UNIVERSITI MALAYSIA PERLIS

International Journal of Nanoelectronics and Materials

IJNeaM

ISSN 1985-5761 | E-ISSN 2232-1535

Selectivity study towards 17α-ethinylestradiol (EE2) detection based on silica microsphere-gold nanoparticle

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Received 22 September 2023, Revised 24 March 2024, Accepted 14 July 2024

ABSTRACT

The widespread use of 17α-Ethinylestradiol (EE2), a synthetic estrogen female hormone with potent physiological effects at lower concentrations than other steroids, poses environmental and health concerns due to its weak electrochemical behavior. Current detection methods lack the requisite sensitivity and selectivity for precise EE2 monitoring, posing potential risks in diverse environments. A critical need exists for a high-performance electrochemical biosensor to address these limitations and selectively detect EE2 with enhanced sensitivity. This development is crucial for accurate monitoring and risk assessment in the context of EE2 exposure. Silica nanoparticles offer advantageous features such as high surface area, excellent stability, large pore volume, adjustable shape, and size, simplicity of production, and biocompatibility. Additionally, gold nanoparticles are effective in facilitating electron transfer processes. Characterization through cyclic voltammogram (CV) and differential pulse voltammogram (DPV) revealed that the anodic peak current of electrodes modified with both silica and gold surpasses that of electrodes modified with silica or gold alone. Notably, the anodic peak current exhibits an upward trend with increasing concentrations of EE2, emphasizing the heightened sensitivity achieved through the incorporation of silica and gold in the electrode modification. The current of the anodic peak was linear within the range 1×10^{-6} -1×10^{-4} M EE2 concentration with linear regression equation Ip_a (μA) = $1.639C_{EE2} + 120.55$ and R^2=0.939. It may be seen that the electrochemical biosensor is superior in performance, due to the presence of the silica and gold that provides sensitivity for EE2 detection. Furthermore, in terms of selectivity, it was found that this sensor possessed acceptable performance in terms of selectivity through E3 which gained an overall percentage change of 0.04%, while E2 gained an overall percentage change of 10.23%, which is considered a good selectivity specifically for E3. The incorporation of silica and gold nanoparticles in electrode modification resulted in a noteworthy outcome: the anodic peak current surpassed that of electrodes modified with silica or gold alone. This increase in current intensity exhibited a direct correlation with the rise in concentrations of EE2, highlighting the enhanced sensitivity achieved through the synergistic combination of silica and gold nanoparticles.

Keywords: ethinylestradiol, silica, gold nanoparticles, biosensor, hormone

1. INTRODUCTION

The health impact of chemical pollutants associated with the disruption of hormonal systems in living organisms, particularly for wildlife and humans, has become a concern for some years. At the beginning of the 1990s, the issue of endocrine disruptors (EDCs) became the focus of considerable scientific concern and attention among researchers (Kasonga et al., 2021; Zoeller et al., 2012). Endocrine-disrupting chemicals (EDCs) are synthetic chemicals that pollute rivers and surface water disrupting the functioning of the human and animal endocrine system by altering the balance of hormones (Nodehi et al., 2020: Yang et al., 2018). EE2 and bisphenol A (BPA) are part of the group of EDCs commonly identified in surface water, as indicated by research from Insyirah et al. (2023), Tang et al. (2021), and Gallo et al. (2018). The presence of EE2 in drinking water has recently emerged as a public health concern since has been detected in treated drinking water at concentrations of up to 0.5 ng/L in Germany [Klaic et al., 2022; Ismail et al., 2019]. It is imperative to detect the presence of EE2 in the drinking water, as EE2 poses a potential threat to public health at large.

Ethinylestradiol (EE2) is a synthetic estrogen female hormone with high estrogenic potency (Tang et al., 2021) that exerts their physiological effects at lower concentrations than other steroids and can be found in the environment in concentrations above their lowest observable effect level (Ismail et al., 2019). Thus, EE2 must be found in water at low concentrations, to prevent health problems such as uncontrolled weight gain (Yang et al., 2018), feminization (Hamilton et al 2022), non-reproductive behavior (Rehberger et al. 2020), and diminished fertility. According to reports by Ismail et al., (2019), high levels of EE2 in estuary water near aquaculture operations in Malaysia which ranged from 0.3 to 6.67 ng/L, aquaculture was most likely a significant source of surface water (Tang et al., 2021).

Many analytical techniques for detecting EE2 in water samples have been developed, including spectrophotometry or liquid and gas chromatography. Despite their great sensitivity, these approaches have a drawback mainly consuming time analysis, which makes them unsuitable for field monitoring. As a result, electrochemical methods are an appropriate alternative since they are a low-cost and adaptable method of detecting materials with electroactive groups, especially since there are only a few works found in the literature reporting the electrochemical behavior of this estrogen. For instance, aptamer-based sensors, electrochemical techniques, solid-phase extraction methods, and molecular-impregnated polymers (MIPs) have been developed to detect and quantify EE2.

To enhance the anodic peak during electrochemical analysis, carbon-based nanoparticles such as gold and non-carbon-based nanomaterials like silica were employed to modify the surface of the working electrode. Gold nanoparticles are known to have remarkable electrochemical qualities (Ting Xiao, 2020), which greatly enhance the performance of the sensor by facilitating the electron transfer process. It also exhibits stability and allows for easy modification (Rui Wu, 2024) with various ligands, biomolecules, or receptors, enabling the customization of biosensors for specific applications. Whereas the silica microspheres provide a high surface area, uniform particle size, and tunable pore size (Shumaila et. al 2022); they also enhance analyte capture and detection sensitivity. Additionally, their chemical stability and biocompatibility ensure reliable performance under diverse experimental conditions without adverse effects on biological samples.

The materials designed in electrochemical sensors affect directly gaining better sensitivity and selectivity of EE2 detection in any medium. There are 4 types of estrogen estrone (E1), estradiol (E2), estriol (E3), and ethinylestradiol (EE2) as illustrated in Figure 1 which can be classified as sterols since all carry hydroxyl group at C-3 (Moss et al., 1989). Moreover, the high potency of EE2 compared to other natural estrogens necessitates its specific detection to accurately assess its environmental and biological impacts.

This research aims to overcome the challenges associated with detecting 17α-ethinylestradiol (EE2), a synthetic, nonpolar, and hydrophobic organic compound characterized by low volatility and resistance to biodegradation (Zaharin et al., 2014). The objective is to develop an electrochemical biosensor capable of detecting EE2 in water samples at low concentrations with high selectivity. To address this, the study proposes the utilization of silica microspheres and gold nanoparticles drop-cast on a carbon sheet as the working electrode for the electrochemical biosensor. This innovative approach introduces a novel aspect to the existing methods, leveraging the unique properties of silica microspheres and gold nanoparticles. The biosensor's electrochemical response will be systematically evaluated to assess its selectivity towards EE2 and its potential interference when coexisting with other estrogen hormones (E1, E2, and E3). This research investigates the effectiveness of the proposed electrochemical biosensor in meeting the specific challenges posed by EE2 detection in water samples.

2. MATERIAL AND METHODS

All the electrochemical measurements were performed using a USB-powered portable Metrohm DropSens potentiostat/galvanostat controlled by DropView 8400 software. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to characterize the electrochemical properties of the modified electrode sensor. A three-electrode cell configuration was used, with the modified sensor as the working electrode, a platinum rod as the counter electrode, and an Ag/AgCl (KCl, 3 M) electrode as the reference electrode.

2.1 Silica microsphere (SiO_2) and Gold (AuNP) working electrode preparation.

Silica microspheres were synthesized using a modified Stober method in ethanol with ammonia solution as a catalyst (Shafawi et al. 2023; Yew et al., 2019). In this study, the working electrode must be prepared to include i) carbon sheet (cs), ii) cs/AuNP iii) cs/ SiO_2, and iv) cs/SiO_2/AuNP. The sensor has been developed based on previous research published by Shafawi et al. (2023). The process of deposition using the drop coat method is shown in Figure 1. Then 5 μL silica microspheres were first deposited on the carbon strip and waited for 5 minutes until dried. Then, 50 μL of gold nanoparticles were dropped and waited for 5 minutes also until dried.

2.2 Selectivity study using Cyclic Voltammogram (CV) and Differential Pulse Voltammetry (DPV)

In this study, the electrochemical response refers to cyclic voltammetry (CV) and different pulse voltammetry (DPV). Since the EE2 oxidation process is irreversible, it is possible to use different pulse voltammetry (DPV) for further testing. To assess selectivity, the biosensor's response was measured concerning the potential peak of EE2 in the presence of other estrogen hormones. This comprehensive evaluation allowed for a thorough examination of the biosensor's ability to discriminate against potential interferents, ensuring its specificity for EE2 detection. The selectivity of the electrode-modified electrochemical biosensor towards EE2 was assessed using different interferences of estrone (E1), estradiol (E2), and estriol (E3) respectively that may usually have the same electrochemical response toward EE2. The analyte of EE2 was prepared at five (5) different concentrations between 1-100 μM for sensitivity analysis. The volume ratio is fixed at 1:1 between EE2 and each solution of E1, E2, and E3 respectively. The interference percentage is calculated using this formula (% current ratio = $(i_{EE2}-i_{interference})/i_{EE2}$ x 100)) (Torrinha et al., 2022).

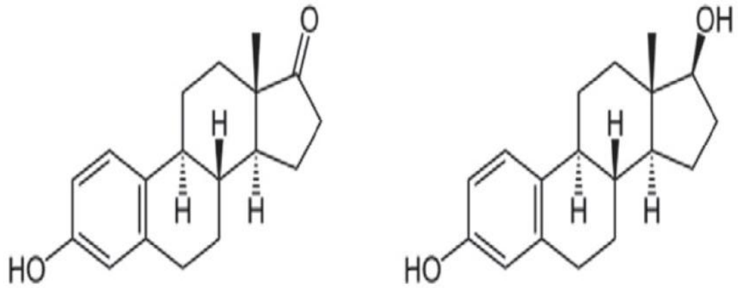
3. RESULTS AND DISCUSSION

The scanning electron microscopy (SEM) analysis reveals the deposition of monodispersed silica microspheres on the carbon sheet, as illustrated in Fig. 3(a) and (b), corresponding to the set of radii of the silica microspheres. The SEM images distinctly showcase well-dispersed silica particles forming homogeneously spherical structures. Specifically, in Fig. 3(b), the initial group of microspheres demonstrates minimal size dispersion, with radii ranging from 0.611 to 0.628 μm. This observation underscores the uniformity and precision achieved in the synthesis of silica microspheres, essential in biosensor development.

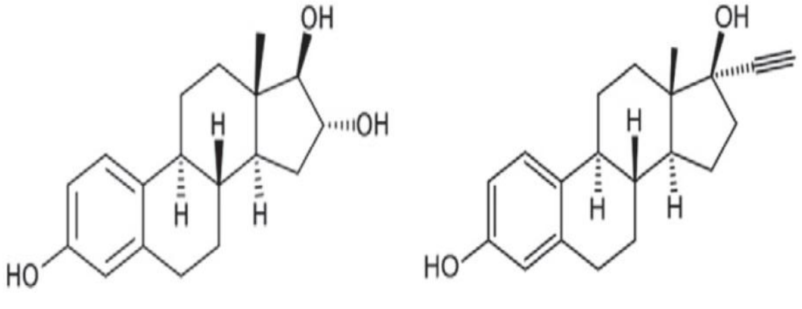
Figure 4 (a) depicts cyclic voltammograms of the modified electrode which is coated with gold nanoparticles in 0.1 M PBS with and without 100 μM EE2. Figure 4 (b) shows the modified electrode coated with silica in 0.1 M PBS with and without 100 μM EE2. Figure 4 (c) depicts the cyclic voltammogram of the modified electrode coated with both silica and gold nanoparticles in 0.1 M PBS with and without 100 μM EE2. The peak potential (Ep_a) of EE2 is recorded at 0.47 V while E1, E2, and E2 were recorded at 0.49 V. The EE2 peak potential of SiO_2/AuNP/cs is towards a more positive peak potential compared to 0.402 V as reported by Zheng et al., (2019). It is in agreement that the EE2 exclusively participated in oxidation, indicating that the process was irreversible and that no reduction peaks were seen when cyclic voltammetry was carried out.

To develop a sensor with good selectivity, it is better to understand the structure of EE2, E1, E2, and E3 as illustrated in Figure 1. The proposed oxidation mechanism of EE2 is proposed by Zheng et al., (2019) as shown in Figure 5. The oxidation occurs most probably at carbon-3 from OH to C=O. The carbon 3 hydroxyl group in this estrogen hormone is the most likely site for electrochemical oxidation due to its high oxidation potential. The analysis of cyclic voltammetry (CV) data indicates significant enhancements in the electrochemical characteristics of the modified working electrode for 17α-Ethinylestradiol (EE2) detection. Notably, the combination of gold nanoparticles and silica yielded the highest peak current, underscoring the pivotal contribution of both materials. These results highlight the enhanced performance of electrodes modified with silica and gold nanoparticles in detecting 17α-Ethinylestradiol (EE2), emphasizing the synergistic effects of these components on improving sensor sensitivity and selectivity.

To understand the linear range detection of this electrochemical sensor, the differential pulse voltammetry was recorded at different concentrations of EE2 between 1 and 100 μM between 0.3 to 0.7 V. Figure 6 (a) shows the DPV of SiO_2/AuNP/cs electrode tested in five different concentrations (1, 25, 50, 75 and 100 μM) of EE2. The current of the anodic peak current (Ip_a) increases with the increase in the range 1×10^{-6} -1×10^{-4} M EE2 concentration. The linear relation of the peak current and EE2 concentration can be expressed as Ip_a (μA) = $1.639C_{EE2} + 120.55$ ($R^2=0.939$) as shown in Figure 6(b). The increase in peak current exhibits comparable tendencies with EE2 concentration and is consistent with Zheng et al. (2019).



Estrone (E1) Estradiol (E2)



Estriol (E3) Ethinylestradiol (EE2)

Figure 1. Structure representation of synthetic and natural hormones of ethinyl estradiol (EE2), estrone (E1), estradiol (E2), and estriol (E3) [Zaharin, 2019].

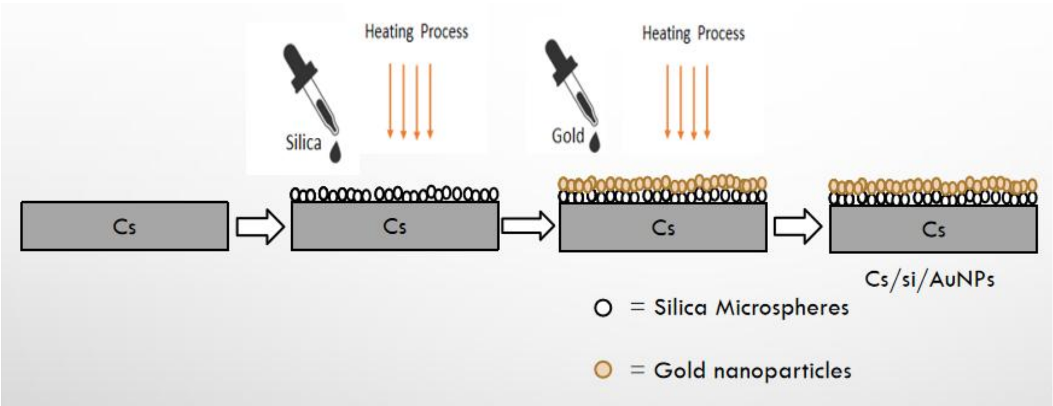
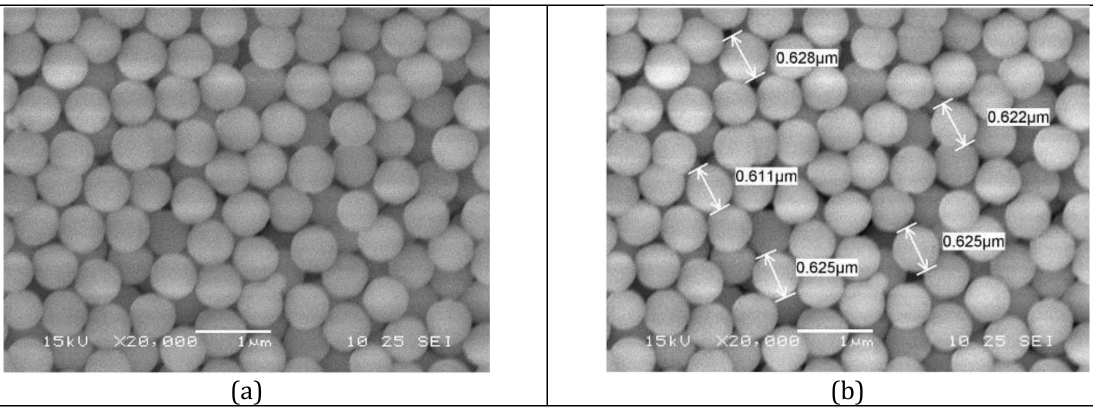
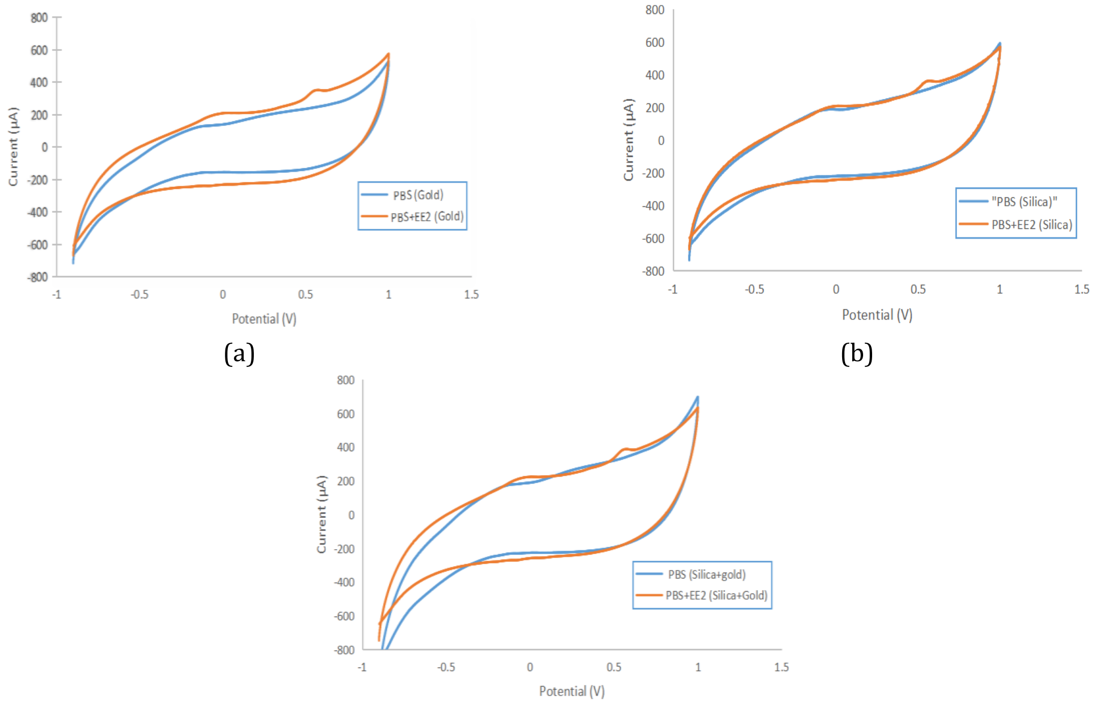


Figure 2. Schematic representation of drop coat method for silica microspheres and gold nanoparticles electrode.



(a) (b)

Figure 3. Scanning electron microscopy (SEM) images for characterization of the dispersed silica microspheres on carbon sheet.



(a) (b)

(c)

Figure 4. Cyclic voltammograms analysis of (a) AuNP/cs (b) SiO_2/cs (c) SiO_2/AuNP/cs in phosphate-buffered saline (PBS) pH 7.0 with 100 μM EE2 and without EE2 in scan rate = 0.05 mV s^{-1}.

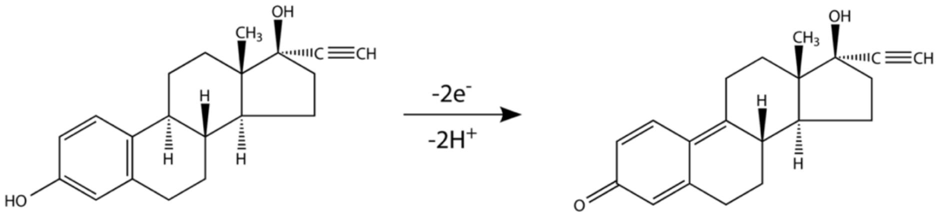
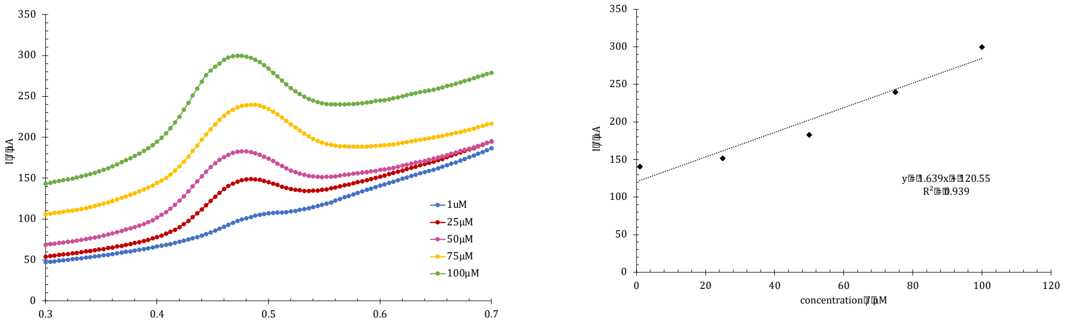


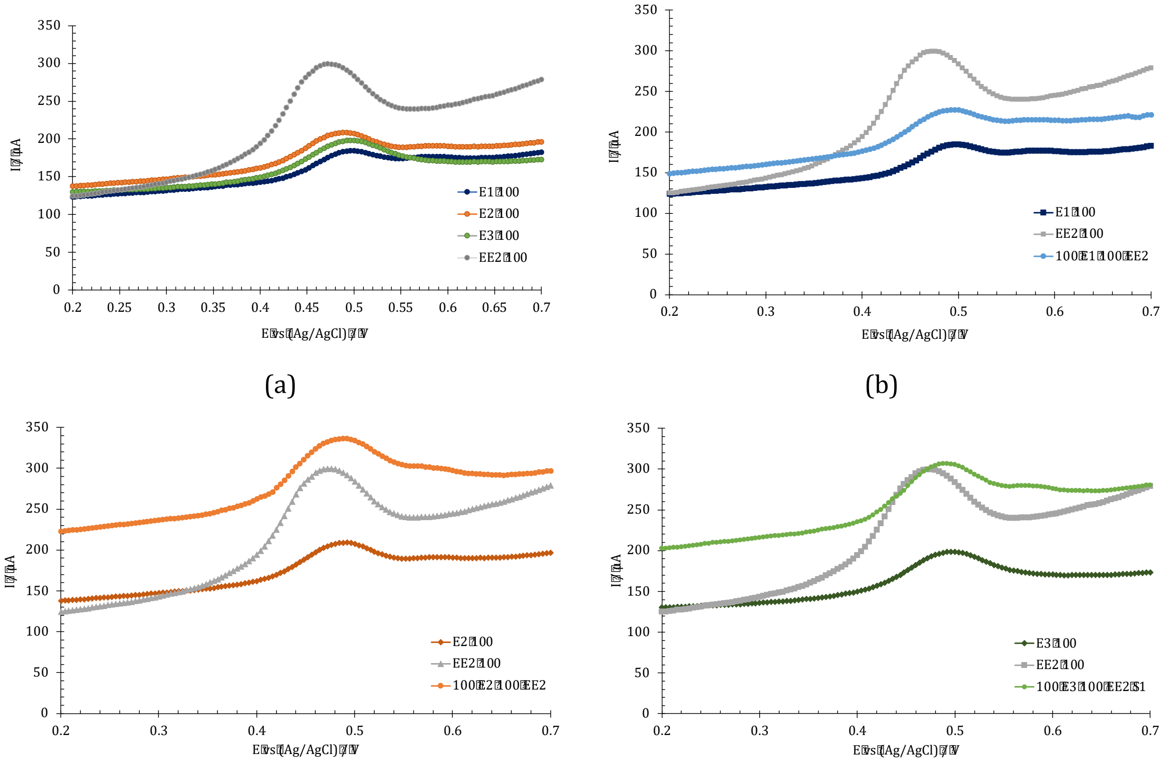
Figure 5. The reaction mechanism of electrochemical oxidation EE2 proposed by (Zheng et al., 2019)



(a)

(b)

Figure 6. (a) Differential pulse voltammetry of AuNP/SiO₂/carbon sheet modified electrode at five different EE2 concentrations. (b) Linear concentration range of EE2 detection with AuNP/SiO₂/cs modified electrode.



(a)

(b)

(c)

(d)

Figure 7. Differential pulse voltammetry (a) individual response of E1, E2, E3 and EE2 (b) E1 mixture E1:EE2 (c) E2 mixture E2:EE2 (d) E3 mixture E3:EE2 at 1:1 ratio in 100 µM concentration.

To understand the linear range detection of this electrochemical sensor, the differential pulse voltammetry was recorded at different concentrations of EE2 between 1 and 100 µM between 0.3 to 0.7 V. Figure 6 (a) shows the DPV of SiO_2/AuNP/cs electrode tested in five different concentrations (1, 25, 50, 75 and 100 µM) of EE2. The current of the anodic peak current (Ip_a) increases with the increase in the range 1×10^{-6} -1×10^{-4} M EE2 concentration. The linear relation of the peak current and EE2 concentration can be expressed as Ip_a (µA) = $1.639C_{EE2} + 120.55$ (R^2=0.939) as shown in Figure 6(b). The increase in peak current exhibits comparable tendencies with EE2 concentration and is consistent with Zheng et al. (2019).

Conversely, the selectivity of the EE2 test was evaluated using differential pulse voltammetry (DPV) with E1, E2, and E3. Figure 7 (a) shows DPV analysis on individual interferences of E1, E2 and E3 and EE2. Fig 7 (b) (c) and (d) show a similar trend when the mixture of interferences was added to EE2, where the potential peak shifted to the right following the interference individual peak potential. The peak potential shifted to the right for E1, E2, and E3 away from the EE2 peak potential showing this modified electrode is capable of distinguishing the EE2 only without interferences added. The peak current of EE2 in a 1:1 mixture with other estrogens is lower than the peak current of only EE2. The addition of interferences significantly affects the response at a 1:1 mixing ratio. It is observed that the peak potential (Ep_a) of E2 is much closer to EE2 than in E1 and E3 to EE2 since they have a similar chemical structure at carbon-17. The hormone with similar characteristic with EE2 is E1, E2, and E3 since 17α-ethynylestradiol (EE2) is derived from 17β-estradiol (E2). The only structural difference between EE2 and the other natural estrogens is the presence of an ethinyl group with two carbon atoms connected by a triple bond at position 17α.

Table 1 summarizes the interference analysis of the EE2 signal when the EE2 solution is mixed with E1, E2, and E3 at a 1:1 ratio, respectively. Based on Table 1, the percentage interference of E3 is the smallest with -0.04% whilst E1 gives the highest interference near to 24%. All undergo similar electrochemical reactions, which means that they can both produce similar electrochemical signals. E2, E3, and EE2 have almost similar hydroxyl groups at carbon 17, which provide similar electrochemical interactions. When E3 is added to EE2, the peak current has little or no significant effect on the overall peak current response of EE2. However, when E1 is added to EE2 at this ratio, it lowers the peak current of EE2 significantly. For E2, it also gives quite high interference signal of -10.23%. In the presence of E1, the current response diminishes and loses its selectivity for EE2. While in E3, the current is maintained and does not lose the selectivity towards EE2. In the case of E3, the current remains consistent and retains its selectivity for EE2 without diminishing. Since there is no bioreceptor is immobilized on this sensor, it is understandable that this sensor has poor selectivity towards EE2.

Table 1. Interference analysis of E1:EE2, E2:EE2 and E3:EE2.

Mixture Ratio (EE2: Interference)	E_{pa} (V)	I_{pa} (µA)	I_{pa} (µA) at 0.472 V peak	% Interferences
EE2 only	**0.472**	**299.50**	**299.50**	
1 EE2: 1 E3	0.492	198.54	299.62	-0.04%
1 EE2: 1 E2	0.488	208.82	330.16	-10.23%
1 EE2: 1 E1	0.488	184.70	227.02	24.20%

4. CONCLUSION

The electrochemical response of the AuNP/SiO2/cs biosensor for detecting 17α-ethinylestradiol (EE2) was systematically investigated, evaluating the impact of varying EE2 concentrations. The electrochemical behavior was characterized through cyclic voltammetry (CV), revealing an irreversible oxidation process. Notably, the anodic peak current exhibited a linear increase corresponding to the rise in EE2 concentration within the range of 1–100 µM. This underscores the superior performance of the electrochemical biosensor, attributed to the synergistic effects of silica and gold, enhancing sensitivity to EE2 detection. The utilization of gold and silica-coated electrodes proved essential, leveraging silica's remarkable properties such as high surface area, stability, large pore volume, adjustable shape, and size. Meanwhile, gold nanoparticles contributed to improved conductivity, reducing electrode surface resistance. This combination facilitated the fabrication of a straightforward biosensor applicable across diverse water matrices. However, it is crucial to acknowledge that while the modified electrode displayed noteworthy selectivity for EE2 in the presence of E3, its specificity diminished in the presence of E1 and E2 in the solution.

To address this limitation, future investigations will focus on incorporating a bioreceptor, such as an aptamer, to enhance the biosensor's selectivity for EE2 detection in real samples. These include enhancing selectivity against potential interferents, assessing performance across diverse sample matrices, improving detection limits, validating real-world applicability, ensuring long-term stability, and mitigating interference effects. Addressing these challenges through continued research efforts can enhance the sensor's overall performance and broaden its utility in environmental monitoring and clinical diagnostics.

ACKNOWLEDGEMENTS

The author would like to acknowledge the support from the Fundamental Research Grant Scheme (FRGS) under the grant number FRGS/1/2019/STG07/UNIMAP/02/5 from the Ministry of Higher Education Malaysia.

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