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ORIGINAL PAPER

Double-walled carbon nanotube based carbon paste electrode as xanthine biosensor

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Abstract We describe the first usage of a double walled carbon nanotube (DWCNT) modified carbon paste electrode as biosensor transducer. Xanthine was chosen as a substrate for evaluation of the electrode performance. Proper amount of DWCNT and xanthine oxidase enzyme were mixed with proper amount of graphite and mineral oil for attaining the xanthine biosensor. Results were compared with previous work that includes multi-walled carbon nanotube and single-wall carbon nanotube based carbon paste electrode xanthine biosensors. A linearity was obtained in the concentration range between 2–50 μM xanthine under the response time of 150 s with the equation of $y=0.0441x + 0.2013$ and RSD value of 4.20%. This system was applied to the determination of xanthine in canned tuna fish samples and recovery was calculated as 99.20%±0.07.

Keywords Double walled carbon nanotube · Xanthine · Amperometric biosensor

Introduction

As the smallest example of multi-walled carbon nanotube (MWCNT), DWCNT consists of two concentric tubes where the outer wall provides an interface with the rest of the system while inner wall can act as 1D nanowire [1, 2]. On the other hand, nitric acid (HNO_3) treatment is widely utilized for purification/and or functionalization of CNTs that can be resulted with improved electrochemical signals for some analytes [3–6]. It has been reported that trace amount of iron within Co catalyst nanoparticles in HNO_3-washed DWCNT provides electrocatalytic activity for the reduction of hydrogen peroxide [3–5].

In terms of electroanalytical and bioanalytical applications, carbon nanotubes (CNT) have been extensively used as electrode material since they have the ability to mediate electron transfer reactions for some analytes [7, 8]. MWCNT and single-walled carbon nanotube (SWCNT) based electrodes were widely utilized as biosensor transducers and for electrochemical detection of various compounds because of their electrocatalytic effect [9–20]. In our previous work, the effect of MWCNT and SWCNT on carbon paste electrode (CPE) performance as xanthine biosensor transducer was investigated [21]. CNT included CPEs were modified with xanthine oxidase (XO) enzyme and resulted composite electrodes were used as xanthine biosensor transducer by following the hydrogen peroxide oxidation at 0.9 V (for MWCNT) and 0.8 V (for SWCNT) [21]. In this study, electroanalytic performance of DWCNT-CPE as a xanthine biosensor transducer was examined. Though immobilization of glucose oxidase enzyme on the DWCNT without using polymer binder was conducted before, the performance of developed system was not tested as a glucose biosensor [22]. So, as far as we know, our work is the first application of DWCNT as part of the biosensor transducer.

Proper amount of HNO_3-treated DWCNT was introduced into CPE and as a result DWCNT-CPE composite electrode was obtained. Then this material was modified with XO enzyme and a biosensor transducer suitable for xanthine detection was attained. Obtained results were

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compared with MWCNT and SWCNT based biosensor including sample application part [21].

Experimental

Reagents

Xanthine oxidase from buttermilk (XO, 0.06 unıts/mg solid) was obtained from sigma (www.sigmaaldrich.com). Double walled carbon nanotube (DWCNT catalog no 637351, purity >90%), graphite powder (| <20 micron) and mineral oil were purchased from Aldrich (www.sigmaaldrich.com). Phosphate buffer (0.05 M, pH 7.0) was used as supporting electrolyte. All solutions were prepared by double distilled water.

Apparatus

Chronoamperometric measurements were carried out with the AUTOLAB PGSTAT 12 electrochemical measurement system from ECO CHEMIE Instruments B.V, (Netherlands) driven by GPES software (www.ecochemie.nl). XO biosensor based on Double walled carbon nanotube carbon paste electrode (DWCNTPE) was used as a working electrode. An Ag/AgCl and platinum electrode were used reference and auxiliary electrode, respectively. The electrodes were immersed into a conventional cell (10 mL) through the Teflon cover.

Treatment procedure

2.4 mg of DWCNT was dispersed into 6 M 60 mL HNO_3 for 5 hours at 80 °C and then cooled in acid solution for overnight. Then it was rinsed with distilled water and dried in room temperature.

Preparation of biosensor

XO-modified DWCNT-CPE was prepared by hand-mixing of % 8 HNO_3 washed DWCNT, % 62 graphite powder, % 30 mineral oil and 0.3 mg XO. Amount of XO was optimized at our previous work [23]. 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 a weighing paper. Surface of the resulting paste electrodes were smoothed and rinsed carefully with double distilled water.

Procedure

All experiments were performed at room temperature. Cyclic voltammetric measurements were conducted in the potential range of −1000 mV to 500 mV at 100 mV/s scan rate in the presence of phosphate buffer that was used as supporting electrolyte.

Chronoamperometric experiments were carried out using stirred solutions by applying proper potential for 150 or 300 sn. The transient current decayed to a steady state value after 50 s in the presence of supporting electrolyte.

Sample application

Canned tuna fish sample (labeled Dardanel) was purchased from a local market. Canned tuna fish was chopped and homogenized until a fine paste was obtained after addition of 5 mL 0.5 M $HClO_4$ for the precipitation of proteins in the sample. Obtained denatured samples 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 two parts. Known amount of xanthine was added at one part while no xanthine was added to the other.

Results and discussions

Electroanalytic performance of the electrode

DWCNT-CPE for H_2O_2 oxidation

It has been claimed that outer wall of oxidized DWCNT can provide active sites for effective oxidation of biomolecules [24]. Also as mentioned above, trace amount of iron within Co catalyst nanoparticles in oxidized DWCNT is responsible for electrocatalytic activity of this material for the reduction of hydrogen peroxide [3–5]. The same catalytic effect was expected to be observed for the oxidation of the hydrogen peroxide. To compare the performances and observe the difference, electrochemical behavior of CPE, MWCNT modified CPE, and DWCNT modified CPE were compared for the oxidation of hydrogen peroxide (Fig. 1). For CPE 14.11 μA at 0.033 V was observed (Fig. 1a) while for treated MWCNT-CPE 23.86 μA at 0.138 V (Fig. 1b) and for DWCNT-CPE 45.00 μA at 0.143 V was attained (Fig. 1, c). The differences in the current values confirm our expectations. For this reason, further experiments were conducted to examine the performance of this electrode as biosensor transducer.

Optimization of electrode structure

Optimum electrode composition directly affects the obtained current values. Thus, it is important to optimize composition amount. Carbon paste composites including various percentages of DWCNT (2, 4, 8, 16, 100%) were

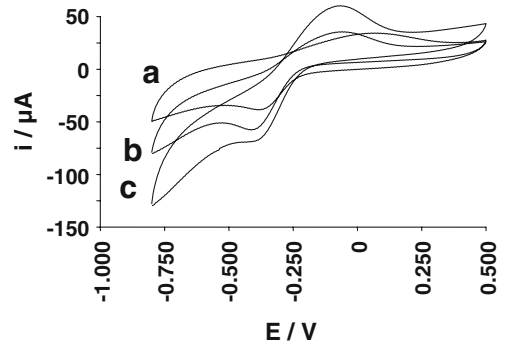

Fig. 1 Cyclic voltammograms of 5 mM H_2O_2 with (a) CPE, (b) %4 CPE-treated MWCNT, (c) %4 CPE-treated DWCNT; scan rate 100 mV/s, 50 mM phosphate buffer supporting electrolyte, pH 7.0

prepared and their electroanalytical performances were examined by recording cyclic voltammograms of 5 mM hydrogen peroxide (Fig. 2). As can be seen from the Figure, best current value was obtained with 8% DWCNT (56 µA). Amount higher than 8% DWCNT causes a decrease in current values. Possessing large surface area is another important advantage of CNTs that can facilitate the modification of this nanotube with biological molecules [25]. On the other hand, large surface areas can cause increment of background current that might decrease the resulted current values as in the case of our work [26]. As a result, further studies were conducted by using % 8 DWCNT-CPE as optimum electrode structure.

DWCNT-CPE as xanthine biosensor transducer

The contribution of MWCNT and SWCNT on CPE as xanthine biosensor transducer was examined in our earlier

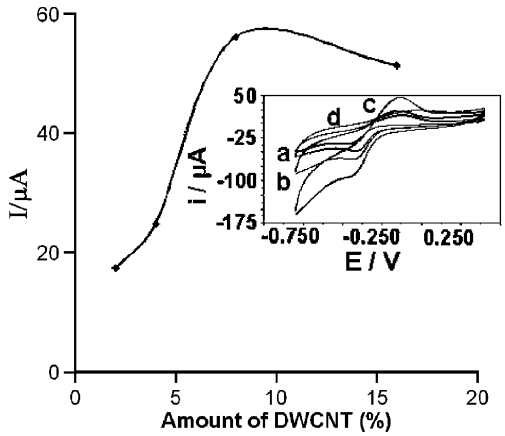

Fig. 2 Effect of DWCNT amount on the current response for 5 mM H_2O_2 with (a) 2% treated DWCNT-CPE, (b) 4% treated DWCNT-CPE, (c) 8% treated DWCNT-CPE, (d) 16% treated DWCNT-CPE, scan rate 100 mV/s, 50 mM phosphate buffer supporting electrolyte pH 7.0; inset cyclic voltammograms of these DWCNT amounts obtained with DWCNT-CPE

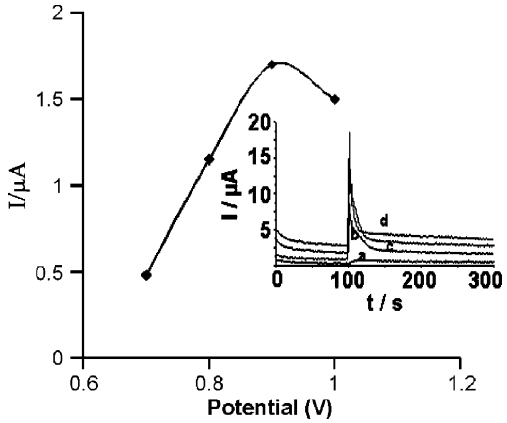

Fig. 3 Effect of working potential (700–1000 mV) on the electrode response in phosphate buffer, 50 mM at pH 7.0 for 25 µM xanthine; inset chronoamperometric responses of the electrode at these potentials

work [21]. Here our aim is to examine the effect of DWCNT on the same transducer and compare the results with previous work.

Developed biosensor's measuring principle is based on the chronoamperometric monitoring of the current that occurs due to the oxidation of the hydrogen peroxide which liberates during the enzymatic reaction as shown below [23].

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

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

Optimum working potential

One of the general and important properties of CNT is its electrocatalytic activity that is expected to decrease the operating potential. In order to examine this effect, the performance of the sensor was tested between 600–

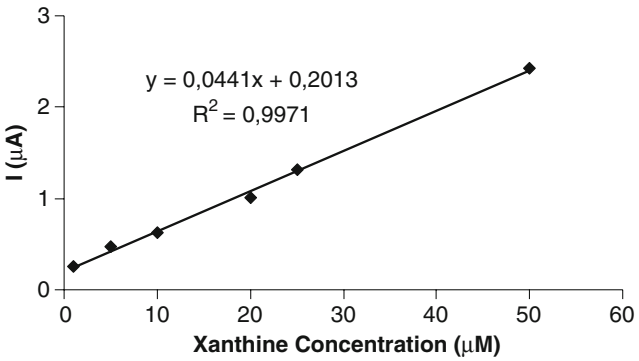

Fig. 4 Calibration curve of the biosensor in the range of 2 µM to 50 µM xanthine concentration. Applied potential: +900 mV; supporting electrolyte: 50 mM phosphate buffer, pH 7.0

1000 mV with increment of 100 mV for 25 μM xanthine solution (Fig. 3). At 600 mV no current value was observed and the highest current value was obtained at 900 mV (1.70 μA). The current value at 800 mV is 1.50 μA which contributes to %88.23 of highest current value. As it is known, other substances like ascorbic acid (AA) and uric acid (UA), may present together with reaction product of xanthine oxidase in real samples[27, 28]. Examination of electrochemical oxidation of these interfering substances which were conducted with different kind of electrode materials, demonstrates that they oxidize at less positive potentials than 900 mV [27, 28]. As a result, in order to avoid the interference of other substances and for obtaining higher current values, 900 mV is selected and utilized as operating potential for the further studies.

Analytical characteristics

The linear range for DWCNT-CPE was found between 2–50 μM xanthine under the response time of 150 s with the equation of $y = 0.0441x + 0.2013$ and R^2 of 0.9971 (Fig. 4). This range is wider than SWCNT-CPE while better linear range was obtained with MWCNT-CPE [21]. At higher concentrations, standard curve showed a deviation from linearity.

The repeatability of the biosensor was tested for 25 μM of xanthine (n=4) and the relative standard deviation (R.S.D) was calculated as 4.20%.

Sample application

After denaturation process of sample that was achieved by means of perchloric acid, centrifugation was applied. For obtaining 10.0 μM xanthine solution, necessary amount of xanthine solution was added to one part of this clear supernatant solution. Other part 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. Xanthine amount in samples was calculated from calibration curve. Tuna fish sample without any xanthine analyte didn't show any significant current value indicating that the nature of sample does not affect the measurement. This situation is confirmed by recovery of xanthine contained sample, 99.20%±0.07.

Conclusion

As far as we know, this work is the first work that includes DWCNT-CPE as biosensor transducer. Proven catalytic activity of HNO_3 washed DWCNT towards hydrogen peroxide results the usage of this material as xanthine biosensor. Though xanthine was chosen as a model substrate to test the applicability of DWCNT modified electrode, the recovery value confirms the utility of this electrode in natural samples as transducer. Application of developed systems to xanthine detection at denatured canned tuna fish sample results with promising recovery value indicating that developed transducer is not being affected by the nature of this sample. In another word, in our opinion, this value confirms that no other substances, like AA or UA, interfere the measurement of xanthine at this natural sample. Moreover, we analyzed xanthine at plasma samples by using standard addition method with MWCNT-CPE at 900 mV in our previous work [21]. In this biological fluid where more effective AA and UA interference is expected, the recovery value was calculated as 100.65±0.54 (n=3), suggesting that under these working conditions, developed sensor is not being affected from the nature of real samples such as biological fluids [21].

Compared to MWCNT-CPE narrow linear range but higher current values was obtained with this electrode [21]. In terms of repeatability, other carbon nanotubes showed slightly better RSD values 3.35% (MWCNT n=4) and 3.50% (SWCNT n=5) respectively [21]. On the other hand, recovery value obtained for tuna fish sample is better compared to one obtained with SWCNT-CPE (103.33%± 0.14) [21]. As a conclusion, it can be suggested that another simple, practical and effective biosensor was obtained when DWCNT introduced into CPE for xanthine detection.

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