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A sensitive biosensor for glucose determination based on the unique catalytic chemiluminescence of sodium molybdate

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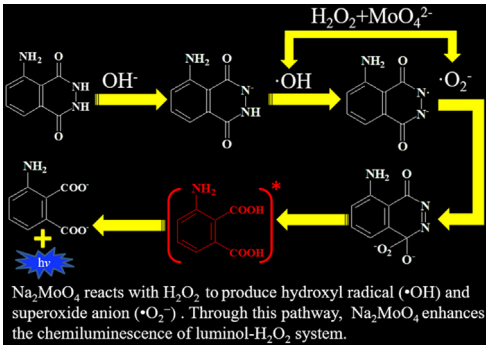
HIGHLIGHTS

- It is the first report that sodium molybdate can dramatically enhance the CL intensity of the luminol-H_2O_2 system.
- A simple and sensitive CL method for the determination of H_2O_2 and glucose is developed.
- The proposed method exhibits low detection limit and wide linear range to H_2O_2 and glucose.
- The catalysis mechanism of sodium molybdate for luminol-H_2O_2 CL reaction has been investigated in detail.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemiluminescent (CL) reaction between hydrogen peroxide (H_2O_2) and luminol was dramatically enhanced by sodium molybdate (Na_2MoO_4) for 284-fold. CL mechanism investigation indicated that Na_2MoO_4 increased the production of hydroxyl radical (•OH) and superoxide anion ($•O_2^-$) in the H_2O_2-luminol system, which could attribute to the enhanced-CL intensity and gave us new insights into the CL-enhanced property of Na_2MoO_4. The CL intensity of Na_2MoO_4-H_2O_2-luminol system increased with the concentration of H_2O_2, based on which, a convenient and sensitive CL determination method could be developed for H_2O_2 in the concentration ranging from 0.5 to 60 μmol/L, with a detection limit of 0.25 μmol/L. Combining with glucose oxidase, the Na_2MoO_4-H_2O_2-luminol system could also be applied for glucose detection. Glucose in human serum has been successfully detected with satisfied recoveries in the range of 96.7 % to 105.4 %.

1. Introduction

Chemiluminescence (CL) is a kind light emission generated through a chemical reaction. CL avoids the background interferences. Hence, it has the advantages of high sensitivity and wide linear range. CL has been widely used in environmental detection, food analysis, clinical diagnosis and some other fields[1]. CL substances include hydrazide compounds (such as luminol, isoluminol and their derivatives), acridine esters and some imidazole compounds[2,3]. The most famouse CL reagent is luminol and its derivatives[4]. Luminol and its derivatives reacted with some oxidants, such as, hydrogen peroxide (H_2O_2), potassium ferricyanide, $NaIO_4$ or peroxinitrous acid to form excited states, which released energy with CL emission. However, the CL emission form binary luminol-oxidant CL system is often weak and could not meet with sensitive determination demand. Natural enzymes, such as horse-

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radish peroxidase (HRP), are always adopted as a catalyst to enhance the CL intensity [5] . However, HRP needs a sophisticated separation process, and its activity is easily affected by environmental condition.

Nowadays, some nanomaterials exhibited excellent catalytic effect and have been used to mimic HRP functions. Cui group firstly found noble element nanoparticles, such as gold[6] or platinum colloids[7] catalyzed the oxygen–oxygen bond of H_2O_2 broken to generate hydroxyl radicals (·OH). ·OH radicals stabilized at the surface of gold or platinum nanoparticles, reacted with luminol anions to form luminol radical ($L^{\bullet-}$). $L^{\bullet-}$further reacted with $\bullet O_2^-$ to produce hydroxy hydroperoxide intermediate (LOO^-). The decomposition of LOO^- generated 3-aminophthalate in its excited-state (3-aminophthalate*). 3-aminophthalate* returns to the ground state with CL emission. Au@Ag NPs[8] could also synergisticly catalyze the oxidation reaction between $L^{\bullet-}$ and $\bullet O_2^-$ to LOO^-. The similar CL enhancement mechanism was illustrated for transition group metal oxide, such as ZnO[9], CuO nanoparticles[10]. Functionalized nanoparticles, such as β-cyclodextrins coated on $CoFe_2O_4$ magnetic nanoparticles[11], copper ion modified graphitic C_3N_4 nanosheets [12], cobalt hydroxide decorated porous graphene[13] and copper-based metal–organic framework[14], were also used to catalyze the CL reaction between H_2O_2 and luminol. The exploration of new kind of catalyst is still a hot and difficult research topic.

In the present study, sodium molybdate (Na_2MoO_4) dramatically increased the CL reaction of H_2O_2 and luminol for 284-fold. The CL enhanced effect of Na_2MoO_4 was much higher than that of transition metal ions and some nanomaterials [15]. Furthermore, Na_2MoO_4 is more stable in alkaline media than transition metal ions. Many techniques, such as CL experiments, electron spin resonance (ESR), ultraviolet–visible (UV–vis) spectroscopy have been utilized to investigate the CL mechanism. The linear relationship between the CL intensity of Na_2MoO_4-luminol-H_2O_2 system and the concentration of H_2O_2 could be developed for H_2O_2 determination. Combining with using of glucose oxidase, the Na_2MoO_4-luminol-H_2O_2 system has been succesfully applied for glucose detection. The method has the advantages of simple operation, easy availability of reagents, low cost, wide detection range and high sensitivity.

2. Experimental section

2.1. Chemicals and materials

Sodium molybdate (Na_2MoO_4), sodium azide (NaN_3) and L-histidine were purchased from Sinopharm Chemical Reagent (Shanghai, China). 1,4-Diazabicyclo[2.2.2]octane (DABCO), starch, H_2O_2, thiourea, luminol, maltose, glucose, sucrose, lactose and sodium hydroxide (NaOH) were from Aladdin Reagent (Shanghai, China). Superoxide dismutase (SOD) and glucose oxidase were purchased from Sigma-Aldrich (Shanghai, China). All reagents were analytical grade. The blood sample was purchased from Beijing Solarbio science and technology Co., Ltd. The distilled water used in the experiment was purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA).

2.2. Apparatus and measurements

UV–vis absorption spectra were collected by a UV-2600 UV–vis spectrophotometer (Shimadzu). The CL spectrum was monitored by a Cary Eclipse spectrophotometer (Agilent, USA). X-ray photoelectron spectra were measured by an ESCALAB 250XI electron spectrometer (Thermo). The ESR spectra were collected by a Bruker EMX electron spin resonance spectrometer. The CL intensity was measured using a luminometer (Centro LB960, Berthold).

2.3. CL from H_2O_2– Na_2MoO_4 -luminol CL system

CL experiments were carried out in the 96 well microplate. 50 μL of Na_2MoO_4 was premixed with 50 μL of luminol. 50 μL of H_2O_2 was then added through a microliter syringe. The microliter syringe was installed in the upper injection port. The reagent addition orders were changed to study the interaction of the reagents.

2.4. Analysis of glucose in human serum sample

Serum samples were centrifuged for 20 min at 10000 rpm firstly and then passed through 0.22 μm pore size membrane filter and stored at 4℃. 250 μL of serum samples, 200 μL of PB (pH 6.0 10 mmol/L) and 50 μL of 100 μg/mL GOx were mixed and incubated at 37 °C for 30 min. The above solution was diluted 100- fold by PB buffer. The solution was mixed with 50 μL of Na_2MoO_4 (0.0125 mol/L) and 50 μL of luminol (10 μmol/L). Finally, the CL emission from the mixture was measured by the 96-well microplate reader.

3. Results and discussions

3.1. CL from H_2O_2– Na_2MoO_4 -luminol CL system

Only weak CL intensity of 19,177 was obtained between the reaction of H_2O_2 and luminol(Fig. 1A). When H_2O_2 was added to the mixed solution of Na_2MoO_4 and luminol, CL intensity as high as 5,454,050 was detected and then decreased slowly over a long time, which showed that Na_2MoO_4 enhanced the CL from H_2O_2-luminol system for 284-fold. There is no CL emission monitored from the reaction of Na_2MoO_4 and H_2O_2 under the present condition (Fig. 1A). Only weak CL intensity of 7547 was obtained between the reaction of Na_2MoO_4 and luminol.

The influences of reagents mixing orders on the CL emission have been investigated (Fig. 1B). The reaction between Na_2MoO_4 and H_2O_2 is rapid. The radicals generated will react quickly with some the other species. If luminol was added into Na_2MoO_4-H_2O_2 mixture, some radicals generated in the Na_2MoO_4-H_2O_2 system could not react with luminol in time. Hence, The CL intensity monitored by adding of luminol into the mixture of Na_2MoO_4 and H_2O_2 is lowest among the three reagents mixing orders in Fig. 1B.

Fig. 1A showed CL intensity from Na_2MoO_4-luminol system was much lower than that from H_2O_2-luminol system. The result indicates the low consumption of luminol in Na_2MoO_4-luminol system. The injection of H_2O_2 into the mixture of Na_2MoO_4 and luminol makes sure the adequate inter-reaction between the luminol and the relative radicals. Hence, The highest CL intensity was detected by adding of H_2O_2 into the mixture of Na_2MoO_4 and luminol.

The CL intensity of Na_2MoO_4-H_2O_2-luminol system increased with the concentration of H_2O_2, based on which, a sensitive CL method could be developed for H_2O_2 determination. Glucose oxidase catalyzed glucose to produce H_2O_2, the generated H_2O_2 then reacted with Na_2MoO_4-luminol to generate CL emission, based on which, a CL method for glucose detection could be developed.

In order to obtain the optimized condition for H_2O_2 and glucose determination, the influences of the concentration of luminol, H_2O_2, Na_2MoO_4 and pH value on the CL analysis were investigated (Fig. 2). pH value of 11.0 is suitable for the luminol-H_2O_2-Na_2MoO_4 CL reaction (Fig. 2A). The CL intensity increased with the concentration of luminol. Considering the measuring range of the CL detector, luminol with a concentration higher than 10 μmol/L has not been used (Fig. 2B). The CL intensity of the Na_2MoO_4-H_2O_2-luminol system was maximum when 1 mmol/L of H_2O_2 and 0.125 mol/L of Na_2MoO_4 were used (Fig. 2 C and D).

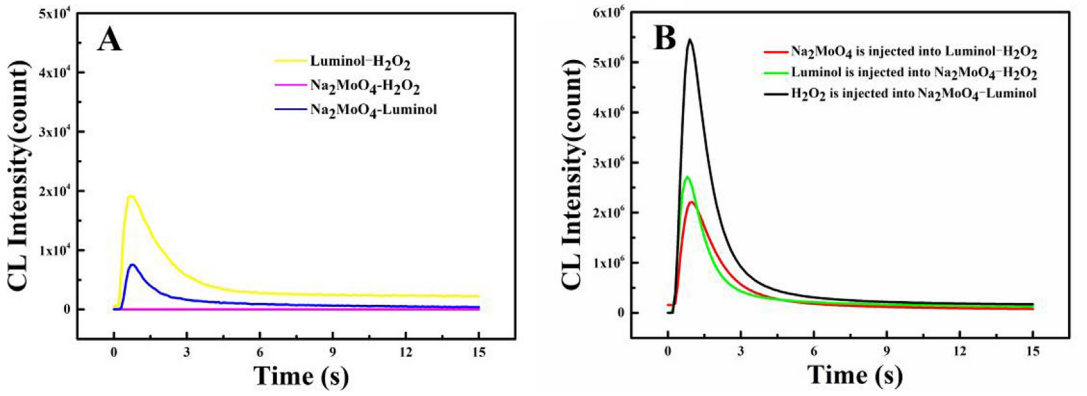


Fig. 1. CL reaction kinetics spectra of Na_2MoO_4-H_2O_2-luminol system under different reagent mixing orders.

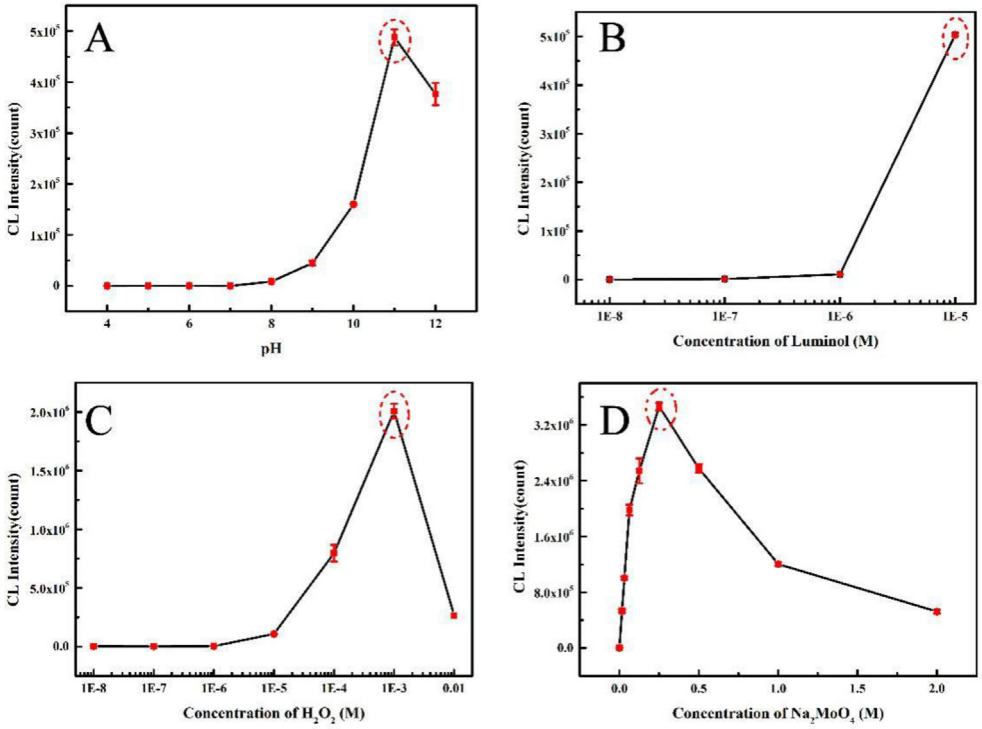


Fig. 2. Effects of pH value (A), luminol concentration (B), H_2O_2 concentration (C), and Na_2MoO_4 concentration (D) on the CL intensity from Na_2MoO_4-H_2O_2-luminol system.

Under the optimized conditions, the CL intensity of Na_2MoO_4-H_2O_2-luminol system was in linear relationship with the concentration of H_2O_2 ranging from 0.5 μmol/L to 60 μmol/L (Fig. 3A). The linear regression equation was $CL_{intensity}$ = =1728.02 + 256.50C_{H2O2}. The correlation coefficient was 0.9937. The detection limit of H_2O_2 was 0.25 μmol/L.

To obtain the optimal conditions for glucose analysis, the effects of pH value, reaction time, reaction temperature and GOx concentration on the oxidation process of glucose catalyzed by glucose oxidase has been studied. It has been found CL intensities are higher than 4.0×10^{-4} count when pH values are ranging from3.0 to 8.0. The result showed the pH range of 3.0 to 8.0 was suitable for the oxidation process (Fig. S1). However, the highest CL signal could be monitored with pH value of 6.0. Hence, pH value of 6.0 is optimized for the reaction. And 100 μg/mL of GOx catalyzed the glucose oxidation reaction at 37 ℃ for 30 min could obtain the highest CL signal. CL intensity has linear relationship with glucose concentrations in the range of 0.15 to 5 μM (Fig. 3B). The linear regression equation was CL = 34.484 + 5805.106$C_{glucose}$. (R^2 = 0.9930) with a detection limit of 0.075 μmol/L, which could meet the practical demand (Table S1). Furthermore, the method has been employed to detect the glucose concentration in human serum. The recoveries of the proposed method were in the range of 92.7～105.4 %. The relative standard deviations (RSDs, n = 3) are ranging from 0.51% to 4.99 % (Table 1).

The selectivity of the method has been assessed. The influences of fructose, maltose, sucrose, starch and some potential saccharides on glucose detection have been studied. The absorbance of the saccharides with their concentrations 5 times higher than that of glucose was negligible (Fig. S2). Ions, including 100 μg/mL of I^-, CH_3COO^-, Cl^-, NO_2^-,NO_3^-, and 10 μg/mL of Ca^{2+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+} , have no influence on the CL emission of the

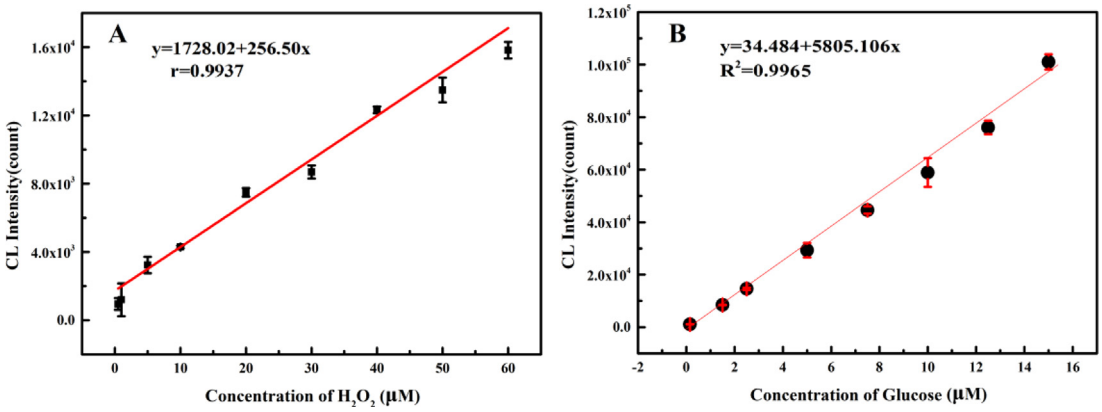


Fig. 3. Standard curves for H_2O_2 (A) and glucose determination (B).

Table 1
Detection results of glucose in spiked human serum samples (n = 3).

Samples	Spiked(μmol/L)	Found(μmol/L)	RSD (%)	Recovery (%)
Human serum 1	0	0.0075	4.6	-
	1.0	0.982	0.51	98.2
	2.0	1.893	3.20	94.3
Human serum 2	0	0.00717	4.86	-
	1.0	0.972	1.34	96.5
	2.0	1.935	1.77	96.4
Human serum 3	0	0.0062	3.22	-
	1.0	1.058	4.99	105.4
	2.0	1.988	1.97	99.1
Human serum 4	0	0.0061	3.22	-
	1.0	0.933	2.46	92.7
	2.0	1.927	1.06	96.4
Human serum 5	0	0.0083	4.82	-
	1.0	0.982	1.83	97.4
	2.0	1.979	2.77	98.5

system (Fig. S3). The result proved the high selectivity of the proposed CL method for glucose detection.

3.2. CL mechanism of the Na_2MoO_4-luminol-H_2O_2CL system

In order to investigate the role of Na_2MoO_4 in luminol-H_2O_2 CL system, room temperature ESR analysis were employed to detect the radicals involved in the CL reaction. The g value of Na_2MoO_4 before and after the CL reaction have been measured and showed little change, which proved the catalysis role of Na_2MoO_4 in the CL system (Fig. 4A). There is no new UV–vis (Fig. 4B) absorption attributing to other Mo species arising after its reaction with H_2O_2 and luminol. Both the XPS spectra of Na_2MoO_4 before and after CL reaction exhibited two well resolved spectral lines with binding energy of 232.1 eV and 235.26 eV. They are corresponding to Mo-$3d_{5/2}$ and Mo-$3d_{3/2}$, which are assigned to Mo^{6+} oxidation state (Fig. 4C and D)[16]. The above result proved the catalysis role of MoO_4^{2-} in the CL system.

DMPO (5, 5-Dimethyl-1-pyrroline-N-oxisde), was employed as specific capture for to detect ·OH and$\cdot O_2^-$ radicals. The ESR signal in Fig. 5 proves that Na_2MoO_4 increased the generation of ·OH and$\cdot O_2^-$ in the Na_2MoO_4-H_2O_2 system (reaction 1–3)[17]. The increased production of ·OH could oxidize luminol to luminol radical, which was then transfered to LOOH by$\cdot O_2^-$ and produced CL emission[18].

$$MoO_4^{2-} + H_2O_2 \rightarrow MoO_4^{3-} + HO_2\cdot + H+ \text{ (Reaction 1)}$$

$$MoO_4^{3-} + H_2O_2 \rightarrow MoO_4^{2-} + OH\cdot + OH^- \text{ (Reaction 2)}$$

$$HO_2\cdot \rightarrow \cdot O_2^- + H+ \text{ (Reaction 3)}$$

The influences of different radical scavengers on the CL intensity of Na_2MoO_4-H_2O_2-luminol system were also studied to further confirm the radicals generated in the systems (Table S2). Thiourea is known as an effective scavenger for •OH[19].Thiourea, with a concentration higher than 1.0×10^{-7} mol/L, showed little effect on the CL. The phenomenon proved that •OH played a major role in the Na_2MoO_4-H_2O_2-luminol CL system (Reaction 1 and 2). Furthermore, 75 U/mL of SOD, as $\bullet O_2^-$ quencher, also inhibited the CL emission (Fig. S4). The results revealed the important role of $\cdot O_2^-$ in the system.

CL spectrum is helpful for us to get further insight into the CL emitter and the roles of the Na_2MoO_4 in the Na_2MoO_4- H_2O_2-luminol CL system. As shown in Fig. S5, the maximum CL spectrum for Na_2MoO_4- H_2O_2-luminol system located around 425 nm, which was characteristic wavelength of excited 3-aminophthalate.

Based on the results above, the enhancement of the Na_2MoO_4 on H_2O_2-luminol system could be explained in Scheme 1. MoO_4^{2-} reacted with H_2O_2 to generate •OH. •OH has strong oxidation abil-

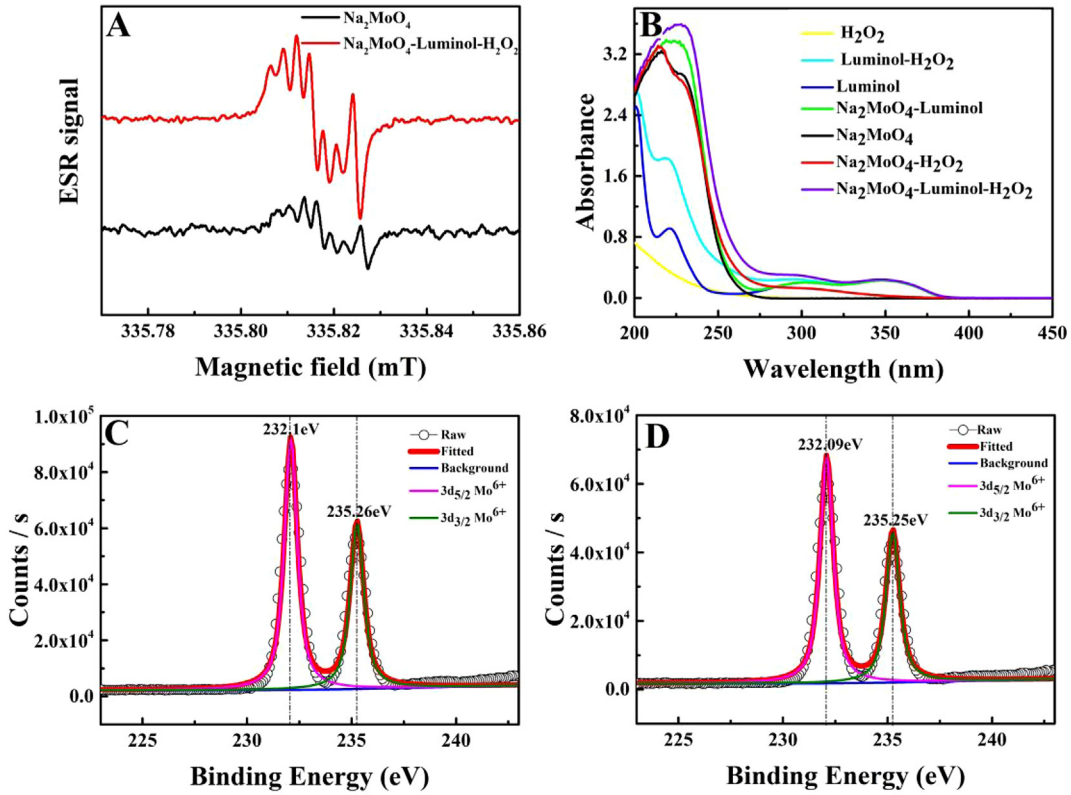


Fig. 4. g value of Na_2MoO_4 before and after the CL reaction (A), UV–vis absorption of Na_2MoO_4-H_2O_2-luminol system (B), The high resolution narrow scan X-ray photoelectron spectrum of Mo-3d core level before and after its reaction with H_2O_2-luminol (C and D).

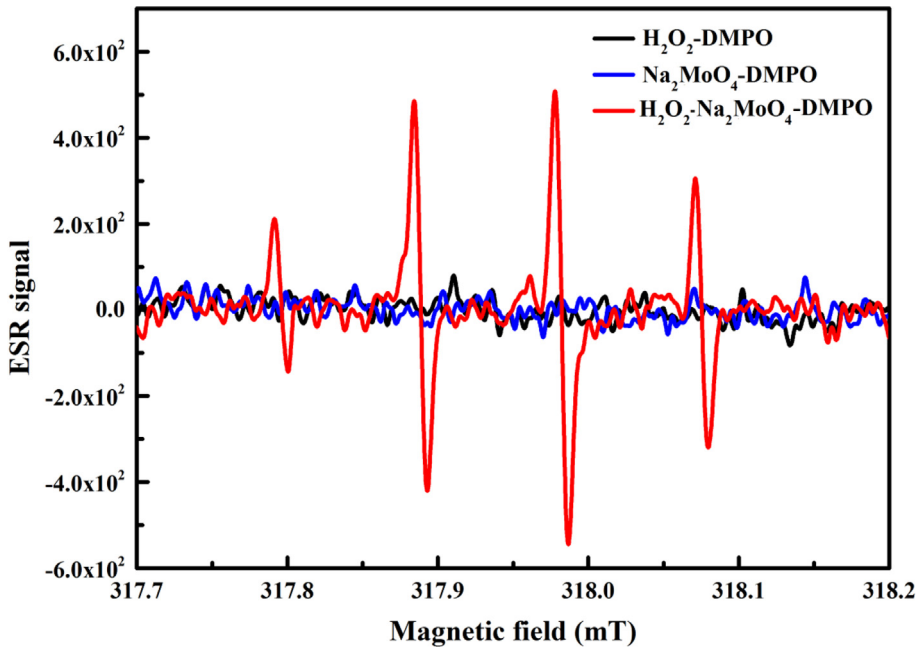
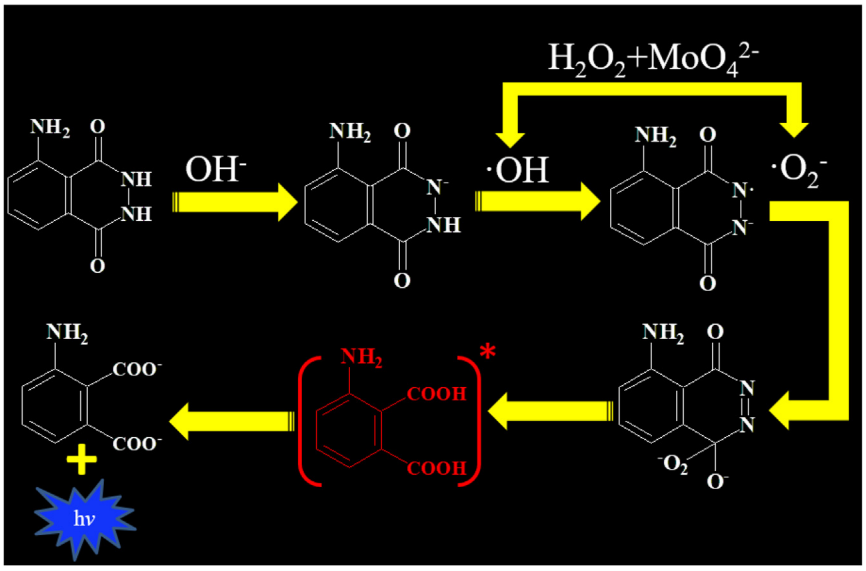


Fig. 5. ESR spectra of •OH and $\bullet O_2^-$ produced by reaction of DMPO probe in Na_2MoO_4-H_2O_2 system.



Scheme 1. Proposed CL mechanism for the H_2O_2-luminol-Na_2MoO_4 system.

ity to oxidize luminol to luminol radicals. Luminol radicals reacted with $\bullet O_2^-$ to be LOOH and produced CL emission[20,21].

4. Conclusions

In conclusion, MoO_4^{2-} dramatically enhanced the CL intensity of the H_2O_2-luminol system. The present study extended the type of the catalyst for luminol system. The H_2O_2-luminol-MoO_4^{2-} system has been further developed as sensitive and facile H_2O_2 and glucose detection. MoO_4^{2-} can be commercially purchased with precise and high purity, resulting in reproducible H_2O_2 and glucose detection results.

CRediT authorship contribution statement

Wensong Yao: Data curation. **Xiaomin Zhang:** Methodology, Data curation. **Zhen Lin:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.120401.

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