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## Original Article

# Fabrication of a novel electrochemical sensor for determination of hydrogen peroxide in different fruit juice samples



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## ABSTRACT

A new hydrogen peroxide ( $H_2O_2$ ) sensor is fabricated based on a multiwalled carbon nanotube-modified glassy carbon electrode (MWCNT-GCE) and reactive blue 19 (RB). The charge transfer coefficient,  $\alpha$ , and the charge transfer rate constant,  $k_s$ , of RB adsorbed on MWCNT-GCE were calculated and found to be  $0.44 \pm 0.01$  Hz and  $1.9 \pm 0.05$  Hz, respectively. The catalysis of the electroreduction of  $H_2O_2$  by RB-MWCNT-GCE is described. The RB-MWCNT-GCE shows a dramatic increase in the peak current and a decrease in the over-voltage of  $H_2O_2$  electroreduction in comparison with that seen at an RB modified GCE, MWCNT modified GCE, and activated GCE. The kinetic parameters such as  $\alpha$  and the heterogeneous rate constant,  $k'$ , for the reduction of  $H_2O_2$  at RB-MWCNT-GCE surface were determined using cyclic voltammetry. The detection limit of  $0.27 \mu M$  and three linear calibration ranges were obtained for  $H_2O_2$  determination at the RB-MWCNT-GCE surface using an amperometry method. In addition, using the newly developed sensor,  $H_2O_2$  was determined in real samples with satisfactory results.

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## 1. Introduction

Hydrogen peroxide ( $H_2O_2$ ) is an essential mediator used in many fields of practice such as food, pharmaceutical, clinical, diagnostic, environmental protection, and in industries [1]. Indeed,  $H_2O_2$  has wide applications in industrial

processes as a universal oxidant and is a very important intermediate agent in environmental and biological reactions. It has also emerged as an important by-product of enzymatic reactions in biosensing processes, because it is released during the oxidation of substrates in the presence of oxygen [2,3]. Monitoring  $H_2O_2$  with a reliable, rapid, and economical method is of great significance for numerous

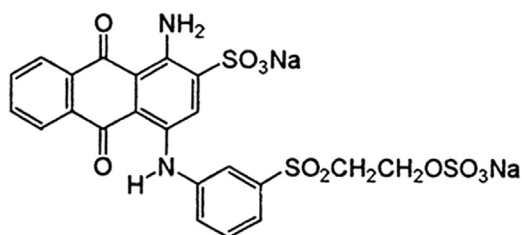
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processes. The determination of  $H_2O_2$  has been an important project over the past century. This is still something to accomplish, especially with the recent realizations about the existence of  $H_2O_2$  in environmental samples. Various techniques including fluorimetry [4], titrimetry [5], chemiluminescence [6], spectrophotometry [7], photometry [8], and electrochemistry [9–11] have been developed for this purpose. Among these techniques, electrochemistry based on simple and low-cost electrodes has been extensively used to determine  $H_2O_2$  [12]. It has been a preferred technique due to its quick response, low cost, simple instrumentation, high sensitivity, and possibility of miniaturization [13]. A number of enzyme electrodes reported for  $H_2O_2$  determination are used due to their simplicity, high sensitivity, and selectivity [14]. Although, many enzymatic  $H_2O_2$  assays possess good sensitivity and selectivity, native enzymes gradually lose their catalytic activity after repeated measurements and they are comparatively expensive. Therefore, the development of enzyme-free  $H_2O_2$  sensors with a low detection limit and a wide responding range is preferred. However, the direct electrochemistry of  $H_2O_2$  requires a higher over-potential and slow electrode kinetics on many electrode materials. Electrodes modified with different electroactive materials have the ability to detect  $H_2O_2$  at low potentials. In recent years, various kinds of electrochemical sensors based on detection of different compounds in the food industry have been developed [15–18]. The study of electrochemical biosensors has been in progress to improve the speed, selectivity, sensitivity, and cost of producing chemical compounds. Immobilization of mediators in charge of electron transfer to electrode surfaces is a key step for the design, fabrication, and performance of sensors and biosensors [19]. Quinones are excellent electron mediators that efficiently reduce the working potentials and eliminate the effects of various electroactive substances in real samples; they have been successfully used in numerous biosensors to detect  $H_2O_2$  [20,21]. Reactive blue 19 (RB) is a derivative of quinone (see its structure in Scheme 1). Owing to the good reactivity of quinone derivative mediators, it seems that the use of RB as a modifier could be important to yield some new information about the catalyzation of slow reactions. In this work, for the first time, RB is introduced as a catalytic compound for electroreduction of  $H_2O_2$ , then its electrochemical behavior and kinetic parameters are investigated. Cyclic voltammetry and amperometry were used to investigate the electrochemical properties and electrocatalytic activity of the modified electrode for determination of  $H_2O_2$ .



Scheme 1 – Structure of reactive blue 19.

## 2. Methods

### 2.1. Reagents and apparatus

$H_2O_2$ , dimethyl formamide, and the other chemicals used to produce the buffer solution were obtained from Merck Company (Darmstadt, Germany) and used as received. RB was obtained from Sigma–Aldrich (St Louis, MO, USA). Multi-walled carbon nanotubes (MWCNTs; with a diameter of 10–20 nm, length of 5–20  $\mu\text{m}$ , and purity of 95%) were purchased from NanoLab Inc. (Waltham, MA, USA). All chemical reagents were of analytical grade. Phosphate buffer solutions (0.1M) were prepared with  $H_3PO_4$ , the pH was adjusted with 2.0M NaOH. All solutions were prepared with doubly distilled water.

Electrochemical experiments were carried out with an Autolab modular electrochemical system (ECO Chemie, Utrecht, The Netherlands) equipped with a PGSTA 30 module and driven by GPES 4.9 software, in conjunction with a three-electrode cell. The cell used was equipped with an RB-MWCNT-modified glassy carbon electrode (GCE) as a working electrode, a platinum electrode (Azar Electrode Co, Tabriz, Iran) as an auxiliary electrode, and a saturated calomel electrode as a reference electrode. All potentials in the text are quoted versus this reference electrode. The pH was measured with a pH/mV meter model 827 (Metrohm, Riverview, FL, USA).

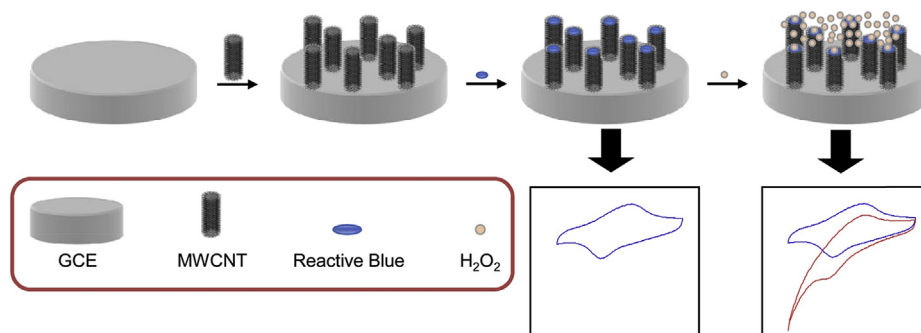
### 2.2. Preparation of modified electrodes

The procedure of fabricating various modified electrodes was as follows. Prior to modification, a bare GCE was polished successively with 0.05  $\mu\text{m}$   $Al_2O_3$  slurry on a polishing cloth and then rinsed with doubly distilled water. To be electrochemically activated, the cleaned electrode was immersed in a 0.1M sodium bicarbonate solution and activated by a continuous potential cycling from  $-1.45$  to  $1.7$  V at a sweep rate of 100 mV/s. To prepare a RB modified GCE (RB-GCE) the activated GCE (AGCE) was rinsed and placed in a 0.10mM solution of RB in a 0.1M phosphate buffer (pH 7.0). It was modified by eight cycles of potential sweep between  $-0.4$  and  $0.5$  V at 20 mV/s. To fabricate MWCNT-GCE, 3  $\mu\text{L}$  of dimethyl formamide-MWCNT solution (1 mg/mL) was placed directly onto the AGCE surface and dried at room temperature for 30 minutes to form a MWCNT film at the GCE surface. An RB-MWCNT-GCE was prepared by immersing the MWCNT-GCE in a 0.10mM solution of RB in a 0.1M phosphate buffer (pH 7.0; Scheme 2). It was modified using the same procedure as for RB-GCE.

## 3. Results and Discussion

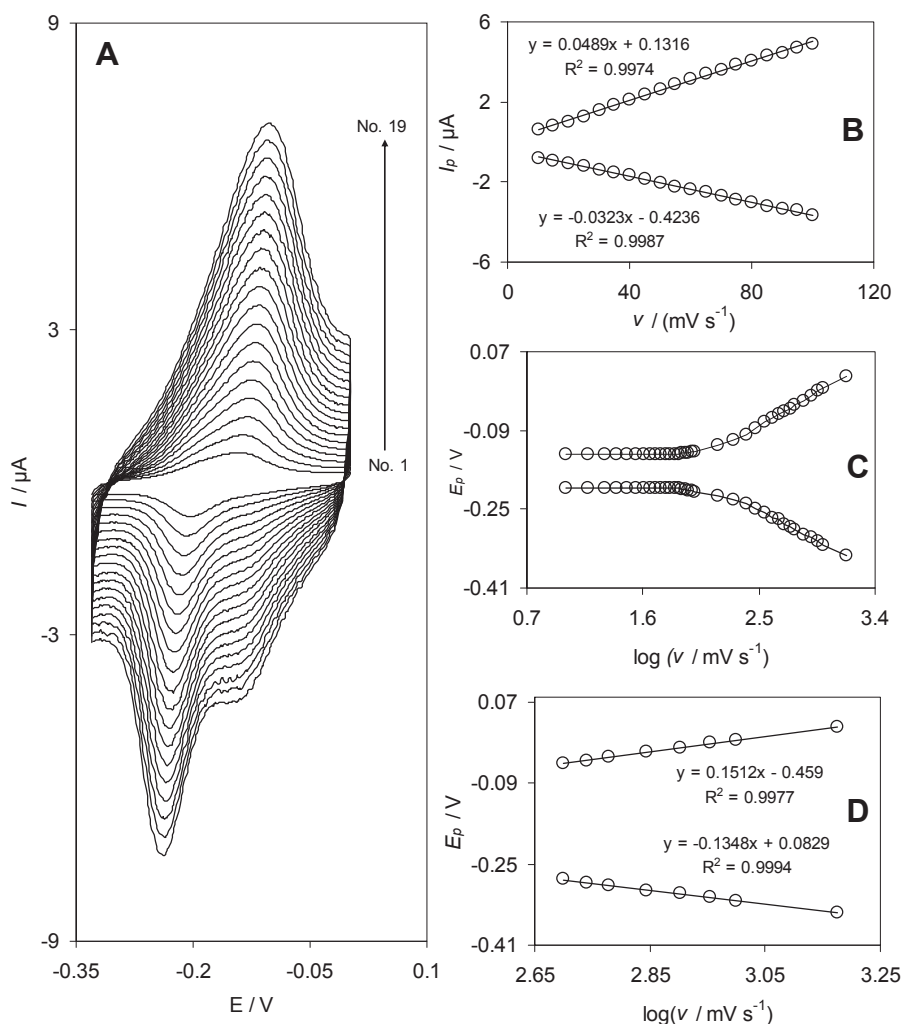
### 3.1. Electrochemical behavior of RB-MWCNT-GCE

In recent years, the mechanism of electrodeposition of *o*- or *p*-hydroquinone derivatives, as modifiers, on the surface of an activated GCE has been a matter for discussion [22–25]. Fig. 1 shows the cyclic voltammograms of the



GCE = glassy carbon electrode; MWCNT = multiwalled carbon nanotube.

**Scheme 2 – Schematic diagram for the sensor fabrication and determination of  $\text{H}_2\text{O}_2$ . GCE = glassy carbon electrode; MWCNT = multiwalled carbon nanotube.**



**Fig. 1 – (A) Cyclic voltammograms of the reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode in 0.1M phosphate buffer solution (pH 7.0) at different scan rates. The numbers 1–19 correspond to 15 mV/s, 20 mV/s, 25 mV/s, 30 mV/s, 35 mV/s, 40 mV/s, 45 mV/s, 50 mV/s, 55 mV/s, 60 mV/s, 65 mV/s, 70 mV/s, 75 mV/s, 80 mV/s, 85 mV/s, 90 mV/s, 95 mV/s, and 100 mV/s, respectively. (B) Plots of anodic and cathodic peak currents vs. the scan rate. (C) Variation of the peak potentials vs. the logarithm of the scan rate. (D) Magnification of the plot inset B for high scan rates.**

**Table 1 – Variations in anodic peak surface coverage,  $\Gamma$ , as a function of reactive blue 19 (RB) modified glassy carbon electrode multiwalled carbon nanotube (MWCNT) amounts, RB solution pH, RB solution concentration, [RB], potential scan rates,  $\nu$ , and number of cycles of potential scans, number of cycles, during the modification step. In all case the scan rate was 20 mV/s and the surface coverage is in  $10^{-11}$  mol/cm.**

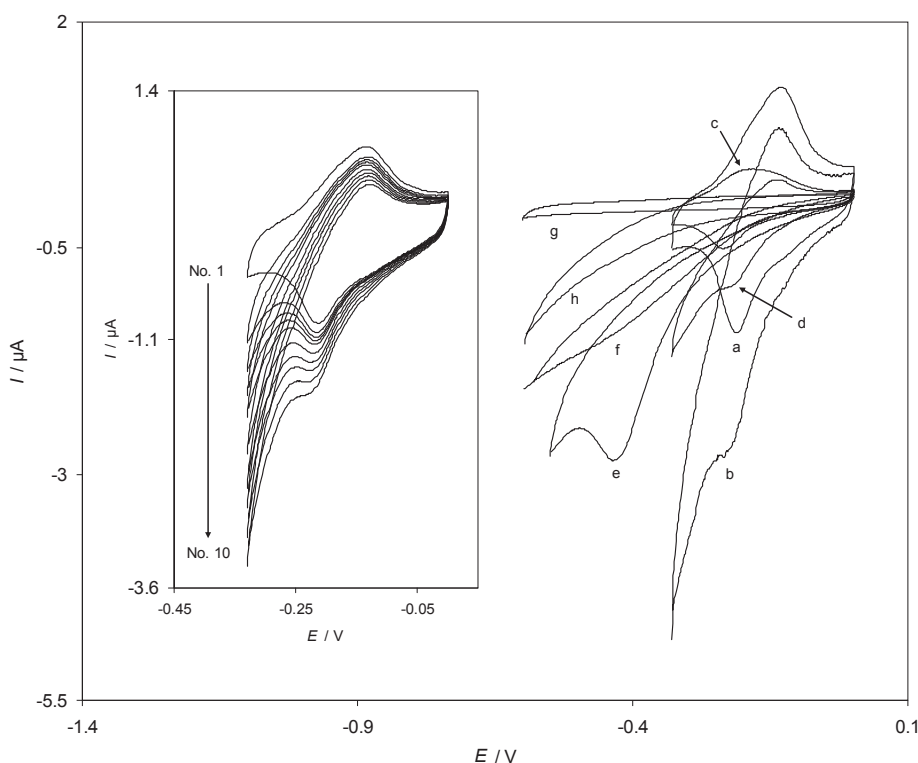
MWCNT amount (mg/cm <sup>3</sup> )	$\Gamma$	pH	$\Gamma$	[RB] (mM)	$\Gamma$	$\nu$ (mV/s)	$\Gamma$	No. of cycles	$\Gamma$
0.2	6.8	3	8.5	0.2	2.1	10	12.9	2	13.3
0.4	8.2	4	9.6	0.4	6.4	15	13.4	4	14.5
0.6	9.5	5	10.2	0.6	10.7	20	15.5	6	15.2
0.8	10.6	6	11.1	0.8	12.6	30	10.7	8	16.1
1.0	11.4	7	12.5	1.0	14.1	40	9.6	10	15.8
1.2	11.4	8	9.8	1.2	13.9	50	6.2	12	15.5
1.4	11.2	9	6.9	1.4	14.0	60	4.9	14	15.7

RB-MWCNT-GCE in a 0.1M phosphate buffer solution (pH 7.0) at various potential scan rates. The plots of the anodic and cathodic peak currents versus the scan rate exhibit a linear relation (Fig. 1, inset B) as predicted theoretically for a surface-immobilized redox couple. This indicates the limitation arising from charge transfer kinetics. In other words, this is the range in which the reaction appears as quasireversible [26].

When the potential was scanned between –330 and 0 mV, a surface-immobilized redox couple with a conditional formal potential,  $E^0$ , of –173 mV was observed. In addition,  $E^0$  was almost independent of any potential scan rate for sweep rates

ranging from 10 mV/s to 100 mV/s, suggesting facile charge transfer kinetics over this range of sweep rates. According to the method described by Laviron [27], the charge transfer coefficient,  $\alpha$ , and the apparent heterogeneous charge transfer rate constant,  $k_s$ , for the electron transfer between an MWCNT-GCE and a surface confined redox couple of RB can be evaluated in cyclic voltammetry by the variation of the anodic and cathodic peak potentials with the logarithm of scan rates.

For high scan rates, this theory predicts a linear dependence of  $E_p$  upon  $\log \nu$ , which can be used to extract the kinetic parameters of  $\alpha$  and  $k_s$  from the slope and intercept of such



**Fig. 2 – Cyclic voltammograms of different electrodes in a 0.1M phosphate buffer (pH 7.0) solution in the absence (a, c, g) and presence (b, d, e, f, h) of 0.40mM H<sub>2</sub>O<sub>2</sub>: (a, b) reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode (RB-MWCNT-GCE), (c, d) RB-GCE, (e) MWCNT-GCE, (f) acquired GCE and (g, h) BGCE. Inset shows the dependence of cyclic voltammetric response at a RB-MWCNT-GCE on H<sub>2</sub>O<sub>2</sub> concentration in 0.1M phosphate buffer (pH 7). The numbers of 1–10 correspond to 0.0–473.7μM H<sub>2</sub>O<sub>2</sub>. In all cases the scan rate was 20 mV/s.**

**Table 2 – Comparison of electrocatalytic reduction of  $H_2O_2$  (0.40mM) on various electrode surfaces at pH 7.0.**

Name of electrode	Reduction potential (mV)	Reduction current ( $\mu A$ )
MWCNT-GCE	–431	–1.353
RB-GCE	–221	–0.397
RB-MWCNT-GCE	–215	–1.73

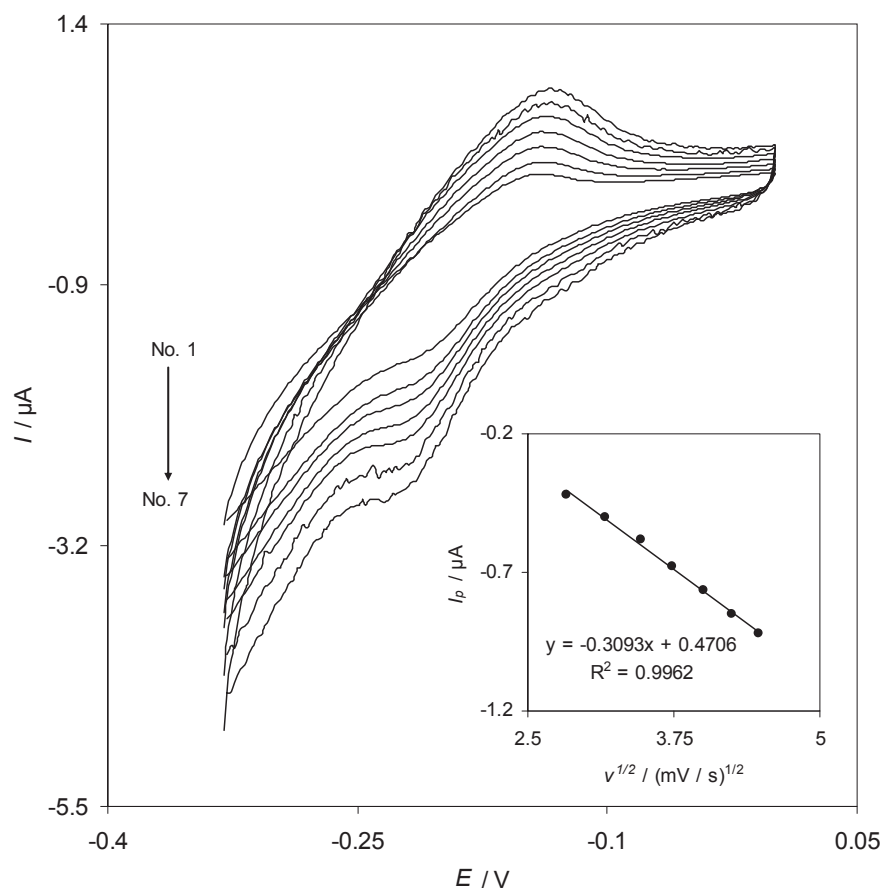
MWCNT-GCE = multiwalled carbon nanotube-modified glassy carbon electrode; RB-GCE = reactive blue modified glassy carbon electrode; RB-MWCNT-GCE = reactive blue multi-walled carbon nanotube-modified glassy carbon electrode.

plots respectively. The results indicate that the values of the peak potentials were proportional to the logarithms of scan rates higher than 500 mV/s (Fig. 1C). Using these plots at pH 7.0, the value of  $\alpha = 0.44 \pm 0.01$  was obtained. From the values of  $\Delta E_p$  corresponding to different sweep rates, an average value of  $k_s$  was found to be  $1.9 \pm 0.05$  Hz. This value of  $k_s$  is comparable to those reported for other modifiers [21,24,27,28]. Since the electron transfer rate constant for the RB is about 1.9 Hz, it can be used as an excellent electron transfer mediator for electrocatalytic processes.

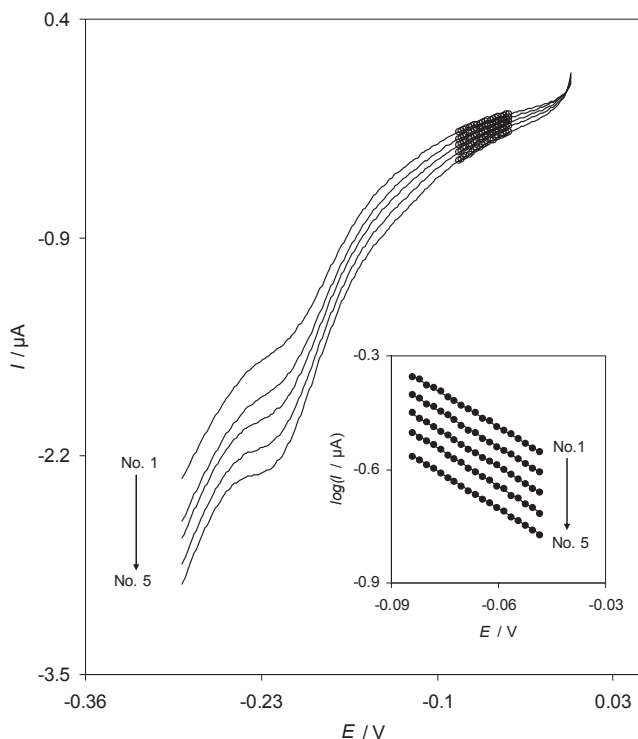
The effects of different experimental variables in the immobilization of RB were investigated to optimize the testing

performance. In other words, the effects of the MWCNT amounts, the modifier solution pH, its concentration, the potential scan rate, and the number of potential recyclings were examined on the performance of modified electrode, and the anodic surface coverage was used as a measure of the surface deposited RB. The surface coverage ( $\Gamma$ ) of the modified electrode was determined from the following equation,  $\Gamma = Q/nFA$ , where  $Q$  is the charge obtained by integrating the anodic or cathodic peak under the background correction and other symbols have their usual meanings, assuming an  $n$  value of 2. Table 1 illustrates the effect of different experimental conditions involved in the fabrication of RB-MWCNT-GCE. However, assuming that no interaction can exist between the variables, the one-at-a-time procedure [29] was used for optimization. The results in Table 1 show that the best surface coverage was obtained when the modification was carried out in a 1.0 mM RB solution with pH 7.0 (0.1M phosphate buffer) and the potential scan rate, was 20 mV/s.

Moreover, the intraday repeatability of RB-MWCNT-GCE was tested by 10 replicate measurements in the buffer solution (pH = 7). The decrease of current signal was about 5%. Also, the long-term stability of RB-MWCNT-GCE was measured for 3 days. When cyclic voltammograms were recorded after the modified electrode was stored in at room



**Fig. 3 – Cyclic voltammograms of reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode in 0.1M phosphate buffer (pH 7.0) containing 0.40mM  $H_2O_2$  at different scan rates. The numbers of 1–7 correspond to scan rates of 8 mV/s, 10 mV/s, 12 mV/s, 14 mV/s, 16 mV/s, 18 mV/s, and 20 mV/s. Inset: variation of the electrocatalytic peak current ( $I_p$ ) versus the square root of sweep rate.**



**Fig. 4 – Linear sweep voltammogram of reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode in 0.1M phosphate buffer solution (pH 7.0) containing 0.40 mM H<sub>2</sub>O<sub>2</sub> at different scan rates (8–16 mV/s). Inset shows the Tafel plots derived from the linear sweep voltammograms.**

temperature, only a 7% decrease was observed in the current response of the modified electrode. The initial decay of the current response of the voltammograms observed in both cases might be due to the release of the modifiers that are weakly bonded to MWCNT deposited on the electrode surface and can be separated somewhat easily.

### 3.2. Electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub> at RB-MWCNT-GCE

To test the electrocatalytic activity of the produced RB-MWCNT-GCE toward H<sub>2</sub>O<sub>2</sub> reduction, cyclic voltammetric responses were obtained in a phosphate buffer solution at pH 7 in the absence and presence of H<sub>2</sub>O<sub>2</sub> (Fig. 2). Fig. 2 indicates the cyclic voltammograms of different electrodes in a 0.1M phosphate buffer (pH 7.0) solution in the absence (a, c, g) and presence (b, d, e, f, h) of 0.40 mM H<sub>2</sub>O<sub>2</sub>. Curves a and b of Fig. 2 show the cyclic voltammograms of RB-MWCNT-GCE in the absence (a) and the presence (b) of 0.4mM of H<sub>2</sub>O<sub>2</sub>. As

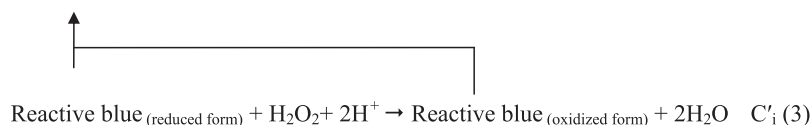
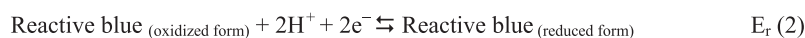
expected for electrocatalytic reduction, there was an increase in the cathodic peak current of RB-MWCNT-GCE<sub>red</sub>/RB-MWCNT-GCE<sub>ox</sub> redox couple at the potential of –215 mV in the presence of H<sub>2</sub>O<sub>2</sub>, whereas the oxidation peak current virtually disappeared. This reflects the efficiency of the catalytic reaction. What occurred is basically because H<sub>2</sub>O<sub>2</sub> in solution diffuses onto the electrode surface and oxidizes RB-MWCNT-GCE<sub>red</sub> into RB-MWCNT-GCE<sub>ox</sub>. This process increases the cathodic peak current, while the anodic peak current is less in the absence of H<sub>2</sub>O<sub>2</sub>. Under the same experimental conditions, the cyclic voltammograms of RB-GCE were recorded in the absence (Fig. 2, curve c) and the presence of 0.40mM of H<sub>2</sub>O<sub>2</sub> (Fig. 2, curve d). Table 2 shows the electrochemical characteristics of H<sub>2</sub>O<sub>2</sub> reduction at various electrode surfaces. The results point to the best electrocatalytic effect for H<sub>2</sub>O<sub>2</sub> reduction observed at RB-MWCNT-GCE. As can be seen in the case of the reduction responses of H<sub>2</sub>O<sub>2</sub> at RB-GCE and RB-MWCNT-GCE, there is a dramatic enhancement in the cathodic peak current at RB-MWCNT-GCE as compared to the value obtained at RB-GCE. Similarly, the peak potential of H<sub>2</sub>O<sub>2</sub> reduction at RB-MWCNT-GCE (Fig. 2, curve b) is less negative compared with that at MWCNT-GCE (Fig. 2, curve e). Also, there is no peak potential observed for H<sub>2</sub>O<sub>2</sub> at AGCE and Reactive Blue modified Glassy Carbon Electrode (RB-GCE) (Fig. 2, curve f, h). The above results clearly show that a combination of MWCNT and RB definitely improves the characteristics of H<sub>2</sub>O<sub>2</sub> reduction. The inset of Fig. 2 shows the dependence of the voltammetric response of RB-MWCNT-GCE on H<sub>2</sub>O<sub>2</sub> concentration. As can be seen, with the addition of H<sub>2</sub>O<sub>2</sub>, there was an increase in the cathodic current.

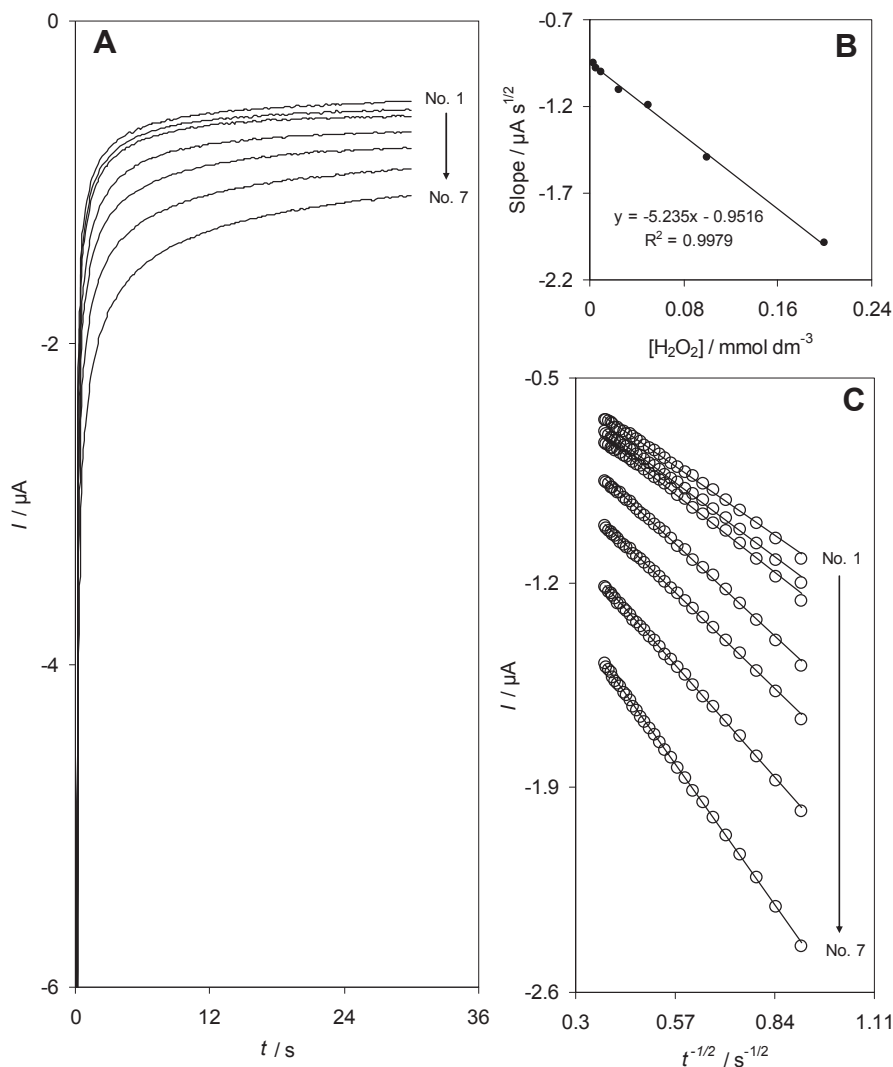
The cyclic voltammograms of a 0.40mM H<sub>2</sub>O<sub>2</sub> solution at different sweep rates are shown in Fig. 3. The inset of Fig. 3 shows that the plot of the catalytic peak current versus the square root of the sweep rate is linear, suggesting that the reaction is diffusion limited. This is an ideal result for quantitative applications [25]. Also, the number of electrons, n, in the overall reduction reaction of H<sub>2</sub>O<sub>2</sub> can be obtained from the plot of slope of I<sub>p</sub> versus v<sup>1/2</sup> (inset of Fig. 3). According to the following equation for a totally irreversible diffusion controlled process:

$$I_p = 3.01 \times 10^5 n[(1 - \alpha)n_a]^{1/2} A C_b D^{1/2} v^{1/2} \quad (1)$$

and considering (1-α)n<sub>a</sub> = 0.7 (see below), D = 2.34 × 10<sup>-6</sup> cm<sup>2</sup>/s, C<sub>b</sub> = 4 × 10<sup>-7</sup> mol/cm<sup>3</sup>, and A = 0.0314 cm<sup>2</sup>, it is estimated that the total number of electrons involved in the reduction of H<sub>2</sub>O<sub>2</sub> is n = 2.02 ~ 2. Similar values were also previously reported for the electroreduction of H<sub>2</sub>O<sub>2</sub> [30–32].

The electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub> at the modified electrode surface can be explained according to an E<sub>r</sub>C<sub>i</sub> catalytic (E<sub>r</sub>C<sub>i</sub>) mechanism. It is shown in the following equations [25]:





**Fig. 5 – (A) Chronoamperometric responses of reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode in a 0.1M phosphate buffer solution (pH 7.0) at a potential step  $-300$  mV for different concentrations of  $\text{H}_2\text{O}_2$ . The numbers of 1–7 correspond to 0.003–0.20mM  $\text{H}_2\text{O}_2$ . Insets: (B) plots of  $I$  versus  $t^{-1/2}$  obtained from chronoamperograms. (C) plot of the slope of straight lines against the  $\text{H}_2\text{O}_2$  concentrations.**

As can be seen,  $\text{H}_2\text{O}_2$  reduces by the reduced form of RB adsorbed at the MWCNT surface, RB-MWCNT-GCE (reduced form). The symbols  $E_r$  and  $C_i$  imply reversible electrochemical and irreversible catalytic chemical reactions. For an  $E_rC_i$  mechanism, Andrieux and Saveant [33] developed a theoretical model and derived the following equation for the relationship between the electrocatalytic peak current and the concentration of the substrate for a case of a slow scan rate,  $\nu$ , and a large catalytic rate constant,  $k'$ :

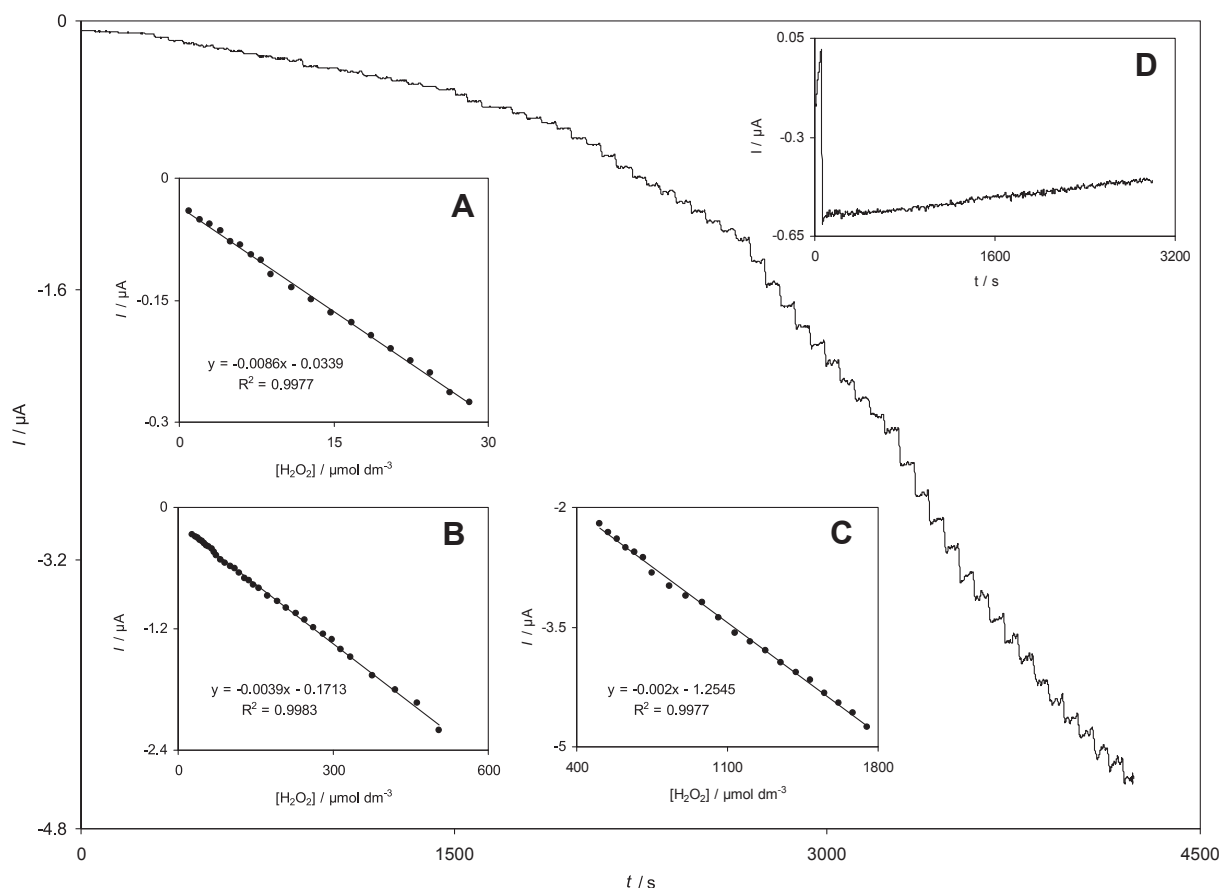
$$I_{\text{cat}} = 0.496nFAC_bD^{1/2}\nu^{1/2}(nF/RT)^{1/2} \quad (4)$$

where  $D$  and  $C_b$  are the diffusion coefficient ( $2.34 \times 10^{-6} \text{ cm}^2/\text{s}$  is obtained by chronoamperometry as below) and the bulk concentration of  $\text{H}_2\text{O}_2$ . Low values of  $k'$  result in coefficient values lower than 0.496 [33]. For low scan rates (8–20 mV/s), we found the value of this coefficient to be 0.3 for the modified electrode based on the slope of the plot in the inset of Fig. 3. An

average value of  $k' = 4.6 (\pm 0.1) \times 10^{-4} \text{ cm/s}$  was calculated by such a working curve.

In order to obtain information about the rate-determining step, the linear sweep voltammograms of 0.40mM  $\text{H}_2\text{O}_2$  were obtained at different sweep rates (Fig. 4). The inset of Fig. 4 shows the Tafel plots drawn from the data of the rising part of the current voltage curves recorded at various scan rates. This part of the voltammogram, known as Tafel region, is affected by the electron transfer kinetics between  $\text{H}_2\text{O}_2$  and the surface-confined RB-MWCNT-GCE. The results of polarization studies for electroreduction of  $\text{H}_2\text{O}_2$  at the modified electrode show that the average of Tafel slope of different plots agree well with the involvement of one-electron transfer process, assuming an average charge transfer coefficient of,  $\alpha_{\text{ave}} = 0.3 \pm 0.01$ . Also, the exchange current density,  $j_0$ , is accessible from the intercept of the Tafel plots and the geometric area.





**Fig. 6 – Amperometric responses at a rotating reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode (rotation speed 2000 rpm) held at  $-300$  mV in different concentrations of  $H_2O_2$ . Insets A, B, and C indicate variations of the amperometric currents vs.  $H_2O_2$  concentrations in the ranges of (A)  $1.99\text{--}28.2\mu\text{M}$ , (B)  $282\text{--}504\mu\text{M}$ , and (C)  $0.504\text{--}1.75\mu\text{M}$ . Inset D shows the stability of the response of the reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode to  $100.0\mu\text{M}$   $H_2O_2$  during 2900 seconds.**

The average value obtained for the exchange current density of  $H_2O_2$  at RB-MWCNT-GCE was found to be  $3.64 \pm 0.1 \mu\text{A}/\text{cm}^2$ .

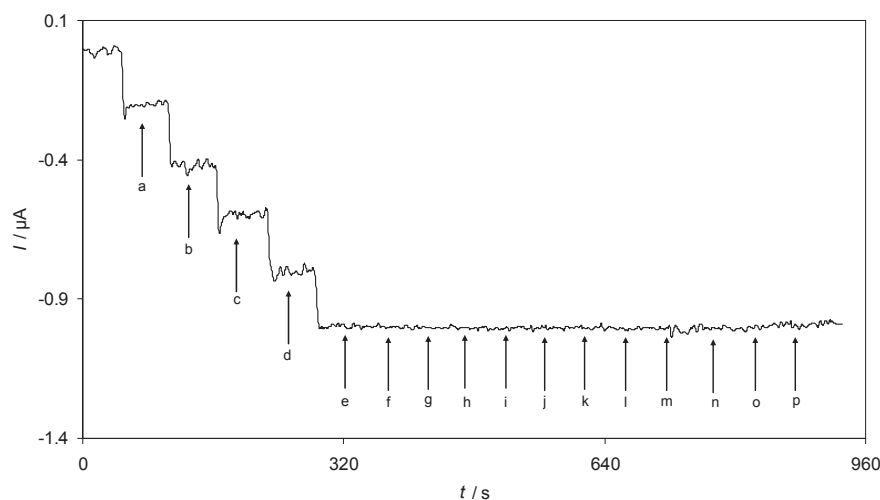
The electrocatalytic reduction of  $H_2O_2$  by RB-MWCNT-GCE was also studied by chronoamperometry. The

chronoamperograms obtained at the potential step of  $-300$  mV are shown in Fig. 5. Inset A of Fig. 5 shows the experimental plots  $I$  versus  $t^{-1/2}$  with the best fits for different concentrations of  $H_2O_2$ . The slopes of the resulting straight lines were then plotted versus the  $H_2O_2$  concentration (Fig. 5,

**Table 3 – Comparison of analytical parameters of several modified electrodes for  $H_2O_2$  determination.**

Modifier	Method	Linear range ( $\mu\text{M}$ )	Detection limit ( $\mu\text{M}$ )	Ref.
Mn-complex-SWCNTs	Amperometry	1–1500	0.2	[3]
[PFeW <sub>11</sub> O <sub>39</sub> ] <sup>4-</sup> polyoxoanion	Amperometry	10–200	7.4	[11]
AgNPs/PMES	Amperometry	0.6–540	0.18	[13]
Nanonickel oxide/thionine	Amperometry	5–20000	1.67	[19]
Nanonickel oxide/celestine blue	Amperometry	1–10000	0.37	[19]
Nano-TiO <sub>2</sub> /DNA/thionin	Amperometry	50–22300	50	[30]
n-octylpyridinium Hexafluorophosphate	Amperometry	20–800	12	[32]
RB-MWCNT-GCE	Amperometry	0.99– $2.8 \times 10^3$ , $2.8 \times 10^2\text{--}5.1 \times 10^2$ , $5.1 \times 10^2\text{--}1.8 \times 10^3$	0.27	This work

AgNPs/PMES = silver nanoparticles/poly(2-(N-morpholine) ethane sulfonic acid); ERGO-Ag nanocomposite = electrochemically reduced graphene oxide (ERGO)-silver nanoparticle hybrid film; Mn-complex-SWCNTs = single wall carbon nanotubes and phenazine derivative of Mn-complex; PB/Pd-Al = aluminum electrode plated by thin layer metallic palladium and modified by Prussian blue; RB-MWCNT-GCE = reactive blue multiwalled carbon nanotubes modified glassy carbon electrode.



**Fig. 7** – Amperometric responses at a rotating reactive blue 19 multiwalled carbon nanotube-modified glassy carbon electrode (rotation speed 2000 rpm) held at  $-300$  mV in buffer solution (pH = 7) for successive addition of  $\text{H}_2\text{O}_2$  (a–e, 20–220  $\mu\text{M}$ ) and 1mM of (f) ascorbic acid, (g) uric acid, (h) glucose, (i) dopamine, (j) oxalic acid, (k)  $\text{NaNO}_3$ , (l)  $\text{Mg}(\text{NO}_3)_2$ , (m) KCl, (n) KI, (o)  $\text{NaNO}_2$  and (p)  $\text{Na}_2\text{SO}_4$ .

inset B), from whose slope and using the Cottrell equation [25] a diffusion coefficient was calculated:  $2.34 \times 10^{-6} \text{ cm}^2/\text{s}$ .

### 3.3. Amperometric determination of $\text{H}_2\text{O}_2$

Amperometry was used to determine the linear ranges and the detection limit of  $\text{H}_2\text{O}_2$  at RB-MWCNT-GCE. The use of amperometry was based on the fact that it has a much higher current sensitivity than cyclic voltammetry. Fig. 6 shows the amperograms obtained for a rotating RB-MWCNT-GCE (rotation speed 2000 rpm), under conditions where the potential was held at  $-300$  mV in a 0.1M phosphate buffer solution (pH 7.0) containing different concentrations (0.99–1.8mM) of  $\text{H}_2\text{O}_2$ .

The insets shown in Fig. 6 demonstrate the linear relationship of the electrocatalytic current vs.  $\text{H}_2\text{O}_2$  concentration in the three linear ranges: (A) 0.99–28  $\mu\text{M}$ ; (B) 28–510  $\mu\text{M}$ ; and (C) 0.51–1.8mM. The decrease of sensitivity

(slope) in the second and third linear range (higher analyte concentrations) is likely to be due to electrode surface pollution.

According to the method mentioned in reference [34], the lower detection limit,  $C_m$ , was found to be 0.27  $\mu\text{M}$  using the equation  $C_m = 3s_{bl}/m$ , where  $s_{bl}$  is the standard deviation of the blank response which is obtained from 14 replicate measurements of the blank solution (0.00077  $\mu\text{A}$ ) and  $m$  is the slope of the calibration plot (0.0086  $\mu\text{A}/\mu\text{M}$ ). Moreover, the limit of quantitation was calculated to be 0.9  $\mu\text{M}$  by limit of quantitation =  $10s_{bl}/m$  [34]. The average amperometric peak current and the precision estimated in terms of the coefficient of variation for repeated measurements ( $n = 10$ ) of 15.0  $\mu\text{M}$   $\text{H}_2\text{O}_2$  at RB-MWCNT-GCE were  $0.167 \pm 0.005 \mu\text{A}$  and 3.02% respectively. It indicated that the sensor possessed good reproducibility. Also the stability of RB-MWCNT-GCE under working conditions was investigated in the presence of 100.0  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$ . Inset D of Fig. 6 indicates the response stability of RB-MWCNT-GCE to 100.0  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  solution during 2900 seconds. As shown, the amperometric current of  $\text{H}_2\text{O}_2$  reduction remained almost constant during the experiment. In Table 3, some of the response characteristics obtained for  $\text{H}_2\text{O}_2$  in this study are compared to those previously reported by others [3,11,13,19,30,32]. As can be seen, the responses of the proposed modified electrode are superior in some cases as compared to the previously reported modified electrodes.

### 3.4. Interference study

An important problem in determining  $\text{H}_2\text{O}_2$  is the effect of potential interfering ions. A survey on the influence of various substances as potential interfering compounds on the  $\text{H}_2\text{O}_2$  determination under optimum conditions at the RB-MWCNT-GCE surface was done. Fig. 7 shows the amperometric response of modified electrode during addition of 20–220  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  and different interferences to buffer solution (pH 7). As

**Table 4** – Determination and recovery of  $\text{H}_2\text{O}_2$  concentration in three fruit juice samples using reactive blue multiwalled carbon nanotube-modified glassy carbon electrode.

Samples	Added ( $\mu\text{M}$ )	Found <sup>a</sup> ( $\mu\text{M}$ )	RSD (%)	Recovery %
Apple juice	–	Not detected	–	–
	10.0	10.3	2.7	103
	20.0	19.5	2.2	97.5
Orange juice	–	Not detected	–	–
	8.0	8.2	2.5	102.5
	16.0	15.7	2.9	98.1
Banana juice	–	Not detected	–	–
	12.0	12.4	2.8	103.3
	24.0	23.5	2.1	97.9

RSD = Relative Standard Deviation.

<sup>a</sup> Three replicate measurements were made on the same samples. Detection limit = 0.27  $\mu\text{M}$ .

illustrated, nearly all compounds such as ascorbic acid, uric acid, glucose, dopamine, oxalic acid,  $\text{NaNO}_3$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{KCl}$ ,  $\text{KI}$ ,  $\text{NaNO}_2$ , and  $\text{Na}_2\text{SO}_4$  do not interfere when present in 1.0 mM concentration. The sensor response toward  $\text{H}_2\text{O}_2$  before and after interference injection is the same. The results indicate that  $\text{H}_2\text{O}_2$  recovery was almost quantitative in the presence of an excess amount of possible interfering species at this sensor.

### 3.5. Analysis of real samples

Sometimes, for the aseptic packaging of fruit juices,  $\text{H}_2\text{O}_2$  is used as a chemical agent for sterilization. However, the  $\text{H}_2\text{O}_2$  residues in higher concentration are skin irritants and may affect the human health. Therefore, determination of  $\text{H}_2\text{O}_2$  in fruit juices is important. The maximum level of  $\text{H}_2\text{O}_2$  residual allowed in the aseptic packaging in accordance with US Food and Drug Administration (21 CFR 178.1005) is 0.5 ppm (14.7  $\mu\text{M}$ ). Since the lower linear range of the proposed sensor is 0.99–28.0  $\mu\text{M}$ , this sensor can be applied for determination of  $\text{H}_2\text{O}_2$  in aseptic packaging.

From the results, it is apparent that RB-MWCNT-GCE possesses a high sensitivity and a good detecting capability to determine  $\text{H}_2\text{O}_2$  in real samples. In order to test its practical application, the modified electrode was used to determine  $\text{H}_2\text{O}_2$  in three fruit juice samples. As the electrochemical techniques are able to determine  $\text{H}_2\text{O}_2$  without pretreatment, therefore the fruit juice samples were directly inserted to the experimental cell. To do the experiment, 2  $\mu\text{L}$  of a fruit juice sample were diluted down to 10  $\mu\text{L}$  with a 0.1 M phosphate buffer solution (pH 7.0). Then, known amounts of  $\text{H}_2\text{O}_2$  were added, and their recoveries were determined using amperometric measurements. The results in Table 4 indicate that the Percentage of relative standard deviation (RSD%) and the recovery rates of the spiked samples are acceptable. Thus, RB-MWCNT-GCE can be efficiently used for  $\text{H}_2\text{O}_2$  determination in different fruit juice samples. It is concluded that the matrix of fruit juice samples does not interfere in determination of  $\text{H}_2\text{O}_2$  at the proposed sensor.

### 3.6. Conclusions

This paper demonstrates the construction of an RB-MWCNT-GCE and its application in determination of  $\text{H}_2\text{O}_2$ . The results of the study show that RB can be immobilized easily at the surface of MWCNT-modified GCE. Kinetic parameters such as the catalytic rate constant,  $k_s$ , transfer coefficient,  $\alpha$ , the number of electrons involved in the rate determining step,  $n_\alpha$ , and the overall number of electrons involved in the catalytic reduction of  $\text{H}_2\text{O}_2$  at the modified electrode surface have also been determined using cyclic voltammetry. The diffusion coefficient of  $\text{H}_2\text{O}_2$  has been found to be  $2.34 \times 10^{-6} \text{ cm}^2/\text{s}$  for experimental conditions using chronoamperometric results. RB-MWCNT-GCE exhibits three wide linear ranges of 0.99–28  $\mu\text{M}$ , 28–510  $\mu\text{M}$ , and 0.51–1.8 mM for  $\text{H}_2\text{O}_2$  determination by amperometry. The lower detection limit of  $\text{H}_2\text{O}_2$  at the modified electrode is 0.27  $\mu\text{M}$ . Also, amperometry can be used as an analytical method for  $\text{H}_2\text{O}_2$  determination in real samples.

### Conflicts of interest

The authors declare no conflicts of interest.

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