Simple method for making MWCNTs/Au-NPs-based biosensor electrodes

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Abstract

Multiwalled carbon nanotubes have great potential when applied as biosensors. Their properties, especially as electrodes with electrochemical characteristics, offer strong benefits for developing biosensors. This research has been able to integrate multiwalled carbon nanotubes (MWCNTs) with Au nanoparticles (Au-NPs) to obtain several new superior properties. Cysteaminium chloride is used to link MWCNTs and Au-NPs while binding to specific antibodies to make them more sensitive to some diseases or viruses. The data on the success of the bonding of MWCNTs/Au-NPs were tested using three characterizations, namely FTIR, SEM, and XRD. Based on the results of testing electrochemical properties using the CV and EIS tests, the capacitance value of 6,363 F g⁻¹ and the Rct value of 717,9 Ω, respectively. This demonstrates good adhesion and electron transfer properties from the electrolyte to the probe and electrode.

1. Introduction

After the carbon nanotube was developed, many scientists were able to apply its unique and special properties to optimizations across a wide variety of fields. Multiwalled carbon nanotubes (MWCNTs) can be described as graphene rolled up to form nanoscale tubes stacked on top of each other to form a wall [1–3]. One of their special properties is electrical [4, 5]. Nanomaterials have a larger surface than bulk material and can lead to better sensitivity and faster electron transfer [6]. Similar to MWCNTs, Au nanoparticles have good electrical properties, so they can be used as sensors or biosensors [7, 8]. The combination of the two materials (MWCNT/Au-NPs) provides new properties that previously did not exist together. These combined materials have better electrochemical performance than when separated, such as better conductivity, enhanced electron transfer, larger sensing surface, and easy immobilization of biomolecules [9–11].

Chemical bonding is very important to develop materials for specific purposes. For biosensors and biomolecules, stable covalent bonding of MWCNTs and Au-NPs is required [12]. Kardimi et al. have used functionalized MWCNT with a mixture of H₂SO₄/HNO₃ and SOCl₂ for the activation process [13]. Chinh et al. have used cysteamine chloride to produce a zwitterionic reaction [14]. Cysteamine chloride is used to replace some toxic chemicals such as carbodiimide hydrochloride or thionyl chloride. These materials produce ionic features that allow electrostatic interactions between MWCNTs and biological molecules and can serve as the basis for developing MWCNT biological probes [15].

From these properties, it can be assumed that MWCNT/Au-NPs is excellent for use as a sensing material [16–19]. Over the past few years, electrochemical detection techniques have grown in popularity. One of them is screen-printed electrodes (SPEs), which are commonly used in electrochemical biosensors. Among electrochemical detection techniques, sensing devices using SPEs are preferred over other types of electrodes because of their simplicity of fabrication and operation methods, ease of preparation, and their ability to be made from a variety of materials [20, 21]. In this study, we have modified the screen-printed electrodes (SPEs) of the GlucoDr diabetic test strip with the MWCNT/Au-NPs we synthesized on the surface of the SPEs’ working electrode. The deposition of the material on the SPE was carried out by the spray deposition method.
2. Experimental

2.1. Material

The functionalized MWCNT used in this study was synthesized at the Bionano Technology laboratory, Diponegoro University, Indonesia. The Au-NPs suspension was provided by our partners from the laser laboratory, Diponegoro University, Indonesia. Dimethylformamide (DMF) was used as solvent for MWCNT, and cysteamine chloride was purchased from Sigma-Aldrich Canada.

2.2. Experimental method

The manufacture of MWCNT/Au-NPs-based material sensors is carried out in two stages. The first step is combining MWCNT and Au-NPs. The second step is coating the material onto the SPE surface. In the first step, the MWCNT functionalization was carried out first. After that, 0.1 gram of functionalized MWCNT was dispersed in dimethylformamide (DMF) with a ratio of 1:50 w:v then stirred using a magnetic stirrer for 30 min at a speed of 1000 rpm. To ensure that the functionalized MWCNT is well dispersed in the DMF, a sonication process was carried out using an ultrasonic bath at 20,000 Hz for 40 min. After that, 200 l of cysteamine chloride 10 mmol was added dropwise while stirring again. The mixture was dried at 90 °C to remove solvent and then washed with distilled water. The dried MWCNT was then mixed with 10 ml of 1 mol Au-NPs for 30 min at 2000 rpm using a magnetic stirrer, then sonicated at 20,000 Hz for 30 min. The deposition of MWCNT/Au-NPs was carried out by spraying onto the surface of the SPEs’ working electrode, which was on a hot plate at a temperature of 90 °C. Spray processing was run using an air brush with 0.8 nozzle, using a twin tube compressor to maintain constant air pressure at 75–85 psi and flowrate at 40 l/min. The distance between material surface and spray nozzle was kept within 30 cm with 2 s spray time and 2 s rest time to ensure constant
flow and air pressure until all liquid was sprayed, covering the working electrode with 7.06 mm² dimension. The working electrode of the SPEs coated with MWCNT/Au-NPs was then dried in an oven at 150 °C for 45 min. Figure 1 shows the synthesis and deposition of MWCNT/Au-NPs on SPEs.

3. Results and discussion

The deposition of MWCNT/Au-NPs material on the surface of the SPEs was carried out by the spray coating method. The deposition is above the working electrode in contact with the sensing object and not on the entire working electrode. In figure 2, it can be seen that the deposition succeeded in covering the top of the SPEs' working electrode.
Figure 3 shows the FTIR characterization used to analyse the functional groups of functionalized MWCNTs and thiolated MWCNTs. In figure 3(a), the FTIR spectrum shows the main peaks in the form of a carboxyl group (COOH) and a hydroxyl group (–OH) in functionalized MWCNTs.

In figure 3(b), the functionalized MWCNTs with the addition of cysteamine have produced a new peak at the wave number of 3445 cm$^{-1}$, referring to the N-H symmetrical amine vibration. In addition, there is also a widening of the peaks around the numbers 1635 cm$^{-1}$ and 1524 cm$^{-1}$, which refers to the overlapping of the COOH and NH$_3^+$ groups. The two peaks indicate the protonation of the amine group, which causes the formation of zwitterionic species through deprotonation of the carboxyl group (COOH). The results of the FTIR spectrum indicate that the addition of cysteamine chloride on the surface of the MWCNTs has been successful.

The morphology of the MWCNTs/Au-NPs material on the surface of SPEs was analysed by scanning with electron microscopy. Figure 4(a) shows that MWCNTs have successfully coated the surface of the SPEs with gold dots, indicating the presence of Au-NPs. Figure 4(b) further shows how Au-NPs (red circle) are present and bound to MWCNTs when the MWCNTs tube size and Au particles are below 100 nm.

Structure characterization was carried out by XRD analysis. As seen in figure 5, each material containing MWCNTs and Au-NPs has 2 peaks that matched with JCPDS No. 04–0784 and JCPDS No. 75–1621. Table 1 shows observation results of 2θ and hkl in each peak.

To better understand the effect of this structure on the redox signal, electrochemistry was employed. This SPE was tested with a PalmSense4 electrochemical instrument. Two differential testing method were used to analyse this SPE’s electrochemical properties.

First, cyclic voltammetry was analysed to determine the difference between the two types of SPEs that were tested. The measurement was carried out at a scanning rate of 0.5V with potential range of −1.0–2.0 V in a H$_2$SO$_4$ 0.1M solution. The resulting CV plots can be seen in figure 6, which show the difference in area of potential (V) versus current (A). SPEs coated with MWCNTs/Au-NPs have larger area than SPEs coated with carbon. As we know, material in nanoparticle sizes have a larger surface area to volume ratio than material in bulk solid sizes. Thus, the SPEs coated with the nanosized material has a larger surface area that affects contact to the specific electrolyte than bulk material, but the area of the resulting product is still the same as carbon material. The effect of having more area was increasing transfer electron between the redox probe and the working electrode, resulting in good adhesion and good conductivity between those two materials. From this test, we can also determine the capacitance, although this aspect was not completely within the scope of this research, as this purpose is for biosensors and not supercapacitors.
The specific capacitance ($C_s$) was affected by the surface area of material and electric conductivity. In this case, the SPE using the nanomaterial for coating has a larger surface area, causing increased capacitance compared to carbon bulk material, and we know that gold and MWCNTs have good electrical conductivity.

| Material                                  | Capacitance (F/g) | Reference |
|-------------------------------------------|-------------------|-----------|
| MWCNTs/Au-NPs-based                       | 6,363             | This study|
| Carbon-based                              | 3,369             | This study|
| Activated carbon/Silver nanocomposite     | 194               | [22]      |

The specific capacitance ($C_s$) was affected by the surface area of material and electric conductivity. In this case, the SPE using the nanomaterial for coating has a larger surface area, causing increased capacitance compared to carbon bulk material, and we know that gold and MWCNTs have good electrical conductivity.
From table 2, in this study of SPEs showed very low capacitance compared to the reference because the CV graph has a very small area. The next electrochemistry test was electrochemical impedance spectroscopy (EIS). The measurement was performed in 0.1M mol H$_2$SO$_4$ solution for the electrolyte. Frequency from 1 Hz to 10k Hz was applied. This technique provided a better understanding by transposing the system into an equivalent electrical circuit. In this curve, the first semicircle represents the charge transfer resistance in parallel with the double layer capacitance, while the second circle, at low frequencies, represents the contribution of the Warburg impedance. Modifying the electrode with MWCNTs/AuNPs caused a large decrease in charge transfer resistance, as shown in table 2. Therefore, the introduction of MWCNTs resulted in a significant change in the electrochemical response of the system. The resistance of $R_s$ and $R_{ct}$ was confirmed due to the fitting and simulation in EIS analysis.

From figures 7(a) and (b), we compared the test material to test the differences in EIS data. The charge transfer resistance between carbon and MWCNTs/Au-NPs had a large gap. This could be caused by fast electron transfer from the MWCNTs. Figure 8 shows the CV curve and Nyquist plot from research before, that actually resemble this study. Indicated our study for SPEs appropriate with other research.

Table 3 shows the result of each index after analysing the Nyquist plot using an EIS spectrum analyser. The reduction in $R_{ct}$ as shown on table 3 can be interpreted as the ease with which electrons are transferred across the interface. It also can be scrib to the enhanced charge transfer kinetics of MWCNTs/Au-NPs as compared to carbon SPE followed via higher separation efficiency of holes and electrons.

4. Conclusion

When focused on developing a good electrochemical material, MWCNTs/Au-NPs performed as good as anything we see in the CV and EIS performance for electrochemical probe instruments. In terms of successfully grafting Au-NPs onto MWCNTs, the data from SEM and x-ray diffraction showed the satisfactory results. The electrochemistry results have two different characteristics. In CV analysis, the MWCNTs/Au-NPs SPEs displayed lower capacitance than previous research due to the small area of the diagram, which affected capacitance results. In EIS analysis, we focused on $R_{ct}$ to better understand electron transfer.

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Data availability statement

The data generated and/or analysed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

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