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The Effect of a Combination of Multiwalled Carbon Nanotubes (MWCNT), Gold Nanoparticles (AuNPs), and Silica Microspheres (Si) on the Detection of 17α -Ethinylestradiol (EE2)

M. Shafawi[1,*], A. H. N. Hamidah[1,3], M. F. M. Fathil[1], Nur Syakimah Ismail[2], A. R. Ruslinda[1], Norhayati Sabani[3], and Mohammad Nuzaihan Md Nor[1]

[1] Institute of Nanoelectronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia
[2] Faculty of Electronic Engineering Technology, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia
[3] Micro System Technology, Centre of Excellence (MiCTEC), Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

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ABSTRACT

17α-ethinylestradiol (EE2) is a female synthetic hormone with high estrogenic activity that can disrupt the environment, which can lead to dire health problems. The current standard method is very time-consuming and expensive, so the objective of this study is to develop chemical sensors that are faster and much cheaper to detect EE2 in water samples. In this work, an electrochemical sensor using a combination of multiwall carbon nanotubes (MWCNT), gold nanoparticles (AuNPs), and silica microspheres (Si) as a modified electrode is reported to demonstrate 17α-ethinylestradiol (EE2) detection using electrochemical analysis. MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs are prepared for the development of this chemical sensor by depositing each material on the carbon electrode by using the drop casting method. The resistance and conductivity of MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs modified electrodes demonstrated stable electrochemical current responses towards the detection of EE2. It demonstrates that when MWCNTs and AuNPs are combined with Si, the sensitivity and R2 value improves at EE2 concentrations ranging from 10 to 50 μM, respectively.

Keywords: Carbon nanotube, electrochemical, ethinylestradiol, gold nanoparticles, sensor

1. INTRODUCTION

Endocrine disruptor chemicals (EDCs) are compounds that can affect with an organism's natural growth and reproduction by interfering its endocrine system [1][2][3]. In the 1990s, researchers gained attention in the implications of wildlife and human exposure to potential endocrine disruptors. EDCs are a diverse group of compounds that have a negative impact on the hormonal system. Most EDCs have been recognized as having estrogenic activities, and several studies have confirmed that low exposure to these estrogenic compounds in the range of nanograms (ng) or nanograms per liter (ng/L) can cause significant effects on aquatic species. Referring to water framework directive, the environmental quality standards derivative was amended in 2013/39/EU and a watch list was established to require monitoring of other substances for which evidence recommends a possible risk to the environment. The first watch list adopted in 2015 identified several substances with a clear estrogenic activity, including two natural hormones, 17β-estradiol (E2) and estrone (E1). This synthetic hormone of interest, 17α-ethinylestradiol (EE2), was also included on the list [4].

* shafawijaafar@gmail.com

The investigation regarding EE2 contamination in water systems around the world, exposing the effect of EE2 and other hormones on living things and aquatic systems, leads to growing interest in the development of technologies with the ability to detect and remove these materials [3]. Researchers from around the world started to develop a biosensor capable of quantifying EE2 at very low concentrations. The advantages of biosensors include a very low limit of detection (LOD), the relative possibility of miniaturization and portability of the devices, and the low cost of biosensor development [5].

Multiwall carbon nanotubes (MWCNT) have attracted attention in biosensing applications and have been labeled as conductive fillers in composites. MWCNT presents outstanding charge transport characteristics with a larger surface area and therefore greatly promotes electron-transfer reactions, which can dramatically improve electrochemical performance. MWCNTs are expected to present a flexible surface chemistry, wide electrochemical window, and biocompatibility [6][7]. Currently, gold nanoparticles play a significant role in the construction of a new generation of biosensors and in specific electrochemical biosensors. The ability of gold nanoparticles to provide a stable surface for biomolecule immobilization while retaining biological activity is a significant advancement in the development of biosensors. Gold nanoparticles (AuNPs) are attributed to improving electron transfer between redox proteins and electrodes due to their high surface-to-volume ratio, high surface energy, ability to reduce the distance between proteins and metal particles, and quality as electron-conducting pathways between compounds and the electrode surface [8][9][10]. Silica nanoparticles have excellent physical, chemical, mechanical, and optical characteristics, making them ideal for use in biosensors, chemical sensors, composite materials, catalysts, photonic crystals, bioimaging markers, nanofillers, and other applications [11]. Mesoporous materials, according to IUPAC, are materials with pores in the range of 2–50 nanometers that have an organized structure and arrangement. Some of the remarkable characteristics of this mesoporous material include its variety of structural features, tunable pore sizes ranging from 2 to 10 nm (2D hexagonal and 3D cubic), controllable particle size, a large surface area and porosity, high pore volume, high chemical stability, easily changeable surface properties, non-toxicity, and strong biocompatibility [12] [13]. Figure 1 below shows the examples of nanomaterial used in chemical sensors.

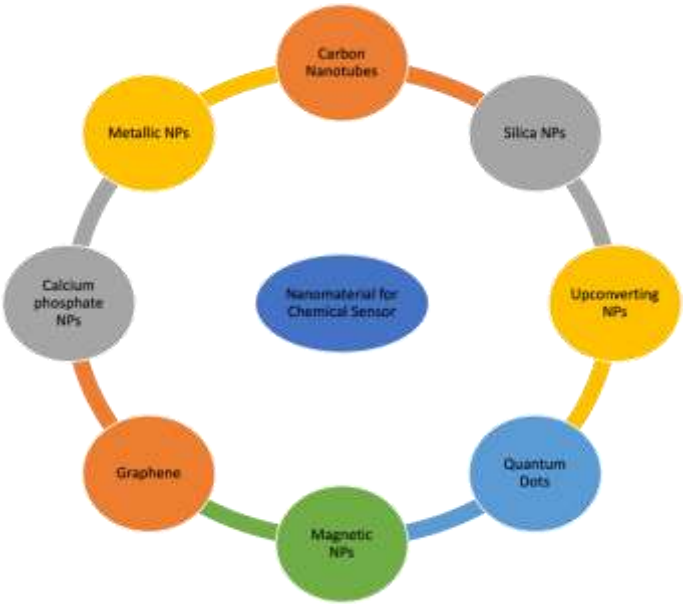


Figure 1. Example of nanomaterial.

The purpose of this study is to study the effect of material combination in EE2 detection. In an electrochemical study, the electrode surface determines optimal performance in electroanalysis. Doping materials aim to increase electrochemical activity [14][15]. This study was conducted using chemical and electrochemical procedures by employing different types of nanomaterial, such as MWCNT, gold nanoparticles, and silica. The materials were combined and deposited on top of each other, then tested at different concentrations to determine which combination could detect EE2 at lower or higher concentrations with a higher current.

2. MATERIALS AND METHODS

All reagents were of analytical or biochemical grade, including ethinyl estradiol, 3-Aminopropyltriethoxysilane (APTS, 99%), tetracholoroauric acid trihydrate, and multiwall carbon nanotube, which were purchased from Sigma Chemical Co., St. Louis, Missouri, USA; tri-sodium citrate dihydrate was provided by Merck, Darmstadt, Germany. The carbon sheets were supplied by STREM (Strem Chemicals, Incorporated, and Mulliken Way, MA, USA). Cyclic voltammetry was performed using an Autolab μStat-I 400s (Metrohm Autolab B.V., The Netherlands). A scanning electron microscope (SEM) (JEOL JSM-6010LY) was used to examine the morphology of the modified electrode surface. Images were taken at 10 kV with various magnifications.

2.1 Materials Preparation

2.1.1 Gold Nanoparticles (AuNPs)

A method proposed by Halim et al. [16] would produce gold nanoparticles (AuNPs) with a diameter of 15 nm by reducing tetrachloroauric acid with trisodium citrate at high temperatures. AuNPs was synthesized using the reduction method using trisodium citrate. In brief, $HAuCl_4$ (0.020g) in 200 ml of DI water. The solution will undergo a heating process and be stirred vigorously until the temperature reaches 97°C. Then, trisodium citrate ($Na_3C_6H_5O_7$) weighed 0.02g, and 1.8 mL of trisodium salt was added to the solution. The mixture is stirred until there are no more color changes; the final color of the AuNPs solution is red wine.

2.1.2 Silica Microsphere Synthesis

Next, for silica microsphere synthesis, a mixture of 2 mL DIW and 5 mL ammonia solution was added to 20 mL of ethanol. Then, the solution was sonicated for 10 minutes before a mixture of 2 ml TEOS and 4 ml ethanol were added to it and it was left for sonication for another 40 minutes. The mixture was centrifuged at 4000 rpm for 20 minutes and washed with DI water three times. 10mg of silica microsphere (Si) was weighed and added to a mixture of ethanol and DI water.

2.1.3 Multiwall Carbon Nanotube Preparation

The preparation of the MWCNT solution starts with 15mg of MWCNT, which is weighed and added to 10 ml of ethanol, which is then sonicated for 20 minutes. The MWCNT and Si solutions were sonicated for 5 minutes before the deposition process to ensure all the particles dispersed in the solution.

2.2 Electrode Surface Modification

With the purpose of increasing the working electrode conductivity, the electrode surface was modified with different combinations of these materials (MWCNT, Si, and Au). Firstly, the carbon sheet was cut into pieces of 0.5 cm by 2.0 cm and washed three times using ethanol and DI water. Once the carbon strips (CS) are prepared, the deposition process using the drop-cast method [17]

is carried out as shown in Figure 2. Briefly, 50 µL or 1.5 mg/mL of MWCNT solution was deposited on top of the CS, followed by 50 µL of AuNPs solution. This process is repeated by depositing MWCNT-Si and Si-Au. Then, the modified electrode will undergo a heating process at 80 °C for 10 minutes to remove the solvent, and all that is left on top of the CS are the nanoparticles. The modified electrode surface was characterized by SEM. Besides, electrochemical characterization was performed by cyclic voltammetry using a solution of EE2 in a 0.1 M PBS buffer solution at a pH of 7.

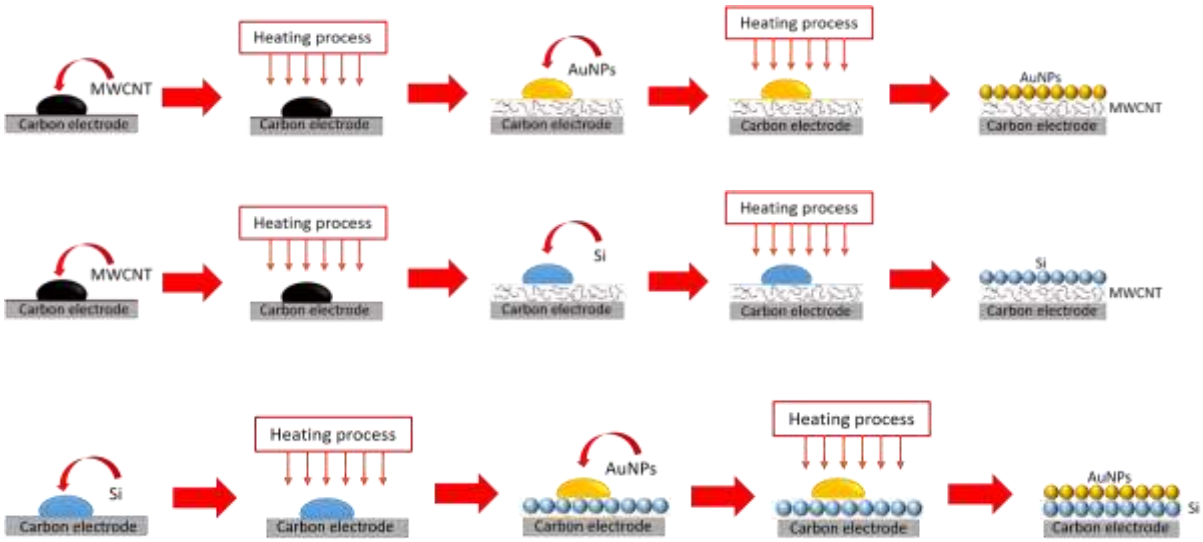
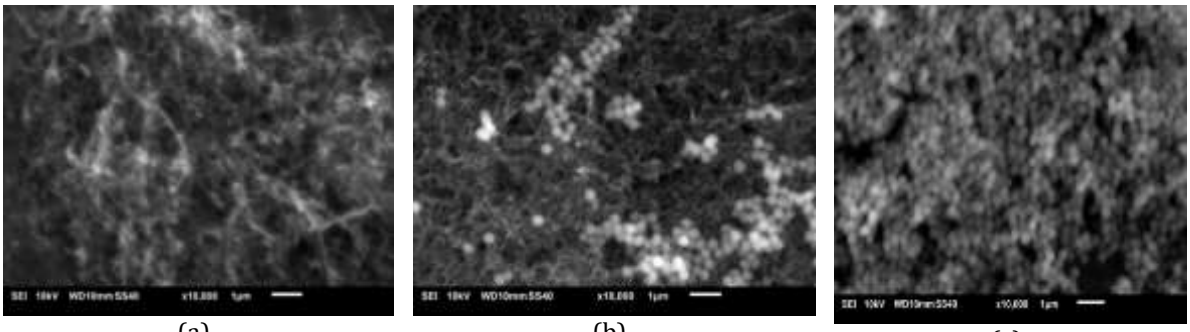


Figure 2. Deposition process of nanomaterial using combination of MWCNT, silica and gold nanoparticles.

3. RESULTS AND DISCUSSION

The morphology and electrochemical response of MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs modified electrode surfaces were characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV), respectively. The electrochemical characterization was performed by CV using EE2 solution in different molarities in PBS buffer solution (0.1M at pH 7 unless stated otherwise). Figure 3 shows the modified electrode, and Figure 3(A) shows the distribution of MWCNT on CS, which were of non-uniform shape with submicron size less than 1m Figure 3(B) shows the MWCNT-Si electrode the silica particles were a uniform shape, but the MWCNT shows good distribution on carbon surface. Figure 3(C) shows the Si-AuNPs combination the silica microsphere particles in uniform shape with size less that 1µm. The gold nanoparticles cannot be seen under scanning electron microscope because the size is around 15nm.



(a) (b) (c)

Figure 3. SEM images of modified electrode a) MWCNT-Au a) MWCNT-Si c) Si-AuNPs

The electrochemical characterization of the modified and bare electrodes was performed by cyclic voltammetry (CVs) obtained for the bare electrode, and the combination of MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs are shown in Figure 4(a), where the peak of EE2 is observed at 0.52 V, which agrees with Carneiro et al. [18]. The oxidation potential of EE2 is around 0.5 V, and the reduction process of EE2 has not occurred due to the tenacious physico-chemical properties of EE2 [3]. These chemical substances are frequently lipophilic, bio accumulative, persistent in their physicochemical qualities, and have low vapor pressure, all of which help them disperse in the environment [19]. First, PBS (0.1M, pH 7) was used to study the characteristics of the modified electrode. Figure 4 (A) shows the voltammograms of the bare and modified electrodes in PBS, and from the observation, there is no peak of oxidation except for PBS at 0V. Figure 4 (B) shows bare electrode in PBS and EE2 from the observation the bare electrode produced a good current response toward EE2 at potential 0.52V. Figure 4 (C), (D) and (E) shows the voltammograms of modified electrode which is MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs respectively. The current is slightly lower when compared to bare electrode.

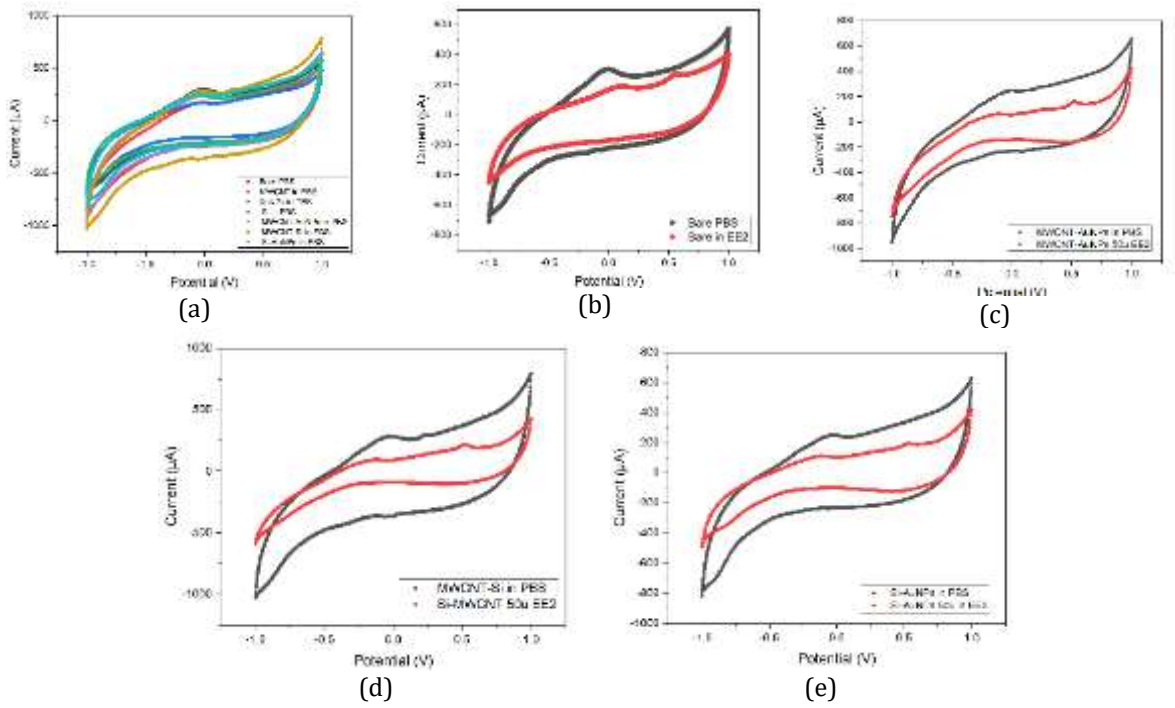
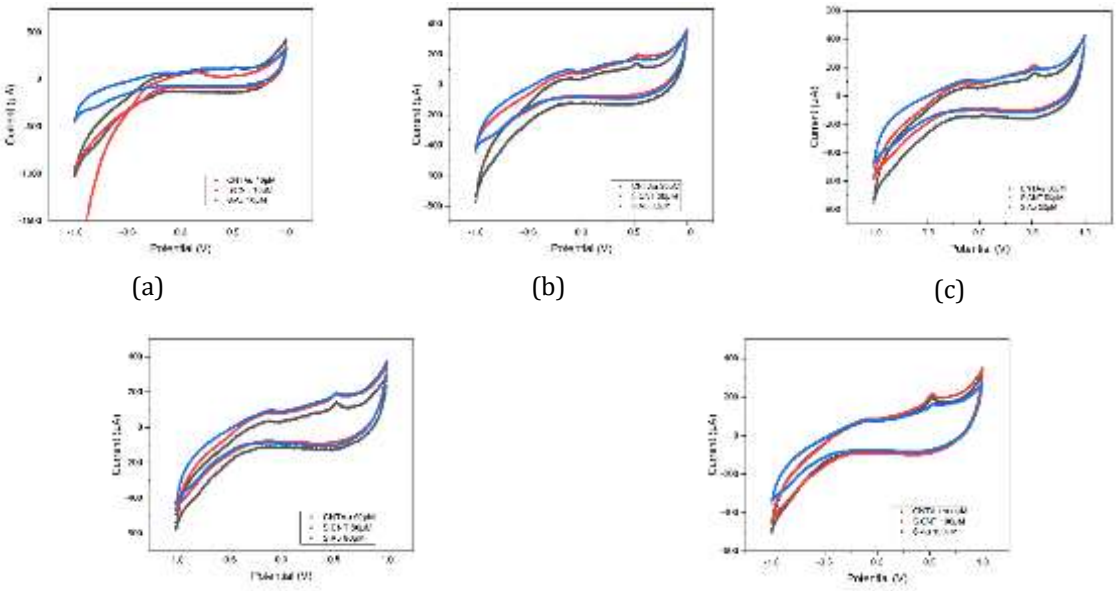


Figure 4. CVs obtained in solutions of PBS and EE2 A) bare and modified electrode in EE2; B) bare tested in PBS and EE2; C) MWCNT-Au in PBS and EE2; D) MWCNT-Si in PBS and EE2; E) Si-Au in PBS and EE2.

The electrochemical responses of each combination were characterized based on the oxidation mechanism of EE2 using cyclic voltammograms (CV). The CV being presented in Figure 5 and the respective peak-to-peak of different materials tested at different concentrations are shown in Figure 6. In terms of peak heights, the MWCNT-Au can detect EE2 in 10 µM with a current of 131.67 µA followed by the Si-Au with a current of 123.63 µA, and the MWCNT-Si is the lowest with a current of 48.33 µA. MWCNT-Au has shown that a perfectly matching material can reduce the resistance on the surface of the electrode. The combination of Si-Au and MWCNT-Si demonstrates that at a certain concentration, the current increased before it remained constant. For MWCNT-Si, the current is increasing directly proportional to the concentration from 10 µM until 50 µM EE2 concentration, but at 100 µM the current is dropping slightly due to the limit of the chemical sensor due to the absence of biomolecules. Based on Zhu et al. [20], by incorporating biomolecules, one can increase the electron transfer and increase the sensitivity and specificity of the sensor.

Nodehi et al. [14] reported a linear range of EE2 detection in concentration range of 0.01-120µM with current response less than 100 µA. However, the current response for MWCNT-AuNPs modified electrode at a concentration of 100 µM of EE2 is given at 200 µA. The difference between this modified electrode and the one developed by Nodehi et al. [14] is that MWCNT-Au is easier to conduct, less expensive, and simpler, but has a higher current output. The Au/Fe_3O_4@TA/MWNT uses a lot of material with magnetic nanoparticles and has a lower current response than MWCNT-Au. Meanwhile, the combination of MWCNT and Si microspheres demonstrated improved current response for EE2 detection, which was most likely due to surface area. However, an electrode proposed by Cincotto et al. [21] shows a better current response with good LOD at a range of 0.1-50 ng/ml at limit of 0.065 ng/ml when compared with MWCNT-Si, which can quantify the value of EE2 at 10µM with a current less than 50µA. The $AgNPs/SiO_2/GO$ has the advantages due the presence of anti-EE2 with the attachment of the biomolecule it enhances the sensitivity and specificity of the modified electrode.



(a) (b) (c)

(d) (e)

Figure 5. CVs obtained for MWCNT-Au, MWCNT-Si, and Au-Si in different concentration range from 10µM to 100µM at 0.05V/S scan rate.

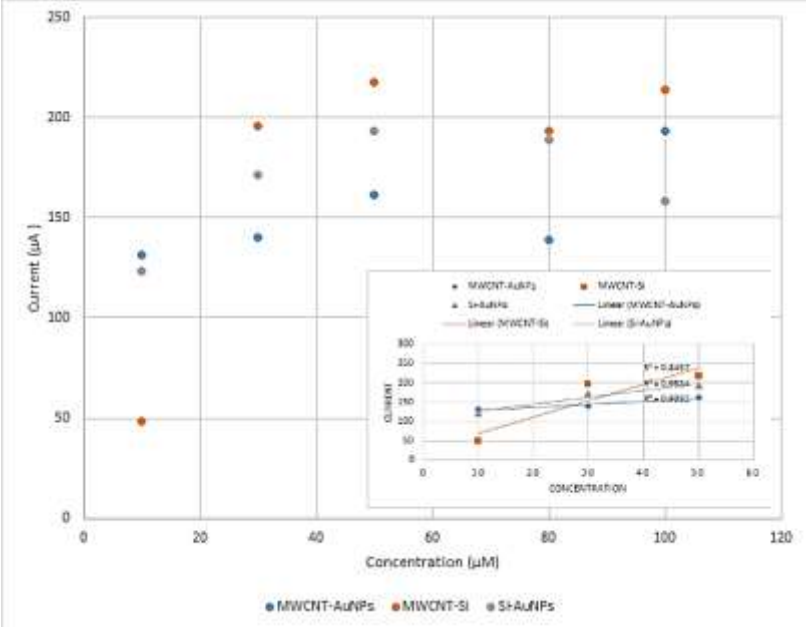


Figure 6. Peak-to-peak of current for MWCNT-AuNPs, MWCNT-Si and AuNPs-Si in 10μM, 30μM, 50μM, 80 μM and 100 μM.

The 17α ethinylestradiol (EE2) concentrations in the range of 10-100 μM were used to determine the ability of the modified electrode in quantifying the EE2. Figure 6 shows the peak-to-peak current of MWCNT-AuNPs, MWCNT-Si, and Si-AuNPs. Based on this material modification, the MWCNT-Si at 10 μM of EE2 shows the lowest current response compared to the other two modified electrodes, and the current response is below 50 μA for MWCNT-AuNPs. In terms of stability and performance, MWCNT-AuNPs show good current response and stability in performance because, at lower concentrations, the current response is still high compared to MWCNT-Si and Si-AuNPs.

4. CONCLUSION

This article describes the development of a chemical sensor for the quantitative discovery of ethinylestradiol (EE2) using cyclic voltammetry as a detection technique. The development of chemical sensors shows the limitations of chemical sensors, which rely on biomolecules to determine the quantitative value of species of interest. The change in current value or the electron transfer due to EE2 binding was monitored by CV in the presence of PBS. The incorporation of nanomaterials allowed us to obtain a large active surface area, reducing resistance by increasing electron transfer. This work demonstrates the effect of different materials on the detection of an analyte. As a conclusion, the Si-AuNPs are more stable compared to other combinations with R^2, the coefficient of determination is 0.9584 for 10–50 μM and this will be the combination chosen to detect EE2. In future work, the Si-AuNPs will use biomolecules to help increase the electron transfer, sensitivity, and specificity of the sensor.

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