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VOLTAMMETRIC STUDIES OF NANO ZIRCONIUM DIOXIDE/CARBON NANOTUBES/CHITOSAN-MODIFIED GLASSY CARBON ELECTRODES

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ABSTRACT

Voltammetric studies of a sensitive electrochemical DNA sensor based on ZrO_2 nanoparticles and multi-walled carbon nanotube (MWNTs) for DNA immobilization is described. Layer deposition technique was used to prepare nano ZrO_2/MWNTs/chitosan-modified glassy carbon electrode (GCE) and oligonucleotides were immobilized to the GCE. The immobilization of DNA on the electrode was monitored by cyclic voltammetry (CV) analysis by measuring the change of peak currents using electroactive methylene blue (MB) as an indicator. Compared with previous DNA sensor with oligonucleotides directly incorporated on carbon electrodes, this carbon nanotubes-based assay with its large surface area and good charge-transport characteristics increased DNA attachment quantity. Parameters used on this study including electrochemical characterization, scan rate study, pH optimization, and scanning electron microscope (SEM) as well as chronoamperometry (CA) and chronocoulometry (CC). The electrochemical reduction and oxidation of the redox couples of methylene blue (as a DNA indicator) can be recognized easily by the solid-phase voltammetry of microparticles. The cyclic voltammograms for three differently modified electrodes, nano ZrO_2/chitosan, MWNTs/chitosan and nano ZrO_2/MWNTs/chitosan, showed 2 major peaks responding to redox couple of methylene blue.

INTRODUCTION

The efficient immobilization of DNA on suitable electrode support is an important issue in the fabrication of a reliable electrochemical genosensor. Glassy carbon and gold surfaces have been demonstrated to be suitable platforms for the covalent immobilization of oligonucleotides. This immobilization method allows the nucleic acid probes retaining their hybridization function and bioaffinity properties [1-4]. Such electrode modifications have the objective of increasing the stability of the electrode response, decreasing the overpotential associated with the electrode process, and/or increasing the oxidative current of the sulphuryl compound. These chemically-modified electrode electrocatalytic systems also are used to minimize problems with poor selectivity and sensitivity commonly associated with the use of solid electrodes [5]. It has been well documented that functionalization of an electrode surface can offer significant analytical advantages in volumetric experiments [6]. Since the discovery of carbon nanotubes in 1991, they have been the target of numerous investigations due to their unique properties [7-10]. The structure of nanotubes can be described as a rolled-

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up tubular shell of graphite sheet with the carbon atoms covalently bound to their neighbors. Ballistic conductivity of metallic nanotubes with a high aspect ratio is extremely attractive for the capture and promotion of electron transfer reactions from analytes. On the other hand, semiconducting nanotubes can be directly used as biosensors and optimized by changing the gate voltage. These properties are the reasons why CNT electrodes have high sensitivity with low detection limits. The key for electrochemical detection of nucleic acids is the immobilization of specific sequence of nucleic acids such as oligonucleotides on nanotubes electrodes. These immobilized oligonucleotides can detect targeted complementary DNA sequences under a hybridization state [11]. Methods for the immobilization of biological species to nanotubes can be separated into covalent bonding and noncovalent bonding or physical adsorption [12-19]. Once nanotubes are oxidized and defects are created, moieties such as carboxylic group and biomolecules can be bonded covalently to the defect sites. In the case of noncovalent bonding, coating or wrapping nanotubes with polymers such as poly(vinyl pyrrolidone), poly(styrene sulfonate) and chitosan makes the nanotubes water soluble and biocompatible. The polymer can also be easily functionalized for protein conjugation (14-15). Zirconia is an inorganic oxide with the thermal stability, chemical inertness, lack of toxicity [20] and affinity for the groups containing oxygen [21], so it is an ideal candidate of materials for immobilization of biomolecules with oxygen groups. The particles of zirconia oxide formed were in the range of few hundred nanometers, which result in a large electrode surface area and the easier attachment of an oligonucleotide with phosphate group at 5' end, thus the DNA immobilization amount could be greatly enhanced [22]. Methylene blue (MB), an organic dye that belongs to the phenothiazine family, is a redox indicator, which due to its interaction with the guanine in DNA, displays significantly different voltammetric signals in the presence of DNA modified electrodes [23-24].

EXPERIMENTAL METHOD

Instrumentation and electro analytical analysis methods

The voltammetric experiments were performed with a BAS (Bioanalaytical Systems, West Lafayette, Indiana, USA): CV-50W electrochemical workstation, which was controlled by an external computer. A conventional three-electrode potentiostated system was used with a 3 mm diameter glassy carbon (GC) disc electrode as the unmodified/bare working electrode, a platinum wire (1 mm diameter) counter electrode and an Ag/AgCl (3M NaCl) reference electrode. Unless otherwise mentioned, the temperature was (25 $\pm$ 2) ^{o}C. All solution was degassed with nitrogen for ten minutes prior to recording the measurement.

Reagents and materials

Single-stranded herring sperm DNA were received from Sigma (Product from United Kingdom). Zirconium (IV) oxide nanopowder (ZrO_2) was obtained from Aldrich at less than 50 nm diameter. Multi-wall carbon nanotubes (MWNTs) were obtained from Dynamic Enterprises Ltd., with 98% purity, with 20-50 nm x 5-20 μm diameter x length, metal salts from Sigma Aldrich. Chitosan (CHIT, medium molecular weight)

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were purchased from Aldrich (USA). Methylene blue (MB) was purchased from HmbG Chemicals. The supporting electrolyte for the determination of redox couple of methylene blue was 0.01molL^{-1} phosphate buffer solution containing 0.1molL^{-1} NaCl (pH 7.0, PBS). Other chemicals were of analytical reagent grade. All solutions were prepared with distilled water.

Preparation of ZrO2/MWNTs/CHIT solution
A 0.2 wt. % CHIT solution was prepared by dissolving appropriate amount of CHIT flakes into 0.05molL^{-1} acetic acid and stirring for 3 h at room temperature until complete dissolution. Appropriate amount of nanoparticles ZrO_2 and MWNTs dispered in 0.1% of chitosan. The mass ratio of ZrO_2: MWNTs: CHIT was 1:2.5:100. The mixture was sonicated for 15 min after stirring 1 hour. Finally, a high dispersed colloidal solution was formed.

Immobilization of DNA on MWNTs-ZrO2-modified GC electrode
A glassy carbon (3mm diameter) electrode was polished before each experiment with α-alumina (>99.5% Al_2O_3) powder successively rinsed thoroughly with absolute alcohol and distilled water in ultrasonic bath and dried in air. Firstly, 10µl of MWNTs/ZrO_2/CHIT casting solution was coated on the glass carbon electrode surface and dried in air. Then 2.5µL of probe DNA at 130nmolL^{-1} was pipetted onto the surface of above modified electrode. The casting solution was allowed to adsorb at room temperature for 1 h. Finally, the DNA electrode was rinsed vigorously with 0.01molL^{-1} PBS (pH 7.0) in order to wash out the unimmobilized DNA from the electrode surface before mesurement.

Scanning electron microscopy (SEM)
A piece of basal plane pyrolitic graphite (BPPG) (Alpha, USA) electrode with a 5 mm diameter and 2-3 mm thickness was cut from a rod. It was then polished with 320CW silicon carbide abrasive paper followed by a further polishing with α-alumina (>99.5% Al_2O_3) powder. Similar methods of cleansing and deposition of and DNA/ZrO_2/MWNTs/CHIT modified glassy carbon electrode were applied to the BPPG electrode experiment. To study the surface morphology of DNA/ZrO_2/MWNTs/CHIT on a BPPG electrode after electrolysis, the electrode, with array of DNA/ZrO_2/MWNTs/CHIT attached on the surface, was joined to a platinum wire that acted as a conductor. The reduction potential was then held at -1.800 V for 1 min in an appropriate electrolyte, using the bulk electrolysis mode of the electrochemical workstation (model BAS 50W). A scanning electron microscope, model JEOL JSM-6400 (Philips), was used to study the surface morphology of DNA/ZrO_2/MWNTs/CHIT before and after electrolysis.

RESULTS AND DISCUSSION

Electrochemical characterization of different film
DNA biosensor with MB as hybridization indicator was never reported in previous literatures [25-26]. Figure 1 displays the CV response of MB in PBS (pH 7.0) obtained

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with above three different electrodes immobilized with DNA. The three electrodes were DNA/ZrO_2/CHIT/GC electrode (a), DNA/MWNTs/CHIT/GC electrode (b) and DNA/ZrO_2/ MWNTs/CHIT/GC electrode (c). As can be seen, compared with the signal obtained with the DNA/ZrO_2/CHIT/GC electrode and DNA/MWNTs/CHIT/GC electrode, DNA/ZrO_2/ MWNTs/CHIT/GC electrode offered a maximal signal. This confirmed the synergetic effects of MWNts/ZrO_2/CHIT composite film which can effectively increased the loading of the DNA, ascribed to the excellent electron-transfer ability of MWNTs and the high surface area of nano ZrO_2 dispersed in CHIT film [27]. The large peak-to peak separation obtained is unexpected for compound attached to a surface and the possible reason are the slow kinetics for the charge transfer, including the slow direct electron transfer between the GC electrode and the DNA/ZrO_2/ MWNTs/CHIT and the transport of the protons between the modified layer and the bulk solution, or the slow electrostatic charge compensation during the redox process [28].

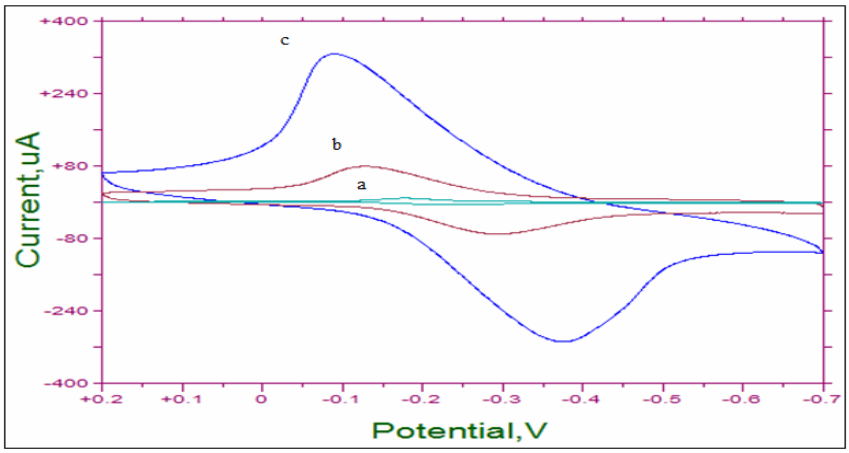

Figure 1: Cyclic voltammogram of three electrodes were DNA/ZrO_2/CHIT/GC electrode (a), DNA/MWNTs/CHIT/GC electrode (b) and DNA/ZrO_2/MWNTs/CHIT/GC electrode (c) in 0.01M phosphate buffer solution (PBS) using MB as indicator.

Scan rate study

Cyclic voltammograms obtained with different scan rates at a DNA/ZrO_2/MWNTs/CHIT/GC electrode in a PBS and MB become as indicator of DNA are shown in Figure 2. The increase of potential scan rate promoted an increase of current peak in anodic and cathodic reactions. At higher scan rates, the signals broadened considerably and peak separation increase as a result of an increase in heterogeneous kinetics and/or IR drops associated with increasing scan rate (see Figure 2)[29]. The relationship between peak current, I_p and scan rate is summarized in a Figure 3. According to the Randless-Sevcik equation (1), the slope of plot log I_p versus log scan rate, yield a straight line with 0.5 indicating diffusion controlled process and the slope of 1.0 indicating that MB is strongly adsorbed to the modified surface [30].

$$I_p = (2.69 \times 10^5)\, n^{3/2}\, D^{1/2}\, v^{1/2}\, A\, C \qquad (1)$$

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Where n = number of electrons transfer
D = diffusion coefficient
v = scan rate in Vs^{-1}
A = electrode surface area
C = concentration of the analyte

Both of the log anodic and cathodic peaks current vary approximately linearly with a slope 0.83 and 0.95 and the log of the scan rate over the range of $10mVs^{-1}$ to $1000mVs^{-1}$, which suggest that the process is a complex surface process.

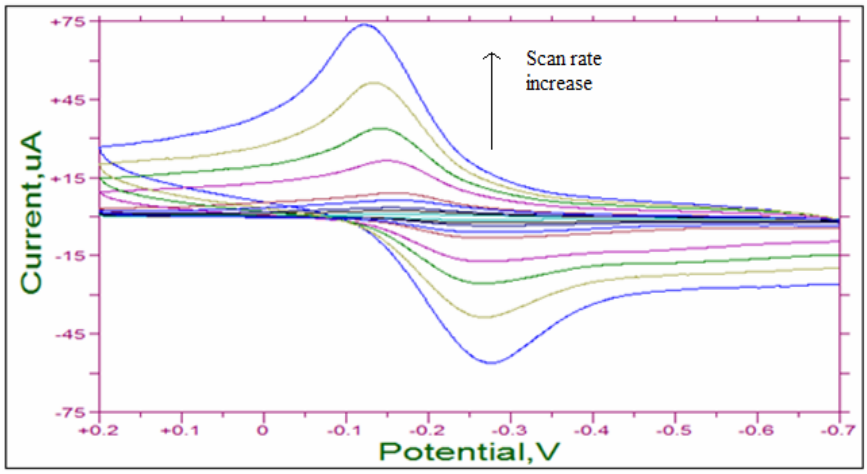

Figure 2: Cyclic voltammograms of different scan rate from $10mVs^{-1}$ to $1000mVs^{-1}$ for MB in 0.01M PBS solution at DNA/ZrO_2/MWNTs/CHIT/GC.

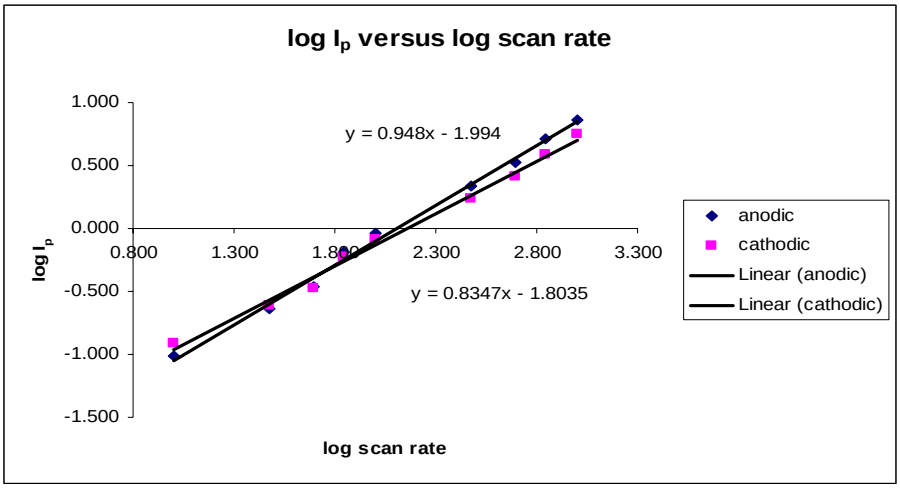

Figure 3: Graph of log I_p versus log scan rate for DNA/ZrO_2/MWNTs/CHIT/GC electrode in 0.01M PBS.

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pH effect

The solution pH was varied from 2.0 to 8.0 in order to determine its effect on the redox reaction of MB at the DNA/ZrO_2/MWNTs/CHIT/GC electrode and using 2.0M HCl and 2.0M NaOH for pH adjustment. From the graph (Figure 4), the increase in pH also resulted in the decrease of peak current. It is clear that in strongly acidic media, the presence of a large concentration of H_3O^+ which brings about a large concentration of oxidant would allow the rate of reduction and dissolution of MB to take place. In addition, the lowering of pH would also bring about a change in diffusion rate affecting the redox reaction. The electrochemical process for MB in hybrid film in the observed pH range can be represented by two-proton, two-electron process [31].

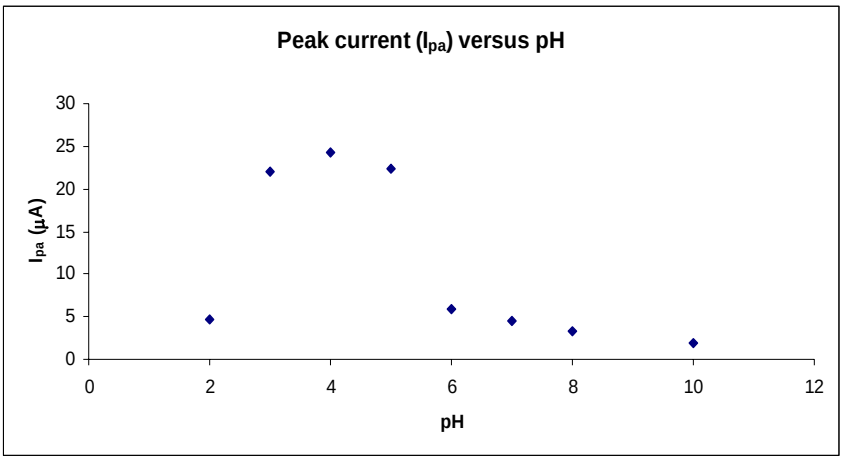

Figure 4: Graph of oxidation current, I_p (μA) versus pH for DNA/ZrO_2/MWNTs/CHIT/GC electrode in 0.01M PBS. (Scan rate: 100mV/s)

Chronoamperometry and Chronocoulometry

Double step potential chronoamperometry and chronocoulometry are useful tools for investigation of the electrochemical processes at chemically modified electrodes [32-35]. The electrocatalytic of MB at the glassy carbon electrode modified with DNA/ZrO_2/MWNTs/CHIT was studied also using double step potential chronoamperometry. Figure 5 shows the current-time curves of the modified DNA/ZrO_2/ MWNTs/CHIT GCE obtained by setting the working electrode potential at 70mV (at first potential step) and 100mV (at second potential step) versus Ag|AgCl. The diffusion coefficient of MB for the modified electrodes can be determined using the Cottrell equation (2):

$$I_p = \frac{nFAD^{1/2}C\,t^{1/2}}{\pi^{1/2}} \qquad (2)$$

Where I_p = current
n = number of electron per molecule
F = Faraday's constant

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A = electrode area
D = diffusion coefficient of electroactive species
C = concentration of electroactive species
t = Time

Using the Cottrell equation, a plot of I_p versus $t^{-1/2}$ shows a linear relation with a slope 3.32 x 10^{-6} A decade^{-1}, and the estimated diffusion coefficient was 8.64 x 10^{-7}cm^{2}s^{-1}. The electrocatalytic behaviour of DNA/ZrO_2/ MWNTs/CHIT modified electrode was also studied by the double potential-step chronocoulometry technique to find the amount of charge, Q that was adsorbed on the electrode surface and from the study it is 16.81μC/cm^2.

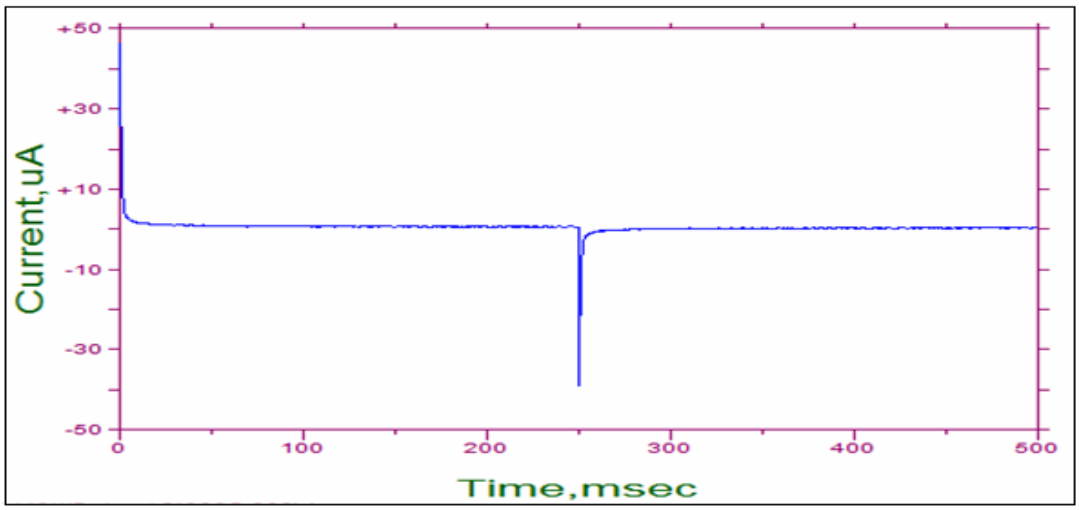

Figure 5: Chronoamperogram for DNA/ZrO_2/MWNTs/CHIT/GC electrode (70mV to 100mV) in 0.01M PBS as supporting electrolyte with pulse width of 250 milliseconds.

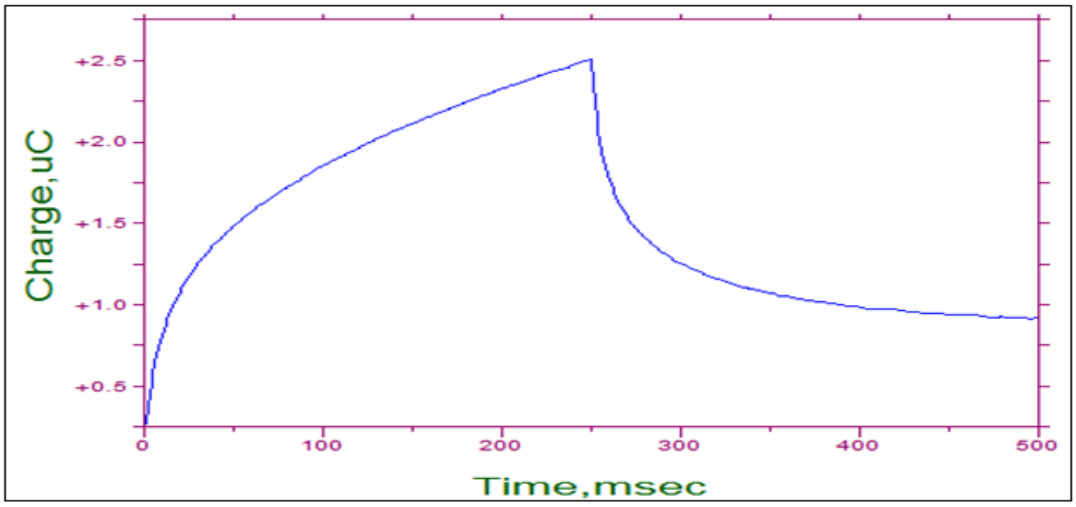

Figure 6: Chronocoulogram for DNA/ZrO_2/MWNTs/CHIT/GC in 0.01M PBS as

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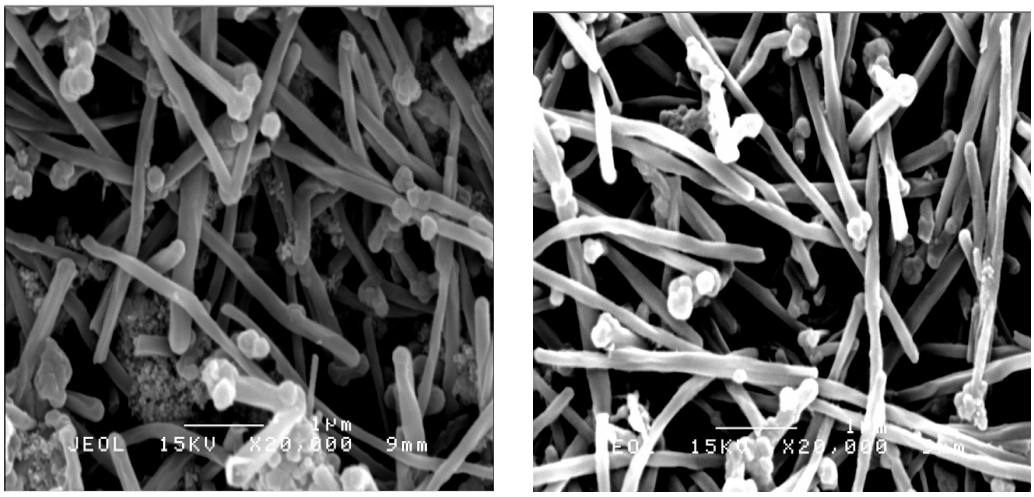
supporting electrolyte.

Characterization of the DNA/MWNTs/ZrO_2/CHIT film

Figure 7 show SEM pictures of ZrO_2/MWNTs/CHIT/GC electrode before (Figure 7a) and after (Figure b) MB electrolysis that shows the existence of MWNTs and ZrO_2 on the surface of modified basal plane graphite electrode (5mm diameter). The Figure 7a and 7b show well defined cylindrical like threads with round shape nano particles attach at the tips. The threads is spread in crisscross manner at along side each other. The morphology of film (Figure 7b) remain quit similar (even after 10^{th} cycles in CV) indicating the stability of film. While Figure 8 show scanning micrographs of a MWNTs/ZrO_2/CHIT mechanically attached to a basal plane graphite electrode (5mm diameter) with immobilized DNA before (a) and after (b) the 10^{th} potential cycling of cyclic voltammetry. The modified electrode shows the presence of DNA over the MWNTs/ZrO2/CHIT-modified electrode (Figure 8a and 8b). SEM results show 2x3µm size microparticles after electrolysis indicating solid to solid conversion process.



(a) (b)

Figure 7: Scanning electron micrographs obtained for a MWNTs/ZrO_2/CHIT mechanically attached to a basal plane graphite electrode (5mm diameter) before (a) and after (b) MB electrolysis with magnification of 20,000 times (a) and magnification of 20,000 times (b).

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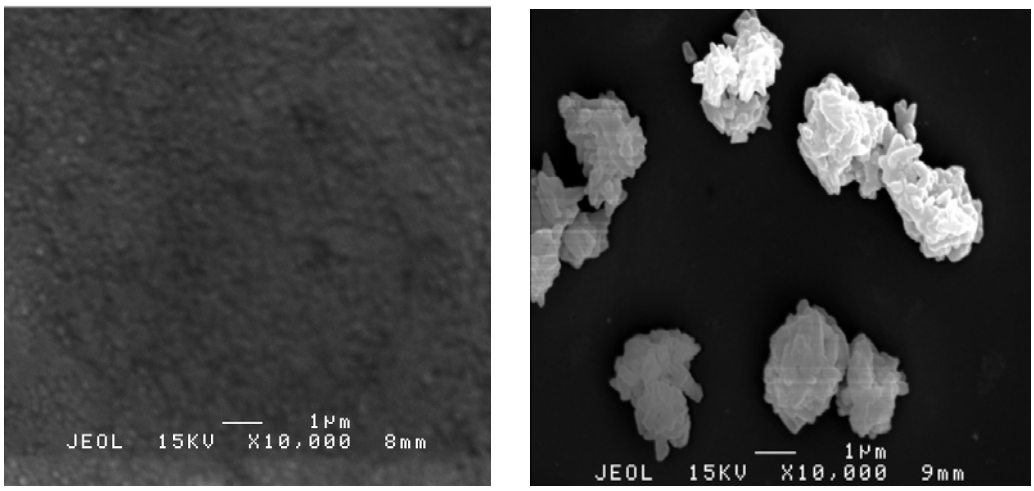

(a) (b)

Figure 8: Scanning electron micrographs obtained for a DNA/MWNTs/ZrO_2/CHIT mechanically attached to a basal plane graphite electrode (5mm diameter) before (a) and after (b) MB electrolysis with magnification of 10,000 times (a) and magnification of 10,000 times (b).

CONCLUSION

The construction of DNA biosensor using MWNTs/ZrO_2/CHIT-modified glassy carbon electrode, as described in this paper, allows the enhancement of the immobilization DNA on the modified surface. The immobilization event is effectively detected by voltammetry of MB intercalated to DNA. The MWNTs/ZrO_2/CHIT modified GC electrode immobilized with DNA based on methylene blue (MB), as indicator is the preferred electrochemical DNA sensor as it produces the best current response. Based on the scan rate and pH studies, the redox reaction of MB in the presence of immobilized DNA observed is a rather complex surface process with optimum response at pH range of 3-5. SEM results show evidence of solid to solid conversion process.

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