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Au NCs-enhanced chemiluminescence from $NaHSO_3$-H_2O_2 and its analytical application

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Abstract:

It was found that the ultra-weak chemiluminescence(CL) emission from sodium bisulfite($NaHSO_3$)-H_2O_2 system could be enhanced by gold nanoclusters (Au NCs). The as-prepared Au NCs was applied to the $NaHSO_3$-H_2O_2 system for the first time. And a decreased CL was observed in the presence of trypsin. This novel CL system based on Au NCs-$NaHSO_3$-H_2O_2 was developed for the trypsin determination. Herein, UV-visible spectroscopy, fluorescence spectra coupled with radical scavengers were used to explore the possible mechanism. The enhanced CL could be attributed to the catalysis of Au NCs and the decreased CL should be ascribed to the decomposition of Au NCs. Finally, the proposed method was successfully utilized to detect trypsin in human urine samples with good accuracy and precision.

Keywords:

Gold nanoclusters; ultra-weak; chemiluminescence; sodium bisulfite; trypsin.

1. Introduction

Protease is an enzyme that can catalyze the breakdown of proteins, which widely exists in the animal internal organs, plant leaves, fruits and microorganisms, and plays an essential and irreplaceable role in biological and physiological processes.[1] As a kind of protease, trypsin is the most important digestive enzyme in the pancreas zymogen, which can be used as a dependable and specific diagnostic biomarker for pancreatitis, cystic fibrosis and cancer.[2-3] Therefore, it is of great importance meaning to detect trypsin in human metabolic processes.

A number of analytical methods have been employed to determine trypsin, such as fluorescence methods,[4-5] raman,[6] colorimetric,[7,8] electrophoresis,[9] electrochemical.[10-12] liquid chromatography.[13] However, the fluorometric detection of trypsin usually needs to interact with a proper fluorescent probe or sensor.[4,5] The electrochemical methods for trypsin detection require a complicated electrodes preparation procedure.[10-12] Liquid chromatography methods for trypsin detection possess a high selectivity, while this method suffers from a tedious sample preparation process. Hence, these conventional methods maybe require sophisticated instrumentation or complicated operation process.

Chemiluminescence (CL) is known as a desirable analytical technique due to its high sensitivity, wide linear range, low detection and simple instrument.[14-17] CL detection technology has been applied to the analysis of many substances.[18-23] The traditional luminescence reagents such as Luminol, TCPO, $Ru(bipy)_3^{2+}$, Acridinium ester were widely used in many CL systems and applied in analytical chemistry. [24-27] Unfortunately, these

reagents are expensive or poisonous to the environment. Thus, it is an attractive research area to develop a new CL system with relatively green and cheap reagents.

In recent years, the potential applications of the ultra-weak CL systems have gradually received research interest.[28-31] Nevertheless, the development of weak chemiluminescence was limited because its intensity was not strong enough for detecting demand. Therefore, it is necessary to find out the ways to increase its sensitivity.

Being an intriguing research field, noble metal nanoclusters (NCs), especially Au NCs have gained great attention because of their remarkable optical properties.[32] Until now, the applications of Au NCs in analytical fields mainly focused on their fluorescence properties.[32-33] Hence, it is highly desirable to find that Au NCs have effect on the ultra-weak CL reaction of $NaHSO_3$ and H_2O_2.

In this paper, we report that the weak CL emission from $NaHSO_3$-H_2O_2 was significantly enhanced by Au NCs. To the best of our knowledge, there is no report about Au NCs-enhanced CL from $NaHSO_3$-H_2O_2. The possible mechanism of enhanced CL signal was also discussed. In the presence of trypsin, the CL signal greatly decreased due to the decomposition of Au NCs. Under optimum conditions, the CL intensity was linear with trypsin concentration, which led to a novel sensing platform based on the system of Au NCs-enhanced $NaHSO_3$-H_2O_2. And then the proposed method has been applied to detect trypsin in human urine samples with desirable accuracy and precision.

2. Experimental

2.1 Reagents and materials.

All chemicals and reagents were of analytical grade and used as received without further purication, and ultrapure water was used throughout. Bovine serum albumin (BSA) and trypsin were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). 30% (v/v) H_2O_2 and nitro blue tetrazolium (NBT) were purchased from Kelong Reagent Co., Chengdu, China. $NaHSO_3$ was purchased from Beijing J & K Chemical Co., Ltd. Thiourea and ascorbic acid (AA) were commercially obtained from Chongqing Chemical Regent Company (Chongqing, China).

Stock solutions of trypsin (1.0×10^{-3} g mL^{-1}) were prepared by dissolving 0.1000g trypsin in 100mL Tris-HCl buffer (0.05mol L^{-1}, PH=8). Trypsin working solution (containing 5×10^{-3} M $CaCl_2$) was prepared by diluting trypsin stock solution with the previous buffer. Stock solutions of $NaHSO_3$ (1 mol L^{-1}) were prepared by dissolving 5.2030g $NaHSO_3$ in 50mL ultra-pure water. Working solutions of H_2O_2 were prepared fresh daily by dilution of 30% H_2O_2 with water.

2.2 Apparatus

The CL experiments were carried out with a Ultra-Weak Luminescence analyzer (Xi'an Remax company, Xi'an, China). CL spectra was obtained by a photomultiplier tube (opened at -900Kv). UV-vis absorption spectra were taken on a Model UV-2550s Spectrophotometer (Shimadzu, Japan). Fluorescence spectra were obtained by means of F-4500 spectrofluorophotometer (Hitachi, Japan). Determination of trypsin was performed based on the net CL intensity of $\Delta I=I_0-I_S$, where I_0 and I_s denote the CL intensity in the absence and presence of trypsin, respectively.

2.3 Preparation of BSA-Au NCs

BSA modified Au NCs were prepared according to the previous literature.[34] $HAuCl_4$ solution (15mL, 10mmol L^{-1}, 37℃) introduced into BSA solution (15mL, 50mg mL^{-1}, 37℃), stirring for 2min. Then NaOH solution (1.5mL, 1 mol L^{-1}) was added to the mixture. The mixture reaction allowed incubating at 37℃ for 24h. The color of the solution changed from light yellow to light brown, and then to deep brown. The as-prepared Au NCs were then dialyzed in ultra-pure water for 48h. The final solution was stored at 4℃ for further work.

2.4 Sample preparation

Human urine sample was analyzed without any pretreatment. The obtained sample was diluted 10-folds with Tris-HCl buffer (pH=8.0). The above solution was then used for trypsin detection.

2.5 CL system based on Au NCs for detection of trypsin

In a typical experiment, 2mL Au NCs was incubated with 500 u L trypsin at 37℃ for 40min to destory BSA-Au NCs and the as-prepared solution was placed to wait for the next measurement. In a quartz glass cuvette, 100 u L Au NCs solution was first mixed with 200uL $NaHSO_3$ solution, and then 200uL H_2O_2 was injected by a syringe. The CL profile and intensity was captured for a 0.2 s interval with the voltage of -900Kv.

3. Results and discussion

3.1 Enhancement of $NaHSO_3$-H_2O_2 CL

In order to evaluate the feasibility of the method, contrast experiment was conducted. As shown in Fig. 1 (curve a), the weak CL from $NaHSO_3$-H_2O_2 was recorded. The enhancement effect of Au NCs on the $NaHSO_3$-H_2O_2 was studied. As show in curve b, the CL intensity of $NaHSO_3$-H_2O_2 could be enhanced remarkably about 11 folds after adding Au NCs. When trypsin exists in Au NCs-enhanced system, the CL intensity decreased significantly (curve c).

3.2 Optimization of the reaction conditions

The reaction conditions were optimized for the $NaHSO_3$–H_2O_2–Au NCs CL system as shown in Fig. 2. The effect of the $NaHSO_3$ concentration on the CL was examined in the range from 0.01 to 0.25mol L^{-1}. The result was shown in Fig. 2a. The maximal signal was obtained at 0.15mol L^{-1}. Therefore, the concentration of 0.15mol L^{-1} was selected for subsequent investigating.

As a basic reaction solution, H_2O_2 reacts with $NaHSO_3$ to produce CL emission. The effect of the H_2O_2 concentration was investigating over the range 0.3 to 0.6mol L^{-1}. As shown in Fig. 2b. The CL intensity increased with increasing H_2O_2 concentration in the range of 0.3 to 0.55mol L^{-1}. At the concentration above 0.55mol L^{-1}, there is no obvious change for CL intensity. Consequently, 0.55mol L^{-1} H_2O_2 was chosen as the optimal for further experiments.

As a CL enhancer, the effect of Au NCs concentration on the CL was studied in the range from 1×10^{-4} to 7.5×10^{-4}mol L^{-1}. As can be seen from Fig. 2c, the CL signal increased

154 on increasing the concentration of Au NCs from 1×10^{-4} to 6×10^{-4}mol L^{-1}. Higher
155 concentration result in an obvious decrease in CL response. Hence, 6×10^{-4}mol L^{-1} Au NCs
156 was recommended.

157 In order to test the effect of the incubating time on the CL intensity, various incubating
158 time ranging from 20 to 100min was compared. As shown in Fig. 2d, with the increase of
159 incubating time, the CL intensity increased sharply and then reached a plateau at 40min.
160 Thus, the incubating time of 40min was selected in this system.

161

162 *3.3 Possible mechanism of CL system*

163 The CL-generation mechanism for $NaHSO_3$-H_2O_2 system had summarized as the
164 following steps by Li et al.[35]

165 (1) HSO_3^- was oxidized by H_2O_2 to produce sulfite radical ($\bullet SO_3^-$), which then
166 dimerized to give $S_2O_6^{2-}$ ion.

167 (2) The emitting species(SO2*), which was generated by the decomposition of $S_2O_6^{2-}$
168 ion. The emission wavelength of SO2* is 260–480 nm. Due to the low emission quantum
169 yield of SO2*, the CL intensity is very low. Thus, it is an extreme difficulty to capture the
170 emission wavelength of SO2*.[36]

171 The UV-visible absorption spectra was conducted in order to confirm the possible
172 catalysis of Au NCs. As shown in Fig. 3, the maximum absorption peaks of Au NCs is
173 observed at around 280nm and $NaHSO_3$-H_2O_2 system at around 204nm. Therefore, the
174 light absorption of the mixed system was approximately equal to the sum of the light

absorption of the two single systems, which suggests that no change was happened between the species after the reaction.

A F-4500 mode fluorescence spectrophotometer has been used to discuss the mechanism of chemiluminescence based on $NaHSO_3$-H_2O_2-AuNCs system in the presence of trypsin. As shown in Fig 4a, the fluorescence intensity of Au NCs decreased clearly after the CL reaction. It was further demonstrated that BSA-Au NCs was not the luminophor of the CL system. The fluorescence spectra of Au NCs and the mixture of Au NCs-trypsin were shown in Fig 4b. It can be seen that the fluorescence intensity of Au NCs decreased in the presence of trypsin, which ascribed to the cleft of BSA molecule from Au NCs.[33] We presumed that the hydrolysis reaction of trypsin with BSA make Au NCs lost the protective effect of BSA. Therefore, trypsin can be detected, making use of our desirable method.

The mechanism was further discussed by the quenching effect of different reactive oxygen species (ROS) on the CL system. Ascorbic acid (AA) is well known as an efficient ROS scavenger, which can terminate active oxygen radicals through electron transfer.[37] AA at a concentration of 0.1 mmol L^{-1} had adverse impact on the CL intensity, the intensity decreased by a factor of ～82.5 (Table 1). Thus, the CL reaction must happen in a radical way. NBT was often used for the detection of $O_2^{\bullet-}$ radicals, which could be reduced to its deep blue diformazan form by $O_2^{\bullet-}$.[38] When 1 mmol L^{-1} NBT was added to the CL system, and then the CL intensity decreased by a factor of ～42.6. The result confirmed that $O_2^{\bullet-}$ was also intermediate in the CL process. Thiourea is an effective radical scavenger for $OH^{\bullet}$.[39] When 1 mmol L^{-1} thiourea was added to CL system, a distinct inhibition is observed by a factor of ～57.6. It indicated that $OH^{\bullet}$ and $O_2^{\bullet-}$ were involved in the CL reaction

process. 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 $OH^{\cdot}$.[40-42] In the same manner, we suggested that the O–O bond of H_2O_2 might be broken up into double $OH^{\cdot}$ radicals by virtue of the catalysis of Au nanoclusters. The $OH^{\cdot}$ reacted with $^{\cdot}SO_3^-$ to form HSO_4^-, and then HSO_4^- was transformed to SO_2*. Finally, SO_2* returned back to ground state with the emission of light. Based on the above results, the whole enhanced mechanism is summarized in Scheme 1.

3.4 Analytical performance

Under the optimum conditions described above, CL intensity versus trypsin concentration shows good linearity ranging from 2.4-48 u g mL^{-1} (Fig.5). The regression equation is ΔI=3024.9+53.94[trypsin]（u g mL^{-1}). The linear correlation coefficient is 0.9973, the limit of detection (LOD) for trypsin is 0.19 u g mL^{-1}. The relative standard deviation (RSD) was less than 3% for 100 u g mL^{-1} trypsin (n=10).

3.5 Selectivity of the method to trypsin over other possible background species

The selectivity of the method for the detection trypsin was examined by compare the CL intensity of trypsin and the mixture of trypsin and background species. As can be seen in Fig 6, the effect of 100 u g mL^{-1} urea, uric acid, glucose, ascorbic acid, dopamine, BSA, Fe^{3+}, NH_4^+, Mg^{2+}, Ca^{2+} and I^- on the detection of 10 u g mL^{-1} trypsin. These background species concentration adopted for interference studies are higher than that in normal urine

samples. Most of the interferences have no influence on the determination of trypsin, indicating that the proposed CL system has good selectivity.

3.6 Detection of spiked trypsin in human urine

In order to test the applicability and reproducibility of the proposed method, recovery experiments were performed in human urine samples. These results were shown in Table 2. Desirable recoveries within the range of 87.5%-104.2% was obtained, showing that this method has good practicability for trypsin detection.

4. Conclusion

In this work, a novel method based on Au NCs-enhanced $NaHSO_3$-H_2O_2 CL system was established for trypsin detection. The added Au NCs could increase the sensitivity of the weak CL system of $NaHSO_3$-H_2O_2. In the presence of trypsin, Au NCs was destroyed because the protein template was enzymatically hydrolyzed, leading to the decreased CL response. The Au NCs-based method was successfully applied to the detection of trypsin in human urine samples with satisfactory accuracy and precision. With trypsin being an index for diseases diagnosis, our method possesses its potential application. Compared with conventional CL systems, the $NaHSO_3$-H_2O_2 CL system is a simple, inexpensive, and relatively nontoxic. What's more, the study on the ultra-weak CL system of $NaHSO_3$-H_2O_2-AuNCs is a new direction to explore a new CL system with nanocluster as catalysts.

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Figure captions

Scheme.1. Possible mechanism for the Au NCs-$NaHSO_3$-H_2O_2 system.

Fig.1. Kinetic curves of CL systems: (curve a) $NaHSO_3$-H_2O_2; (curve b) $NaHSO_3$-H_2O_2-Au NCs; (curve c) $NaHSO_3$-H_2O_2-Au NCs-trypsin. $NaHSO_3$: 0.15 mol L^{-1}, H_2O_2: 0.55 mol L^{-1}, Au NCs: 6×10^{-4} mol L^{-1}, trypsin: 50 ug mL^{-1}.

Fig.2. Effects of the reaction conditions on the $NaHSO_3$-H_2O_2-AuNCs system. (a) Effect of $NaHSO_3$ concentration: H_2O_2: 0.5 mol L^{-1}; Au NCs: 7.5×10^{-4} mol L^{-1}; Incubating time: 60min. (b) Effect of H_2O_2 concentration: $NaHSO_3$: 0.15 mol L^{-1}; AuNCs: 7.5×10^{-4} mol L^{-1}; Incubating time: 60min. (c) Effect of Au NCs concentration: $NaHSO_3$: 0.15 mol L^{-1}; H_2O_2: 0.55 mol L^{-1}; Incubating time: 60min. (d) Effect of incubating time: $NaHSO_3$: 0.15 mol L^{-1}; H_2O_2: 0.55 mol L^{-1}; Au NCs: 6×10^{-4} mol L^{-1}.

Fig.3. UV-vis absorption spectra of (a) Au NCs; (b) $NaHSO_3$-H_2O_2; (c) $NaHSO_3$-H_2O_2-Au NCs.

Fig.4. (a) Fluorescence spectra of BSA-Au NCs before and after the CL reaction. (b) Fluorescence spectra of BSA-Au NCs with and without trypsin.

Fig.5. The calibration curve for trypsin. Error bars represent the standard deviation of three paralleled measurements.

Fig.6. Relative CL intensity of (a) 10 u g mL^{-1} trypsin (b-l): the mixture of trypsin and potential background species (100 u g mL^{-1}). From left to right: urea, uric acid, glucose, ascorbic acid, dopamine, BSA, Fe^{3+}, NH_4^+, Mg^{2+}, Ca^{2+} and I^-. Error bars represent the standard deviation of three paralleled measurements.

Tables:

Table.1 Effect of different radical scavengers on the CL of $NaHSO_3$-H_2O_2 in the presence of Au nanoclusters [a]

Scavengers	Intermediates	Concentration	Percent inhibition[b] (%)
Ascorbic acid	$OH^{\cdot}$ $O_2^{\cdot-}$	0.1mmol L^{-1}	82.5
Thiourea	$OH^{\cdot}$	1mmol L^{-1}	57.6
NBT	$O_2^{\cdot-}$	1mmol L^{-1}	42.6
NaN_3	$^1O_2$	5mmol L^{-1}	8

[a] Solution condition: 0.55mol L^{-1} H_2O_2, 6.0×10^{-4} mol L^{-1} Au NCs, 0.15mol L^{-1} $NaHSO_3$.

[b] Average value of three determination.

Table 2 Detection of spiked trypsin in human urine

Samples	Added (u g mL^{-1}) trypsin	Found (u g mL^{-1}) trypsin	Recovery (%) (n=3)
1	2.4	2.1	87.5%
2	7.2	6.9	95.8%
3	14.4	15	104.2%

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Fig.1

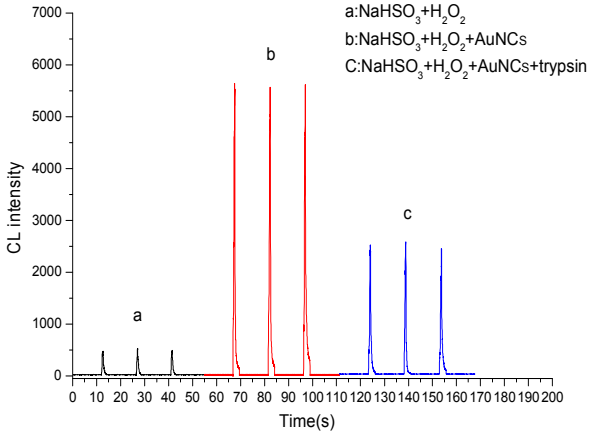
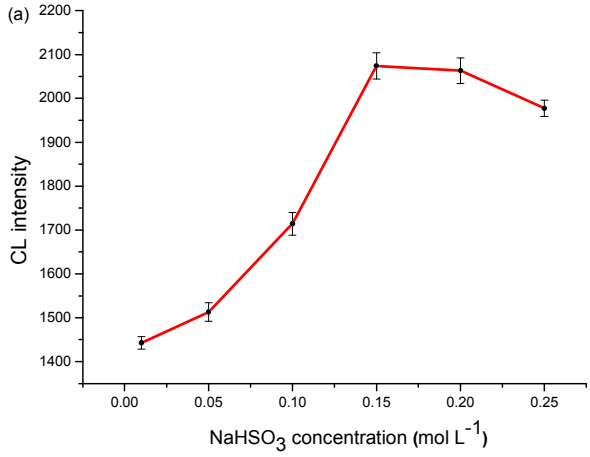


Fig.2(a)



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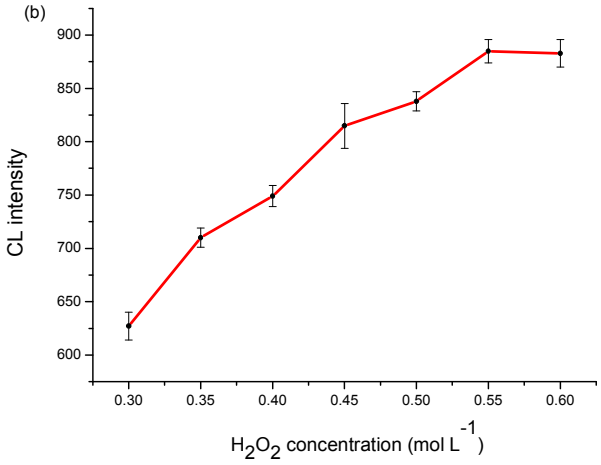

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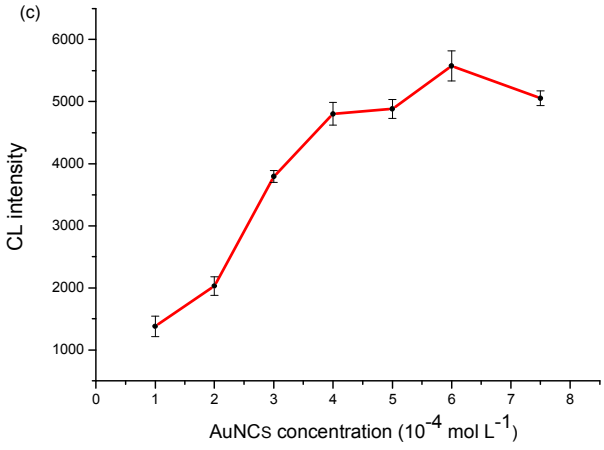

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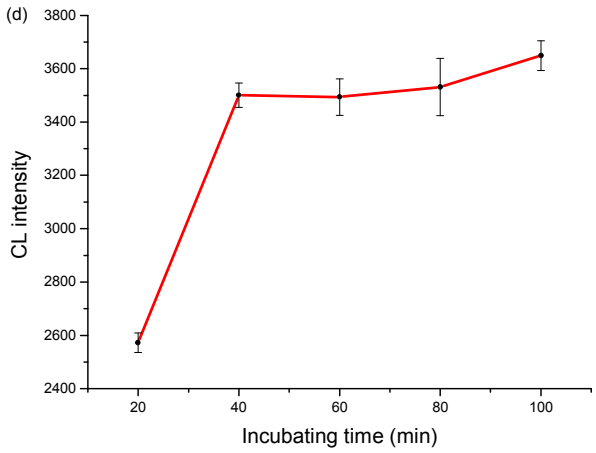


Fig.3

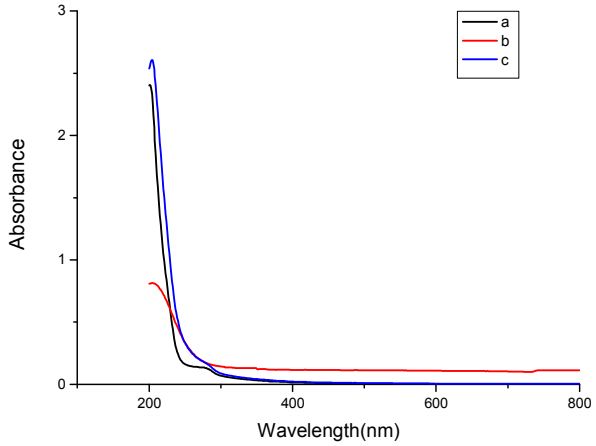
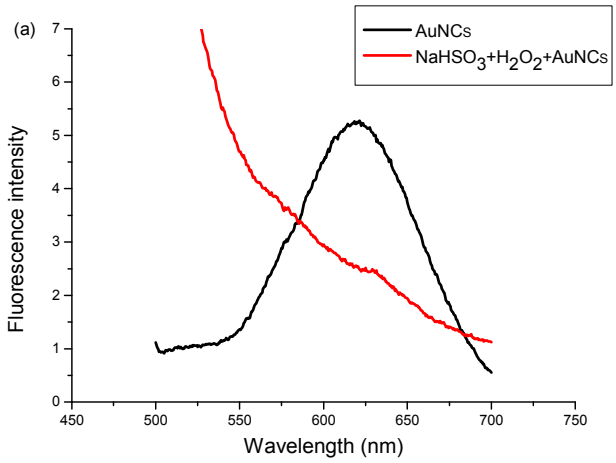


Fig.4(a)



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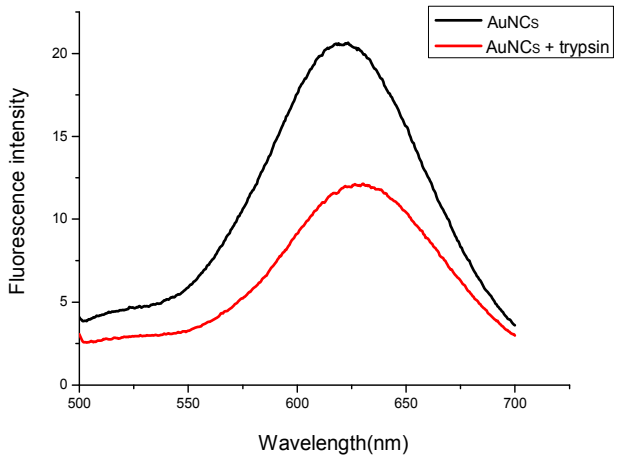

Scheme.1

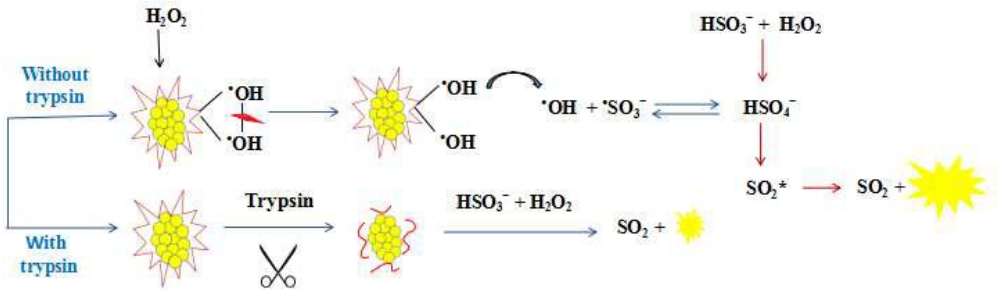

Fig.5

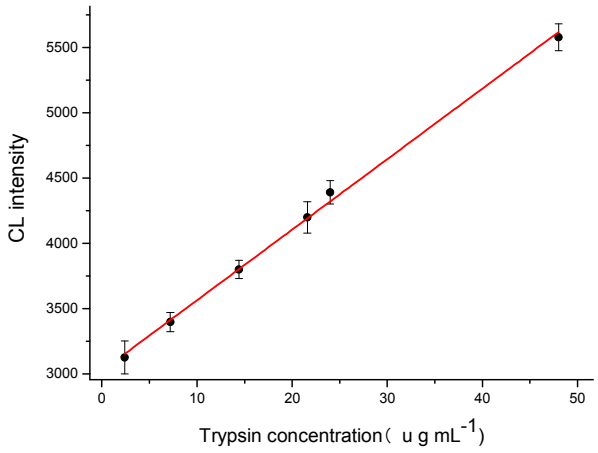

Fig.6