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Research Article

Catalase-Based Modified Graphite Electrode for Hydrogen Peroxide Detection in Different Beverages

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A catalase-based (NAF/MWCNTs) nanocomposite film modified glassy carbon electrode for hydrogen peroxide (H_2O_2) detection was developed. The developed biosensor was characterized in terms of its bioelectrochemical properties. Cyclic voltammetry (CV) technique was employed to study the redox features of the enzyme in the absence and in the presence of nanomaterials dispersed in Nafion® polymeric solution. The electron transfer coefficient, α, and the electron transfer rate constant, k_s, were found to be 0.42 and 1.71 s^{-1}, at pH 7.0, respectively. Subsequently, the same modification steps were applied to mesoporous graphite screen-printed electrodes. Also, these electrodes were characterized in terms of their main electrochemical and kinetic parameters. The biosensor performances improved considerably after modification with nanomaterials. Moreover, the association of Nafion with carbon nanotubes retained the biological activity of the redox protein. The enzyme electrode response was linear in the range 2.5–1150 μmol L^{-1}, with LOD of 0.83 μmol L^{-1}. From the experimental data, we can assess the possibility of using the modified biosensor as a useful tool for H_2O_2 determination in packaged beverages.

1. Introduction

In recent years, many researchers focused their activity on developing new tools to detect H_2O_2, not only as an oxidases reaction byproduct but also as a conservative compound in food and drugs [1, 2]. Indeed, hydrogen peroxide finds significant employment in industrial processes as an oxidant [3]: in particular, hydrogen peroxide is released into the environment in either small or large amounts, since it is used as oxidant, whitening, or sterilant tool in packaging materials owing to its sporicidal and bactericidal features [4–6]. Nevertheless, high H_2O_2 concentrations would be dangerous for human beings [7–10].

Several analytic methods as chemiluminescence [11–17], photometry [18], fluorimetry [19–21], titrimetry [22, 23], spectrophotometry [23–26], high-performance liquid chromatography (HPLC) [27], and especially electrochemistry [3, 28–37] are reported in the literature for detection of hydrogen peroxide.

The electrochemical techniques provide some interesting advantages in comparison to the other ones mentioned above like fast, specific, and cheap monitoring of hydrogen peroxide [37–43]. The direct reduction of H_2O_2at a bare sensor is not suitable for analytical measures due to its slow kinetics and high potentials required for redox reactions [44]. To overcome these problems, several modified electrochemical sensors were developed. Electrochemical biosensors based on the biocatalytic activity of immobilized enzymes towards the substrate $H_2O_2$are helpful because of their high sensitivity, selectivity, and ease of use [45, 46]. Some authors, in the recent years, have applied different modified biosensors, based on various redox proteins, to realize interesting tools for the monitoring of H_2O_2 [45, 47–55].

Catalase (CAT) belongs to oxidoreductase family class and has a heme prosthetic group at its active site with ferric ion (Fe(III)) [48, 50, 56–59]. The catalytic ability of CAT to reduce hydrogen peroxide was used in the developing of biosensors [50, 56, 60]. To investigate CAT catalytic activity,

it is important to study its capacity to perform direct electron transfer (DET) to the electrode surface. It is usually difficult to observe the DET because the heme groups are buried deeply inside in the large structure of the protein [61, 62]. Also, denaturation of the redox protein could occur on the sensor surface due to the immobilization method and to the matrix composition. To overcome these problems and promote the DET carbon nanotubes (CNTs), modified electrodes are widely employed as support for the physical immobilization of biological molecules to promote the DET thanks to their high surface/volume ratio and conductivity and also to enhance sensors and biosensors performances [63–67]. A drawback on the use of CNTs to modify electrode surface is their insolubility [68, 69]. However, some authors have obtained good results in the CNTs modification of the electrode surface by using polymers as dispersing support [70, 71]. Nafion is a perfluorinated polymer resistant to chemical attack and the CNTs dispersion in its film has been investigated [72–74].

In the present study, we report the development of a biosensor for H_2O_2monitoring based on the immobilization of catalase in a Nafion film containing dispersed functionalized MWCNTs-COOH. The Nafion film ensures efficient immobilization of the protein in its native configuration. The DET of catalase was investigated either on modified or on bare electrode to identify the optimal conditions for H_2O_2 detection. In view of the possible practical application, the same modification steps were performed on screen-printed electrodes (SPEs) with a working electrode based on mesoporous graphite (MG-SPE). Finally, the obtained biosensor was applied for the determination of hydrogen peroxide in beverages samples.

2. Experimental

2.1. Materials and Reagents. Catalase from bovine liver (CAT, EC 1.11.1.6, activity ≥ 10,000 U mg^{-1} protein) was supplied by Sigma-Aldrich (Switzerland) and stored at −20°C. All chemicals used were of analytical grade. In particular, Na_2HPO_4, NaH_2PO_4, $HOC(COOH)(CH_2COOH)_2$, KCl, ($K_3[Fe(CN)_6]$), Nafion 117 solution (NAF, purum, ~5% solution in a mixture of lower aliphatic alcohols and water), CH_3CH_2OH (~96% v/v), and H_2O_2 (30 wt.% in H_2O) were purchased from Sigma-Aldrich (Switzerland). High purity deionized water (resistance: 18.2 MΩ × cm at 25°C; TOC: <10 $\mu g\ L^{-1}$) obtained from a Millipore Direct-Q 3 UV system (France) was used throughout the experiments. The working solutions were prepared by diluting the stock solution with 0.1 mol L^{-1} phosphate buffer solution and 0.1 mol L^{-1} KCl, pH 7.0 (PBS buffer solution), and then deoxygenated by bubbling $N_2$for about 20 min. Multiwalled carbon nanotubes modified with carboxylic groups (MWCNTs-COOH) were obtained from DropSens (Spain).

2.2. Electrochemical Measurements. All electrochemical measurements were performed with μ-Autolab type III potentiostat (EcoChemie, Netherlands) controlled using the GPES Manager program (EcoChemie, Netherlands) at room temperature in N_2 atmosphere. Batch electrochemical experiments were performed in a 5 mL thermostated glass cell (model 6.1415.150, Metrohm, Switzerland) containing PBS buffer solution, with a conventional three-electrode system. Different working electrodes were used, in particular glassy carbon electrode (GCE, cat. 6.1204.300, Metrohm, Switzerland, ϕ = 3 mm) and a mesoporous graphite screen-printed electrode (MG-SPE, model DRP-110MC, ϕ = 4 mm, DropSens, Spain). A saturated calomel electrode (SCE, cat. 303/SCG/12, Amel Instruments, Italy) as the reference electrode and a carbon rod (cat. 6.1248.040, Metrohm, Switzerland) as the counter one were employed. For SPEs, the counter electrode was carbon and the reference one was silver, respectively. All the reported potentials are referred to as saturated calomel electrode (E = 0.241 V versus NHE). All pH measures were performed using a digital pH meter (827 pH lab, Metrohm, Italy). The morphology of the samples was observed using high-resolution field emission scanning electron microscopy (HR FESEM, Zeiss Auriga Microscopy) equipped with Microanalysis EDS ≤ 123 Mn-Kα eV (Bruker).

2.3. Procedures. The GCE surface was polished with 0.3 and 0.05 μm alumina slurry on polishing silk cloth (SIEM, Italy) and rinsed with deionized water. Then, the electrode was sonicated in deionized water to remove trace of alumina from the surface (Sonicator AU-32, ArgoLab, Italy).

The physical immobilization of the enzyme was realized by dropping onto the working electrode surface 2 μL of 0.5 wt.% Nafion solution containing 1 mg mL^{-1} of redox protein either in the presence or in the absence of 1 mg mL^{-1} of MWCNTs-COOH. The electrode surface was finally air-dried for about 20 min at room temperature. The biosensors were stored in PBS buffer solution at 4°C before use.

The analysis protocol of real beverages is described as follows: 2.5 mL of different beverages sample was diluted to 10 mL with PBS buffer solution. Then, a certain amount of H_2O_2 (15 μmol L^{-1}) was added and the solutions were deoxygenated. Then, the samples were analyzed directly by cyclic voltammetry (CV) method and finally the recoveries were evaluated. For the study of pH dependance, the McIlvaine buffer was used at different pH values.

3. Results and Discussion

3.1. Electrochemical Characterization of Glassy Carbon Electrode after Steps of Modification. The effect on the improvement of electrochemical performances by using nanomaterials as MWCNTs-COOH was evaluated with cyclic voltammetry measurements of the electroactive area (A_e) and of the heterogeneous standard rate constant (k^0) of the different electrodes. The cyclic voltammograms (not shown) were recorded in a solution of 1. 1 mmol L^{-1} potassium ferricyanide in PBS buffer solution. A_e was determined from the Randles-Sevčik equation: $I_p = 2.686 \times 10^5 n^{3/2} A_e D^{1/2} C v^{1/2}$ [95], where I_p is current in amps (A), n is number of electrons transferred of $K_3[Fe(CN)_6]$ by cyclic voltammetry (CV) in the redox event (usually 1), A_e is electroactive area (cm^2), D is

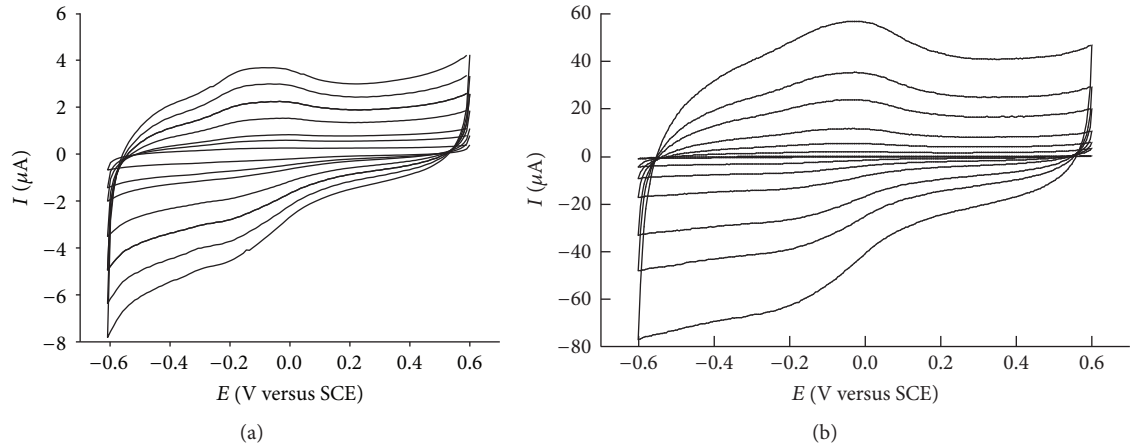

Figure 1: CVs for NAF-GCE-CAT (a) and NAF-MWCNTs-COOH-GCE-CAT (b) at different scan rates (10–500 mV s^{-1}) in deoxygenated PBS buffer solution.

Table 1: Electroactive area and heterogeneous standard rate constant of bare sensor and after modification steps.

Sensor	A_e/mm^2	$k^0 \times 10^{-4}$/cm s^{-1}
Bare-GCE	4.89	9.3
NAF-GCE	2.16	6.5
NAF-MWCNTs-COOH-GCE	16.42	13.5

diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}), C is concentration (mol cm^{-1}), and v is scan rate (Vs^{-1}). k^0 was calculated by an extended method [96], a combination of Nicholson [97] and Klingler and Kochi treatments [98], by CV data using the same solution described above, in the scan rate range 5–100 mV s^{-1}.

By comparing the results (see Table 1) arising from the several modification steps of the sensor, two aspects can be pointed out: (i) the parameters obtained for the Nafion modified sensor (NAF-GCE) are lower than both the bare sensor (bare-GCE) and the nanomaterial modified sensor (NAF-MWCNTs-COOH-GCE): presumably, this is due to the Nafion film that hinders the charge transfer and slows down the substrate rate towards the sensor surface; (ii) the use of carbon nanotubes enhances hugely the electrochemical signal increasing A_e (about 4 and 8 times compared to the bare-GCE and NAF-GCE, resp.) and improves k^0 of the ferricyanide ion towards the sensor surface despite the ion exchange polymer presence (about 1.5 and 2 times compared to the bare-GCE and NAF-GCE, resp.): this could be ascribed to their excellent properties of increasing area/volume ratio and high electron conductivity and of facilitating the electron transfer [99–104]. The association of these nanomaterials with Nafion (as solubilizing agent) does not impair the electrocatalytic features of carbon nanotubes. This aspect was also observed in our previous work where the use of NAF/MWCNTs composite film has greatly increased the transfer charge rate [105].

3.2. Biosensor Voltammetric Behavior before and after Nanomaterial Modification. The comparison of electrocatalytic performances was evaluated by using catalase as model redox protein and comparing the voltammetric behavior (Figures 1(a) and 1(b)) measuring several electrochemical parameters (see Section 3.4). The catalase was immobilized by a Nafion film onto the GCE surface, in the absence and in the presence of MWCNTs-COOH; the electrochemical behavior of the modified electrodes has been investigated in N_2 saturated PBS buffer solution, using CV. The cyclic voltammograms were recorded at NAF-GCE-CAT and NAF-MWCNTs-COOH-GCE-CAT modified GCEs in the potential range from 0.6 V to −0.6 V. In the absence of MWCNTs-COOH, catalase immobilized in a Nafion film onto GCE surface showed a quasi-reversible signal (see Figure 1(a)) with a midpoint potential of $E^{0\prime} = -128$ mV; the separation of cathodic and anodic peak potential $\Delta E_p = 80$ mV (at scan rates lower than 100 mV s^{-1}) indicated a fast electron transfer reaction according to the literature [106]. For the other modified electrode, when the redox protein is in the presence of carbon nanotubes, CV experiments yielded evidence of a prominent increase (about 20 times) of faradic current (Figure 1(b)) and also an enhancement of electron transfer kinetic was observed at a constant amount of immobilized protein. In particular, $E^{0\prime}$ shifted to a more negative potential value (−140 mV) and ΔE_p was 70 mV, assuming that carbon nanotubes play an important role in the rising of the system reversibility.

3.3. Study of pH Dependence on the Modified Electrode. The effect of pH solution on the modified NAF-MWCNTs-COOH-GCE-CAT electrode was also tested. In Figure 2(a), the peak currents at different pH values are shown. The maximum of anodic current occurred at pH 7.0. This value was consistent with that reported for catalase enzyme [60, 76–78]. Based on these results, pH 7.0 for PBS buffer solution was used as the optimal pH for further experiments. Also, the influence of pH solution on the oxidation peak potentials was investigated. The oxidation peak potential was reported versus solution pH values in the range 3.5–8.0 (Figure 2(b)). The obtained slope (0.044 V) suggests that the reaction at

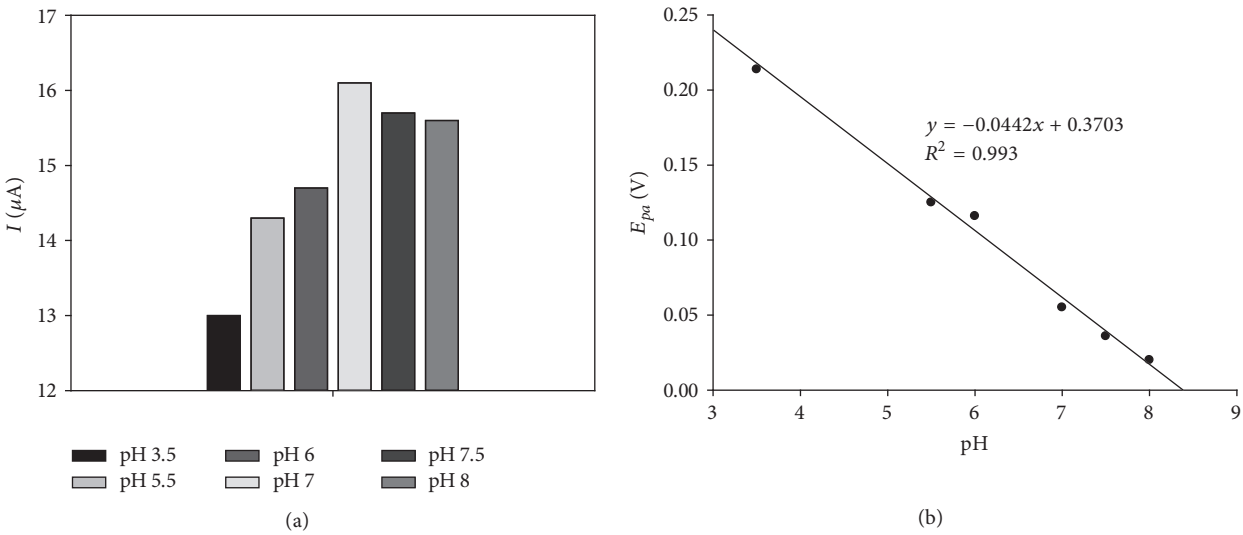

Figure 2: The effect of pH on the redox peak currents of NAF-MWCNTs-COOH-GCE-CAT in various buffer solutions with pH values 3.5, 5.5, 6.0, 7.0, 7.5, and 8.0 (a); E_{pa} versus pH plot (b).

the electrode surface is accompanied by proton transfer. The slope value is slightly smaller than Nernst's value of $0.059\,\mathrm{V\,pH^{-1}}$ for the reaction of one electron coupled to one proton [76]. This is probably ascribable to the influence of protonation states of trans ligands of the heme iron and amino acids around the heme, or the protonation of H_2O molecule coordinated to the coordinated iron [107, 108].

3.4. Cyclic Voltammetric Studies of Direct Electron Transfer of Catalase before and after Nanomaterial Modification of the Biosensor. Figure 3(a) shows typical cyclic voltammograms of NAF-MWCNTs-COOH-GCE-CAT biosensor at different scan rates (10–1400 mV s^{-1}). The dependence of peak currents and peak potentials on the scan rate is also observed in Figures 3(b) and 3(c), respectively. As is obvious from Figure 3(b), the peak currents change linearly with scan rate over a range of 10 to 1400 mV s^{-1} (with correlation coefficients of 0.9924 and 0.9914), as expected for thin layer electrochemistry [35, 109] and according to a surface-controlled process. The slope of corresponding $\log I_p$ versus $\log \nu$ linear plot, with a correlation coefficient of 0.9949, was found to be 1.115, very close to the theoretical slope 1 for thin layer voltammetry [109].

The surface concentration of electroactive redox protein (Γ) can be estimated using Faraday law (see (1)) and calculated from the slope of peak current/scan rate plot [76, 109]:

$$\Gamma = \frac{4I_pRT}{n^2F^2A\nu}, \quad (1)$$

where ν is the scan rate, A is the electrode surface area (0.07 cm^2), T is the temperature, n is the number of electrons, and R and F are gas and Faraday constants, respectively. Thus, the average surface concentration Γ of catalase was found to be 4.76×10^{-10} mol cm^{-2}, which indicates that the immobilized enzyme is in the form of an approximate monolayer on the surface of the modified electrode [63, 75].

Table 2: Electrochemical parameters for immobilized catalase either in the absence or in the presence of nanomaterials.

Biosensor	$E^{0\prime}$/mV	α	k_s/s^{-1}	Γ/mol cm^{-2}
NAF-GCE-CAT	−128	0.89	1.03	2.30×10^{-10}
NAF-MWCNTs-GCE-CAT	−138	0.38	1.65	3.50×10^{-10}
NAF-MWCNTs-COOH-GCE-CAT	−140	0.42	1.71	4.76×10^{-10}

Moreover, the peak-to-peak separation at a scan rate of 10 mV s^{-1} was approximatively 70 mV, indicating a quasi-reversible electron transfer process. Based on the Laviron theory [109], the transfer coefficient (α) and the electron transfer rate constant (k_s) for immobilized catalase either in the absence or in the presence of nanomaterials can be estimated by measuring the variation of peak potential separation with scan rate (at higher scan rates, as shown in Figure 3(c)) and reported in Table 2.

Besides, by comparing our proposed biosensor to other similar ones in the literature [60, 77–83], all based on CAT modified GCEs by using MWCNTs, it is evident that the amount of our electroactive catalase is higher, probably due to the simple NAF/MWCNTs matrix that could increase the exposure extent of the heme group in the catalase enzyme (see Table 3). The formal potential $E^{0\prime}$ of our biosensor is much less negative than those proposed by other authors [63, 76–83, 108, 110]. The formal potential value is dependent on the protein structure [111, 112], so a change of the heme protein in the NAF/MWCNTs composite film results in a shift of $E^{0\prime}$ to positive potential values. Moreover, partial denaturation of the enzyme could cause heme leakage and then a negative shift of the redox peaks (change in the coordination sphere) [113].

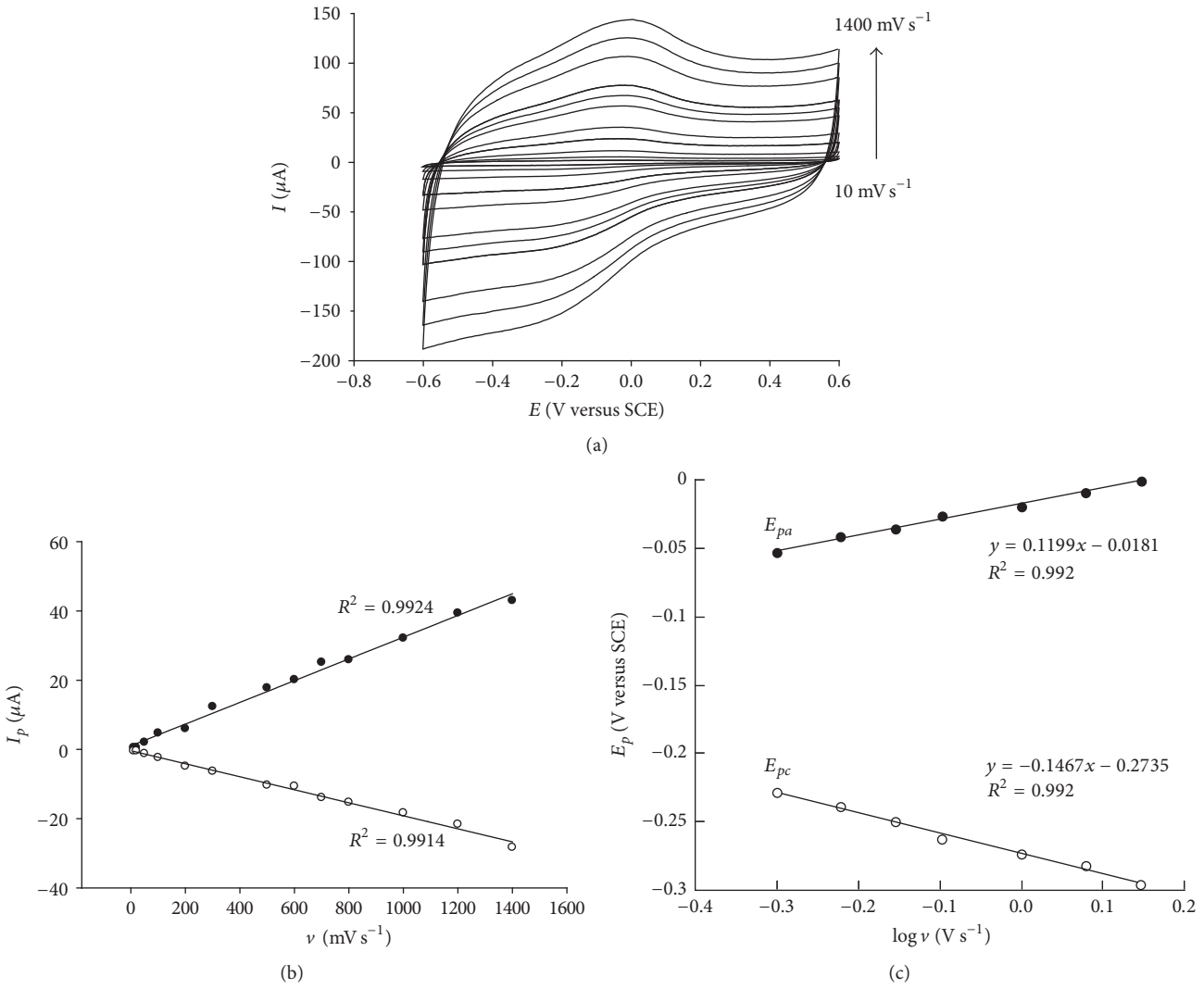

Figure 3: CVs for NAF-MWCNTs-COOH-GCE-CAT in deoxygenated PBS buffer solution at various scan rates (a). Relationship between the anodic and cathodic peak currents and scan rates (b). Relationship between peak potential separation and logarithm of scan rates (c).

3.5. Catalytic Activity of Catalase. The voltammetric characterization of the hydrogen peroxide reduction by means of the developed NAF-MWCNTs-COOH-GCE-CAT biosensor was performed in PBS buffer solution, at a scan rate of 50 mV s^{-1} (Figure 4(a)).

An increase in the cathodic peak with the hydrogen peroxide concentration and a decrease in the anodic peak during the scan reversal have been observed. Conversely, in the absence of catalase, no current change has been detected by the NAF-MWCNTs-COOH-GCE electrode. From our experiments, we confirm the EC mechanism previously reported in the literature [77, 95]:

$$\begin{aligned} &\text{Cat-Fe (III)} + e^- + H^+ \rightleftharpoons \text{Cat-Fe (II) } H^+ \\ &\qquad\qquad\qquad\qquad\qquad\qquad \text{at the electrode surface} \\ &H_2O_2 + \text{Cat-Fe (II) } H^+ \longrightarrow \text{Cat-Fe (III)} + H^+ + H_2O \\ &\qquad\qquad\qquad\qquad\qquad\qquad \text{in solution} \end{aligned} \quad (2)$$

Figure 4(b) reports the catalytic efficiency (I_c/I_d) changes versus H_2O_2 concentration; I_c and I_d are the cathodic peak currents in the presence and in the absence of hydrogen peroxide, respectively.

As can be observed, the catalytic efficiency increases with the H_2O_2concentration up to 298 μM, and then a plateau is reached. This is probably due to the denaturing effect of hydrogen peroxide at high concentration values.

Based on these results obtained using a classical GCE electrode and employing a very simple and easy immobilization procedure, the same modification system has been developed on screen-printed electrodes in view of a possible application for determination of hydrogen peroxide in real samples.

3.6. Morphological Characterization of Screen-Printed Electrodes and Electroanalytical and Kinetic Characterization. The surface morphology of the modified screen-printed electrodes (SPEs) was obtained by scanning electronic

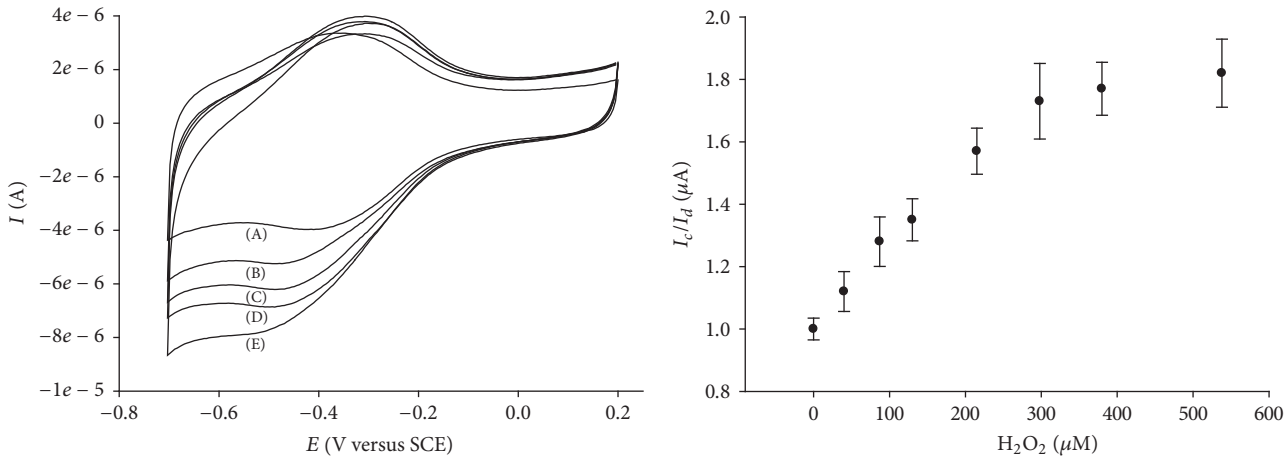

Figure 4: CVs of NAF-MWCNTs-COOH-GCE-CAT modified biosensor in the absence (A) and in the presence of 130 μM (B), 215 μM (C), 298 μM (D), and 538 μM (E) of the substrate H_2O_2 (a). Catalytic efficiency changes versus hydrogen peroxide, where I_c and I_d are the cathodic peak currents in the presence and in the absence of H_2O_2, respectively (b). Experimental conditions: deoxygenated PBS buffer solution, $\nu = 50\,\text{mV s}^{-1}$.

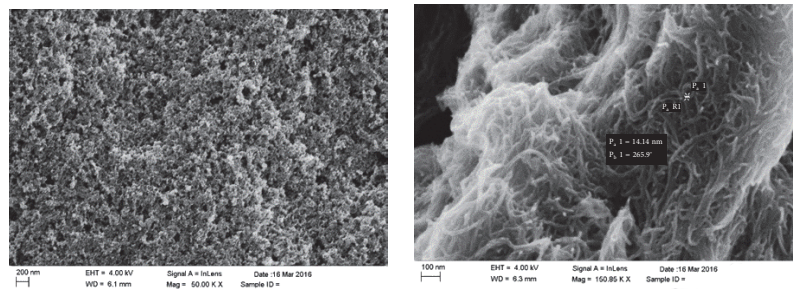

Figure 5: SEM images of electrodes surfaces: MG-SPE bare (a) and NAF-MWCNTs-COOH-MG-SPE modified electrode (b).

Table 3: Comparison of electrochemical parameters of the catalase modified glassy carbon electrodes by using MWCNTs recently developed for H_2O_2 determination.

Catalase modified GCE	$E^{0\prime}$/mV	k_s/s^{-1}	Γ/mol cm^{-2}	Ref.
[bmim][PF_6]-MWCNTs	~−100[a,d]	1.95	3.31×10^{-10}	[75]
Ionic-liquid-MWCNTs-NH_2	−460[a,d]	2.23	2.88×10^{-10}	[76]
MWCNTs-NF-DTAB	−279[a,d]	10.71	2.6×10^{-11}	[77]
CA-MWCNTs	−559[a,d]	1.22	1.49×10^{-10}	[78]
PEI-MWCNTs-NF	−450[a,e]	1.05	2.10×10^{-10}	[79]
MWCNTs-NF-DDAB	−380[a,c]	11.0	73×10^{-12}	[80]
PLL-f-MWCNTs	−471[a,c]	5.48	4.072×10^{-10}	[81]
NAF-MWCNTs-COOH-CYS-AuNPs	−441[a,d]	8.72	2×10^{-9}	[82]
NAF-MWCNTs-COOH-GCE	−140[b,d]	1.71	4.76×10^{-10}	This work

[a]Versus Ag/AgCl; [b]versus SCE; [c]pH 6.5; [d]pH 7.0; [e]pH 7.5.

microscopy (SEM). In Figure 5(a), mesoporous graphite SPE (MG-SPE bare) surface, without modification, is shown. Figure 5(b) reveals the presence of a cross-linked structure of multiwalled carbon nanotubes modified with carboxylic groups dispersed in a Nafion film (NAF-MWCNTs-COOH-MG-SPE surface). Moreover, the diameter of the carbon nanotubes (~14 nm) is indicated. In the presence of the enzyme, the highly porous architecture that is formed between the MWCNTs-COOH and the Nafion film is suitable for immobilization of catalase that is confirmed in the following electrochemical measures.

Also, electrochemical characterization of these SPEs was carried out and the results are reported in Table 4. Also, for these electrodes, the feature of nanomaterials to increase the sensor performances considerably is confirmed, so the following studies were performed using the NAF-MWCNTs-COOH-MG-SPE sensor.

Successively, the main electrochemical parameters of our proposed biosensor NAF-MWCNTs-COOH-MG-SPE-CAT were evaluated (see Table 5).

The electrochemical response of the obtained biosensor for different concentrations of $H_2O_2$was studied. The current-concentration dependence of hydrogen peroxide was modeled by using Michaelis-Menten nonlinear fitting thus

Table 4: Electroactive area and heterogeneous standard rate constant of bare screen-printed sensor and after the modification step.

Sensor-SPE	A_e/mm^2	$k^0 \times 10^{-4}$/cm s^{-1}
MG-SPE bare	7.93	16.5
NAF-MWCNTs-COOH-MG-SPE	11.65	30.2

Table 5: Electrochemical parameters for immobilized catalase in the presence of nanomaterials on mesoporous graphite SPE.

Biosensor	$E^{0\prime}$/mV	α	k_s/s^{-1}	Γ/mol cm^{-2}
NAF-MWCNTs-COOH-MG-SPE-CAT	−254	0.37	0.60	2.87×10^{-10}

Table 6: Comparison of analytical and kinetic parameters for H_2O_2 detection for different redox protein modified electrodes using H_2O_2 as substrate.

K_M^{app}, mmol L^{-1}	Slope, μA μmol^{-1} L	Linear range, μmol L^{-1}	LOD, μmol L^{-1}	R	Ref.
0.26	0.0112	0.21–3000	0.08	0.999	[34]
0.21	287.98	10–3200	3.33	0.995	[78]
0.224	0.392	1–3600	0.008	0.998	[81]
—	—	200–5000	1.0	0.997	[83]
2.61	—	5–5130	1.7	0.999	[84]
—	—	10–1130	0.65		[85]
0.51	369.2	6–1010	0.39	0.996	[86]
51.7	—	0.0067–8000	0.0022	0.998	[87]
—	—	9.8–6000	4.9	0.999	[88]
—	0.9103	0.1–100	0.05	0.997	[89]
—	0.61	0.3–1000	0.1	0.999	[88]
0.21	0.0281	1–140	0.93	0.998	[90]
0.29	0.315	50–1800	4.0	0.997	[91]
0.010		1–600	7.3		[92]
0.089		50–135	1.67		[93]
2.81		0.3–600	0.05		[94]
1.5	0.38	2.5–1150	0.83	0.999	This work

allowing the calculation of the main kinetic parameters; data obtained are reported in Table 6. It is clear that the biosensor has a good LOD of 0.83 μmol L^{-1} and a good sensitivity to determine H_2O_2 concentrations. Moreover, a comparison of analytical and kinetic parameters for H_2O_2 detection for different redox protein modified electrodes is summarized in Table 6 [34, 81, 83, 85–94, 110, 114].

Also, the reproducibility of the developed biosensor was calculated as RSD = 5.0% by using 500 μmol L^{-1} H_2O_2 in a series of six experiments. By the data achieved, the following can be assessed: (i) the immobilized enzyme retained good biocatalytic activity; (ii) the carbon nanotubes dispersed in the Nafion film provided an optimal microenvironment; (iii) the nanocomposite was a good matrix for catalase immobilization and biosensing preparation; (iv) the redox protein maintained active site accessibility and exchanged electrons with the sensor surface. This platform was applied for H_2O_2 sensing in real samples.

3.7. Determination of H_2O_2 in Beverages. Based on the results declared in the previous sections and in order to test the reliability of the proposed biosensor for practical application, different commercial beverages were chosen (tea, juice, and milk). Every sample was pretreated as reported in Section 2.3. The concentration of 15 μmol L^{-1} was chosen because an FDA regulation currently limits residual H_2O_2 to 0.05 ppm (corresponding to 15 μmol L^{-1}), leached into distilled water, in finished food packages [115]. The results show good recoveries, in the range 100.3–105.7%, for our modified NAF-MWCNTs-COOH-MG-SPE-CAT biosensor (Table 7).

3.8. Stability of NAF-MWCNTs-COOH-MG-SPE-CAT Biosensor. The shelf lifetime of our modified biosensor was tested by measuring its current response obtained for 500 μmol L^{-1} H_2O_2 concentration during a period of 21 days. The biosensor was stored in PBS buffer solution at 4°C before and after use. During the first week, a 4% decrease was observed, reaching a 15% decrease after three weeks. This result can be ascribable to the presence of the nanomaterials, which avoid the fouling phenomena of the surface which could affect the biosensor performances, and also the use of NAF/MWCNTs composite film provides a strong and

Table 7: Determination of H_2O_2 in several commercial beverages, spiked with H_2O_2 15 μmol L^{-1}, using NAF-MWCNTs-COOH-MG-SPE-CAT as biosensor.

Beverages samples	Found/μmol L^{-1}	Recovery %
Peach tea	15.9	105.7
Lemon tea	15.3	102.3
Green tea	14.8	101.0
Apple juice	14.9	100.3
Blood orange juice	15.7	104.8
Pineapple juice	14.7	102.0
Lactose-free milk	15.6	103.8

biocompatible microenvironment for stabilizing the catalase activity.

4. Conclusion

In this study, an electrochemical biosensor was developed for the determination of hydrogen peroxide concentration in packaged beverages. To this aim, direct electrochemical properties of catalase, confined in a Nafion film on the surface of a glassy carbon electrode, were studied. The electron transfer coefficient, α, the electron transfer rate constant, k_s, and the surface concentration of electroactive redox protein, Γ, were evaluated by cyclic voltammetry studies. The modification of the electrode surface by using nanostructured materials dispersed in Nafion polymeric solution resulted in an enhancement of the overall bioelectrochemical properties of the developed biosensor. The biocatalytic activity towards catalase substrate hydrogen peroxide confirmed that the immobilization procedure allowed a good microenvironment for catalase and facilitated the electron exchange to the electrode surface. Hence, based on these interesting results obtained, the same modification procedure was applied to screen-printed electrodes. Also, this platform of the modified biosensor was entirely characterized and was applied to detect H_2O_2 in spiked real samples of different commercial beverages obtaining good recoveries.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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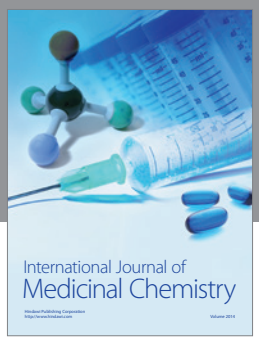

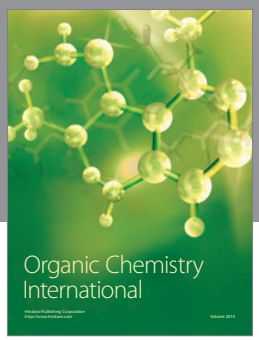

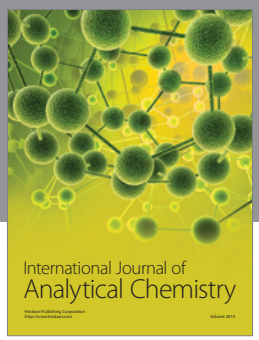

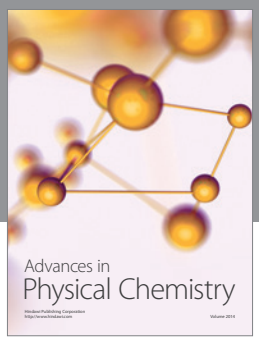

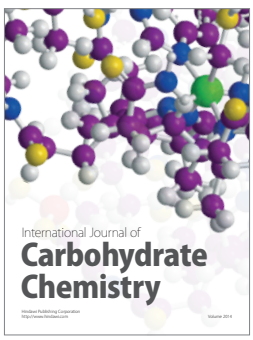

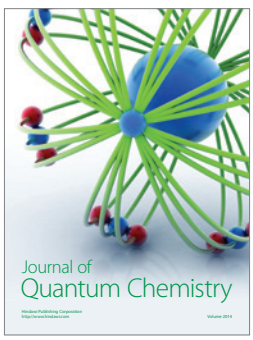

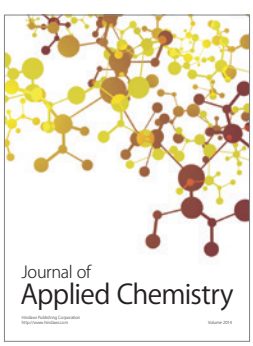

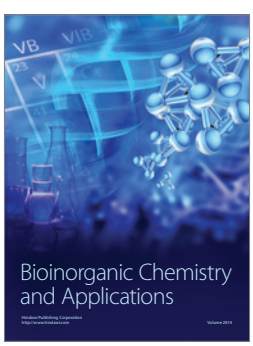

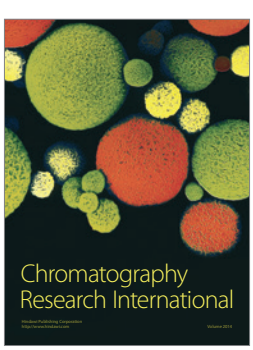

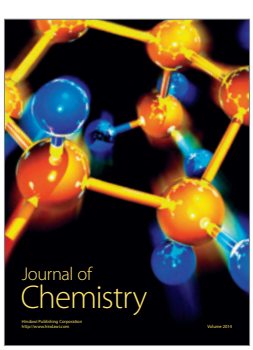

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