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Self-assembly electrode based on silver nanoparticle toward electrogenerated chemiluminescence analysis of glucose

Ali Shokuhi Rad*,†, Mehdi Ardjmand, Mohsen Jahanshahi***, and Ali-Akbar Safekordi***

*Chemical Engineering Department, Faculty of Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
**Chemical Engineering Department, Faculty of Engineering, Tehran South Branch, Islamic Azad University, Tehran, Iran
***Nanobiotechnology Research Group, Babol Noshirvani University of Technology, Babol, Iran
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Abstract−Electrogenerated Chemiluminescence (ECL) involves applying a certain electric potential to a chemical reaction, resulting in the oxidation or reduction of the substance which reacts to produce light. We determined the amount of glucose by its reaction to glucose oxidase (GO_X) on the surface of the proposed modified electrode, which results hydrogen peroxide (H_2O_2) as side product. After that the reactions between luminol and H_2O_2 under oxidizing conditions generate dependent light which can be used to analyze. In the current article at first we proposed a convenient method to obtaining a self-assembly modified electrode. A nano based modified glassy carbon (GC) electrode (Glucose oxidase/Ag nanoparticles/cysteamine (CA)/p-aminobenzene sulfonic acid/GC electrode) was prepared, and the ECL behavior of luminol in the presence of glucose was examined. Compared to the bare GC electrode, the modified electrode incorporating glucose oxidase significantly enhanced the response of the ECL biosensor to glucose due to the enhanced specificity of the modified surface to enzymatic reaction, and the sensitivity of the luminol ECL reaction. Under optimal conditions, the electrode was established to respond linearly to glucose in the concentration range 5.0×10^{-7} to 8.0×10^{-3} mol/L, and the detection limit was established to be a glucose concentration of 4.0×10^{-8} mol/L.

Key words: ECL, Luminol, Glucose, Modified Electrode, Ag Nanoparticle

INTRODUCTION

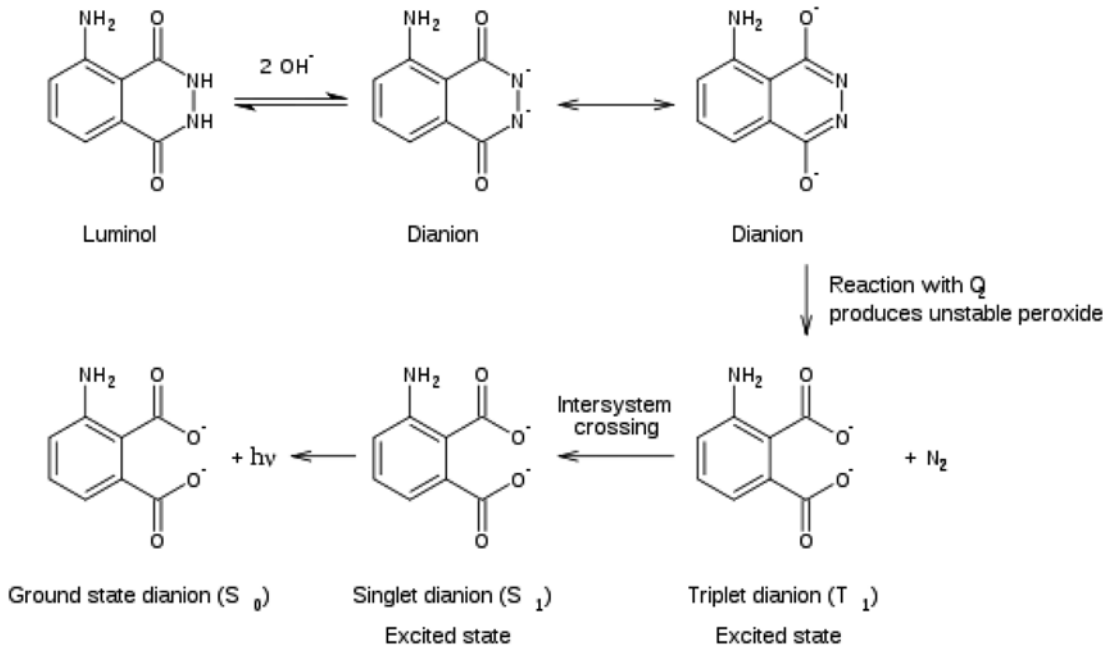
Electrogenerated chemiluminescence (ECL) involves applying electric potential to a chemical reaction, resulting in the oxidation or reduction of the substance which reacts to produce light. As an analytical technique, ECL has been paid more and more attention since it not only retains the advantages of chemiluminescence (CL) sensor, such as excellent sensitivity, wide dynamic concentration response range, but also has some additional advantages [1].

ECL can be used to measure the amount of a given chemical in solution. The amount of analyte is ideally proportional to the amount of light detected. ECL measurements enabled the achievement of highly sensitive biosensors, free of matrix interference, for the detection of glucose. Successful and extensive applications of ECL for the determination of various species reveal that ECL is an important and valuable detection method in the field of biosensors [2].

Luminol ($C_8H_7N_3O_2$) as an inexpensive classic organic reagent is considered as an efficient system in ECL measurement for the detection of H_2O_2 and one of the most commonly used ECL luminophores or labels, especially in the field of bioanalytical application such as enzyme biosensors [3]. Luminol generates strong CL and ECL signals in alkaline media, but its ECL signals in neutral media are relatively weak. Recently, several efforts have been devoted to sensitize and apply luminol ECL reaction for the determination of biologically important compounds in their physiological conditions [4-6]. Well-organized and highly sensitive detection of glucose is of great interest from various aspects ranging from medical applications in blood glucose sensing to ecological applications. On the basis of the reaction between luminol and H_2O_2, an ECL sensor of glucose can be fabricated based on H_2O_2 produced during the oxidation of glucose in the presence of glucose oxidase (GO_X). This kind of biosensor shows low oxidation potential, low-cost reagent use and high emission yields. There are various methods for determination of glucose, such as enzymatic methods [7,8]. Other ways are based on modification of electrode surface using nano materials which enhance ECL signals compared to the unmodified electrode [10,11]. In general, with regard to works done on this topic, the enzyme can be immobilized on a appropriate support, such as nafion film or sol-gel film [12], which were immobilized on the electrode. There are diverse mechanistic pathways for the ECL reaction of luminol, which partially depend on the applied potential, electrode materials and the electrode surface status [13]. Disregarding the above factors, in general we can consider the following mechanism for ECL reaction of luminol: a dianion is formed after luminol reacts with the hydroxide salt, the oxygen generated from the H_2O_2 then reacts with the luminol dianion. The product of this reaction, organic peroxide, is extremely unstable and is made by losing nitrogen, electrons going from excited state to ground state, and energy emitted as a photon. This emitting of the photon is what ultimately gives off the blue light. The proposed mechanism is shown in Scheme 1.

So we can expect light-generation during a reaction between luminol and H_2O_2 under oxidizing conditions. It is necessary to know that luminol is capable of generating light by itself under ECL con-

†To whom correspondence should be addressed.
E-mail: A.Shokuhi@gmail.com



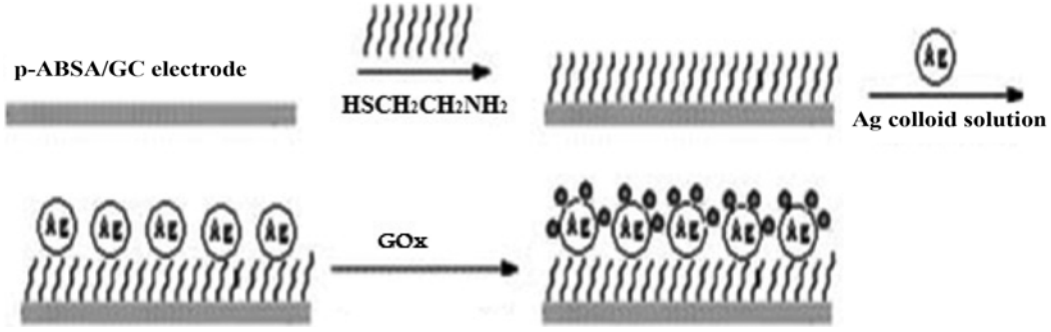
Scheme 1. The proposed mechanism ECL reaction of luminol.

ditions [14], but enhanced ECL will be seen in presence of H_2O_2. It is well known that H_2O_2 is one of the by-products of many enzymatic reactions. One of these reactions is between that of glucose and glucose oxidase. When this reaction occurs, the amount of H_2O_2 generated is proportional to the amount of glucose originally in solution. H_2O_2 and luminol then react under alkaline and oxidizing conditions to produce light [15,16].

$$\text{D-Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{gluconate} + H_2O_2$$

$$\text{Luminol} + 2H_2O_2 \xrightarrow{\text{Oxidation reaction}} \text{3-aminophthalate} + N_2 + 3H_2O + h\nu$$

It is important to immobilize GO_X on the surface of electrodes in the fabrication of the ECL sensor of glucose. There are many techniques for immobilization of GO_X such as adsorption system [17], the embedding system [1], the covalent cross-linking method [18, 19] and finally electrochemical polymerization [20]. But these methods used in the preparation of the ECL biosensor not only need a unwieldy and complicated process but also would result in an troubled renewable sensitive membrane on the electrode surface. Nano material not only increases the stability and the amount of the immobilized enzyme but also improves the catalytic activity of the enzyme and the responsibility of the sensor [21]. In the present research, we made a nano-based self-assembly modified electrode (Glucose oxidase/Ag nanoparticles/CA/p-aminobenzene sulfonic acid/GC electrode) and used it as biosensor for ECL analysis of glucose. For doing this at the first stage we electropolymerized p-ABSA to obtain negatively charged poly-(p-ABSA) film to immobilize CA. Here, immobilization of CA was attributed to the electrostatic force between positively charged CA and the negatively charged sulfonic acid groups in p-ABSA polymer. Silver nanoparticle attached to the CA monolayer through electrostatic adsorption and finally GO_X adsorbed on AgNP in three dimensional. GO_X was selected as a model enzyme which immobilized on AgNP adsorbed on a CA monolayer-modified GC to prepare the biosensor. AgNP has large specific surface area and good biocompatibility. Additionally, it can act as tiny conduction centers which facilitate the transfer of electrons.



Scheme 2. Preparation process for the GO_X/silver particles/CA/GC electrode.

The preparation process of the electrode is shown in Scheme 2.

EXPERIMENTAL

1. Chemicals

D (+)-glucose (97%), horseradish peroxidase type II (HRP) and P-ABSA were obtained from Sigma (St. Louis, MO, USA). Glucose oxidase (GO_X; 256 U mg^{-1}) and was purchased from Aldrich (Steinheim, Germany). Cysteamin (CA) and $AgNO_3$ were obtained from Merck (Germany). Sodium borohydride and sodium citrate were from Fluka. A 1.0×10^{-2} M stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving luminol in a small amount of 0.1 M NaOH. Working solutions of luminol were prepared by diluting the stock solution with phosphate buffer solution (PBS, 0.1 M, pH 7). A stock solution of glucose (1 M) was prepared in PBS and stored at 4 °C when it was not in use. The stock solution of glucose was allowed to mutarotate at room temperature for 24 h before use. Double distilled water was used throughout. All other reagents were of analytical grade and were used as received. Phosphate buffer solutions (PBS) with different pH values were prepared by mixing aqueous solutions of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 and adjusting the pH with 0.1 M H_3PO_4 or NaOH.

2. Apparatus and Procedure

Cyclic voltammetry (CV) was performed using an IVIUMSTAT & IVIUMSTAT.XR (The Netherlands) with a conventional three-electrode set-up in which modified GC electrode included Glucose oxidase/Ag nanoparticles/CA/p-aminobenzene sulfonic acid, an saturated calomel electrode and a platinum wire, respectively, served as the working, reference and auxiliary electrodes. The ECL was performed by an MPIA ECL analytical system (Xian Remax Electronic Science Tech. Co., Ltd., Xian, China) with an R456 photomultiplier (PMT) (Xian Remax Electronic Science Tech. Co., Ltd., Xian, China) for transforming ECL emission into electrical signals. The PMT was operated at −800 V, and the ECL cell was placed in front of the PMT. The photodetector and ECL cell were enclosed in a light-tight black box. A Metrohm 691 pH meter was used for pH adjustments. All measurements were performed at room temperature.

3. Preparation of Silver Nanoparticles

Silver nanoparticles were prepared by reducing metallic ions in aqueous solution with freshly-prepared NaBH4 solution in ice-cold conditions, and stored at 4 °C. An aqueous solution of 2 mM $NaBH_4$ (2 mL) was cooled with ice, and then 1 mL of 2 mM $AgNO_3$ aqueous solution was added to it under vigorous stirring, resulting in the light-brown Ag colloidal solution. The average nanoparticle diameter is about 80 nm measured by TEM (figure not shown).

4. Preparation of Modified Electrode as Biosensor

The bare GCE was polished with 0.05 μm Al_2O_3 before modification, rinsed with double-distilled water, and sonicated in acetone and double-distilled water for 5 min, respectively.

After electrochemically cleaning the freshly polished GC electrode by sending it through several potential cycles between −0.5 and 1.2 V versus SCE in 0.1 M H_2SO_4, the electrode was immersed in 2.0×10^{-3} mol/L p-ABSA solution containing 0.1 mol/L NaCl and was conditioned by cyclic sweeping between −1.5 and +2.5 V at 100 mV/s for 10 scans. After that, the electrode was washed by water and dried in air, immersed in a solution of 2 mM CA for 12 h. After being cleaned with distilled water, the electrode was immersed in Ag colloids for 8 h to form a nano Ag layer. Subsequently, the resulting electrode was immersed in GO_X solution (1 mg/ml, 0.1 mol/L pH 6.0 PBS) for 12 h to obtain the glucose biosensor, which was stored in a refrigerator (4 °C) until further use.

RESULT AND DISCUSSION

Atomic force microscopy (AFM) was used to monitor the self-assembly process and to examine the distributions of the silver nanoparticles. Fig. 1 shows AFM images of unmodified and modified GC electrode. As shown in Fig. 1(a), the GC substrate was very flat after the CA had assembled on the GC electrode surface, which indicated that the CA had formed a uniform monolayer on the GC electrode substrate. As shown in Fig. 1(b), after the substrate was immersed in GOx solution, the number of particles on the substrate surface increased markedly than before.

1. Characterization and ECL Ability of AgNPs/CA/PABSA/GC Electrode

Cyclic voltammogram measurements of luminol was made by

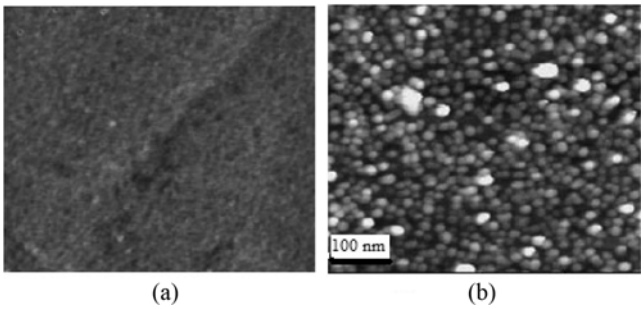


Fig. 1. AFM images of CA/GCE (a) and self-assembled modified GCE (b).

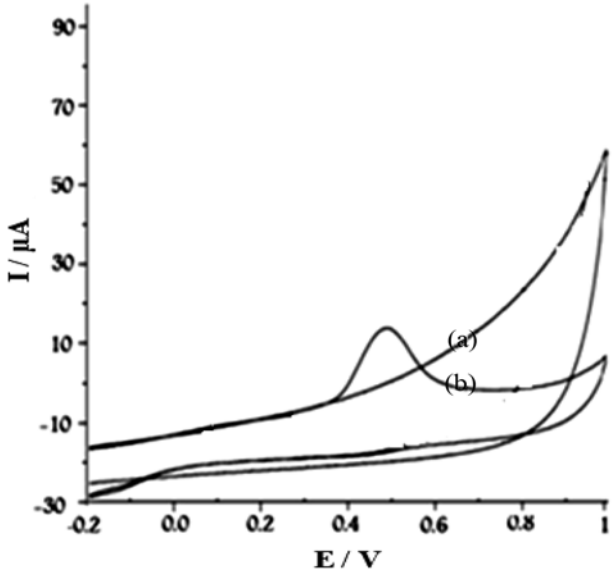


Fig. 2. Cyclic voltammograms of luminol behavior at bare GC electrode (a) and modified electrode (AgNPs/CA/PABSA/GCE). Conditions: Luminol, 50 μM; supporting electrolyte, phosphate buffer solution (0.1 M and pH 7.0); potentialscanrate, 100 mVs^{-1}.

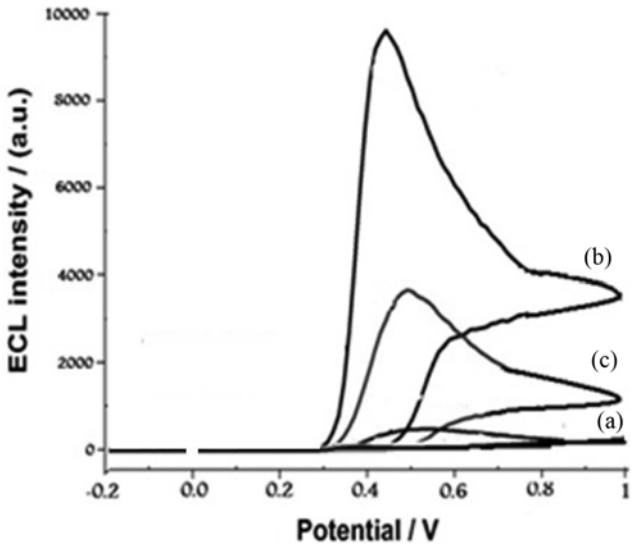


Fig. 3. Corresponding ECL responses of luminol at bare GC electrode* (a), modified electrode (AgNPs/CA/PABSA/GCE)* (b) and modified electrode (AgNPs/CA/PABSA/GCE) (c). *. Swiping potential from −0.2 V to 1 V. **. Swiping potential from 0 V to 1 V. Other conditions: Luminol, 50 μM; supporting V. electrolyte, phosphate buffer solution (0.1 M and pH 7.0); potentialscanrate, 100 mVs^{-1}.**

using modified and unmodified electrodes in pH 7.0 phosphate buffer solutions in order to determine the optimum applied potential for the luminol ECL. For luminol, there is a distinct oxidation peak at 0.47 V versus SCE as shown in Fig. 2. This peak is related to the oxidation process of luminol to diazasemiquinone. No cathodic peak was detected in the reverse scan.

The ECL responses of luminol at the mentioned different electrodes (Fig. 3(a)) passed over a maximum at about 0.47 V, which suggested that the luminol oxidation product generates ECL emission. The ECL intensity of luminol increased by modification of the GCE with AgNPs/CA/PABSA/GC (Fig. 3(b)). The increase of charging currents at modified electrode in comparing with that of GC electrode was related to the increase of electrode surface area because of the presence of AgNPs. Moreover, the enhancement of ECL response intensity, as a result of luminol oxidation, at modified electrode in comparing with that of unmodified was related to the increase of electrode surface area because of the presence of AgNPs.

It seems that the initial potential in the cyclic voltammetry has a significant function in ECL intensity. Further investigation was carried out on ECL behavior of luminol at above electrodes by sweeping potential from 0 to 1 V instead of −0.2 to 1 V (Fig. 2(c)). The ECL intensity in this case is much smaller than that of observed previously. As a result, sweeping potential in negative direction caused the making of species, most likely reactive oxygen species such as HO_2•, O_2•− and OH•, at the electrode surface which could develop the intensity of luminol ECL response [22].

Luminol is oxidized to the luminol radical anion during the sweeping potential in positive direction with the maximum peak current intensity at about 0.47 V. Afterwards, the chemical reaction of luminol radical anions with reactive oxygen species creates the ECL signal, which AgNPs/CA/PABSA/GC electrode significantly enhances the ECL signal by increasing the amount of different electrogenerated reactive oxygen species from the reduction of dissolved oxygen at negative potential and also by increasing the amount of luminol oxidation product, luminol radical anions, via increasing the surface of electrode. The recent reports on luminol ECL mechanism [13,23] are in accord with the proposed mechanistic pathway.

Therefore, as a result, the great enhancement of luminol ECL response indicates that AgNPs not only increases the rate of luminol oxidation by their catalytic effect but also increases the amount of reactive oxygen species at the electrode surface by increasing the rate of oxygen reduction.

2. Electrochemical and ECL Behaviors of Luminol in the Present of H_2O_2

The electrocatalytic property of AgNPs on H_2O_2 oxidation reaction has been investigated by Huang et al. [24]. It seems luminol ECL reaction can be sensitized by H_2O_2 (by itself) and also by the generated oxygen and superoxide radical anion during the oxidation of H_2O_2 at the electrode surface [23]. Therefore, the observed enhancements in both CV and ECL responses at AgNPs/CA/PABSA/GCE are attributed to the electrocatalytic activity of AgNPs on luminol and H_2O_2 oxidation reactions.

AgNPs/CA/PABSA/GC electrode was further modified with GO_X to manufacture an ECL glucose biosensor based on the electrocatalytic effect of AgNPs on luminol-H_2O_2 ECL reaction. It is well known that GO_X catalyzes the oxidation of glucose to produce H_2O_2. Under sweeping potential in positive direction, the immobilized AgNPs on the surface of GCE catalyze the electrochemical oxidation of luminol and the enzymatically produced H_2O_2 (not shown). The produced luminol radical anions are further oxidized by the enzymatically produced H_2O_2 directly and by electrochemically produced reactive oxygen species, as the products of H_2O_2 oxidation at the electrode surface, to give the excited state 3-amino phthalate anions that emit light at about 425 nm.

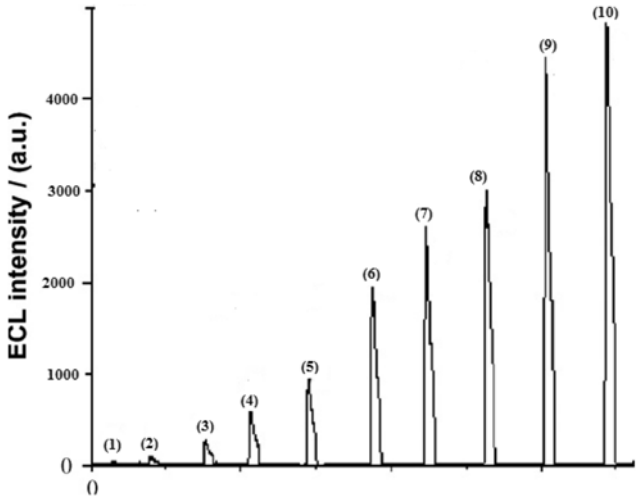


Fig. 4. The calibration curve for glucose [luminol]=50 μM, buffer solution (0.1 M and pH 7.0). ECL curves with various glucose concentration: (1) 0.0, (2) 5×10^{-7}, (3) 5.0×10^{-6}, (4) 5×10^{-5}, (5) 2×10^{-4}, (6) 6×10^{-4}, (7) 9×10^{-4}, (8) 1×10^{-3}, (9) 2×10^{-3}, (10) 8×10^{-3} mol L^{-1}.

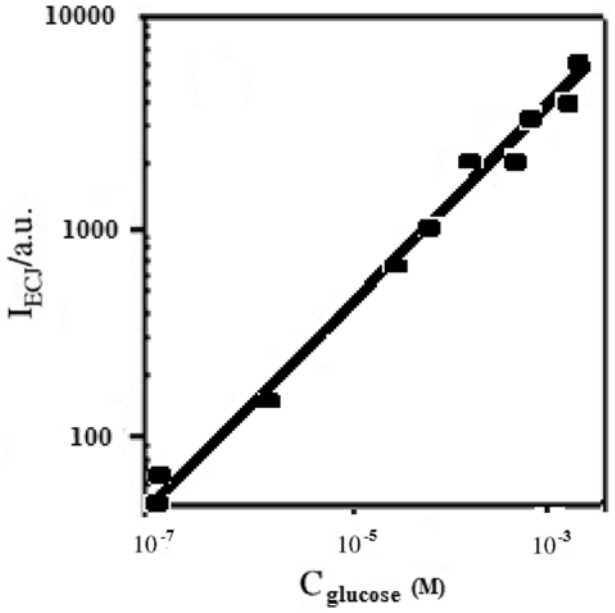


Fig. 5. Linear relationship between ECL intensity and glucose concentration with respect to Fig. 5.

For determination of glucose in aqueous solution, a calibration curve was obtained under the optimal conditions. As shown in Figs. 4 and 5, the intensity of ECL was linear with the concentration of glucose in the range of 5.0×10^{-7} to 8.0×10^{-3} mol L^{-1}. The detection limit was calculated 4.0×10^{-8} mol L^{-1}.

3. The Effect of pH

In reality, the intensity of ECL is more efficient in alkaline media (pH=8.5-10), but the optimum pH condition for the activity of GO_X was usually below neutral pH. And higher pH value may cause the enzyme to lose its activity. Therefore, in this study, the effect of pH value on the enhanced ECL intensity was investigated in the range of 5.0-9.0. As shown in Fig. 6, the ECL intensity increased as the pH changed from 5 to 7 and decreased when the pH was above 7. This result most likely was a compromise between the optimum for enzyme activity and the optimum luminol chemiluminescent reaction around pH 9. So the optimal value of 7 was chosen for subsequent glucose assays.

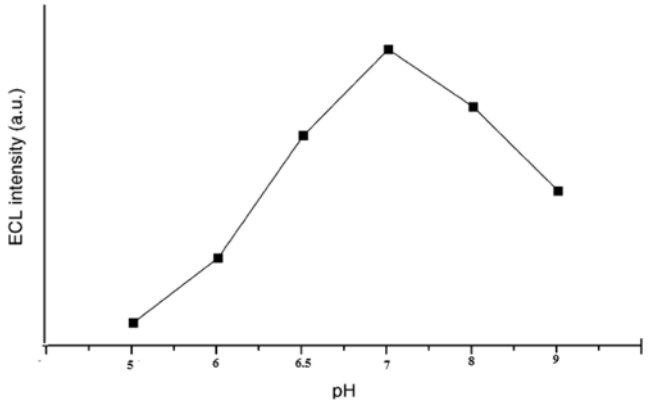


Fig. 6. The Effect of pH of buffer solution on ECL intensity. Luminol concentration: 50 μM; glucose concentration: 10 μM.

CONCLUSION

Light-emitting surfaces and electrochemiluminescence (ECL) reactions are the topics of the current research. Glucose oxidase/Ag nanoparticles/CA/p-aminobenzene sulfonic acid/GC electrode was presented as a novel nano-hybrid material with attractive enhancement and sensitizing effect on luminol-H_2O_2 ECL systems at neutral media. The observed enhancement effect on luminol ECL signal intensity was attributed the electrocatalytic activity of AgNPs toward H_2O_2 oxidation and also luminol oxidation reactions. The proposed ECL systems presented a number of attractive features such as wide linear range, low limit of detection, excellent reproducibility and satisfactory stability and selectivity. Application of Glucose oxidase/Ag nanoparticles/CA/p-aminobenzene sulfonic acid/GC electrode for modification of the electrode surface may create a new opportunity for the development of new sensitized ECL sensors and biosensors that can be used in a variety of fields such as clinical diagnostics, immunological analysis and environmental monitoring due to their simplicity and high efficiency.

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