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Novel CA-125 antigen determination in serum by electrochemical methods with onion oil-containing organo-hydrogels

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ABSTRACT

CA-125 antigen is a glycoprotein that can be found at distinct levels in blood samples according to the phases of ovarian cancer. Herein, we designed novel onion oil-organo-hydrogels (OOHGs) to detect CA-125 antigen at high sensitivity and selectively via electrochemical methods. OOHGs produced are characterized by swelling analysis and Fourier Transform Infrared Spectroscopy (FT-IR). Cyclic voltammetry (CV), Electro impedance spectroscopy (EIS), and Differential pulse voltammetry (DPV) techniques in the potentiostat triple electron system are used for performing the electrochemical measurements. Performances and electron transfer resistances of OOHGs and OOHG+CA-125s are researched via CV and EIS, and the sensitivity properties such as LOD and LOQ of the sensor are determined via DPV. OOHG-2 among OOHGs produced exhibited the highest performance with 0.8151 mA/cm^2 (815.1 μA/cm^2) value at determining CA-125 in serum medium. Moreover, this electrode is found that exhibit a wide linear range like a 1-500 ng/mL concentration range. The limit of quantification (LOQ) and the lowest of detection (LOD) for the OOHG-2 electrode are calculated as 0.531 μU/mL and 0.265 μU/mL (S/N=3), respectively. Further, the CA-125 antigen of the OOHG-2 electrode in interference results is observed that can be detected with high selectivity. With these results, it can be noted that the OOHG-2 electrode holds great hope for detection ovarian cancer by electrochemical methods.

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1. Introduction

Cancer markers could be proteins, mRNA, DNA, metabolites, and they are fabricated either by other tissues or the tumors themselves in response to conditions such as inflammation or the presence of cancer. Biomarkers may be found in cell lines, tissues, or a variety of body fluids such as nipple discharge, saliva, urine, and effusions. They are playing an important role in the screening of cancers, assessing prognosis, aiding in diagnosis, monitoring patients with cancer, and predicting response to treatment [1-3]. Biomarkers can be helpful in symptomatic patients in diagnosing whether the tumors are benign or malignant [4]. The development of strategies such as cost-effective, reliable, high-sensitivity monitoring, and strong detection for cancer screenings, cancer diseases are of great importance especially due to recurrence rates, potential lethality, and disease prevalence. Systems that can be developed over biomarkers in cancer screening offer great potential for early detection of cancer and personalized treatment methods [5-7].

Ovarian cancer is a gynecological disease with high mortality rates that all benign and malignant tumors occur from one of the germ cells, epithelial cells, and stromal cells, generally [8]. Ovarian cancer is one of the most difficult cancers to detect early due to the lack of symptoms, specific signs, and reliable screening methods. CA-125, which is a glycoprotein in the MUCIN-16 family, normal level in the blood between 0-35 U/mL, and molecular weight 200 kDa, is the only biomarker used in the screening, in the progression of the disease, and in the monitoring of relapses for ovarian cancer [9-11]. Distinct methods such as mass spectrometry [12], surface plasmon resonance [13], fluorescence [14], and colorimetry [15] have been used for the screening of ovarian cancer. While these methods have provided some benefits, they have some disadvantages such as low sensitivity, complex test procedures, and long detection time, which can greatly affect

sensitivity [16]. However, the electrochemical sensors (ESs) that use voltammetric, amperometric, impedimetric, or capacitive signals to characterize antigen and antibody binding are particularly promising for use in strong, free-label, sensitive, and rapid diagnostic fields [17-19]. Moreover, these sensors have superior properties such as low cost, fast response, and are easily miniaturized [20-22].

Recently, to increase sensitivities and selectivity's for CA-125 antigen of the electrochemical sensors (ESs) have been studied on materials such OHCOs [23], Ppy nanowire [24], CS–PDDA–PB nanoparticles [25], Ag-RGO/CysA-Au NPs [26], thionine/CA125/CNF/GCE [27], Au-VBG/BDD [28], and benzothiophene derivates [29-32]. In addition, Hasanzadeh et al. reported that developed an electrochemical sensor for the detection of CA-125 with Cys-AuNPs/ERGO probes, and they expressed that this sensor is exhibited high sensitivity and good stability to detect CA-125 with 0.1 U/mL LOQ value and at a wide concentration range as 0.1-400 U/mL linear range [33]. In another study, Zheng et al. reported that enhanced the electrochemical sensor with AuNP-PB-PtNP-PANI hydrogel material to detect CA-125 antigen, and this sensor was exhibited high sensitivity for determining CA-125 with quite a wide linear range among 0.01-5000 U/mL and low detection limit as 0.0044 U/mL values [34]. Apart from these studies, the features of distinct ESs summarized in the literature to detect CA-125 are given in Table 1.

Table 1. *Performances of dissimilar ESs reported in the literature for determining of CA-125 antigen.*

Biomarker	Sensor	LOD	Linear range	Ref.
CA-125	anti-CA125/Au–Thi-CPE	1.8 U/mL	10-30 U/mL	[35]
CA-125	MIP based Au-SPE	0.01 U/mL	0.01-500 U/mL	[36]
CA-125	M-Pt NPs	0.002 U/mL	0.05-20 U/mL	[37]
CA-125	GCE/FA@H-PANI@CS-HCl/Ab-Ag@Co_3O_4	0.25 pg/mL	0.001-25 ng/mL	[38]
CA-125	AgNPs-GQDs/Ab/BSA/Ag	0.01 U/mL	0.01-400 U/mL	[39]
CA-125	Au electrode	5.5 U/mL	10-100 U/mL	[40]
CA-125	MOF-808/CNT	0.0005 ng/mL	0.001-30 ng/mL	[41]
CA-125	ZnO NRs-Au NPs NHs based matrix	2.5 ng/μL	---	[42]
CA-125	OOHG-2	0.265 μU/mL	1-500 ng/mL	This study

The literature reported that studies were carried out on different materials to monitor the level of CA-125 antigen with different methods. However, studies on OOHGs weren't conducted on the follow-up of cancers. Herein, we enhanced an electrochemical sensor (ES) with onion oil-based OOHG's (Agar, glycerol, and MBA) for determining at high sensitivity and selectivity of CA-125 antigen in serum medium. Hydrogels are special kinds of polymers with tremendous capacity that can absorb large amounts of water, and these have used in many applications in technology and science. In particular, hydrogels offer great potential for applications in healthcare and diagnostic care due to their biodegradability, biocompatibility, and non-toxicity [43-46]. Organo-hydrogels can identify as hydrogels formed from synthetic or naturally derived molecules with physical or chemical crosslinkers [47-49]. Onion oil is contained at high concentrations from 3-((ethyltrisulfanyl)methyl)-3,4-dihydro-2H-thiopyran, 1,3-dipropyltrisulfane, and 1-methyl-2-propyldisulfane structures, and this oil is known properties as antibacterial, antitoxigenic, and antidermatophytic [50, 51].

2. Materials and Methods

All materials used in the realization of the measurements and the synthesis of OOHGs are given in detail in S1. The characterization methods and synthesis phases of OOHGs and fabricated steps of ESs with OOHGs have been given in S2 and S3. In addition, preparation steps of OOHGs for ESs were demonstrated in Fig. 1.

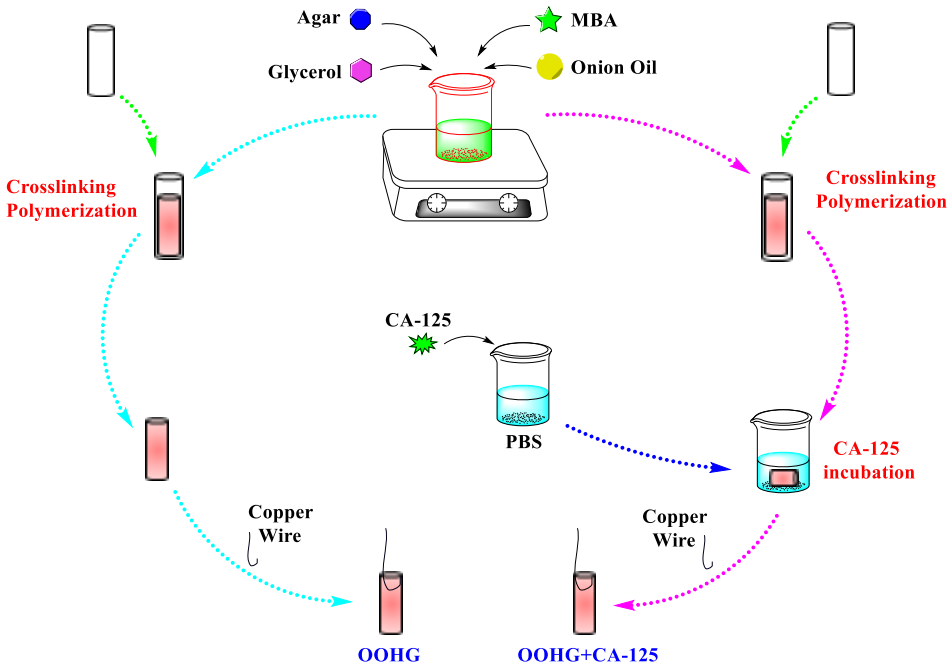


Figure 1. *Measurements and fabricate steps of ES prepared with onion oil-based OOHGs.*

Measurements for ES were performed with techniques such as EIS, CV, and DPV in potentiostat triple electrode system. CV measurements were firstly taken at 50 mV/s scan rate in pH: 7.4 PBS that contains 5.0 mM $Fe(CN)_6^{3-/4-}$ (prepared solution) over OOHGs and OOHG+CA-125s fabricated with CA-125 (500 ng/mL) for 30 min. OOHG-2+CA-125 electrode were exhibited the best activity in measurements taken over OOHG+CA-125s.

Secondary, CV measurements for the best concentration value were obtained at 50 mV/s scan rate in pH: 7.4 PBS that contains 5.0 mM $Fe(CN)_6^{3-/4-}$ (prepared solution) over OOHG-2+CA-125s produced with distinct amounts (1~5000 ng/mL) for 30 minutes. 500 ng/mL CA-125 amount value was determined as the best amount among concentrations prepared. Thirdly, CV measurements for the best incubation time were received at 50 mV/s scan rate in prepared solution over OOHG-2+CA-125s produced with CA-125 (500 ng/mL) during distinct incubation times among 10-90 min at room temperature. 30 min incubation time was found as the best time for incubation of CA-125 antigen over OOHG-2s.

To understand electrooxidation reaction between CA-125 antigen and OOHG-2, CV measurements at varying scan rates (5-1000 mV/s) and EIS measurements at varying potentials among -0.6~0.5 V were performed in prepared solution over OOHG-2+CA-125s produced with determined conditions (30 minute and 500 ng/mL CA-125).

To research properties such as LOD, LOQ, and linear range of ES, DPV measurements were taken in prepared solution over OOHG-2+CA-125s produced with different CA-125 amounts between 0.001~5000 ng/mL during 30 minutes.

The effects on the electrochemical reaction between CA-125 and OOHG-2 of structure molecules such as ascorbic acid, uric acid, dopamine, and glucose that found in serum medium were researched via CV technique at 50 mV/s scan rate and EIS technique at 0.2 potential in prepared solution+0.1 mM Ascorbic acid, prepared solution+2.5 mM Uric acid, prepared solution+0.1 mM Dopamine, prepared solution+4.7 mM Glucose, respectively. Measurements were performed over OOHG-2s and OOHG-2+CA-125s fabricated with determined conditions (30 minute and 500 ng/mL CA-125).

Finally, the effect on electrooxidation reaction among OOHG-2 and CA-125 antigen of the salts that found in serum medium were researched via EIS and CV. CV measurements at 50 mV/s scan rate and EIS measurements at 0.2 potential were performed in artificial and 0.9% isotonic NaCl serums over OOHG-2+CA-125 produced with determined conditions (30 minute and 500 ng/mL CA-125). Artificial serum was performed with $MgCl_2$ (1.6 mM), D-glucose (4.7 mM), $CaCl_2$ (5.0 mM), urea (2.5 mM), and KCl (4.5 mM).

3. Results and Discussion

CV measurements that performed in prepared solution at 50 mV/s scan rate over OOHGs and OOHG+CA-125 to detect CA-125 antigen in serum at room temperature were presented in Fig. 2. CV results, initially, were obtained over OOHGs produced in the absence of CA-125 antigen. Forward and backward peaks that express the electrooxidation process were not clearly observed, and the current densities on total currents were very lowest (Fig. 2a). Secondary, CV results were obtained on OOHGs produced by incubating CA-125 antigen. The maximum current density of OOHG-2 synthesized with 0.2 mL onion oil was high than the maximum current densities of OOHG-1 and OOHG-3 synthesized with 0.1 mL and 0.3 mL onion oil (Fig 2b). These

results show that 0.2 mL is the ideal onion oil amount in the OOHGs to can synthesize with the MBA crosslinker for the detection of CA-125. OOHG-2+CA-125 was exhibited the best electrochemical activity with 0.8151 mA/cm^2 (815.1 μA/cm^2) at 0.24 V of forward peak and 0.8258 mA/cm^2 (825.8 μA/cm^2) at -0.31 V of backward peak values (Fig. 2c). OOHG-3+CA-125 was shown lowest activity with 0.6461 mA/cm^2 (646.1 μA/cm^2) at 0.35 V of forward peak and 0.6212 mA/cm^2 (621.2 μA/cm^2) at -0.38 V of backward peak values (Fig. 2b). These results could say that was a higher performance than the studies for the detection of CA-125 antigen [40, 52, 53]. When comparing OOHG-2 and OOHG-2+CA-125, it can clearly see that OOHG-2 has a high activity to the CA-125 antigen (Fig. 2c).

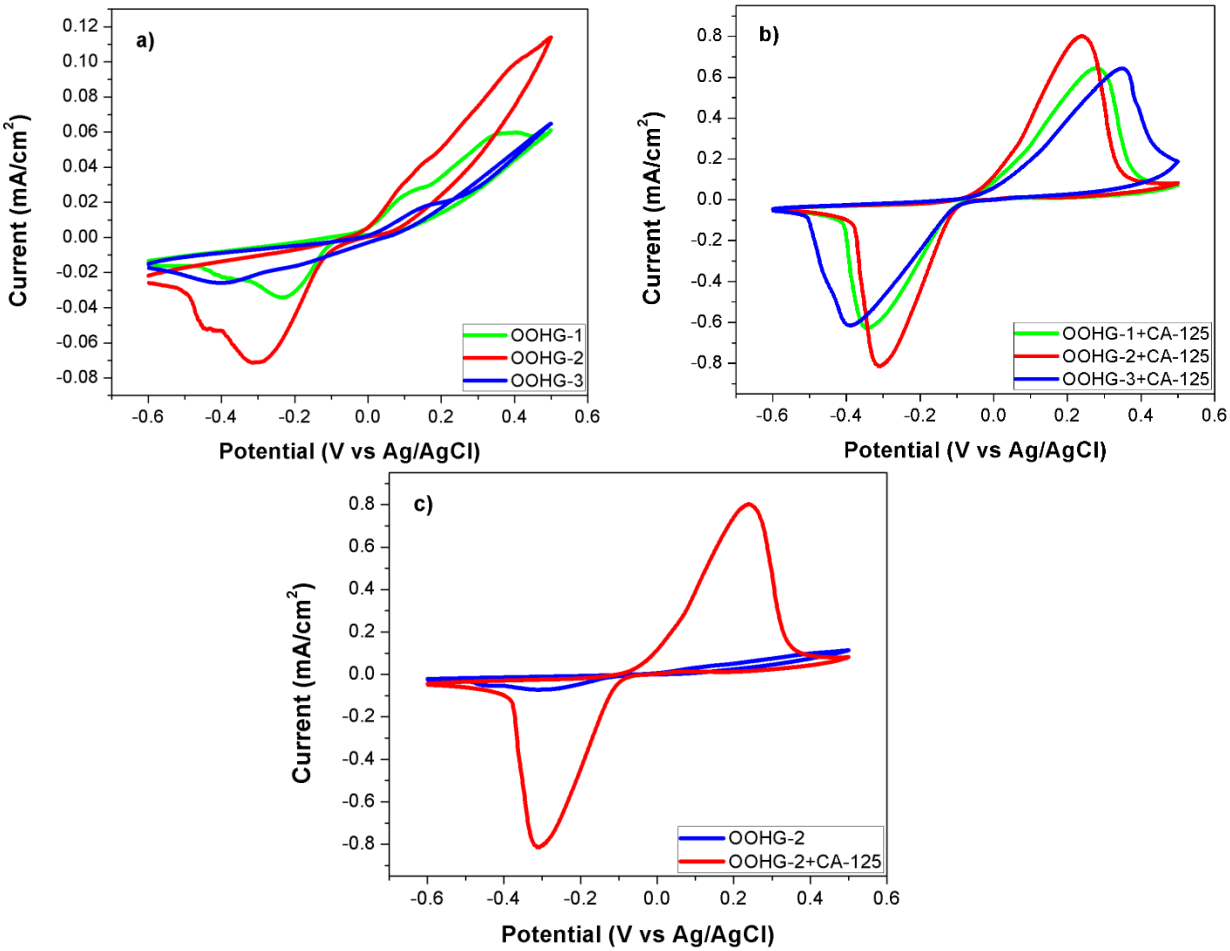


Figure 2. *CV results at 50 mV/s scan rate in prepared solution on a) OOHGs without CA-125 antigen, b) OOHG+CA-125s produced with determined conditions (30 minute and 500 ng/mL CA-125), and c) OOHG-2 and OOHG-2+CA-125 compare.*

To determine the optimum operating conditions of ES, CV measurements were received in prepared solution over OOHG-2+CA-125s produced with on distinct CA-125 concentrations and then distinct incubation times. All results are presented in Fig. 3. Concentration measurements were performed over OOHG-2+CA-125 produced with varying CA-125 amounts among 1~5000 ng/mL (Fig. 3a). Electrooxidation peaks were observed for all concentrations ratios. A gradual increase from 1 ng/mL to 500 ng/mL and a gradual decrease from 500 ng/mL to 5000 ng/mL were observed in maximum current densities. The highest the electrochemical activity was obtained on 500 ng/mL with 513.8 μA/cm^2 at 0.24 V of forward peak and 506.1 μA/cm^2 at -0.31 V of backward peak values. The incubation times measurements were taken over OOHG-2+CA-125 produced at varying times between 10-90 minutes (Fig. 3b). The lowest electrooxidation peaks were obtained in the measurements taken on the OOHG-2+CA-125 produced with 10 min and 90 min incubation times. The highest activity with 542.0 μA/cm^2 at 0.26 of forward peak and 513.3 μA/cm^2 at -0.32 of backward peak values was obtained from OOHG-2+CA-125 produced with 30 min. As a result, these data show that the determined conditions (30 minute and 500 ng/mL CA-125) for ES produced with OOHG-2.

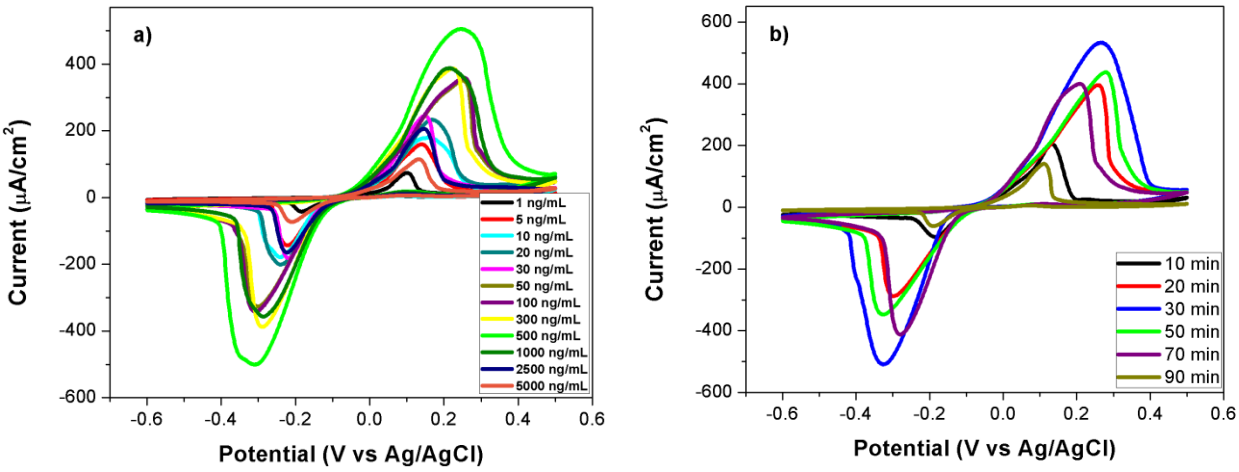


Figure 3. *CV results at 50 mV/s scan rate in prepared solution over a) OOHG-2+CA-125s produced with distinct CA-125 amounts (1~5000 ng/mL) for 30 minutes and b) OOHG-2+CA-125s produced with CA-125 (500 ng/mL) for distinct incubation times among 10 min and 90 min at room temperature.*

To investigate the electrooxidation process among OOHG-2 and CA-125 antigen, CV measurements at distinct scan rates and EIS measurements at distinct potentials were performed in prepared solution. CV results and the Nyquist plots obtained from EIS data are given in Fig. 4. OOHG-2+CA-125s that were used in measurements were produced at determined conditions (30 minute and 500 ng/mL CA-125). A gradual increase in the maximum current intensity from 5 mV/s to 1000 mV/s was observed in CV measurements taken at varying scan rates (5~1000 mV/s) (Fig. 4a). This regular increase indicates that a diffusion-controlled reaction has occurred over the OOHG-2 surface at the presence of CA-125 antigen. EIS is an electrochemical technique that expresses resistance to the flow of alternating current (AC). This technique is a powerful measurement method that can be used in a wide range of fields such as materials science, biology, medicine, and sensors. The Nyquist plots obtained from EIS data occurs a semi-circular area showing the charge transfer and linear sections, which expresses a diffusion-controlled process [54-58]. EIS measurements over OOHG-2+CA-125 were performed in prepared solution at varying potentials (-0.6~0.5 V) (Fig. 4b). When the diameter of the semicircles is large, the electron transfer resistance (Rct) is high, and when the diameter of the semicircles is small, the electron transfer resistance (Rct) is low also [59, 60]. It was seen a linear decrease in electron transfer resistance at potentials among -0.6~0.2 V, and a linear increase in electron transfer resistance at potentials between 0.2 V and 0.5 V. The lowest electron transfer resistance was observed on 0.2 potential. 0.2 potential was the potential at which the maximum current density was seen in the CV results. Therefore, EIS data and CV results are in agreement.

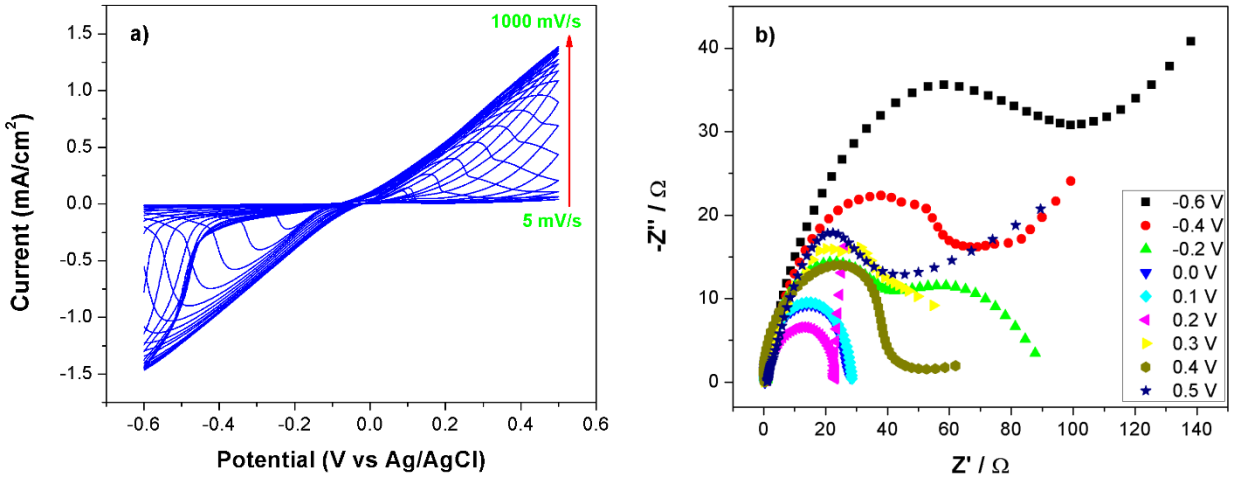
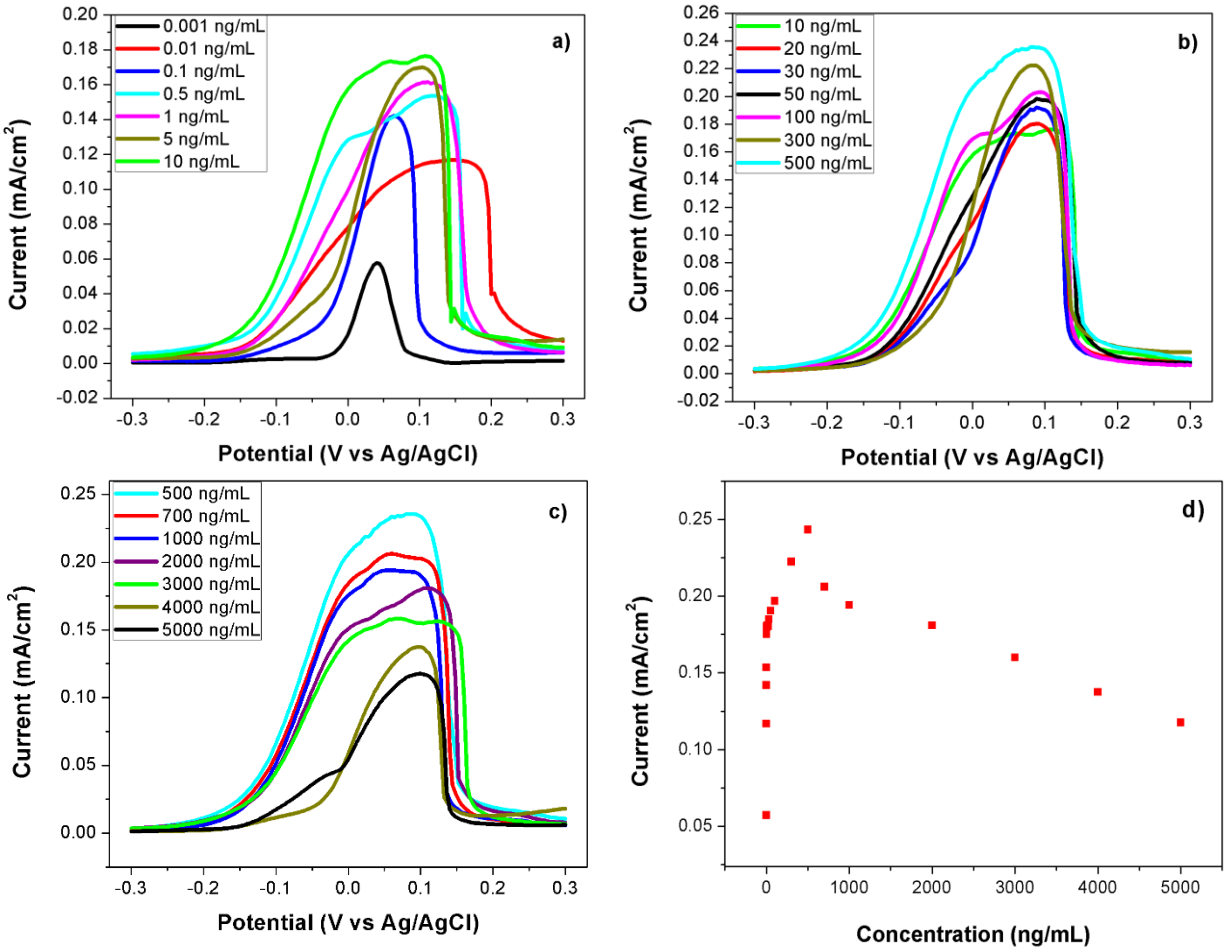


Figure 4. *a) CV results at distinct scan rates among 5 mV/s and 1000 mV/s and b) the Nyquist plots of EIS data at distinct potentials among -0.6 V and 0.5 V in prepared solution over OOHG-2+CA-125s produced determined conditions (30 minute and 500 ng/mL CA-125).*

Sensitivity of OOHG-2 electrodes produced with varying CA-125 concentrations for 30 minutes were investigated DPV technique. DPV plots that measurements received in prepared solution are presented in Figure 5a-c, and the calibration plots of concentration ratios vs. maximum current densities belonging DPV curves are demonstrated in Fig. 5d-e. As clearly seen in Fig. 5a and 5b, it could note that displayed a linear relationship among 1-500 ng/mL concentrations, and R^2 of this linear range was calculated as 0.9826. These concentration range value are wide a linear range than reported in the literature (Table 1). The limit of quantification (LOQ) and lowest detection limit (LOD) values for OOHG-2 electrode produced were determined by receiving measurements over 10 blank electrodes without CA-125 antigen, and these results of measurements are demonstrated in Figure 5f. LOD and LOQ values were calculated as 0.265 μU/mL and 0.531 μU/mL (S/N=3), respectively. LOD value that reckons for OOHG-2 electrode is lower than noticed in the literature (Table 1).



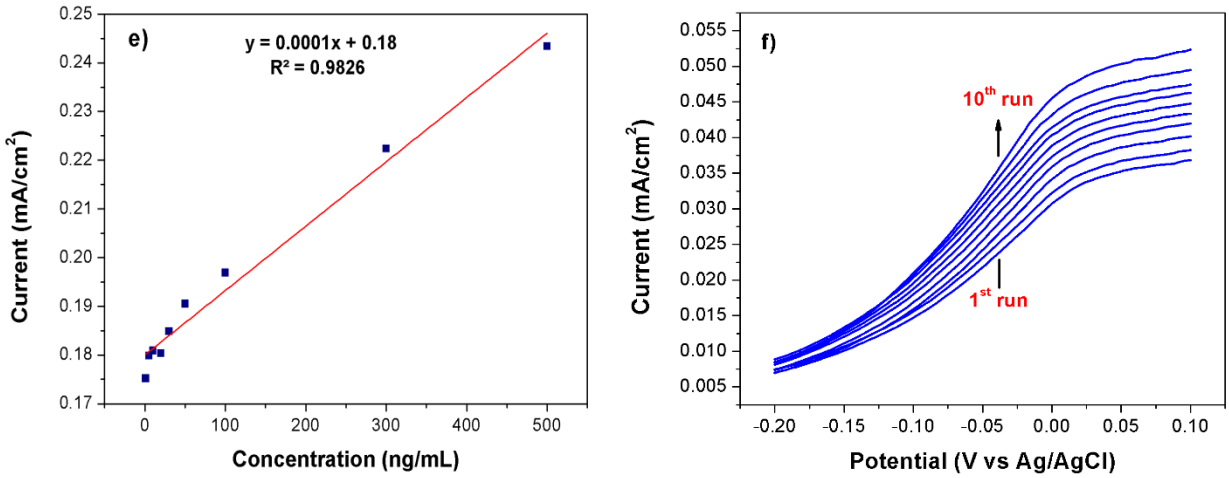
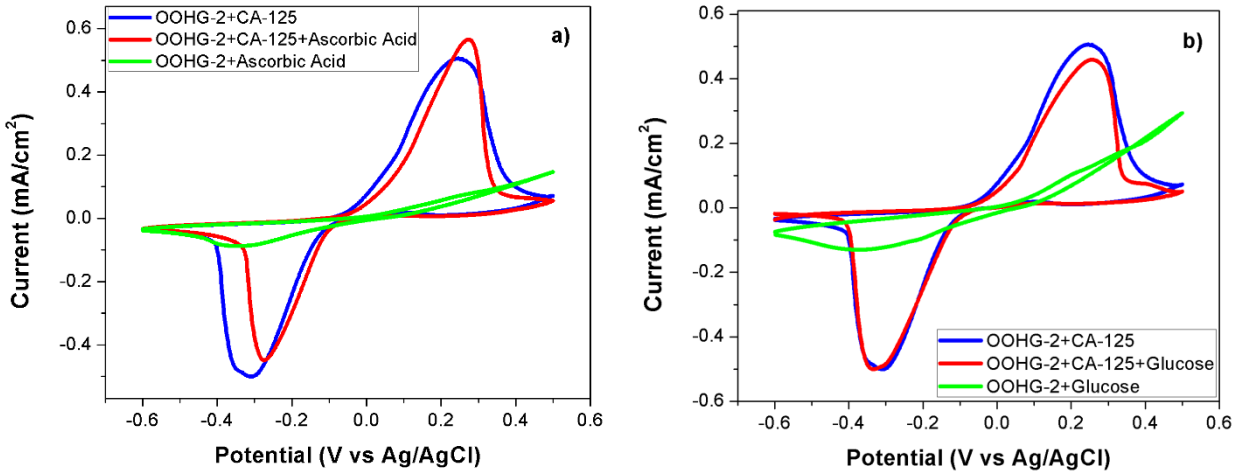


Figure 5. *DPV results at 50 mV amplitude in prepared solution over OOHG-2+CA-125 produced with distinct CA-125 amounts among a) 0.001-10 ng/mL, b) 10-500 ng/mL, c) 500-5000 ng/mL during 30 minutes, d-e) the calibration plots of maximum current densities vs. concentration ratios, and f) results of 10 blank measurements.*

Interference effects of structure molecules such as glucose, urea, dopamine, and ascorbic acid in serum over the electrooxidation process among OOHG-2 and CA-125 antigen were researched in distinct solutions prepared with urea, ascorbic acid, D-glucose, and dopamine. Measurements were performed with CV technique at 50 mV/s scan rate and EIS technique at 0.2 potential over OOHG-2s and OOHG-2+CA-125s produced at determined conditions (30 minute and 500 ng/mL CA-125). CV results and EIS data are presented in Fig.6 and Fig. 7, respectively. The electrooxidation peaks weren't seen in CV results that were achieved over OOHG-2s without CA-125 antigen (Fig. 6). One could see that causes a small increase over the maximum current densities of ascorbic acid, urea, and dopamine structures (Fig. 6a-c-d). It can be said that the glucose structure has almost no effect on the electrooxidation process (Fig. 6b). In addition, it was appeared that causes a slight shift towards the up field of dopamine structure and towards the downfield of the uric acid structure over potential (Fig. 6c-d). As a result, these structures may be clearly stated that there was no significant effect on the electrooxidation peaks of CA-125 antigen in CV results. Electron transfer resistances were high found according to the presence of CA-125 in the Nyquist plots obtained from EIS data at the absence of CA-125 antigen for all structures (Fig. 7). For all structures were no observed a significant difference in electron transfer resistances in the presence of CA-125 antigen, these results were in agreement with the CV results. All CV and EIS results prove that ascorbic acid, uric acid, glucose, and dopamine structures do not have any interference effects at the electrooxidation of CA-125 on OOHG-2 structure.



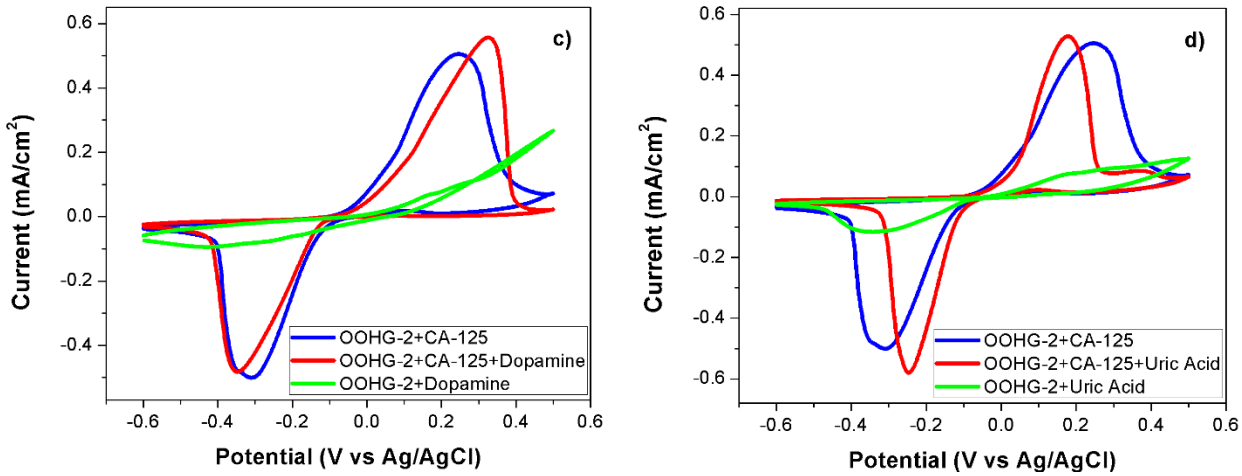


Figure 6. *CV results at 50 mV/s scan rate in a) prepared solution+Ascorbic acid, b) prepared solution+Glucose, c) prepared solution+Dopamine, and d) prepared solution+Uric acid over OOHG-2+CA-125 produced with determined conditions (30 minute and 500 ng/mL CA-125).*

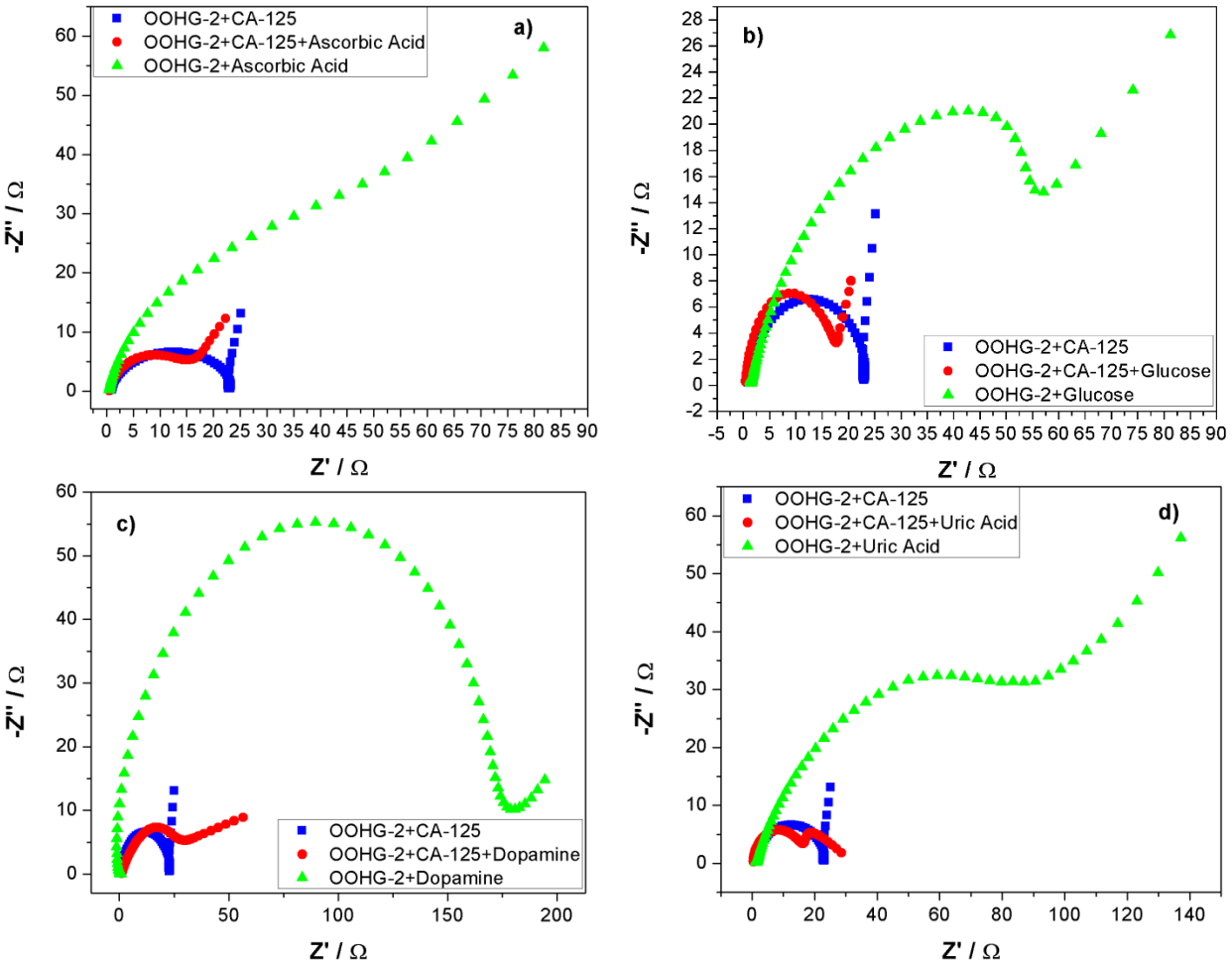


Figure 7. *The Nyquist plots of EIS data at 0.2 potential in a) prepared solution+Ascorbic acid, b) prepared solution+Glucose, c) prepared solution+Dopamine, and d) prepared solution+Uric acid over OOHG-2+CA-125 produced with determined conditions (30 minute and 500 ng/mL CA-125).*

Finally, the effect over the electrooxidation process among OOHG-2 and CA-125 of salts found in serum was examined in artificial and 0.9% isotonic NaCl serums via CV at 50 mV/s scan rate and EIS at 0.2 potential. OOHG-2+CA-125s that used in measurements were produced at determined conditions (30 minutes and 500 ng/mL CA-125). Results are given in Fig. 8. As clearly seen in Fig. 8a the salts in artificial and isotonic serums were found that don't have important effects over the electrooxidation reaction of CA-125. Likewise, the electron transfer resistances in these serum mediums were found that be very close to each other (Fig. 8b), and these data were compatible with CV results. As a result, CV results and EIS data prove that do not the effects over the electrooxidation process among OOHG-2 and CA-125 of the salts that found in serum medium.

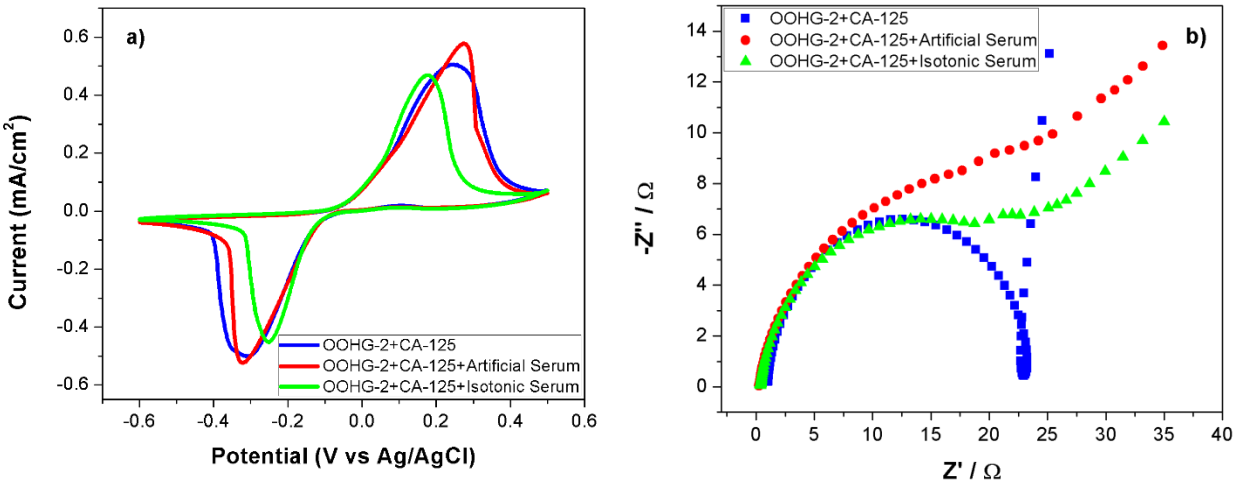


Figure 8. *a) CV results at 50 mV/s scan rate and b) the Nyquist plots of EIS data at 0.2 potential in artificial and isotonic serums over OOHG-2+CA-125 produced with determined conditions (30 minute and 500 ng/mL CA-125).*

4. Conclusions

In this study, onion oil-basic OOHGs were characterized by synthesizing to detect CA-125 in serum. FT-IR results and swelling tests were shown that OOHG structures synthesized successfully. Then, CA-125 at determined conditions was incubated over OOHG structures, and CV measurements were performed in the absence and presence of CA-125. While the electrooxidation peaks were not observed in measurements over OOHGs, Forward and backward peaks were clearly seen in measurements over OOHG+CA-125. OOHG-2+CA-125 among OOHGs prepared was displayed the highest performance with 0.8151 mA/cm^2 at 0.24 V of forward peak and 0.8258 mA/cm^2 at -0.31 V of backward peak values. Moreover, the linear range, LOD, and LOQ values for OOHG-2+CA-125 were found as 1-500 ng/mL concentrations (R^2 = 0.9826), 0.265 µU/mL, and 0.531 µU/mL, respectively. In addition, the electrooxidation peak belonging to CA-125 antigen was observed not affected in interference measurements of structures such as ascorbic acid, dopamine, glucose, and uric acid that could be found in the blood. These results show carries major hope for detecting at high sensitivity and selectivity, fast, and reliability of CA-125 in serum via electrochemical methods over OOHG-2 structure.

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