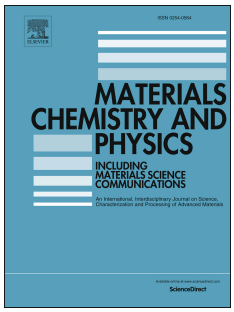


Novel Cacao oil-based organo-hydrogels to detect carcinoma antigen 125 in serum medium; synthesis, characterization, and electrochemical measurements

Omer Faruk Er, Duygu Alpaslan, Tuba Ersen Dudu, Nahit Aktas, Hilal Kivrak

PII: S0254-0584(22)01101-4

DOI: https://doi.org/10.1016/j.matchemphys.2022.126795

Reference: MAC 126795

To appear in: *Materials Chemistry and Physics*

Received Date: 11 April 2022

Revised Date: 13 September 2022

Accepted Date: 15 September 2022

Please cite this article as: O.F. Er, D. Alpaslan, T.E. Dudu, N. Aktas, H. Kivrak, Novel Cacao oil-based organo-hydrogels to detect carcinoma antigen 125 in serum medium; synthesis, characterization, and electrochemical measurements, *Materials Chemistry and Physics* (2022), doi: https://doi.org/10.1016/j.matchemphys.2022.126795.

CRediT authorship contribution statement

Hilal Kivrak: Conceptualization, Methodology, Writing and Supervision, Writing – review & editing.
Omer Faruk Er: Visualization, Investigation, Electrochemical measurements.
Duygu Alparslan: Visualization, Investigation
Tuba Erşen Dudu: Visualization, Investigation
Nahit Aktas: Conceptualization, Methodology, Writing and Supervision

Novel Cacao oil-based organo-hydrogels to detect carcinoma antigen 125 in serum medium; synthesis, characterization, and electrochemical measurements

Omer Faruk Er[a], Duygu Alpaslan[a], Tuba Ersen Dudu[a], Nahit Aktas[b], Hilal Kivrak[c,d]

[a]*Department of Chemical Engineering, Faculty of Engineering, Van Yuzuncu Yil University, Van, 65000, Turkey*

[b] *Department of Chemical Engineering, Faculty of Engineering, Kyrgyz-Turkish Manas University,, BİSHKEK, KYRGYZSTAN*

[c]*Department of Chemical Engineering, Faculty of Engineering and Architectural Sciences, Eskisehir Osmangazi University, Eskisehir, 26040, Turkey*

[d]*Translational Medicine Research and Clinical Center, Eskisehir Osmangazi University, 26040 Eskisehir, Turkey*

Corresponding Author: hilalkivrak@gmail.com

Novel Cacao oil-based organo-hydrogels to detect carcinoma antigen 125 in serum medium; synthesis, characterization, and electrochemical measurements

Omer Faruk Er[a*], Duygu Alpaslan[a], Tuba Ersen Dudu[a], Nahit Aktas[b], Hilal Kivrak[c,d++]

[a]*Department of Chemical Engineering, Faculty of Engineering, Van Yuzuncu Yil University, Van, 65000, Turkey*

[b] *Department of Chemical Engineering, Faculty of Engineering, Kyrgyz-Turkish Manas University,, BİSHKEK, KYRGYZSTAN*

[c]*Department of Chemical Engineering, Faculty of Engineering and Architectural Sciences, Eskisehir Osmangazi University, Eskisehir, 26040, Turkey*

[d]*Translational Medicine Research and Clinical Center, Eskisehir Osmangazi University, 26040 Eskisehir, Turkey*

*Corresponding Author 1: omerfaruker56@gmail.com

+Corresponding Author 2: hilaldemir.kıvrak@ogu.edu.tr, hilalkivrak@gmail.com

ABSTRACT

In present study, cacao oil-based organo-hydrogels (OHCOs) are synthesized to detect carcinoma antigen 125 (CA-125) in serum medium with electrochemical methods such as cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). OHCOs are prepared by the free radical polymerization reaction with agar, glycerol, and distinct ratios of cacao oil with glutaraldehyde (GA) crosslinker or methylene bisacrylamide (MBA) crosslinker. OHCOs are characterized via Fourier Transform Infrared Spectroscopy (FT-IR), in different solvent environments and pHs. Electrochemical measurements are performed on OHCOs at the presence and absence of CA-125 antigen in serum medium. For the electrochemical sensor, two distinct linear ranges are determined as 0.00083-41.5 U/mL and 83.0-2075 U/mL. LOD and LOQ values are found as 0.34 μU/mL and

1.01 μU/mL, respectively. These results clearly show that OHCOs is a promising sensor material for the determination of CA-125 in human serum, sensitively.

Keywords: ovarian cancer, CA-125, cacao oil, electrochemical, sensor

1. Introduction

Ovarian cancer is one of the most common gynecological cancers with the highest mortality rate. The reason of the high mortality for ovarian cancer is due to the fact that asymptomatic and secret growth of the tumor leads th emergence of symptoms in the late stages [1-4]. Ovarian cancer can be treated with chemotherapy or surgery in the early stages without metastasis [5-7].

Markers are indicators that can be used to evaluate biological processes at biological states and drug responses [8, 9]. Biological markers such as DNA, antibody, enzymes, RNA, peptide, or receptors structures found in secretions such as serum, urine, blood, saliva, and nipple discharge could help in early screening, monitoring, and diagnosis of cancers [10-15].

CA-125 is the only marker approved as a tumor marker to monitor stages and response to treatment for ovarian cancer. CA-125 has a high molecular weight protein in the MUCIN 16 family, and it is found on the cell surface of ovarian tumors. Levels in blood samples and production of CA-125 are known to be associated with the growth of cancer cells [16, 17]. In healthy individuals, CA-125 levels are at a threshold of less than 35 U/mL, and higher ratios than from this value of CA-125 are usually associated with ovarian cancer. Apart from that, the level of CA-125 can increase in cancer types such as lung, gastrointestinal, breast, and endometrial cancers [18, 19].

In literature, in order to detect CA-125 more sensitively, studies have been made on different types of sensors such as chemiluminescence [20, 21], fluorescence [22, 23], electrochemical sensor [24, 25], colorimetric [26, 27], resonance [28, 29], and photoluminescence [30].

Electrochemical sensors are of great importance for screening and following cancers due to having very sensitive detection limits to monitor the level of markers in patients and normal serums. In addition, these sensors are rapid, cheap, simple, and reliable devices [31]. PAA/GSPE [32], SPE/ Au–AgNPs [33], benzothiophene derivates [34-37], Ab_1/Au-rGO/GCE [38], Cat@AMQDs-GCE [39], HRP [40], Ppy nanowire [41], MOF-808/CNT/GCE [42], and $Co(bpy)_3^{3+}$/MWNTs–Nafion/GC [43] materials were studied to measure CA-125 level sensitively with electrochemical methods. In addition, Rebelo et al. reported that they developed molecular imprinting polymers (MIP) to detect CA-125. They indicated that this sensor has a good selectivity in 0.01-500 U/mL concentration range and with a 0.01 U/mL detection limit [44]. In another study, Torati et al. developed a gold nanostructures modified electrode (GNs) for the detection of CA-125 and they found that this GNs electrode has a linear range of 10-100 U/mL and a low detection limit of 5.5 U/mL [45]. Moreover, Ravalli et al. reported that they prepared a screen-printed graphite electrode modified with gold nanoparticles for the detection of CA-125, which varies in a linear concentration range of 0-100 U/mL and allows for a clear identification of CA-125 with a detection limit of 6.7 U/mL [46]. Apart from these studies, the LOD and concentration ranges of electrochemical sensors compiled from the literature were given in Table 1.

Table 1. Detection limits and concentration ranges of different electrochemical sensors reported from the literature

Tumor Marker	Sensor	Detection Limit	Concentration range	Ref.
CA-125	AuNP-PB-PtNPPANI Hydrogel	4.4 mU/mL	0.01-5000 U/mL	[47]
CA-125	Au–Thi-CPE	1.8 U/mL	10-30 U/mL	[48]
CA-125	CA125/colloidal AuNPs/CA-GCE	1.73 U/mL	0-30 U/mL	[49]
CA-125	Ag NPs-GQDs/Ab/BSA/Ag	0.01 U/mL	0.01-400 U/mL	[50]
CA-125	thionine/CA125/CNF-GCE	1.8 U/mL	2-75 U/mL	[51]
CA-125	FA@H-PANI@CS-HCl	0.25 pg/mL	0.001-25 ng/mL	[52]
CA-125	Ab_2–Ag–Ab_1/Au-VBG/BDD/Ta	0.09 mU/mL	0.5-100 U/mL	[53]

CA-125	BSA/Ab/Au NPs/Cys A/ERGO-P(DA)-GCE	0.1 U/mL	0.1-400 U/mL	[54]
CA-125	OHCO-2	0.34 μU/mL (LOD) 1.01 μU/mL (LOQ)	0.00083-41.5 and 83.0-2075 U/mL	**This Study**

Herein, OHCOs were synthesized by free radical polymerization reaction to detect CA-125 sensitively in serum medium. Agar, glycerol, and MBA or GA crosslinkers were used in the preparation of OHCOs. Cacao oil used in the preparation of OHCOs can include structures molecules in ratios 25.6% palmitic acid, 34.6% stearic acid, 34.7% oleic acid, 3.3% linoleic acid, and 1.8% others [55-59].

2. Materials and Methods

2.1. Materials

Chemicals such as agar, dopamine, methylene bisacrylamide (MBA), glutaraldehyde (GA), glycerol, D-glucose, calcium chloride ($CaCl_2$), uric acid, potassium chloride (KCl), magnesium dichloride ($MgCl_2$), ethanol, acetone, ascorbic acid, sodium hydrogen phosphate (Na_2HPO_4), potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$), potassium hydrogen phosphate (K_2HPO_4), and sodium chloride (NaCl) were used for the electrochemical sensor by supplying from Sigma-Aldrich. 0.9% isotonic sodium chloride solution was purchased from the pharmacy. DI water was obtained from the Milli-Q water purification system. All glassy materials was rinsed with DI water, ethanol, and acetone.

2.2. Characterization and Synthesis of organo-hydrogels

Cacao oil-based organo-hydrogels (OHCOs) were synthesized as described by Alpaslan et al. [60]. Briefly, 2 mL of agar solution and 0.04 mL of glycerol were added to the 20 mL flask and of different amounts (0.1, 0.2 and 0.3 mL) Cacao oil was added to the reactions mixture. Or-hydrogel mixture was stirred at 800 rpm for 15 min until the formation of a clear

93 homogeneous solution emulsion and then MBA (0.1%) or glutaraldehyde reagent was added
94 as a crosslinker and further homogenized. In 100 µL DI water, the polymerization process was
95 started by adding the initiator solution ammonium persulfate (APS). The solution was pipetted
96 into a 6 mm diameter pipe and allowed to polymerize for 4 hours before being cut into 6 mm
97 long cylinders. The oven at 40 ^{0}C until a constant weight was achieved and stored at 4 ^{0}C for
98 further uses.

99 **Table 2.** Contents of cacao oil-based or-hydrogels (OHCOs)

OHCO No	Amount of Cacao Oil (mL)	Crosslinker	Mixture
1	0.1	Methylene bisacrylamide (MBA)	Agar (2 mL) + Glycerol (0.04 mL
2	0.2	Methylene bisacrylamide (MBA)	
3	0.3	Methylene bisacrylamide (MBA)	
4	0.1	Glutaraldehyde (GA)	
5	0.2	Glutaraldehyde (GA)	
6	0.3	Glutaraldehyde (GA)	

100

101 The swelling analysis methods described in the literature were used for analyses. Swelling tests
102 were performed at room temperature of 25 ^{0}C [61, 62]. The Fourier Transform Infrared
103 Spectroscopy were measured with a Fourier Transform Infrared Spectrometer at a frequency
104 range of 4000-650 cm^{-1}.

105 **2.3. Fabrication of the electrochemical sensor**

106 OHCOs were synthesized, cut into suitable sizes to prepare electrodes, and then CA-125 was
107 incubated on prepared electrodes at certain times in varying concentrations. Finally, OHCOs
108 and OHCO+CA-125 electrodes by using a thin copper wire were prepared as working
109 electrodes. Electrochemical measurements were obtained with CV, EIS, and DPV by a
110 potentiostat device with a triple electrode system. Pt wire and Ag/AgCl (3 M KCl) in the triple
111 electrode system were used as counter electrode and reference electrode, respectively. All
112 preparation steps of the electrochemical sensor are given in Figure 1.

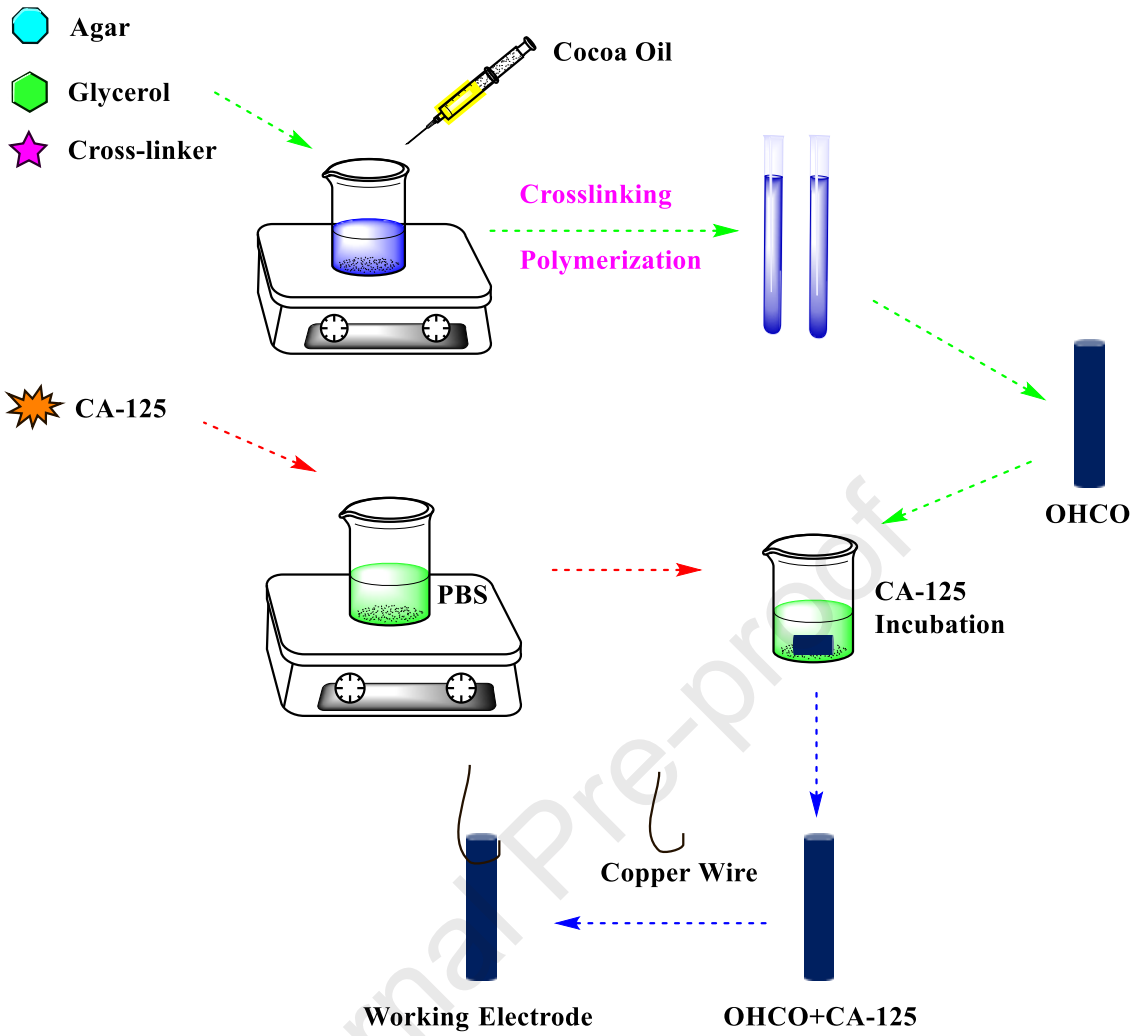


Figure 1: Synthesis of OHCOs and preparation steps of the electrochemical sensor.

2.4. Electrochemical Measurements

Electrochemical measurements were performed with CV, EIS, and DPV methods on OHCO based electrodes. Firstly, CV measurements were obtained at room temperature at 50 mV/s scan rate in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCOs and OHCOs+CA-125 prepared by incubating 1000 ng/mL CA-125 for 30 min.. OHCO-2+CA-125, containing 0.2 mL cacao oil, exhibited the highest current value. The effect of experimental parameters such as concentration of CA-125 and incubation time on OHCO-2+CA-125 electrode. The effect of CA-125 concentration (1-50000 ng/mL CA-125) was examined with CV at room temperature and 50 mV/s scan rate in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) over OHCO-2+CA-125.

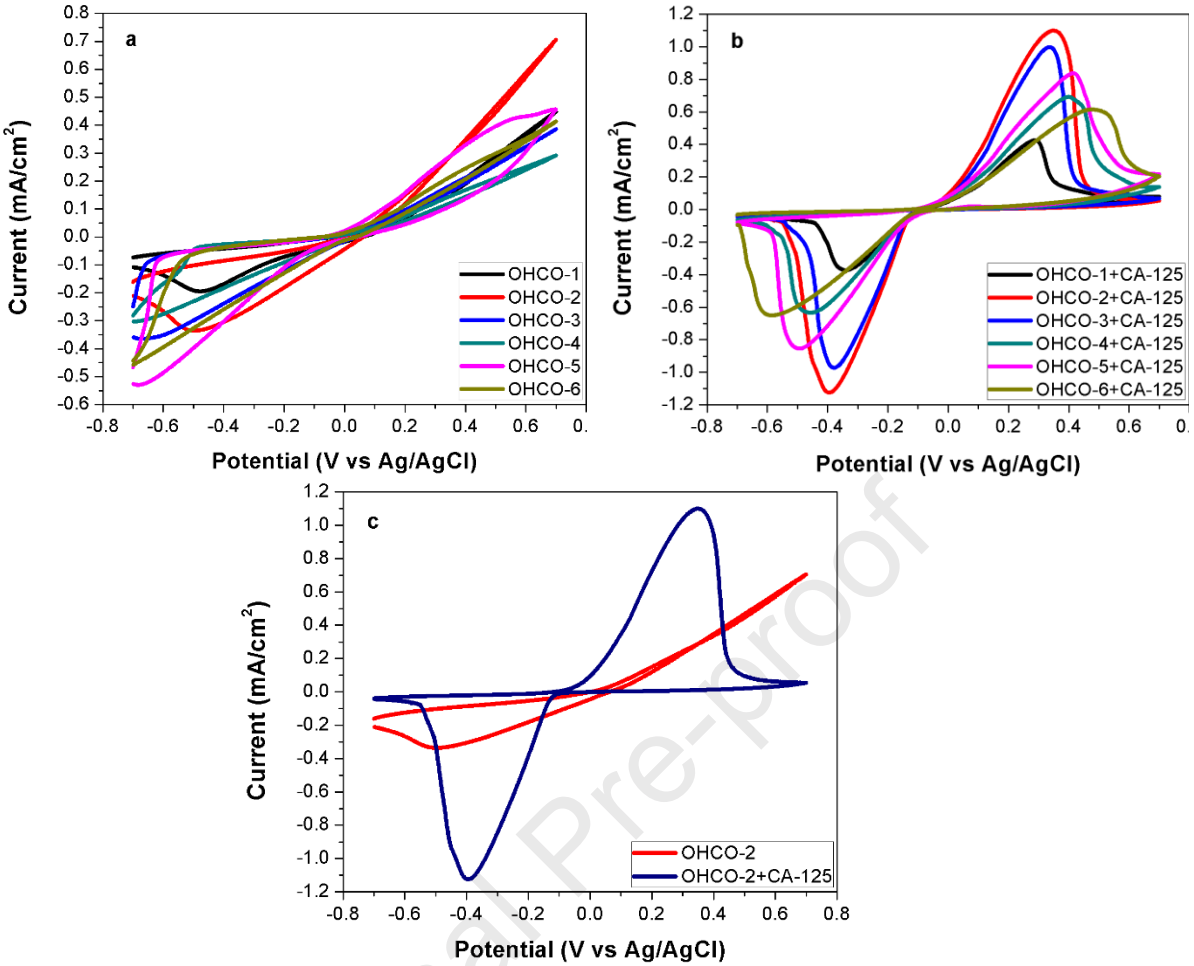
Results revealed that 1000 ng/mL CA-125 concentration incubated electrode had the best current value. The incubation time that the best important parameter for a sensor was investigated via CV at room temperature and 50 mV/s scan rate in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) over OHCO-2+CA-125s, which prepared at varying incubation times among 10-110 min. with 1000 ng/mL CA-125 amount. The best incubation time was determined as 30 min.. Further, the effect of the scan rate on the electrooxidation process was researched with CV at varying scan rates as 5-1000 mV/s at room temperature in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125 electrode prepared by incubating of 1000 ng/mL CA-125. In addition, the electrooxidation process was investigated by receiving measurements with EIS at room temperature and at varying potentials -0.6 V-0.6 V in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125 prepared by incubating for 30 min. of 1000 ng/mL CA-125. The sensitivity of the sensor was determined by taking measurements with DPV at room temperature in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125 prepared by incubating for 30 min. at varying amounts among 0.001-50000 ng/mL CA-125. The sensitivity of the sensor was approximated from the calibration plot slope of DPV curves.

Interference effects on the electrooxidation process on OHCO-2 in serum medium were investigated with CV and EIS at room temperature and 50 mV/s scan rate on OHCO-2 and OHCO-2+CA-125 prepared by incubating for 30 min. of 1000 ng/mL CA-125 in 0.1 M PBS+4.7 mM Glucose, 0.1 M PBS+0.1 mM Dopamine, 0.1 M PBS+0.1 mM Ascorbic acid, and 0.1 M PBS+2.5 mM Uric acid.

Finally, the effects of distinct salt on the electrooxidation process on OHCO-2 electrode in serum medium were examined by taking measurements with CV and EIS at room temperature and 50 mV/s scan rate on OHCO-2 and OHCO-2+CA-125 prepared with 1000 ng/mL CA-125 for 30 min., in 0.9% isotonic NaCl solution and artificial serum solution (included 5 mM $CaCl_2$, 4.7 mM D-glucose, 1.6 mM $MgCl_2$, 4.5 mM KCl, and 2.5 mM uric acid).

3. Results and Discussion

OHCOs were successfully synthesized according to the results of the swelling tests (Fig. S1) and FT-IR (Fig. S2). OHCOs were used to detect CA-125 more sensitively with electrochemical methods such as CV, EIS, and DPV in serum medium without anti CA-125. Firstly, CV measurements were taken at room temperature and 50 mV/s scan rate in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCOs in the absence of CA-125. Following this, similar measurements were taken in the presence of CA125. CV results were shown in Figure 2. Electrooxidation peaks were not observed in measurements received on OHCOs without CA-125, but these peaks were clearly observed at 0.0~0.7 V potentials at measurements obtained on OHCO+CA-125s prepared by incubating 1000 ng/mL CA-125 amount for 30 min. (Fig. 2a-b). Moreover, as can be clearly seen in Fig. 2c, the electrooxidation peaks of CA-125 were clearly observed in the measurements taken in the presence and absence of CA-125. In the measurements obtained on OHCO-1, OHCO-2, OHCO-3 synthesized with MBA crosslinker, the electrooxidation peaks came at lower potentials compared to OHCO-4, OHCO-5, OHCO-6 synthesized with GA crosslinker (Fig. 2b and Table 2). OHCO-2+CA-125 synthesized with 0.2 mL cacao oil and MBA crosslinker exhibited the highest performance with forward peak 1.114 mA/cm^2 (1114.0 $\mu A/cm^2$) at 0.35 V and backward peak 1.125 mA/cm^2 (1125.0 $\mu A/cm^2$) at -0.39 V. In addition, OHCO-1+CA-125 synthesized with 0.1 mL cacao oil and MBA crosslinker had the lowest performance with forward peak 0.4277 mA/cm^2 (427.7 $\mu A/cm^2$) at 0.29 V and backward peak 0.3766 mA/cm^2 (376.6 $\mu A/cm^2$) at -0.33 V. Similar behavior for the CV measurements in the presence of CA-125 was observed for the OHCO-4, OHCO-5, OHCO-6 electrodes synthesized with GA crosslinker (Fig. 2b). These results obtained by CV technique on OHCOs without anti-CA125 are promising for sensitively detecting CA-125 in serum medium.



172 **Figure 2.** CV results that received at room temperature and 50 mV/s scan rate in 0.1 M PBS
173 (included 5 mM $Fe(CN)_6^{3-/4-}$) on **a)** OHCOs electrodes, **b)** OHCOs+CA-125 electrodes, and **c)**
174 compare of OHCO-2 and OHCO-2+CA-125 electrodes.

175 CV measurements were performed at room temperature and 50 mV/s scan rate in 0.1 M PBS
176 (included 5 mM $Fe(CN)_6^{3-/4-}$) to examine the effect of CA-125 concentration on the
177 electrooxidation process between OHCO-2 and CA-125. Results are presented in Figure 3a.
178 Different OHCO-2+CA-125 electrodes were prepared at varying concentrations between 1-
179 50000 ng/mL by incubating CA-125 for 30 min. at room temperature for these measurements.
180 One could note that a gradual increase in current density was observed in measurements
181 obtained on OHCO-2+CA-125 between 1-1000 ng/mL and a gradual decrease in current
182 density was observed in measurements taken on OHCO-2+CA-125 between 1000-50000
183 ng/mL. From these results, one could understand that the electrochemical sensor had the

highest current density at 1000 ng/mL CA-125 concentration. Following this, CV measurements were taken to investigate the effect of CA 125 incubation time (10-110 min. on OHCO-2+CA-125 at room temperature and 50 mV/s scan rate in 0.1 M PBS over OHCO-2+CA-125 (Fig. 3b). Results revealed that 30 min was the best incubation time of CA-125 on OHCO-2.

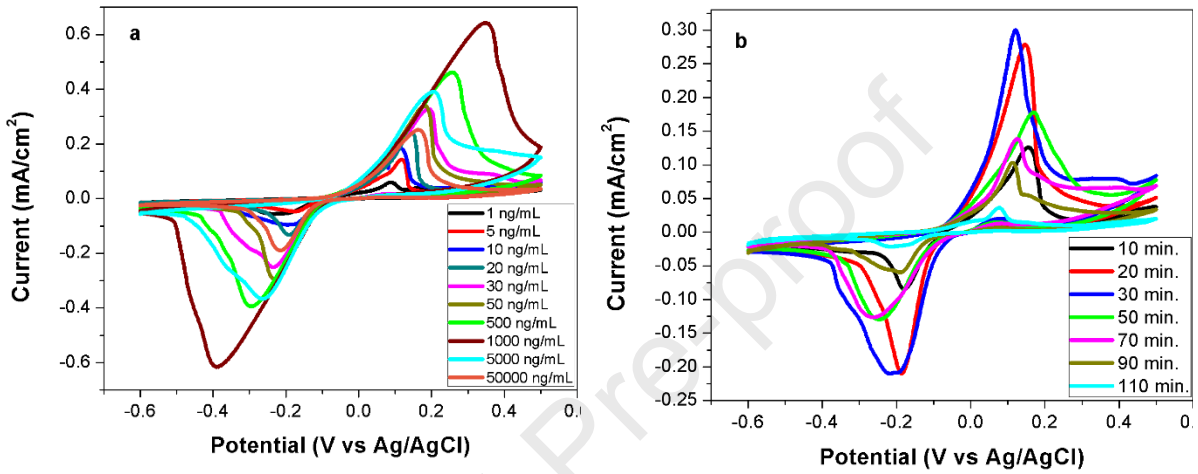


Figure 3. CV results that received at room temperature and 50 mV/s scan rate in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$); **a)** on OHCO-2+CA-125s prepared with varying rates at 1-50000 ng/mL CA-125 concentrations by incubating 30 min.; and **b)** on OHCO-2+CA-125s prepared with 1000 ng/mL by incubating 10-110 min..

The scan rate effect on electrooxidation process on OHCO-2+CA-125 prepared at optimum conditions (1000 ng/mL CA-125 amount and 30 min incubation time) was investigated by taking measurements at varying the scan rates among 5-1000 mV/s at room temperature. Results of these measurements were demonstrated in Fig. 4. It was observed that the current density increased with increasing scan rate (5-1000 mV/s), indicating that a diffusion-controlled electrochemical reaction took place on OHCO-2.

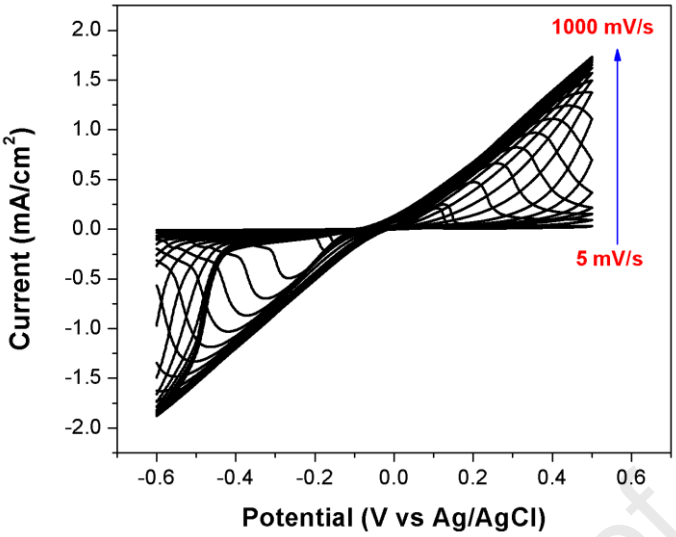


Figure 4. CV results taken at varying scan rates at room temperature in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125s prepared with 1000 ng/mL CA-125 amount by incubating 30 min.

EIS method was employed to investigate the electrooxidation process on the prepared electrodes. Nyquist plots from the EIS data consist of a semicircular field expressing linear divisions with the charge transfer resistance (R_{ct}) denoting the diffusion process. When the radius of these semicircles is small, R_{ct} is small, and when their radius is also large, R_{ct} is large [63-66]. To understand of electrochemical process between CA-125 and OHCO-2, EIS measuremenst were performed at varying potentials at room temperature in 0.1 PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125 electrode prepared by incubating 1000 ng/mL CA-125 for 30 min. Results and the equivalent circuit model were presented in Fig. 5. The measurements taken between -0.6 V (220.7 ohm) and 0.6 V (836.2 ohm) had different semi-circles at each potential. The electrooxidation of CA-125 on OHCO-2 was slow when the semicircle diameter of the Nyquist plots was large, but the electrooxidation of CA-125 on OHCO-2 was fast when the semicircle diameter of the Nyquist plots was small. According to Fig. 4b, CA-125 electrooxidation reaction on OHCO-2 was slow at -0.6 V (220.7 ohm), -0.4 V

(310.3 ohm), 0.5 V (346.8 ohm), and 0.6 V (836.2 ohm), but CA-125 electrooxidation was fast at -0.2 V (232.1 ohm), 0.0 V (114.8 ohm), 0.1 V (107.5 ohm), 0.2 V (364.1 ohm), and 0.3 V (129.3 ohm). The rapid kinetics of CA-125 electrooxidation on OHCO-2 was at the lowest possible level at 0.1 V (107.5 ohm). These results are also compatible with results obtained from DPV and CV.

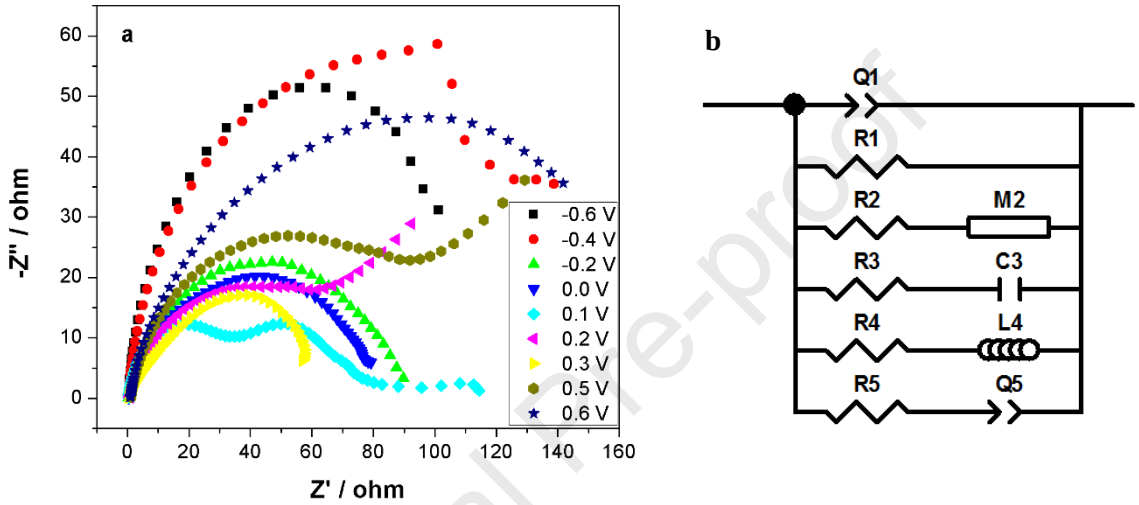
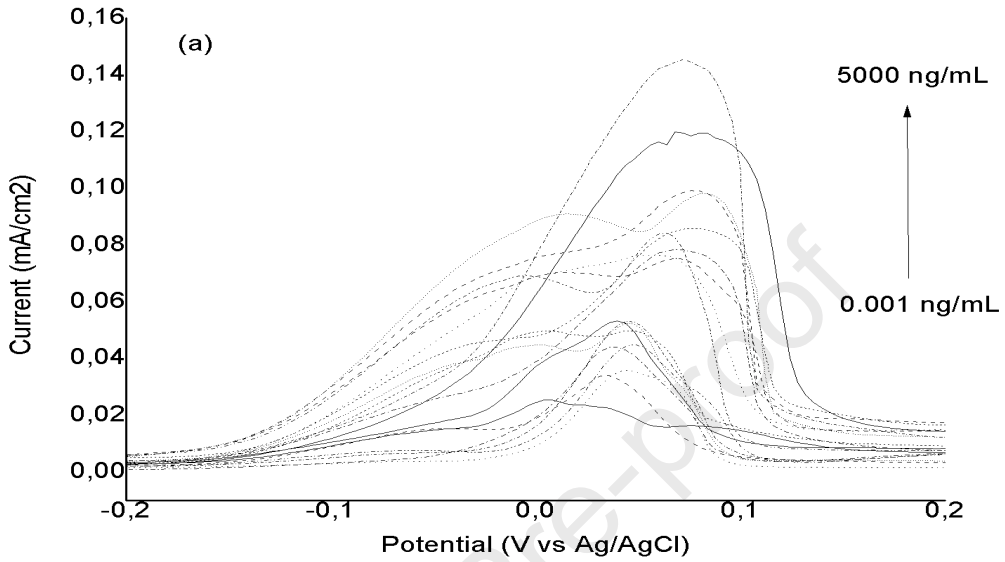


Figure 5. a) EIS results that taken at varying potentials between -0.6 V to 0.6 V at room temperature in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125s prepared with 1000 ng/mL CA-125 for 30 min. and **b)** equivalent circuit model obtained for OHCO+CA-125 electrodes.

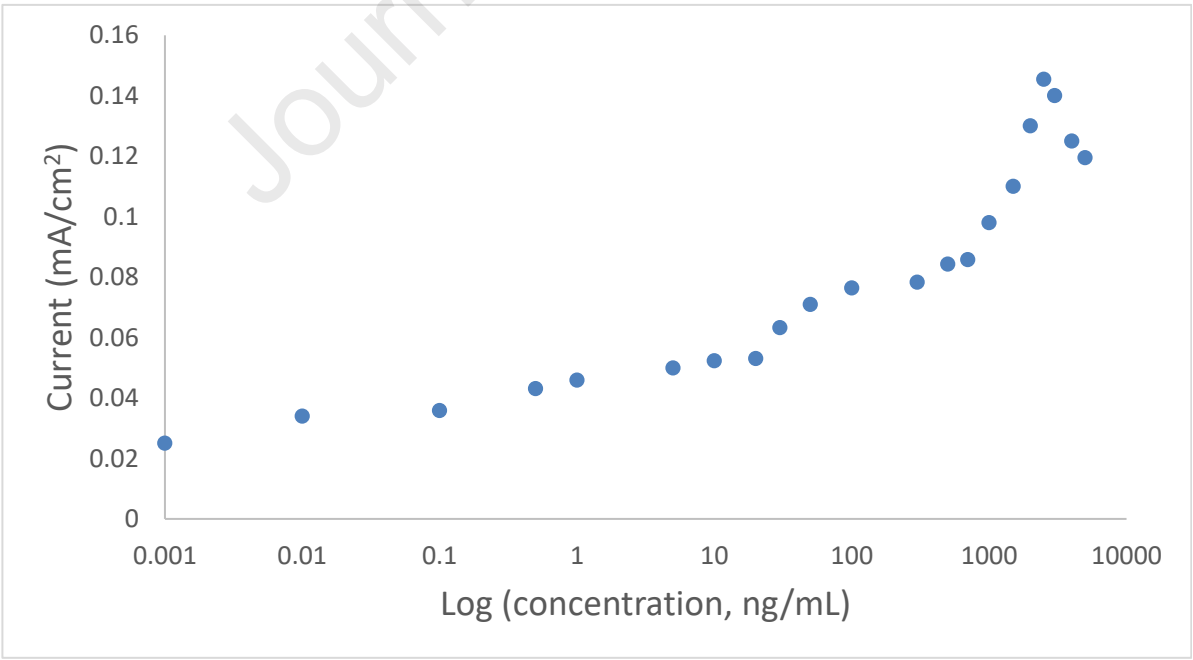
The lowest detection limit (LOD) and limit of quantification (LOQ) values were calculated with OHCO-2 and OHCO-2+CA-125 electrodes prepared with distinct CA-125 concentrations at 0.001-5000 ng/mL for 30 min incubation time. Initially, 10 blank DPV measurements were performed on OHCO-2s without CA-125 and then DPV measurements were taken on OHCO-2s at the presence of CA-125. In order to obtain sensitivity value, the maximum current values versus concentration values were plotted and presented in Fig. 6. One could observe that there were two distinct linear ranges as 0.001-50 ng/mL (0.00083-41.5 U/mL) and 100-2500 ng/mL (83.0-2075 U/mL). LOD and LOQ values for the sensor were found as 0.00041 ng/mL (0.34

234 μU/mL) and 0.00122 ng/mL (1.01 μU/mL), respectively. LOD value found for the sensor was

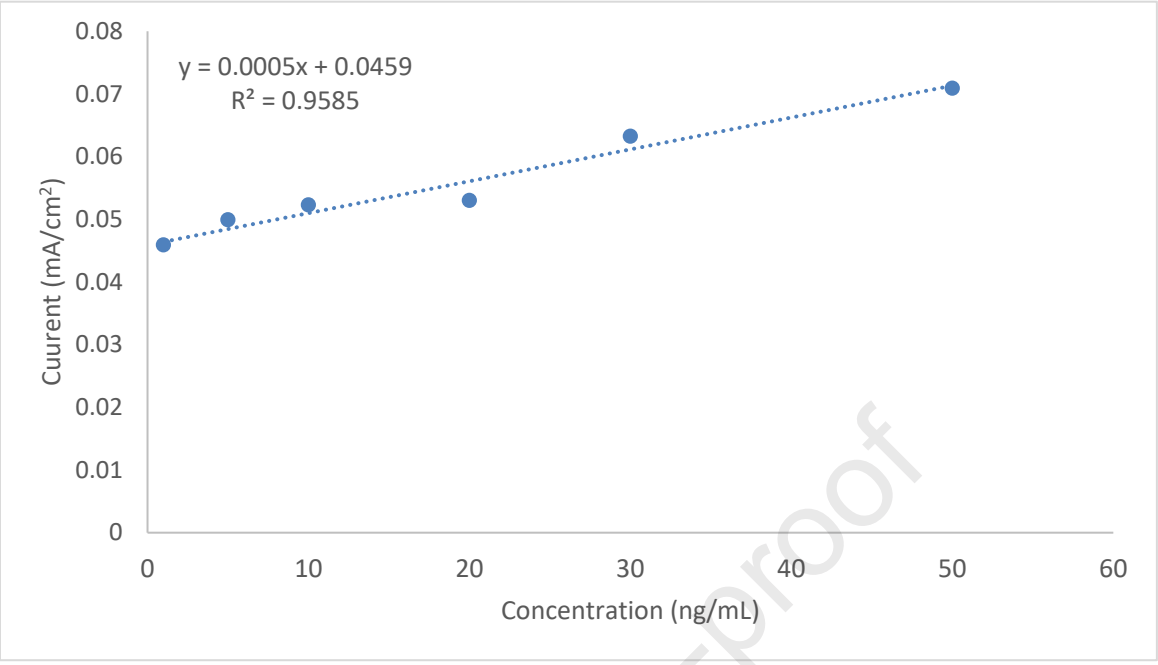
235 lowest than reported in the literature (Table 1).



b



c



d

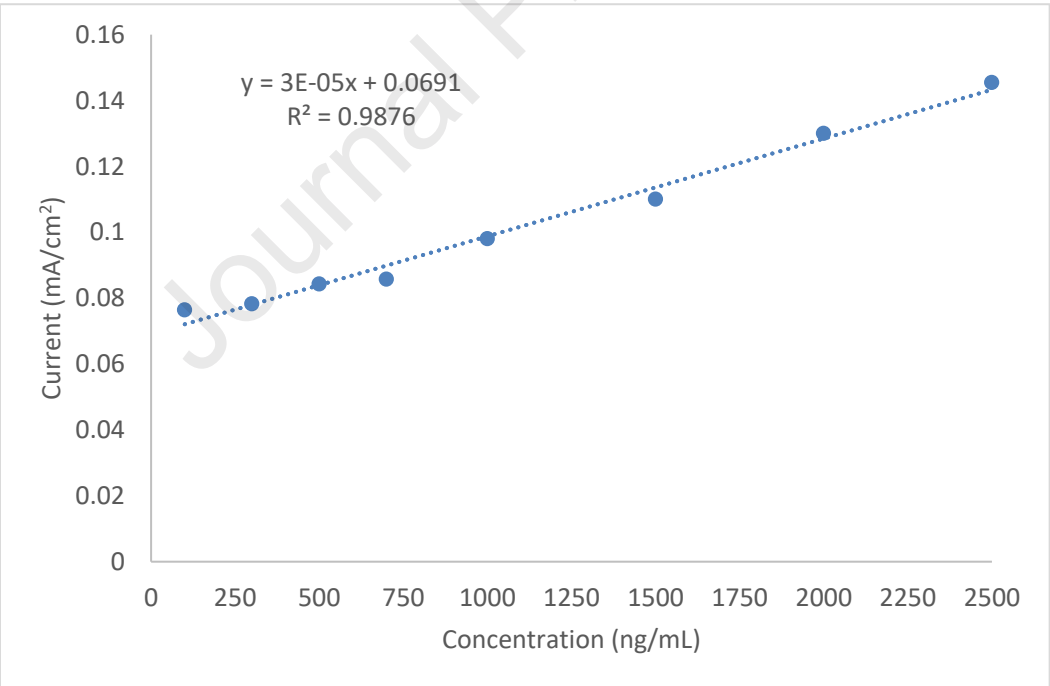
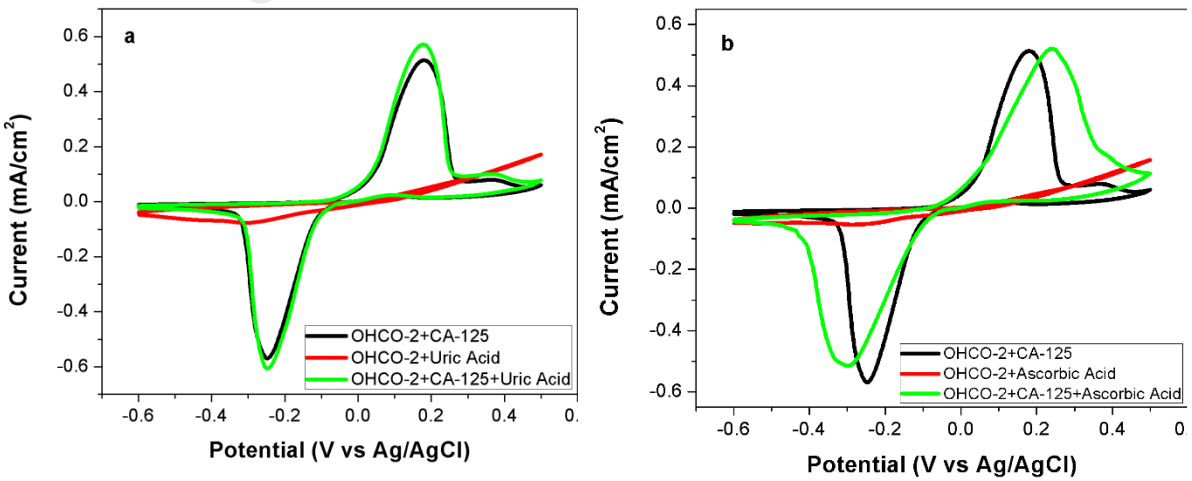
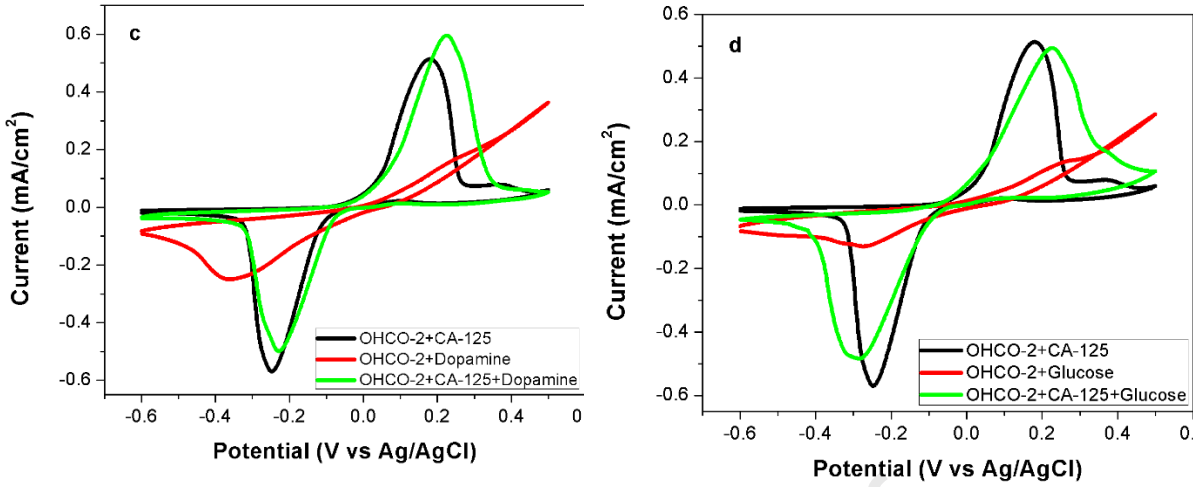


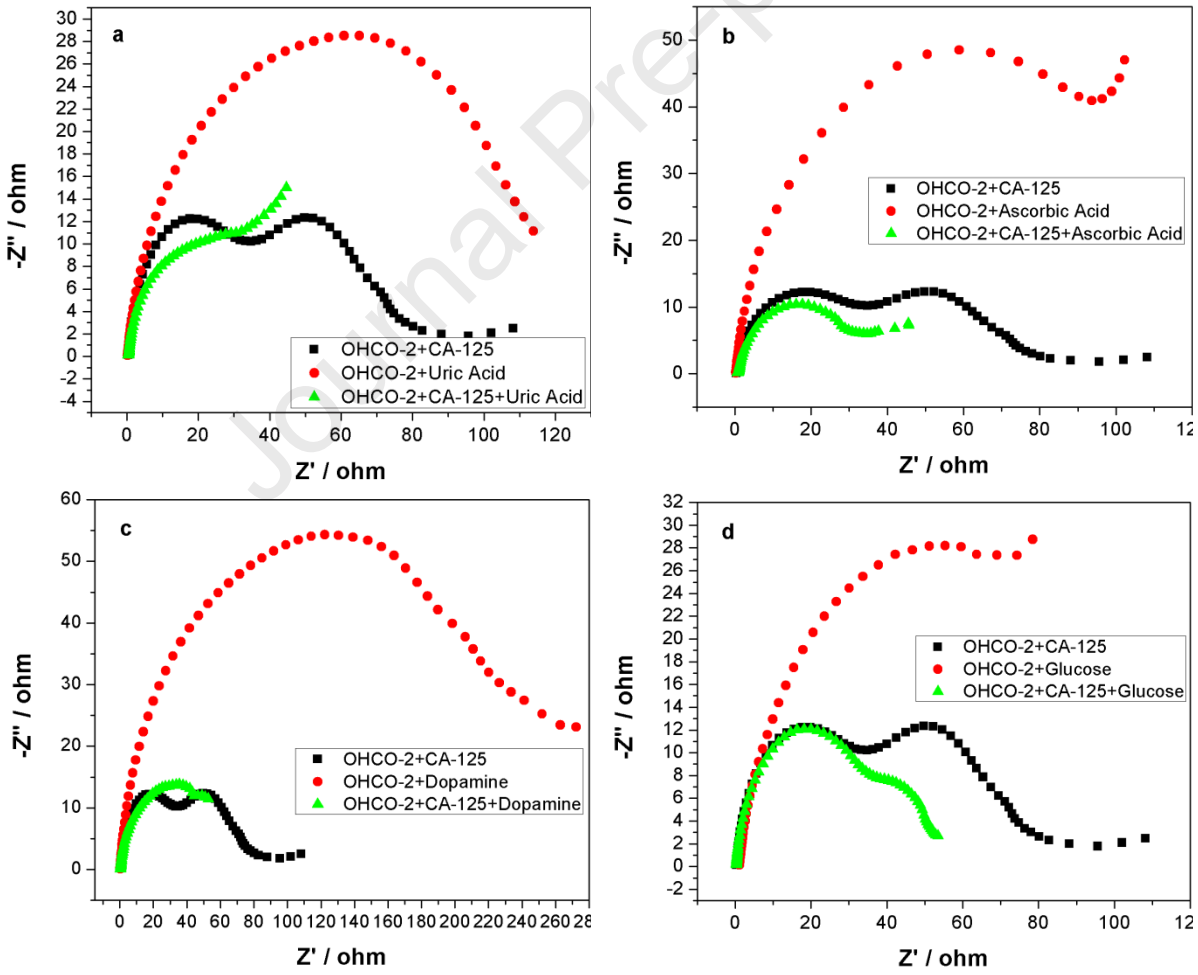
Figure 6. DPV results in 0.1 M PBS (included 5 mM $Fe(CN)_6^{3-/4-}$) on OHCO-2+CA-125 produced with varying concentrations between **a)** 0.001, 0.1, 0.5, 10, 30, 50, 100, 300,500, 700, 1000, 1500, 2000, 2500, 3000, 4000, 5000 ng/mL, **b)** maximum current versus log CA-125 concentration, **c)** maximum current against 0.001-50 ng/mL CA-125 concentration values, and **d)** maximum currents vs. 100-2500 ng/mL CA-125 concentration values.

Interference measurements were taken in the presence of uric acid, ascorbic acid, dopamine, and glucose on OHCO-2 + CA-125 electrodes by CV and EIS. CV and EIS results of these measurements are presented in Fig.7 and Fig.8, respectively. These measurements were performed on OHCO-2 and OHCO-2+CA-125 prepared by incubating 1000 ng/mL CA-125 for 30 min at room temperature. In CV measurements received over OHCO-2s without CA-125, any electrooxidation peaks were not observed for uric acid, ascorbic acid, dopamine, and glucose (Fig. 7). However, CA125 electrooxidation peaks were clearly observed on OHCO-2+CA-125s electrodes. These peaks were almost the same as the peak obtained over OHCO-2+CA-125s without interfering molecules. These results show that the interfering molecules in the serum samples have no effect on the CA-125 electrooxidation reaction on OHCO-2. One could note that OHCO-2 was only sensitive to CA-125 antigen (Fig. 7). Likewise, EIS measurements on OHCO-2 at 0.1 potential in the absence of CA-125 showed that the charge transfer resistance (R_{ct}) was very large compared to the measurements obtained in the presence of CA-125. It can clearly see that the load transfer resistances (R_{ct}) of results obtained on OHCO-2+CA-125s in the presence and absence of structural molecules are close to each other as CV results (Fig. 8).





257 **Figure 7.** CV results of interference measurements that received at room temperature in **a)** Uric
258 Acid+PBS, **b)** Ascorbic Acid+PBS, **c)** Dopamine+PBS, and **d)** Glucose+PBS on OHCO-2 and
259 OHCO-2+CA-125s that prepared with 1000 ng/mL CA-125 amount for 30 min.



260 **Figure 8.** EIS results of interference measurements that received at room temperature 0.1
261 potential in **a)** Uric Acid+PBS, **b)** Ascorbic Acid+PBS, **c)** Dopamine+PBS, and **d)**

Glucose+PBS on OHCO-2+CA-125s that prepared with 1000 ng/mL CA-125 amount for 30 min. and OHCO-2.

Finally, CV and EIS measurements in isotonic (0.9% isotonic NaCl solution) and artificial serums were performed at room temperature at 50 mV/s scan rate and 0.1 V to investigate the effects of different salts in serum samples on the CA-125 electrooxidation reaction over OHCO-2+CA-125s prepared by incubating 1000 ng/mL CA-125 for 30 min. Comparative CV and EIS results are given in Fig. 9. Artificial serum was prepared with 5 mM $CaCl_2$, 4.7 mM D-glucose, 1.6 mM $MgCl_2$, 4.5 mM KCl, and 2.5 mM uric acid. As seen clearly in Fig. 9a, it is understood that the salts in isotonic and artificial serum do not have any effect on the CA-125 electrooxidation reaction. Likewise, similar load transfer resistances were obtained in the EIS results and it was compatible with these CV results (Fig. 9b).

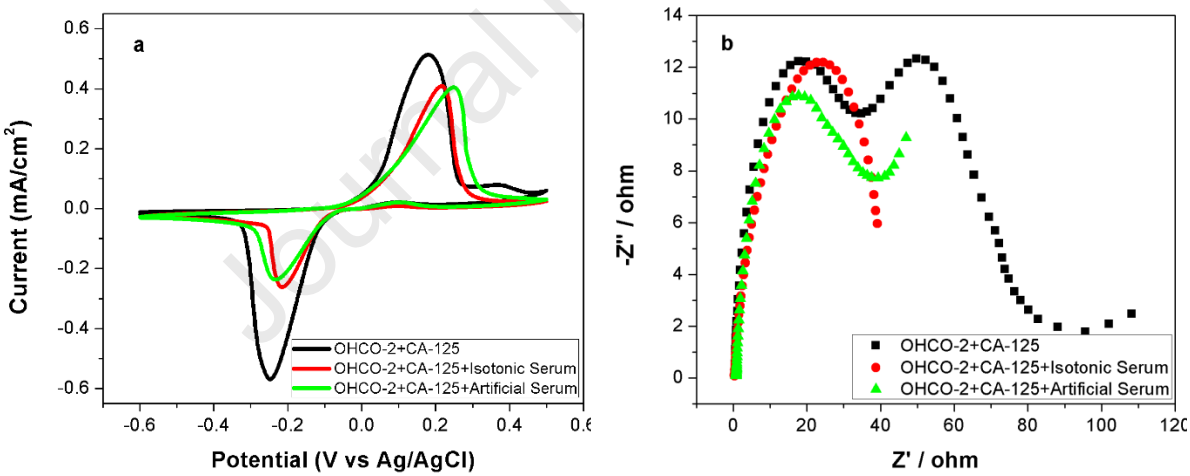


Figure 9. **a)** CV results and **b)** EIS results at 0.1 V that received at room temperature in isotonic serum and artificial serum on OHCO-2s and OHCO-2+CA-125s prepared with 1000 ng/mL CA-125 amount for 30 min.

4. Conclusions

In this study, cacao oil-based or-hydrogels (OHCOs) were prepared with free radical polymerization reaction to detect CA-125 in serum medium. Firstly, CV measurements were taken in the presence and absence of CA-125 with different OHCOs synthesized and OHCO-2 that show the best performance with forward peak 1.114 mA/cm^2 (1114.0 μA/cm^2) at 0.35 V and backward peak 1.125 mA/cm^2 (1125.0 μA/cm^2) at -0.39 V values were determined. OHCOs were incubated with 1000 ng/mL CA-125 for 30 min. Secondly, the effect of parameters such as concentration, incubation time, scan rate over OHCO-2 on the electrooxidation process between CA-125 and OHCO-2 were investigated. Moreover, EIS measurements were found that the charge transfer resistance between CA-125 and OHCO-2 reached to the lowest level at 0.1 V (107.5 ohm). The interfering effect of ascorbic acid, dopamine, glucose, uric acid and different salts in serum medium on the electrooxidation process between CA-125 and OHCO-2 were investigated in artificial serum measurements. The findings showed that these interfering molecules have little effect on the electrooxidation process. As result, this study for the future gives great hope for the detection of CA-125 in serum medium with cacao oil-based or-hydrogels.

Acknowledgements

O. F. ER thank the Scientific and Technological Research Council of Turkey (TUBITAK) for the 2211-A scholarship and the Council of Higher Education (YOK) for 100/2000 scholarship.

References

[1] Z. Momenimovahed, A. Tiznobaik, S. Taheri, H. Salehiniya, Ovarian cancer in the world: epidemiology and risk factors, International journal of women's health, 11 (2019) 287.
[2] I.J. Jacobs, U. Menon, Progress and challenges in screening for early detection of ovarian cancer, Molecular & Cellular Proteomics, 3 (2004) 355-366.
[3] A. Yoneda, M.E. Lendorf, J.R. Couchman, H.A. Multhaupt, Breast and ovarian cancers: a survey and possible roles for the cell surface heparan sulfate proteoglycans, Journal of Histochemistry & Cytochemistry, 60 (2012) 9-21.

[4] D. Badgwell, R.C. Bast Jr, Early detection of ovarian cancer, Disease markers, 23 (2007) 397-410.
[5] B. Deb, A. Uddin, S. Chakraborty, miRNAs and ovarian cancer: An overview, Journal of cellular physiology, 233 (2018) 3846-3854.
[6] U.A. Matulonis, A.K. Sood, L. Fallowfield, B.E. Howitt, J. Sehouli, B.Y. Karlan, Ovarian cancer, Nature Reviews Disease Primers, 2 (2016) 1-22.
[7] G.C. Jayson, E.C. Kohn, H.C. Kitchener, J.A. Ledermann, Ovarian cancer, The Lancet, 384 (2014) 1376-1388.
[8] F. Bahavarnia, A. Saadati, S. Hassanpour, M. Hasanzadeh, N. Shadjou, A. Hassanzadeh, Paper based immunosensing of ovarian cancer tumor protein CA 125 using novel nano-ink: a new platform for efficient diagnosis of cancer and biomedical analysis using microfluidic paper-based analytical devices (μPAD), International journal of biological macromolecules, 138 (2019) 744-754.
[9] B.V. Chikkaveeraiah, A.A. Bhirde, N.Y. Morgan, H.S. Eden, X. Chen, Electrochemical immunosensors for detection of cancer protein biomarkers, ACS nano, 6 (2012) 6546-6561.
[10] A. Rasooly, J. Jacobson, Development of biosensors for cancer clinical testing, Biosensors and Bioelectronics, 21 (2006) 1851-1858.
[11] M. Nagpal, S. Singh, P. Singh, P. Chauhan, M.A. Zaidi, Tumor markers: A diagnostic tool, National journal of maxillofacial surgery, 7 (2016) 17.
[12] M. Duffy, Clinical uses of tumor markers: a critical review, Critical reviews in clinical laboratory sciences, 38 (2001) 225-262.
[13] M. Schwartz, A pragmatic review of tumor markers risk assessment analysis (screening), JOURNAL OF CLINICAL LIGAND ASSAY, 20 (1997) 152-154.
[14] M.J. Duffy, Tumor markers in clinical practice: a review focusing on common solid cancers, Medical Principles and Practice, 22 (2013) 4-11.
[15] N. Husseinzadeh, Status of tumor markers in epithelial ovarian cancer has there been any progress? A review, Gynecologic oncology, 120 (2011) 152-157.
[16] I. Diaconu, C. Cristea, V. Hârceagă, G. Marrazza, I. Berindan-Neagoe, R. Săndulescu, Electrochemical immunosensors in breast and ovarian cancer, Clinica Chimica Acta, 425 (2013) 128-138.
[17] R. Lahoud, A. O'Shea, C. El-Mouhayyar, I. Atre, K. Eurboonyanun, M. Harisinghani, Tumour markers and their utility in imaging of abdominal and pelvic malignancies, Clinical Radiology, (2020).
[18] K. Sjövall, B. Nilsson, N. Einhorn, The significance of serum CA 125 elevation in malignant and nonmalignant diseases, Gynecologic oncology, 85 (2002) 175-178.
[19] R.C. Bast Jr, T.L. Klug, E.S. John, E. Jenison, J.M. Niloff, H. Lazarus, R.S. Berkowitz, T. Leavitt, C.T. Griffiths, L. Parker, A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer, New England journal of medicine, 309 (1983) 883-887.
[20] Z. Yang, Z. Xie, H. Liu, F. Yan, H. Ju, Streptavidin-Functionalized Three-Dimensional Ordered Nanoporous Silica Film for Highly Efficient Chemiluminescent Immunosensing, Advanced Functional Materials, 18 (2008) 3991-3998.
[21] I. Al-Ogaidi, H. Gou, Z.P. Aguilar, S. Guo, A.K. Melconian, A.K.A. Al-Kazaz, F. Meng, N. Wu, Detection of the ovarian cancer biomarker CA-125 using chemiluminescence resonance energy transfer to graphene quantum dots, Chemical Communications, 50 (2014) 1344-1346.
[22] X. Xu, J. Ji, P. Chen, J. Wu, Y. Jin, L. Zhang, S. Du, Salt-induced gold nanoparticles aggregation lights up fluorescence of DNA-silver nanoclusters to monitor dual cancer markers carcinoembryonic antigen and carbohydrate antigen 125, Analytica Chimica Acta, 1125 (2020) 41-49.
[23] M.K. Pal, M. Rashid, M. Bisht, Multiplexed magnetic nanoparticle-antibody conjugates (MNPs-ABS) based prognostic detection of ovarian cancer biomarkers, CA-125, β-2M and ApoA1 using fluorescence spectroscopy with comparison of surface plasmon resonance (SPR) analysis, Biosensors and Bioelectronics, 73 (2015) 146-152.
[24] J. Das, S.O. Kelley, Protein detection using arrayed microsensor chips: tuning sensor footprint to achieve ultrasensitive readout of CA-125 in serum and whole blood, Analytical chemistry, 83 (2011) 1167-1172.

[25] S. Chen, R. Yuan, Y. Chai, Y. Xu, L. Min, N. Li, A new antibody immobilization technique based on organic polymers protected Prussian blue nanoparticles and gold colloidal nanoparticles for amperometric immunosensors, Sensors and Actuators B: Chemical, 135 (2008) 236-244.
[26] O. Hosu, A. Ravalli, G.M.L. Piccolo, C. Cristea, R. Sandulescu, G. Marrazza, Smartphone-based immunosensor for CA125 detection, Talanta, 166 (2017) 234-240.
[27] W. Hong, S. Lee, Y. Cho, Dual-responsive immunosensor that combines colorimetric recognition and electrochemical response for ultrasensitive detection of cancer biomarkers, Biosensors and Bioelectronics, 86 (2016) 920-926.
[28] S. Suwansa-ard, P. Kanatharana, P. Asawatreratanakul, B. Wongkittisuksa, C. Limsakul, P. Thavarungkul, Comparison of surface plasmon resonance and capacitive immunosensors for cancer antigen 125 detection in human serum samples, Biosensors and Bioelectronics, 24 (2009) 3436-3441.
[29] S. Hamd-Ghadareh, A. Salimi, F. Fathi, S. Bahrami, An amplified comparative fluorescence resonance energy transfer immunosensing of CA125 tumor marker and ovarian cancer cells using green and economic carbon dots for bio-applications in labeling, imaging and sensing, Biosensors and Bioelectronics, 96 (2017) 308-316.
[30] H. Jin, R. Gui, J. Gong, W. Huang, Aptamer and 5-fluorouracil dual-loading Ag2S quantum dots used as a sensitive label-free probe for near-infrared photoluminescence turn-on detection of CA125 antigen, Biosensors and Bioelectronics, 92 (2017) 378-384.
[31] E.B. Bahadır, M.K. Sezgintürk, Applications of electrochemical immunosensors for early clinical diagnostics, Talanta, 132 (2015) 162-174.
[32] Z. Taleat, A. Ravalli, M. Mazloum-Ardakani, G. Marrazza, CA 125 immunosensor based on poly-anthranilic acid modified screen-printed electrodes, Electroanalysis, 25 (2013) 269-277.
[33] R. Raghav, S. Srivastava, Core–shell gold–silver nanoparticles based impedimetric immunosensor for cancer antigen CA125, Sensors and Actuators B: Chemical, 220 (2015) 557-564.
[34] O.F. Er, H. Kivrak, O. Ozok, S. Çelik, A. Kivrak, A novel electrochemical sensor for monitoring ovarian cancer tumor protein CA 125 on benzothiophene derivative based electrodes, Journal of Electroanalytical Chemistry, (2021) 115854.
[35] O.F. Er, H. Kivrak, O. Ozok, A. Kivrak, Superior and Novel Carbohydrate Antigen 125 Electrochemical Sensor Based on 4-(2-(Naphthalen-1-Yl) benzo [b] thiophen-3-Yl) benzaldehyde, Available at SSRN 3863113, (2021).
[36] O.F. Er, H. Kivrak, O. Ozok, A. Kivrak, Novel 5-(2-phenylbenzo [b] thiophen-3-yl) furan-2-carbaldehyde based ovarian cancer carbohydrate antigen 125 electrochemical sensor, Materials Chemistry and Physics, (2022) 126560.
[37] H. Kivrak, O.F. Er, O. Ozok, S. Celik, A. Kivrak, Synthesis and characterization of 4-(2-(4-methoxyphenyl) benzo [b] thiophen-3-yl) benzaldehyde for carbohydrate antigen 125 electrochemical detection and molecular docking modeling, Materials Chemistry and Physics, 281 (2022) 125951.
[38] X. Liang, H. Han, Z. Ma, pH responsive amperometric immunoassay for carcinoma antigen 125 based on hollow polydopamine encapsulating methylene blue, Sensors and Actuators B: Chemical, 290 (2019) 625-630.
[39] B. Fatima, D. Hussain, S. Bashir, H.T. Hussain, R. Aslam, R. Nawaz, H.N. Rashid, N. Bashir, S. Majeed, M.N. Ashiq, Catalase immobilized antimonene quantum dots used as an electrochemical biosensor for quantitative determination of H2O2 from CA-125 diagnosed ovarian cancer samples, Materials Science and Engineering: C, 117 (2020) 111296.
[40] Z. Dai, F. Yan, J. Chen, H. Ju, Reagentless amperometric immunosensors based on direct electrochemistry of horseradish peroxidase for determination of carcinoma antigen-125, Analytical chemistry, 75 (2003) 5429-5434.
[41] M.A. Bangar, D.J. Shirale, W. Chen, N.V. Myung, A. Mulchandani, Single conducting polymer nanowire chemiresistive label-free immunosensor for cancer biomarker, Analytical chemistry, 81 (2009) 2168-2175.

[42] S. Biswas, Q. Lan, Y. Xie, X. Sun, Y. Wang, Label-Free Electrochemical Immunosensor for Ultrasensitive Detection of Carbohydrate Antigen 125 Based on Antibody-Immobilized Biocompatible MOF-808/CNT, ACS Applied Materials & Interfaces, 13 (2021) 3295-3302.
[43] S. Chen, R. Yuan, Y. Chai, L. Min, W. Li, Y. Xu, Electrochemical sensing platform based on tris (2, 2′-bipyridyl) cobalt (III) and multiwall carbon nanotubes–Nafion composite for immunoassay of carcinoma antigen-125, Electrochimica acta, 54 (2009) 7242-7247.
[44] T.S. Rebelo, R. Costa, A.T. Brandão, A.F. Silva, M.G.F. Sales, C.M. Pereira, Molecularly imprinted polymer SPE sensor for analysis of CA-125 on serum, Analytica chimica acta, 1082 (2019) 126-135.
[45] S.R. Torati, K.C. Kasturi, B. Lim, C. Kim, Hierarchical gold nanostructures modified electrode for electrochemical detection of cancer antigen CA125, Sensors and Actuators B: Chemical, 243 (2017) 64-71.
[46] A. Ravalli, G.P. Dos Santos, M. Ferroni, G. Faglia, H. Yamanaka, G. Marrazza, New label free CA125 detection based on gold nanostructured screen-printed electrode, Sensors and Actuators B: Chemical, 179 (2013) 194-200.
[47] Y. Zheng, H. Wang, Z. Ma, A nanocomposite containing Prussian Blue, platinum nanoparticles and polyaniline for multi-amplification of the signal of voltammetric immunosensors: highly sensitive detection of carcinoma antigen 125, Microchimica Acta, 184 (2017) 4269-4277.
[48] D. Tang, R. Yuan, Y. Chai, Electrochemical immuno-bioanalysis for carcinoma antigen 125 based on thionine and gold nanoparticles-modified carbon paste interface, Analytica chimica acta, 564 (2006) 158-165.
[49] L. Wu, J. Chen, D. Du, H. Ju, Electrochemical immunoassay for CA125 based on cellulose acetate stabilized antigen/colloidal gold nanoparticles membrane, Electrochimica Acta, 51 (2006) 1208-1214.
[50] M. Jafari, M. Hasanzadeh, E. Solhi, S. Hassanpour, N. Shadjou, A. Mokhtarzadeh, A. Jouyban, S. Mahboob, Ultrasensitive bioassay of epitope of Mucin-16 protein (CA 125) in human plasma samples using a novel immunoassay based on silver conductive nano-ink: A new platform in early stage diagnosis of ovarian cancer and efficient management, International journal of biological macromolecules, 126 (2019) 1255-1265.
[51] L. Wu, F. Yan, H. Ju, An amperometric immunosensor for separation-free immunoassay of CA125 based on its covalent immobilization coupled with thionine on carbon nanofiber, Journal of immunological methods, 322 (2007) 12-19.
[52] X. Ren, H. Wang, D. Wu, D. Fan, Y. Zhang, B. Du, Q. Wei, Ultrasensitive immunoassay for CA125 detection using acid site compound as signal and enhancer, Talanta, 144 (2015) 535-541.
[53] H. Li, J. Qin, M. Li, C. Li, S. Xu, L. Qian, B. Yang, Gold-nanoparticle-decorated boron-doped graphene/BDD electrode for tumor marker sensor, Sensors and Actuators B: Chemical, 302 (2020) 127209.
[54] M. Hasanzadeh, R. Sahmani, E. Solhi, A. Mokhtarzadeh, N. Shadjou, S. Mahboob, Ultrasensitive immunoassay of carcinoma antigen 125 in untreated human plasma samples using gold nanoparticles with flower like morphology: a new platform in early stage diagnosis of ovarian cancer and efficient management, International journal of biological macromolecules, 119 (2018) 913-925.
[55] T. Yoshida, K. Nakanishi, T. Yoshioka, Y. Tsutsui, A. Maeda, H. Kondo, K. Sako, Oral tacrolimus oil formulations for enhanced lymphatic delivery and efficient inhibition of T-cell's interleukin-2 production, European Journal of Pharmaceutics and Biopharmaceutics, 100 (2016) 58-65.
[56] T. Olak, A. Turan, D. Alpaslan, T.E. Dudu, N. Aktaş, Developing poly (Agar-co-Glycerol-co-Thyme Oil) based organo-hydrogels for the controlled drug release applications, Journal of Drug Delivery Science and Technology, 60 (2020) 102088.
[57] D. Alpaslan, T.E. Dudu, N. Aktaş, Synthesis and characterization of novel organo-hydrogel based agar, glycerol and peppermint oil as a natural drug carrier/release material, Materials Science and Engineering: C, 118 (2021) 111534.
[58] T.E. Dudu, D. Alpaslan, N. Aktas, Application of Poly (Agar-Co-Glycerol-Co-Sweet Almond Oil) Based Organo-Hydrogels as a Drug Delivery Material, Journal of Polymers and the Environment, (2021) 1-11.

[59] M.E. Helgeson, S.E. Moran, H.Z. An, P.S. Doyle, Mesoporous organohydrogels from thermogelling photocrosslinkable nanoemulsions, Nature materials, 11 (2012) 344-352.
[60] D. Alpaslan, T.E. Dudu, N. Aktaş, Synthesis and characterization of novel organo-hydrogel based agar, glycerol and peppermint oil as a natural drug carrier/release material, Materials Science and Engineering: C, 118 (2021).
[61] D. Alpaslan, T.E. Dudu, N. Aktas, Synthesis and characterization of novel organo-hydrogel based agar, glycerol and peppermint oil as a natural drug carrier/release material, Mater Sci Eng C Mater Biol Appl, 118 (2021) 111534.
[62] D. Alpaslan, T. Olak, A. Turan, T. Ersen Dudu, N. Aktas, A garlic oil-based organo-hydrogel for use in pH-sensitive drug release, Chem Zvesti, (2021) 1-14.
[63] B.-Y. Chang, S.-M. Park, Electrochemical impedance spectroscopy, Annual Review of Analytical Chemistry, 3 (2010) 207-229.
[64] Ö.F. ER, B. ULAŞ, H.D. KIVRAK, Remarkable bismuth-gold alloy decorated on MWCNT for glucose electrooxidation: the effect of bismuth promotion and optimization via response surface methodology, Turkish Journal of Chemistry, 45 (2021) 1173-1188.
[65] H. Kivrak, K. Selçuk, O.F. Er, N. Aktas, Electrochemical Cysteine Sensor on Novel Ruthenium Based Ternary Catalyst, (2021).
[66] O.F. Er, B. Ulas, O. Ozok, A. Kivrak, H. Kivrak, Design of 2-(4-(2-pentyllbenzo [b] thiophen-3-yl) benzylidene) malononitrile based remarkable organic catalyst towards hydrazine electrooxidation, Journal of Electroanalytical Chemistry, 888 (2021) 115218.

Highlights

- cacao oil-based organo-hydrogels are employed as sensor to detect CA-125 antigen.
- cacao oil-based organo-hydrogels are promising materials for CA-125 detection.
- Sensor has fairly wide linear range as 0.00083-41.5 U/mL and 83.0-2075 U/mL

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: