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PAPER

Intensification of electrochemiluminescence of luminol on TiO_2 supported Au atomic cluster nano-hybrid modified electrode

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With TiO_2 nanoparticles as carrier, a supported nano-material of Au atomic cluster/TiO_2 nano-hybrid was synthesized. It was then modified onto the surface of indium tin oxide (ITO) by Nafion to act as a working electrode for exciting the electrochemiluminescence (ECL) of luminol. The properties of the nano-hybrid and the modified electrode were characterized by XRD, XPS, electronic microscopy, electrochemistry and spectroscopy. The experimental results demonstrated that the modification of this nano-hybrid onto the ITO electrode efficiently intensified the ECL of luminol. It was also revealed that the ECL intensity of luminol on this modified electrode showed very sensitive responses to oxygen and hydrogen peroxide. The detection limits for dissolved oxygen and hydrogen peroxide were 2 $\mu g\ L^{-1}$ and 5.5×10^{-12} M, respectively. Besides the discussion of the intensifying mechanism of this nano-hybrid for ECL of luminol, the developed method was also applied for monitoring dissolved oxygen and evaluating the scavenging efficiency of reactive oxygen species of the *Ganoderma lucidum* spore.

1. Introduction

Electrochemiluminescence (ECL) consists of a rapid light emission process from a species of exciton which occurs during an electron transfer reaction and/or concomitant chemical reactions.[1] It has been widely applied for analytical purposes in recent years. In general, the advantages of ECL over conventional chemiluminescent (CL) or fluorescent (FL) analysis include wider dynamic range, higher sensitivity, greater controllability, simpler instrumentation and lower detection cost.[2] It has attracted increasing interest, especially in fields such as trace determination of organic or biochemical compounds, immune diagnostics and biochemical analysis.[3] Of all the luminescent reagents, luminol is one of the most important and has been intensively adopted in ECL applications.[4] However, the application of luminol has been limited by the need for strong alkaline solutions and a high exciting potential of at least 1.5 V.[5] These requirements make it difficult for direct application in bio-analytical situations. Much work in our group has demonstrated that with the help of nanomaterials, the intensification of luminol's ECL can be achieved in weak alkaline medium under lower potential.[6] Also, our previous studies revealed that reactive oxygen species (ROS) played an important role in the intensification of luminol's ECL.[7] These studies established a basis for ECL research of biomaterials and hence extended the scope of ECL research into the physiological field. However, methods to further intensify the ECL of luminol have significant importance to the practical application of ECL in bio-detection.

Titanium dioxide (TiO_2), an important semiconductor material, has been previously studied for the photo-assisted degradation of toxic chemicals.[8] It is also an attractive material for chemical modification of electrodes due to its semi-conductive properties, insertion host capacity and long-term chemical stability.[9] Much research has focused on the mechanism of the oxygen reduction reaction (ORR) on TiO_2 modified electrodes, hence different mechanisms have been proposed.[10] Our previous study also showed that the ECL intensity of luminol on a nano-TiO_2 modified electrode was enhanced and this enhancement was directly related to ROS.[11] Another nano-material, gold nanoparticles, has also been applied in CL and ECL intensification.[12] Electrodes modified by gold nanoparticles have been applied as electrochemical and electrochemiluminescence sensors with excellent results.[13] There are reports on the success of gold nanoparticle-modified electrodes for intensification of luminol's ECL in alkaline solution.[14]

Oxygen and hydrogen peroxide (H_2O_2) are forms of ROS which play pivotal roles in the metabolism of organisms or as the natural byproducts. H_2O_2 is also a general enzymatic product of oxidases and a substrate of peroxidases.[15] The detection of ROS is of great significance in biomedical and other fields. Various methods have been developed for this purpose, such as electrochemical[16] and fluorescent[17] sensors for oxygen, and titrimetry,[18] spectrophotometry,[19] chemiluminescence[20] and electrochemistry[21] for H_2O_2. The biosensing technology based on oxidoreductase[22] is another important research field in H_2O_2 detection.

In the present work, a TiO_2 supported nano-hybrid was synthesized, in which TiO_2 nanoparticles were adopted as

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a carrier to support the Au atomic clusters. This nano-hybrid was then immobilized onto ITO together with Nafion and acted as the working electrode to excite the ECL of luminol. The results indicated that the nano-hybrid showed more effective intensification of the ECL of luminol than nano-TiO_2 or nano-Au alone. Furthermore, this intensification largely reduced the limitations of the ECL of luminol, such as the requirements for alkaline medium and high exciting potential. Strong and stable ECL signals were obtained under lower potential and even in a nearly neutral medium. In particular, the ECL behavior of luminol on this modified electrode was very closely related to the ROS. It provides a very sensitive and practical approach for the detection of low levels of O_2 or H_2O_2.

The as-prepared modified electrode was thereafter applied for real-time monitoring of the distribution of oxygen between gaseous and aquatic phases, and to evaluate the antioxidant efficiency of the spore powder of *Ganoderma lucidum*, an oriental fungus. *Ganoderma lucidum* has been widely used as a remedy for promotion of health and longevity in China and other Asian countries.[23] The fruiting bodies and cultured mycelia of *Ganoderma lucidum* were reported to be effective in the treatment of chronic hepatopathy, hypertension, hyperglycemia and neoplasia. This medical fungus has also attracted great attention due to the fact that its polysaccharide fractions have anti-tumor activity.[24] It was demonstrated that some components of *Ganoderma lucidum* have strong antioxidant potency according to the *in vitro* evaluation of its free radical-scavenging and self-oxidation of 1,2,3-phentriol inhibitory activities, which are comparable to those of vitamin C.[25] The results reported in this paper reveal the great potential of this modified electrode for further applications.

2. Experimental

2.1 Apparatus and reagents

The ECL experiments were carried out on a lab-built system. An RST600 Electrochemical Workstation (custom-built, RST Instrument Co. Ltd., Suzhou, P. R. China) was used to provide the electrolytic potential to excite and record the ECL signals. A R212 photomultiplier tube (PMT, Hamamatsu, Japan) acted as an ECL detector with a −800 V bias potential. A 10 mL cuvette was used as an ECL cell. It was completely shielded with a silver-mirror film, reserving only a small window in the middle of its wall to allow the transmission of light. A piece of ITO glass served as the working electrode while a platinum wire and a saturated calomel electrode were employed as the auxiliary and reference electrodes, respectively. The ECL cell was mounted on the front of the window of the PMT, meanwhile the cell and PMT were shielded in a black box.

Scanning electron microscopy (SEM) was taken with an S-4700 scanning electron microanalyzer (Hitachi, Japan) under an accelerating voltage of 15 kV. A transmission electron microscope (TEM, FEI, USA) was applied to study the morphology and size of nanoparticles at an accelerating voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) was obtained using a Kratos AXIS ultra-DLD ultrahigh vacuum system (at a base pressure of 3×10^{-10} Torr) with monochromatic Al Kα source (1486.6eV).The UV-Vis absorption spectra were obtained on a 2810 UV-Vis spectrophotometer (Hitachi, Japan). A SG-9 dissolved oxygen (DO) meter (Mettler Toledo Instrument Shanghai Co. Ltd, Shanghai, China) was used for DO calibration. The quantification of Au in the nano-hybrid was carried out with an AA-Duo atomic absorption spectrometer (AAS) (Varian, USA).

5-Amino-2,3-dihydro-1,4-phthalazinedione (luminol) was purchased from Fluka (USA). Nafion (5 wt%) solution was from Alfa Aesar (Tianjin, P.R.China). TiO_2 nanoparticles were obtained from Nanjing High Technology Nano Material Co. Ltd (Nanjing, China). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), trisodium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), ammonia, ethanol, phosphate ($NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$) and NaOH were from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All reagents were of analytical grade and used without further purification. Ultrapure water was used in all aqueous solutions.

2.2 Synthesis of Au/TiO_2 nano-hybrid

All glassware used in the procedures was firstly washed with freshly prepared HNO_3/HCl (1 : 3, v/v), then rinsed thoroughly with water and dried. TiO_2 nanoparticles were dispersed in 50 mL 1×10^{-4} g mL^{-1} $HAuCl_4$ with an optimized content of 10 mg mL^{-1} under sonication for 10 min, and then refluxed at 130 °C on an oil bath with stirring. A purple suspension was obtained when 1 mL of 1×10^{-2} g mL^{-1} sodium citrate solution was rapidly injected into the boiling solution under vigorous stirring. After an additional 15 min of stirring, the Au/TiO_2 nano-hybrid had been synthesized.

2.3 Fabrication of Au/TiO_2/ITO modified electrode

The ITO glass was cut into 0.8 cm × 5 cm pieces, these cut pieces were then covered with insulating tape to reserve a 0.8 cm × 1 cm rectangle to act as the working area of the electrode. After that, it was cleaned in an ultrasonic bath sequentially with diluted ammonia, ultrapure water, ethanol and ultrapure water (15 min each), then dried under nitrogen flow. 2 mL of Au/TiO_2 suspension was mixed together with 100 μL of 0.05% Nafion solution, and then 30 μL of the mixture was poured onto the surface of the working area. Finally, the electrode was air-dried and stored at room temperature.

2.4 The manipulation of ECL measurement and important factors

The ECL was excited by consecutive right-rectangle pulsed electrolytic potential. The duty factor, upper and lower limiting potentials of the pulse are the most important electrical factors which greatly influence the ECL performance, and also reflect the mechanism of the ECL response, especially for oxygenic matters. In these experiments, the duty factor of the pulse was selected as 1 : 5, and the limiting potentials were optimized for the best ECL response.

2.5 ECL monitoring of dynamic oxygen distribution

After the test solution was transferred into a capped ECL cell and thoroughly deoxygenated by N_2 purging for 15 min, the ECL

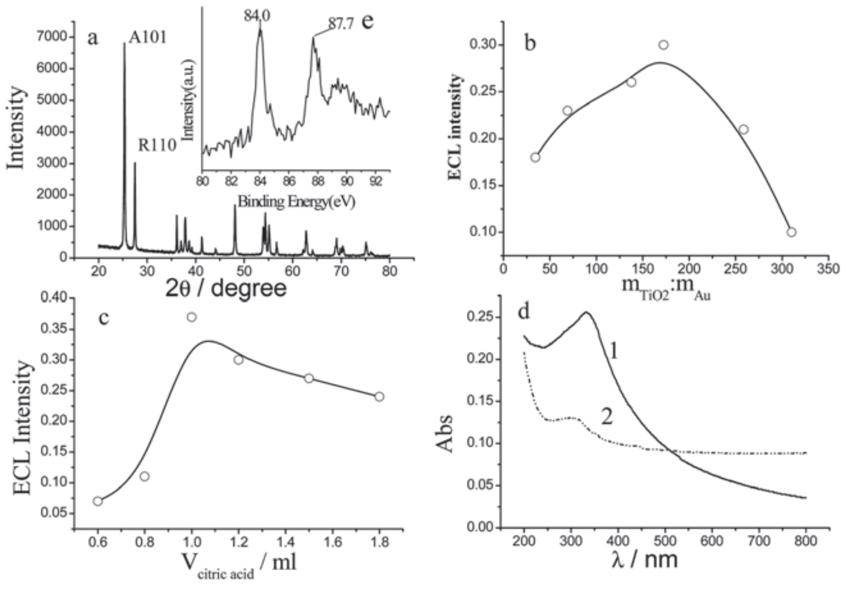


Fig. 1 (a) The XRD of nano-TiO_2, (b) the effect of the mass ratio of TiO_2 to Au on intensification performance, (c) the optimal dosage of sodium citrate for nano-hybrid synthesis, (d) UV-Vis spectra of (1) nano-TiO_2 and (2) Au/TiO_2 nano-hybrid and (e) characteristic region of the XPS of Au/TiO_2.

detection was started, and oxygen was introduced at a constant flow rate into the cell but above the surface of solution. The recorded ECL signal is an index for monitoring the dissolving of oxygen into the solution according to the partial pressure of oxygen. When the ECL signal stabilized, the oxygen flow was stopped, and the dissolved oxygen was released from the solution causing a decrease of the ECL signal.

2.6 Sample preparation and detection

The *Ganoderma lucidum* spore powder was purchased from Dejian Biotechnology Co., Ltd (Shanghai, P. R. China). 10 g *Ganoderma lucidum* spore powder was heated at reflux for 6 h with 100 mL water or ethanol, and centrifuged to obtain the supernatant for further detection.

3. Results and discussion

3.1 Characterization of Au/TiO_2 nano-hybrid and Au/TiO_2/ITO electrode

The X-ray diffraction (XRD) pattern (Fig. 1a) of the nano-TiO_2 shows the typical diffraction peaks of A101 from rutile and R110 from anatase. The proportion of rutile is 75.24% according to $X = 1/(1 + 0.8I_R/I_A)$. So the nano-TiO_2 is a mixed crystal of the two phases—rutile and anatase—which have been demonstrated to have high intensifying efficiency for the ECL of luminol.[11] For the best performance of the nano-hybrid, which was predicted to be related to the dosage of Au and TiO_2, the mass ratio of Au to TiO_2 was optimized by experiment. By quantitative detection of the content of gold in the as-obtained nano-hybrid, after the gold was dissolved in aqua regia and detected by AAS, for 69.7% of the total dosage, the mass ratio was optimized as about 1 : 172 (see Fig. 1b). Therefore, 1×10^{-4} g mL^{-1} of $HAuCl_4$ and 10 mg mL^{-1} of nano-TiO_2 were adopted in the synthesis of the nano-hybrid. Sodium citrate was used as a reducing agent and also as the protective agent in the synthesis of the nano-hybrid; its dosage also strongly affects the reduction procedure of $HAuCl_4$ to obtain the nano-hybrid. Fig. 1c shows that the optimal dosage of sodium citrate was 1 mL of 1×10^{-2} g mL^{-1} solution in a total of 50 mL of the mixture of $HAuCl_4$ and TiO_2, to obtain the best performance of the nano-hybrid. The UV-Vis spectrometric investigation of the as-obtained nano-hybrid (Fig. 1d) revealed important information about its structure. There is no trace of the absorption peaks of Au nanoparticles around 540 nm, which indicates no presence of any independent Au nanoparticles in the nano-hybrid. Additionally, the blue shift of the absorption peak of TiO_2 by about 28 nm demonstrates the strong interaction between the Au and nano-TiO_2, which is the

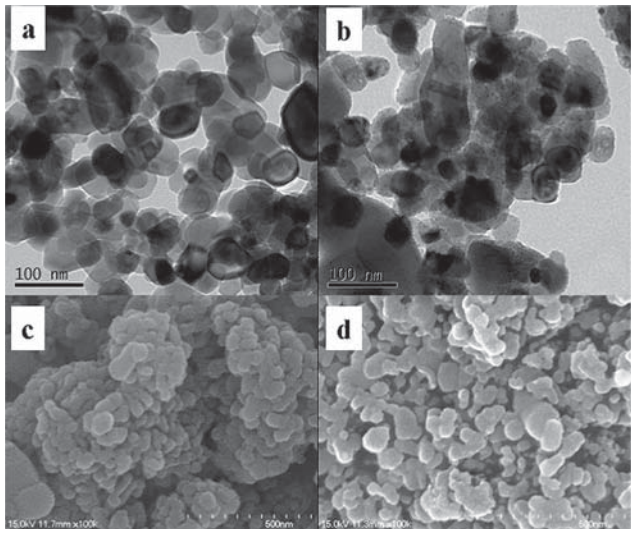


Fig. 2 The TEM images of (a) nano-TiO_2 and (b) Au/TiO_2 nano-hybrid, and the SEM images of (c) TiO_2/ITO electrode and (d) Au/TiO_2/ITO electrode.

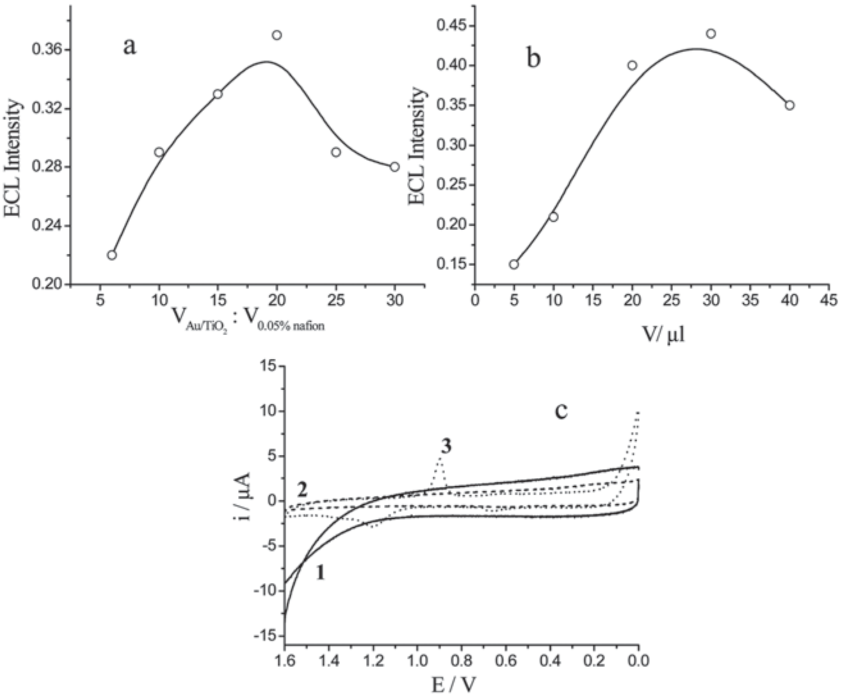


Fig. 3 The effect of (a) volume ratio of Au/TiO_2 dispersion to Nafion and (b) total volume of the mixture on performance of the resulting modified electrode. (c) The cyclic voltammograms on electrodes of (1) ITO, (2) TiO_2/ITO and (3) Au/TiO_2/ITO in 0.5 M H_2SO_4 with a scan rate of 0.05 V s^{-1}.

result of the enlarged band gap of TiO_2.[26] Fig. 1e shows the characteristic region of the XPS of the Au/TiO_2 film; it clearly displays the definite binding energies of Au $4f_{7/2}$ and $4f_{5/2}$ of 84.0 eV and 87.7 eV, respectively, in very good agreement with the standard binding energy of zero-valent gold,[27] demonstrating the presence of atomic gold.

The TEM images (pictures a and b in Fig. 2) show the morphology of nano-TiO_2 and the as-prepared Au/TiO_2

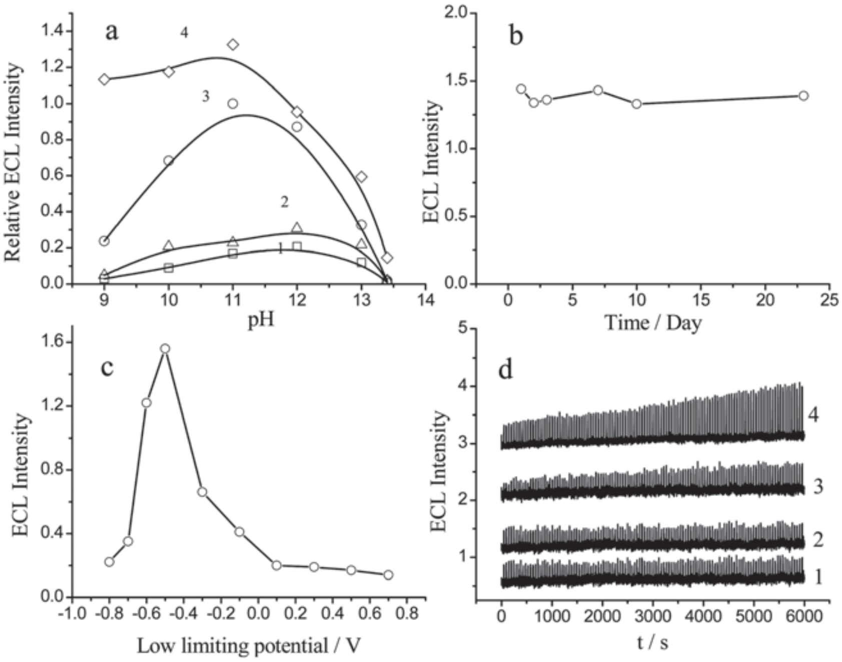


Fig. 4 (a) The effect of pH on ECL intensity of luminol on (1) ITO, (2) TiO_2/ITO and (3) Au/TiO_2/ITO electrodes in oxygen equilibrated solution and (4) Au/TiO_2/ITO electrode in oxygen saturated solution ($C_{\mathrm{Luminol}} = 1 \times 10^{-8}$ M). (b) The stability of Au/TiO_2/ITO electrode. (c) The effect of lower limiting potential and (d) upper limiting potential at (1) 0.8 V, (2) 1.0 V (3) 1.2 V, (4) 1.5 V on the ECL of luminol on Au/TiO_2/ITO electrode.

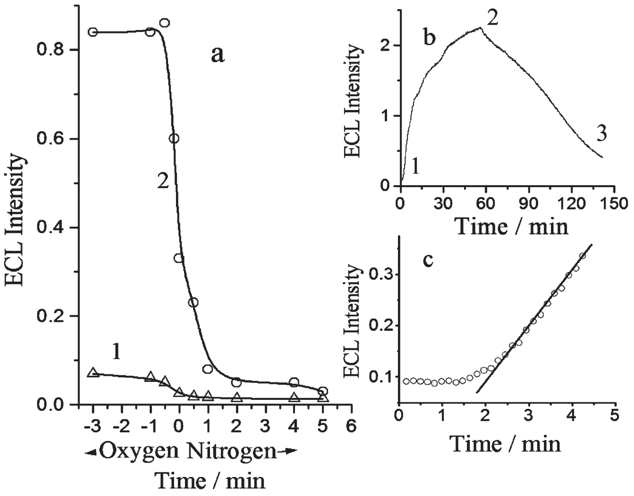


Fig. 5 (a) The influence of dissolved oxygen on the ECL intensity of luminol, (b) the real-time ECL response to dissolved oxygen and (c) the linear relationship between ECL intensity and dissolved oxygen.

nano-hybrid. It could be seen that the nano-TiO_2 was approximately spherical and about 50 nm in diameter. Regarding the TEM of the as-prepared hybrid, there are slight dots mounted on the surface of the nano-TiO_2. The size of these dots was estimated to be less than 1 nm from this TEM image, therefore they could be rationalised as the cluster which was stacked with about ten atoms; also the TEM image suggests the accretion of isolated clusters on the surface of nano-TiO_2. Meanwhile, it is believable that the size of clusters was dependent upon the dosage of $AuCl_4^-$ to TiO_2, the optimization process resulted in the most suitable size of those Au clusters for ECL intensification. All these results demonstrated that the synthesized Au/TiO_2 nano-hybrid was a supporting structure, and the Au atomic clusters were loaded on the surface of nano-TiO_2 in a dispersive way.

The SEM images (Fig. 2 c, d) show the improved homogeneity of the Au/TiO_2 modified layer as compared with the single TiO_2 modified layer. Fig. 3c shows the CV curves of ITO, TiO_2/ITO and Au/TiO_2/ITO in 0.5 M H_2SO_4, and two redox couples of Au appear on the CV curve of Au/TiO_2/ITO. These results revealed that the novel structure of the as-prepared Au/TiO_2 nano-hybrid provided an auriferous surface which resulted in better dispersion and electrochemical activity.

The quantities of Au/TiO_2 nano-hybrid and Nafion for modification on ITO have been studied. As displayed in Fig. 3 a and b, the experimental results show the optimal conditions as 30 μL of the mixture solution of nano-hybrid and Nafion with a 20 : 1 volume ratio.

3.2 The function of immobilized Au/TiO_2 nano-hybrid for intensifying luminol ECL

It is well known that the ECL intensity of luminol depends heavily on the pH of the buffer solution. In phosphate buffer solutions (0.2 M) of pH ranging from 9.0 to 13.4, the ECL behavior of luminol was examined. The results revealed that the ECL responses on all the electrodes were related to the pH, but the optimal pH values were different (see Fig. 4a). The optimal pH value on the bare ITO electrode or TiO_2-modified electrode is 12, and on the Au/TiO_2 modified electrode is 11. This difference could be attributed to the fact that the intensification of Au/TiO_2 for luminol ECL was related to oxygen, which was more efficient under lower pH conditions, as can be seen in curve 4 in Fig. 4a, where the ECL intensities were detected in an oxygen saturated solution. A detailed discussion will be presented in a later section of this paper. Also from this test, it was demonstrated that the ECL intensity of luminol on the Au/TiO_2/ITO electrode was much higher than that on the ITO or the TiO_2/ITO electrode in the tested pH range, with about 15 times enhancement over the bare ITO electrode. The stability and repeatability of the modified electrode was also investigated. Fig. 4b reveals that the intensity decreased by only 3.5% over 23 days, suggesting a good stability. Also, the RSD values for seven consecutive pulses of ECL emission, seven repetitive detections, and the average ECL intensity on seven electrodes for 2×10^{-7} M luminol in phosphate buffer of pH 9.0, were evaluated as 2.4%, 3.4% and 4.4% respectively. These results evidenced the good repeatability of the modified electrode.

Fig. 4 c, d show the effect of the applied pulse potential on the ECL intensity of luminol on the Au/TiO_2/ITO electrode. Fig. 4c displays the alteration of ECL intensity upon variation of the lower limiting potential; it is clear that the greatest intensity appeared at −0.5 V. This means that the ECL must be related to the reduction of some concomitant species in solution. A detailed discussion will appear in a later section of the paper. Fig. 4d illustrates the ECL signal at different upper limiting potentials. It suggests that 1.0 V (curve 2) and even 0.8 V (curve 1) of upper limiting potential are favorable for ECL emission for both intensity and stability, but a higher upper limiting potential is not favorable for stability although the intensity is greater (curves 3 and 4). The results absolutely demonstrate the action of nano-hybrid for reducing the requirement of high potential for ECL.

From the experimental results mentioned above, it is revealed that the Au/TiO_2 nano-hybrid not only intensifies the ECL strength of luminol, but also extends its practicable pH window and reduces the required exciting potential to avoid some possible interference.

Table 1 Comparison of the performance of various detection methods for DO

Method	Detection limit	References
ECL–CdS modified screen-printed carbon electrode	0.02 mg L^{-1}	R. J. Zhang *et al.* (30)
Pulsating potentiometric titration technique	10 μg L^{-1} level	P. Sahoo *et al.* (31)
Optical fiber dissolved oxygen sensor	0–40 mg L^{-1}	C. S. Chu *et al.* (32)
ECL–TiO_2/Nafion/GCE electrode	0.12 mg L^{-1}	Z. Y. Lin *et al.* (33)
ECL–Au/TiO_2/ITO electrode	2 μg L^{-1}	Present work

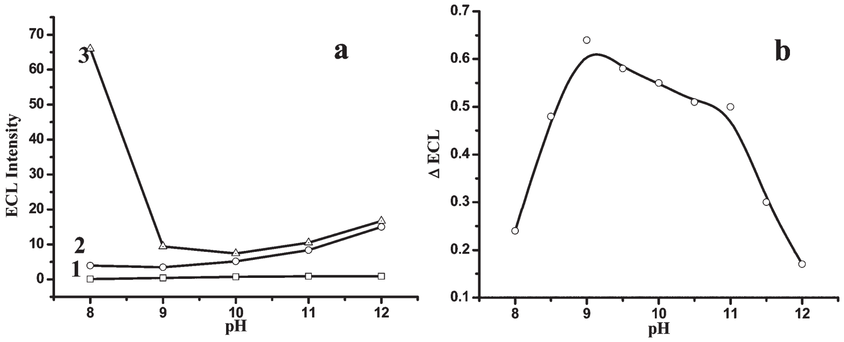


Fig. 6 (a) The responses of ECL on (1) ITO and (2) Au/TiO_2/ITO electrodes towards H_2O_2 at different pH values, (3) is the enhanced multiples. (b) The optimization of pH condition on the quenching effect of extractions of *Ganoderma lucidum* spore in oxygen equilibrated solution.

Table 2 The detection performances for H_2O_2 on modified and bare ITO electrode

pH		8	9	12
ITO	Linear limit (mol L^{-1})	4×10^{-6}	2×10^{-7}	2×10^{-8}
	Linear equation	$I = -1.858 + 4.915E5\ C$	$I = 0.213 + 1.285E6\ C$	$I = 0.293 + 1.35E6\ C$
	Detection limit (mol L^{-1})	3.96×10^{-6}	1.61×10^{-7}	1.73×10^{-8}
Au/TiO_2/ITO	Linear range (mol L^{-1})	2×10^{-10}–1×10^{-8}	1×10^{-10}–1×10^{-8}	2×10^{-11}–1×10^{-8}
	Linear equation	$I = 0.072 + 4.6 \times 10^{8}\ C$	$I = 0.141 + 0.194\ \lg\ [C \times 10^{-11}]$	$I = 0.36 + 8.5 \times 10^{9}\ C$
	Detection limit (mol L^{-1})	1.58×10^{-10}	2.38×10^{-11}	5.54×10^{-12}
	RSD ($n = 6$)	3.8%	3.9%	4.2%

3.3 The ECL response of O_2 and H_2O_2 on modified electrode

Fig. 5a shows that the ECL intensity on both the Au/TiO_2/ITO and ITO electrodes strongly depends upon the presence of oxygen, and the response on the Au/TiO_2/ITO electrode (curve 2) was greatly enhanced compared with that on the bare ITO electrode (curve 1). The results indicate that the modified electrodes are very sensitive to dissolved oxygen. It had been noted that the CL intensity of luminol becomes higher in the presence of O_2 in alkaline solutions,[28] and the ECL of luminol at a gold electrode had been found to be dependent on the presence of O_2 under conventional cyclic voltammetric conditions in alkaline solutions.[29] These results suggested a similar mechanism in that the light emission on this modified electrode was also related to the presence of O_2.

This response to DO can be applied for sensitive real-time monitoring of oxygen dissolution. Fig. 5b shows the dynamic distribution of oxygen between the gaseous and aquatic phases. The ECL intensity gradually rose when blowing oxygen above the surface of the deoxygenated solution (from 1 to 2). Once the oxygen flow was stopped, the ECL intensity decreased until reaching equilibrium (from 2 to 3), which came from the release of oxygen from the solution. At a low level of DO, calibrated with a DO meter, the ECL intensity linearly responded to the content of oxygen (Fig. 5c) with a regression equation of $I = -0.1661 + 3.55 \times 10^{-4}\ C\ (r = 0.9976)$ and a detection limit of 2 μg L^{-1} (S/N = 3). A comparison with different reported methods for DO detection is shown in Table 1.

H_2O_2 is another ROS and is also capable of enhancing the ECL intensity of luminol. As shown in Fig. 6a, over a wide pH range, the response to H_2O_2 on the modified electrode is significantly higher than that from a bare ITO electrode. Here curve 3 is the enhanced multiples, and it indicates approximately 70 times enhancement under the condition of pH 8.0. Table 2 shows the detection performance for H_2O_2 on this modified electrode, with a decrease of almost four orders of magnitudes in the detection limit for this pH range. Table 3 shows the comparison with different reported methods. It demonstrates that the present method is very sensitive compared with previous ones.

3.4 The ROS scavenging effect of *Ganoderma lucidum* spore

Based on the ECL response towards the ROS, the presented electrode could be applied to evaluate the antioxidant efficiency of some scavengers. The extracts of *Ganoderma lucidum* spore powders were tested in experiments.

Table 3 Comparison of the performance of various detection methods towards H_2O_2

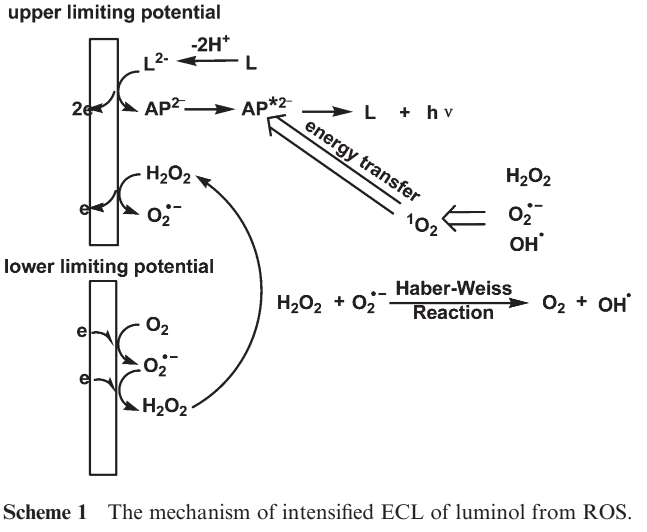
Method	Detection limit	Reference
ECL–TiO_2/ITO electrode	1×10^{-8} M	T. X. Zhou *et al.* (6)
Biosensor–Hb/Ti/nano-Au network electrode	2×10^{-8} M	A. K. M. Kafi *et al.* (34)
Chemiluminescence	1×10^{-7} M	S. Hanaoka *et al.* (35)
Fluorimetry	1.1×10^{-8} M	J. Z. Li *et al.* (36)
ECL–Au/TiO_2/ITO electrode	5.5×10^{-12} M	Present work

In oxygen equilibrated solution, under the pH conditions optimized by the experiments (displayed in Fig. 6b), the quenching degree of ECL (ΔECL) responded linearly to the amount of extract added. Within the extract range of 20 mg L^{-1} to 120 mg L^{-1}, the ΔECL and amount of the added extract followed the equations $y = 0.347 + 0.0901x$ or $y = 0.11 + 0.09x$ for ethanol or water as extraction solvent. By calibration with the DO meter ($I = -0.403 + 2.428DO$, $r = 0.9992$), the ECL quenching caused by *Ganoderma lucidum* spore extract can be converted into the gross ROS scavenging efficiency of 19.0 mg O_2 g^{-1} with ethanol as the extraction solvent or 13.7 mg O_2 g^{-1} with water as the extraction solvent.

3.5 The mechanism of intensification of ECL of luminol on Au/TiO$_2$/ITO electrode

It has been reported that TiO_2 can accelerate electron transfer,[37] and then the dissolved oxygen could be reduced to yield superoxide anions ($O_2^{\cdot-}$). The effect of lower limiting potential on ECL behavior of luminol is also meaningful for understanding the intensification mechanism. This potential is adequate to reduce the oxygen to superoxide anions or H_2O_2, and thereafter the H_2O_2 could be re-oxidized to generate superoxide anions under the upper limiting potential. Meanwhile, a Haber–Weiss reaction will take place between the superoxide anions and H_2O_2 to yield the more oxidative hydroxyl free radicals. Finally, singlet oxygen ($^1O_2$), the entity of excited oxygen, will be produced from the reactions of those ROS[38] and transfer the energy to intensify the ECL. According to the discussion in our previous papers,[39] there only demanded lower potential to oxidize the luminol to its intermediate and to induce the oxygen transformation, so the applied potential for this nano-hybrid intensified ECL emission could be lower; also the intensification is more efficient at lower pH.[7] Scheme 1 describes the reaction mechanism clearly.

As discussed in our research,[39] the moderate width of the band gap of TiO_2 (3.2 eV) is favorable for the generation of an electron–hole couple (e^-/h^+) through the electron transition from the valence band to the conduction band. These as-generated couples could gain electrons from the externally absorbed species on the surface to cause their redox reactions. Comparatively, the holes are more reactive to induce highly oxidative radicals which would catalyze the oxidation of luminol to produce an excited state AP^{2-*}, to enhance its ECL. The Au atomic cluster on the surface of TiO_2 plays another important role in this ECL intensification. As is well known, as a poorly reactive metal, gold has been regarded as a poorly active heterogeneous catalyst. However, when gold is deposited on selected metal oxides as ultra-fine particles, its chemistry dramatically changes. The highly dispersed gold catalysts are in fact very active in many important reactions.[40] On the other hand, even though oxygen was also adsorbed onto the TiO_2 without Au, the presence of Au has a positive effect on O_2 adsorption. The Au promotes the formation of superoxide ($O_2^{\cdot-}$) bound to Ti^{4+}.[41] The third function of the Au cluster is to boost the generation of electron–hole couples in TiO_2 due to the depressed Fermi level pinning effect.[26]



Scheme 1 The mechanism of intensified ECL of luminol from ROS.

4. Conclusion

In conclusion, a TiO_2 supported Au atomic cluster nano-hybrid was synthesized and modified on the ITO to act as the working electrode for the excitation of luminol ECL. It enhances the ECL intensity and meanwhile enlarges the practicable pH range and reduces the required potential. The developed Au/TiO_2/ITO modified electrode exhibits high sensitivity towards trace amounts of H_2O_2 or oxygen. It was then applied to monitor the DO and detect the ROS scavenging efficiency of *Ganoderma lucidum* spores with satisfactory results. The intensification for ECL of luminol on this nano-hybrid modified electrode showed great potential for the sensitive detection of reactive oxygen species and further substances in biological systems.

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