Improved electrogenerated chemiluminescence of luminol by cobalt nanoparticles decorated multi-walled carbon nanotubes

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Abstract

Multi-walled carbon nanotubes (MWCNTs) were decorated with cobalt nanoparticles (Co-NPs) by a simple thermal decomposition method. The prepared nanohybrid material (nanoCo-MWCNTs) was then cast on the surface of a glassy carbon electrode (GCE) as a novel electrocatalyst for the construction of a sensitive electrochemiluminescence (ECL) sensor. The fabricated sensor (GCE/nanoCo-MWCNTs/Nafion) showed excellent electrocatalytic activity toward luminol and H₂O₂ oxidation reactions at neutral media. Under optimal experimental conditions, the ECL signal intensity of the sensor was linear with the concentration of luminol in the range between 80 nM and 140 μM ($r = 0.9963$) and also with the concentration of H₂O₂ in the range between 1 nM and 240 μM ($r = 0.9980$). Detection limits ($S/N = 3$) for luminol and H₂O₂ were 8.7 and 0.2 nM, respectively. The relative standard deviations (RSD) for repetitive

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measurements of 100 μM luminol ($n = 10$) and 10 μM H_2O_2 ($n=11$) were 1.6% and 2.0%, respectively. Also, the prepared sensor was further modified with glucose oxidase (GOx) to fabricate a glucose ECL based biosensor (GCE/nanoCo-MWCNTs/GOx/Nafion). The fabricated ECL biosensor exhibited excellent performance toward glucose detection in the concentration range between 0.5 and 600 μM with a satisfactory detection limit (50 nM) and reproducibility (2.3%).

Keywords: Electrochemiluminescence; Multi-walled carbon nanotubes; Co nanoparticles; Luminol; Hydrogen peroxide; Glucose.

1. Introduction

Electrogenerated chemiluminescence or electrochemiluminescence (ECL) is an attractive analytical tool with broad applications in different research areas. ECL is a hybrid technique which provides electrochemical and spectral information, simultaneously [1]. Simple and low-cost instrumental set up, excellent sensitivity and low background signal are the most important features of ECL [2, 3]. A increased interest is observed for ECL investigations and this fact has been reflected in the number of reviews [4-9] which report the effectiveness of ECL detection method and its wide analytical applications.

Luminol (2, 3-aminophthalhydrazide) is the most popular organic luminescent species which can be oxidized at the electrode surface. The subsequent chemical reaction between the oxidized luminol and H_2O_2 (or O_2) produces ECL signal. Since the first report on the observation of ECL response for luminol at the electrode surface in alkaline solution [10], numerous attempts have been done to find effective approaches for the enhancement of ECL signal intensity of luminol in neutral medium [11-14]. It has been shown that the ECL reaction of luminol with oxygen or hydrogen peroxide is more efficient in alkaline medium through modification of the electrode surface with nano-materials [12, 15].

With the development of nanotechnology, various types of nano-structured materials with outstanding properties are being synthesized and applied for the construction of novel sensors and biosensors. Nano-structured materials provide higher effective surface area and higher catalytic activity in comparison with their respective bulk materials. The catalytic effect of some nano-structured materials such as multi-walled carbon nanotubes (MWCNTs) [16, 17], Au nanoparticles (NPs) [18-20]; Pt-NPs [21], Ag-NPs [22-24] and TiO_2 -NPs [25] on chemiluminescence (CL) and ECL reactions have been investigated for luminol- H_2O_2 and

luminol-O₂ systems in alkaline and neutral media. Discovering of carbon nanotubes (CNTs) by Iijima [26] and surface functionalization of CNTs with nanoparticles (so called “decoration process”) [27] revealed a new class of nanohybrid materials with the integrated properties of two components [28]. It seems, the application of this class of nanohybrid materials for ECL measurements provides novel opportunities for developing a new generation of (bio)sensors. Our previous studies [13, 29, 30] obviously demonstrated that this class of nanohybrid materials noticeably improved the sensitivity of detection. The observed enhancement was attributed to the surface area and electrocatalytic activity of the nanohybrid materials which promote the rate of electron transfer reactions of luminol, oxygen and hydrogen peroxide at the electrode surface and also catalyze the generation of reactive oxygen species (ROSs) [15]. ROSs are strong oxidizing radicals and generally highly reactive species due to the presence of unpaired valence shell electron. Singlet oxygen (¹O₂), superoxide hydrogen (HO₂[•]), superoxide radical (O₂^{•-}), hydroxyl radical (OH[•]) and inorganic or organic peroxides are the most well-known ROSs.

Glucose is an important carbohydrate which is introduced as a source of energy for living organisms. Its determination is vital because of its importance in clinical and industrial purposes. Rapid glucose monitoring is essential for treatment and control of diabetes. Among numerous methods which have been developed for the fast and accurate determination of glucose, electrochemical and optical based methods are the most common methods. In this context, ECL can be proposed as a powerful analytical tool for glucose detection due to its inherent sensitivity and selectivity. In an enzymatic ECL glucose biosensor, glucose oxidase (GOx) catalyzes the oxidation of glucose to gluconic acid by the reduction of molecular oxygen to the hydrogen peroxide. The enzymatically produced H₂O₂ reacts with luminol radical anion, the oxidation product of luminol at the electrode surface, and gives the excited state 3-aminophthalate anion

which its light emission at about 425 nm is used for quantification of hydrogen peroxide or indirectly for glucose detection. Various nano-structured materials such as CNTs film [17], CNTs paste [31] and Au-NPs self-assembled onto a silica sol-gel network [20], Fe₃O₄-NPs [32], Au-NPs and Pd-NPs decorated CNTs [13, 30], graphene Pt nanoflower/graphene oxide [33], C₆₀ embedded in tetraoctylammonium bromide [34], poly(luminol-aniline) nanowires [35] and hollow gold nanospheres [36] have been applied for the fabrication of glucose biosensor based on the monitoring of ECL signal of luminol.

Nano-structured Co and cobalt oxide have been widely used for the determination of biological and environmental species such as glucose [37], cysteine [38], methanol [39, 40], arsenic [41], nitrite [42] and hydrogen peroxide [43]. Our previous study revealed that cobalt nanoparticles decorated multi-walled carbon nanotubes (nanoCo-MWCNTs) is an effective nanohybrid material for the cathodic ECL measurement of luminol and dissolved oxygen [44]. In the present work, the electrocatalytic activity of nanoCo-MWCNTs on luminol ECL reaction is explored to show the feasibility of the proposed nanohybrid material for the fabrication of novel ECL (bio)sensors for luminol, H₂O₂ and glucose detection.

2. Experimental

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Nafion perfluorinated ion-exchange (5% solution in 90% light alcohol) was obtained from Fluka (Buchs, Switzerland). Luminol, Cobalt (II) acetate, sodium hydroxide, dimethylformamide (DMF) were obtained from Merck (Darmstadt, Germany). Multi-walled carbon nanotubes (95% purity, OD = 10-30 nm, ID = 5-10 nm and length = 0.5-500 μm) was purchased from Aldrich

(Steinheim, Germany). Co-NPs decorated MWCNTs nanohybrid (nanoCo-MWCNTs) was prepared according to the method reported previously via manual mixing without modifications {Lin, 2009 #27}.

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=7.4). Analytical grade O₂ and Ar gases were used for the preparation of solutions saturated with oxygen and argon gases, respectively. Phosphate buffer solution (0.1 M, pH=7.4) saturated with oxygen and argon gases were prepared separately by sparging of O₂ and Ar into the PBS for at least 30 min.

2.2. Apparatus and procedure

Electrochemical and ECL measurements were carried out in a 4 mL homemade Teflon ECL cell [30]. The working electrode was mounted in the horizontal position in the ECL cell where its surface was exactly in front of the window of a Hamamatsu photomultiplier module (H7468) (Hamamatsu city, Japan). The photodetector and ECL cell were enclosed in a light-tight black box. The electrochemical system consisted of a conventional three-electrode set-up in which nanoCo-MWCNTs modified glassy carbon electrode, a platinum wire, and an Ag|AgCl|KCl_{sat} electrode served as the working, auxiliary and reference electrodes, respectively. The working potential was applied to the working electrode in the standard way using an Autolab potentiostat-galvanostat model PGSTAT30 (Utrecht, The Netherlands) and the output cyclic voltammograms and ECL signals were acquired using Autolab NOVA software and a

home-written data acquisition program, respectively. A Metrohm 691 pH meter was used for pH adjustments. All measurements were performed at room temperature.

2.3. Fabrication of luminol, H₂O₂ and glucose ECL based (bio)sensor

The surface of a glassy carbon electrode (GCE) was polished with 0.3, 0.1 and 0.05 μm alumina past (Struers, Copenhagen, Denmark), consecutively to obtain a mirror like surface and then cleaned in water-ethanol mixture using ultrasonic agitation for 3 minutes. One milligram of nanoCo-MWCNTs was dispersed in 1 mL DMF with ultrasonic agitation to achieve a well-dispersed suspension. 2 μL of the prepared nanoCo-MWCNTs suspension was cast on the surface of GCE and dried in an oven at 50 $^{\circ}\text{C}$ to prepare GCE modified with nanoCo-MWCNTs (GCE/nanoCo-MWCNTs). The prepared sensor, GCE/nanoCo-MWCNTs, was directly used for luminol detection. Then, 5 μL of Nafion solution (0.1%) was cast on the surface of GCE/nanoCo-MWCNTs to fabricate H₂O₂ sensor (GCE/nanoCo-MWCNTs/Nafion). The prepared sensor was stored at room temperature in air when not in use. Furthermore, 2 μL of PBS containing desired amount of GOx (2.11 U μL^{-1}) was placed on the surface of GCE/nanoCo-MWCNTs and allowed to dry at room temperature. Then, 5 μL of Nafion solution (0.1%) was cast on the surface of GCE/nanoCo-MWCNTs/GOx to fabricate glucose biosensor (GCE/nanoCo-MWCNTs/GOx/Nafion). The prepared glucose biosensor was stored at 4 $^{\circ}\text{C}$ in PBS when not in use. For comparison GCE/MWCNTs, GCE/Nafion and GCE/MWCNTs/Nafion were prepared through similar procedures.

Nafion solution was used for the fabrication of H₂O₂ sensor and also for the immobilization of GOx at the electrode surface because of its outstanding biocompatibility and excellent thermal and mechanical properties. Moreover, Nafion can induce permselectivity property against some

compounds such as ascorbic acid, uric acid and dopamine which co-exist in the biological fluids and may interfere with glucose determination. Also, Nafion film prevents the passivation of electrode surface by side products of ECL reaction and improves the reproducibility of the ECL system.

3. Results and discussion

3.1. Characterization of GCE/nanoCo-MWCNTs

Energy dispersive X-ray spectroscopy (EDS) and transmission electron microscopy (TEM) were used to characterize the prepared nanoCo-MWCNTs (Fig. S1). The analysis of EDS profile revealed that the calculated atomic levels of carbon and cobalt were approximately similar to the applied amounts of MWCNTs and cobalt for the preparation of nanohybrid. Also, the formation of Co-NPs with an average diameter of 5 nm on the outer surface of MWCNTs was approved by TEM images.

The presence of Co-NPs in the prepared nanohybrid material was approved by cyclic voltammetry (CV) studies in alkaline solution (pH=12) at a scan rate of 20 mV s⁻¹. Noticeable redox peaks were observed for GCE/nanoCo-MWCNTs and no redox peaks for GCE/MWCNTs and GCE (Fig. S2). The appearance of several redox peaks at different potentials was attributed to the conversion between different forms of cobalt which were stable at alkaline pH [38, 39, 43, 45-47].

Charge transfer property of GCE/nanoCo-MWCNTs was also examined by CV and electrochemical impedance spectroscopy (EIS) using 5 mM Fe(CN)₆^{3-/4-} as redox couple in 0.1 M KCl (Fig. S3). Well-defined redox peaks with peak-to-peak separation (ΔE_p) values of 191, 151 and 122 mV versus Ag|AgCl|KCl_{sat} were observed for Fe(CN)₆^{3-/4-} couple at GCE,

GCE/MWCNTs and GCE/nanoCo-MWCNTs, respectively. Also, charge transfer resistance (R_{ct}) values for GCE/nanoCo-MWCNTs (35 Ω) was much less than those observed for GCE/MWCNTs (75 Ω) and GCE (280 Ω). The CV and EIS results clearly demonstrated that rate of electron transfer at GCE/nanoCo-MWCNTs was much faster than at GCE/MWCNT and GCE.

3.2. Electrochemical and ECL investigations on luminol ECL based sensor

Cyclic voltammograms and corresponding I_{ECL} -E curves (ECL responses) of 100 μ M luminol in PBS (0.1 M, pH=7.4) were recorded in the potential range between -0.3 and 0.7 V versus (Ag|AgCl|KCl_{sat}) with the scan rate of 100 mV s⁻¹. As shown in Fig. 1, the redox peaks current, charging current and ECL response intensities recorded for luminol at GCE/nanoCO-MWCNTs were much higher than those observed for luminol at GCE/MWCNTs and GCE. The value of formal potential ($E^{\circ'} = (E_{pc} + E_{pa})/2$, mean value of cathodic and anodic peak potentials) for luminol at the mentioned three electrodes was about 0.5 V, where the ECL responses of luminol at the mentioned electrodes passed over a maximum. The anodic peak current intensity of luminol at GCE/nanoCo-MWCNTs was 2 and 6 times of that observed at GCE/MWCNTs and GCE, respectively. Also, the ECL response intensity of luminol at GCE/nanoCo-MWCNTs was 3 and 5 times of that observed at GCE/MWCNTs and GCE, respectively. The observed enhancement in peak current and ECL response intensities for luminol at GCE/MWCNTs compared with GCE was attributed to the increase of electrode surface area because of the presence of MWCNTs. But, the presence of Co-NPs on the surface of the MWCNTs in the prepared nanohybrid material, nanoCo-MWCNTs, provided many active sites with higher electrocatalytic activity and sensitizing effect for the catalytic oxidation of luminol and caused to

observe a more enhancement in peak current and ECL response at GCE/nanoCo-MWCNTs compared with GCE/MWCNTs.

Further investigation was performed on ECL behavior of luminol at the mentioned three electrodes to propose a possible mechanism for the observed ECL reaction. Potential was swept in the range between -0.3 and 0.7 V with the scan rate of 100 mV s^{-1} and ECL responses of $100 \text{ }\mu\text{M}$ luminol in PBS (0.1 M, pH=7.4) were recorded in the absence and presence of oxygen. It was found that ECL response intensity of luminol decreased remarkably by purging of oxygen and increased significantly by sparging of oxygen, suggesting the participation of molecular O_2 in ECL reaction. Also, the increase of ECL response intensity of luminol in the presence of O_2 at GCE/nanoCo-MWCNTs was much higher than those of observed at GCE/MWCNTs and GCE, suggesting the catalytic action of Co-NPs in ECL reaction. Moreover, potential was swept in the range between 0 and 0.8 V and ECL responses of luminol were recorded at the same experimental conditions as mentioned before. It was found that the ECL response intensity at GCE/nanoCo-MWCNTs was similar to that recorded for GCE/MWCNTs with no enhancement.

Based on the results obtained in this study and those reported previously [13, 29, 44], it is proposed that the molecular oxygen is reduced by applied negative (reduction) potential and various reactive oxygen species (ROSs) is produced at the electrode surface. The chemical reaction of ROSs with the luminol radical anion, the oxidation product of luminol at 0.5 V, produces ECL signal. It seems that the electrocatalytic activity of Co-NPs in nanoCo-MWCNTs nanohybrid material towards O_2 reduction and luminol oxidation enhances the rate of production of ROSs and luminol radical anions and consequently enhances the intensity of ECL response. The proposed mechanism was in good agreement with those reported for luminol ECL mechanism [15, 48-50].

The effect of nanoCo-MWCNTs loading and potential scan rate on ECL response of luminol in PBS (0.1 M, pH=7.4) was explored at the fixed concentrations of luminol (100 μ M) and dissolved oxygen (0.29 mM). The results showed that the ECL response intensity of luminol passed over a maximum with increasing the amount of nanoCo-MWCNTs on the electrode surface. But, the ECL response intensity of luminol enhanced and reached a plateau with increasing the scan rate. The maximum ECL response was observed for 2 μ L of nanoCo-MWCNTs (1 mg mL⁻¹) loading (Fig. 2A) and the scan rate of 100 mV s⁻¹ (Fig. 2B).

Under optimized conditions and by the sweep of potential in the range between -0.3 and 0.7 V, the proposed GCE/nanoCo-MWCNTs was used as a sensor for determination of luminol at pH of 7.4 (0.1 M PBS). The ECL response of the proposed sensor was linear with the concentration of luminol in the range between 80 nM and 140 μ M with a correlation coefficient of 0.9963 (Fig. 3A). The limit of detection (S/N=3) for luminol was 8.7 nM. The relative standard deviations (RSD) for repetitive measurements (n = 10) of 100 μ M luminol was 1.6% (Fig. 3B).

3.3. Electrochemical and ECL investigations on H₂O₂ ECL based sensor

Electrochemical and ECL behaviors of luminol were investigated at GCE/nanoCO-MWCNTs/Nafion in the presence H₂O₂ and in the potential range where the reduction of molecular oxygen was negligible. For this purpose, potential was swept in the range between 0 and 0.8 V with the scan rate of 100 mV s⁻¹ and cyclic voltammograms and corresponding ECL responses of 100 μ M luminol were recorded in PBS (0.1 M, pH=7.4) and in the presence of 10 μ M H₂O₂. As shown in Fig. 4, the oxidation peak current intensity and ECL response of luminol in the presence of H₂O₂ at GCE increased by modification of GCE with MWCNTs and nanoCo-

MWCNTs nanohybrid. The ECL responses of luminol at GCE/MWCNTs/Nafion and GCE/nanoCo-MWCNTs/Nafion were 4 and 10 times of that observed at GCE/Nafion. It has been cited that the oxidation products of H_2O_2 (e.g. oxygen and superoxide radical anions) which are generated during the oxidation of H_2O_2 at the electrode surface [15] react with the luminol radical anion and produce ECL signal. In the other words, under sweeping potential in positive direction (oxidation), the immobilized nanoCo-MWCNTs on the surface of GCE catalyze the electrochemical oxidation of luminol and hydrogen peroxide. The produced luminol radical anions are further oxidized by hydrogen peroxide directly and by the oxidation products of H_2O_2 (e.g. oxygen and superoxide radical anions) to give the excited state 3-aminophthalate anions that emit light at about 425 nm.

The effect of nanoCo-MWCNTs and Nafion loadings and potential scan rate on ECL response of luminol (100 μM) was explored in PBS (0.1 M, pH=7.4) and in the presence of 10 μM H_2O_2 . The intensity of ECL response passed over a maximum with increasing nanoCo-MWCNTs and Nafion loadings. The maximum ECL response was observed for 2 μL of nanoCo-MWCNTs (1 mg mL^{-1}) and 5 μL Nafion (0.1%) loadings. The effect of potential scan rate on ECL response of luminol- H_2O_2 system was similar to that observed for luminol- O_2 system with a maximum ECL response at the scan rate of 100 mV s^{-1} .

Under optimum experimental conditions, the proposed GCE/nanoCo-MWCNTs/Nafion was used as a sensor for determination of H_2O_2 by the sweep of potential in the range between 0 and 0.8 V. The ECL signal of the sensor was linear with the concentration of H_2O_2 in the range between 1 nM and 240 μM ($r^2=0.9980$) (Fig. 5A). The limit of detection ($S/N=3$) for H_2O_2 was 0.2 nM. The relative standard deviations (RSD) for repetitive measurements ($n=11$) of 10 μM H_2O_2 was 2.0% (Fig. 5B).

3.4. Electrochemical and ECL investigations on glucose ECL based biosensor

For the fabrication of glucose ECL based biosensor (GCE/nanoCo-MWCNTs/GOx/Nafion), the proposed H_2O_2 ECL based sensor, GCE/nanoCo-MWCNTs/Nafion, was further modified with glucose oxidase (GOx). Preliminary investigation showed (Fig. 6) that the oxidation peak current and ECL signal intensities of luminol increased with the addition of glucose in the reaction medium. The observation of ECL emission at 425 nm was attributed to the reaction of luminol radical anion with H_2O_2 (the product of the enzymatic reaction of GOx and glucose) at the electrode surface. Therefore, at the optimized conditions for H_2O_2 detection, i.e. 2 μL of nanoCo-MWCNTs (1 mg mL^{-1}) and 5 μL Nafion (0.1%) loadings, the effect of GOx loading and pH on ECL response of luminol (100 μM) at GCE/nanoCo-MWCNTs/GOx/Nafion was explored in the presence of 100 μM glucose and in the potential range between 0 and 0.8 V with the scan rate of 100 mV s^{-1} .

The results showed that the ECL response of the proposed glucose biosensor in PBS (0.1 M, pH=7.4) increased with increasing the amount of GOx and then reached a plateau when 2 μL of $2.11 \text{ U } \mu\text{L}^{-1}$ GOx was cast at the electrode surface (Fig. 7A). Investigation on the effect of supporting electrolyte pH (PBS, 0.1 M) revealed that the ECL response of the glucose biosensor increased with increasing of pH, reached a maximum at pH of 9.0 and then decreased (Fig. 7B). The decline of ECL response at pH more than 9.0 was attributed to the decrease of GOx activity. It should be mentioned that the sensitivity of the proposed glucose biosensor in PBS (0.1 M) at pH of 9.0 was higher than that observed at pH of 7.4. But, glucose monitoring at pH of 7.4, i.e. physiological pH, is more favorable because of possible application of the biosensor for in vivo monitoring.

The fabricated biosensor, GCE/nanoCo-MWCNTs/GOx/Nafion, was used for the determination of glucose at pHs of 7.4 and 9.0. The ECL response of the biosensor was linear with the concentration of glucose in the range between 0.5 - 600 μM ($r^2=0.9998$) at pH of 7.4 (Fig. 8A) and in the range between 0.1 - 250 μM ($r^2=0.9988$) at pH of 9.0 (Fig. 8B). The limits of detection ($S/N=3$) for glucose were 50 and 20 nM at pHs of 7.4 and 9.0, respectively. The relative standard deviations (RSD) for repetitive measurements ($n = 17$) of 100 μM glucose at pH of 7.4 was 2.3% (Fig. 8C). The sensitivity of the proposed ECL glucose biosensor at pH of 9.0 was about 3 times of its sensitivity at pH of 7.4. The analytical features of some reported luminol based ECL glucose biosensors are summarized in Table 1 and compared with those obtained in this study using GCE/nanoCo-MWCNTs/GOx/Nafion.

The effects of potential interfering biological compounds such as uric acid, dopamine and ascorbic acid on the ECL response of the proposed glucose biosensor were examined. A series of solutions containing 100 μM glucose plus potent interfering compounds at different concentrations were prepared. The results of the prepared test solutions were collected and compared with that obtained for the standard glucose solution. A 5 % error criterion was adopted. The analysis of results showed that the tolerance of ECL signal for 100 μM glucose at pH of 7.4 was less than 5% in the presence of 50 μM uric acid, 10 μM dopamine and 5 μM ascorbic acid. Uric acid, dopamine and ascorbic at the concentrations more than 50 μM decreased the ECL intensity of glucose. The obtained results are summarized in Table 2.

The applicability of the proposed glucose ECL biosensor was also examined by the determination of glucose in normal human serum sample. 3 μL of normal human serum sample was mixed with 3 mL PBS (0.1 M, pH=7.4) containing 100 μM luminol and certain amount of glucose (0, 10, 30, 50 or 100 μM). The prepared solutions were then examined using

GCE/nanoCo-MWCNTs/GOx/Nafion. The concentration of glucose in normal human serum determined by the use of proposed glucose biosensor and by local hospital was 4.71 and 4.83 mM, respectively. The recovery of the analysis was about 97.5%. Recoveries for the five spiked samples were between 97% and 105%.

4. Conclusions

An economical nanohybrid material, nanoCo-MWCNTs, was prepared using a facile and rapid approach. The prepared nanohybrid material was successfully applied for the fabrication of a novel ECL based (bio)sensor for the determination of luminol, H_2O_2 and glucose. The fabricated ECL (bio)sensor showed strong sensitizing effect on the ECL response of luminol due to the excellent electrocatalytic activity of nanoCo-MWCNTs towards O_2 reduction and luminol and H_2O_2 oxidation reactions. The analysis of results indicated that the proposed nanohybrid material and fabricated (bio)sensor have a great potential for practical applications.

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Legend for figures

Fig. 1. A) Cyclic voltammograms and B) corresponding ECL responses of luminol at a GCE (dotted line), GCE/MWCNTs (dashed line) and GCE/nanoCO-MWCNTs (solid line). Conditions: luminol, 100 μM ; supporting electrolyte, PBS (0.1M and pH 7.4); potential scan rate, 100 mV s^{-1} .

Fig. 2. Effect of A) nanoCo-MWCNTs loading and B) scan rate on the ECL response of luminol at GCE/nanoCO-MWCNTs. Conditions: luminol, 100 μM ; supporting electrolyte, PBS (0.1M and pH 7.4); potential scan rate for A, 100 mV s^{-1} .

Fig. 3. A) Calibration plot of ECL response versus luminol concentration and B) reproducibility of ECL responses for 100 μM luminol at GCE/nanoCo-MWCNTs/Nafion. Conditions: supporting electrolyte, PBS (0.1 M and pH 7.4); potential scan rate, 100 mV s^{-1} .

Fig. 4. A) Cyclic voltammograms and B) corresponding ECL responses of luminol at a GCE/Nafion (dotted line), GCE/MWCNTs/Nafion (dashed line) and GCE/nanoCO-MWCNTs/Nafion (solid line) in the presence of 10 μM H_2O_2 . Conditions: luminol, 100 μM ; supporting electrolyte, PBS (0.1M and pH 7.4); potential scan rate, 100 mV s^{-1} .

Fig. 5. A) Calibration plot of ECL response versus H_2O_2 concentration and B) reproducibility of ECL responses for 10 μM H_2O_2 at GCE/nanoCo-MWCNTs/Nafion. Conditions: Luminol, 100 μM ; supporting electrolyte, PBS (0.1 M and pH 7.4); potential scan rate, 100 mV s^{-1} .

Fig. 6. A) Cyclic voltammograms and B) corresponding ECL responses of luminol at GCE/nanoCo-MWCNTs/GOx/Nafion in the absence (dashed line) and presence (solid

line) of 100 μM glucose. Conditions: luminol, 100 μM ; supporting electrolyte, PBS (0.1M and pH 7.4); potential scan rate, 100 mV s^{-1} .

Fig. 7. Effect of **A)** GOx loading and **B)** pH on the ECL response of luminol at GCE/nanoCO-MWCNTs/GOx/Nafion. Conditions: glucose, 100 μM ; luminol, 100 μM ; supporting electrolyte, PBS (0.1M and pH 7.4); potential scan rate for A, 100 mV s^{-1} .

Fig. 8. A) Calibration plot of ECL response versus glucose concentration in 0.1 M PBS at pH of 7.4, B) at pH of 9 and C) reproducibility of ECL responses for 100 μM glucose at GCE/nanoCo-MWCNTs/GOx/Nafion. Conditions: Luminol, 100 μM ; supporting electrolyte, PBS (0.1 M and pH 7.4 for reproducibility); potential scan rate, 100 mV s^{-1} .

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Table 1. Analytical features reported for some luminol based ECL glucose biosensors.

Modified electrode	LDR (μM)	DL (μM)	pH	Reference
CNTPE	1 - 2000	0.5	7.0	[31]
GCE/CNTs/Nafion	5 - 80	2	7.4	[17]
Au/sol-gel/AuNPs	1 - 5000	0.2	7.5	[20]
GCE/MWCNTs/polyNiTSPc/Nafion	1 - 100	0.08	7.4	[16]
SCPE/ Fe_3O_4	10 - 10000	1	8.0	[32]
GCE/Graphene/Nafion	2 - 100	1	7.4	[51]
GE/PLANC	0.1 - 50	0.03	9.0	[35]
GCE/collagen membrane	0.6 - 3000 ^a	0.06 ^a	8.5	[52]
GCE/polyamide membrane	1.5 - 300 ^a	1.5 ^a	8.5	[52]
GCE/sol-gel matrix	50 - 10000	26	9.0	[53]
CCE	10 - 10000	8.16	8.5	[54]
SP graphite electrode microarrays	3 - 3000	3	nr	[55]
GCE/PDDA-CS	0.0005 - 40	0.0001	7.5	[56]
GCE/AuNPs-MWCNTs/CS	1 - 1000	0.5	7.4	[29]
	0.5 - 110	0.06	8.5	
GCE/nanoPd-MWCNTs/Nafion	0.1 - 1000	0.054	7.4	[13]
GCE/PtNFs/GO	5 - 1000	2.8	7.4	[33]
GCE/nanoCo-MWCNTs/Nafion	0.5 - 600	0.05	7.4	This work
	0.1 - 250	0.02	9.0	

LDR, linear dynamic range; DL, detection limit; AuNPs, gold nanoparticles; CCE, ceramic carbon composite electrode; CNTPE, carbon nanotubes paste electrode; CNTs, carbon nanotubes; CS, chitosan; GCE, glassy carbon electrode; GCF, glassy carbon foil; GE, graphite electrode; MWCNTs, multi-walled carbon nanotubes; PDDA, poly(diallyldimethylammonium chloride); PLANC, Poly(luminol-aniline) nanowires composite; polyNiTSPc, poly(nickel(II)tetrakisulfophthalocyanine); SCPE, solid paraffin carbon paste electrode; SP, screen printed; nanoPd: palladium nanoparticle, PtNFs/GO: platinum nanoflowers/graphene oxide, nanoCo: cobalt nanoparticle, nr: not reported. ^a nmol.

Table 2. The effect of potential interfering compounds on the ECL signal intensity of 100 μ M glucose.

Interfering compounds	[interference] (μ M)	Average ECL signal (arbitrary unit)	Recovery (RSD for n=5) (%)
Ascorbic acid	0	50.5	100 (0.7)
	1	49	98 (0.6)
	5	48	95 (0.9)
Dopamine	0	51.1	100 (0.4)
	1	50.7	99 (0.3)
	5	50.1	98 (0.7)
	10	48.6	95 (0.5)
Uric Acid	0	49.9	100 (0.4)
	1	49.6	99 (0.2)
	5	49.4	99 (0.4)
	10	48.6	97 (0.7)
	50	47.5	95 (0.8)

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8