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Electrochemiluminescence Analysis of Levodopa using Luminol at MWCNT-Modified Electrode

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Abstract- Levedopa was sensitively determined based on electrogenerated chemiluminescence (ECL) mechanism through enhancing the weak ECL of electro-oxidized luminal in presence of levodopa, on a multiwall carbon nanotubes (MWCNTs)-modified electrode. Under optimal conditions, the relative ECL emission/levodopa concentration plot was found to be linear from 1.0×10^{-9} to 1.7×10^{-7} M and a detection limit of 6.7×10^{-10} M was recorded. The modified electrode was found applicable to the analysis of levodopa in urine samples.

Keywords- Multiwalled carbon nanotube, Electrochemiluminescense, Gold electrode, Luminol, Levodopa

1. INTRODUCTION

As a well-established phenomenon, electrogenerated chemiluminescence (ECL), has been used in the highly sensitive sensors in analytical purposes [1,2]. ECL sensors not only enjoy the advantages offered by chemiluminescence (CL) devices (e.g. high sensitivity, and linear response windows), enjoy further advantages like the need for less sample quantities and enhanced selectivity, in amperometry and constant potential electrolysis procedures [3–15].

One of the first compounds known to have CL and ECL effects is 3-aminophthalhydrazide (luminal). However, many promising and rapidly developing area of analytical ECL still concerns the luminol ECL system [16–21]. This can be attributed to the quantum yield, low oxidizing potential, and reasonable costs of the regent as well as technical fact that its reactions can simply occur in buffered aqueous media even containing impurities like oxygen, etc. other hand, (–)-3-(3, 4-dihydroxylphenyl)-L-alanine, also known as levedopa, is a neurotransmitter that is used for treating conditions caused due to the lack of dopamine, like Parkinson's disease. This is because levodopa is absorbed and changed to dopamine by decarboxylase, after administration, and can hence treat such symptoms as muscular rigidity and tremors. The monitoring and determination of levodopa is hence a critical area of research [22,23]. Levodopa has traditionally been analyzed through techniques such as spectrophotometry [24,25], HPLC [26], spectrofluorimetry [27], GC [28], electrochemical [29-35] radio immunoassay [36], capillary electrophoresis with chemiluminescence detection [37] and potentiometry [38] techniques.

In the course of the present study, it was found a weak ECL signal generated by the electrochemical oxidation of luminol on the surface of a gold electrode (GE) modified by MWCNTs-COOH in the borax buffer solution can be greatly enhanced by addition of Levedopa to the luminol solution. This phenomenon was used to develop a very sensitive and selective ECL electrode for levodopa.

2. EXPERIMENTAL

2.1. Reagents and chemicals

The chemicals used were all of analytical reagent grade purity and were used as received from the supplier. Levodopa was procured from Iran Hormone (a local pharmaceutical company). Luminol, NaOH, and dimethylformamide (DMF) were from Merck. The Nafion perfluorinated ion-exchange (5% solution in 90% light alcohol) was supplied by Fluka and the 95% pure MWCNT (10-40 nm in diameter, 1-25 μm in length, 5-10 nm in core diameter; SBET: 40-600 m^2/g, V_{total}: 0.9 cm^3/g, bulk density 0.1 g/cm^3, true density 2.1 g/cm^3) was obtained from a local company (Iran).

All luminal solutions used were prepared from a 1.0×10^{-2} M stock solution of 3-aminophthalhydrazide, which was prepared by dissolving the compound in a small volume of

a 0.1 M sodium hydroxide solution. Dilution of the stock solution was performed with a Borate buffer solution (BBS, 0.1 M, pH= 9). The 0.01 M stock solutions of levodopa were prepared using distilled water, which shall be simply referred to as water from hereon.

2.2. Instruments

All experiments were performed under ambient temperature and pressure. Cyclic voltammetric (CV) measurements were conducted on a PalmSens PC handheld potentiostat–galvanostat (Netherlands). The experiments were performed using a three-electrode cell including a GE/MWCNTs-COOH/Nafion working electrode, an Ag|AgCl|KCl(sat) reference electrode and a platinum wire as the auxiliary electrode. To record the ECL signals the GE/MWCNTs-COOH/Nafion electrode was equatorially set up in a 4-mL quartz cell so that its exactly faced the window of a Perkin Elmer FL-win photomultiplier. This assembly was enclosed in a black box.

To perform the electrochemical impedance spectroscopy (EIS) experiments 1 mM $Fe(CN)_6^{3-/4-}$ solution further containing 0.1 M of KCl was used and the tests were performed at a amplitude of 10 mV of in a frequency window ranging from 1 to 10,000 Hz. The pH measurements were performed using a Metrohm pH meter.

2.3. Functionalization of the MWCNT

The typical procedure included dispersing 6 mg of the MWCNTs in a 10 mL aqueous solution of 35% HNO_3 for 6 hours under sonication. The resulting suspension was next diluted with water and then filtered with a 0.45 μm polytetrafluoroethylene (PTFE) filter (Schleicher & Schuell). The MWCNTs were then washed with water, until the pH of the water was above 6 and dried under an IR lamp. During the experiments usually, 2 mg of the resulting carboxy functionalized CNTs was added to 1 mL of DMF and dispersed under sonication**.**

To prepare the working electrodes, a gold electrode was polished with 0.3 and 0.1 μm alumina and then cleaned in water under sonication. In the meantime 1mg of MWCNTs–COOH in 1 mL of DMF was sonicated for 60 minutes to form a uniform suspension. In a next step 5 μL of resulting mixture was taken and cast on the gold electrode and allowed to dry under IR light to form the modified electrode (i.e. GE/MWCNTs–COOH). This was followed by casting 5 μL of a 0.5% nafion solution on the GE/MWCNTs–COOH to form the final electrode (i.e. GE/MWCNTs–COOH /Nafion).

3. RESULTS AND DISCUSSION

EIS results, obtained under the operating conditions mentioned above, were used to evaluate the effect of modification on the behavior of the electrode surface [9,39]. The Z_{im} *vs.* Z_{re} plots (Nyquist plots) acquired for GE/Nafion and GE/MWCNTs–COOH/Nafion are presented in Fig. 1.

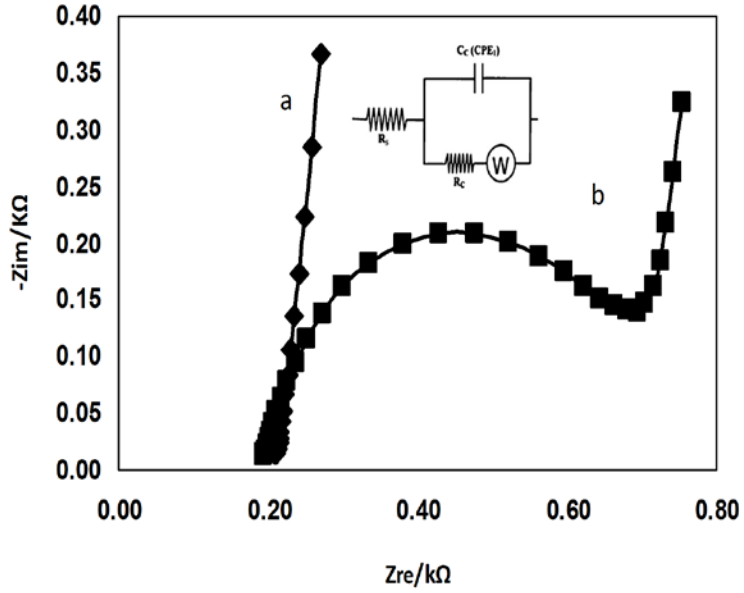


Fig. 1. Nyquist plots of 1 mM $Fe(CN)_6^{4-/3-}$ couple in 0.1 M KCl solution at GE/Nafion (a); GCE/MWCNTs-COOH/Nafion (b)

These results reveal serious differences in the electron transfer resistances (R_{ct}) behaviors of the bare and modified electrodes. This is reflected by the fact that the plots obtained for GE/Nafion do not include a semicircle and are instead in the form of a rather straight line, which correspond to a diffusion limiting step in the electrochemical process. The R_{ct} of the modified electrode shows an increase because the MWCNTs-COOH modified gold electrode were absorbed on the surface of that. The EIS results prove the successful absorption of the MWCNTs-COOH on the gold electrode.

3.1. Electrochemical and ECL behaviors of luminol on GE/MWCNTs-COOH/Nafion

The CVs obtained for luminol using GE and the GE/MWCNTs-COOH/ Nafion working electrodes in the potential range of 0.0 to 0.8 V (versus Ag/AgCl, KCl $_{(sat)}$) at 100 mVs^{-1} are presented in Fig. 2(a). The voltammograms recorded using the bare gold electrode does contain any peaks for luminol, while in the case of the modified electrode (Fig. 2 (b)) strong peaks in terms of redox current and charging current can be observed.

The high surface to volume and quantum chemical properties of nanoscale catalytic materials such as MWCNTs, have changed them to attractive materials. Other advantages of such materials are their considerable mechanical strength and electrical conductivity, good chemical stability and inertness, and wide applicable potential window in a wide range of electrolyte solutions. Functionalized CNTs, further, offer enhanced interfacial bonding strengths, surface activity and the higher possibility of bonding to other functional groups. If used to modify electrodes, the materials can enhance the electron transfer reactions.

The considerable charging currents observed using GE/MWCNTs-COOH/Nafion could be due to the increase in the surface of the electrode, and the resistive Nafion coating present on its surface [9]. Further, the amino-group of luminol can interact with the carboxyl groups of the functionalized MWCNTs, which enhances the adsorption phenomena on the surface of the electrode. A similar enhancement can be observed in the ECL signals of the electrode (Fig. 2B) and the ECL for luminol was increased 2.2 fold in the case of the modified electrode.

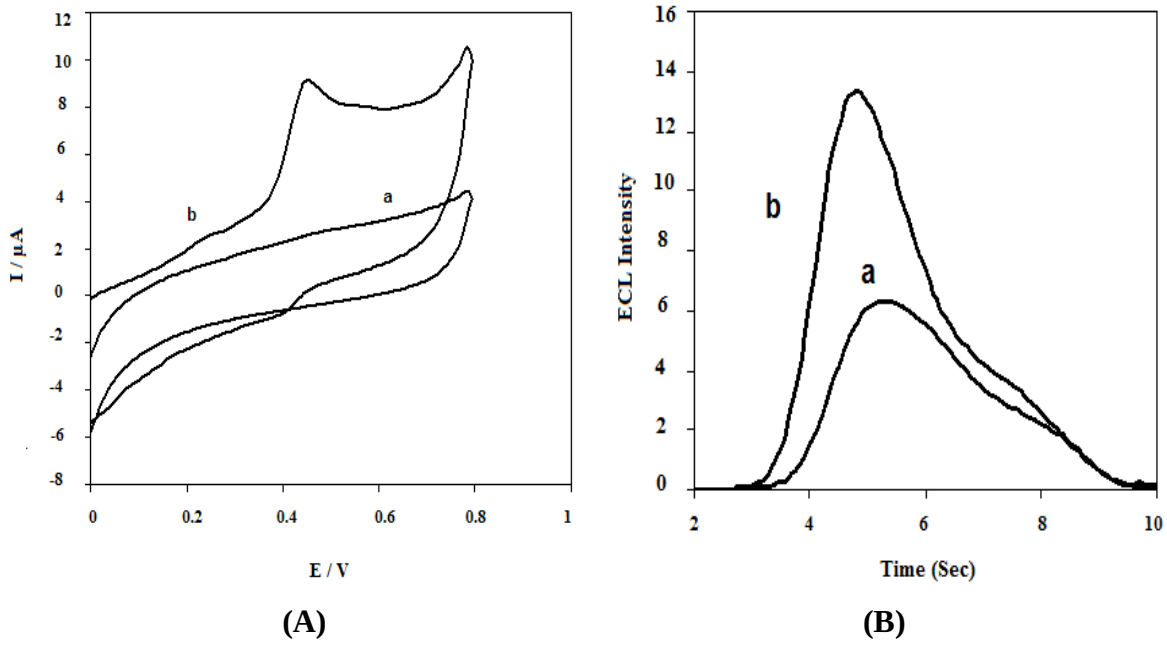


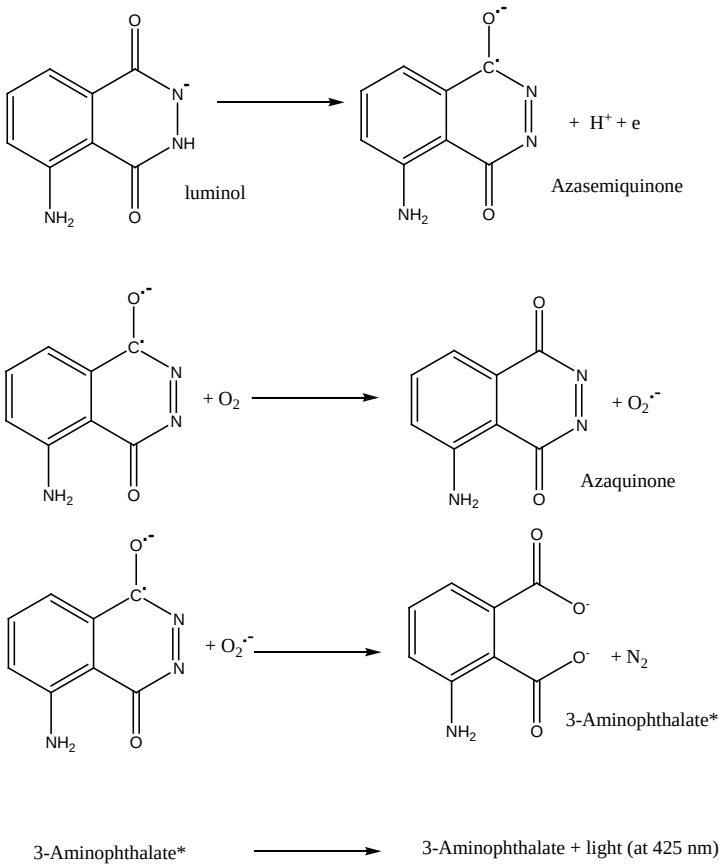
Fig. 2. (A) Cyclic voltammograms and (B) corresponding ECL responses of luminol at a GE/ Nafion (a) and GE/MWCNTs-COOH/Nafion (b). Conditions: luminol, 100µM; supporting electrolyte, 0.1M Borate buffer (pH 9); potential scan rate, 100 mVs^{-1}

Further the electrochemical and ECL behaviors of luminol were evaluated using the GE/MWCNTs-COOH/Nafion in the presence and absence of Levodopa. When levodopa is not present in the solution, luminol is oxidize and the resulting product has an emission at

425 nm, while in the presence of Levodopa, both species oxidize and products of their oxidations interact with each other, enhancing the ECL signal. The ECL is created according to the following mechanism, i.e. the oxidation of luminol form the luminol radical anion (Azasemiquinone), which next reacts with the O_2 produced through the oxidation of the hydroxide ion. As a result excited 3-aminophthalate anion (AP^{2-*}) is produced, which emits the ECL at 425 nm (Scheme 1):

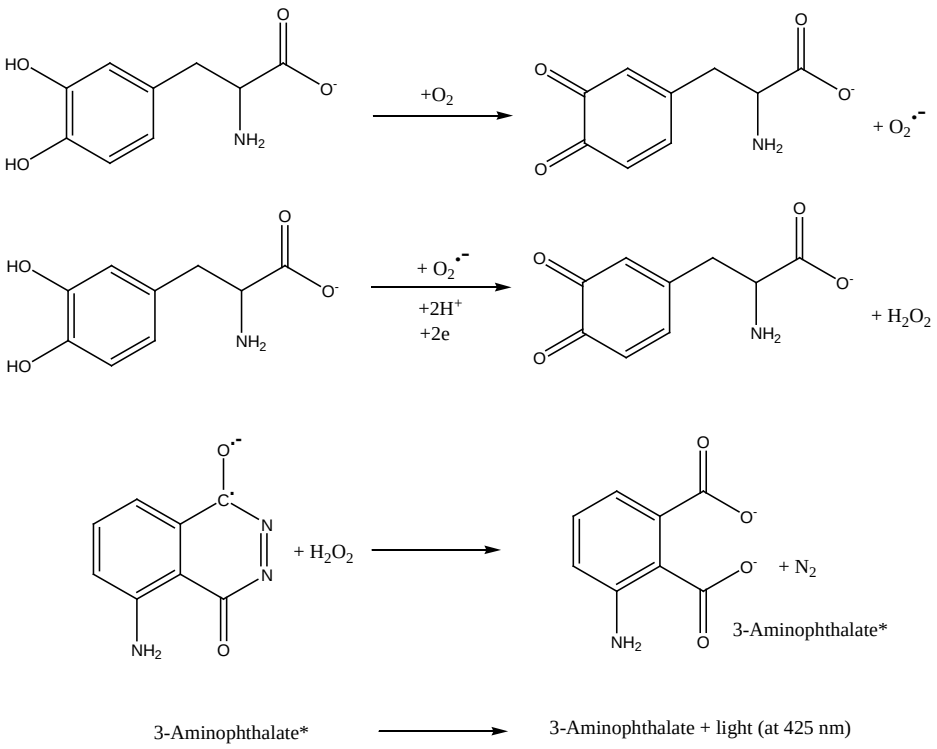
$2OH^- \rightarrow [O]_{ads} + H_2O + 2e$

$([O]_{ads}) \rightarrow O_2$



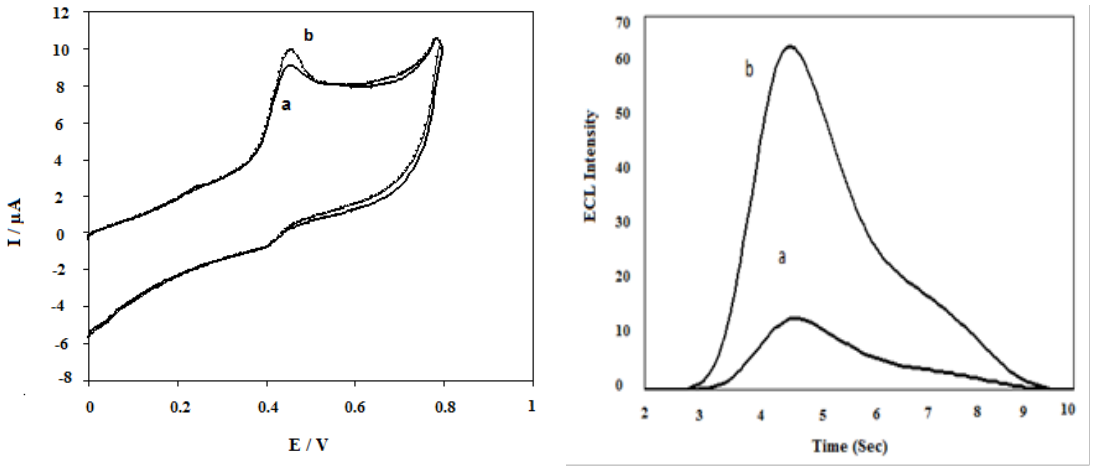
Scheme 1. Response mechanism of luminol ECL signal

When levodopa is present in the solution, a subsequent reaction occurs, in which the luminol radical anion and the hydrogen peroxide produced due to the oxidation of levodopa, leads to the formation of excited state 3-aminophthalate anion. This enhances the ECL signal at 425 nm, based on a previously reported mechanism, Scheme 2 [40,41].



Scheme 2. Response mechanism of luminol ECL signal

According to Fig. 3, the presence of levedopa enhances both the oxidation (Fig. 3A (b)) and ECL (Fig. 3B (b)) signals. The MWCNTs-COOH particles present on the surface GE seem to create more active sites for the redox reaction between luminal and Levodopa, and hence catalyze it.



(A) **(B)**

Fig. 3. (A) Cyclic voltammograms and (B) corresponding ECL responses of luminol in the absence (a) and presence (b) of levodopa at GE/MWCNTs-COOH/Nafion. Conditions: luminol, 100 µM; levodopa, 0.1 µM; supporting electrolyte, 0.1 M borate buffer (pH 9); potential scan rate, 100 mVs^{-1}

Further although the modified electrode has enhanced electron-transfer properties, given the low amounts of levodopa as opposed to the high quantum yield of the luminol, ECL measurements offer higher levels of sensitivity.

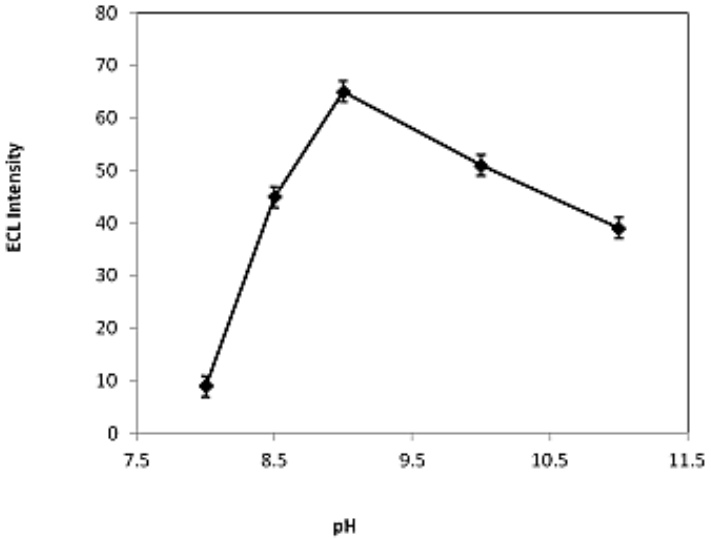


Fig. 4. Effect of pH on the ECL Intensity

3.2. Optimizing the ECL measurements

To optimize the function of the ECL sensor for levodopa the effects of pH, amounts of MWCNTs-COOH and Nafion, luminal concentration, and scan rate on the signal were evaluated.

3.3. Effect of pH

The ECL intensity/pH behavior observed was studied in the pH range of 8.0 to 11.0 and the result is illustrated in Fig. 4, clearly indicating that the ECL signal considerably increased by changing the solution from 8.0 to 9.0, while over 9.0 it decreased. Hence the optimal pH of 9.0 was used in the rest of experiments.

3.4. Amount of MWCNTs-COOH and Nafion

To investigate the effect of the amount of MWCNTs-COOH on the ECL suspensions containing various amounts of MWCNTs-COOH were cast on the GE surface and the results (Fig. 5) were recorded. Based on the results, increasing the amount of MWCNTs-COOH loading up to 5 µL (1 mg mL^{-1}), where the signal stayed unchanged, enhanced the ECL

signal. Similar studies on the effect of Nafion revealed an increase in the ECL intensity of the sensor due to increasing the concentration of Nafion up to a maximum about 0.5% (v/v).

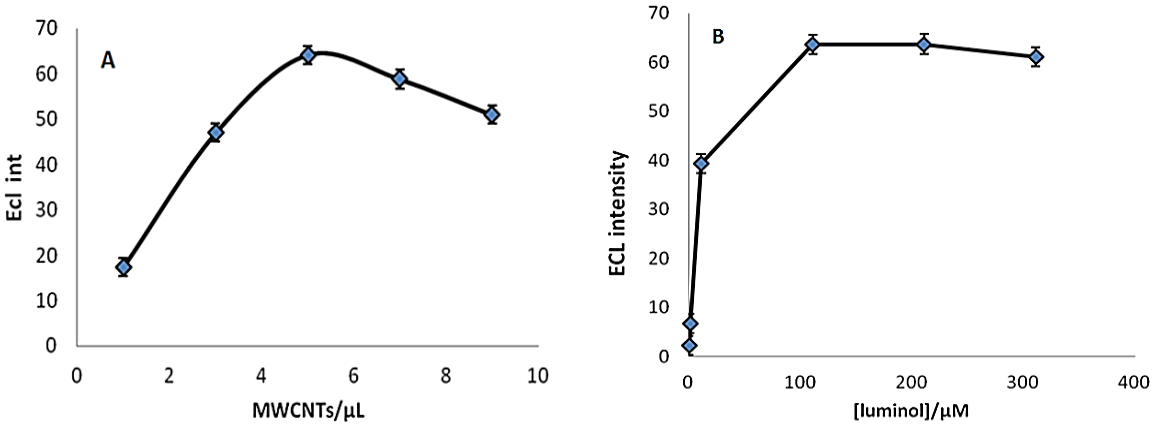


Fig. 5. (A) Effect of volume of MWCNTs-COOH of electrode surface on the ECL signal; (B) effect of concentration of luminol on ECL signal; in pH=9 of 0.1 M borate buffer and 0.1 µM of levodopa at scan rate of 100 mV s^{-1}

3.5. Effect of luminal concentration

The results in Fig. 5 clearly show that the ECL signal observed for a 0.1 µM levodopa solution linearly increased with increasing the luminol concentration from 0.1 to 100 µM, while further increases in the concentration up to 200 µM did not lead to show any effect. The undesirable background ECL signal of luminal, on the other hand, constantly increased by increasing the luminal concentration from 1 to 200 µM, and hence 100 µM was chosen as the optimal value.

3.6. Effect of scan rate

The studies on the effect of the scan rate (v) on the ECL and CV signals of luminal under optimal conditions revealed that upon increasing v the ECL intensity to luminol passed over a maximum about 100 mVs^{-1}. CV, studies performed between 10 and 100 mVs^{-1} are presented in Fig. 6. The results indicate that the anodic peak current is linearly proportional with $v^{1/2}$, reflecting a diffusion-controlled redox process, and based on the observations a scan rate of 100 mVs^{-1} was chosen as the optimal value.

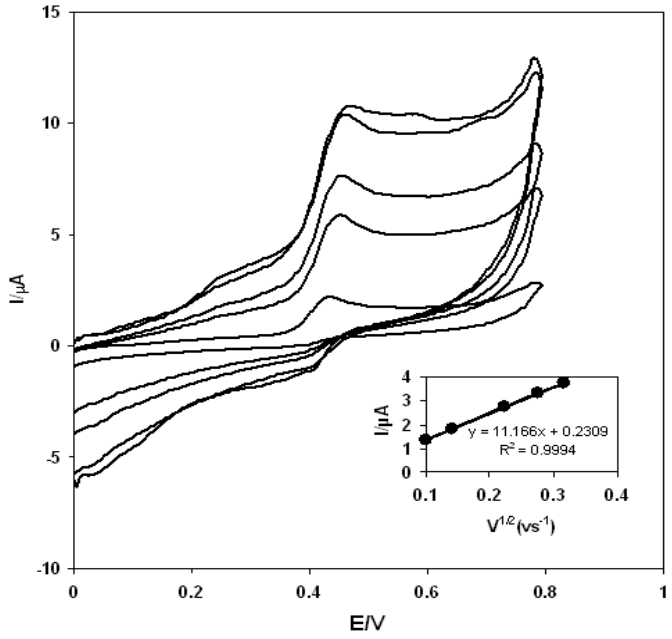


Fig. 6. Cyclic voltammograms of luminol (100 μM) in the presence of levodopa (0.1 μM) on GE/MWCNTs-COOH/Nafion at different scan rates and inset: plot of peak current versus the square root of scan rate. Conditions: supporting electrolyte, 0.1 M Borate buffer (pH 9); scan rates from inner to outer, 10–20–50–75 and 100 mVs^{-1}

3.7. Interference studies

To evaluate the effect of some commonly occurring foreign species on the analysis result under optimal conditions, different concentrations of the selected species were added to the analyte solution, and the measurements were performed. Considering a maximum of ±5% as the acceptable error level, no interference was observed in the presence of up to 1000 folds of Mg^{2+}, K^{+}, Cl^{-}, 500 folds of glucose, and 300 folds of L-methionine, Citric acid, glycine, L-alanine, and 100 folds of ascorbic acid.

3.8. Analytical performance the electrodes

The response of the electrode to levedopa was found to be linear from 1.0×10^{-9} to 1.7×10^{-7} M under optimal conditions and a detection limit of 6.7×10^{-9} M (S/N=3, and $\Delta ECL=5.0\times10^{8}C+65.77$ ($R^2=0.9999$)) were observed. Fig. 7. illustrates a typical calibration plot for the ECL-based analysis of levedopa. A relative standard deviation (RSD) of less than 4% was also determined for the analysis of 1.0×10^{-8} M of (n=7) (Fig. 8).

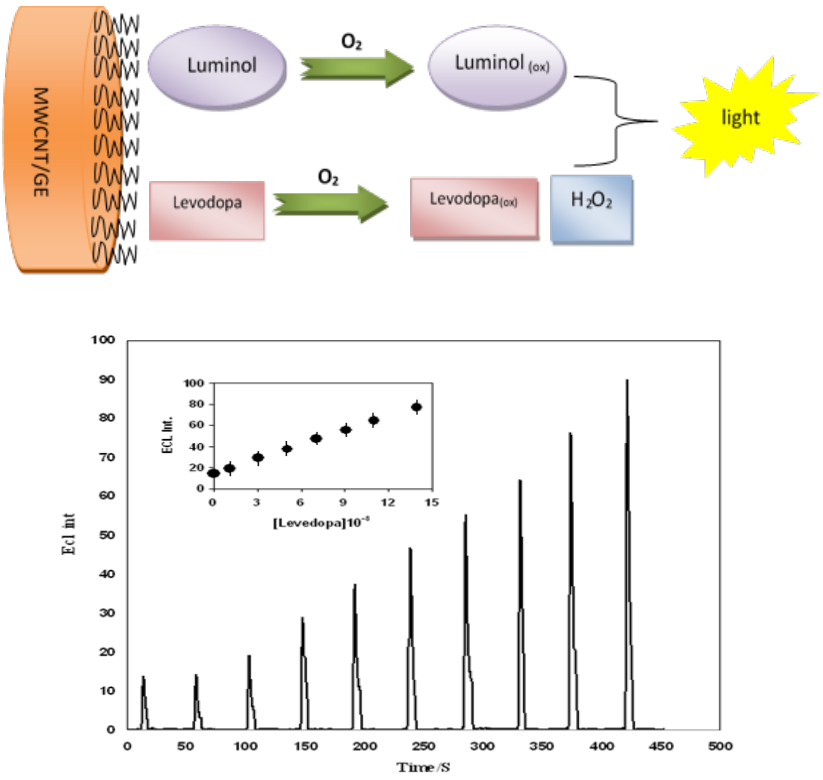


Fig. 7. ECL responses of luminal (100) in the presence of 1.0×10^{-9} M, 1.1×10^{-8} M, 3.1×10^{-8} M, 5.1×10^{-8} M, 7.1×10^{-8} M, 9.1×10^{-8} M, 1.1×10^{-7} M, 1.4×10^{-7} M levedopa, Inset shows linear relationship between the ECL intensity and the concentration of levodopa

The reproducibility of the results was also evaluated using six similar electrodes for the analysis of samples for different days, and the value was found to be 8%. Further using a sensor for 1 h/day (based on an identical protocol according to which after each measurement the electrode was dried and stored under ambient conditions), the proposed sensor was found to be capable of being used for at least 6 weeks, without any considerable changes in its ECL intensity.

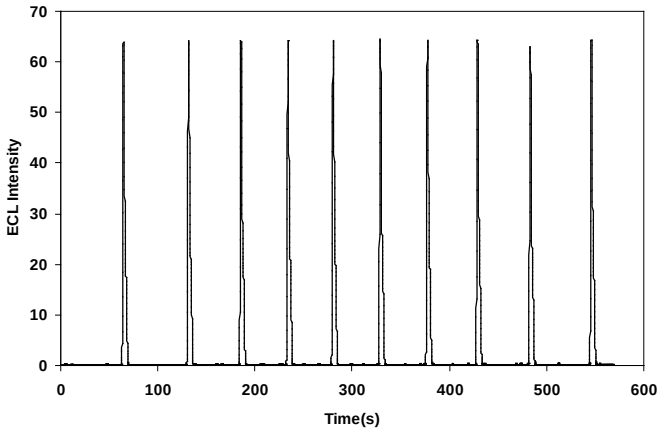


Fig. 8. The successively cyclic ECL responses of the sensor towards 0.1 µM levodopa for 10 times; Conditions: supporting electrolyte, 0.1 M borate buffer (pH= 9); scan rate, $100mVs^{-1}$

3.9. Analytical Application

The proposed and optimized method was also evaluated in the analysis of levedopa in urine samples, through a standard addition approach and the results, presented in Table 1, were found to be in good agreement with the added amounts.

Table 1. The application of proposed method for determination of LOD in urine samples

sample	Spiked(M)	Found [a] (ppm)	Relative error (%)
1	0.03	(0.029±0.003)	5.0
2	0.05	(0.051±0.005)	3.3
3	0.09	(0.088±0.003)	3.3

a: The results are based on five replicate measurements

An electrochemiluminescence, using modified ECL electrode based enhancing the on the weak ECL emission created using the electrochemical oxidization of luminol at a MWCNTs/Nafion-modified gold electrode was developed and optimized. Under optimal conditions, the relative ECL emission signal observed was found to be linearly proportional to the levodopa concentration from 1.0×10^{-9} to 1.7×10^{-7} M and a detection limit of 6.7×10^{-10} M could be reached. The method was also successfully used to the determination of levodopa in urine specimens.

4. CONCLUSION

In this work, describes a novel ECL sensor for levodopa detection with high sensitivity and selectivity. Levedopa, a well-known neurotransmitter agent has been found to have strong ECL activity in the $Ru(bpy)_3^{2+}$/ co-reactant ECL system. Under optimal conditions, the relative ECL emission/levodopa concentration plot was found to be linear from 1.0×10^{-9} to 1.7×10^{-7} M and a detection limit of 6.7×10^{-10} M was recorded. The proposed method was also evaluated in the analysis of levedopa in urine samples.

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