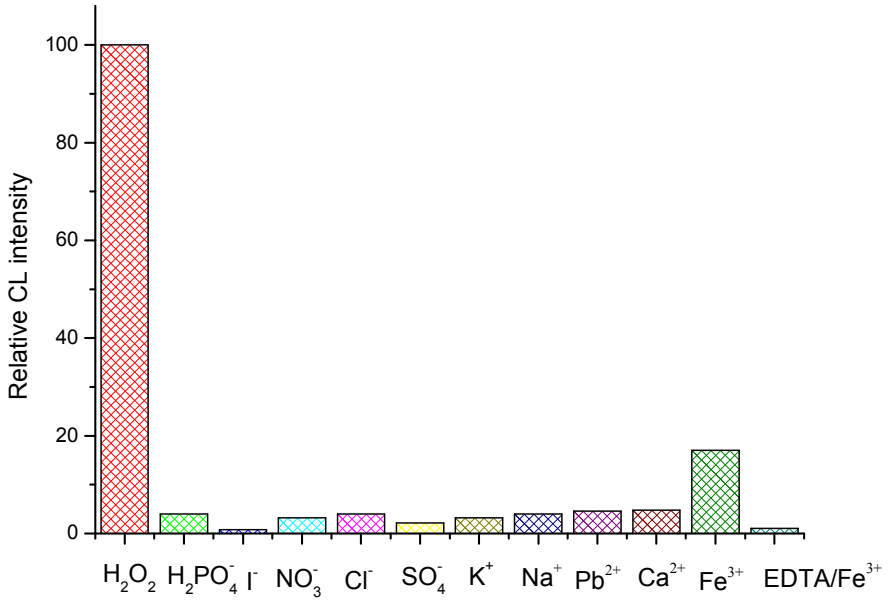
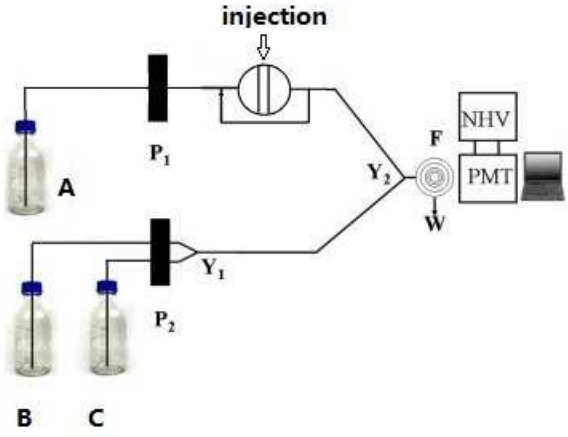
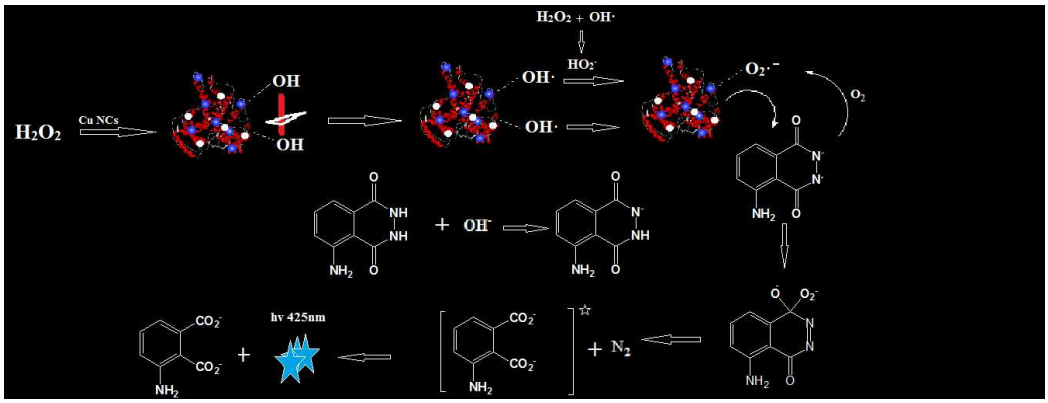


Figure 6. Selectivity for H_2O_2 assay against other common cations. H_2O_2: 1 mM; The concentration of each cations was 1M.



Scheme1. Diagram of the flow-injection chemiluminescence detection system



Scheme 2. Possible mechanism for the luminol-H_2O_2-Cu NCs CL system.

Luminol chemiluminescence enhanced by copper nanoclusters and its analytical application

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Abstract

It was found that Cu nanoclusters could enhance the chemiluminescence (CL) emission from the luminol-hydrogen peroxide system in an alkaline medium. Herein, the CL spectra, UV-visible spectroscopy and radical scavengers were conducted to explore the possible enhancement mechanism. The enhanced CL should attribute to the catalysis of Cu nanocluster, which effectively catalyzed the decomposition of H_2O_2 to produce double hydroxyl radical. The inhibiting effects of some organic compounds were also investigated. Then, the proposed method has been successfully applied to determine H_2O_2 in enviroment water samples with satisfactory accuracy and precision.

Keywords

Cu nanoclusters; chemiluminescence; luminol; hydrogen peroxide;

Introduction

In recent years, chemiluminescence and related analytical techniques have attracted extensive interest and have been developed as important and powerful tools in different fields,[1-9]because of its inherent strengths: high sensitivity, a wide linear range, simple instrumentation, and, in many cases, lack of background scattering light interference.

However, resulting from the weak CL emission of traditional CL system, people centered their interest on some new material for the purpose of enhancement of the CL intensity. Catalysts, such as transition metal ions and peroxidases, have been applied for that purpose.[10-13] Lately, much attention has been paid to the chemiluminescence of nanomaterials system, providing amplified CL emission. Many researches have demonstrated that use of nanoparticles in CL reactions has proposed new methods to enhance the inherent sensitivity and expand new applications in detection. For example, Cui and co-workers have reported many prominent works about noble metal nanoparticles-catalyzed CL systems, such as Au, Ag, and Pt nanoparticles, which significantly enhanced many traditional CL systems.[14-16] Yu et al. have decorated Pt-Co bimetallic alloy nanoparticles on graphene to catalyze luminol CL system for sensing glucose.[17] In other situations, metal oxide nanoparticles, such as Fe_2O_3, ZnO, Co_2O_3, $CoFe_2O_4$, CeO_2, ZnS and CuO, have also used in the CL reaction.[18-25] However, the application of metal nanocluster as catalysts for the CL system has not yet been reported, to the best of our knowledge.

Metal nanoclusters (NCs) consisting of several to tens atoms have recently attracted

much attention.[26-28] Because their unique physical, electrical, and optical properties have made metal NCs as promising candidates in the fields of catalysis, chemical sensors, electronic devices, and biological imaging.[29-32] Until now, the application of metal NCs in analytical fields is mainly focus on their fluorescence properties and very little on their catalytic properties for biological or chemical sensing application.[33-35] Therefore, it is very meaningful to investigate novel sensing platforms based on their catalytic activitys of metal NCs.

In this paper, we report the catalytic property of copper (Cu) NCs in luminol CL system for the first time. Compared with the noble metals Au and Ag, the metal Cu is relatively abundant, inexpensive, and readily available from commercial sources. It was found that Cu NCs could enhance greatly CL from Luminol-H_2O_2 system. A possible enhancement mechanism of Cu NCs on luminol CL was exploited. The effect of Cu NCs on the luminol-H_2O_2-Cu NCs CL system was studied. Experimental results suggested that some organic compounds containing –OH, -NH_2, -SH groups could inhibit the CL signal of luminol-H_2O_2-Cu NCs system. It indicated that the proposed system had great potential for the determination of such compounds. Meanwhile, the feasibility of the present method for H_2O_2 detection was also researched. Under optimum conditions, the CL intensity was linear with H_2O_2 concentration.

Experimental

Reagents and materials

All chemicals and reagents were of analytical grade and used as received without further purification, and ultrapure water was used throughout. Bovine serum albumin (BSA) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). $CuSO_4 \cdot 5H_2O$ was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 30% (v/v) H_2O_2, sodium hydroxide and nitro blue tetrazolium (NBT) were purchased from Kelong Reagent Co., Chengdu, China. Thiourea, ascorbic (AA) were commercially purchased from Chongqing Chemical Regent Company (Chongqing, China).

A 1.0×10^{-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_2O_2 were prepared fresh daily by dilution of 30% (v/v) H_2O_2.

Synthesis of BSA-Cu nanoclusters

BSA modified Cu NCs were prepared in aqueous solution following a previous method[36]. In a typical experiment, 1mL aqueous $CuSO_4 \cdot 5H_2O$ solution (20 mM) was added to the BSA solution (5 mL, 15mg/mL) under vigorous stirring for 5min at room temperature. Then, The solution PH was adjusted to 12 by adding NaOH solution and the mixture was allowed to proceed under vigorous stirring at 55℃ for 8 h. The solution was

111 then dialyzed in ultra-pure water for 48 h to remove unreacted Cu^{2+}. The final solution was
112 stored at 4℃ in refrigerator when not in use.
113
114 *General procedure for CL analysis*
115
116 The chemiluinescence detection was conducted on a laboratory-built flow injection CL
117 system（Xi'an Remax Company, Xi'an, China）, consisting of two peristaltic pumps to
118 deliver the reactants to the flow cell. (Scheme 1) One delivered Cu NCs and H_2O_2 (or
119 samples) with two channels at a flow rate (per tube) of 1.9 mL/min. The other pump was
120 used to carry luminol solution at the same flow rate. The PTFE tubing (0.8 mm i.d.) was
121 used to connect all components in the flow system. A six-way injection valve equipped
122 with an 8 cm long sampling loop was used to inject. The CL signal produced was detected
123 by a photomultiplier tube (operated at −550 V), and was then recorded by a computer
124 equipped with a data acquisition interface. Data acquisition and treatment were performed
125 with BPCL software running under Windows XP. When the CL system was used to study
126 the effect of organic compounds and the free radical scavengers, one peristaltic pump was
127 used to deliver Cu NCs and the mixture of H_2O_2 and luminol, and the other was used to
128 carry organic compounds or free radical scavenger at 1.9 mL/min, respectively.
129
130 *Sample preparation*
131 For hydrogen peroxide determination, the tap water samples were chosen for
132 investigation in this study. The water sample was filtered through a 4.5μm micropore
133 membrane before experiment.

Results and discussion

Enhancement of luminol CL

The effects of Cu NCs on the luminol-H_2O_2 chemiluminescence system were studied. As show in Fig. 1, the oxidation of luminol by H_2O_2 generates weak CL in alkaline media. However, the CL signal intensity could be enhanced significantly up to about 70 folds as soon as adding the Cu NCs. Compared with other nano-catalysts reported in the literatures (Table 1), the CL enhancement factor on luminol- H_2O_2 CL system of Cu nanoclusters is much higher than that of most catalysts mentioned. Though the catalytic activities of Au and Pt nanoparticles are little higher than Cu nanoclusters, they are costly. Therefore, Cu nanocluster could be an outstanding catalyst on the luminol- H_2O_2 CL system.

Optimization of the reaction conditions

The reaction conditions were optimized for the luminol-H_2O_2-Cu nanoclusters CL system shown in Fig. 2. The pH of luminol solution is of great importantance in the CL reaction, so the effect of pH on the CL was tested in the range of pH 11.4–12.6 (Fig. 2A). The optimized PH condition for luminol-H_2O_2-Cu nanoclusters CL system was pH 11.8 When the pH of luminol solution was lower than 11.8, the CL intensity increased with increasing the pH. The effect of luminol concentration on the CL was investigated in the range from 1.0×10^{-6} to 2.0×10^{-4} M (Fig. 2B), the CL intensity increased with increasing

156 luminol concentration in the range of 1.0×10^{-6} to 5.0×10^{-5} M. However, when the
157 concentration of luminol was above 5.0×10^{-5} M, only slight changes in the light intensity
158 were observed. Therefore, 5.0×10^{-5} M was selected as the optimal luminol concentration
159 in the present study. The effect of H_2O_2 concentration on the CL was studied in the range of
160 0.01-0.5 M (Fig. 2C), the CL intensity increased with increasing H_2O_2 concentration in the
161 range of 0.01-0.15 M and decreased when the concentration of H_2O_2 is larger than 0.15 M.
162 The effects of the concentration of Cu NCs and the flow rate were also discussed (Fig. 2D,
163 2E). Considering the CL intensity and the consumption of the regents, the optimized
164 conditions for the luminol- H_2O_2-Cu NCs system were as follows: 5.0×10^{-5} M luminol in
165 NaOH solution (PH=11.8), 0.15 M H_2O_2, 12.8 mg/L Cu nanoclusters and 1.90 mL/min
166 flow rate.
167
168 *Mechanism Discussion*
169
170 A F-7500 mode fluorescence spectrophotometer has been used to discuss the CL
171 mechanism of luminol- H_2O_2-Cu nanoclusters sysyem. The CL spectra was obtained after
172 turning off the light entrance slot. As shown in Fig. 3, the maximal emission peak located at
173 425nm clearly, indicating that the luminophor was still the excited-sate 3-aminophthalate
174 anions (3-APA*).[37-38] Therefore, the adding of Cu nanoclusters did not result in forming a
175 new luminophor for this CL system. The enhanced CL signals were thus attributed to the
176 possible catalysis from Cu nanoclusters. In order to further confirm the possible catalysis of
177 Cu nanocluster, the UV–visible absorption spectra was recorded. As shown in Fig. 4, the

maximum absorption peaks of Cu NCs and luminol- H_2O_2-Cu NCs system are observed at around 325 nm and 346 nm, respectively. Therefore, the light absorption of the mixed system was approximately equal to the sum of the light absorption of the two individual systems, which implied that no change was taken between the species after the reaction. As a result, the enhancement of CL signals had derived from the catalytic effects of Cu NCs.

The CL-generation mechanism for luminol oxidation in aqueous solution has been extensively studied. It was reported that H_2O_2 decomposition on supported metal catalysts such as Au NPs, Ag NPs and CuO NPs involved the formation of hydroxyl radicals OH·. Furthermore, Xu et al has found Cu NCs could exhibit significant peroxidase-like activity.[35] Similarly, we suggested that the O-O bond of H_2O_2 might be broken up into double OH· radicals by virtue of the catalysis of Cu nanocluster. Then the OH· radicals reacted with luminol anion and HO_2^- to form luminol radical ($L\cdot^-$) and superoxide radical anion $O_2\cdot^-$, which further reacted with each other to form the excited 3-aminophthalate anion (3-APA*).

To acquire further insight into the mechanism of the CL system, the effects of various active oxygen radical scavengers on the CL were studied. (Table 2) AA is well known as an efficient ROS scavenger, and it can terminate active oxygen radicals by electron transfer. The influence of AA on the CL signal was investigated, and the results showed it could quench the CL even at a relatively low concentration. Therefore, we confirmed that the CL reaction must happen in a radical way, in which the generation of free radicals appeared to be the key factors.

For purpose of identifying the generation of $O_2\cdot^-$ and OH· in the CL reaction, NBT was frequently used for the detection of $O_2\cdot^-$ radicals. $O_2\cdot^-$ can reduce NBT to its deep blue

diformazan form. The color changed from yellow to blue when 1mM mol/L NBT was added to the CL system, and then the CL intensity decreased by a factor of ~52.3. The result confirmed that $O_2\cdot^-$ was involved in the CL process. OH· is always supposed to be one of the most potent oxidizers among the oxgen-centered free radicals. Thiourea is an effective radical scavenger for OH·. When 1.0 mM thiourea is added to CL system, a distinct inhibition is observed by a factor of ~61. It indicated that OH· is generated in the CL process.

Based on the above results, the whole enhanced mechanism is summarized in Scheme 2.

Inhibition effects of organic compounds

Some organic compounds containing hydroxyl (OH), amino (NH_2), or mercapto (SH) groups were found to inhibit the CL from the luminol-H_2O_2 system-Au NPs/Ag NPs system. It also has been reported that the reducing groups of OH, NH_2, or SH are possible to compete with luminol for active oxygen intermediates, giving rise to a decrease in CL intensity. [14,39] Moreover, such compounds may interact with Cu NCs to interrupt the formation of luminol radicals and hydroxyl radicals taking place on the surface of nanoclusters, causing a decrease in the CL intensity. Therefore, the effects of such organic compounds on the luminol-H_2O_2 system-Cu NCs were studied as list in Table 3. As expected, for 10^{-4} M tested compounds, the CL signals were obviously inhibited. In addition, the inhibition percentage varied with the spcies and concentration of the compounds. The results demonstrate that the luminol-H_2O_2 system-Cu NCs system has the

potential to respond such compounds. Nevertheless, low selectivity does be the main disadvantage of the CL detection, but this weakness can be overcome by implementation of a separation unit. As a result, it is perfect to design a CL detector in HPLC and high-performance capillary electrophoresis for the simultaneous determination of numerous compounds.

Analytical performance

Hydrogen peroxide is of vital importance for medical diagnosis, because it is involved in many detection processes as an intermediate product. The possibility of using the proposed method to detect hydrogen peroxide is studied (Fig. 5). Under the optimum conditions described above, the linear calibration range prolonged over 3 orders of magnitude from 0.1 mM to 150 mM. The regression equation is $\Delta I=54.39 + 30117.8[H_2O_2]$ (mol/L), r=0.9984 (n=9). The limit of detection (LOD, 3σ) for hydrogen peroxide was 0.03 mM. The relative standard deviation (RSD) was 3.1% for 60 mM H_2O_2 (n=7).

Interference study

The selectivity of the proposed method was evaluated by analyzing a standard solution of 1.0 mM H_2O_2, to which varying amounts of possible interference were added. With respect to 1.0 mM H_2O_2, the tolerable limit of each exotic species was considered as a relative error less than the 5% level. As shown in Fig. 6, most of the ions had no essential effect on the detection of 1.0 mM H_2O_2. Though Fe^{3+} is the main interference for determination, the

interference could be eliminated for adding the EDTA. The experimental result suggested that the addition of EDTA could realise the quantitative recovery of H_2O_2 from the water samples as compared to that without EDTA. Therefore, the results indicated that the proposed CL system is highly selective for hydrogen peroxide

Analytical applications

The CL method based on Cu nanocluster catalysis was applied to the determination of H_2O_2 in tap water. From Table 4, it can be seen that the recovery of H_2O_2 in tap water sample ranged from 85.0 to 110.0% through standard addition experiments, which demonstrated the proposed CL system was satisfactory for H_2O_2 analysis. Meanwhile, as shown in Table 5, the concentration of the H_2O_2 was in excellent agreement with that obtained by spectrophotometric method.

Conclusion

In summary, Cu NCs were found to enhance greatly the Luminol- H_2O_2 CL signals. The enhancement of CL was suggested to attribute to the catalysis of Cu NCs on the radical generation and electron-transfer processes during the luminol CL reaction. Some organic compounds containing OH, NH_2, or SH groups interacting with Cu NCs were found to inhibit the CL signals of the luminol-H_2O_2-Cu NCs system under the optimized experimental conditions, which could be potentially used to detect these compounds.

Moreover, the proposed method was successfully applied for H_2O_2 detection in water sample. This work was of great importance for the investagation of new and efficient catalysts for CL system and helpful for understanding of CL mechanism correspondingly.

Acknowledgement

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Table 1 Enhancement factor of various nano-catalysts on luminol-H_2O_2 CL system

Nano-catalyst	Enhancement factor	Literature
Ag NPs	3-10	15
Au-Ag alloy NPs	5	40
ZnO NPs	18	19
CeO_2 NPs	22.5	22
Co Fe_2O_4 MNPs	50	21
Fe_2O_3 NPs	18	18
Au NPs	100	14
Pt NPs	120	16
Cu NCs	70	This work

Table 2. Effect of different radical scawenger on the CL of Luminol-H2O2 in the presence of Cu nanocluster[a]

scavengers	Intermediates	Concentration	Percent inhibition(%)[b]
H_2O			0
Ascorbic acid	OH·, O_2·−	0.1mM	70.1
NBT	O_2·−	1mM	52.3
Thiourea	OH·	1mM	61.0

[a]Solution condition: Luminol, 5×10^{-5} M in pH 11.8 (sodium hydroxide solution); H_2O_2, 0.15 M; Cu NCs, 12.8 mg L^{-1}

[b]Average value of three determination

Table 3 Inhibition effects of organic compounds (1.0×10^{-4}M) on luminol-H_2O_2-Cu NCs CL system.

Organic compounds	Quenching (%)	Organic compounds	Quenching (%)
Ascorbic acid	70.1	L - alanine	55.7
L- leucine	25.4	L - phenylalanine	24.7
Resorcinol	73.4	L - glycine	36.8
L – aspartate	43.0	L - histidine	16.2
L - tryptophan	17.4	L - valine	43.7
hydroquinone	84.6	Butylated hydroxytoluene	57.2
L – glutamic acid	18.3	L - cysteine	42.6
L - serine	39.7	L-Proline	42.9

[a] The percentage of quenching was calculated as I/I_0. The blank CL signal I_0 was obtained by luminol-H_2O_2-Cu NCs CL system without the tested organic compounds

Table 4 Analytical results of H_2O_2 in tap water (n=3)

Tap water	Detected	Added (mM)	Found (mM)	Recovery (%)
Sample1	ND[a]	0.20	0.17	85.0
Sample2	ND[a]	10	11	110.0
Sample3	ND[a]	150	148	98.6

[a] ND (not detected)

Table 5 Determination of H_2O_2 in tap water (n=3)

Tap water	Proposed method	Spectrophotometric method
	H_2O_2 (mM)	H_2O_2(mM)
Sample1	2. 0±0.1	1.9±0.1
Sample2	10.0±0.2	10.3±0.1
Sample3	23.0±0.4	22.6±0.4