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Examination of the Electroanalytic Performance of Carbon Nanotube (CNT) Modified Carbon Paste Electrodes as Xanthine Biosensor Transducers

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The effect of multi-walled carbon nanotube (MWCNT) and single-wall carbon nanotube (SWCNT) on carbon paste electrode (CPE) electrochemical response was examined by introducing various portions of CNT into the CPE. The optimum electrode structure was determined by comparing the prepared electrodes' electroanalytical performance towards ferricyanide. Then these optimum compositions were modified with xanthine oxidase (XO) enzyme for obtaining a xanthine biosensor. After the optimization of biosensor working conditions, the developed systems were characterized for xanthine. Linearity was obtained in the concentration range between 1 and 100 μM xanthine with an RSD value of 3.35% for MWCNT-CPE, while with SWCNT-CPE these values were 1-20 μM and 3.5%, respectively.

The developed biosensors were also applied for the detection of xanthine in denatured plasma samples (MWCNT-CPE) and canned tuna fish samples (SWCNT-CPE), and very good recoveries were obtained.

Key Words: MWCNT, SWCNT, xanthine, amperometric biosensor

Introduction

As a relatively novel nanomaterial, carbon nanotube (CNT) has received significant attention due to its dimension and structure sensitive properties. In terms of electroanalytic and bioanalytic applications, the high electrical conductivity and electrocatalytic activity of CNT allow this nanoparticle to be used as electrode material with the ability to mediate electron transfer reactions.[1,2]

CNTs include both single-walled and multi-walled structures. Single wall CNTs (SWCNTs) comprise a cylindrical graphite sheet of nanoscale diameter capped by hemispherical ends. These nanotubes have been found to be metallic or semiconducting, depending on their structure. They are both immensely strong (a tensile strength similar to that of steel) and mechanically flexible.[3] The closure of the cylinder is

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a result of pentagon inclusion in the hexagonal carbon network of the nanotube walls during the growth process. SWCNTs have diameters typically 1 nm, with the smallest diameter reported to date of 0.4 nm. On the other hand, multi-wall CNTs (MWCNTs) comprise several layers of graphene cylinders that are concentrically nested like rings of a tree trunk with a layer spacing of 0.3-0.4 nm. MWCNTs tend to have diameters in the range 2-100 nm. MWCNT can be considered a mesoscale graphite system, whereas the SWCNT is truly a single large molecule.[4,5]

CNTs have been utilized for electroanalytical examination of a wide range of compounds such as neurotransmitters,[6–9] NADH,[1,10–12] hydrogen peroxide,[6,11–13] ascorbic[6–8] and uric acid,[4] cytochrome *c*,[14] hydrazines,[15] hydrogen sulfide,[16] amino acids,[17] and DNA.[18] As a result, it is found that besides its small size with larger surface area, when used as an electrode material CNTs offer better sensitivity, fast response, good reversibility,[19] enhanced electron transfer,[20] and easy immobilization of biological substances with retention of activity.[20,21] The reported electrocatalytic properties of CNT are claimed to originate from the open ends and possible defects of the structure.[1]

The present work is composed of 2 sections. In the first part, the electroanalytic performance of carbon paste electrodes (CPEs) containing various portions of multi-wall carbon nanotube (MWCNT) and single-wall carbon nanotube (SWCNT) separately was examined for 2 mM ferricyanide. After finding the optimum electrode structure, CPEs including CNT were modified with xanthine oxidase (XO) enzyme and the resulting composite electrodes were used as xanthine biosensor transducers. After the optimization and characterization of the developed biosensors, they were applied for the detection of xanthine in canned tuna fish and plasma samples.

Experimental

Apparatus

Cyclic voltammetric and chronoamperometric measurements were carried out with the AUTOLAB PGSTAT 12 electrochemical measurement system from ECO CHEMIE Instruments B.V., (Netherlands) driven by GPES software. The experiments were conducted in a 10 mL voltammetric cell (Metrohm), at room temperature (25 ^{o}C), using a 3-electrode configuration. A platinum electrode served as an auxiliary electrode and an Ag/AgCl electrode as a reference electrode. Electrodes were inserted into the cell through the Teflon cover.

Reagents and materials

Xanthine oxidase (XO, 0.06 units/mg solid) was obtained from Sigma. Graphite powder, multiwall (90%), and single wall (90% short) carbon nanotubes and mineral oil were purchased from Aldrich. Potassium ferricyanide was obtained from Sigma. Phosphate buffer (0.05 M, pH 7.0) served as the supporting electrolyte. All solutions were prepared using double distilled water.

Electrode preparation

XO-based MWCNT and SWCNT modified CPEs were prepared in different portions (70:30 (w/w) % graphite powder/mineral oil, appropriate amount of carbon nanotube and 0.3 mg of XO) by hand-mixing of graphite powder with enzyme and mineral oil using a spatula. A portion of the resulting paste was then packed

firmly into the electrode cavity (3.0 mm diameter and 5 mm depth) of a PTFE sieve. Electrical contact was established via a copper wire. The paste surface was smoothed on weighing paper. The surface of the resulting paste electrodes was smoothed and rinsed carefully with double distilled water.

Procedure

Chronoamperometric measurements were carried out in 0.05 M phosphate buffer (pH 7.0) medium under the operating potential of +0.9 V for MWCNT and +0.8 V for SWCNT while the solution was being stirred. The duration of each analysis was 150 s and the transient current decayed to a steady state value after 50 s in the presence of supporting electrolyte. After completion of the measurement, the electrode was rinsed with distilled water and allowed to equilibrate before another measurement.

Cyclic voltammetric measurements were conducted in the potential range of 550 or 600 mV and -400 mV, at 100 mV/s scan rate in the presence of the same supporting electrolyte.

Sample application

Canned Tuna Fish

A canned tuna fish sample (Dardanel brand) was purchased from a local market. The fish was chopped and homogenized until a fine paste was obtained after addition of 5 mL of 0.5 M $HClO_4$ for the precipitation of proteins in the sample. The denatured samples obtained were mechanically stirred for 10 min and then centrifuged at 4000 rpm for 5 min. The pH of the supernatant was adjusted to pH 7.0 with concentrated NaOH and diluted 10 times. Then these denatured sample solutions were divided into 2 parts. A known amount of xanthine was added to one part while no xanthine was added to the other.

Amperometric measurements in stirred solutions with SWCNT-CPE were obtained after transferring the corresponding analytical solution to the electrochemical cell with a potential of +800 mV versus Ag/AgCl. Determination of xanthine was made using a standard addition method that involves the addition of 2 or 3 successive denatured sample solutions not containing any xanthine analyte and then denatured samples that included a known amount of xanthine (5 μM) consecutively.

Plasma sample

Plasma samples were obtained from a local hospital and again denatured by adding 5 mL of 0.5 M $HClO_4$. The obtained plasma sample was diluted 100 times and standard addition was used for analytical assays. The other pretreatment procedures were like those described for the tuna fish sample.

Amperometric measurements were conducted under the operating potential of 0.9 V with MWCNT-CPE. Determination of xanthine involved 2 or 3 successive additions of samples without any xanthine inside and denatured samples that included a known amount of xanthine (100 μM) consecutively.

Results and Discussion

CNT modified electrodes are extensively used nowadays due to their low detection limits, high sensitivity, decreased overpotentials, and resistance to surface fouling.[22] The developed biosensor's measuring principle is based on the chronoamperometric monitoring of the current that occurs due to the oxidation of the hydrogen peroxide liberated during the enzymatic reaction as shown below.[23]

$$\text{Xanthine } O_2 \xrightarrow{\text{XOD}} \text{Uric acid } + H_2O_2$$

The aim of this work was to observe the difference in biosensor response when MWCNT and SWCNT were introduced into the structure of carbon paste electrodes. The electrocatalytic contributions of SWCNT and MWCNT were also compared based on the results obtained with this biosensor.

Electroanalytic Performance of Electrode

Optimization of Electrode Composition

The first step of the work consisted of finding the optimum CNT amount. In order to decide about the optimum composition of the developed electrode, carbon paste composites including various percentages of MWCNT and SWCNT (2%, 4%, 8%, and 100%) were prepared and their electroanalytical performances were examined by recording cyclic voltammograms of 2 mM ferricyanide. The results are given in Figures 1 and 2 and Tables 1 and 2. The best current values and hence optimum structures correspond to electrodes that contain 4% MWCNT and 4% SWCNT (Figures 1 and 2, Tables 1 and 2). A plain CPE was also prepared and compared with the SWCNT and MWCNT modified CPE (Figure 3, Table 3). The voltammograms show that the plain CPE had a current value of 14.5 μA while the CNTs including MWCNT and SWCNT had current values of 22.20 μA and 19.5 μA, with Ep values of 0.344 V (CPE), 0.327 V (MWCNT-CPE), and 0.354 V (SMWCNT-CPE), respectively. The slight increase in current value and the decrease in peak potential (as in the case of MWCNT) can be attributed to the electrocatalytic activity of CNT, which can mediate the electron transfer reactions.[1,2] However, as the amount of CNT is increased in the composite, the current value decreases. Large surface area is another important advantage of CNT that can facilitate the modification of this nanoparticle with biological molecules.[24] However, large surface areas can cause increments in background current, which might decrease the resulting current values as in the case of our work.[25] As a result, further studies were conducted using a 4% MWCNT/SWCNT modified CPE.

Table 1. Obtained current values of 2 mM ferricyanide solution at MWCNT modified CPE.

Composite	Ip (μA)
CPE + 2% MWCNT	19.6 0
CPE + 4% MWCNT	22.20
CPE + 8% MWCNT	13.30
100% MWCNT	2.52

Table 2. Obtained current values of 2 mM ferricyanide solution at SWCNT modified CPE.

Composite	Ip (μA)
CPE + 2% SWCNT	19.0
CPE + 4% SWCNT	77.5
CPE + 8% SWCNT	14.4
100% SWCNT	14.2

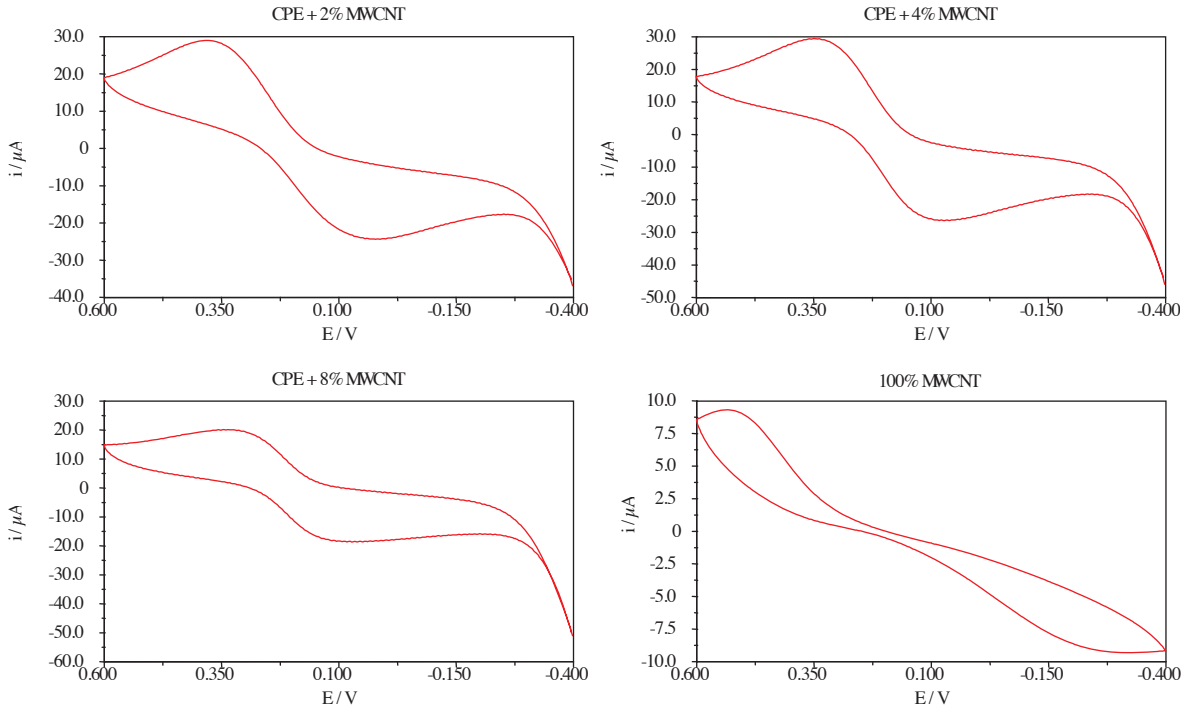

Figure 1. The effect of MWCNT amount on cyclic voltammograms of 2 mM ferricyanide, in the potential range of 600 to -400 mV, scan rate 100 mV/s, 50 mM phosphate buffer supporting electrolyte pH 7.

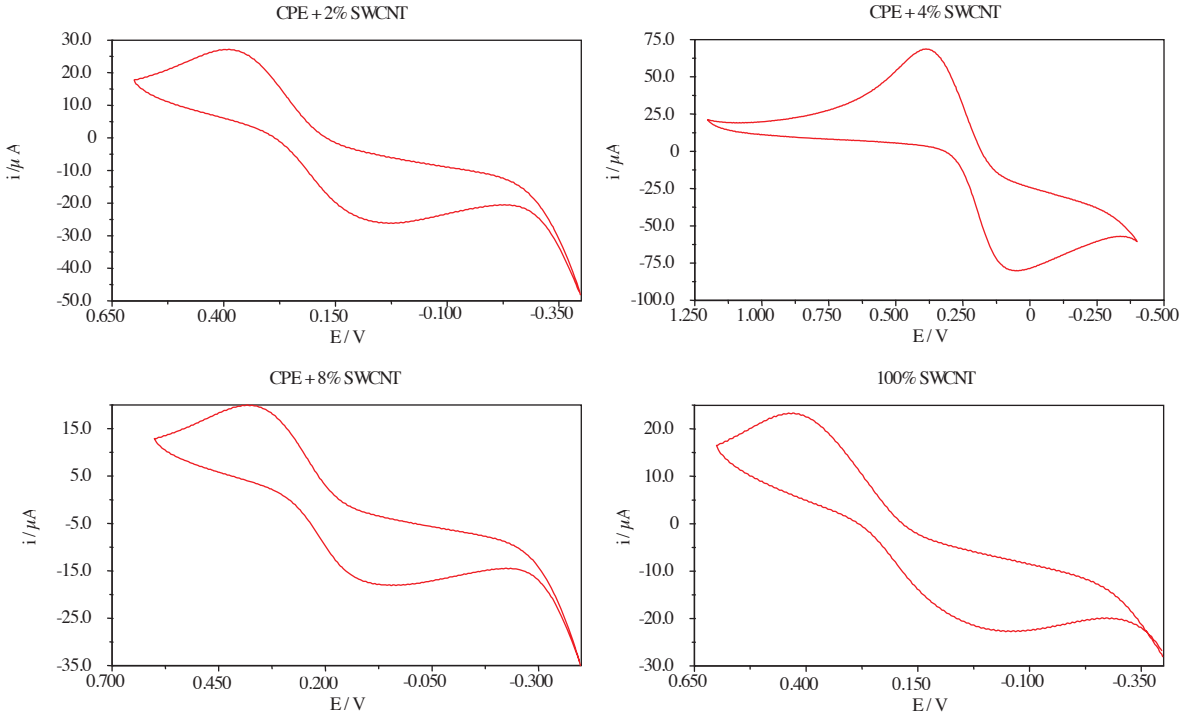

Figure 2. The effect of SWCNT amount on cyclic voltammograms of 2 mM ferricyanide, in the potential range of 600 to -400 mV, scan rate 100 mV/s, phosphate buffer supporting electrolyte pH 7.

Table 3. Comparison of CPE, MWCNT-CPE, and SWCNT-CPE in terms of electrocatalytic activity.

Composite	E_p(V)	I_p(μA)
CPE	0.344	14.5
4% MWCNT	0.327	22.2
4% SWCNT	0.354	19.5

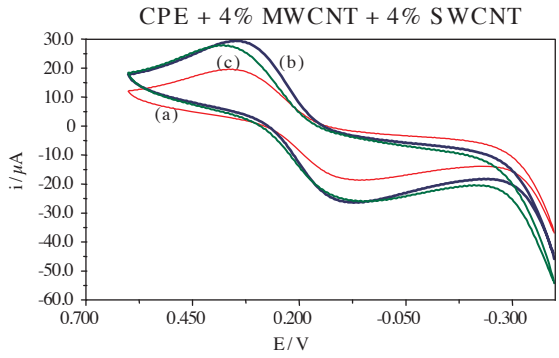

Figure 3. The cyclic voltammograms of 2 mM ferricyanide with (a) CPE, (b) 4% MWCNT-CPE, (c) 4% SWCNT-CPE; all other conditions as in Figure 2.

Analysis of the ferricyanide faradaic current as a function of the scan rate resulted in a linear Ip versus $\nu^{1/2}$ relationship over the 5-250 mVs^{-1} range for MWCNT and CPE indicating that the current is controlled by semi-infinite linear diffusion. SWCNT linearity continued up to 1000 mVs^{-1}, indicating the diffusion-controlled nature of this reaction (data not shown).

Use of the Developed Electrodes as Xanthine Biosensor Transducers

Optimum working potential

After finding the optimum electrode structure, this composite was modified by xanthine oxide (XO) enzyme for obtaining a xanthine biosensor. The system's pH, XO amount, and temperature were optimized before and presented in our previous paper.[23]

One of the attractive properties of CNT is its electrocatalytic activity, which is expected to decrease the operating potential. In order to examine this effect, the performance of the sensor was tested between 600 and 900 mV with increments of 100 mV for 1 mM hydrogen peroxide solution (Figure 4a and b). With MWCNT higher current values were obtained as the potential was increased and the highest value was obtained at 900 mV. Almost the same thing was happened for SWCNT, except that the difference between current values at 800 and 900 mV was not as high as it was for MWCNT. For this reason, as a 68% higher current value was attained at 800 mV, further experiments were conducted using 800 mV for the SWCNT and 900 mV for the MWCNT modified CPE.

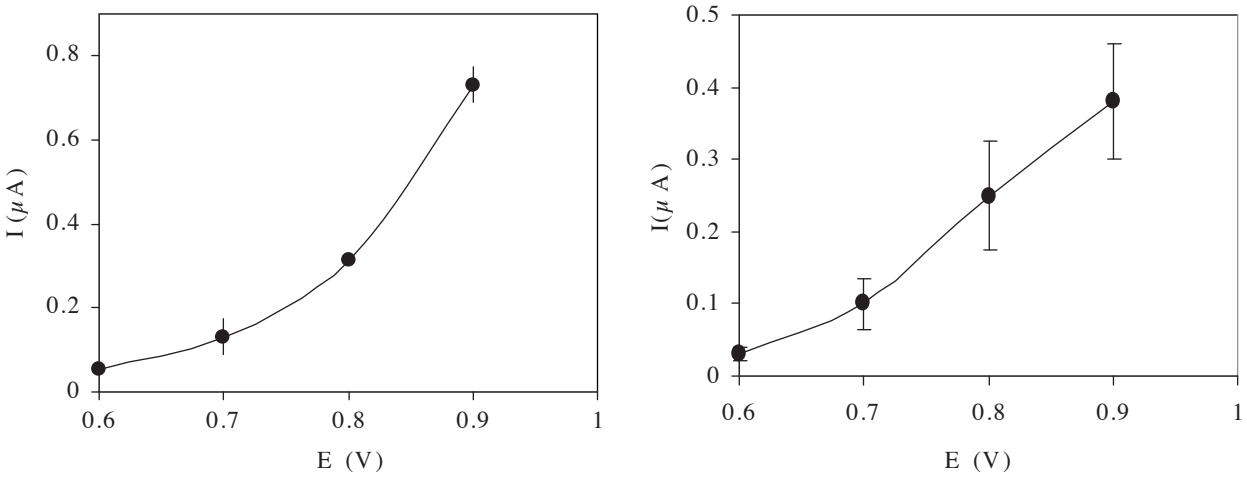

Figure 4. The effect of operating potential on current values for 1 mM H_2O_2 at (A) 4% MWCNT modified, (B) 4% SWCNT modified CPE.

Analytical Characteristics

The MWCNT modified biosensor gave a linear range between 1 and 100 μM xanthine under the response time of 150 s with the equation y = 0.0455 x + 0.205 (R^2 = 0.99), while the linear range and equation were 1-20 μM*and*y = 0.0688 x + 0.1071 (R^2 = 0.99) for the SWCNT modified CNT. At higher concentrations, the standard curve showed a deviation from linearity.

The repeatability of the biosensor was tested for 100 μM of xanthine (n = 4) and the relative standard deviation (RSD) was 3.35% for MWCNT. For SWCNT this value was tested for 5 μM of xanthine (n = 5) and the RSD was 3.50%.

Sample Application

a) Canned Tuna Fish Sample

Xanthine level in meat and marine products is important as a food quality control index. Canned tuna fish samples were used for xanthine detection by applying a standard addition assay with SWCNT-CPE.

As already mentioned in the experimental section, perchloric acid was used for deproteinization of samples by providing denaturation of proteins. After centrifugation, clear supernatant solution was obtained. Standard xanthine solution was added to one part of this supernatant solution to obtain a final concentration of 5.0 μM, while the other part was used without any xanthine standard solution inside. Both solutions were subjected to standard addition; in other words, samples with and without standard xanthine analyte were used as stock substrate solutions and added to the reaction cell after equilibration. The xanthine amount in samples was calculated from the calibration curve. The tuna fish sample without any xanthine analyte did not show any significant current value, indicating that the nature of the sample does not affect the measurement. This situation is confirmed by the closeness of the recovery of the sample containing xanthine, 103.33% $\pm$ 0.14, to 100%.

b) Plasma Sample

The above procedure was followed for the detection of xanthine in plasma samples with MWCNT-CPE. Denatured plasma samples that included a known amount of xanthine (100 μM) and plasma sample

without any xanthine analyte inside were used as stock substrate solutions. They were diluted with working buffer solution and added to the reaction cell after equilibration. Plasma sample solution that did not contain any xanthine analyte did not show any significant current value, proving that the nature of the sample does not affect the measurement. From the calibration curve xanthine amounts in samples were found and the recovery value was 100.65 ± 0.54 (n = 3).

Conclusion

In the present work, the effects of MWCNT and SWCNT on CPE as a xanthine biosensor transducer were investigated. For the optimum composite structure, the electroanalytic behavior of electrodes containing various portions of CNT was examined for ferricyanide solution and compared with the plain CPE. As a result, higher current values with lower peak potentials (with MWCNT-CPE) were obtained with CNT modified CPEs. The repeatability of both electrodes to xanthine substrate was almost the same, while MWCNT-CPE indicated a wider linear range compared to SWCNT-CPE. On the other hand, from the voltammograms, it is clear that MWCNT-CPE became a more effective transducer as the operating potential was increased. Although higher current values were obtained at +900 mV, it is shown that the SWNT-CPE can perform at +800 mV (from the sample application).

The application of the developed systems to xanthine detection in denatured canned tuna fish and plasma samples provided promising results, indicating that the developed transducers are not affected by the nature of the samples and they can be used for different types of samples. As a result, it can be concluded that a simple and practical biosensor with better sensitivity is obtained when CNT is introduced into CPE for xanthine detection.

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