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Preparation of DNA biosensor application from fuel oil waste by functionalization and characterization of MWCNT

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ABSTRACT

The potential of using a multi-wall carbon nanotube (MWCNT) synthesized from a fuel oil waste of power plants has discovered for the first time for DNA biosensors application. The MWCNT surface morphologies were examined by field emission scanning electron microscopy (FE-SEM) and atomic force microscopy (AFM). The thickness of the MWCNT was found 203 nm and confirmed by FESEM. The electrochemical DNA biosensor was successfully developed using a MWCNT modified on SiO_2 thin films. The capacitance measurements were performed to detect the sensitivity of DNA detection. The change in capacitance before and after immobilization of the DNA was measured in the frequency range of 1 Hz to 1 MHz. The results indicate that bare device exhibited the lowest capacitance value, which was 32.7 μF. The capacitance value of the DNA immobilization increase to 52 μF. The permittivity and conductivity also were examined to study the effect of the DNA immobilization toward the MWCNT modified surface. This present demonstrated that the MWCNT modified SiO_2 a thin film was successfully fabricated for DNA biosensor detection.

1. Introduction

In the past 10 years, the carbon nanotubes has been witnessed significant progress in both carbon nanotube synthesis and investigations on their electrical, mechanical, and chemical properties [1]. Carbon nanotubes (CNT) rank among the most exciting new developments in modern science and engineering. They offer several advantages such as small dimensions, extremely high strength, lightweight, elasticity, air stability, high electric and thermal conductivity [2]. The CNT comprises of single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) which is depending on their tube walls either one layer or more [3].

MWCNT is a materials used in many fields due to their unique properties which have high tensile strength, small size, good electric conductivity, mechanical, thermal properties and high electron transfer rate etc. [4]. The strong dependence of the properties of MWCNT on surface modification, mechanical deformation, doping, coating, etc. makes them have unique properties and very attractive material in different field such as chemical, biological, and physical sensors. In addition, small changes in the environment of the MWCNT can lead to significant variation to its electrical properties [5]. Further, many different applications have been suggested to exploit these unique properties, including: energy storage [6], molecular electronics [7], nano-probes [8], nano sensors [5], nanotube composites and nanotube templates etc. [6,8].

MWCNT could be synthesized via using different techniques, which involves gas phase processes. These processes provide access to the high synthetic temperatures that required for carbon nanotube production. The most common methods of producing carbon nanotubes are the electric arc discharge [6], laser vaporization, chemical vapour deposition (CVD) [5], electrolytic synthesis and solar production method. However, the conventional methods that have been mentioned above are costly and less reproducible to obtain the MWCNT [9]. Therefore, in this present work, we will present the ultrasonic probe method to produce MWCNT that prepared by fuel oil waste from more unique economical ways, environment technology friendly, cost effective, and safe comparable with novel materials.

DNA detection technology has been developed rapidly due to its extensive application. Moreover, the main issue in molecular bio diagnostics [10], is determination of genetic diversity [11], food analysis [12,13] criminal investigation in forensics and immigration [14] and environmental monitoring [10]. Different techniques have been

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proposed for the detection of target DNA, such as electrochemical sensing [15], fluorescence [16], chromatography in tandem with mass spectrometry [17], surface plasma resonance [18], and oligonucleotide microarray and DNA [10]. The detection of DNA is an area that taking attention interestingly due to it plays a main role in clinical, pharmaceutical and forensic applications. Electrochemical transducers offer several advantages, such as high sensitivity, simple, inexpensive and accurate specificity for converting DNA hybridization results into useful analytical signals [19–22]. An electrochemical DNA biosensor was successfully applied for detection of not only human diagnostic but also transgenic plants [15,23–33]. The sensitivity of DNA detection of combining with MWCNT surface may be increased the electrochemical signal. In this study, we develop a MWCNT synthesis from fuel oil waste by ultrasonic technique for DNA detection. The ultrasonic technique is considering a low-cost and the chemical composition, which can be controlled. The modification of the SiO_2 thin films surfaces with MWCNT have been improved of the label-free DNA detection. The surface morphology of MWCNT was examined and the electrical performances were evaluated using capacitance measurements.

2. Materials and methods

2.1. Preparation of MWCNT by sonication probe

The samples of fuel oil residue were collected from the power plant in Anbar area, Iraq, and refining according to the procedure mentioned elsewhere [34].

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 0.1 g activated carbon sample was placed in 250 ml vessel containing 100 ml of deionized water. Samples were placed into thermostated with circulating water bath at 25 ± 1 °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 °C for 24 h.

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 h at room temperature. Then the samples were washed several times with deionized water. For getting extra purification, the prepared materials were dissolved in 5 M nitric acid for 3 h at 70 °C. After that, the washing step was repeated as mentioned above for the HNO_3 treatment process. Treated MWCNT were dried at 120 °C. In order to eliminate non-carbon elements, all of the purified materials were placed in the furnace at 400 °C for 30 min, cooled in a desiccator and then identified using FE-SEM, and AFM.

2.2. Preparation and fabrication of MWCNT electrode

MWCNT were prepared from fuel oil waste by a sonication technique which is performed in a probe-type operating at room temperature. The surface morphology of the samples were examined by field emission scanning electron microscopy (FESEM); FEI Nova Nano-SEM 450 and atomic force microscopy (AFM) using Nano scope analysis version1.20 (Veeco), respectively [34]. A p-type silicon (100) wafer (1 cm × 1 cm) was cleaned by using acetone and isopropanol in ultrasonic for about 15 min and was immersed into the buffered oxide etch (BOE) solution and washed with deionized water followed by oxidation process for 30 min. After oxidation, the aluminium (99.99% of purity) was deposited on the backside of the Si using thermal evaporator. A 1.0 mg of MWCNT added into 5 ml of polyethyl eneimine branch (PEI-b), and then the 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, which tested by using electrochemical measurement were performed in a three-electrode Teflon cell (volume of the solution employed 10 mL of 10 μM potassium hexacyanoferrate III, $K_3Fe(CN)_6$ in 0.1 M KCl). The cell was hooked up to a potentiostat system (Autolab, PGSTAT30/GPES, Netherlands). An electrode was used as the working electrode, Ag/AgCl as the reference electrode and Pt wire has been used as the counter electrode. Scan rate used was 100 mV/s and the voltage was 1.5 V–1.8 V.

2.3. Probe DNA immobilization

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 h at 4 °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 h. After 2 h, 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. Electrochemical measurements

Electrochemical measurement was performed by using dielectric analyzer. The tests were conducted by using Ag/AgCl as the reference electrode and COOH-MWCNT modified electrode as a working electrode. The Al acts as a back gate. The responses of the DNA immobilization were investigated at 10 μM Potassium Hexacyanoferrate III, $K_3Fe(CN)_6$ aqueous solution containing 0.1 M KCl as electrolyte. The schematic view of testing measurement for DNA immobilization as shown in Fig. 1.

3. Results and discussion

3.1. Surface morphologies of MWCNT

3.1.1. The field emission scanning electron microscopy

Fig. 2 shows typical surfaces of MWCNT prepared by fuel oil waste with the uniform morphology. 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. The MWCNT are well dispersed and shows sheet which regards as an entangled MWCNT network. The MWCNT sheets have high flexibility and not easily damaged during oxidation, washing and drying processes.

3.1.2. Atomic force microscopy (AFM)

AFM is an important biophysical technique for studying the morphology of MWCNT and biomolecules. Quantitative information from individual MWCNT can be generated from software-based image

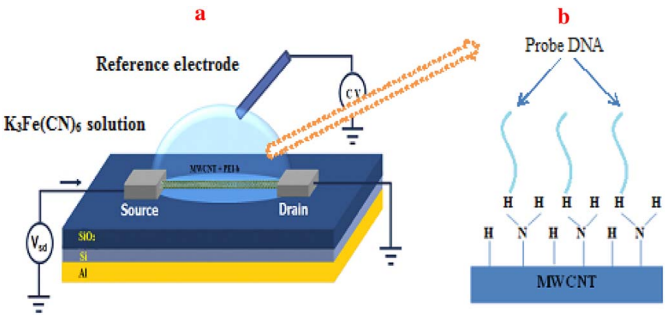

Fig. 1. The schematic illustration of testing measurement (a) The modified COOH-MWCNT electrode and (b) The immobilization of DNA using PEI-b.

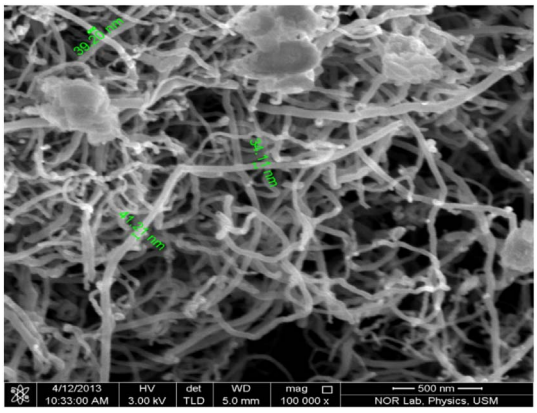

Fig. 2. FE-SEM image of MWCNT by fuel oil waste preparation using an ultrasonic probe technique with the diameter of MWCNT is around 30–40 nm.

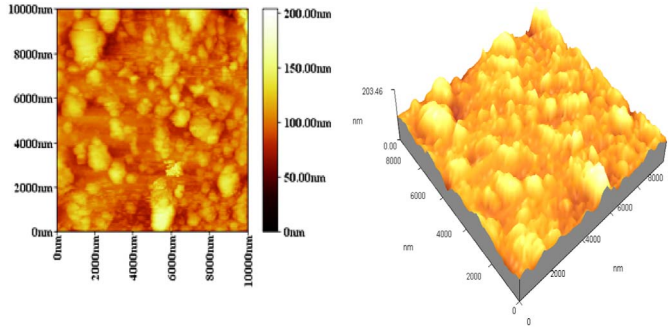

Fig. 3. AFM images of 2-D and 3-D of MWCNT.

processing of AFM data. Size information (length, width, and height) and other physical properties (morphology and surface texture) can be measured for individual particles. AFM can be performed both in liquid or gas media. This capability can be highly advantageous for MWCNT characterization. AFM is applied to analyse the morphology of particle size and topography of the surface structure of MWCNT. Fig. 3 shows AFM of the images that present a two- dimensional and three-dimensional view of the surface structure of the MWCNT, which has grown by ultrasonic probe technique. The images indicate that the MWCNT have a roughness surface of 203 nm and small particles size distribution. The surface roughness results indicated that MWCNT plays an important role in increasing the surface area.

3.2. Capacitance measurement

The dielectric behaviour of MWCNT that modified surface for DNA detection was investigated by using dielectric analyzer. Fig. 4 demonstrates the capacitance-frequency (C-F) characterization by connecting two points probe. The change in capacitance before and after immobilization of the DNA onto the MWCNT-modified electrode was carried out in the frequency range of 1 Hz to 1 MHz on the same sample. The result shows that the capacitance values of the bare, MWCNT modified surface and immobilization of DNA were 32.7 μF, 40 μF, 52 μF, respectively at 1 Hz. The capacitance value for MWCNT modified surface is higher than bare device. This could be due to the PEI-b is a conducting polymer material that performs a better capacitance signal compared with the bare device. The immobilization of DNA was successfully detected by showing the highest capacitance value of 52 μF onto the modified MWCNT electrode. The capacitance value between MWCNT-modified surface and DNA immobilization have been achieved successfully using a MWCNT that prepared by fuel oil waste as a potential material for DNA detection for the first time. M. M. Ahmed et al. [35] the aim of this study, synthesis DNA biosensor by (3-

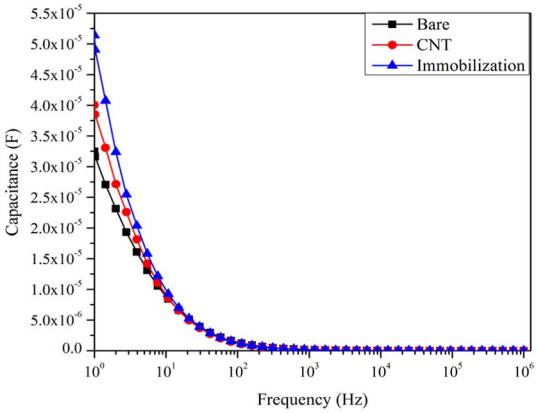

Fig. 4. The capacitance as a function of frequency for MWCNT-modified SiO_2 thin films for DNA immobilization.

aminopropyl) tri-ethoxysilane on a thermally oxidized SiO_2 thin film. The SiO_2 thin films surface was chemically modified with a mixture of APTES and GNPs for DNA detection in different time periods of 30 min, 1 h, 2 h, and 4 h, respectively. The DNA immobilization and hybridization were conducted by measuring the differences of the capacitance value within the frequency range of 1 Hz to 1 MHz. The differences of the capacitance value between the DNA immobilization and hybridization revealed that the modified SiO_2 thin films using APTES and GNPs were successfully developed for DNA detection. M. M. Ahmed [36] reported on the synthesis of a biosensor using silicon oxide for biomedical applications, and its effective use for the detection of target DNA hybridization. An electrochemical DNA biosensor was successfully fabricated by using (3-aminopropyl) tri-ethoxysilane (APTES) as a linker molecule combined with gold nanoparticles (GNPs) on a thermally oxidized SiO_2 thin film. The size of the GNPs was calculated by utilizing UV–vis data with an average calculated particle size within the range of 30 ± 5 nm, and characterization by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The GNP-modified SiO_2 thin films were electrically characterized through the measurement of capacitance, permittivity and conductivity using a low-cost dielectric analyzer. The capacitance, permittivity and conductivity profiles of the fabricated sensor clearly differentiated DNA immobilization and hybridization.

3.3. Permittivity measurement

Measurements of the permittivity were also carried out in the same device dielectric analyzer (Alpha-A High Performance Frequency Analyzer, Novocontrol Technologies, Germany) Fig. 5. These measurements have the same direction at which it gave the largest changes in permittivity with probe DNA immobilization. However, it clearly

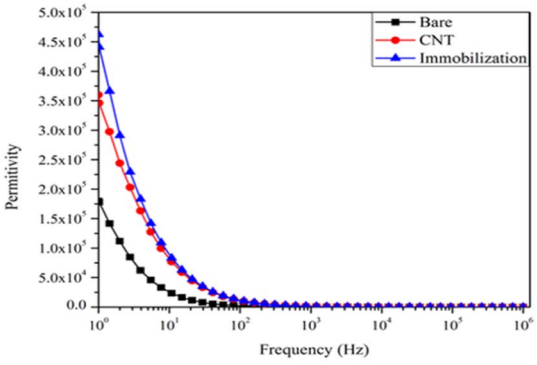

Fig. 5. The permittivity as a function of frequency for MWCNT-modified SiO_2 thin films for DNA immobilization.

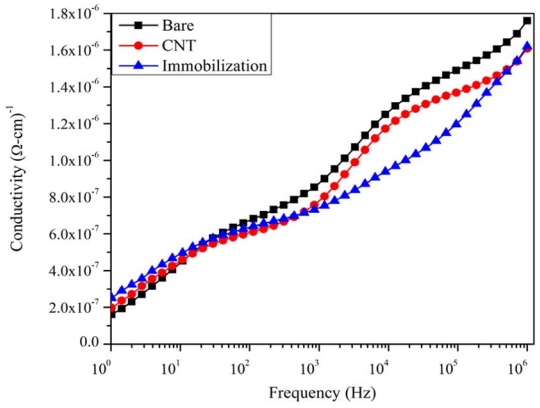

Fig. 6. The conductivity as a function of frequency for MWCNT-modified SiO_2 thin films for DNA immobilization.

displayed that permittivity increased dramatically from 188 × 10^3 to 468 × 10^3 at the frequency range of ~200 Hz to 1 Hz for bare and DNA immobilization respectively, whereas the capacitance profile started significantly higher from a frequency ~ 1 Hz and tend to decrease as the frequency increases. The results reveal that permittivity measurement has giving more sensitivity at lower frequency. The results also indicate that from both measurements, displayed considerable changes in capacitance and permittivity value of the MWCNT-modified electrode after probe DNA immobilization.

3.4. Conductivity measurement

Conductivity measurements were also performed to investigate the effect of probe DNA immobilization on the MWCNT-modified SiO_2 thin films. The measured conductivity values for bare, MWCNT and immobilized device was 1.6 × 10^{-7}, 2.0 × 10^{-7} and 2.57 × 10^{-7} Ω-cm^{-1}, respectively. It can be observed from Fig. 6, the conductivity was increased after MWCNT deposited on the bare electrode; therefore the resistivity of the device was decreased. On the other hand, after DNA probe was immobilized onto the MWCNT modified SiO_2 thin films, conductivity increases as the resistivity of the device 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 [37]. Furthermore, the differences value of conductivities between MWCNT-modified surface and DNA immobilization has shown the electron transfer occurred during the immobilization of DNA. Therefore, the conductivity measurement enhanced the reaction of the DNA immobilization onto MWCNT modified surface, which were realized the characteristic of the electrolyte solution.

4. Conclusions

It has been shown that the MWCNT-modified electrode in electrolyte solution exhibits a good performance in capacitance, permittivity and conductivity measurements. This study provides clear evidence that the DNA were successfully immobilized onto MWCNT-modified surface to assemble label-free biosensors. 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 was achieved on MWCNT modified SiO_2 thin films by controlling the surface chemistry with $K_3Fe(CN)_6$ solution. These MWCNT modified electrode were found to be an exemplary material and holds a great potential for future medical diagnostics.

Conflict of interest

The authors have declared no conflict of interest.

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