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Synthesis of MWCNT Functionalized SiO_2 Thin Film for Biosensor Application

Ahmed Mishaal Mohammed [1, 2]

[1] Chemistry Department, College of Sciences, Anbar University Ramadi, Iraq

[2] Institute of Nano Electronic Engineering, University Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

Abstract: ***The improvement of sensor sensitivity is the quest of every researcher in the recent time, sensor developed using multi-walled carbon nanotubes (MWCNT) has been used in many different applications, and however, the device is not quite sensitive to go for lower concentration in medical diagnostic. Hence, to improve the sensitivity of this device we synthesized MWCNT from a fuel oil waste of power plants. The MWCNT surface morphologies were examined by field emission scanning electron microscopy (FE-SEM), the elemental analysis performed with an energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The electrochemical DNA biosensor was successfully fabricated by depositing polyethyl-eneimine branch (PEI-b) solution via MWCNT on SiO_2 electrode. Cyclic voltammetry (CV) was done to study the electrochemical behaviour of the device using potassium hexacyanoferrate characterization on the MWCNT electrode. For a better performance, MWCNT was modified onto SiO_2 to enhance the stability and high signal of MWCNT electrode better when PEI-b was used as the binder compared to bare electrode. It shows the highest electrical performance of DNA immobilization and hybridization on MWCNT modified SiO_2 surface, hence, this successfully electrochemically and significantly increased the sensitivity of the biosensor.***

Keywords: Biosensor; DNA Immobilization; DNA Hybridization; MWCNT.

1. Introduction

Carbon nanotubes (CNT) rank between exciting new developments in modern science and engineering, they have attracted particular interest because they are predicted, and indeed observed, such small dimensions as extremely high strength, lightweight, elasticity, high thermal and air stability, high electric and thermal conductivity, and high aspect ratio offer crucial advantages. The potential utility of carbon nanotubes in a variety of technologically and some important applications [1].

This has been largely driven by the exciting science involved and numerous proposed applications of carbon nanotubes due to their unique chemical and electronic properties and nanometre sizes [2]. The two main of carbon nanotubes exist, the single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) depending on whether the tubes walls are made of one layer (graphene tubes) or more than ones (graphitic tubes) [3].

Small changes in the environment of the MWCNT can cause drastic changes to its electrical properties [4]. Many different applications have been proposed to exploit these unique properties, including energy storage [5], molecular electronics [6], nano-probes [7], nano sensors [4], nanotube composites and nanotube templates etc.[5], [7].

In recent years, DNA sequence analysis have been intensively investigated and studied. DNA sensor with the advantages of label-free, high sensitivity, specificity, simple and low-cost is currently attracting great amount of attentions with research efforts. This DNA sensor is increasingly important with its application and widely used in determination of genetic variation [8], forensic application [9] and food analysis [10], [11]. Various techniques have been studied for the detection of DNA, such as electrochemical [12], surface plasma resonance [13], fluorescence [14], mass spectrometry [15], enzymatic [16], atomic force microscopy [17] and electrophoretic [18], was developed to detect DNA hybridization, however, the electrochemical method better than other methods because it's sensitivity, stability, selectivity and cheap. An electrochemical DNA biosensor was successfully applied for detection of not only human diagnostic but also even transgenic plants [19]. Thus, the immobilization and hybridization of DNA to the surface of the working electrode was prepared and used recently for the fabrication of the DNA biosensor [20]-[25]. MWCNT is a promising material is successfully bind with DNA through covalent bond [26], [27]. MWCNT surface can increased the electrochemical signal of the DNA indicator and rise sensitivity for DNA detection but has not been investigated previously. However, conventional MWCNT electrodes are expensive due to the complicated fabrication method. As compare to the conventional method of fabricating MWCNT electrodes.

In this work, we developed an electrode with a low cost using MWCNT manufactured from fuel oil waste MWCNT/SiO_2/Si/Au by ultrasonic technique for DNA detection. The ultrasonic technique is considering a low-cost and the chemical composition can be controlled. Immobilization and hybridization of DNA was performed using electrochemical detection with potassium hexacyanoferrate for sensitivity of the DNA detection.

2. Materials and Methods

2.1. Preparation of MWCNT by Sonication Probe

MWCNT prepared by a sonication technique which is performed in a probe-type operating at a fixed frequency of

22 KHz, amplitude of 100 µm and a power value of 100 W. Weight of 0.1g activated carbon sample was placed in 250 ml vessel containing 100 ml of deionized water. Samples were placed in thermostated circulating water bath at 25 ±1 ^{o}C during sonication for (1, 2, 3, 4 and 5) hours. Then the solution was centrifuged for 15 min at 6000 rpm, dried at 110-120 ^{o}C for 24 hours.

The synthesized materials were purified as follows. In order to obtain pure MWCNT, and removing the metal catalysts, the products were dissolving in 10% HCl solution for about 16 hours at room temperature. Then the samples were washed several times with deionized water. In order to achieve extra purification, the prepared materials were dissolved in 5 M nitric acid for 3 hours at 70 ^{o}C. After that, the washing step was repeated as mentioned above for the HNO_3 treatment process. Treated MWCNT were dried at 120 ^{o}C. In order to eliminate non-carbon elements, all of the purified materials were placed in the furnace at 400 ^{o}C for 30 min, cooled in a desiccator and then identified using FE-SEM, EDX, TEM, and AFM.

2.2. Modification of SiO_2 with MWCNT

A p-type silicon (100) wafer was used as a substrate for the deposition of the SiO_2 thin films. The substrates were 1st cleaned with acetone and isopropanol using ultrasonic cleaner. Then immersed into the buffered oxide etch (BOE) solution and washed with deionized water followed by oxidation process for 30 minutes. After oxidation, the silicon oxide (SiO_2) layer of thickness ~50 nm, the gold was deposited on the backside of the Si using thermal evaporator. A 1.0 mg of MWCNT was added into 5 ml of polyethyl eneimine branch (PEI-b) solution was prepared by adding 0.667 ml of 1% w/v PEI-b into 20 ml of water and sonicated for one hour to disperse the MWCNT. After that, 10 µl of the MWCNT/PEI-b mixture solution was dropped onto the sample and dried. Then the sample was tested using electrochemical measurement.

2.3. Probe DNA Immobilization on Modified MWCNT

Probe DNA were purchased from Sigma-Aldrich Co. The probe DNA sequences were 5'-5AmM C6/CCA CTA CCA GGG CAG GT-3. The solution were prepared by mixing 40 mM EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), 20 mM NHS (N-hydroxy succinimide) solution and 100 mM of phosphate buffer solution (PBS). The MWCNT electrode was immersed into solution for 3 hours at 4 ^{o}C to activate the COOH-MWCNT surfaces. The sample was washed with deionized water and dried at room temperature. 10 µl of 10 µM aminated probe DNA used and dropped onto the MWCNT electrode for immobilization incubated for 2 hours. After 2 hours, the electrode was carefully rinsed using deionized water to remove any unbound DNA probe and dried at room temperature. The COOH-MWCNT electrode is ready for electrochemical measurements.

2.4. DNA Hybridization Detection Using Modified MWCNT

Hybridization of DNA used in this project was purchased from 1st BASE Pte Ltd (Malaysia). Hybridization with complementary DNA sequences was 5'-CTA CGG TCA TCA CAA ATC TAC TAT CAG-3'. Hybridize the DNA, 10 µl of 10 µM complementary DNA was dropped onto MWCNT electrode and incubated for 2 hours. After that, the MWCNT electrode was washed by using deionized water to remove any non-hybridized DNA and dried at room temperature. 10 µl of 0.5 µM methylene blue then dropped onto the MWCNT electrode and incubated again for 3 minutes. Finally, the MWCNT electrode will once again wash with deionized water to remove any excess of methylene blue and the MWCNT electrode is ready to be electrical measured once it has dried.

2.5. Electrochemical Measurements

Electrochemical measurement was performed by using dielectric analyser. The tests were conducted by using Ag/AgCl as the reference electrode and COOH-MWCNT modified electrode as a working electrode. The Au act as a back gate. The responses of the DNA immobilization and hybridization were investigated in 10 µM Potassium Hexacyanoferrate III, $K_3Fe(CN)_6$ aqueous solution containing 0.1 M KCl as electrolyte.

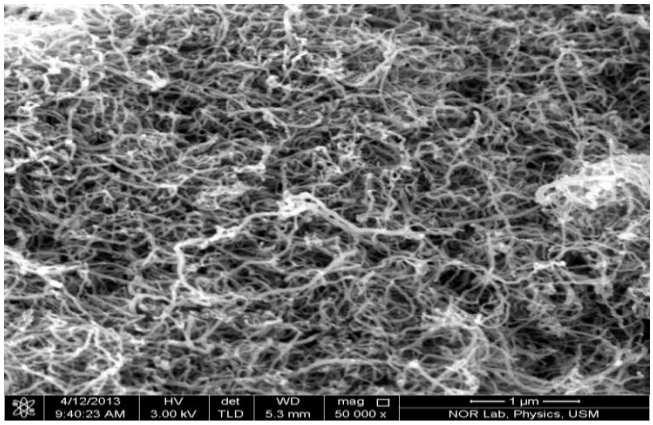
2.6. Characterization

The morphology of the MWCNT was characterized using a field emission scanning electron microscope (FESEM, Hitachi SU-70). The elemental analysis performed with an energy-dispersive X-ray spectroscopy (EDX). Transmission electron microscopy (TEM, Libra 120-Carl Zeiss) and atomic force microscopy (AFM) was used to study the structural properties of the MWCNT. DNA immobilization and hybridization was tested using a dielectric analyzer (Nova Control, Germany).

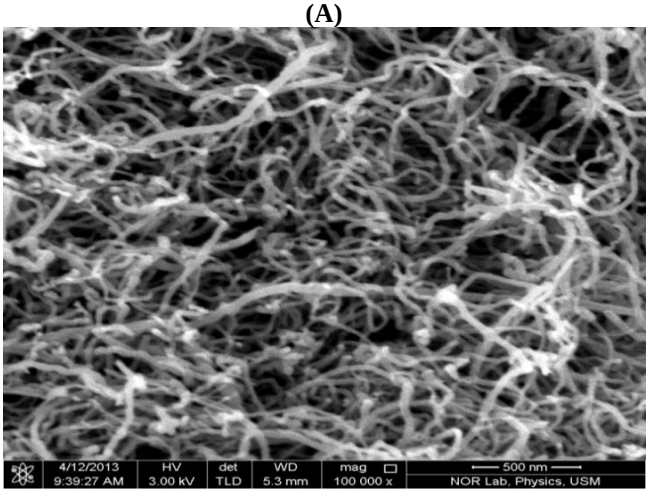
3. Results and Discussion

3.1. Characterization of Surface Morphology

Morphology and microstructure of the MWCNT prepared by fuel oil waste were investigated by field emission scanning electron microscopy (FE-SEM). Figure (1) shows typical surface morphologies of MWCNT as obtained from FE-SEM. The MWCNT can be clearly recognized without showing any preferred direction. The individual MWCNT have a bamboo-like structure which is a typical feature of relatively 30-40 nm in diameter.



(A)



(B)

Figure1: FE-SEM of of MWCNT (**A**) and (**B**)

3.2. Elemental Composition of MWCNT

The elemental and the chemical characterization MWCNT had been analyzed using EDX which is shown in Fig. (2). The spectrum for MWCNT demonstrated strong peaks for O and C, suggesting the chemical purity of the prepared MWCNT.

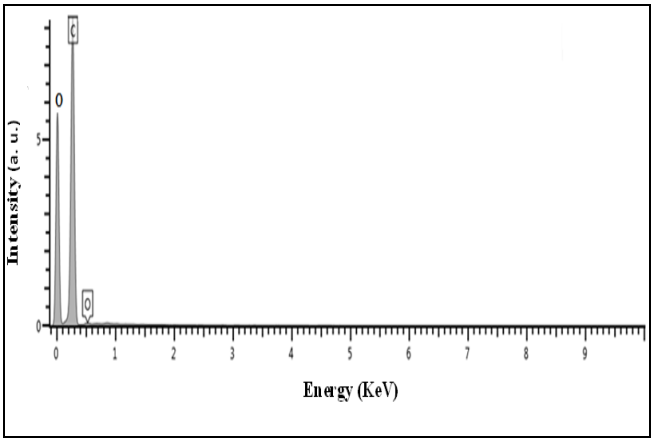


Figure 2: EDX spectrum MWCNT

3.3. Transmission Electron Microscopy (TEM) Analysis

TEM is applied to analyze the particle size and structure of MWCNT. Fig. (3) shows TEM image of the synthesized MWCNT as a powder grown by ultrasonic probe technique after purification.

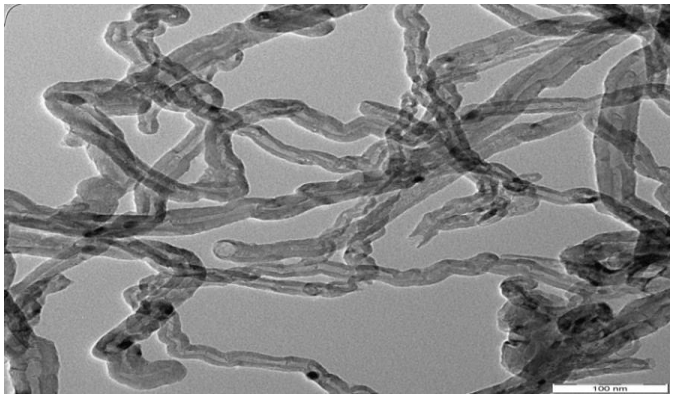
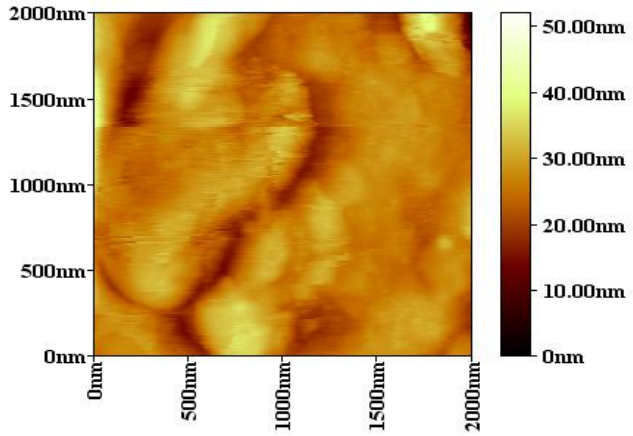


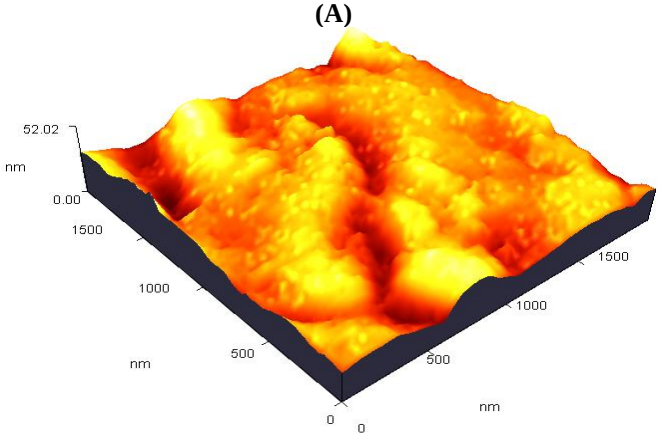
Figure 3: TEM of MWCNT

3.4. Atomic Force Microscopy (AFM)

AFM demonstrated the particle size and structure of MWCNT. Figure (4) shows AFM images which present a two-dimensional and three-dimensional view of the surface structure of the MWCNT prepared by ultrasonic probe technique. The images confirmed that the MWCNT have a roughness surface of 52.02 nm and small particles size distribution.



(A)



(B)

Figure 4: AFM images of 2-D (A) and 3-D (B) of MWCNT

3.5. Capacitance Measurement

The change of dielectric properties capacitance before and after immobilization and target DNA hybridization at different frequencies was measured using a dielectric

analyzer Figure (5). The capacitance measurement was also performed when the DNA-probe was immobilized onto the MWCNT-modified SiO_2thin films and hybridization was tested at frequency range of 1Hz to 1MHz on the same device. The result shows that the capacitance values of the bare, MWCNT modified surface and immobilization and target DNA hybridization were 43×10^{-12}, 17 µF, 27 µF and 22 µF respectively at 1 Hz. The capacitance value for MWCNT modified surface is higher than bare device. This is because the PEI-b is a conducting polymer material that performs a better capacitance signal compared with the bare device.

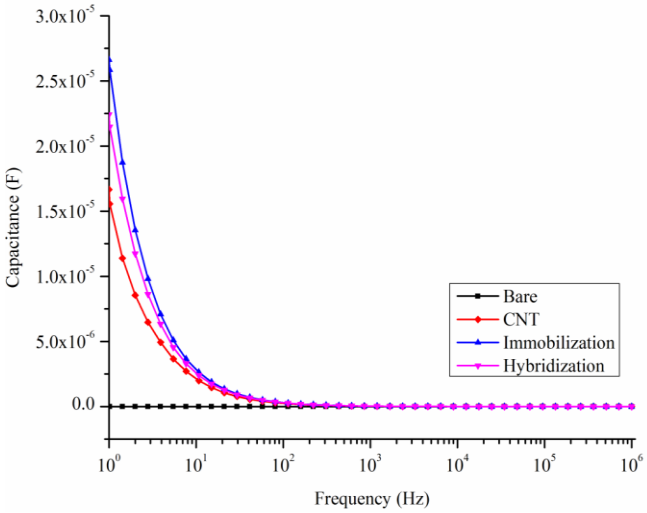


Figure 5: The capacitance versus frequency of MWCNT-modified SiO_2 thin films for DNA immobilization and hybridization detection.

3.6 Permittivity Measurement

The permittivity measurements were performed on the same sample Fig. (6). These measurements have the same trend whereby it gave the largest changes in permittivity with probe DNA immobilization and target DNA hybridization. However, it clearly displayed that permittivity measurement increased from 388×10^{-3} to 239×10^{3} and 201×10^{3} at the frequency range of ~200 Hz to 1 Hz for bare and DNA immobilization and hybridization respectively whereas the capacitance profile started significantly high from a frequency ~1 Hz and tend to decrease as the frequency increases. The result revealed that permittivity measurement giving more sensitivity at lower frequency. The results obtained from both measurements, displayed considerable changes in capacitance and permittivity value of the MWCNT-modified electrode after probe DNA immobilization and hybridization detection.

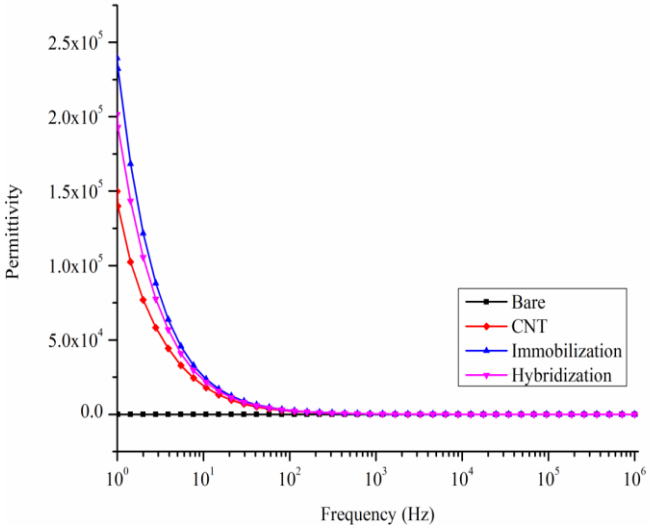


Figure 6: The permittivity versus frequency of MWCNT-modified SiO_2 thin films for DNA immobilization and hybridization detection

3.7. Conductivity Measurement

Conductivity measurements were also carried out to further investigate the effect of probe DNA immobilization and target DNA hybridization on the MWCNT-modified SiO_2 thin films. The measured conductivity values for bare, MWCNT and immobilized and hybridization device was 4.0×10^{-14}, 3.3×10^{-8}, 1.12×10^{-7} and 1.08×10^{-7} S-cm $^{-1}$, respectively. It can be observed from Fig. (7) that the conductivity was increased after MWCNT was deposited on the bare electrode; therefore the resistivity of the device was decreased. On the other hand, after DNA probe was immobilized and hybridization on the MWCNT modified SiO_2 thin films, conductivity was increased and therefore the resistivity of the device was decreased. This might be due to a strong interaction occurred between Potassium Hexacyanoferrate III, $K_3Fe(CN)_6$ and the unpaired guanine base in the probe DNA [28].

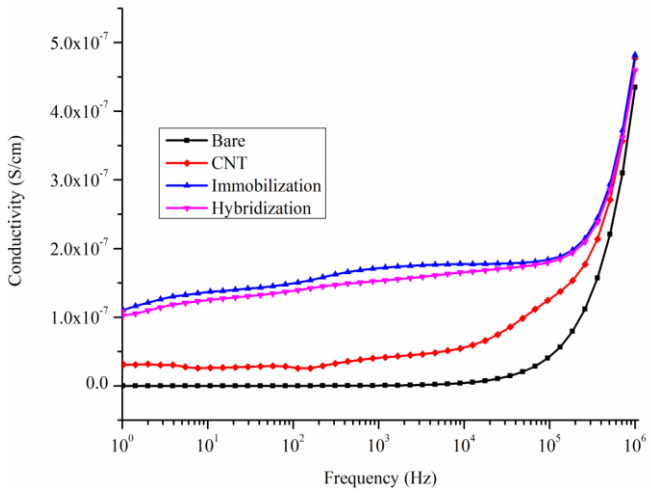


Figure 7: The conductivity versus frequency of MWCNT-modified SiO_2 thin films for DNA immobilization and hybridization detection.

4. Conclusions

DNA biosensor was successfully fabricated using MWCNT-modified SiO_2 thin films in electrolyte solution displays a good performance in capacitance, permittivity and conductivity measurements. The characterization of MWCNT using FE-SEM, EDX, TEM and AFM demonstrated good quality nanostructured. The MWCNT prepared by fuel oil waste ultrasonic probe technique provide a simple and promising method for DNA detection. The detection of the DNA immobilization and hybridization was achieved on MWCNT modified SiO_2 thin films by controlling the surface chemistry with $K_3Fe(CN)_6$ solution.

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Author Profile

Dr. Ahmed Mishaal: 2002, 2014: received the Masters and Ph. D degrees in Chemistry from Anbar University. **2004-2015:** worked as Faculty member and currently working as Assistant Professor in Chemistry Department of Faculty of Sciences, Anbar University, (Iraq). **Teaching Specializations:** Physical chemistry, Nano chemistry, Sono chemistry, Surface chemistry, Photo chemistry, and Pesticides chemistry.