Zinc Layered Hydroxide-Sodium Dodecyl Sulphate-Isoprocarb Modified Multiwalled Carbon Nanotubes as sensor for Electrochemical Determination of Dopamine in Alkaline Medium

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A sensitive and selective electrochemical sensor for detection of dopamine (DOP) using zinc layered hydroxide-sodium dodecyl sulphate-isoprocarb (ZLH-SDS-I)/multiwalled carbon nanotubes (MWCNT) in 100 mM phosphate buffer solution (PBS) and at the pH 8.0 was developed. The morphology and electrochemistry properties of ZLH-SDS-I/MWCNT nanocomposites were characterized using square wave voltammetry (SWV), transmission electron microscopy (TEM), cyclic voltammetry (CV), electrochemical impedence spectroscopy (EIS) and scanning electron microscopy (SEM). Under optimized conditions, the linear analytical curve for determination of dopamine was 1.0 µM to 0.3 mM with the detection limit of 0.43 µM. Besides that, the proposed sensor also exhibited good selectivity, stability, sensitivity and was found to be applicable for dopamine detection in the commercial dopamine hydrochloride injection samples.

1. INTRODUCTION

3,4-dihydroxyphenylethylamine or also known as dopamine (DOP) is an important catecholamine neurotransmitters that play significant roles in the cardiovascular, central nervous and hormonal systems [1-3]. Normal level of DOP in human blood is approximately 10.0 nM to 1.0 µM [4]. Low level of DOP will cause neurodegenerative disorders such as Parkinson, schizophrenia and Alzheimer's diseases while high level of DOP often cause pleasurable and sometimes even euphoria [5-7]. Therefore, the development of a sensitive and simple determination of DOP oxidation for early diagnosis of various neurological disorders has received a great attention. In recent years, various approaches including fluorimetry [8], high performance liquid chromatography (HPLC) [9,10], chemiluminescence [11] and capillary electrophoresis [12] have been used for determination of DOP. Consequently, these methods have some limitations such as time consuming treatment process, complicated sample preparation, expensive solvent or equipments and consumption of environmental unfriendly solvents [13]. Compared with methods listed above, electrochemical techniques have gain considerable attention due to time effectiveness, simplicity, rapid detection, low cost and high sensitivity [14,15].

However, the direct detection of DOP using conventional electrode generally gives the poor results due to the fouling of the electrode surface by the products' oxidation. To overcome this problem, various nanomaterials have been applied to enhance the selectivity and sensitivity of the electrode. Carbon nanotubes (CNT) became one of the most popular and extensively used in electrode due to their extraordinary and specific properties including larger surface area, high stability and superb electrocatalytic activity [16-20].

To date, there have been no reports on the using of ZLH-SDS-I/MWCNT as a sensor for the electrochemical determination of DOP. Herein, a simple approach for the fabrication of ZLH-SDS-I modified MWCNT sensor was developed. The analytical performances were studied by characterizing the sensitivity, selectivity, calibration curve, limit of detection and reproducibility of the sensor.

2. EXPERIMENTAL

2.1. Reagents

MWCNT (diameter: 15nm and purity: 99.9%) was purchased from Timesnano, China. Dopamine hydrocloride (DOP), acetaminophen, KCl were acquired from Sigma-Aldrich, USA. Fructose, sucrose, lactose and ascorbic acid were obtained from Merck, Germany. Phosphate buffer solution (PBS) was employed as supporting electrolyte by mixing the stock solution of 100 mM K_2HPO_4 and 100 mM KH_2PO_4 and then adding NaOH or diluted HCl for adjusting the pH values. Unless otherwise indicated, all reagents were analytical grade without any further purification.

2.2. Instrumentations

Electrochemical impedance spectroscopy (EIS) and Cyclic voltammetry (CV)/square wave voltammetry (SWV) were performed using Galvanostat/Potentiostat model Ref 3000 (USA) and Potentiostat Series-G750 (USA), respectively. Surface morphology of the ZLH-SDS-I and MWCNT were carried out on the FESEM model SU8020 UHR from Hitachi, Japan. Electrochemical measurements were obtained using a standard of three electrode cells with an Ag/AgCl electrode MF-2052 (Bioanalytical system, USA) as a reference electrode, a platinum wire as a counter electrode and the ZLH-SDS-I/MWCNT or bare MWCNT paste electrodes as a working electrode.

2.3. Fabrication of the ZLH-SDS-I/MWCNT

The ZLH-SDS-I nanocomposites were synthesized similar to the method reported in the literature [21]. The ZLH-SDS-I/MWCNT sensor was fabricated by mixing 95 mg of MWCNT and 5 mg of the ZLH-SDS-I with 3 drops of paraffin oil and ground carefully until paste was homogenized. Then, the prepared carbon paste was packed firmly into Teflon tube and then polished on a weighing paper. One of the end of the sensor was inserted with copper wire to establish the electrical contact. For comparison purpose, the sensor of bare MWCNT was prepared according to the same procedure without adding of the ZLH-SDS-I.

2.4. General measurement procedure

The electrochemical behaviour of DOP at the sensors were performed from - 0.2 to + 0.4 V using SWV with conditions as follows: frequency = 30 Hz; step increment = 1 mV and pulse height = 60 mV. All electrochemical experiments were carried out in 100 mM PBS (pH 8.0) as a supporting electrolyte and at 24 ± 1 °C, unless otherwise stated. The study of EIS was conducted in 4 mM $[Fe(CN)_6]^{3-}$ containing 100 mM KCl. The data for optimization of experiments variables and calibration curve were the mean of three measurements.

3. RESULTS AND DISCUSSION

3.1. Characterization of the ZLH-SDS-I/MWCNT

The MWCNT and ZLH-SDS-I surface morphology were characterized by TEM and SEM. Fig. 1 (A) shows clearly the top view images of the ZLH-SDS-I as an irregularly flakes. The ZLH-SDS-I was distributed uniformly onto MWCNT indicating that ZLH-SDS-I was successfully modified with MWCNT as shown in Fig. 1 (B) and (C), which could significantly enhance the surface area of the modified electrode that effectively improve the peak currents of DOP oxidation.
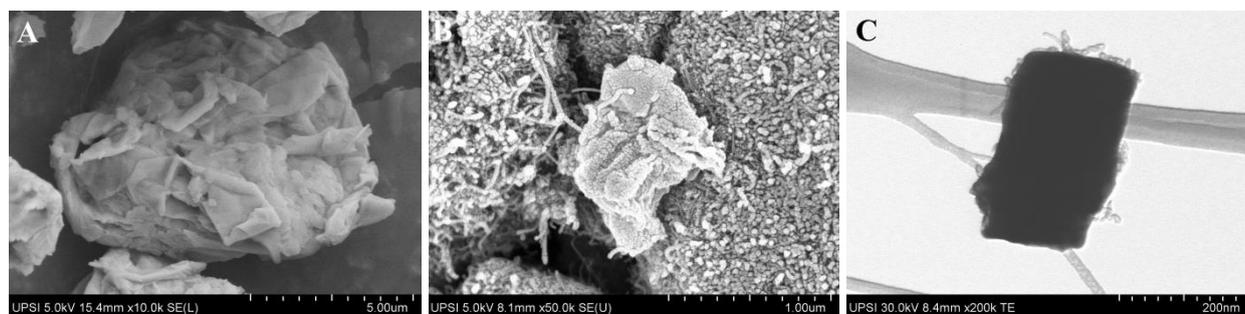
Figure 1. SEM images for (A) ZLH-SDS-I; (B) ZLH-SDS-I/MWCNT and (C) TEM image of the ZLH-SDS-I/MWCNT.

Electrochemical impedance spectroscopy (EIS), a facile and powerful technique has been widely used to study the interface properties of the modified electrodes. The linear part at lower frequency was corresponded to the diffusion process while the diameter of semicircle at higher frequency was related to the electron charge transfer resistance (R_{ct}) [22]. This study was carried out in 4 mM $[Fe(CN)_6]^{3-}$ consisting 100 mM KCl and the Nyquist plot recorded for the bare MWCNT and ZLH-SDS-I/MWCNT is illustrated in Fig. 2. By fitting the Randle's equivalent electrical circuit system (inset of Fig. 2), R_{ct} value of the bare MWCNT was 6.121 kΩ. Compared to the bare MWCNT, the R_{ct} value of ZLH-SDS-I/MWCNT was dramatically decreased to 2.858 kΩ indicating that the introduction ZLH-SDS-I into MWCNT improved the conductivity by facilitating the rate of electron transfer.
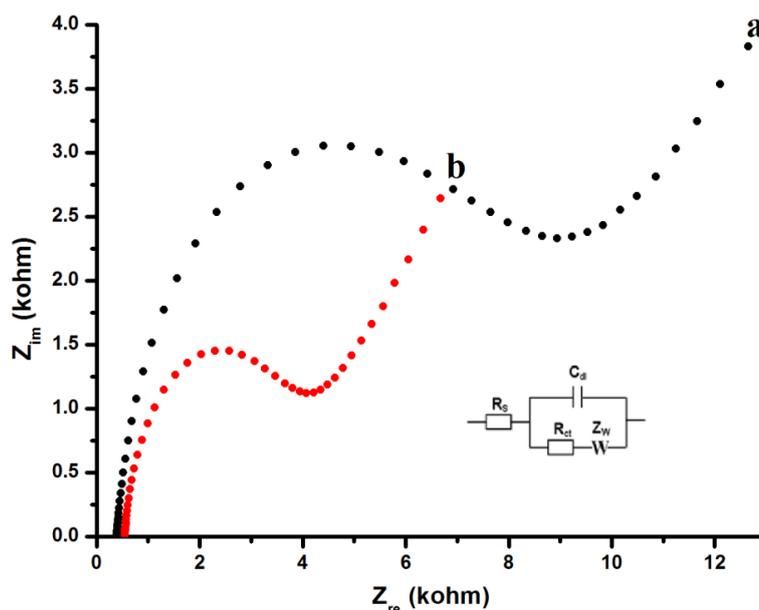
**Figure 2.** Nyquist plots of the (a) bare MWCNT and (b) ZLH-SDS-I/MWCNT in 4 mM $[Fe(CN)_6]^{3-}$ + 100 mM KCl solution.

3.2. Electrochemical characteristic of the ZLH-SDS-I/MWCNT

To compare electroanalytical performance of the ZLH-SDS-I/MWCNT and bare MWCNT, CV measurements of 0.1 mM DOP were carried out in 100 mM PBS (pH 8.0) and the scan rate of 100 mV

s^{-1}. Cyclic voltammogram in Fig. 3 (A) shows the low peak currents and the peak potential of oxidation (E_{pa}) and reduction (E_{pc}) of DOP at 247.8 mV and 109.9 mV, respectively with high peak to peak separation (ΔE_p = 137.8 mV) for bare MWCNT indicating quasi-reversible electrode process. On ZLH-SDS-I/MWCNT, the peak currents of DOP increased about 2.7 times compared to the bare MWCNT and improved the reversibility of the redox couple of DOP with the smaller peak to peak separation (ΔE_p = 56.0 mV). The results suggested that the ZLH-SDS-I had the ability to accelerate the transfer of electrons between DOP and electrode.

SV voltammogram in Fig. 3 (B) displays the peak current of DOP oxidation on ZLH-SDS-I/MWCNT is higher by the factor of 7 (I_{pa} = 22.56 µA) compared to the peak currents of bare MWCNT (I_{pa} = 3.168 µA). Thus, the electrochemical sensor based on ZLH-SDS-I/MWCNT is an interesting alternative to DOP electroanalysis. SWV appears to be more sensitive for determining DOP than CV so that SWV was used in this work.
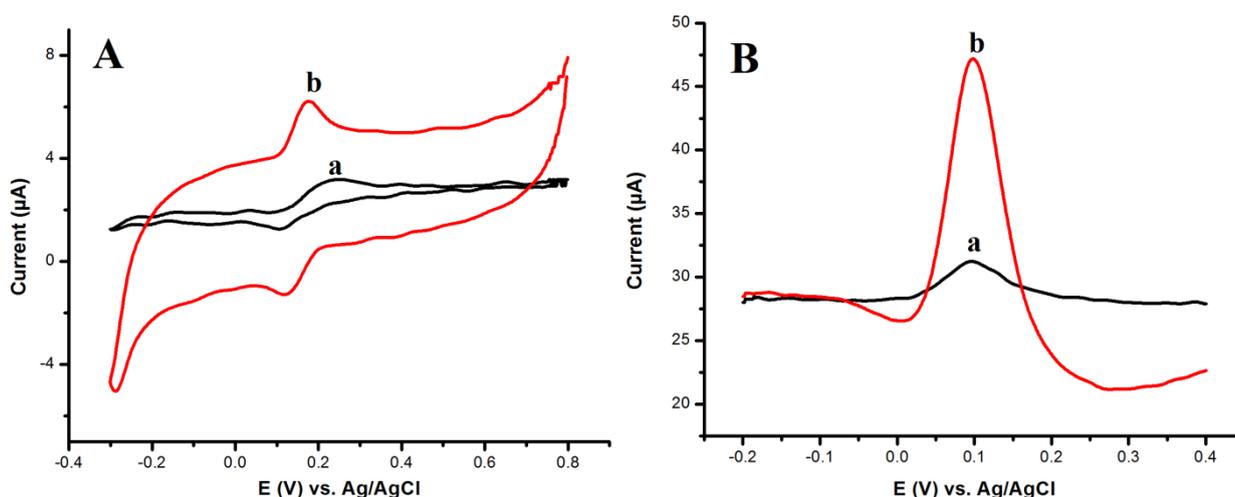
Figure 3. The cyclic voltammogram (A) and SW voltammogram (B) of 0.1 mM DOP were recorded at the (a) bare MWCNT and (b) ZLH-SDS-I/MWCNT in 100 mM PBS at pH 8.0.

3.3. Chronocoulometry study

In order to determine the surface coverage of the ZLH-SDS-I/MWCNT and bare MWCNT, double potentials step chronocoulometry experiment was carried out towards 4 mM [Fe(CN)$_6$]$^{3-}$ consisting 100 mM KCl. The calculation was given by Anson's equation (Eq. 1):

$$Q\,(t) = 2\,n\,F\,A\,C\,D^{1/2}\,t^{1/2}\,/\,\pi^{1/2} + Q_{dl} + Q_{ads} \tag{1}$$

where D = the standard diffusion coefficient of [Fe(CN)$_6$]$^{3-}$ (7.6 × 10^{-6} cm^2 s^{-1} at 25 °C [23]), Q_{dl} = double layer charge which could be eliminated by background subtraction, F = Faraday's constant (96485 C), Q_{ads} = Faradic charge, n = number of electron and C = concentration of the substrate (mol cm^{-3}). From the slope of Q vs. $t^{1/2}$, the effective surface area (A) were 0.0325 and 0.0476 cm^2 for the bare MWCNT and ZLH-SDS-I/MWCNT, respectively indicating the modification of

MWCNT with ZLH-SDS-I improved the effective surface area of the electrode, which then enhanced the current response (Fig. 4A).

The diffusion coefficient (D) and Faradic charge (Q_{ads}) of DOP at the ZLH-SDS-I/MWCNT can be determined by double potentials step chronocoulometry experiment towards 1.0 mM DOP in 100 mM PBS (pH 8.0). From the plot of charge (Q) *vs.* square root of time ($t^{1/2}$), the slope and intercept (Q_{ads}) were found to be 523.8 µC s$^{-1/2}$ and 269 µC, respectively as can be seen in Fig. 4 B. As a result, D was calculated to be 2.55×10^{-3} cm^2 s^{-1}. The adsorption capacity (Γ) of the ZLH-SDS-I/MWCNT can be measured based on the Eq. 2:

$$Q_{ads} = n\,F\,A\,\Gamma \qquad (2)$$

Based on the data obtained, the Γ value of the ZLH-SDS-I/MWCNT was calculated as 29.3 nmol cm^{-2} which suggesting high adsorption capacity for detection of DOP [24].
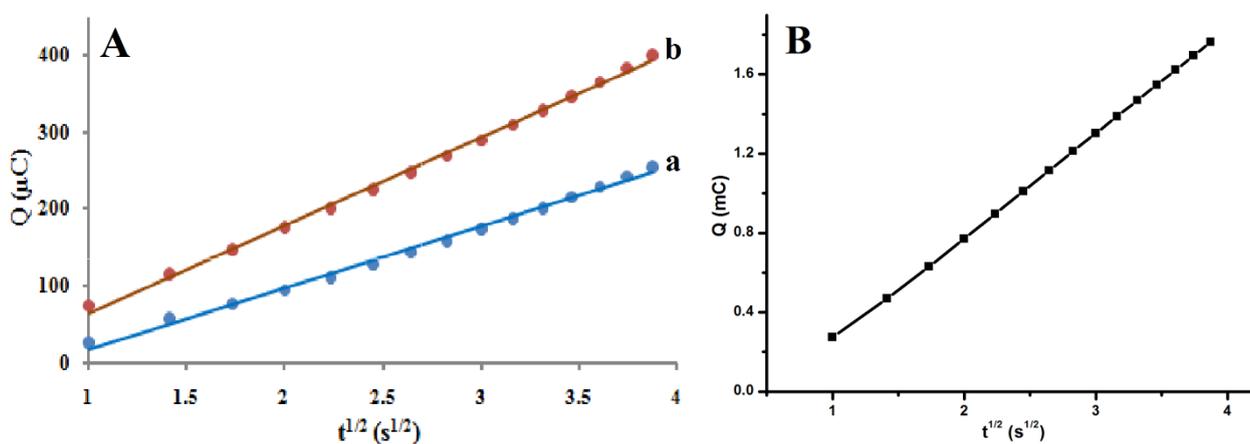

Figure 4. Plot of Q *vs.* $t^{1/2}$ on the (A) bare MWCNT (a) and the ZLH-SDS-I/MWCNT (b) towards 4 mM [Fe(CN)$_6$]$^{3-}$ + 100 mM KCl and (B) the ZLH-SDS-I/MWCNT (b) towards 1.0 mM DOP in 100 mM PBS (pH 8.0).

3.4. The effect of scan rate on DOP peak currents

The effects of scan rate (υ) on the electrochemical behaviours of 1.0 mM DOP in 100 mM PBS (pH 8.0) at the ZLH-SDS-I/MWCNT was investigated in detail to study the reaction kinetics. According to equation of Randles-Sevick, the scan rate is proportional to the peak current. Peak currents of DOP increased gradually with increasing in scan rate (υ) from 10 to 200 mV s^{-1} and shifted to positive in peak potential, indicating kinetic limitation in the reaction as shown in Fig. 5 (A) [25]. In order to determine the process of electrode, the graph of peak currents versus scan rate (υ) was plotted as can be seen in Fig. 5 (B) and it displays straight line with the linear regression equations, Current (I_{pc}) = 0.1046υ + 1.9177 and Current (I_{pa}) = -0.0651υ - 0.2612. The obtained correlation coefficients were 0.9939 and 0.9923, respectively. As can be seen in Fig 5 (C), the plot of peak currents versus

square root of scan rate ($v^{1/2}$) showed a straight line with the linear equations as, Current (I_{pc}) = $1.79v^{1/2}$ - 4.402 (R^2 = 0.9934) and Current (I_{pa}) = $-1.1469v^{1/2}$ + 3.5167 (R^2 = 0.9920). These results suggested that the overall processes were controlled by DOP adsorption on the surface of electrode [26].
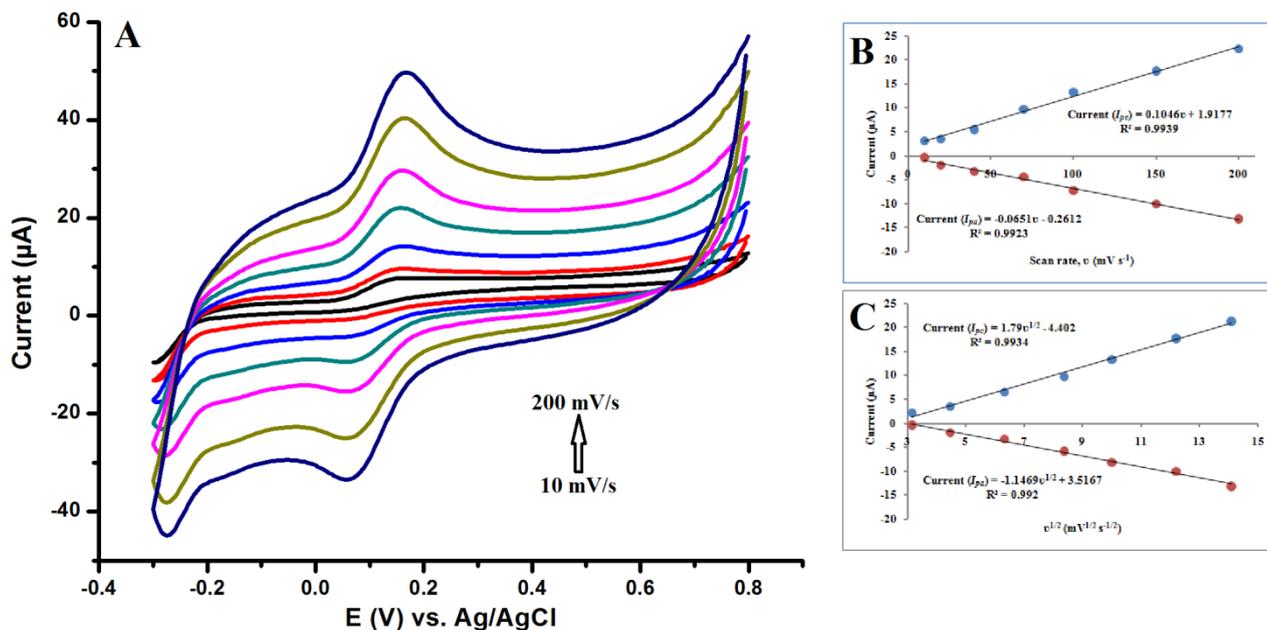
Figure 5. CV of 1.0 mM DOP at the ZLH-SDS-I/MWCNT in 100 mM PBS (pH 8.0) at scan rates of 10, 20, 40, 70, 100, 150 and 200 mV s^{-1} (A), plot of peak currents versus scan rate (B) and the graph of peak currents versus square root of scan rate (C).

3.5. The effect of pH

The effect of pH values of 100 mM PBS towards electrochemical determination of 0.1 mM DOP on the ZLH-SDS-I/MWCNT was determined by SWV in the range of pH 6.0 to 8.5 because PBS is more effective in the pH range of 6.2 to 8.0 [27]. The oxidation of peak current of DOP increased with increasing the pH from 6.0 to 8.0, as can be seen in Fig. 6 (a). Thus, pH 8.0 was chosen as the optimum pH for detection of DOP in this experiment. The effect of pH towards peak potential (E) of DOP was also studied. As can be seen in Fig. 6 (b), the peak potential of DOP oxidation negatively shifted with the increasing of pH buffer indicating involvement of protons in the oxidation of DOP, and the dependence of peak potential of DOP towards pH can be expressed as E_p = -0.0572 pH + 0.6148 (R^2 = 0.9965) suggesting the same number of electrons and protons involved in the reaction of DOP due to the close slope of 57.2 mV pH^{-1} compared to the 59 mV pH^{-1} (Nernst value).
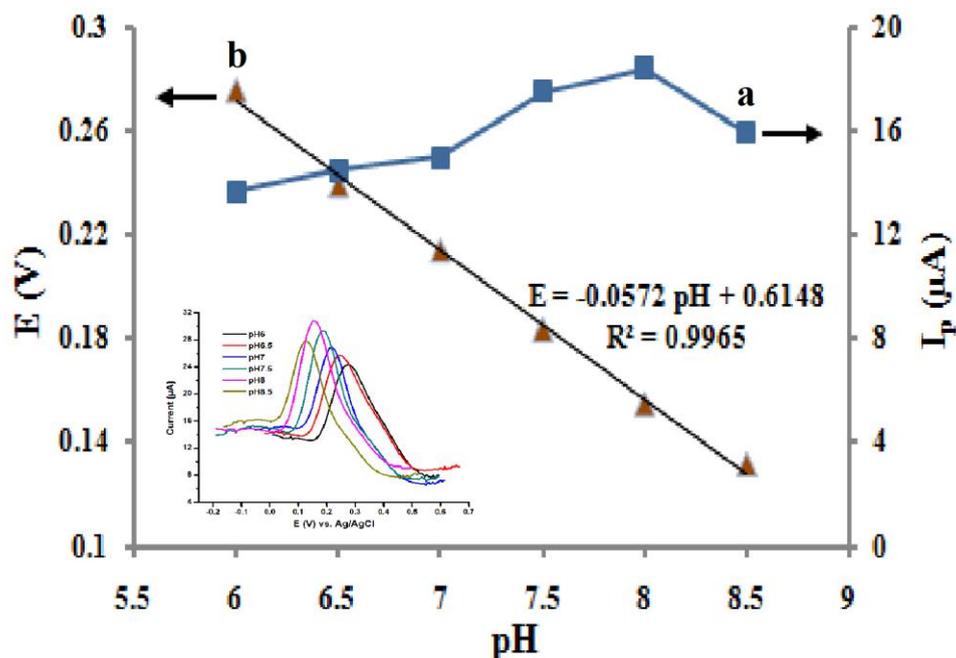
Figure 6. Effect of pH towards peak current (a) and peak potential (b) for determination of DOP at the ZLH-SDS-I/MWCNT (Inset: SW voltammogram at differents pH).

3.6. Calibration curve and limit of detection

In order to get the best sensitivity in determination of DOP oxidation, the specific conditions of SWV parameters' (frequency: 30 Hz; pulse size: 60 mV; step size: 1 mV) were selected. The electrocatalytic oxidation of DOP was studied on the ZLH-SDS-I/MWCNT by varying its concentration. The plot of peak current (µA) *vs.* -Log [DOP] as shown in Fig. 7 shows linear relationship in the DOP working range between 1.0 µM to 0.3 mM with linear regression equation as Current (µA) = -10.898 (-Log [DOP]) + 67.083 (R^2 = 0.9971). The limit of detection (LOD) was estimated to be 0.43 µM which was calculated by using Eq. (2):

$$LOD = 3\sigma / m \tag{2}$$

where, m = slope obtained from calibration curve and σ = standard deviation of five blank measurements. The result reveals that the ZLH-SDS-I/MWCNT showed relatively wider calibration curve with lower limit of detection compared with several electroanalytical methods for determination of DOP as presented in Table 1.
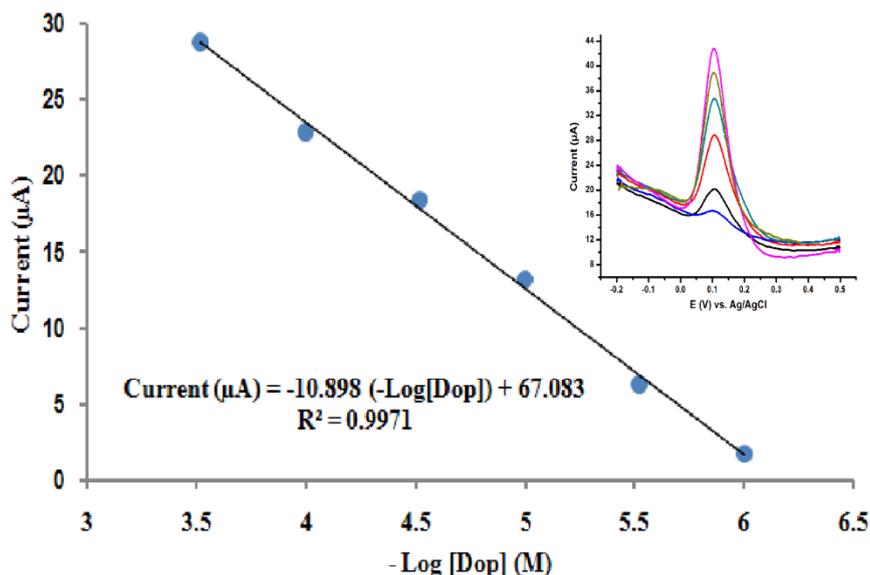
Figure 7. Calibration curve of DOP. Inset shows the SW Voltammogram responses to the different concentrations of DOP.

3.7. Reproducibility and stability of the ZLH-SDS-I/MWCNT

Table 1. Comparison of analytical performance of the fabricated electrode in the electrocatalysis of DOP.

Electrode / Modifier	Method	Calibration curve (μM)	LOD (μM)	Ref.
Ni/C/GCE	DPV	1.0 – 55.0	0.05	[28]
CuO NPlts/Nf /GCE	DPV	10.0 – 80.0	8.25	[29]
SDS/CPE	CV	1.0 – 1000.0	0.1	[30]
$(P_2W_{16}V_2$-AuPd/PEI$)_8$	DPV	2.1 – 2060	0.83	[31]
PG/GCE	CV	5.0 – 710	2.0	[32]
CeO$_2$/Au/GCE	CV	10.0 – 500.0	0.056	[33]
[Co(phen)$_3$]$^{2+}$/MWCNTs/GCE	Amp*	5.0 – 453.0	1.76	[34]
AuNCs/AGR/MWCNT/GCE	CV	1.0 – 210.0	0.08	[35]
3DGH-Fc/GCE	DPV	10.0 – 180.0	0.042	[36]
ZLH-SDS-I/MWCNT/CPE	SWV	1.0 – 300.0	0.43	This work

*Amp: Amperometry

The reproducibility of the ZLH-SDS-I/MWCNT was examined over three electrodes fabricated with the same procedure. The relative standard deviation (RSD) of these three electrodes obtained was 5.83%. In addition, 10 successive measurements with a similar electrode yielded 3.89% of RSD. The stability of the ZLH-SDS-I/MWCNT that was stored at room temperature was tested towards 0.1 mM DOP in 100 mM PBS (pH8.0) every 5 days in one month. After a month, the ZLH-SDS-I/MWCNT maintaining about 90% from its initial value which demonstrates highly stable over a long period [37]. These results suggesting that the ZLH-SDS-I/MWCNT has good stability and reproducibility, making it attractive for practical applications.

3.8. Interference study of the ZLH-SDS-I/MWCNT

The effects of foreign substances such as K^+, Mg^{2+}, NO_3^-, SO_4^{2-}, Cl^-, glucose, ascorbic acid (AA), , fructose, *L*-lysine and uric acid (UA) on the determination of 0.1 mM DOP were investigated by SWV. Tolerance limit of below than 10% was selected as a maximum concentration of foreign substances that caused relative error on detection of DOP. As can be seen in Fig. 8, 50-fold of all substances did not interfere the detection of DOP suggesting that the ZLH-SDS-I/MWCNT has excellent selectivity.
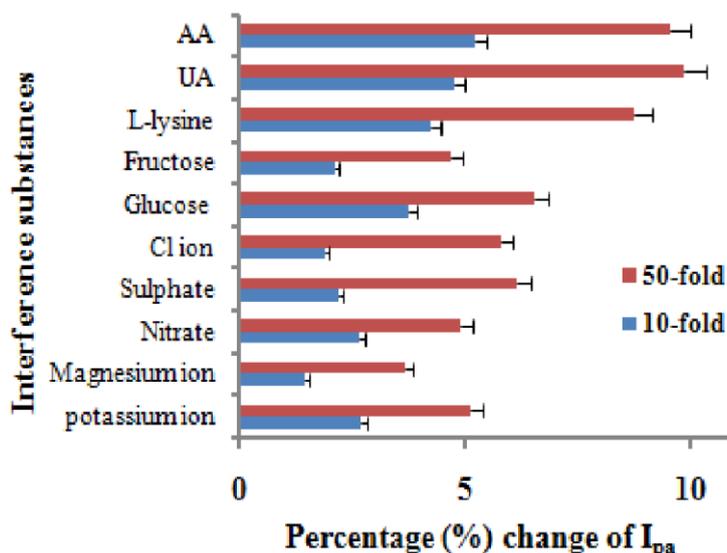
Figure 8. The influence of various interfering substances to 0.1 mM DOP at the ZLH-SDS-I/MWCNT.

3.9. Analytical applications

Table 2. The detection of DOP in sample of dopamine hydrochloride injection.

No.	Sample (µM)	Added (µM)	Detected (µM)	Recovery (%)
1	21.5	0.00	21.1	98.14
2	21.5	50.0	73.1	102.2
3	21.5	100.0	119.3	98.19

Under optimum experimental conditions, the DOP content in dopamine hydrochloride injection was examined by SWV at the ZLH-SDS-I/MWCNT to evaluate the reliability and potential application. The standard addition method was used and the results obtained are shown in Table 2. The results displayed satisfactory recovery with the range of 98.14% to 102.2%, which suggesting the ZLH-SDS-I/MWCNT can be efficiently applied for detection of DOP.

4. CONCLUSION

This work presents a novel and sensitive electrochemical sensor combining high catalytic activity of the ZLH-SDS-I nanocomposites and excellent electrical conductivity of MWCNT for determination of DOP. It also offers a platform for applying the ZLH-SDS-I/MWCNT as an alternative modifier for the DOP analysis.

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