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# Electrochemical determination of serotonin in urine samples based on metal oxide nanoparticles/MWCNT on modified glassy carbon electrode

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## ABSTRACT

The electrochemical response of serotonin on the modified electrode based on multiwalled-carbon-nanotube (MWCNT) doped respectively with nickel, zinc and iron oxide nanoparticles coating on glassy carbon electrode (GCE) at physiological pH 7 was determined using cyclic voltammetry (CV) and square wave voltammetry (SWV). The modified GCE/MWCNT-metal oxide electrodes exhibited excellent electrocatalytic activity towards the detection of serotonin at large peak current and lower oxidation potentials compared to other electrodes investigated. The dynamic range for the serotonin determination was between  $5.98 \times 10^{-3} \mu\text{M}$  to  $62.8 \mu\text{M}$  with detection limits 118, 129 and 166 nM for GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> sensors respectively. GCE-MWCNT-NiO was the best electrode in terms of serotonin current response, electrode stability, resistance to fouling and limit of detection towards the analyte. The developed sensors were found to be electrochemically stable, reusable, economically effective due to their extremely low operational cost, and have demonstrated good limit of detection, sensitivity and selectivity towards serotonin determination in urine samples.

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## 1. Introduction

It is an established fact that carbon nanotubes possess unique structural, electronic and mechanical properties that make them to be useful for very attractive and wide range of applications. They have high capacitive ability to promote electron - transfer in a reaction when used as electrodes due to the nanometer dimensions, electronic structure and topological defects present on the tube surfaces [1–5]. Based on these outstanding properties of CNT, they have been widely and successfully used in preparation of chemical or electrochemical sensors [6–9]. On the other hand, CNT with different functionalities has also been used for detection of biological analytes such as glucose [10], reduction of peroxide [11], and oxidation of hydrazine [12]. Studies also showed that fabrication of electrodes with functionalized carbon nanotubes exhibited huge capacitive-current in some electrolytes [13,14].

5-Hydroxytryptamine (serotonin (SE)) is an important and major biogenic monoamine neurotransmitter as well as neuromodulator [15], which are largely distributed in human brain. 5-Hydroxytryptamine (serotonin (SE)) makes an important contribution

in wide variety of biological, physical, psychopathological processes including sleep regulation, depression, eating disorder, alcoholism, infantile autism, anxiety disorders, muscle contraction, liver regeneration, endocrine regulation, obsessive-compulsive disorder and psychosis [16,17]. Therefore on this note the determination of SE is instructive in the diagnosis of various diseases and hence makes it the subject of biologically and pharmacologically oriented research [18]. To date, a variety of analytical methods such as spectrophotometry [19], fluorometry [20–22], enzyme immunoassay [23,24], radioimmunoassay [25], capillary electrophoresis (CE) [26] and GC-MS [27], have been developed for the determination of serotonin. Serotonin is usually assayed by HPLC with either electrochemical or fluorometry detection [28,29]. However, these reported techniques are expensive and require time-consuming derivatization steps, and also in some cases low sensitivity and selectivity makes them unsuitable for a routine analysis coupled with the fact that the concentration of SE is very low in biological systems. The other problem which must be solved in the electrochemical detection of SE is the co-existence of many interfering compounds in biological systems. Among these interfering compounds, ascorbic acid (AA), and dopamine (DA) are particularly important because they can all be oxidized at similar potentials resulting in overlap of voltammetric responses. On unmodified glassy carbon electrodes (using Ag/AgCl, sat'd KCl as reference electrode), the oxidation potential of SE (0.38 V at pH 7) is close to that of DA (0.22 V) and AA (0.2 V). To overcome

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these problems, one of the most effective ways to improve the measuring sensitivity of serotonin (SE) detection and remove the interference of ascorbic acid (AA) and dopamine (DA) is using a modified electrode.

There are several literatures on the electro-oxidation of serotonin on modified electrodes [30–36]. For example, Goyal et al. developed a gold nanoparticles modified ITO (Au/ITO) electrode for the successful detection of dopamine and serotonin in the presence of a high concentration of ascorbic acid by using electrochemical method [30]. Wu et al. reported a chemically modified electrode based on the carbon nanotube film-coated glassy carbon electrode (GCE) for the simultaneous determination of dopamine (DA) and serotonin (5-HT) at lower oxidation over potentials [31].

Despite this huge literature, the use of carbon nanotubes decorated with metal oxide nanoparticle for electrochemical detection of serotonin is limited. To date there is no report on the comparative comprehensive study detailing the extent of electron transport of the different metal oxides (NiO, ZnO and Fe<sub>3</sub>O<sub>4</sub>) and their interaction with serotonin at physiological pH as reported in this study.

In this work, we developed and studied electrochemical sensors for the detection of serotonin (SE) in the presence of DA and AA using multiwalled carbon nanotubes doped with nickel, zinc and iron oxides nanoparticles. The synthesized nano materials were characterized with field emission scanning electron microscope (FESEM), transmission electron microscopy (TEM), fluorescence, cyclic voltammetry and electrochemical impedance spectroscopy (EIS) techniques. GCE-MWCNT-NiO, GCE-MWCNT-ZnO and GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites modified electrodes demonstrated faster electron transport properties in ferricyanide probe compared with other fabricated sensors investigated. The sensors electrocatalytic behaviours towards SE oxidation were evaluated by cyclic voltammetry and square wave voltammetry. The GCE-MWCNT-MO electrodes exhibit excellent electrocatalytic activity towards the electrochemical oxidation of serotonin with GCE-MWCNT-NiO demonstrating the best sensing properties. The proposed serotonin sensors have proved to be economically effective due to their extremely low operational cost, and have demonstrated good limit of detection, sensitivity and selectivity towards serotonin determination.

## 2. Experimental

### 2.1. Reagents and materials

All chemicals used were of analytical-reagent grade. Serotonin hydrochloride, ascorbic acid and dopamine hydrochloride were obtained from Sigma Aldrich and they were all used as received. Solutions were prepared using distilled-deionised water. All electrochemical measurements were performed at ambient temperature.

### 2.2. Equipment and procedure

The following characterization techniques were used for the synthesized nanocomposites: Field emission scanning electron microscopy (FESEM) images were obtained from JEOL JSM 5800 LV (Japan) while the energy dispersive X-ray spectra (EDX) were obtained from NORAN VANTAGE EDX (USA). TEM experiment was performed using a Model JEOL JEM-2100F field emission transmission electron microscope, Tokyo (Japan). Fluorescence spectra were measured on full automatic microplate reader MB-580, Heales at excitation wavelengths of 450 and 492 nm. Electrochemical experiments were carried out using an AUTOLAB Potentiostat PGSTAT 302 (Eco Chemie, Utrecht, and The Netherlands) driven by the GPES software version 4.9. Electrochemical impedance spectroscopy (EIS) measurements were performed with an AUTOLAB frequency response analyser (FRA) software between 1.0 Hz and 10 kHz using a 5 mV rms sinusoidal modulation with a solution of 5 mM of K<sub>4</sub>Fe(CN)<sub>6</sub> and a 5 mM K<sub>3</sub>Fe(CN)<sub>6</sub> (1:1) mixture in phosphate buffer solution (PBS) of pH 7.0 and at the E<sub>1/2</sub> of the [Fe(CN)<sub>6</sub>]<sup>4−</sup>/[Fe(CN)<sub>6</sub>]<sup>3−</sup> (0.3 V vs. Ag/AgCl in sat'd KCl). Bare or modified glassy

carbon electrode (GCE) disk (*d* = 3.0 mm in Teflon) was used as the working electrode and Ag/AgCl, sat'd KCl as reference electrode. All solutions were de-aerated by bubbling nitrogen gas prior to each electrochemical experiment. Experiments were performed at 25 ± 1 °C.

### 2.3. Electrode modification procedure

Before each experiment, the working electrode was polished with slurry containing 0.3 μm and then with 0.05 μm sized aluminum oxide particles for 5 min. After each treatment, the electrode was washed and ultra sonicated in distilled water for 5 min to remove retained aluminum oxide particles on the electrode surface. GCE/MO, GCE/MWCNT and GCE/MWCNT-MO were prepared by a drop-dry method. About 20 μL drop of MO, MWCNT and MWCNT-MO (2 mg acidified MWCNTs and 2.5 mg MO respectively mixed and sonicated in 1 mL DMF) were dropped on the bare GCE and dried in an oven at 50 °C for about 2 min [37,38] to give GCE/MWCNT-NiO, GCE/MWCNT-ZnO, GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes. Other developed sensors are GCE/MWCNT, GCE/NiO, GCE/ZnO and GCE/Fe<sub>3</sub>O<sub>4</sub>.

### 2.4. Real sample analysis

The real sample analysis for the detection of serotonin was conducted as follows: Urine samples were collected from laboratory personnel in clean vials and preserved in the refrigerator at 4 °C in readiness for estimation of serotonin in the samples. The samples were diluted 200 times with 0.1 M phosphate buffer of pH 7.0 prior to analysis in order to reduce matrix complexity. Diluted urine samples were then spiked with different amounts of SE and the amounts of the analyte at GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> were determined without any further treatment. All samples and experiment were conducted at room temperature.

## 3. Result and discussion

### 3.1. Characterization of nanocomposite with SEM and TEM

The surface morphology of the material using scanning electron microscope (SEM) and transmission electron microscope (TEM) have been reported [39]. Unlike the TEM micrograph of the MWCNT, which gave a well dispersed and de-bundled nanotubes due to the repulsion between negative charges generated through the carboxyl moieties, the TEM images of the metal oxide (MO) nanoparticles were porous, crystalline and somewhat aggregated, possibly due to ineffective dispersing capability of the solvent [39]. The TEM images of MWCNT-MO nanocomposite (not shown) showed formation of clusters of metal oxides around the nanotubes which can be attributed to the strong ionic interaction between the metal oxide nanoparticles and the MWCNT. The average particle size distribution of the MO and MWCNT-MO nanocomposite from the TEM image was estimated to be 10–50 nm. The SEM images indicate the formation of thick film structure of MWCNT-NiO, MWCNT-ZnO and MWCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites with some form of particles agglomeration observed for MWCNT-NiO and MWCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites [39].

### 3.2. Characterization of nanocomposite with EDX

EDX spectra Fig. 1 gives insights to the elemental compositions of nanocomposite formed when the MWCNT was doped with nickel, zinc and iron oxide nanoparticles respectively. The EDX profile of MWCNT Fig. 1(i) was purely carbon (~100%) as would be expected for pure MWCNT. However, after doping with the MO nanoparticle, the prominent peaks of nickel, zinc, iron and oxygen proves the successful doping of MWCNT with the NiO, ZnO and Fe<sub>3</sub>O<sub>4</sub> nanoparticles Fig. 1(ii–iv). In addition, the atomic ratio of Fe to O obtained for the EDX analysis of MWCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite was approximately 3:4

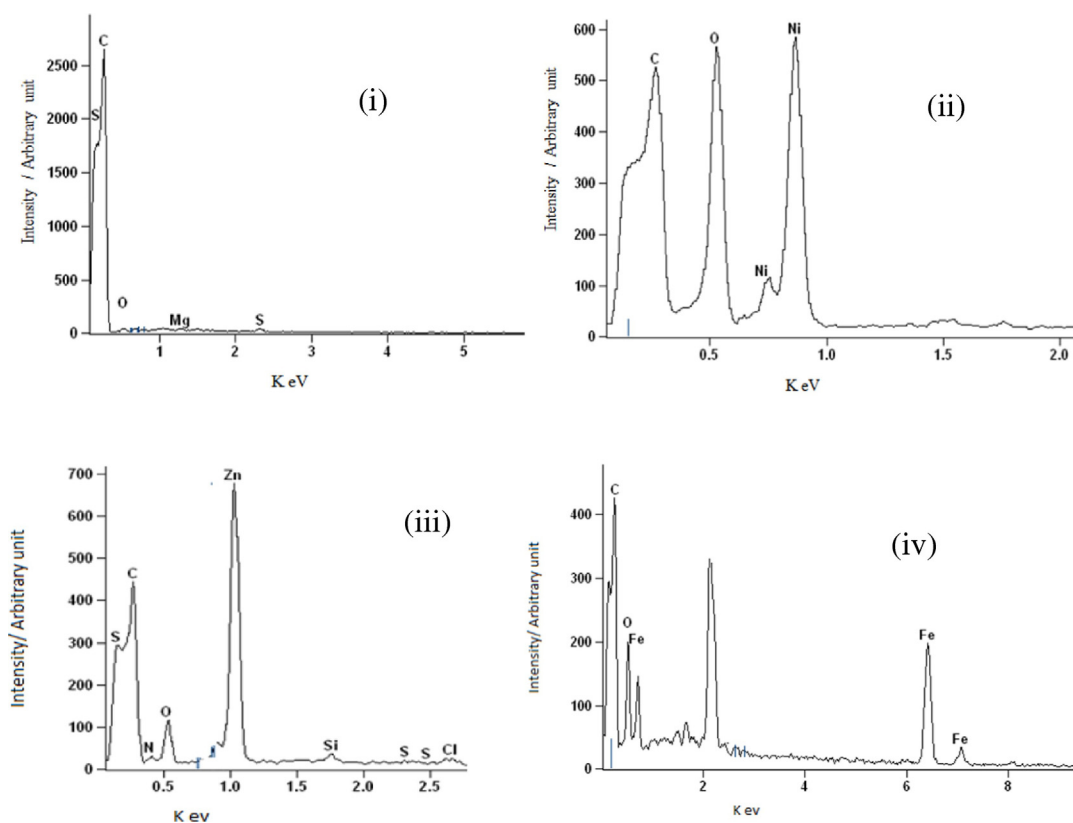


Fig. 1. Typical EDX spectra of (i) MWCNT (ii) MWCNT-NiO (iii) MWCNT-ZnO and (iv) MWCNT-Fe<sub>3</sub>O<sub>4</sub> nanoparticle.

which further confirms the presence of Fe<sub>3</sub>O<sub>4</sub> nanoparticles in the nanocomposite.

### 3.3. Fluorescence spectroscopy

Fluorescence occurs when a molecule absorbs light photons from the UV-visible light spectrum (200–900 nm), known as excitation, and then rapidly emits light photons as it returns to its ground state. The intensity of the fluorescence collected directly corresponds to the magnitude of electron-hole pair recombination, in which lower fluorescence intensity indicates better photoreactivity with less de-excitation of electron-hole pairs. In order to probe the photoexcited-state interactions between the metal oxides nanoparticles and the MWCNTs, the steady state fluorescence spectra of MWCNT, NiO, ZnO, Fe<sub>3</sub>O<sub>4</sub>, MWCNT-NiO, MWCNT-ZnO and MWCNT-Fe<sub>3</sub>O<sub>4</sub> were measured at the same wavelength  $\lambda = 450$  and 492 nm. The result is presented in Fig. 2. From Fig. 2, following excitation, emission intensity of the MWCNT-MO nanocomposites (where MO: NiO, ZnO and Fe<sub>3</sub>O<sub>4</sub>) decreased compared to the MO at equivalent absorption therefore suggesting that MWCNT-MO nanocomposites undergoes fluorescence quenching which is indicative of photoinduced electron/energy transfer from MO to MWCNT [40,41]. Thus, the MWCNT-MO could be potential photocatalyst for photochemical reactions. The enhancement in photocatalytic activity of the MWCNT-MO nanocomposites over MO or MWCNT alone is attributed to synergy between the two conducting nanomaterials (MWCNT and MO), and the excellent electronic property of MWNTs. This is because MWNTs can capture the photon-excited electrons from the conduction band of the metal oxides nanoparticles because of its large electron-storage capacity.

### 3.4. Electrochemical characterization of modified electrodes

Comparative current response of the bare GCE and GCE/MWCNT, GCE/NiO, GCE/ZnO, GCE/Fe<sub>3</sub>O<sub>4</sub>, GCE/MWCNT-NiO, GCE/MWCNT-ZnO,

GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrodes in 0.1 M PBS (pH 7.0) containing 5 mM [Fe(CN)<sub>6</sub>]<sup>4-</sup>/[Fe(CN)<sub>6</sub>]<sup>3-</sup> was investigated using cyclic voltammetry technique as reported in our earlier study [39]. The study showed that the GCE/MWCNT-MO modified electrodes gave a higher current response compared with the bare GCE, GCE/MWCNT, GCE/NiO, GCE/ZnO and GCE/Fe<sub>3</sub>O<sub>4</sub> electrodes, which agreed with similar study in literature [39]. The remarkable enhancement in the peak current at the GCE/MWCNT-MO modified electrodes is a clear evidence of the high electron transfer properties of the MWCNT in the nanocomposite [42,43]. Using the Randles-Sevcik equation, the areas of the modified electrodes were calculated to be 1.88, 1.5 and 0.5 cm<sup>2</sup> for GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> respectively which are about 7–27 times higher than the bare GCE geometric area (0.07 cm<sup>2</sup>). Modification of bare electrodes therefore increase the surface area and electroactive properties of the electrode as also reported in other studies in literature [44–46].

### 3.5. Effect of pH on the electrochemical detection of serotonin

The influence of buffer pH on the peak current and peak potential of serotonin detection was investigated using GCE/MWCNT-NiO modified electrode at scan rate 10 and 25 mV s<sup>-1</sup>. In Fig. 3(a–c), it was observed that the peak current of serotonin decreased with the increase of pH, while a maximum value was obtained at pH 7.0 and at scan rate 25 mV s<sup>-1</sup>. Therefore, the subsequent experiments were performed using 0.1 mM SE prepared in 0.1 M PBS of pH 7.0 and scan rate 25 mV s<sup>-1</sup>.

The oxidation peak potential of serotonin shifted to negative with the increase of pH (Fig. 3c) which indicates that protons take part in the electrode reaction. The regression equation for the GCE/MWCNT-NiO at 25 mV s<sup>-1</sup> is  $E \text{ (V)} = -0.051 \text{ pH} + 0.74$  with a correlation coefficient of 0.9979. The slope value is close to the Nernstian theoretical value of 59 mV which suggest that the electrochemical detection of

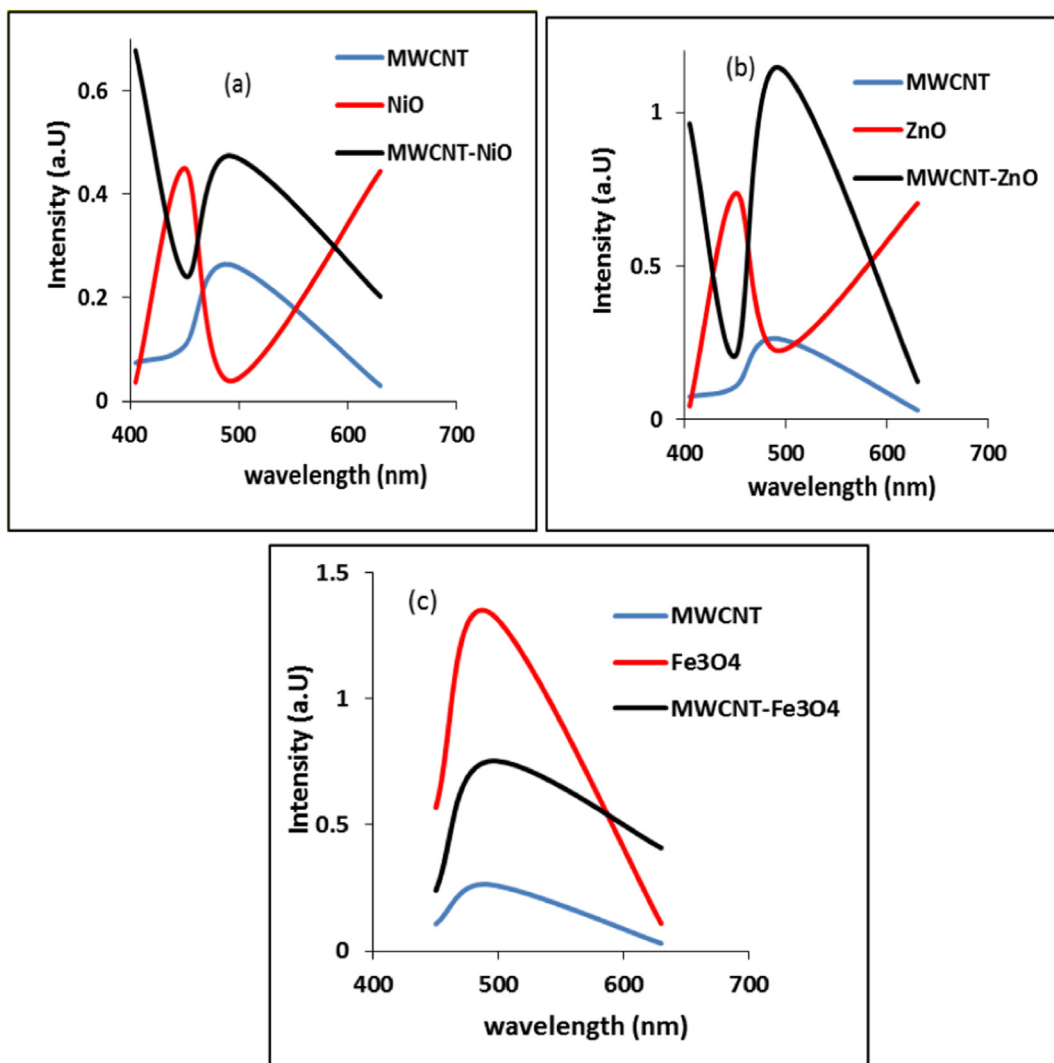


Fig. 2. Fluorescence spectra of MWCNT and (a) NiO, MWCNT-NiO (b) ZnO, MWCNT-ZnO and (c) Fe<sub>3</sub>O<sub>4</sub>, MWCNT-Fe<sub>3</sub>O<sub>4</sub>.

serotonin at GCE/MWCNT-MO modified electrode is a two-electron transfer process coupled to two-proton transfer steps [47].

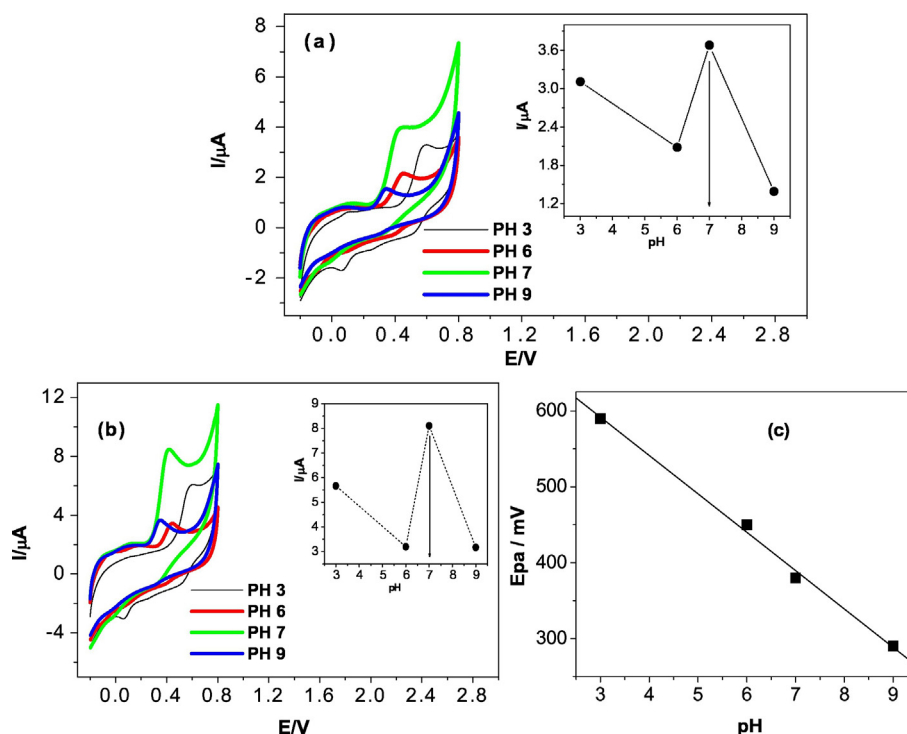
### 3.6. Electrocatalytic response of the modified electrodes towards the oxidation of serotonin (SE)

The electrochemical behaviour of different modified electrodes in the presence of 0.1 mM SE in 0.1 M PBS (pH 7.0) was investigated by CV technique at a scan rate of 25 mV s<sup>-1</sup> and presented in Fig. 4. All the electrodes showed well defined serotonin oxidation peaks at approximate potential of 0.35 V. From the CV obtained, serotonin oxidation current (and oxidation potential) are 4  $\mu$ A (0.38 V) at the bare GCE, 5.8  $\mu$ A (0.36 V) at GCE/NiO, 5  $\mu$ A (0.38 V) at GCE/ZnO and 5  $\mu$ A (0.38 V) at GCE/Fe<sub>3</sub>O<sub>4</sub> electrodes respectively. The results clearly indicate that there is no significant difference in SE oxidation current at bare GCE electrode compared with the GCE/MO modified electrodes. However the GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> showed higher current response (at around 0.38 V) than that of the bare and other modified electrodes and these results indicate that the GCE/MWCNT-MO electrodes have better electrocatalytic activity and demonstrated enhanced current response towards SE Fig. 4(a–c). Another peak was observed at potential around 0.6 V on the modified GCE-MWCNT-NiO and GCE-MWCNT-ZnO electrodes can be attributed to the oxidation of nickel (II) and zinc (II) ions in their respective

metal oxides nanoparticles present in the nanocomposites of MWCNT-NiO and MWCNT-ZnO respectively. The non-appearance of this peak on GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrode could be attributed to the capacitive nature of the electrode as indicated by its CV (Fig. 4c), thus favouring storage of charges than rapid transfer of charges during oxidation of Fe (II) in the Fe<sub>3</sub>O<sub>4</sub> nanoparticles. After background current subtraction shown in Fig. 4(d), SE current response on the GCE-MWCNT-MO electrodes follow the order: GCE/MWCNT-NiO (309.85  $\mu$ A) > GCE/MWCNT-ZnO (128.53  $\mu$ A) > GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> (118.7  $\mu$ A). These current are in the order of 70, 32 and 30 times higher than SE current at bare GCE electrode. Thus, GCE/MWCNT-NiO electrode demonstrated the best electrocatalytic behaviour towards SE oxidation. Similar results have been obtained and reported in literature for modified electrodes [30–32]. For example, Wu et al. reported simultaneous determination of dopamine (DA) and serotonin (5-HT) at lower oxidation over potentials using carbon nanotube film-coated glassy carbon electrode (GCE) [31].

The enhanced SE current at the nanocomposite modified electrodes can be attributed to the high electrical conductivity of the metal oxide nanoparticles and the MWCNT, large porous surface area created by the MWCNT for easy diffusion of the analyte and electrolyte in and out of the electrode surface, uniform structure of the nanocomposite, improved catalytic properties of the surface electroactive materials due to the synergy between MWCNT and the metal oxide nanoparticles



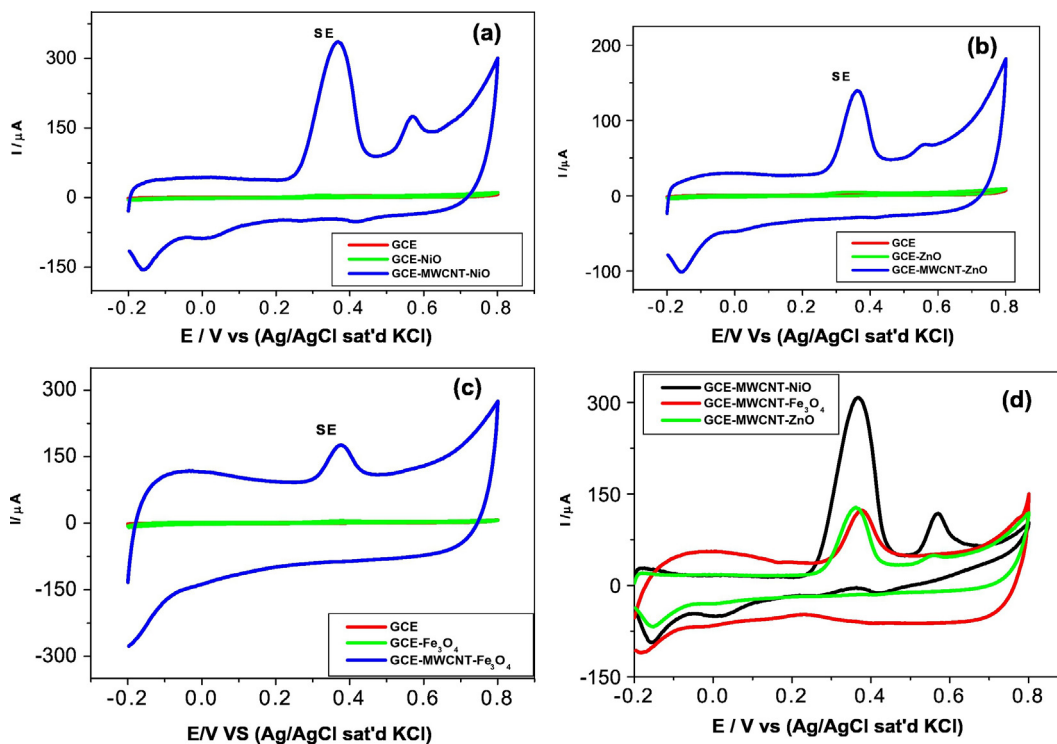


**Fig. 3.** The effect of buffer pH on the peak current of 0.1 mM SE prepared in 0.1 M PBS at pH: 3.0, 5.0, 7.0 and 9.0. (a) Scan rate 10 mV s<sup>-1</sup> and (b) 25 mV s<sup>-1</sup>. Inset: is the relationship between the oxidation peak current and the pH (c) is the relationship between the peak potential and pH at 25 mV s<sup>-1</sup> using GCE/MWCNT-NiO modified electrode.

and biocompatibility of the nanocomposite with the analyte [32–36]. It is believed that the existence of MWCNT is an ideal support material which acts as an effective electron promoter for electrocatalytic oxidation of SE [3,31]. Since GCE/MWCNT-MO electrodes gave better SE

electrochemical response, further studies are carried out using these electrodes platform.

Impedance studies were also carried out to monitor the mechanism of the electron transfer process at the electrode-electrolytes interface



**Fig. 4.** Cyclic voltammograms of GCE and (a) GCE/NiO, GCE/MWCNT-NiO, (b) GCE/ZnO, GCE/MWCNT-ZnO and (c) GCE/Fe<sub>3</sub>O<sub>4</sub>, GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> in pH 7.0 PBS containing 0.1 mM SE at a scan rate of 25 mV s<sup>-1</sup>. (d) Comparative current response of GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes after background current subtraction (scan rate: 25 mV s<sup>-1</sup>).

during the electrocatalytic oxidation of  $10^{-4}$  M SE at fixed potential 0.2 V and frequencies between 10 kHz and 1.0 Hz. The impedance spectra (Nyquist plots) obtained for the electrodes are presented in Fig. 5(a–c), while Fig. 5(d) shows the equivalent circuits used in the fitting of the impedance data. The GCE modified electrodes were successfully fitted with minimum error values (Table 1). In the circuit model Fig. 5 (d),  $R_s$  is the solution resistance,  $C_{dl}$  is the double layer capacitance,  $R_{ct}$  is the charge transfer resistance and  $Q$  is the constant phase element. The  $R_{ct}$  value is lower at GCE/MWCNT-NiO (54.2  $\Omega$ ), GCE/MWCNT-ZnO (57.2  $\Omega$ ) and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> (63.4  $\Omega$ ) electrodes compared with the bare GCE (100.2  $\Omega$ ), GCE/NiO (115.2  $\Omega$ ), GCE/ZnO (144.4  $\Omega$ ) and GCE/Fe<sub>3</sub>O<sub>4</sub> electrodes. The result further confirms that MWCNT-MO nanocomposite can form a good electron transport pathway between electrode and electrolyte/analyte during electrocatalytic oxidation of serotonin compared with the bare GCE or GCE/MO modified electrodes. Therefore, the electron transfer process is fastest at GCE/MWCNT-NiO electrode and follow the order, GCE/MWCNT-NiO > GCE/MWCNT-ZnO > GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> for the GCE/MWCNT-MO modified electrodes. This result is also in good agreement with SE oxidation peak current values obtained from the CVs experiment discussed above where GCE/MWCNT-NiO > GCE/MWCNT-ZnO > GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub>. The MWCNT creates a porous and large surface area that mediates the analyte/catalyst electrocatalytic process. Similar results have been reported for chemically modified electrodes [48]. Since GCE/MWCNT-MO electrodes gave better SE electrochemical response, further studies are carried out using these electrodes platform.

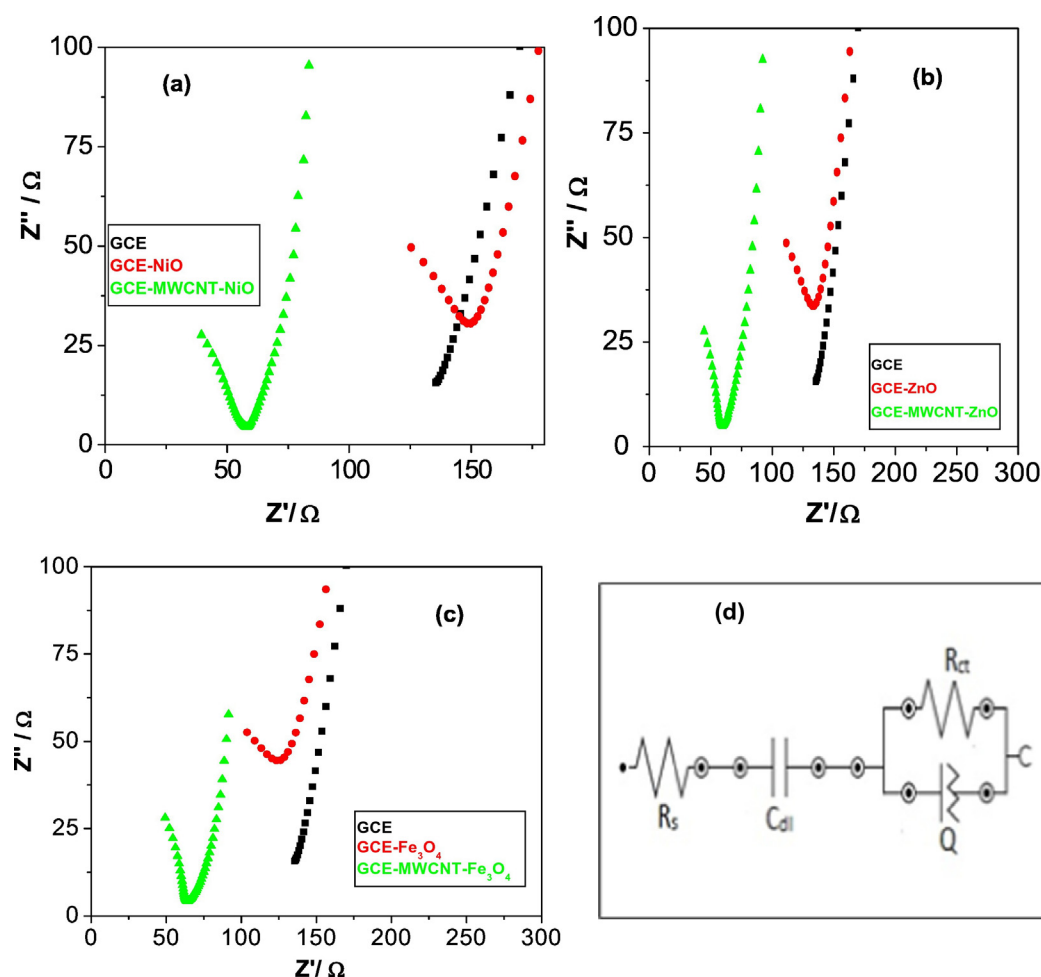
**Table 1**

Charge transfer resistance values for the electrodes in 0.1 mM SE in pH 7.0 PBS (at Ag/AgCl saturated KCl). Note that the values in parenthesis are percentage errors of the data fitting.

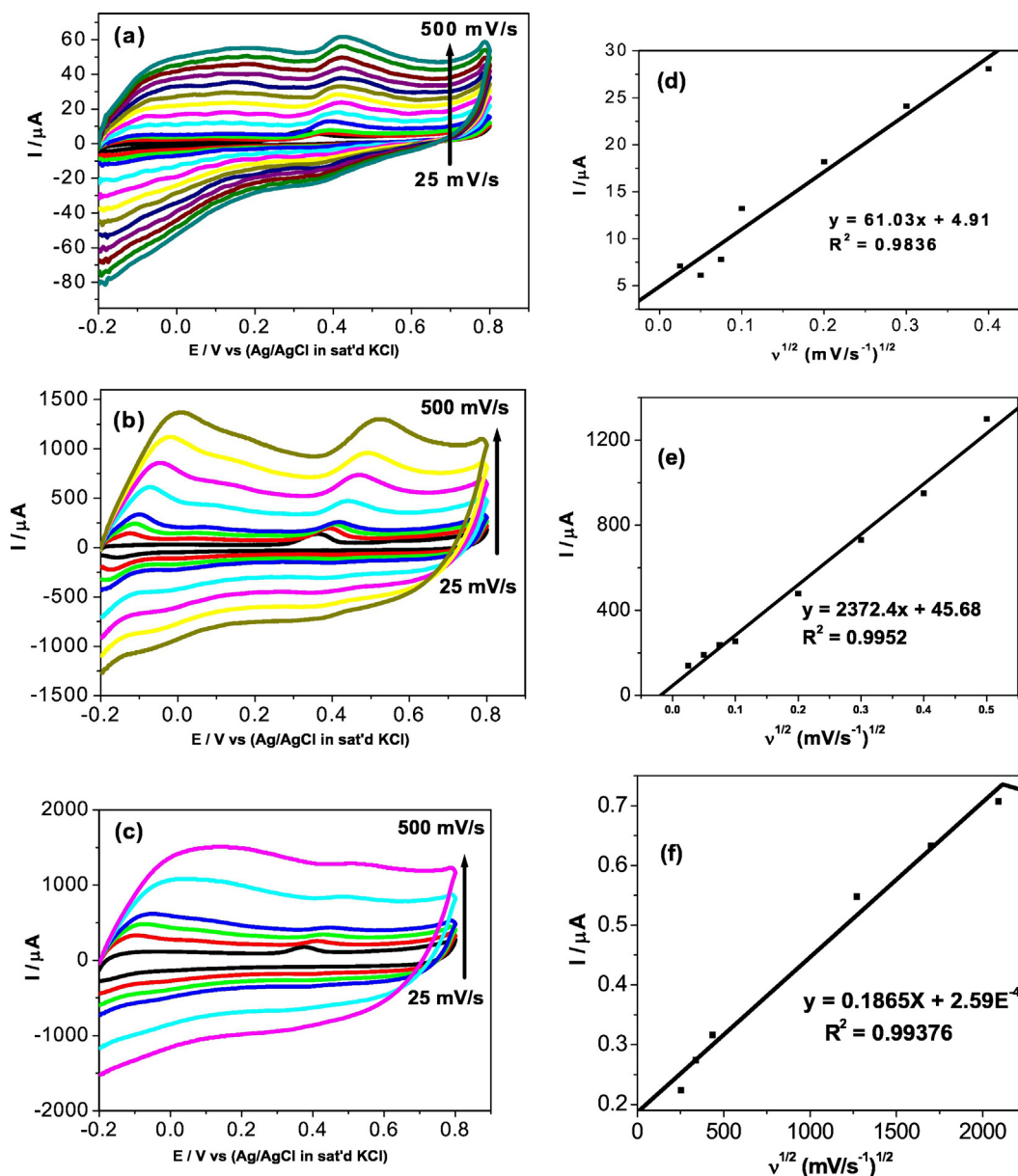
Electrodes	$R_{ct}$ ( $\Omega$ )
GCE	100.2 (16.23)
GCE/NiO	115.2 (8.912)
GCE/ZnO	144.4 (11.64)
GCE/Fe <sub>3</sub> O <sub>4</sub>	99.2 (4.59)
GCE/MWCNT	7.18 (7.29)
GCE/MWCNT-NiO	54.2 (7.19)
GCE/MWCNT-ZnO	57.2 (10.46)
GCE/MWCNT-Fe <sub>3</sub> O <sub>4</sub>	63.4 (8.56)

### 3.7. Effect of scan rate on the electrocatalytic oxidation of serotonin (SE) at modified electrode

The influence of scan rate on the electrochemical response of SE at GCE/MWCNT-MO electrodes was investigated by cyclic voltammetry, and the results are shown in Fig. 6. The oxidation peak currents gradually increased with increasing scan rate in the range of 25–500 mV s<sup>-1</sup> Fig. 6(a–c). When the peak current ( $I_{pa}$ ) was plotted against the square root of scan rate ( $v^{1/2}$ ), a linear relationship with a regression coefficient > 0.98 was obtained for the GCE/MWCNT-MO electrodes Fig. 6(d–f). This behaviour suggests that the oxidation of SE at the modified electrodes GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/



**Fig. 5.** Typical Nyquist plots (a–c) obtained for the electrodes in 0.1 mM SE solution (PBS pH 7.0) at a fixed potential of 0.2 V (vs. Ag/AgCl, saturated KCl) (d) represents the circuit used in the fitting of the EIS data (a–c).



**Fig. 6.** Effect of scan rate ( $25\text{--}500\text{ mV s}^{-1}$ ) on the cyclic voltammograms of (a) GCE/MWCNT-NiO (b) GCE/MWCNT-ZnO and (c) GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> in 0.1 mM SE. The plots of current ( $I$ ) against square root of scan rate ( $v^{1/2}$ ) are presented in Fig. 5(d–f) for GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes respectively.

MWCNT-Fe<sub>3</sub>O<sub>4</sub> is diffusion controlled. A positive shift of the anodic peak potential ( $E_{pa}$ ) with increase in scan rate was also observed. The apparent electron transfer constant ( $k_s$ ) and the electron transfer coefficient ( $\alpha$ ) of a surface-confined redox couple can be evaluated from cyclic voltammetric results, and by using the variation of anodic peak potentials with scan rate. It was found that  $E_{pa}$  increased linearly with  $\ln v$  (graph not shown). The number of electrons involved in the reaction can be calculated according to the Laviron's equation [49–52]. Accordingly, the slope ( $b$ ) of the  $E_p$  vs.  $\ln(v)$  plot is represented in Eq. (1).

$$b = 2.3RT/n\alpha F \quad (1)$$

where  $b$  is the slope. The values of the charge transfer coefficient ( $\alpha$ ) of GCE-MWCNT-NiO, GCE-MWCNT-ZnO and GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes were calculated as 0.55, 0.96 and 0.49 respectively. The obtained value for  $n$  is approximately 1, which indicates that one electron is involved in the oxidation of serotonin. The electrode

reaction standard rate constant ( $k_s$ ) for the modified electrodes were obtained by using Eq. (2) to be equal to 0.27, 0.47 and  $0.52\text{ cm s}^{-1}$  for GCE-MWCNT-NiO, GCE-MWCNT-ZnO and GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes respectively.

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log(RT/nFn) - \alpha(1-\alpha)nFE/2.3RT \quad (2)$$

According to the kinetics of electron process, when the rate constant is larger than  $10^{-2}\text{ cm s}^{-1}$ , the electron transfer process is very fast, and the electrode reaction is reversible, and when  $10^{-4} < k_s < 10^{-2}\text{ cm s}^{-1}$ , the electrode reaction is a quasireversible process [49].

The peak potential,  $E_p$ , is proportional to  $\log v$  (graph not shown). The slopes of  $E_p$  vs.  $\log v$  are 61.7, 108.3 and 121.7 mV for the modified electrodes GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> respectively. The Tafel slopes ( $b$ ) may be estimated according to the Eq. (2) for totally irreversible diffusion controlled process [53]. So, the values of  $b$  for GCE/MWCNT-NiO, GCE/MWCNT-ZnO



and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes are 123, 216 and 243 mV dec<sup>-1</sup> respectively.

$$E_p = (b/2) \log(v/\text{mVs}^{-1}) + \text{constant} \quad (3)$$

These Tafel values are higher than the theoretical value of 118 mV dec<sup>-1</sup> for a one-electron process involved in the rate-determining step. This suggests the occurrence of either adsorption or involvement of reaction intermediates on the electrode surface [54]. The result further confirm the porous nature of the GCE/MWCNT-MO modified electrodes which despite their porous and adsorptive nature gave better performance towards SE oxidation in terms of oxidation potential, current recovery, stability and resistance to fouling effects.

### 3.8. Electroanalysis of serotonin (SE)

Concentration study was carried out in PBS (pH 7.0) containing  $5.98 \times 10^{-3}$  μM to 62.8 μM serotonin (SE) using square wave voltammetry (SWV) measurement. The result obtained for the square wave

voltammograms of the GCE/MWCNT-MO electrodes is presented in Fig. 7. The dependence of the oxidation peak currents on the concentration of SE showed that the anodic peak current increased as the concentration of serotonin (SE) increases. The detection limit was calculated based on the relationship  $\text{LoD} = 3.3 \delta/m$  where  $\delta$  is the relative standard deviation of the intercept of y-coordinates from the line of best fit, and  $m$  is the slope of the same line [55]. The analysis was conducted three times ( $n = 3$ ) and the standard deviation accompanying the LoD values were evaluated and found to be 0.36, 1.15 and 0.71 nM for modified electrodes GCE-MWCNT-NiO, GCE-MWCNT-ZnO and GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> respectively. Therefore using the IUPAC formula, the obtained limit of detection and the sensitivity of the modified electrodes are  $118 \pm 0.36$  nM (9.96 μA/μM),  $129 \pm 1.15$  nM (29.53 μA/μM) and  $166 \pm 0.71$  nM (13.93 μA/μM) for GCE-MWCNT-NiO, GCE-MWCNT-ZnO and GCE-MWCNT-Fe<sub>3</sub>O<sub>4</sub> sensors respectively. The performances of the present electrochemical sensors were compared with those reported in the literature (Table 2). The low-detection-limit (at nano molar SE concentration) obtained in the present study compare favourably and even better than previously reported limit of detection for SE using various electrochemical methods with different modified

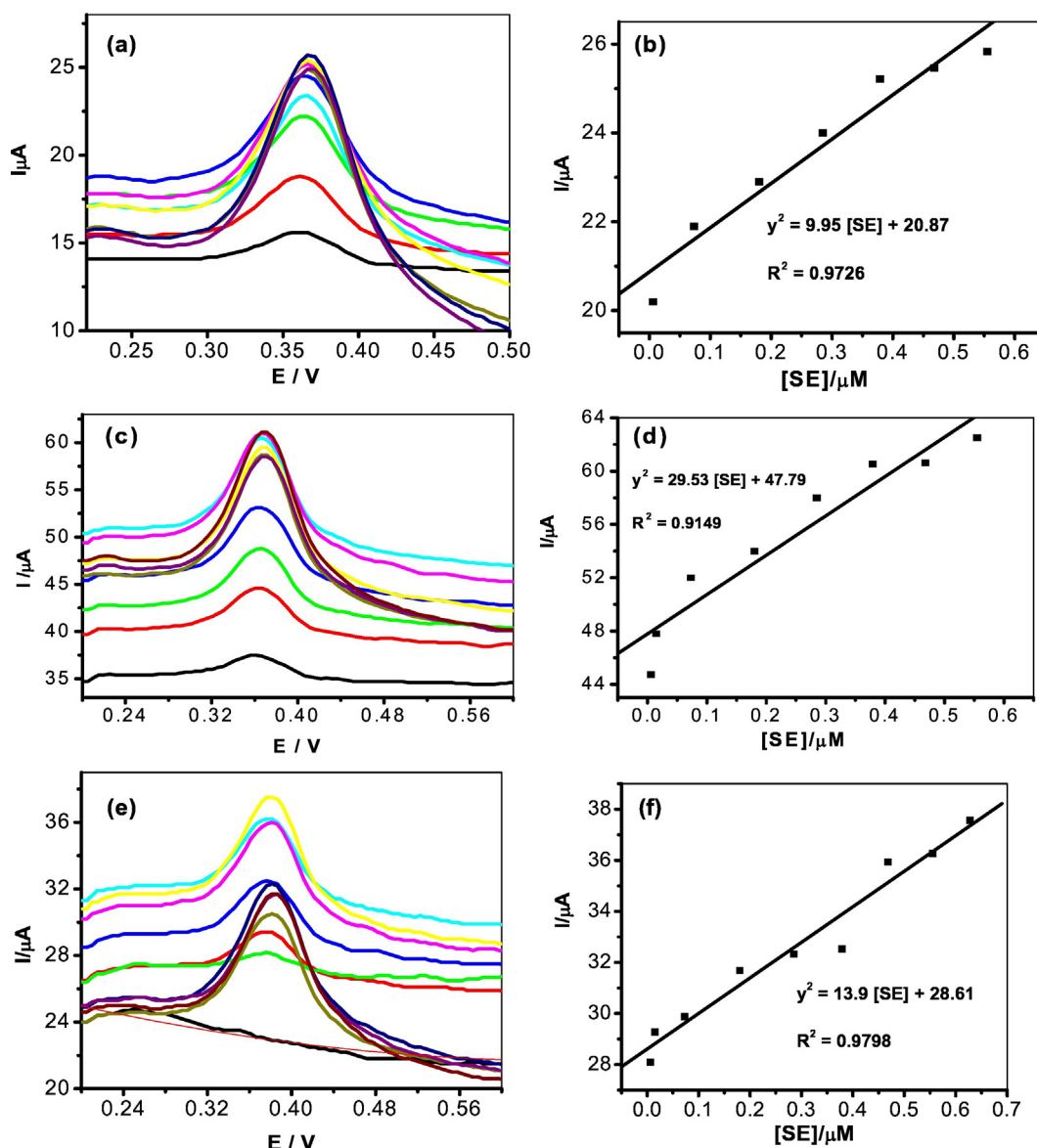


Fig. 7. Square wave voltammograms of (a) GCE/MWCNT-NiO (c) GCE/MWCNT-ZnO and (e) GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> in PBS pH 7 containing  $5.98 \times 10^{-3}$  μM to 62.8 μM SE concentration and (b, d and f) are the plots of current ( $I$ ) against concentration of SE for modified electrodes GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> respectively.

**Table 2**  
Comparison of different electrochemical sensors for the determination of serotonin.

Electrode material	Techniques	Ep(V) (Ag/AgCl)	Detection limit (M)	References
TPyP-31P/FTO	CVs	0.2	$2.2 \times 10^{-4}$	[56]
F-MWCNTs/BR9	DPV	0.21	$9.0 \times 10^{-6}$	[57]
GN-SPEs	CVs	0.28	$1.0 \times 10^{-4}$	[58]
GCE/MWCNT-NiO	SWV	0.36	$(118 \pm 0.36) \times 10^{-9}$	This work
GCE/MWCNT-ZnO	SWV	0.37	$(129 \pm 1.15) \times 10^{-9}$	This work
GCE/MWCNT-Fe <sub>3</sub> O <sub>4</sub>	SWV	0.38	$(166 \pm 0.71) \times 10^{-9}$	This work

electrodes (Table 2) [56–58]. Since the concentration of serotonin in healthy human is in the range of 0.5 to 1.4 mM [59], the present sensor system with a low-detection-limit (at nano concentration) especially GCE/MWCNT-NiO and GCE/MWCNT-ZnO are well suitable for the analysis of serotonin in diluted physiological samples. The present sensor system was then subjected to stability and interference studies and for the determination of serotonin directly from physiological samples (urine).

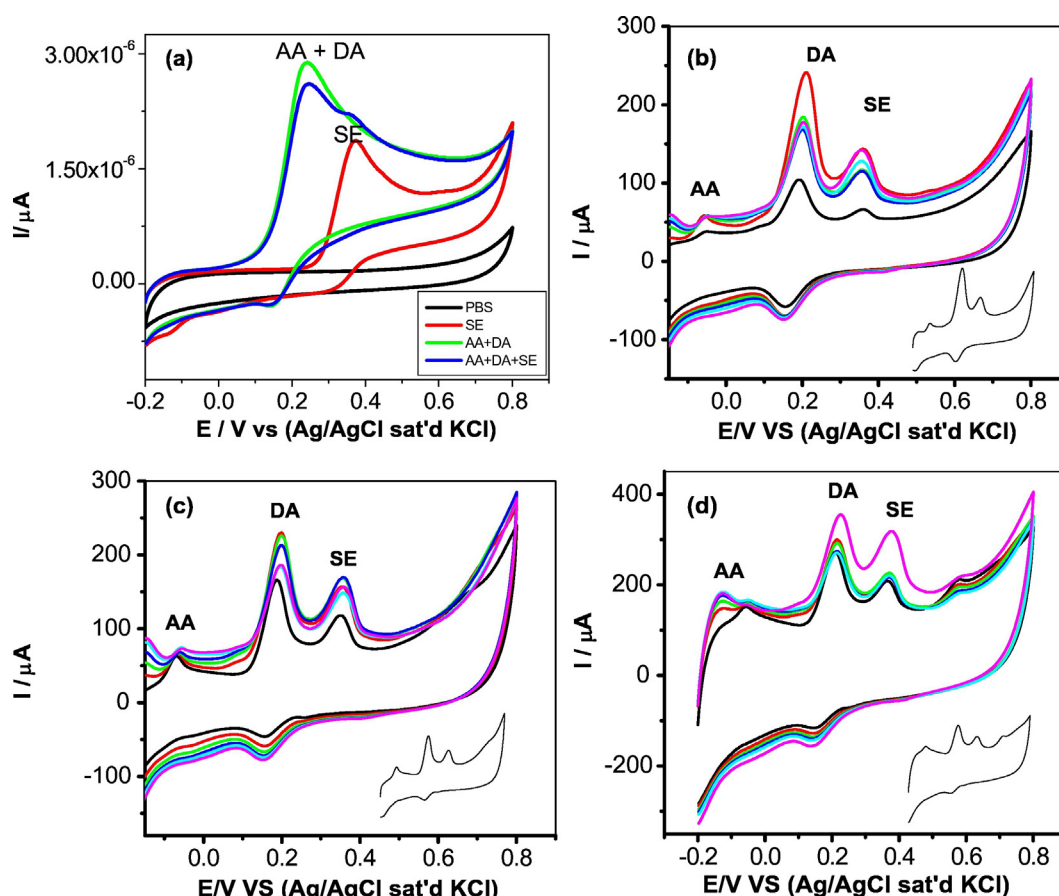
### 3.9. Stability and reproducibility of the modified electrodes

The stability of the modified electrodes (GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub>) towards determination of serotonin (SE) was examined by measuring the current response at fixed concentration of SE ( $2 \times 10^{-4}$  M) in pH 7.0 PBS (not shown). The electrodes were repetitively scanned (20 scans) in a  $2 \times 10^{-4}$  M serotonin at  $25 \text{ mV s}^{-1}$ . A decrease in peak currents ( $\sim 10\%$ ) was observed for the three composite modified electrodes after the first scan, which is a typical behaviour for a poisoned electrode. However, on rinsing the electrodes in a fresh PBS (pH 7.0) solution, the electrode surface was

renewed and more than 95% of the initial catalytic current was obtained, indicating the electrochemical stability and reusability of the electrode after analysis.

### 3.10. Interference study

Since serotonin (SE), dopamine (DA) and ascorbic acid (AA) have similar oxidation potentials at most solid electrodes, separate determination of these species is a great problem due to their overlapping signals. In order to establish a sensitive and selective discrimination between SE, DA and AA on our developed sensor, the electrochemical oxidation of the mixture containing these three species at the MWCNT-MO modified GCE was studied. As shown in Fig. 8, the CV of the mixture solution containing DA and AA shows broad and overlapped anodic peaks at bare GCE, so the peak potentials for SE, DA and AA are indistinguishable and therefore, it is impossible to separate the analytes from the broad and overlapped voltammetry peak Fig. 8(a). However, at the GCE/MWCNT-MO, the overlapped voltammetry peak is resolved into three well-defined anodic peaks at about 0.37, 0.2 and 0.07 V corresponding to the oxidation of SE, DA and AA respectively Fig. 8(b–d). The result obtained for interference studies of serotonin in the presence of dopamine and ascorbic acid suggests an interaction between the nanoparticles and the different analytes (AA, DA and SE). The metal oxide oxidation peaks observed at the anodic potential of around 0.6 V in Fig. 4(a,b) disappears as the concentration of serotonin in the analytes increases as shown in Fig. 8(b,c) indicating successful catalysis of the analytes. In Fig. 8d, the presence of the three analytes peaks, and reappearance of the catalyst peak around 0.6 V suggest a faster electron transport properties (less capacitance) of the electrodes during multiple oxidation process, this in turn affects the Fe(II)/Fe(III) electronic transition of the Fe<sub>3</sub>O<sub>4</sub> nanoparticles. It could also mean strong resistance of



**Fig. 8.** Cyclic voltammograms of  $10^{-4}$  M of AA, DA and SE (in PBS pH 7.0) at (a) Bare GCE (b) GCE/MWCNT-NiO (c) GCE/MWCNT-ZnO and (d) GCE/MWCNT-Fe<sub>3</sub>O<sub>4</sub> electrodes.

the  $\text{Fe}_3\text{O}_4$  nanoparticles to any form of poisoning effect resulting from the oxidation intermediates during AA, DA and SE oxidation. The separations of peaks were 300 mV, 170 mV and 130 mV between SE and AA, SE and DA, and DA and AA, respectively, which were large enough to determine SE, DA and AA individually and simultaneously. Thus, the GCE/MWCNT-MO electrodes can conveniently detect SE in physiological medium without interference from DA or AA.

### 3.11. Determination of serotonin (SE) in human urine sample

Since SE concentration in urine is an important marker for various diseases and ageing related physiological conditions, it is considered necessary to detect these biomolecules in biological fluids [60] and therefore there are several literatures on its determination in urine samples [61–64]. Also, whenever a compound is traceable in urine, sampling of urine always gets preference over blood sample due to its non-invasive nature. The results obtained are presented in Table 3. GCE/MWCNT-NiO and GCE/MWCNT-ZnO gave better recovery as compared with GCE/MWCNT- $\text{Fe}_3\text{O}_4$ , and the result agreed with their detection limits towards SE. This result further confirmed the suitability and reliability of GCE/MWCNT-MO electrodes as a potential sensor for the analysis of SE.

## 4. Conclusions

A highly sensitive electrochemical sensor was fabricated for selective determination of serotonin at physiological pH. The modified GCE/MWCNT-metal oxide nanocomposite electrodes (GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT- $\text{Fe}_3\text{O}_4$ ) exhibit excellent electrocatalytic activity towards the electrochemical oxidation of serotonin at large peak current and lower oxidation potentials compared to other electrodes investigated. Serotonin (SE) showed a well-defined oxidation peaks at these electrodes. The limits of detection of the GCE/MWCNT-MO electrodes are 118 nM, 129 nM and 166 nM for GCE/MWCNT-NiO, GCE/MWCNT-ZnO and GCE/MWCNT- $\text{Fe}_3\text{O}_4$  sensors

**Table 3**  
Recovery data for serotonin determination in human urine samples at modified GCE electrodes.

(A)			
Sr.no/electrode GCE/MWCNT-NiO	Amount added (mmol L <sup>-1</sup> )	Amount detected (mmol L <sup>-1</sup> )	Recovery %
1	2.00	2.28	114
2	2.00	2.20	110
3	2.00	2.08	104
4	2.00	2.20	110
The RSD % for n = 4 is 3.77%			
(B)			
Sr.no/electrode GCE/MWCNT-ZnO	Amount added (mmol L <sup>-1</sup> )	Amount detected (mmol L <sup>-1</sup> )	Recovery %
1	2.00	2.08	104
2	2.00	2.00	100
3	2.00	1.92	96
4	2.00	1.96	98
The RSD % for n = 4 is 3.43%			
(C)			
Sr.no/electrode GCE/MWCNT- $\text{Fe}_3\text{O}_4$	Amount added (mmol L <sup>-1</sup> )	Amount detected (mmol L <sup>-1</sup> )	Recovery (%)
1	2.00	1.92	96
2	2.00	1.84	92
3	2.00	1.92	96
4	2.00	1.96	98
The RSD % for n = 4 is 2.64%			

respectively. GCE/MWCNT-NiO electrode gave the best limit of detection and faster electron transfer process towards serotonin determination. The nanocomposites modified electrodes were highly stable for multiple analyses over a long period due to the unique binding character of MWCNT towards the metal oxide nanoparticles. Simultaneous voltammetric determination of serotonin (SE), ascorbic acid (AA) and dopamine (DA) was successful at the MWCNT-MO modified GCE electrodes without AA and DA signals interfering with serotonin. The present sensor system exhibited very good recovery limits for the determination of SE directly from urine sample. The method developed is simple, fast and accurate and opens new avenues for quick simultaneous estimation of physiologically important compounds.

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