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Stainless steel electrode for sensitive luminol electrochemiluminescence detection of H_2O_2, glucose, and glucose oxidase activity

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ABSTRACT: The electrogenerated chemiluminescence (ECL) application of stainless steel, a robust and cost-effective material, has been developed for the first time. The type 304 stainless steel electrode shows appealing ECL performance for luminol-H_2O_2 system. It enables the detection of H_2O_2 with a linear range from 1 to 1000 nM and a limit of detection of 0.456 nM (S/N = 3). The ECL method based on type 304 stainless steel electrode is more sensitive, more cost-effective, and much simpler than other ECL methods reported before. Because the stainless steel electrode has excellent performance for H_2O_2 detection and H_2O_2 participates in many important enzymatic reactions, the applications of stainless steel electrode-based ECL for the detection of enzyme activities and enzyme substrates were further investigated using glucose oxidase (GODx) and glucose as representative enzyme and substrate. The concentrations of glucose and the activity of GODx were directly proportional to the ECL intensities over a range of 0.1 – 1000 μM and 0.001 – 0.7 U/mL with a limit of detections of 0.076 μM and 0.00087 U/mL (S/N = 3), respectively. This method was successfully used for determining glucose in honey. Because of its remarkable performance and user-friendly features, stainless steel electrode holds great promise in various electroanalytical applications, such as biosensing, disposable sensors, and wearable sensors.

Electrogenerated chemiluminescence (ECL) is one of the most powerful analytical techniques and has widespread applications in various research areas. ECL is a well-known method depending on the generation of an optical signal by an electrochemical reaction.[1-5] An increased interest is observed for ECL investigations because of outstanding advantages, such as high sensitivity, selectivity, the absence of background signal, easiness to control the reaction by applying potential at the electrode, wide linear range and so on.[6-8] Due to this fact, ECL method has been used for wide analytical applications.[9-11]

Luminol is one of the well known luminophores.[12-17] Because of high sensitivity for H_2O_2, most of the applications of luminol-H_2O_2 ECL are used to detect H_2O_2 generating label enzymes, H_2O_2 enzymatic precursors, and luminol-labelled molecules.[12,13,18,19] Luminol can generate strong ECL signal with H_2O_2 which takes part in many enzymatic reactions.[14-16,20] Due to this, luminol ECL plays significant role in the determination of H_2O_2, enzymes, and substrates that can be converted to H_2O_2 enzymatically and H_2O_2-related biosensors.[21-23] For example, luminol ECL has been used for the determination of glucose and glucose oxidase (GODx) since glucose can react with dissolved oxygen to produce H_2O_2 in presence of GODx. ECL of luminol has been studied at different electrodes, such as glassy carbon[24], platinum[24-26], gold[27,28], copper[28,29], paraffin-impregnated graphite[30] electrodes. These previous works clearly indicated that the electrode material plays highly important role in the ECL of luminol.

Stainless steels are iron alloys which contain more than 10% chromium by mass and other alloying elements to enhance the properties of steel and corrosion resistance.[31,32] Besides its corrosion resistance, stainless steel has interesting features, such as environmentally friendly, low-cost, good electrical conductivity, high mechanical strength, and commercial availability.[33,34] To the best of our knowledge, stainless steel has never been used as working electrode for ECL study.

In this study, stainless steel has been explored as working electrode for ECL study for the first time. The ECL behaviors of luminol at stainless steel electrode were studied. Its use for the detection of luminol, H_2O_2, glucose and GODx were investigated. The use of stainless

steel electrode significantly enhances the sensitivity for the detection of H_2O_2, glucose and GODx.

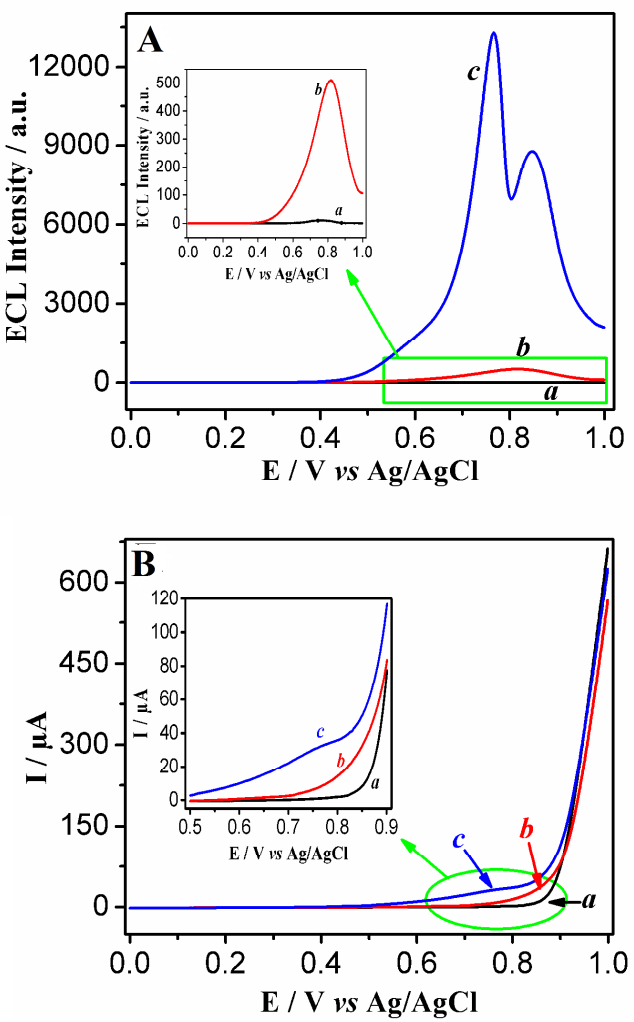


Figure 1. ECL intensity curves (A) and Linear sweep voltammograms (B) of blank (a), 0.5 mM luminol (b) and 0.5 mM luminol and 0.5 mM H_2O_2 (c) measured at the type 304 stainless steel electrode in 0.1 M carbonate buffer solution pH 11.0; Scan rate: 100 mV s^{-1}; PMT voltage = 600.

EXPERIMENTAL SECTION

Chemicals

We bought types 201, 304, and 316 stainless steel from Suzhou Qiangda Fastener Co., Ltd. Luminol and hydrogen peroxide were obtained from Beijing Chemical Reagent Company. Glucose oxidase was purchased from Sigma. β-D-Glucose (>99.8%) was purchased from Amresco. Ethiopian highland honey was used for real sample analysis. 0.1 M phosphate buffer solution (pH 8 and 9) and 0.1 M carbonate buffer solution (pH 10, 11 and 12) were prepared by mixing a balanced amount of 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 and 0.2 M Na_2CO_3 and $NaHCO_3$, respectively. Stock solution of 10 mM luminol was prepared by dissolving luminol in 0.1 M NaOH. Luminol working solutions were prepared by diluting the stock solution with 0.1 M carbonate buffer solution (pH = 11). Glucose (10 mM) and GODx (10 mg/mL) were prepared in phosphate buffer solution (PBS) (pH = 7.4) and double distilled water, respectively and stored at 4 °C when not in use. Working solutions of glucose were prepared by diluting the stock solutions with PBS. Working solution in the cell was changed with fresh solution at each time. All experiments were carried out at room temperature.

Apparatus

Electrochemical measurements were carried out in a conventional three-electrode system with a CHI 660C electrochemistry workstation and ECL measurements were performed with a BPCL-1-KIC luminescence analyzer, and unless noted otherwise, the photomultiplier tube (PMT) was biased at 900 V. Stainless steel electrode (3 mm in diameter), Ag/AgCl (saturated KCl) electrode and a gold wire were used as working, reference and auxiliary electrode, respectively. The stainless steel electrode was fabricated by tightly pack stainless steel rode with 3 mm in diameter into the electrode cavity of Teflon tube. The stainless steel electrode was polished with abrasive papers and slurry of 0.03 µm alumina then sonicated and rinsed with double distilled water. The ECL emission spectrum was obtained by collecting the ECL data during amperometric technique with filters at 400, 425, 440, 460, 490, 535, 555, 575, 620, and 640 nm, respectively.

ECL measurement for glucose biosensing

A desired amount of glucose (pH 7.4) and GODx were mixed in a test tube and allowed to react for 5 minutes. Then 200 µL of 5 µM luminol (pH 11) was added and the volume was adjusted to 1 mL by adding carbonate buffer solution (pH 11). A desired volume of the working solution was added into the ECL cell. ECL signals were generated, when the potential was applied to the working electrode. The ECL intensity *vs* time (I_{ECL}/t) and current *vs* potential (i/E) curves were recorded.

RESULTS AND DISCUSSION

ECL and electrochemical studies of luminol–H_2O_2 system at the stainless steel electrode

Figure 1A shows the ECL curve of luminol with and without H_2O_2. The ECL emission was observed approximately at 0.70 V *vs* Ag/AgCl for luminol-H_2O_2 and 0.8 V *vs* Ag/AgCl for luminol system. The ECL intensity of the luminol-H_2O_2 system is approximately 26 times stronger than that of only luminol at type 304 stainless steel electrode. This is because H_2O_2 could generate reactive oxygen species (ROS) upon the anodic potential scanning at the electrode, which can accelerate the ECL reaction of luminol with enhanced ECL intensity. As shown in Figure 1B the anodic peak current of luminol-H_2O_2 system is greater than the anodic peak current of only luminol. Generally, anodic luminol ECL is weak in the absence of H_2O_2 due to the difficulty to produce ROS, and requires the addition of H_2O_2. To give insight into the ECL on the stainless steel electrode, we have also tested ECL at other type stainless steel electrodes. As shown in Figure S1, the ECL intensities at types 201, 304 and 316 stainless steel electrodes are similar. All the stainless

steels contain much chromium. It is well-documented that the surface of stainless steel is covered with a thin layer of chromium (III) oxide. It suggests that the thin layer of chromium (III) oxide of stainless steel play critical roles for the enhanced ECL properties on stainless steel electrode. Since type 304 stainless steel is cost-effective and widely used, we employed type 304 stainless steel electrode for following experiments.

The effect of scan rate on the oxidation peak current and ECL intensity of luminol-H_2O_2 system was also studied. As shown in Figure 2A and B, the oxidation currents and ECL intensities are proportional to the square root of the scan rate over a range of 10 to 400 mVs^{-1}, demonstrating that the electron transfer reaction is controlled by the diffusion of luminol and H_2O_2.

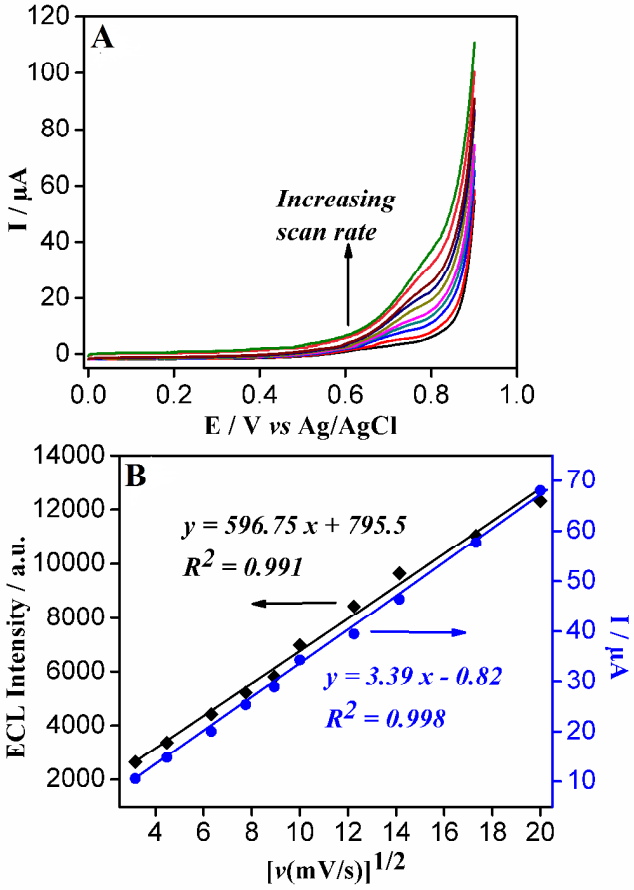


Figure 2. Linear sweep voltammograms of 0.5 mM luminol and 0.5 mM H_2O_2 at scan rates: 10, 20, 40, 60, 80, 100, 125,150, 200, 300 and 400 mV s^{-1} (A) and the linear relationship between the ECL intensity or the anodic current and the square root of the scan rate ($v^{1/2}$) (B) measured at the type 304 stainless steel electrode in 0.1 M pH 11.0 carbonate buffer; PMT voltage = 600 V.

ECL mechanism

The emission spectrum of luminol-H_2O_2 system at type 304 stainless steel electrode was investigated and the result is illustrated in Figure S2. The maximum emission wavelength is at about 425 nm, suggesting that the luminophore of the luminol-H_2O_2 system is luminol.[9,35] Since the luminol is the luminophore, the emission mechanism was proposed as follows. Luminol is first oxidized at the electrode to luminol anion (Equation 1 of Scheme S1) upon the anodic potential scanning at type 304 stainless steel electrode. Then, luminol anion reacted with ROS generated by H_2O_2 (Equation 3 of Scheme S1) to produce the excited state luminol anion which emits light with λ_{max}~425 nm (Equation 4 of Scheme S1).

Effect of pH for luminol – H_2O_2 ECL

The ECL intensities of the 10 μM luminol and 10 μM H_2O_2 was studied in phosphate buffer solution (pH 8.0 and 9.0), and carbonate buffer solution (pH 10.0, 11.0 and 12.0) at type 304 stainless steel electrode. As shown in Figure S3A, the ECL intensities increase with increasing pH value from 8.0 to 11.0 and then decrease as the pH increases further. ECL of luminol-H_2O_2 system reactions are involved in the electrochemical oxidation of luminol and electrogeneration of ROS from H_2O_2. Therefore, the increase of ECL intensities from pH 8.0 to 11.0 is attributed to the faster generation of ROS and the deprotonation of luminol.

Detection of luminol and H_2O_2

The ECL intensity has a linear relationship over luminol in range of 10 to 1000 nM when the concentration of H_2O_2 is 10 μM. Also, under optimal conditions, the ECL intensity has a linear relationship over H_2O_2 in the concentration range between 1 to 1000 nM when the concentration of luminol is 10 μM and the detection limit of luminol and H_2O_2 is 2.41 and 0.456 nM (S/N = 3), respectively (Supporting Information Figure S4A and B). The linear range and the detection limit of H_2O_2 determination at type 304 stainless steel electrode were compared with other reported ECL methods in Table S1. It can be seen from the table that our method has wider linear range and higher sensitivity. The relative standard deviations (RSD) for 5 repetitive measurements of 10 nM luminol and 10 nM H_2O_2 were of 5.76 and 3.23%, respectively.

ECL behaviors of luminol–glucose–GODx system at the stainless steel electrode

Because glucose can be oxidized by GODx to generate H_2O_2, and this enzymatic reaction has been extensively used for the detection of glucose and GODx, the ECL intensities of luminol at type 304 stainless steel electrode with and without glucose-GODx are compared and the results are shown in Figure 3. The ECL intensity of the luminol-glucose-GODx system is approximately 27 times stronger than that of only luminol at type 304 stainless steel electrode. As shown in Scheme 1. First, H_2O_2 is generated from the oxidation of glucose in the presence of GODx. Second, under positive potential scan luminol and enzymatically generated H_2O_2 undergoes oxidation to produce luminol radical anion and ROS, respectively. Finally, the produced luminol radical anion and ROS

react to generate the excited-state 3-aminophthalate anion which emit light (λ_{max} = 425 nm) on relaxation to the ground state.

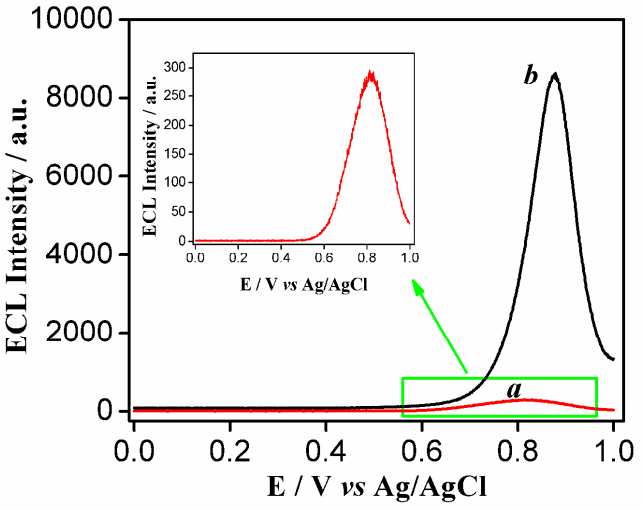
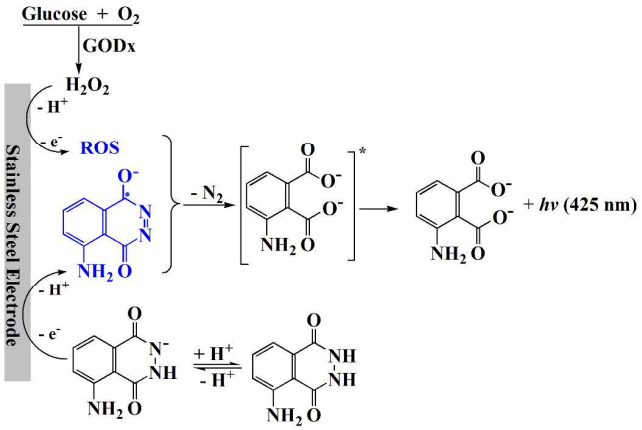


Figure 3. ECL intensity curves of 1 μM luminol (a) and 1 μM luminol – 1 mM glucose – 1 mg/mL GODx (b) measured at the type 304 stainless steel electrode.

Optimizing the performance of the ECL biosensor

In order to optimize the performance of the proposed ECL biosensor towards glucose and GODx detection, the effect of pH, the effect of reaction time, and the effect of concentration of GODx were studied.

Scheme 1. Possible mechanism of the luminol-glucose-GODx system on the stainless steel electrode



Effect of pH for glucose–GODx reaction

The effect of solution pH of glucose–GODx was studied from 7.0 to 8.0 on the biosensor performance. The ECL intensities increase with increasing pH value from 7.0 to 7.4 and then decrease as the pH increases further to 8.0 (Figure S3B). It is well-known that the pH of a solution has significant effect on the activity of GODx. Due to less activity of GODx at higher pH values, the amount of H_2O_2 produced from the catalytic reaction of glucose and dissolved oxygen will be less and thus the ECL intensity decreased. Therefore, a PBS at pH 7.4 was selected as the supporting electrolyte for the reaction of glucose and GODx in the further studies.

Effect of reaction time for glucose – GODx ECL

The effect of reaction time of glucose and GODx on the ECL intensity is also studied (Figure S3C). With increasing the reaction time, ECL intensity increased and did not show significant increase after 5 minutes of reaction time. To obtain good sensitivity and save measurement time, a reaction time of 5 minutes was selected for the following experiments.

Effect of GODx activity on Glucose–GODx ECL and Determination of GODx activity

Figure 4 and Figure S3D show the effect of activity of GODx on the ECL signals. There is a good linear relationship between the ECL intensities and GODx activity from 0.001 U/mL to 0.7 U/mL with a regression equation I_{ECL} = 7407.5 x + 367.5 and a correlation coefficient of 0.991 (n= 8), where x is the GODx activity in U/mL. The detection limit is 0.00087 U/mL. The method is more sensitive than the previous studies.[36-38] The ECL intensities increase further with increasing GODx activity and level off at an GODx activity of 5 U/mL. Thus, 5 U/mL of GODx was chosen for the determination of glucose.

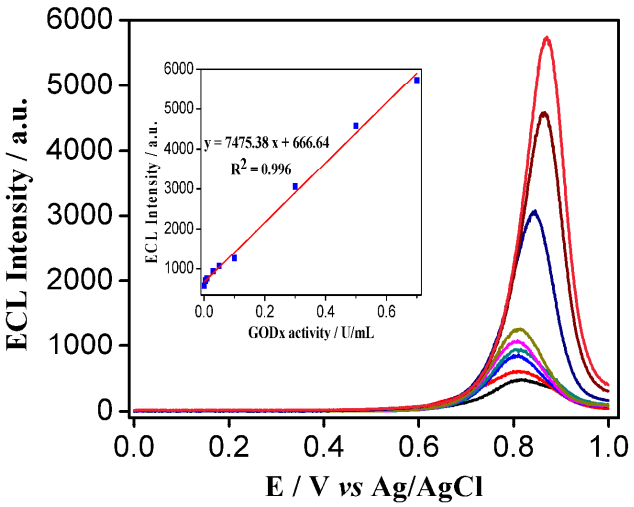


Figure 4. ECL intensity curves of type 304 stainless steel electrode at different activities of GODx and the inset is the calibration curve for GODx activity.

Glucose detection based on ECL biosensor

The ECL biosensor was evaluated under the optimized experimental parameters by determining standard glucose solutions. As shown in Figure 5, the ECL intensities increase with increasing glucose concentrations. The linear calibration range is from 0.1 μM to 1000 μM. The regression equation is I_{ECL} = 8.724 C + 660.406 with a correlation coefficient of 0.989 (n=9), where C is the glucose concentration in μM. The detection limit (S/N=3) for glucose is 0.076 μM. The relative standard deviation of the biosensor response to 0.3 mM glucose was 4.23% for eight successive measurements (Figure S5). As shown in Table 1, this method exhibits higher sensitivity than most previous reported methods. Moreover, the electrode used is much

simpler, cheaper and more robust than that used in other ECL methods.

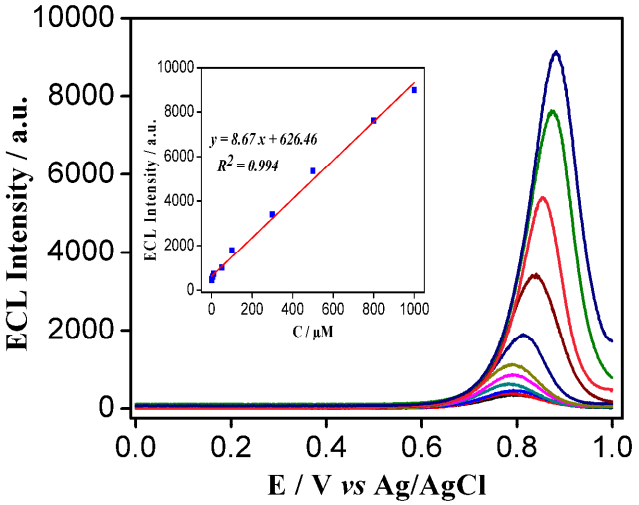


Figure 5. ECL intensity curves of type 304 stainless steel electrode at different concentrations of glucose and the inset is the calibration curve of glucose.

Interference study of the ECL biosensor

Some species which co-exist and possibly interfere with glucose detection were tested. Increasing amounts of interfering species were added into the solution containing 0.3 mM of glucose. An interfering specie was considered not to interfere if it caused a relative error < 5% during the measurement of 0.3 mM glucose sample. Figure S6 shows that 10 μM ascorbic acid, 5 μM dopamine, 10 μM uric acid, 5 mM fructose, 5 mM galactose and 5 mM phenylalanine have negligible interference with the determination of 0.3 mM glucose.

Table 1: Comparison of the analytical performance of proposed glucose detection method with some luminol based ECL glucose biosensors

ECL System	Linear range (μM)	LOD (μM)	Ref.
PLANC	0.1 – 50	0.08	[39]
SG/GCE	50 - 10000	26	[40]
MWNT/ NF/GCE	5 – 800	2	[41]
PtNFs/GO/GODx	5 – 80	2.8	[42]
AuNPs/SG/Au electrode	1 – 5000	0.2	[43]
Pd NP/MWCNT/GCE	0.1 – 1000	0.054	[44]
GR/NF/GOD/GCE	2 – 100	1	[45]
MWCNT/AuNps/GOx /CS/GCE	1 – 1000	0.5	[46]
CNP	1 - 2000	0.5	[47]
CCCE	10 - 10000	8.16	[48]
Type 304 stainless steel electrode	0.1 – 1000	0.076	This work

PLANC-Polyluminolaniline nanocomposite, SG/GCE- Sol-gel/GCE, MWNT/NF/GCE-multi-walled carbon nanotubes/Nafion, PtNFs/GO/GODx-Pt nanoflowers/graphene oxide/glucose oxidase, AuNPs/SG/Au–Au nanoparticles/sol gel/ gold electrode, Pd NP/MWCNT/GCE-Paladium nanoparticles/multi-walled carbon nanotubes, GR/NF/GOD/GCE-Graphine/Nafion/glucose oxidase, MWCNTs/AuNPs/GOx/CS/GCE-multi-walled carbon nanotubes/Au nanoparticles/ glucose oxidase/chitosan, CNP-Carbon nanotube paste, CCCE-Ceramic carbon composite electrode

Table 2: Determination results of glucose in real sample (n=5)

Sample	Added (mM)	Found (mM)	%RSD	Recovery, %
Honey	-	0.309	6.07	-
	0.125	0.434	3.92	100.0
	0.20	0.524	2.76	102.9
	0.35	0.634	4.89	96.2

Real sample analysis

The proposed method has been applied to evaluate glucose content in honey samples without sample pretreatment except a dilution step. ECL of 1 mg/mL honey solution in 0.1 M PBS pH 7.4 was used to determine glucose under optimized condition and the results are shown in Table 2. The percent recovery values range from 96% to 103%, indicating that the present methods is feasible for real sample analysis.

Conclusion

The ECL application of stainless steel working electrode has been demonstrated for the first time using luminol-H_2O_2 system as representative system. The stainless steel working electrode shows superior sensitivity for H_2O_2 detection in comparison with ECL methods reported. By coupling with enzymatic reaction, it allows sensitive detection of glucose and GODx activity. Since stainless steel has excellent performance, as well as is cheap, robust and user-friendly, it is a highly promising electrode for ECL applications, such as ECL biosensing, disposable sensors, and wearable sensors.

ASSOCIATED CONTENT

Supporting Information

Additional information about ECL intensity curves luminol and luminol-H_2O_2 measured at the 201, 304 and 316 stainless steel electrodes, Reaction mechanism for the luminol–H_2O_2 ECL system, the ECL spectrum of luminol-H_2O_2 system at stainless steel electrode, effect of experimental parameters, linear calibration curve for luminol and H_2O_2, comparison of linear range and detection limit with different ECL system toward H_2O_2 and interference study. (pdf)

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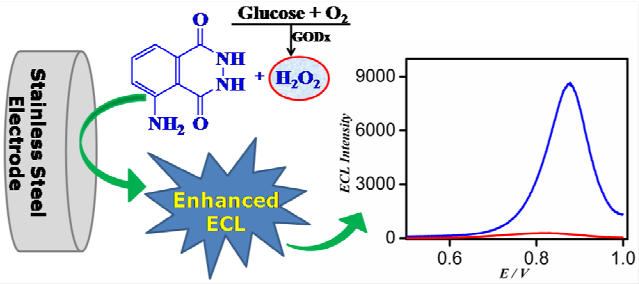
REFERENCES

(1) Mahdi, H.; Zhifeng, D. *J. Electrochem. Soc.* **2016**, *163* H3116-H3131.
(2) Liu, Z.; Qi, W.; Xu, G. *Chem. Soc. Rev.* **2015**, *44*, 3117-3142.
(3) Miao, W. *Chem. Rev.* **2008**, *108*, 2506-2553.
(4) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003-3036.
(5) Zhang, H.-R.; Wang, Y.-Z.; Zhao, W.; Xu, J.-J.; Chen, H.-Y. *Anal. Chem.* **2016**, *88*, 2884-2890.
(6) Hong, J.; Ming, L.; Tu, Y. *Talanta* **2014**, *128*, 242-247.
(7) Jiang, X.; Chai, Y.; Wang, H.; Yuan, R. *Biosens. Bioelectron.* **2014**, *54*, 20-26.
(8) Xie, S.; Dong, Y.; Yuan, Y.; Chai, Y.; Yuan, R. *Anal. Chem.* **2016**, *88*, 5218-5224.
(9) Liu, X.; Qi, W.; Gao, W.; Liu, Z.; Zhang, W.; Gao, Y.; Xu, G. *Chem. Commun.* **2014**, *50*, 14662-14665.
(10) Li, G.; Yu, X.; Liu, D.; Liu, X.; Li, F.; Cui, H. *Anal. Chem.* **2015**, *87*, 10976-10981.
(11) Cui, C.; Chen, Y.; Jiang, D.; Zhu, J.-J.; Chen, H.-Y. *Anal. Chem.* **2017**, *89*, 2418-2423.
(12) Marquette, C. A.; Blum, L. J. *Anal. Bioanal. Chem.* **2006**, *385*, 546-554.
(13) Marquette, C. A.; Blum, L. J. *Anal. Bioanal. Chem.* **2008**, *390*, 155-168.
(14) Haghighi, B.; Tavakoli, A.; Bozorgzadeh, S. *J. Electroanal. Chem.* **2016**, *762*, 80-86.
(15) Khan, P.; Idrees, D.; Moxley, M. A.; Corbett, J. A.; Ahmad, F.; Figura, G. v.; Sly, W. S.; Waheed, A.; Hassan, M. I. *Appl. Biochem. Biotechnol.* **2014**, *173*, 333-355.
(16) Yuanyuan, C.; Yifeng, T. *Electrochim. Acta* **2014**, *135*, 187-191.
(17) Gu, W.; Deng, X.; Gu, X.; Jia, X.; Lou, B.; Zhang, X.; Li, J.; Wang, E. *Anal. Chem.* **2015**, *87*, 1876-1881.
(18) Ou, X.; Tan, X.; Liu, X.; Chen, H.; Fan, Y.; Chen, S.; Wei, S. *RSC Adv.* **2015**, *5*, 66409-66415.
(19) Wu, L.; Ding, F.; Yin, W.; Ma, J.; Wang, B.; Nie, A.; Han, H. *Anal. Chem.* **2017**, *89*, 7578-7585.
(20) Lou, F.; Lu, Z.; Hu, F.; Li, C. M. *J. Electroanal. Chem.* **2017**, *787*, 125-131.
(21) Doroftei, F.; Pinteala, T.; Arvinte, A. *Microchim. Acta* **2014**, *181*, 111-120.
(22) Shimeles Addisu, K.; Birhanu Desalegn, A.; Tesfaye Refera, S. *J. Serb. Chem. Soc.* **2013**, *78*, 701–711
(23) de Poulpiquet, A.; Diez-Buitrago, B.; Dumont Milutinovic, M.; Sentic, M.; Arbault, S.; Bouffier, L.; Kuhn, A.; Sojic, N. *Anal. Chem.* **2016**, *88*, 6585-6592.
(24) Sun, Y. G.; Cui, H.; Lin, X. Q. *Chin. J. Anal. Chem.* **1999**, *27*, 497–503.
(25) Sun, Y. G.; Cui, H.; Lin, X. Q. *Acta Chim. Sin.* **2000**, *58*, 567-571.
(26) Sun, Y. G.; Cui, H.; Lin, X. Q. *Acta Chim. Sin.* **2000**, *58,* , 1151-1155.
(27) Cui, H.; Zhang, Z.-F.; Zou, G.-Z.; Lin, X.-Q. *J. Electroanal. Chem.* **2004**, *566*, 305-313.
(28) Yu, H.-X.; Cui, H. *J. Electroanal. Chem.* **2005**, *580*, 1-8.
(29) Huertas-Pérez, J. F.; García-Campaña, A. M.; González-Casado, A.; Gámiz-Gracia, L. *Luminescence* **2004**, *19*, 222-224.
(30) Cui, H.; Zou, G.-Z.; Lin, X.-Q. *Anal. Chem.* **2003**, *75*, 324-331.
(31) Zheng, Z. J.; Gao, Y.; Gui, Y.; Zhu, M. *J. Solid State Electrochem.* **2014**, *18*, 2201-2210.
(32) Gui, Y.; Meng, X. B.; Zheng, Z. J.; Gao, Y. *Appl. Surf. Sci.* **2017**, *419*, 512-521.
(33) Lo, K. H.; Shek, C. H.; Lai, J. K. L. *Mater. Sci. Eng.: R- Rep.* **2009**, *65*, 39-104.
(34) Hedberg, Y.; Karlsson, M.-E.; Blomberg, E.; Odnevall Wallinder, I.; Hedberg, J. *Colloids Surf. B.* **2014**, *122*, 216-222.
(35) Saqib, M.; Gao, W.; Lai, J.; Qi, L.; Majeed, S.; Gilani, M. R. H. S.; Xu, G. *Chem. Commun.* **2015**, *51*, 6536-6539.
(36) Luong, J. H. T.; Masson, C.; Brown, R. S.; Male, K. B.; Nguyen, A. L. *Biosens. Bioelectron.* **1994**, *9*, 577-584.
(37) Kroger, S.; Setford, S. J.; Turner, A. P. F. *Biotec. Techn.* **1998**, *12*, 123-127.
(38) Hu, L.; Han, S.; Liu, Z.; Parveen, S.; Yuan, Y.; Xu, G. *Electrochem. Commun.* **2011**, *13*, 1536-1538.
(39) Li, G.; Lian, J.; Zheng, X.; Cao, J. *Biosen. Bioelectron.* **2010**, *26*, 643-648.
(40) Zhu, L.; Li, Y.; Zhu, G. *Sensor. Actuat. B: Chem.* **2002**, *86*, 209-214.
(41) Lin, Z.; Chen, J.; Chen, G. *Electrochim. Acta* **2008**, *53*, 2396-2401.
(42) Tian, X.; Lian, S.; Zhao, L.; Chen, X.; Huang, Z.; Chen, X. *J. Solid State Electrochem.* **2014**, *18*, 2375-2382.
(43) Liu, X.; Niu, W.; Li, H.; Han, S.; Hu, L.; Xu, G. *Electrochem. Commun.* **2008**, *10*, 1250-1253.
(44) Haghighi, B.; Bozorgzadeh, S. *Anal. Chim. Acta* **2011**, *697*, 90-97.
(45) Chen, X.; Ye, H.; Wang, W.; Qiu, B.; Lin, Z.; Chen, G. *Electroanalysis* **2010**, *22*, 2347-2352.
(46) Haghighi, B.; Bozorgzadeh, S.; Gorton, L. *Sensor. Actuat. B-Chem.* **2011**, *155*, 577-583.
(47) Chen, J.; Lin, Z.; Chen, G. *Anal. Bioanal. Chem.* **2007**, *388*, 399-407.
(48) Zhu, L.; Li, Y.; Tian, F.; Xu, B.; Zhu, G. *Sensor. Actuat. B: Chem.* **2002**, *84*, 265-270.

For TOC only:


Glucose + O2
GODx
Stainless Steel Electrode
NH
NH
NH2
O
O
+ H2O2
Enhanced ECL
9000
6000
3000
0
ECL Intensity
0.6
0.8
1.0
E / V