

Electrogenerated Chemiluminescence of Luminol on a Gold-Nanorod-Modified Gold Electrode

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Electrogenerated chemiluminescence (ECL) of luminol on a gold-nanorod-modified gold electrode was studied, and five ECL peaks were obtained under conventional cyclic voltammetry in both neutral and alkaline solutions. Among them, four ECL peaks (ECL-1–4) were also observed on a gold-nanosphere-modified gold electrode, but the intensities of these ECL peaks were enhanced about 2–10-fold on a gold-nanorod-modified gold electrode in neutral solution. One new strong ECL peak (ECL-5) was obtained at -0.28 V (vs SCE) on a gold-nanorod-modified gold electrode in both neutral and alkaline solutions and enhanced with an increase in pH. In strong alkaline solutions, ECL-1 and ECL-2 on a gold-nanosphere-modified electrode were much stronger than those on a gold-nanorod-modified gold electrode, while ECL-3–5 appeared to only happen on a gold-nanorod-modified gold electrode. The emitter of all the ECL peaks was identified as 3-aminophthalate. The ECL peaks were found to depend on the scan direction, the electrolytes, the pH, and the presence of O_2 and N_2 . The reaction pathways for ECL-4 have been further elucidated, and the mechanism of the new ECL peak (ECL-5) has been proposed. The results indicate that a gold-nanorod-modified gold electrode has a catalytic effect on luminol ECL different from that of a gold-nanosphere-modified gold electrode, revealing that the shape of the metal nanoparticles has an important effect on the luminol ECL behavior. The strong ECL of luminol in neutral solution obtained on a gold-nanorod-modified electrode may be used for the sensitive detection of biologically important compounds in physiological conditions.

Introduction

Colloidal gold nanoparticles have received considerable interest in recent years because of their unique catalytic and optical properties, chemical reactivity, and excellent biocompatible property.^{1–5} Recently, special attention has been paid to electrogenerated chemiluminescence (ECL) involving gold nanoparticles.^{6–12} In our group, it was found that, on a 16 nm gold nanosphere self-assembled gold electrode, two anodic ECL peaks and one cathodic ECL peak of luminol in neutral aqueous solution were greatly enhanced and a new cathodic ECL peak was initiated compared with a bare gold electrode, whereas, in alkaline solution, two anodic ECL peaks much stronger than those on a bulk gold electrode were obtained.⁹ In addition, two enhanced cathodic ECL peaks of lucigenin were also observed on such a modified electrode.¹⁰

The gold nanorods are of great importance among gold nanoparticles due to their shape-dependent optoelectronic properties.¹³ Some unique properties of gold nanorods such as the appearance of a longitudinal plasmon resonance, strong surface-enhanced Raman scattering, fluorescence, and anisotropic chemical reactivity can be exploited for various applications.^{4,14,15} Previous work demonstrated that ECL behavior depended on the electrode material as well as the surface state of the electrode.^{16–20} As the catalytic effect of nanoparticles depends on their sizes and shapes, it is reasonable to deduce that the ECL signal of some traditional ECL systems might be improved significantly by use of a gold-nanorod-modified electrode. However, to the best of our knowledge, there is no

report concerning the effect of a gold-nanorod-modified electrode on traditional ECL systems. In this work, gold nanorods synthesized by the wet chemical method^{21–26} were fabricated onto the cysteine monolayer self-assembled on the surface of the gold electrode through electrostatic interaction. The surface state of a gold-nanorod-modified gold electrode was characterized by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). Luminol ECL was explored on such a modified electrode under conventional cyclic voltammetry (CV) conditions in neutral and alkaline solutions. It was found that five ECL peaks were generated. Among them, four ECL peaks were also observed on a gold-nanosphere-modified gold electrode, but the intensities of these ECL peaks were significantly enhanced on a gold-nanorod-modified gold electrode in neutral solution. One new strong ECL peak was obtained on the gold-nanorod-modified electrode in both neutral and alkaline solutions. The effects of various factors such as the potential scan direction and range, N_2 , O_2 , the pH, the electrolytes, and the concentration of KBr on luminol ECL peaks were examined. Finally, the reaction pathways for ECL-4 have been further elucidated, and a possible mechanism for the new ECL peak has been proposed.

Experimental Methods

Chemicals and Solutions. A 1.0×10^{-2} mol/L stock solution of luminol was prepared by dissolving luminol (Sigma) in 0.1 mol/L sodium hydroxide solutions without purification. Working solutions of luminol were prepared by diluting the stock solution. $HAuCl_4 \cdot 4H_2O$ (48% w/w) was obtained from Shanghai Reagent (Shanghai, China). A 1.0 g/L $HAuCl_4$ stock solution was prepared by dissolving 1 g of $HAuCl_4$ in 1 L of redistilled water

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and stored at 4 °C. All other reagents used in these experiments were of analytical grade, and doubly distilled water was used throughout. All glassware were cleaned by aqua regia and rinsed with redistilled water prior to experiments.

Synthesis of Gold Nanorods. Two kinds of gold nanorods with different aspect ratios were synthesized according to the seed-mediated protocols to compare the effect of the aspect ratio of gold nanorods on luminol ECL. Gold nanorod-1 was synthesized as described in ref 25, and gold nanorod-2 was synthesized as described in ref 26.

The resulting gold nanorods were characterized by transmission electron microscopy (TEM) (Hitachi H-800, Japan) and UV-vis spectroscopy (Shimadzu UV-2401 PC spectrophotometer, Japan). Statistical analysis of TEM data revealed that the average aspect ratio was 3.2 ± 0.5 for gold nanorod-1 and 7.5 ± 1.1 for gold nanorod-2, respectively. The UV-vis spectra exhibited two well-separated surface plasmon absorption peaks at 520 and 670 nm for gold nanorod-1 and 530 and 810 nm for gold nanorod-2, corresponding to transverse and longitudinal plasmon bands, respectively.

Fabrication of Gold Nanorods on a Gold Electrode. A 0.1 mol/L concentration of cysteine was prepared by dissolving cysteine in pH 7 0.1 mol/L phosphate buffer solutions (PBSs) to ensure that cysteine was negatively charged in the solution. A 6.8×7.0 mm² bar gold electrode was polished with abrasive paper and rinsed with ethanol and redistilled water to remove the trace remainder. Then, the electrode was cycled between 0 and 1.5 V (vs SCE) in 0.5 mol/L sulfuric acid at a scan rate of 100 mV/s. This potential cycling was continued until a reproducible voltammogram for gold oxide formation/reduction was obtained, indicating a clean surface of the gold electrode was obtained. The electrode was again rinsed with redistilled water and cleaned in an ultrasonic bath. The cleaned gold electrode was immersed in pH 7 cysteine solution for 2 h at room temperature in darkness to form a monolayer of cysteine and dried under air conditions. A 25 μ L sample of the gold nanorod solution was dropped onto the modified gold electrode and dried under air conditions. The morphology of the gold-nanorod-modified electrode was characterized by SEM (JEOL JSM-6700F, Japan).

Electrochemical, ECL, and ECL Spectral Measurements. ECL and electrochemical (EC) measurements were performed by a homemade ECL/EC system, including a model CHI832 electrochemical working station (Chenhua Inc., China, i.e., the Chinese distributor of CH Instruments Inc., Austin, TX), an H-type electrochemical cell (homemade), a model 1P21 photomultiplier tube (PMT) (Binsong Electronic Co., China), a model GD-1 luminometer (Ruimai Electronic Science Co., China), and a computer. A gold-nanorod-modified gold electrode served as the working electrode, a platinum wire as the counter electrode, and a silver wire as the quasireference electrode (AgQRE). Although the potential of the AgQRE was found to be essentially stable during an experiment, measurements of $\Delta E = E_{\text{Ag}/\text{Ag}^+} - E_{\text{SCE}}$ in different solutions were taken for potential calibrations.

To the working compartment and the auxiliary compartment of the ECL cell were added 2.0 mL portions of the sample solution and the blank solution without luminol, respectively. When a potential was applied to the working electrode, an ECL signal was generated. The curves of ECL intensity versus applied potential (I_{ECL}/E) and the curves of current versus applied potential (i/E) were recorded simultaneously.

An RF-5301 PC spectrofluorophotometer (Shimadzu, Japan) was used to record the ECL spectra of various peaks at different

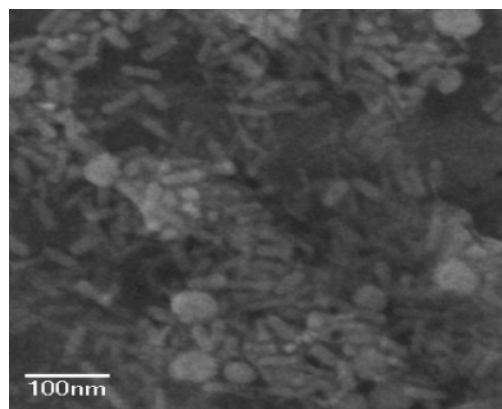


Figure 1. SEM image of a gold-nanorod-modified gold electrode.

potentials under CV conditions. The time needed for recording ECL spectra was 4 s, and the adopted potential range for the measurement of ECL-1–5 was 0.56–0.72, 0.83–0.99, –0.40 to –0.56, –1.44 to –1.60, and –0.38 to –0.22 V, respectively. In the potential range, the ECL intensity did not change significantly with the potential.

ac Impedance Measurements. A model CHI 760B electrochemical working station (Chenhua Inc., China) was used for EIS. ac impedance measurements were carried out at an amplitude of 5 mV in the frequency range from 10 kHz to 0.01 Hz. Complex impedance plots were obtained at 0.28 V with the $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ pair acting as a probe.

Results and Discussion

Surface State of a Gold-Nanorod-Modified Gold Electrode. Gold nanorods were self-assembled on a gold electrode by using cysteine as a link reagent. The surface state of the electrode modified with gold nanorods was characterized by SEM as shown in Figure 1. It was found that gold nanorods could form a relatively dense film on the surface of a gold electrode. Gold nanorods synthesized by wet chemical methods are positively charged for the existing cetyltrimethylammonium bromide (CTAB) bilayer.²⁷ It was reported that cysteine could be adsorbed on a gold electrode through the formation of S–Au bonds, with –COOH and –NH₂ groups extending into the surrounding solution freely.²⁸ When the pH of the solution was higher than the isoelectric pH of cysteine, the carboxyl was negatively charged through deprotonation, which adsorbed positively charged gold nanorods by virtue of electrostatic interaction and constructed the gold nanorod monolayer.

Electrochemical Impedance Spectroscopy of the Gold Nanoparticle Monolayer. It is well-known that EIS is an effective tool for studying the interface properties of surface-modified electrodes.^{29–31} To better understand the properties of a gold nanorod self-assembly film in the ECL process, EIS was recorded as shown in Figure 2. The impedance spectrum of a bare gold electrode was a semicircle in all frequency domains, indicating that the electrode process was controlled only by electron transfer. When gold nanoparticles were self-assembled on the gold electrode, the impedance spectra consisted of a semicircle at high ac modulation frequency and a line at low ac modulation frequency, demonstrating that the electrode process was controlled by electron transfer at high frequency and controlled by diffusion at low frequency. The diameter of the Nyquist circle on a bare gold electrode was larger than that on a gold-nanoparticle-modified gold electrode, indicating that the charge transfer through the gold nanoparticle film was relatively facile. It was reported that the assembly of gold

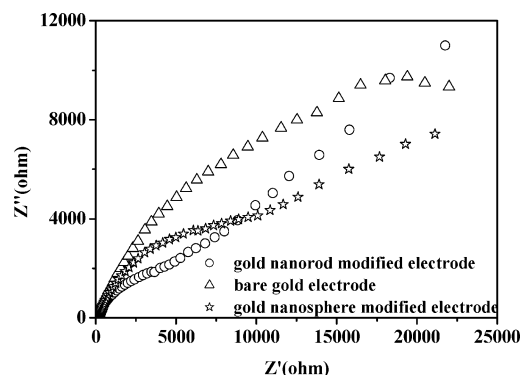


Figure 2. Electrochemical impedance spectroscopy of a bare gold electrode (triangles), of a gold-nanosphere-modified gold electrode (stars), and of a gold-nanorod-modified gold electrode (circles) ($\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$, 1×10^{-3} mol/L; KBr , 0.05 mol/L).

nanoparticles on a gold electrode behaved as an array of randomly distributed nanoelectrodes.³² The fast electrode charge-transfer kinetics on a gold-nanoparticle-modified gold electrode originated from the enlarged surface area of the gold nanoparticle monolayer.³³ Even though electrostatic repulsion between the negatively charged gold nanosphere and the $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ pair existed, charge transfer on a gold-nanosphere-modified electrode was still more facile than that on a bare gold electrode. Meanwhile, the diameter of the Nyquist cycle on a gold-nanorod-modified gold electrode was smaller than that on a gold-nanosphere-modified gold electrode, indicating that the charge transfer through the gold nanorod monolayer was easier than that through the gold nanosphere monolayer. It is known that gold nanorods are positively charged while gold nanospheres are negatively charged.²⁷ Therefore, it was easier for the $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$ pair to reach the gold nanorod monolayer than the gold nanosphere monolayer for the existence of electrostatic interaction, and as a result, charge-transfer resistance of the gold nanorod film was smaller than that of the gold nanosphere film.

Electrochemistry and ECL of Luminol in Neutral Solution under Air-Saturated Conditions. Cyclic voltammograms and I_{ECL}/E curves of luminol in pH 7 solution containing KBr on a

gold-nanorod-modified gold electrode, on a gold-nanosphere-modified gold electrode, and on a bare gold electrode under air-saturated conditions are shown in Figure 3. In cyclic voltammograms of a gold-nanorod-modified gold electrode (Figure 3A), three anodic peaks (cvp1, cvp2, cvp3) were observed at 0.63, 0.81, and 1.20 V (vs SCE), respectively, on the positive scan. Three cathodic peaks (cvp4, cvp5, cvp6) were observed during the reverse potential scan at 0.41, -0.41 , and -1.20 V, respectively. Among them, cvp1 disappeared in blank solution without luminol (inset a in Figure 3A), which suggested that cvp1 corresponded to the oxidation process of luminol. cvp2 could be observed on a gold-nanorod-modified gold electrode, but not on a bare gold electrode or a gold-nanosphere-modified gold electrode (Figure 3A,C), indicating that cvp2 was contributed by the oxidation of the gold nanorod. cvp3 could be observed on a gold-nanorod-modified gold electrode, on a gold-nanosphere-modified gold electrode, and on a bare gold electrode, which could be attributed to the oxidation of the gold substrate. When KBr was replaced by KNO_3 , the intensity of cvp3 decreased greatly, but cvp3 could still be observed. It was reported that Br^- could also be oxidized to BrO^- at this potential;¹⁶ therefore, cvp3 probably included the oxidation of Br^- . However, it was difficult to distinguish the oxidation peak of Br^- from that of gold under this condition. cvp4 with a shoulder probably contained two electrochemical processes, which could be distinguished clearly on a gold-nanosphere-modified gold electrode and on a bare gold electrode (Figure 3C), corresponding to the reduction of the oxidized gold substrate and BrO^- , respectively.⁹ cvp5 was for the reduction of dissolved oxygen in solution to HOO^- according to our previous studies on luminol ECL at a glassy carbon electrode.¹⁷ cvp6 was for the reduction of PO_4^{3-} , which was proven in previous work.⁹ Finally, the onset of H_2 was at about -1.35 V.

In I_{ECL}/E curves on a gold-nanorod-modified electrode (Figure 3B), ECL-1 and ECL-2 were observed at 0.65 and 0.92 V, respectively, on the positive scan, corresponding to the potentials of cvp1 and cvp3, respectively. The ECL-3 peak, corresponding to the electrochemical process of cvp5, was obtained at -0.50 V, where dissolved oxygen was reduced to HOO^- . ECL-4 was initiated at -1.30 V, where hydrogen evolution started. The

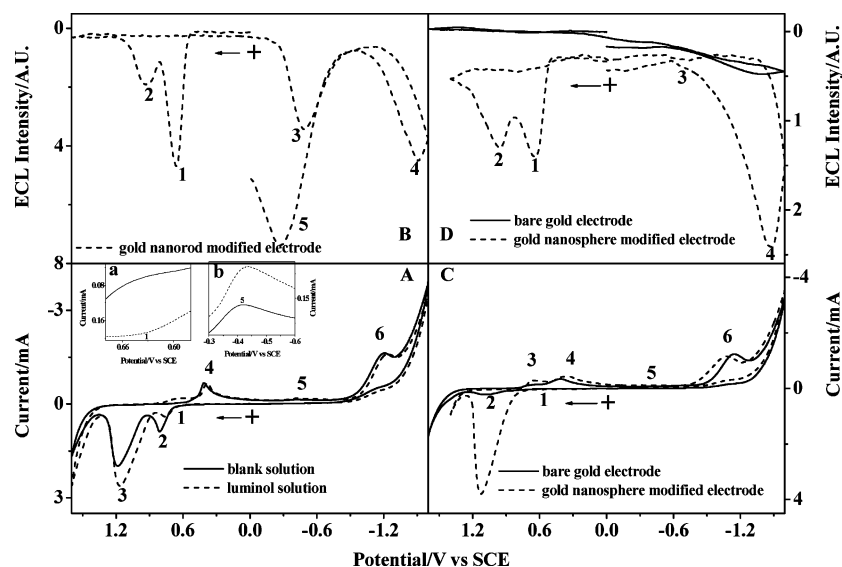


Figure 3. (A) CV curves of a gold-nanorod-modified gold electrode, (B) I_{ECL}/E curves of luminol on a gold-nanorod-modified gold electrode in a blank solution (solid line) and in a sample solution (dashed line), and (C) CV curves and (D) I_{ECL}/E curves of luminol on a gold-nanosphere-modified gold electrode (dashed line) and on a bare gold electrode (solid line) under air-saturated conditions (KBr , 0.1 mol/L; pH 7; PBS , 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s). Inset a shows the enlarged cvp1, and inset b shows the enlarged cvp5. If not mentioned additionally, all high voltages applied to the PMT were maintained at -550 V.

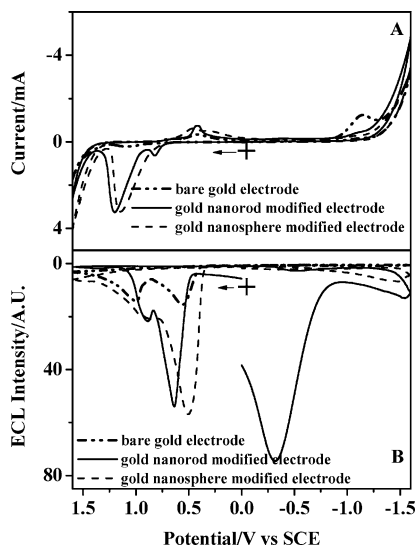


Figure 4. (A) CV curves and (B) I_{ECL}/E curves of luminol on a bare gold electrode (dashed–dotted–dotted line), a gold-nanorod-modified gold electrode (solid line), and a gold-nanosphere-modified gold electrode (dashed line) under air-saturated conditions (KBr, 0.1 mol/L; pH 8; PBS, 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

ECL-5 peak was obtained at -0.28 V on the reverse scan, where no corresponding electrochemical peak was observed in cyclic voltammograms.

ECL-1–4 could also be obtained on a gold-nanosphere-modified gold electrode, and all ECL peaks were almost invisible on a bare gold electrode (Figure 3D). ECL-5 could only be observed on a gold-nanorod-modified gold electrode, implying that gold nanorods were necessary for the generation of ECL-5. The intensities of the ECL peaks were enhanced by ~ 2 –3 orders of magnitude on gold-nanoparticle-modified gold electrodes compared with a bare gold electrode in neutral solution. The intensities of ECL-1–4 on a gold-nanorod-modified gold electrode were obviously enhanced compared with those on a gold-nanosphere-modified gold electrode. ECL-1 and ECL-3 were enhanced about 5-fold and 10-fold, respectively, and ECL-2 and ECL-4 about 2-fold. The results demonstrate that a gold-nanorod-modified gold electrode has a stronger catalytic effect on luminol ECL than a gold-nanosphere-modified gold electrode in neutral solution. It was concluded from the result of EIS (Figure 2) that anions could easily approach a gold nanorod monolayer. Therefore, the charge transfer of luminol anions through a gold nanorod film was more facile than that through a gold nanosphere film, and stronger ECL peaks were obtained on a gold-nanorod-modified gold electrode.

Effect of pH on Luminol ECL. The effect of the pH on luminol ECL was examined in the pH range of 8.0–10.0. The behavior of luminol ECL in pH 8 solution containing KBr on a gold-nanorod-modified gold electrode, on a gold-nanosphere-modified gold electrode, and on a bare gold electrode under air-saturated conditions is shown in Figure 4. In pH 8 solution, ECL-1–5 were also observed on a gold-nanorod-modified gold electrode, while ECL-1–4 and ECL-1,2 could be obtained on a gold-nanosphere-modified gold electrode and on a bare gold electrode, respectively (Figure 4B). ECL-5 could only be observed on a gold-nanorod-modified gold electrode, just like that in neutral solution. ECL-1 and ECL-2 were enhanced about 4-fold and 2-fold, respectively, on two gold-nanoparticle-modified gold electrodes compared with a bare gold electrode. The intensities of ECL-3 on two gold-nanoparticle-modified gold

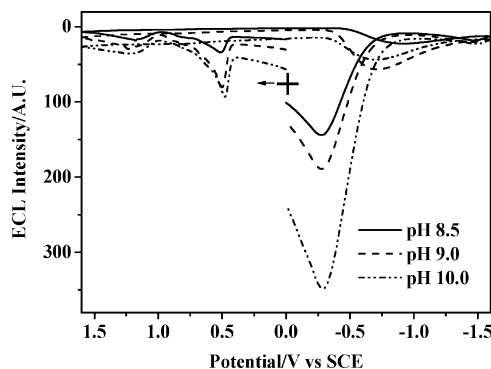


Figure 5. Effect of pH on luminol ECL: pH 8.5 (solid line), pH 9 (dashed line), pH 10 (dashed–dotted line) (PBS, 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

electrodes were almost the same. The results indicated that gold nanorods and gold nanospheres had the same effect on ECL-1–3 in pH 8 solution. ECL-4 was stronger on a gold-nanorod-modified gold electrode than that on a gold-nanosphere-modified gold electrode. Therefore, both gold nanospheres and gold nanorods could catalyze ECL-1–4 significantly, and gold nanorods were better than gold nanospheres for ECL-4. Additionally, gold nanorods could initiate ECL-5.

In the pH range of 8.0–10.0 (Figure 5), the intensities of ECL-1, ECL-2, and ECL-5 increased consistently with an increase in pH on a gold-nanorod-modified gold electrode. ECL-3 increased with an increase in pH in the range of 8.0–9.0, reached its maximum at pH 9.0, and then decreased with a further increase of pH. However, ECL-4 changed slightly in the pH range, which was probably due to the fact that ECL-4 was generated in the potential range of hydrogen evolution accompanied by the formation of a lot of OH^- anions and had almost no relationship with the solution pH. It could also be found from Figure 5 that a new ECL peak appeared at about 1.4 V in the higher pH solution, which was probably due to the oxidation of OH^- to H_2O_2 , which could react with luminol anions to generate chemiluminescence.¹⁸ When the pH exceeded 11.0, the gold-nanorod-modified gold electrode was damaged; therefore, only pH values lower than 11.0 were studied.

The maximal emission was obtained at pH 10.0 for most of the ECL peaks on a gold-nanorod-modified gold electrode, whereas the maximal emission occurred at pH 11.0 on a gold-nanosphere-modified gold electrode.⁹ ECL-1 and ECL-2 appearing in the positive potential region at pH 11.0 on a gold-nanosphere-modified gold electrode were much stronger than those at pH 10.0 on a gold-nanorod-modified gold electrode. ECL-3–5 appearing in the negative potential region only happened on a gold-nanorod-modified gold electrode in strong alkaline solution. The results reveal that gold nanospheres are favorable for ECL emission in the positive potential region and gold nanorods are advantageous for ECL emission in the negative potential region in strong alkaline solution.

It was reported that gold nanoparticles could adsorb radicals.³⁴ Therefore, luminol anion radicals ($\text{L}^{\bullet-}$) might be adsorbed on gold nanoparticles on a gold electrode in the positive potential region. In mild alkaline solution, on one hand, a gold nanorod film could accelerate the electron transfer, causing an increase in the ECL intensity; on the other hand, OH^- could also be adsorbed on positively charged gold nanorods through electrostatic interaction, which would compete with luminol anion radical adsorption on gold nanorods and lead to a decrease in ECL intensity. As a result, the catalytic effect of gold nanorods and gold nanospheres on ECL-1,2 was almost the same, which

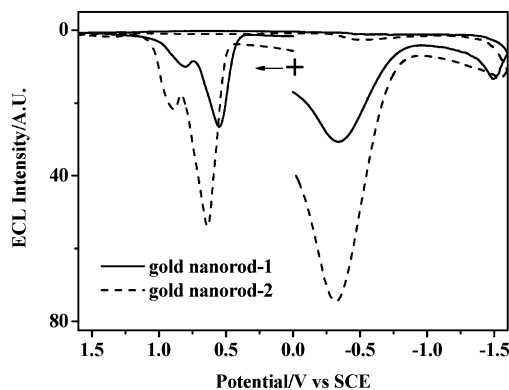


Figure 6. I_{ECL}/E curves of luminol on a gold-nanorod-1-modified gold electrode (solid line) and on a gold-nanorod-2-modified gold electrode (dashed line) in alkaline solution under an air-saturated atmosphere (KBr, 0.1 mol/L; pH 8; PBS, 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

was different from that in neutral solution. In strong alkaline solution, the competitive adsorption of OH^- with luminol anion radicals for positively charged gold nanorods plays a predominant role in the existence of abundant OH^- anions in solution. As a result, ECL-1 and ECL-2 obtained on a gold-nanorod-modified gold electrode, which related to luminol anion radicals directly, were much weaker than those on a gold-nanosphere-modified gold electrode in strong alkaline solution. ECL-3–5 had no direct relationship with luminol anion radicals, were not influenced by the competitive adsorption of OH^- , and could be obtained on a gold-nanorod-modified gold electrode in strong alkaline solution.

Luminol ECL on a Gold Electrode Modified with Gold Nanorods of Different Aspect Ratios. A gold electrode modified with gold nanorod-1 and gold nanorod-2 was used to study luminol ECL as shown in Figure 6. It was found that gold nanorods with larger aspect ratios had a stronger catalytic effect on luminol ECL than those with smaller aspect ratios. Jana and co-workers studied the anisotropic chemical reactivity of gold nanorods, suggesting that there were many defect sites along the length of the nanorods, which would be the sites for chemical attack.¹⁴ Therefore, longer nanorods might have more defect sites and might more easily adsorb luminescent reagent to generate chemiluminescence, and as a consequence, a stronger ECL intensity was obtained on a larger aspect ratio gold-nanorod-modified gold electrode.

Effect of Various Electrolytes and the Concentration of KBr. The luminol ECL was studied in pH 8 PBS solution containing electrolytes such as KCl, KBr, KI, and KNO_3 . It was found in Figure 7 that the four electrolytes exhibited different effects on luminol ECL. For halide ions, the intensity of ECL-1 increased in the following order: $\text{KI} < \text{KBr} < \text{KCl}$, being in good agreement with the quenching effect of luminescence by heavier halide ions.⁹ ECL-2 was not apparent and became a shoulder of ECL-1 in the solutions other than KBr. ECL-3 could be obtained in KCl and KBr solutions and was hard to observe in KNO_3 and KI solutions. ECL-4 could be observed in the solutions containing halogen ions just like that on a gold-nanosphere-modified gold electrode, indicating that ECL-4 was related to halogen ions, which was demonstrated in previous work.⁹ ECL-5 could be found in KI and KBr solutions and was extremely weak in KCl and KNO_3 solutions, indicating that I^- and Br^- might play an important role in the formation of ECL-5.

The effect of the concentration of KBr was also studied as shown in Figure 8. When the intensity of ECL-1 was very

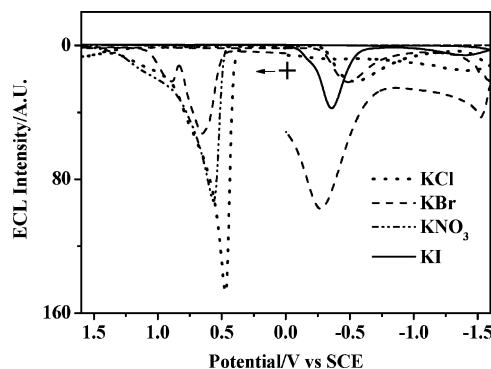


Figure 7. I_{ECL}/E curves of luminol under air-saturated conditions in various electrolytes such as KCl (dotted line), KBr (dashed line), KI (solid line), and KNO_3 (dashed–dotted–dotted line) (concentration of the electrolytes, 0.1 mol/L; pH 8; PBS, 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

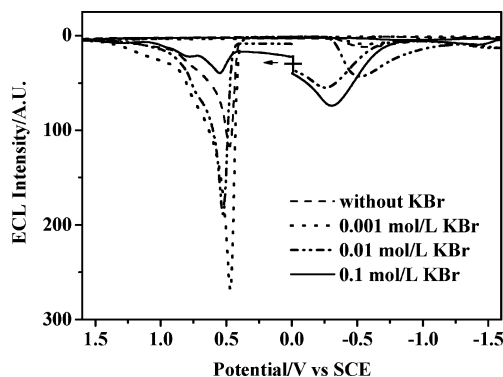


Figure 8. Effect of the concentration of KBr on luminol ECL: 0.1 mol/L KBr (solid line), 0.01 mol/L KBr (dashed–dotted–dotted line), 0.001 mol/L KBr (dotted line), without KBr (dashed line) (pH 8; PBS, 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

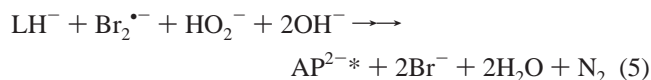
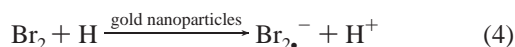
strong, ECL-2 dwindled to a shoulder peak. The intensities of ECL-1 and ECL-2 decreased when the concentration of KBr increased from 0.001 to 0.1 mol/L. However, when luminol solution did not contain KBr, the intensities of ECL-1 and ECL-2 were lower than those in 0.001 mol/L KBr solution. The strongest intensities of ECL-3 and ECL-4 were obtained in 0.01 mol/L KBr solution, while the strongest ECL-5 intensity was obtained in 0.1 mol/L KBr solution. When the concentration of KBr was too low, ECL-4 and ECL-5 disappeared. KBr acted both as a reactant for ECL-2–5 and as a supporting electrolyte in the ECL system. As a reactant, the higher the concentration of KBr, the stronger the intensity of the ECL peaks. However, too high a concentration of KBr would lead to the quenching of luminescence.⁹ As a supporting electrolyte, the concentration of KBr influences the ion strength of the solution. It was reported that some intermediates called “encounter complexes” could be generated first during the ECL process, which could be stabilized by lowering the ion strength of the solution and favor light emission subsequently.^{35,36} However, when the concentration of electrolyte was too low, the conductivity of the solution was damaged, and as a result, the ECL intensity decreased. Therefore, the intensity of the ECL peak depends on the co-action of the factors above, and there is an optimal concentration of KBr for different ECL peaks.

Effect of N_2 and O_2 . Under an O_2 atmosphere, ECL-1 and ECL-2 increased slightly, whereas ECL-3, ECL-4, and ECL-5 increased significantly. Under a N_2 atmosphere, the intensities of all ECL peaks decreased at least 1 order of magnitude. The results suggest that O_2 takes part in the reactions of five ECL peaks.

ECL Spectra of Various Peaks. The ECL spectra of ECL-1–5 in I_{ECL}/E curves were analyzed under air-saturated conditions using a fluorescence spectrometer as shown in Figure 9. The results show that the maximum emission of all ECL peaks is ~ 430 nm, corresponding to the light emission of 3-aminophthalate (AP^{2-}). It is concluded that all ECL peaks are initiated by the luminol reactions.

Mechanism of Luminol ECL on a Gold-Nanorod-Modified Gold Electrode. ECL-1–4 could be obtained on a gold-nanorod-modified gold electrode as well as on a gold-nanosphere-modified gold electrode under the same experimental conditions, with only a difference in the ECL intensity, and the emitter of all the ECL peaks was identified as 3-aminophthalate. Moreover, the electrochemical processes corresponding to ECL-1–4 and the species involved in ECL-1–4 on a gold-nanorod-modified gold electrode according to the studies above were the same as those on a gold-nanosphere-modified gold electrode. Therefore, we could conclude that the mechanisms of ECL-1–4 on a gold-nanorod-modified gold electrode were the same as those on a gold-nanosphere-modified gold electrode. Because the mechanism of ECL-1–3 reactions has been elucidated in detail in our previous work,⁹ only the mechanism of ECL-4 and ECL-5 will be discussed in the following.

The previous and present work show that ECL-4 is related to the luminol anion (LH^-), Br_2 , HO_2^- , and the hydrogen atom, but the reaction pathway of ECL-4 still remained unknown in our previous work. Therefore, the mechanism of ECL-4 will be discussed further in the present work. It was reported that Br_2 could react with a hydrogen atom generated at the hydrogen evolution potential to form $\text{Br}_2^{\bullet-}$, which could enhance luminol CL.^{37,38} Meanwhile, gold nanoparticles could adsorb hydrogen atoms and catalyze the subsequent reactions involving hydrogen atoms.³⁹ Therefore, we deduced that gold nanoparticles assembled on the gold electrode could adsorb hydrogen atoms. The adsorbed hydrogen atom reacted with Br_2 to form $\text{Br}_2^{\bullet-}$ by virtue of the catalysis of gold nanoparticles. Finally, the luminol anion reacted with HO_2^- and $\text{Br}_2^{\bullet-}$ to generate the excited-state 3-aminophthalate (AP^{2-*}), emitting light. The pathways for ECL-4 could be as follows:



ECL-5 was enhanced under an O_2 atmosphere and suppressed under a N_2 atmosphere, indicating that the O_2 was necessary for ECL-5. The dissolved oxygen is readily electroreduced to OOH^- at negative potential.⁴⁰ When the scan direction was reversed, OOH^- was oxidized to $\text{O}_2^{\bullet-}$ at -0.1 V on a Pt–C electrode.⁴¹ Therefore, we deduced that $\text{O}_2^{\bullet-}$ might be generated upon the reversal scan from -1.6 V in the present system. It is well-known that superoxide dismutase can scavenge $\text{O}_2^{\bullet-}$ efficiently. To discern whether $\text{O}_2^{\bullet-}$ was involved in the ECL-5 reaction, superoxide dismutase (SOD) was added to the working solution. The result showed that ECL-5 was suppressed significantly in the presence of superoxide dismutase, which

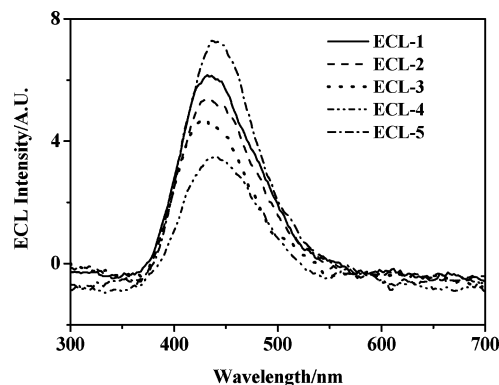
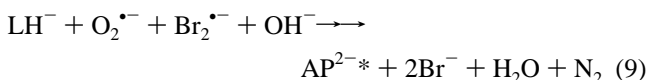
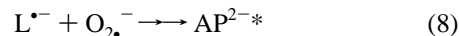
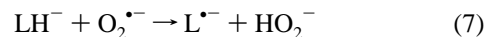
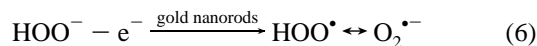


Figure 9. ECL spectra of ECL-1–5: ECL-1 (solid line), ECL-2 (dashed line), ECL-3 (dotted line), ECL-4 (dashed–dotted line), ECL-5 (dashed–dotted–dotted line) (KBr , 0.1 mol/L; pH 8; PBS , 0.1 mol/L; luminol, 1×10^{-4} mol/L; scan rate 40 mV/s).

confirmed that $\text{O}_2^{\bullet-}$ participated in ECL-5 reactions. $\text{O}_2^{\bullet-}$ is often quite stable in aprotic solvents but not in aqueous solution.⁴² Accordingly, ECL-5 was not observed in aqueous solutions on a bare gold electrode or on a gold-nanosphere-modified gold electrode. It was reported that metal cations acting as Lewis acids could react with $\text{O}_2^{\bullet-}$ to form a superoxo complex, which could stabilize $\text{O}_2^{\bullet-}$.⁴² We deduced that positively charged gold nanorods could stabilize $\text{O}_2^{\bullet-}$ in aqueous solution just like metal cations and ECL-5 could be generated on a gold-nanorod-modified gold electrode. Besides $\text{O}_2^{\bullet-}$, Br^- seems also to be involved in the ECL-5 reactions. When the initial scan direction was negative in the presence of KBr or the initial scan direction was positive in the absence of KBr such as in KNO_3 medium, ECL-5 could not be observed. Moreover, ECL-5 could not be observed when the positive reversal potential was lower than 0.8 V. However, when the reversal potential exceeded 1.0 V, a stronger ECL-5 could be obtained where Br^- could be oxidized to BrO^- during the positive scan,⁹ indicating that BrO^- was necessary for the generation of ECL-5. When the potential was reversed from $+1.6$ to -1.6 V, BrO^- was reduced to Br_2 at 0.42 V. Br_2 is likely to react further with a hydrogen atom generated at the hydrogen evolution potential to produce $\text{Br}_2^{\bullet-}$ just like ECL-4 because an extremely weak ECL-5 could be obtained when the reversal potential was more positive than -1.0 V, where $\text{O}_2^{\bullet-}$ was generated and the hydrogen evolution did not occur, and ECL-5 was enhanced greatly when the negative reversal potential was at -1.6 V, where the hydrogen evolution occurred. According to the experimental results above, ECL-5 is related to LH^- , $\text{Br}_2^{\bullet-}$, and $\text{O}_2^{\bullet-}$. The pathway of ECL-5 might be as follows:



Conclusions

Gold nanorods could be self-assembled on the surface of a gold electrode to form a compact monolayer through electrostatic interaction, making the charge transfer through the surface of the electrode facile. Five luminol ECL peaks were obtained

on a gold-nanorod-modified gold electrode either in alkaline or in neutral conditions. ECL-1–4 could also be found on a gold-nanosphere-modified gold electrode, while ECL-5 could only be initiated on a gold-nanorod-modified gold electrode. ECL-4 was further elucidated due to the reaction of the luminol anion with various electrogenerated species such as OOH^- and $\text{Br}_2^{\bullet-}$. ECL-5 is likely due to the reaction of the luminol anion with various electrogenerated species such as $\text{O}_2^{\bullet-}$ and $\text{Br}_2^{\bullet-}$. The gold nanoparticle as a nanosized reaction platform and a catalyst was very important for the formation of $\text{Br}_2^{\bullet-}$, and the gold nanorod was very important for the stabilization of $\text{O}_2^{\bullet-}$. In strong alkaline solution, ECL-1 and ECL-2 were stronger on a gold-nanosphere-modified gold electrode, while ECL-3–5 were stronger on a gold-nanorod-modified gold electrode. In neutral solution, all ECL peaks were enhanced about 2-fold to 10-fold on a gold-nanorod-modified gold electrode compared with a gold-nanosphere-modified gold electrode and by ~ 3 –4 orders of magnitude compared with those on a bare gold electrode, implying that luminol ECL on the gold-nanorod-modified gold electrode is of great potential for the sensitive detection of biologically important compounds in physiological conditions. This work shows that luminol exhibits different ECL properties on a gold-nanorod-modified gold electrode and on a gold-nanosphere-modified gold electrode, revealing that the shape of the metal nanoparticles has an important effect on the luminol ECL behavior. This provides new knowledge about the physical chemistry properties of nanoparticle-modified electrodes.

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