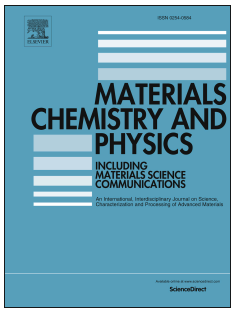

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CRediT authorship contribution statement

Hilal Kivrak: Conceptualization, Writing-original draft, Methodology, Review, Supervision. Omer Faruk Er: Visualization, Data curation, Review & editing. Omruye Ozok: Visualization, Investigation. Sabahattin Çelik: Visualization, Investigation. Arif Kivrak: Conceptualization, Methodology, Supervision.

Synthesis and characterization of 4-(2-(4-methoxyphenyl)benzo[b]thiophen-3-yl)benzaldehyde for carbohydrate antigen 125 electrochemical detection and molecular docking modeling

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ABSTRACT

4-(2-(4-methoxyphenyl)benzo[*b*]thiophen-3-yl)benzaldehyde (**MPBB**)-based chemical antibody is developed to detect CA125 (MUC-16) level more effectively via electrochemical methods. Novel **MPBB** is synthesized by using Pd-catalyst coupling reactions and electrophilic cyclization reactions. New benzothiophene structure is dispersed in Nafion solution and CA125 antigen was incubated on this electrode. Optimum conditions are found as 3 μL MPBB loading, 30 ng/mL CA125 antigen concentration, and 90 min incubation time. Two distinct linear ranges of the electrode prepared at optimum conditions are determined by DPV as 5-50 ng/ml and 100-500 ng/ml. Limit of detection and limit of quantification values have been obtained as 0.03385 ng/mL and 0.10155 ng/mL, respectively. Interference and artificial serum results reveal that this electrode is a promising electrode for CA125 antigen determination for ovarian cancer. Binding energy is investigated by employing a molecular docking modeling study between CA125 antigen and **MPBB**. Electrochemical results and molecular docking results revealed that the **MPBB** is a promising alternative to marketed CA125 immunoassays.

KEYWORDS: Cancer; Carbohydrate antigen 125; ovarium; benzothiophene; molecular docking; sensor

1. Introduction

Cancer is one of the leading causes of disease-related deaths worldwide. The reasons for the high loss of life from cancer are the lack of biomarkers providing early diagnosis and the inability to develop targeted therapies.[1-4] One of the main points of interest in cancer research is the discovery of biological markers that distinguish cancer cells from other cells. The biomarker can be defined as molecules that show a normal or abnormal process of change in blood or other body fluids. Biomarkers including proteins, enzymes, receptors, DNA,

antibody, RNA, and peptides molecules are often referred to as tumor markers. These altered molecules can be detected in secretions such as whole blood, plasma, serum, urine, nipple discharge, and saliva.[1, 2, 5-9] Carbohydrate markers (CAs) are released by the tumor cell or antigens on the tumor cell surface. They are more specific than naturally-released markers such as carbohydrate markers, enzymes, and hormones.[2, 10-13]

Ovarian cancer (OC) is the 4th most common cause of cancer-related deaths in women. Cancer antigen 125 (CA125) is a protein found in OC cells. The CA125 blood test measures the amount of 125 cancer antigens in the bloodstream. CA125 test can be used to monitor specific cancers. For example, the CA125 test may be used for the determination of the OC which has a very high risk for women. Normal values on the CA125 test are between 0-35 U/mL. When between 35-50 U/mL, the patient should be checked with surveillance. If the value is above 50 U/mL, OC is suspected and the necessary tests should be done. A variety of noncancerous conditions such as menstruation and uterine fibroids causes to increase in the CA125 level, so the CA125 test is not generally accurate enough to be used for OC screening. Moreover, some cancers can increase the CA125 levels like endometrial, peritoneal, and fallopian tube cancers. Despite this, CA125 remains the best standard for OC determination without new or additional markers. Employing CA125 has high importance to determine whether the treatment is sufficient or not and for the detection of disease recurrence.[1, 5, 7, 9, 14, 15]

CA125 level is usually measured by the CA125 Enzyme-linked Immunosorbent Assay (ELISA) method; an immunoenzymatic colorimetric method for the quantitative determination of tumoral antigen CA125 concentration in human serum or plasma. Electrochemical determination of CA125 could be most effective for the determination of CA125 in human serum or plasma due to the fact that electrochemical methods are fast and

save time.[16-31] There are several studies performed in literature for the determination of CA125 by electrochemical methods, compiled in **Table 1**.

Recently, the gold NP-zinc-oxide (ZnO)-based nanohybrid was fabricated for label-free CA125 determination and showed a limit of detection (2.5 ng/μL) with high reproducibility, specificity, and notable durability by Gasparotto et al.[19] In addition, Gazze et *al*.[32] reported a graphene-polyaniline-based biosensor for label-free recognition of CA125. This electrode was then functionalized with anti-CA125Ab via polyaniline with covalent cross-linking. The determination concentration of CA125 with this modified electrode was found as 0.923 ng/μL over the linear range of 0.92 pg/μL, 15.20 ng/μL. It was also found that the ferrocene carboxylic acid, HCl-doped polyaniline, chitosan hydrochloride composite, and Ag-Co_3O_4 nanosheets were used as an immunosensor with high antibody capacity. This study was improved in that it is good recovery percentage in the serum samples ensures the suitability of this sensor in the clinical diagnosis with acceptable stability and reproducibility. This biosensor displayed detection limit of 0.25 pg/mL over the concentration range of 0.001-25 ng/mL[33] (**Table 1**). Wang et al.[34] was prepared a 3D microfluidic immune device with molybdenum disulfide (MoS_2) as support for CA125 (Ab1), and the gold nanoflowers were used as the supporter of glucose, glucose oxidase (GOx), and CA125 secondary Ab (Ab2). Screen-printed electrodes were used for the preparation of the microfluidic system. While MoS_2 enhanced the reduction of H_2O_2, GOx catalyzed glucose oxidation. These microfluidic paper-based analytical devices have good sensitivity and low detection limit (0.36 pg/mL) with excellent linearity over the CA125 concentration range (0.001-50 ng/mL). Moreover, polymer-based immunosensors were designed and tested for the detection of CA125. For example, the combination of polymerization-aided signal amplification with μ-PAD was synthesized by using screen-printing methods.[35]

Table 1: Electrode materials employed for electrochemical determination of CA125.

OC biomarker	Sensor	Linear range	LOD or LOQ	Ref
CA125	Gold NP-ZnO nanorods	2.5 ng/μL-1 ng/μL	2.5 ng/μL	19
CA125	Graphene polyaniline	0.92 pg/μL-15.2 ng/μL	0.923 ng/μL	32
CA125	FA-HCl-doped polyaniline-chitosan and Ag-Co_3O_4 nanosheets	0.001-25 ng/mL	0.25 pg/mL	33
CA125	MoS_2-gold-nanoflowers	0.01-50 ng/mL	0.36 pg/mL	34
CA125	GO-chitosan-PGMA	0.05-100 ng/mL	0.05 ng/mL	35
CA125	Three dimensional gold electrode	1 fg/mL-1 μg/mL	1 pg/mL	36
CA 125	**GCE+Nafion+MPBB antibody**	**5-50 ng/ml and 100-500 ng/ml**	**0.03385 ng/mL (LOD) 0.10155 ng/mL (LOQ)**	**This work**

CA125 is a heterogenous mucin-like transmembrane glycoprotein, with a molecular weight range of 200-1000 kDa. The CA125 extracellular domain consists of numerous (>60) highly conserved tandem repeats, consisting of 157 amino acids and surrounded by glycosylated motifs.[37] To detect CA125 levels in human serum and plasma, antibodies are employed against CA125 to recognize two main epitope regions of OC 125 and M 11. These epitope regions are both localized inside tandem repeats. The first-generation immunoassays employed antibodies specific to the OC 125 region as capture monoclonal antibodies (MAb) and as the tracer. On the other hand, they noted that second-generation assays utilized antibodies against both M 11 epitope as a capture antibody and OC 125-related antibodies as the tracer.[38] Today, commercially available CA125 immunoassays have acceptable performance; however, inconsistencies can observe between test results for some samples because there are different antibodies in the assays. Novel antibodies may help to improve existing assays for the detection of cancer. In addition, utilization of locally produced antibodies may help to cost savings for cancer diagnostics. Thus, the antibody should have an amine layer that allows the subsequent surface functionalization and electron flow for the

detection of CA125. We developed a benzothiophene-based chemical antibody with amine groups to detect CA125 levels more effectively via electrochemical methods.

Heteroaromatic compounds including thiophenes, benzothiophenes, and indoles play very critical roles not only in medicine but also in material applications. Benzothiophene derivatives are well-known pharmacologically active compounds, as well as commercially available drugs such as raloxifene, used in the treatment of postmenopausal osteoporosis. They were also used as antimicrobial, antitumor, anti-inflammatory, and antihypertensive agents. Therefore, the synthesis of novel benzothiophene derivatives has high importance due to the discovery of new properties.[39-41]

In the present study, a modified biomarker sensor capable of detecting CA125 at low concentrations was developed. **MPBB** was synthesized by using Pd-catalyst coupling reactions and electrophilic cyclization reactions. The immunosensor was constructed by the modification of a glassy carbon electrode with **MPBB** as an antibody against CA125 for the detection of CA125. The sensor showed two distinct linear ranges as 5-50 ng/mL and 100-500 ng/ml. A limit of detection (LOD) of 0.03385 ng/mL was achieved, which is lower than other current biosensing techniques and delivers the level of sensitivity suitable for early OC detection and screening of at-risk individuals.

2. Experimental Section

2.1 Reagents and materials

HRMS, ^{1}H-NMR, and ^{13}C-NMR were used for the determination of isolated compounds. ^{1}H and ^{13}C NMR spectra were obtused for the ained by using an Agilent NMR (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from an internal trimethylsilane (TMS) reference. Thick-walled glass columns and ‘flash grade’ silica (Merck 230–400 mesh) were used for flash chromatography. A commercially-prepared 0.25

mm silica gel plate was applied for thin-layer chromatography (TLC). The mass analysis was performed by using Thermo Q Exactive LCMS/MS. All glassware was washed and dried in an oven prior to use. Carbonhyde Antigen 125 (CA125), Rabbit was obtained from ELISA Kit with ARP American Research Products brand. Nafion solution was purchased from Sigma-Aldrich.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) measurements were carried out using CHI 660E potentiostat/galvanostat system connected to a three-electrode system consisting of 3 mm diameter GCE as the working electrode, Ag/AgCl as a reference electrode, and a Pt wire as a counter electrode.

*2.3. Synthesis and characterization of 4-(2-(4-methoxyphenyl)benzo[b]thiophen-3-yl)benzaldehyde (**MPBB**)*

*2.3.1 Synthesis of (2-((4-methoxyphenyl)ethynyl)phenyl)(methyl)sulfane **3***

To a solution of the 2-iodothianisole **1** (7 mmol, 1.7 g) in tetrahydrofuran (THF) (10 mL), triethylamine (15 mL) and bis (triphenylphosphine) palladium (II) dichloride ($PdCl_2(PPh_3)_2$) (0.13 mmol, 98 mg) were added 4-ethynylanisole **2** (9.6 mmol, 1.1 g) and copper(I) iodide (CuI) (0.07 mmol, 13 mg) under argon atmosphere. Then, the mixture was stirred at room temperature for 3 hours. After the reaction was done, the reaction mixture was washed with saturated brine (NaCl) and extracted with ethyl acetate (EtOAc) (3x15 mL). The combined organic phase was dried with anhydrous magnesium sulfate ($MgSO_4$) and filtrate. The organic solvent was removed under reduced pressure and the residue purified by using flash column chromatography on silica gel Hexane/Ethyl acetate (100:1) to give (2-((4-

methoxyphenyl)ethynyl)phenyl)(methyl)sulfane **3** (88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.9 Hz, 2H), 7.47 (dd, *J* = 7.6; 1.5 Hz, 1H), 7.28 (td, *J* = 7.4; 1.5 Hz, 1H), 7.18 (dd, *J* = 8.1 Hz, 1H), 7.10 (td, *J* = 7.5; 1.2 Hz, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 3.82 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 141.3, 133.0, 132.0, 128.3, 124.2, 124.1, 121.7, 117.3, 113.9, 95.9, 85.6, 55.2, 15.0.

*2.3.2 Synthesis of 3-iodo-2-(4-methoxyphenyl)benzo[b]thiophene **4***

To a solution of the (2-((4-Methoxyphenyl)ethynyl)phenyl)(methyl)sulfane **3** (5.3 mmol, 1.35 g) and molecular iodine (10.6 mmol, 2696 mg) were stirred at room temperature for 1 hour in dichloromethane (15 mL). After the reaction was done, the mixture was washed with saturated sodium thiosulfate ($Na_2S_2O_3$) and extracted with dichloromethane (3x20 mL). The organic phase was dried over $MgSO_4$, filtered, and removed under reduced pressure. The residue was purified by using flash column chromatography on silica using Hexane/Ethyl acetate (50/1) to give 3-iodo-2-(4-methoxyphenyl)benzo[b]thiophene **4** (78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.74 (m, 2H), 7.68- 7.60 (m, 2H), 7.42 (d, *J* = 7.1 Hz, 2H), 7.04-6.98 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 142.1, 141.9, 138.7, 131.2, 126.8, 126.0, 125.3, 125.2, 122.0, 113.9, 78.9, 55.3.

*2.3.3 Synthesis of **MPBB***

The solution of 3-iodo-2-(4-methoxyphenyl)benzo[b]thiophene **4** (0.68 mmol, 250 mg) in methanol (MeOH) (4 mL) and tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) (0.13 mmol, 157 mg) was added 4-formylphenylboronic acid **5** (0.81 mmol, 122 mg) and lithium carbonate (Li_2CO_3) (1.3 mmol, 100 mg) under argon atmosphere at room temperature. The reaction was stirred at room temperature overnight. After the reaction was done, the reaction mixture was washed with brine (NaCl) and extracted with EtOAc (3x20 mL). The organic phase was dried

with anhydrous $MgSO_4$, filtered, and the organic solvent was removed under reduced pressure. The residue was purified by using flash column chromatography on silica gel Hexane/EtOAc (50:1) as the eluent to give **MPBB** (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.89-7.85 (m, 1H), 7.59-7.55 (m, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.40-7.33 (m, 2H), 7.21 (d, *J* = 8.9 Hz, 2H), 6.80 (d, *J* = 8.9 Hz, 2H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.8, 159.5, 142.4, 141.0, 140.1, 138.7, 135.1, 131.1, 130.9, 130.8, 130.0, 125.9, 124.7, 124.5, 122.6, 122.1, 114.0, 55.2. FT-IR: 2962.66, 2926.01, 2846.93, 2835.36, 2748.56, 1687.71, 1600.92, 1573.91, 1556.55, 1544.98, 1508.33, 1490.97, 1460.11, 1435.04, 1413.82, 1390.68, 1350.17, 1290.38, 1238.30, 1209.37, 1163.08, 1138.00, 1101.35, 1068.56, 1028.09, 1014.56, 767.67, 732.35. LCMS calculated $C_{22}H_{17}O_2S$ 345.0943 $[M+H]^+$, found 345.0943 $[M+H]^+$.

2.4 Fabrication of the immunosensor

GCE was polished using alumina (0.05, 0.3, and 1.0 mm) and then washed with deionized water and ultrasonicated in 1:1 deionized water and ethanol solution. Finally, the electrode was dried by nitrogen gas flow. Following this, 3 mg **MPBB** was dispersed in 1 mL of Nafion, an ink was obtained. 1-5 μL from this ink was transferred onto GCE and dried. Subsequently, different concentrations at 5-50000 ng/mL of CA125 antigen were incubated on the surface of the modified electrode at 37 ^{0}C for 5-30 min.

2.5 Electrochemical measurements

Electrochemical measurements were performed by CV, DPV, and EIS in 0.1 M PBS (pH=7.4) + 5.0 mM $Fe(CN)_6^{3-/4-}$ (PBS). Cyclic voltammograms were taken using the fabricated immunosensor electrodes in PBS solution at room temperature (scan rate=50 mV/s) on GCE, Nafion-modified GCE, Nafion + GCE + MPBB, and Nafion + GCE + MPBB +

CA125 at 5000 ng/mL CA125 antigen concentration. During the fabrication of the immunosensor, the surface of the electrode was exposed to different concentrations of CA125 antigen of 0.1-50000 ng/mL. To determine the effect of CA125 antigen concentration, CV measurements were also taken in PBS solution at room temperature (scan rate=50 mV/s). Optimum CA125 antigen concentration was obtained as 30 ng/mL and following this, the incubation time of CA125 antigen on the electrode for the construction of the immunosensor was investigated by varying time from 10-150 min. At optimum CA125 concentration of 30 ng/mL CA125 antigen and 90 min incubation time, the loading effect of the **MPBB** for the CA125 antigen determination was investigated with 1-5 μL **MPBB** loading in PBS. To determine the effect of scan rate on the CA125 antigen determination, cyclic voltammograms were taken on the electrode fabricated with 30 ng/mL CA-125 antigen at varying scan rates of 5-1000 mV/s. Furthermore, DPV measurements were taken on the fabricated immunosensor electrode consisting of 30 ng/mL CA125 antigen. The calibration plot of DPV curves was obtained by plotting the maximum current versus concentration values. From the slope of this calibration plot, the sensitivity of sensor was predicted.

In order to understand the CA125 electrooxidation process, EIS measurements were performed on the fabricated sensor with 3 μL **MPBB** antibody + 30 ng/mL CA125 antigen for 90 min incubation time at varying potentials.

For the interference study, CV (scan rate=50 mV/s) and EIS (0.2 V) measurements were taken on the fabricated immunosensor consisting of 30 ng/mL CA125 antigen, 3 μL **MPBB**, and prepared at 90 min incubation time. Interference of glucose (4.7 mM), uric acid (2.5 mM), dopamine (0.1 mM), ascorbic acid (0.1 mM), arginine (0.1 mM), glutamine (0.1 mM), asparagine (0.1 mM), hystidine (0.1 mM), D-L vanil (0.1 mM), glycine (0.1 mM), proline (0.1 mM), L-methionine (0.1 mM), leucine (0.1 mM), and serine (0.1 mM) in the absence and

presence of 30 ng/mL CA125 antigen and 30 ng/mL CA125 was determined by in PBS at room temperature.

The CA125 antigen-antibody interactions with the artificial serum sample and isotonic serum were measured by CV. For artificial serum, a solution containing 4.7 mM glucose, 2.5 mM urea, 0.1% BSA and 145 mM NaCl, 4.5 mM KCl, 5 mM $CaCl_2$, and 1.6 mM $MgCl_2$ was prepared. CV (scan rate=50 mV/s) and EIS (0.2 V) measurements for the fabricated sensor were performed at optimum conditions both in artificial serum and isotonic serum.

2.6 Molecular docking

The **MPBB** was drawn by using ChemDraw and energy minimization was examined using Avogadro and saved in CML format before converting to pdbqt via the OpenBabel program. The crystal structure of CA125 (MUC16) (PDB: 1IVZ) was downloaded from the Protein Data Bank (https://www.rcsb.org) and used as a target in docking studies (Model 1). The binding mode of the benzothiophene for CA125 was determined by AutoDock Vina 1.1.2. The analysis and visualization of the docking results were performed using PyMOL2.

3. Results and Discussion

Initially, compound **3** was prepared from 2-iodothioanisole via Sonogashira cross coupling reaction. When 2-iodothianisole was allowed to react with 4-ethynylanisole **2** in the presence of $PdCl_2(PPh_3)_2$ and CuI with a base, compound **3** was obtained with **88%** yields. Then, the electrophilic cyclization reaction (ECR) was used for the formation of compound **4**. In recent decades, ECRs have gained large importance due to many advantages such as; high regioselectivity, mild reaction conditions, and shorter reaction time. Compound **3** underwent a cyclization reaction with molecular iodine, and the desired intermediate **4** was formed (**78%** yield). After isolation of intermediate **4**, the Suzuki-Miyaura cross coupling reaction was applied for the synthesis of the final **MPBB** (**76%**) (**Fig. 1**).

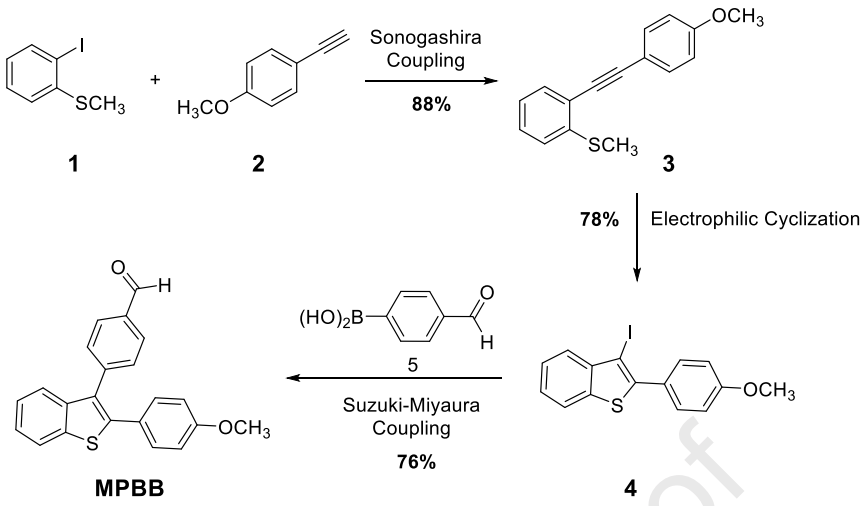


Figure 1: Synthesis of **MPBB**.

The **MPBB** was used as an antibody to detect CA125 antigen by electrochemical methods of CV, DPV, and EIS. CV measurements were performed on GCE, Nafion-modified GCE, Nafion + GCE + MPBB, and Nafion + GCE + MPBB + CA125 at 5000 ng/mL CA125 antigen concentration in PBS at room temperature (scan rate=50 mV/s). Results of these measurements are presented in **Figure 2.** One can note that cyclic voltammograms taken in PBS at room temperature (scan rate=50 mV/s) for Nafion-modified GCE electrode before and after CA125 immobilization have similar behavior. However, MPBB modified GCE electrode had significant effect on CA125 antigen determination. The current density values observed in forward scan at 0.25 V with 100 $\mu A/cm^2$ and backward scan at -0.25 V with 140 $\mu A/cm^2$ belongs to CA125 antigen peaks. These current density values are greater than the literature reported values.

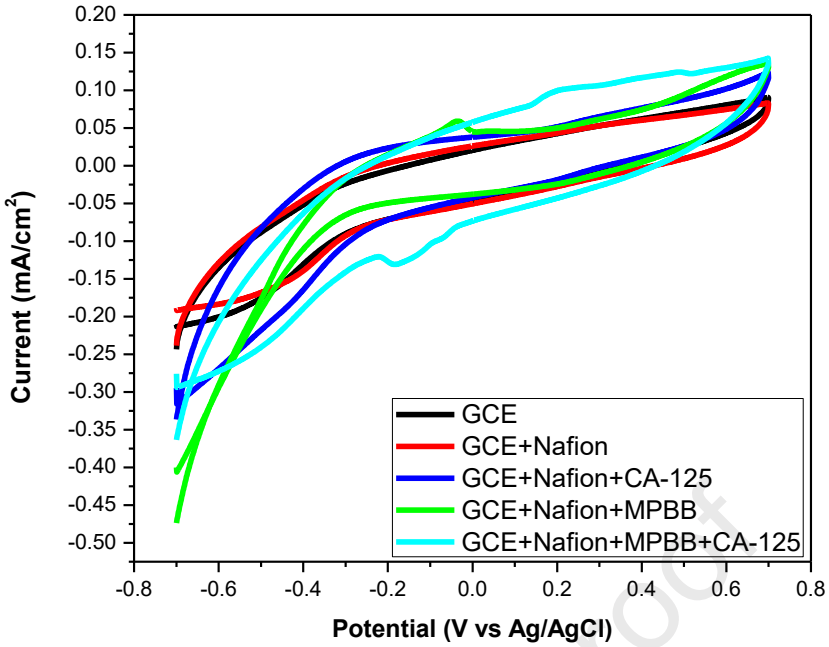


Figure 2: Cyclic voltammograms for GCE, Nafion-modified GCE, Nafion + GCE + MPBB, and Nafion + GCE + MPBB + CA-125 at 5000 ng/ml CA-125 antigen concentration in PBS at room temperature (scan rate=50 mV/s)

CV measurements were taken in PBS at room temperature (scan rate=50 mV/s) to investigate the effect of CA125 antigen concentration employed during the fabrication of the immunosensor, presented in **Figure 3.** For these measurements, different immunosensor electrodes were fabricated by using 3 μL + 0.1-50000 ng/mL CA125 antigen at 30 min incubation time. One can note that the current increased stepwise with successive additions of CA125 antigen, ascribed to the sensitive and rapid response of the electrodes to CA125 antigen oxidation. Results revealed that the immunosensor fabricated using 3 μL MPBB + 30 ng/mL CA125 antigen at 30 min incubation time has the highest current density.

Optimum CA125 antigen concentration was obtained as 30 ng/mL and following this, the incubation time of CA125 antigen on the electrode was investigated for construction of the immunosensor by varying time from 10-150 min. These measurements are given in **Figure 4**. Results reveal that 90 min incubation time is the optimum incubation time.

At optimum conditions, CA125 concentration of 30 ng/mL CA125 antigen and 90 min incubation time, the loading effect of the **MPBB** antibody on the CA125 antigen determination was investigated with 1-5 µL benzothiophene loading in PBS and these results are presented in **Figure 5.** The results indicate that the sensor fabricated with 3 µL **MPBB** antibody + 30 ng/mL CA125 antigen at 90 min incubation time had the highest current density.

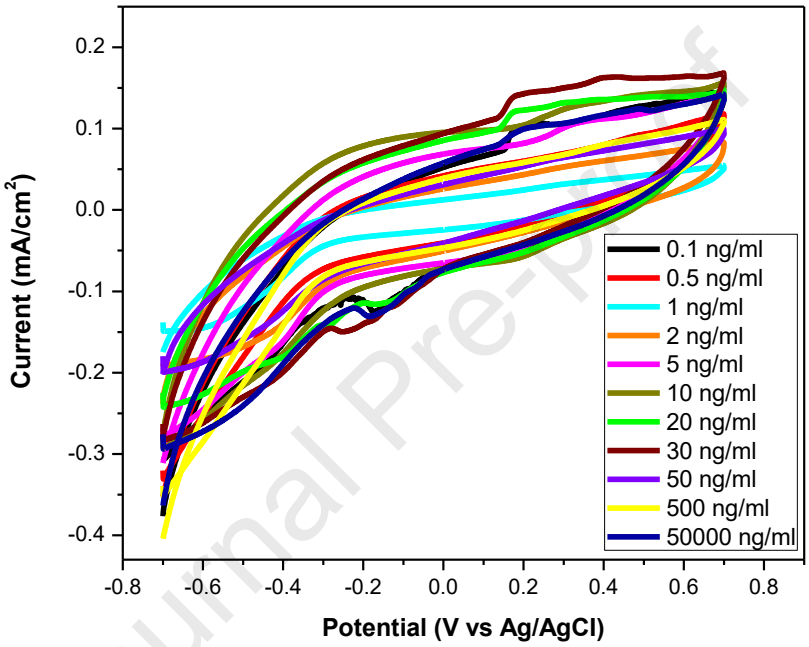


Figure 3: Cyclic voltammograms taken at immunosensor electrode fabricated with 3 µL MPBB antibody + 0.1-50000 ng/ml CA-125 antigen at 30 min incubation time in PBS at room temperature (scan rate=50 mV/s).

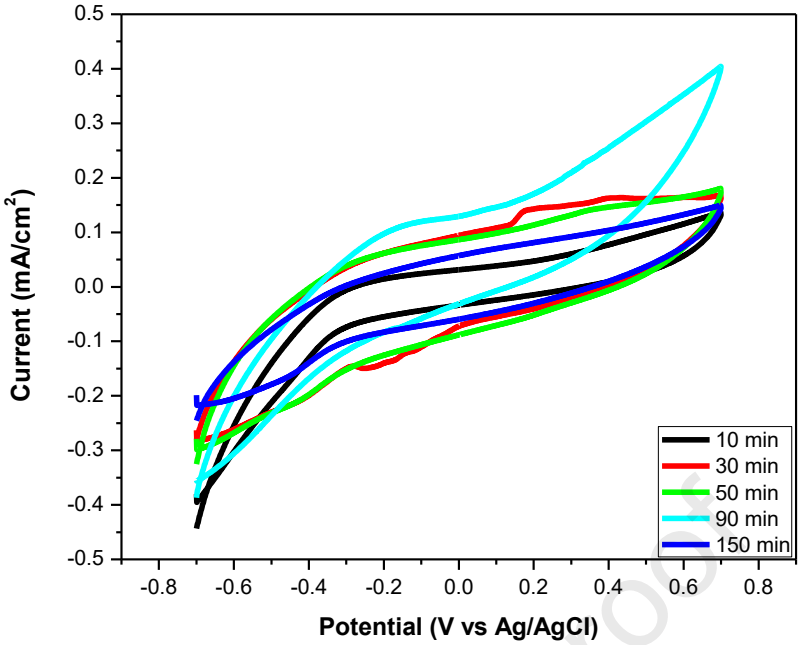


Figure 4: Cyclic voltammograms taken at immunosensor electrode fabricated with 3 µL MPBB antibody + 30 ng/ml CA-125 antigen at varying incubation times of 10-150 min in PBS at room temperature (scan rate=50 mV s/1).

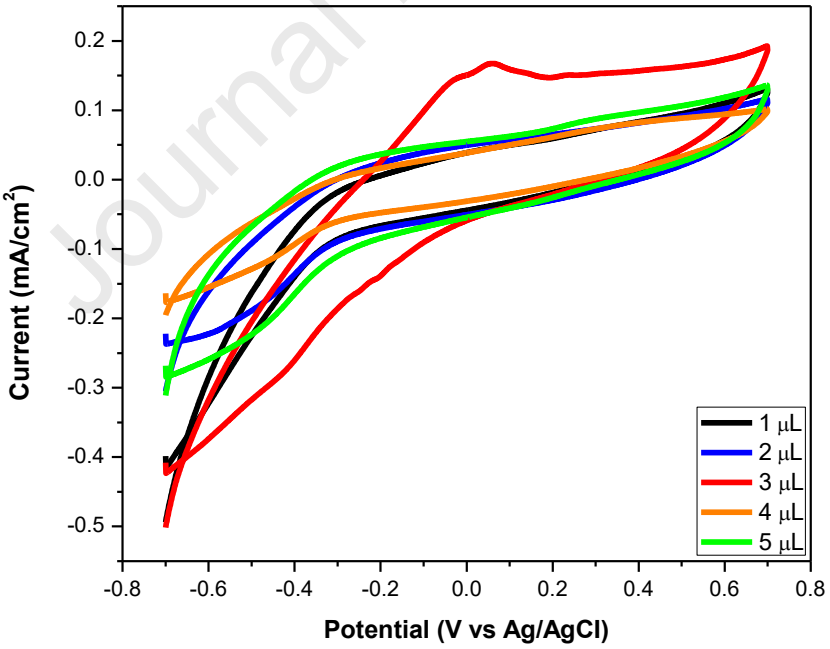


Figure 5: Cyclic voltammograms taken at immunosensor electrode fabricated with 1-5 µL MPBB antibody + 30 ng/ml CA-125 antigen at 90 min incubation time in PBS at room temperature (scan rate=50 mV/s).

The effect of scan rate on the electrocatalytic activity of CA125 antigen was investigated on the sensor fabricated with 3 μL **MPBB** antibody + 30 ng/ml CA125 antigen at 90 min incubation time. Results of these measurements are given in **Figure 6**. One can note that current density increased by increasing the scan rate, representing a good linear relationship. This phenomenon could be attributed to the fact that this reaction is controlled by diffusion.

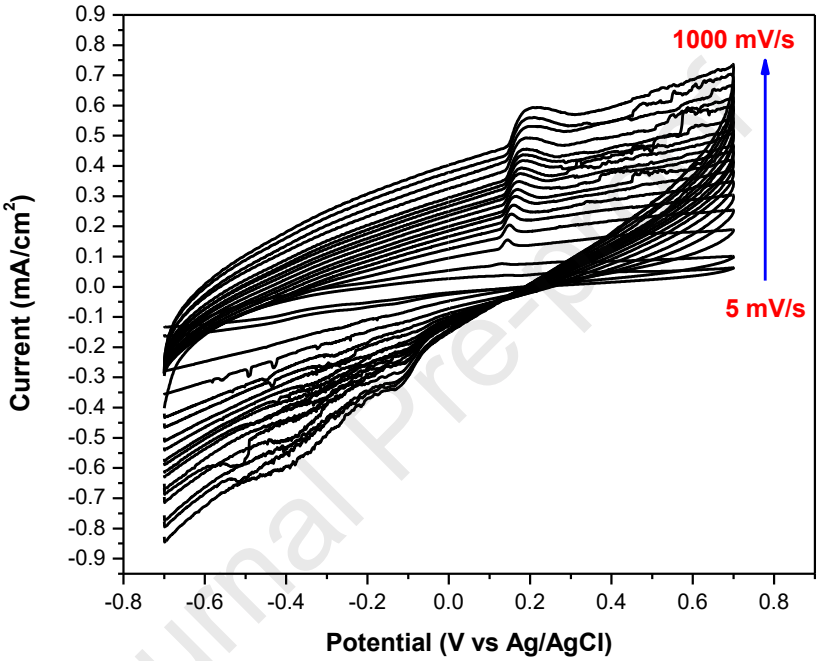
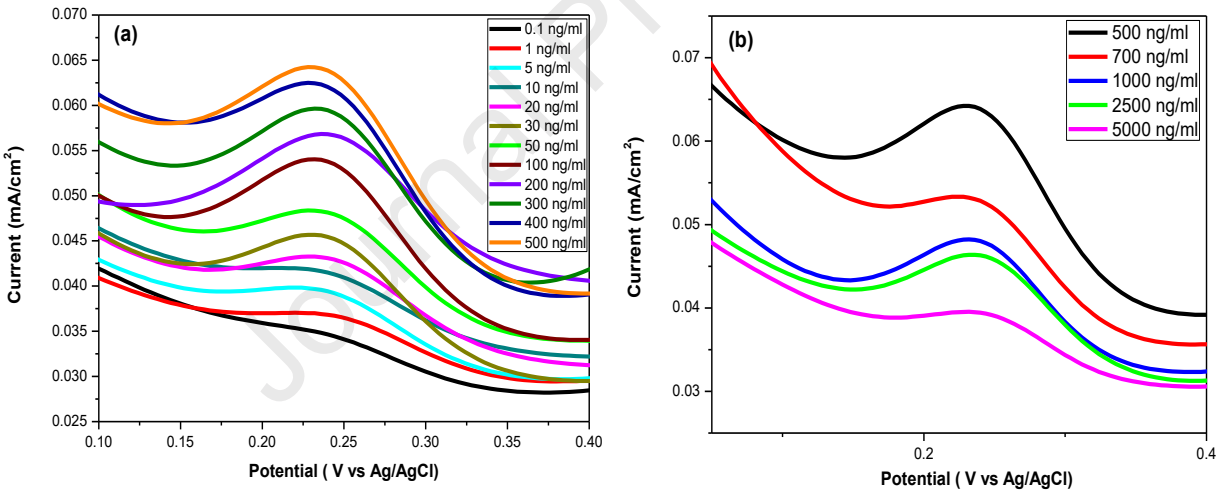


Figure 6: Cyclic voltammograms taken at immunosensor electrode fabricated with 3 μL MPBB antibody + 30 ng/ml CA-125 antigen at 90 min incubation time in PBS at room temperature (scan rate=5-1000 mV/s).

Sensitivity of the immunosensor electrode fabricated with 3 μL **MPBB** antibody + various concentrations of CA125 antigen at 90 min incubation time was determined by the DPV method in PBS. The DPV curves for these electrodes in 0.1 M PBS at varying concentrations (0.01-5000 ng/mL CA 125 antigen) are depicted in **Figure 7a-b**. The calibration plot of the DPV peak current densities versus concentration of CA125 antigen is also illustrated in **Figure 7c-e.** One can note that the DPV current densities versus CA125 antigen concentrations plot exhibited a linear relationship within the ranges of 5-50 ng/mL and 100-

500 ng/mL, and R^2 of these linear ranges were determined as 0.97 and 0.99. These linear ranges values are higher than those reported in the literature (**Table 1**).

Limit of Blank (LOB) and lowest detection limit (LOD) for CA125 antigen measured at acceptable statistical certainty and lowest concentration of analyte called the limit of quantification (LOQ) determined at acceptable sensitivity were calculated for the immunosensor electrode fabricated with 3 µL **MPBB** antibody. To determine LOB, 10 blank electrode responses without analyte were taken, and then the standard deviation of 10 blank electrode responses and these blank DPV measurements are presented in **Figure 8**. LOB, LOD, and LOQ were found as 0.01692, 0.03385, and 0.10155 ng/mL at (S/N=3), respectively. It is clear that the LOD of this sensor is lower than the sensors reported in literature (**Table 1**).



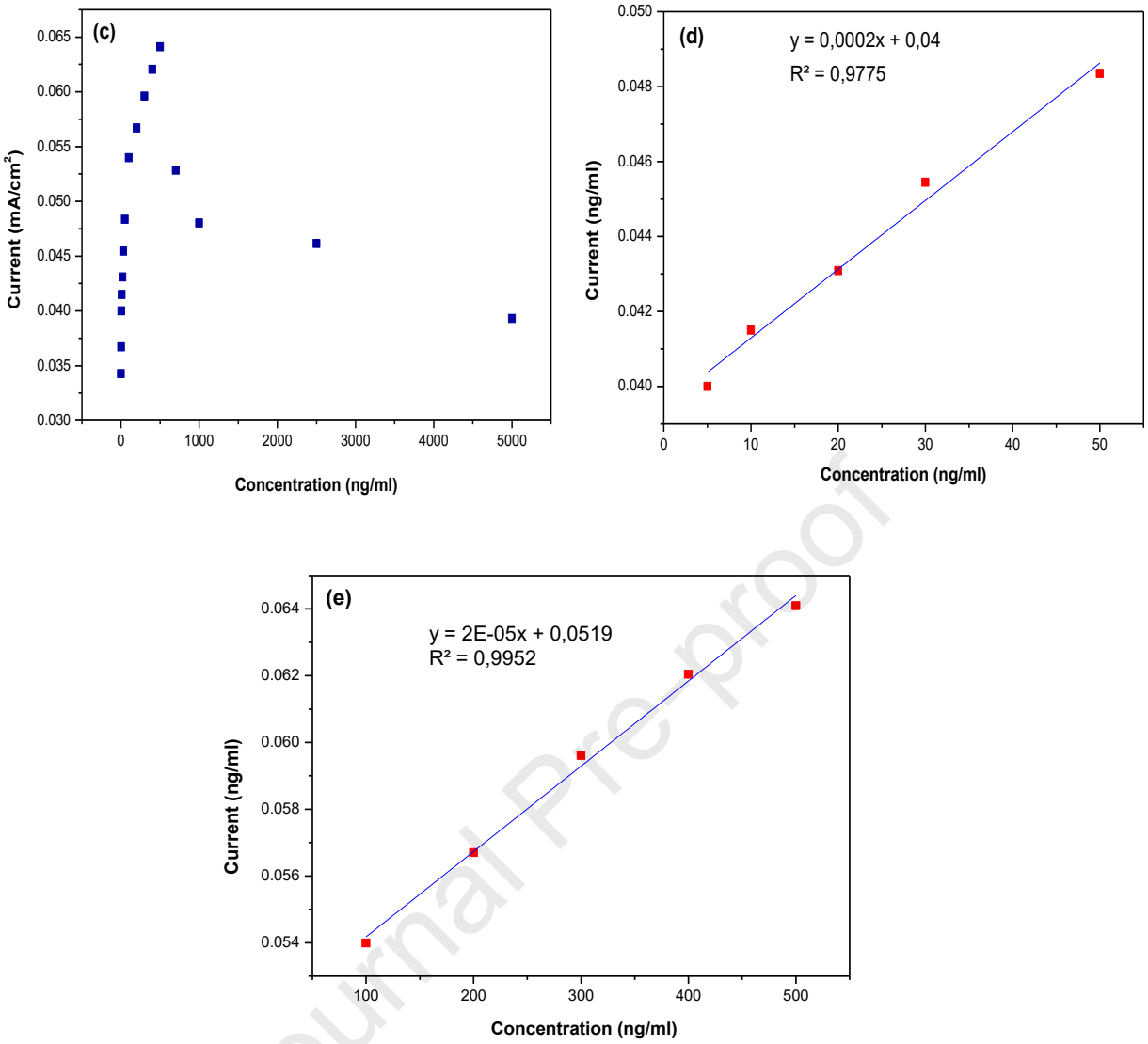


Figure 7: Differential pulse voltammetry measurements taken at immunosensor electrode fabricated at 90 min incubation time with 3 μL MPBB antibody and **(a)** 0.1-500 ng/ml CA-125, **(b)** 500-5000 ng/ml CA-125 in PBS at room temperature, and **(c, d, e)** maximum currents vs. CA125 concentration, insert: calibration plot obtained from differential pulse voltammetry measurements.

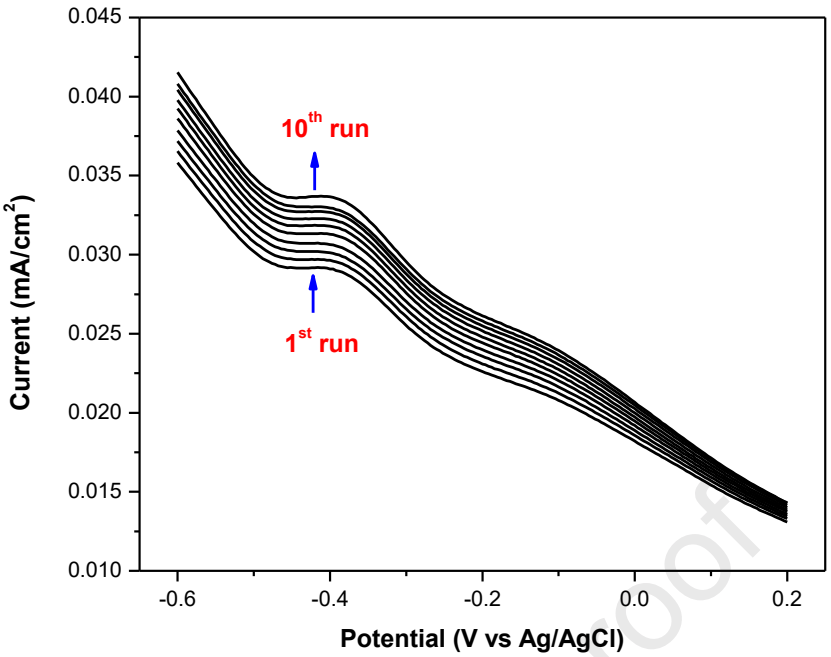


Figure 8: Differential pulse voltammetry measurements taken to measure 10 blank electrode responses without analyte at immunosensor electrode fabricated with 3 μL MPBB antibody in PBS at room temperature.

Impedance is a measure of the resistance to flow of an alternating current (AC). Impedance spectroscopy is a powerful measurement method used in many application areas such as electrochemistry, materials science, biology and medicine, the semiconductor industry, and sensors. Using complex impedance at various frequencies increases the information base that can be obtained during a measurement. It helps to distinguish the different effects contributing to a measurement, and, inaccessible quantities can be calculated with advanced mathematical methods. The Nyquist plot of the electrochemical impedance spectrum consists of a semi-circular area giving the charge transfer and linear sections corresponding to the controlled diffusion process. In order to understand the CA125 electrooxidation process, EIS measurements were performed on the sensor fabricated with 3 μL MPBB antibody + 30 ng/mL CA125 antigen at 90 min incubation time with various potentials. Results are given in **Figure 9.** The EIS results indicate that the sensor fabricated with 3 μL MPBB antibody + 30 ng/mL CA125 antigen at 90 min incubation time have

different EIS plots at various potentials. As mentioned above, the semicircular diameter of the impedance spectra is equal to the electron transfer resistance (Rct) and the large arc reveals that CA125 antigen electrooxidation is slow but a small arc is a sign of fast electro-oxidation kinetics of CA125 antigen electrooxidation. At **Figure 9**, one could note that the arc diameter decreases with increasing potential up to 0.2 V and then starts to increase, attributed to the fact that the resistance to charge transfer of CA-125 antigen electrooxidation decreases. At 0.2 V, the charge transfer is the lowest which could be related the fast oxidation kinetics of CA125 antigen electrooxidation. This result is in agreement with the CV and DPV results.

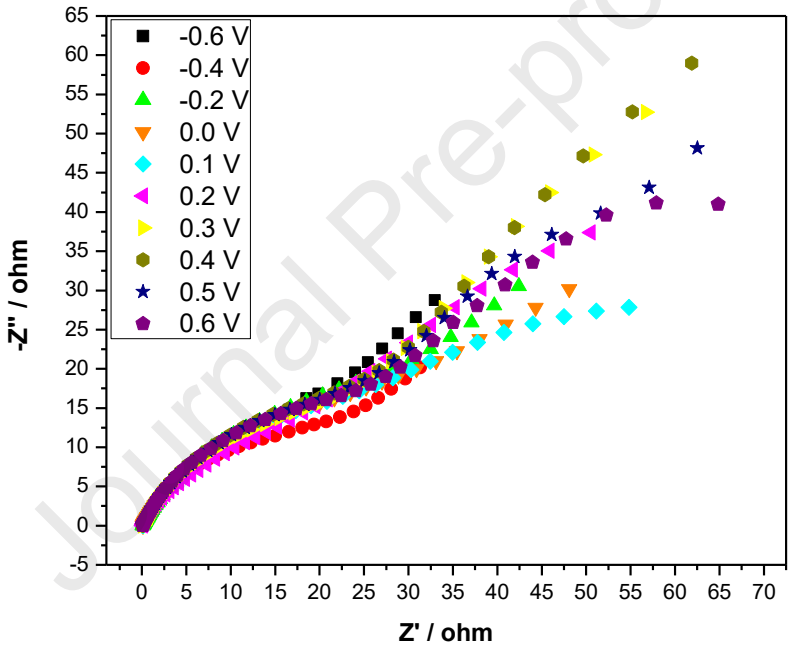


Figure 9: Electrochemical impedance spectra on sensor fabricated with 3 µL MPBB antibody + 30 ng/ml CA-125 antigen at 90 min incubation time at various potentials in PBS at the frequency range (0.02–100,000 Hz) with signal amplitude (10 mV).

The effects of some interfering species in blood samples on the determination of CA125 antigen were investigated on fabricated immunosensor consisting of 30 ng/mL CA125 antigen, 3 µL **MPBB** antibody, and prepared at 90 min incubation time. These selectivity measurements of the sensor were examined by CV and EIS. Interference of D-glucose (4.7

mM), uric acid (2.5 mM), dopamine (0.1 mM), and ascorbic acid (0.1 mM) in the absence and presence of 30 ng/ml CA-125 antigen was determined by CV in PBS at room temperature. These results are presented in **Figure S1a-d**. For CV, the response was measured through anodic peak current increase given in **Fig. S1a-d**. The charge transfer resistances (Rct) of D-glucose (4.7 mM), uric acid (2.5 mM), dopamine (0.1 mM), ascorbic acid (0.1 mM), arginine (0.1 mM), asparagine (0.1 mM), D-L vanil (0.1 mM), glutamine (0.1 mM), glycine (0.1 mM), hystidine (0.1 mM), L-methionine (0.1 mM), leucine (0.1 mM), proline (0.1 mM), and serine (0.1 mM) in the absence and presence of 30 ng/ml CA-125 antigen was determined by EIS at 0.2 potential in pH: 7.4 PBS. Results are presented in **Fig. S2** and **Fig. S3**. The interference effect of dopamine was greater than uric acid, glucose, and ascorbic acid. Similarly, impedance results of interfering species are given in **Figure S2a-d**. In addition, the charge transfer resistance in arginine solution was found to be higher in the presence of CA-125 according to in the absence of CA-125 (**Fig S3a**). However, the charge transfer resistances in asparagine, D-L vanil, glutamine, glycine, hystidine, leucine, proline, and serine solutions were observed to be low in the presence of CA-125 according to in the absence of CA-125 (**Fig. S3b-i**). Finally, it was seen that charge transfers were very close each other in L-methionine solution (**Fig. S3j**). EIS results are in generally agreement with results showing that CA125 antigen-modified electrode had the lowest charge transfer. In this context, it is clear that the current and impedance responses of interfering species could be ignored, revealing that the immunosensor fabricated with 30 ng/mL CA125 antigen, 3 μL **MPBB** antibody, and prepared at 90 min. incubation time has promising selectivity for CA125 antigen determination.

After obtaining sensitivity, LOD, and LOQ values and interference effects, artificial serum and isotonic serum measurements were performed. The antigen-antibody interactions with the artificial serum sample are affected by salts in the serum. Thus, artificial serum and

isotonic serum measurements were performed on the fabricated immunosensor consisting of 30 ng/mL CA125 antigen, 3 μL MPBB antibody, and prepared at 90 min incubation time and these results were compared with the results obtained in PBS by CV and EIS measurements and presented in **Figure 10**. A solution containing 4.7 mM (D+) glucose, 2.5 mM urea, 0.1% BSA and 145 mM NaCl, 4.5 mM KCl, 5 mM $CaCl_2$, and 1.6 mM $MgCl_2$ was prepared. CV and EIS results reveal that antibody and CA125 antigen dilutions with artificial serum and isotonic serum samples do not interact with the salts in the serum.

Electrochemical results revealed that **MPBB** antibody is a promising antibody with good sensitivity and LOD and LOQ values were obtained that are higher than the literature values given in Table 1. This phenomenon could be attributed to the fact that **MPBB** antibody and CA125 antigen were bonded like antibody and antigen pairs. CA125 is composed of 157 amino acids and surrounded by highly glycosylated motifs. In order to understand how CA125 antigen and **MPBB** antibody bind to each other and the amino acid part they interact through, molecular docking modelling was performed. Molecular docking results showed that **MPBB** had more potent activity for CA125. In-silico studies showed that binding energy between **MPBB** and CA125 is -6.16 kcal/mol (**Figure 11**). Moreover, estimated inhibition constant (Ki) was found as 30.28 uM (micromolar) at 298.15 K. According to the analysis of docking results, the interactions between CA-125 and binding sites are highly consistent with that of **MPBB** which formed hydrogen bonds with HIS13 (histidine).

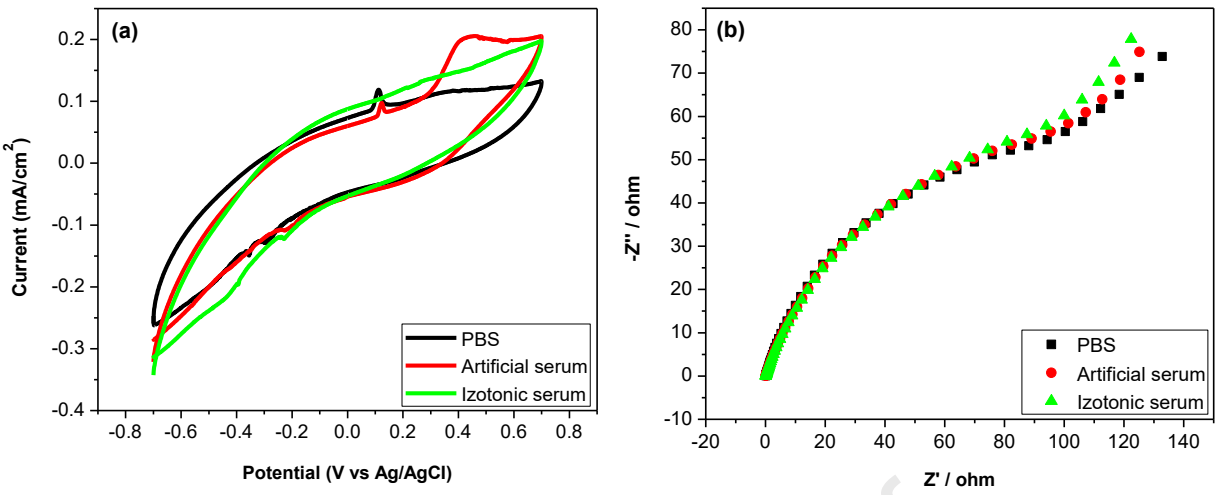


Figure 10: Comparison of MPBB antibody and CA-125 antigen dilutions with PBS, artificial serum, and isotonic serum with (a) CV and (b) EIS at 0.2 V.

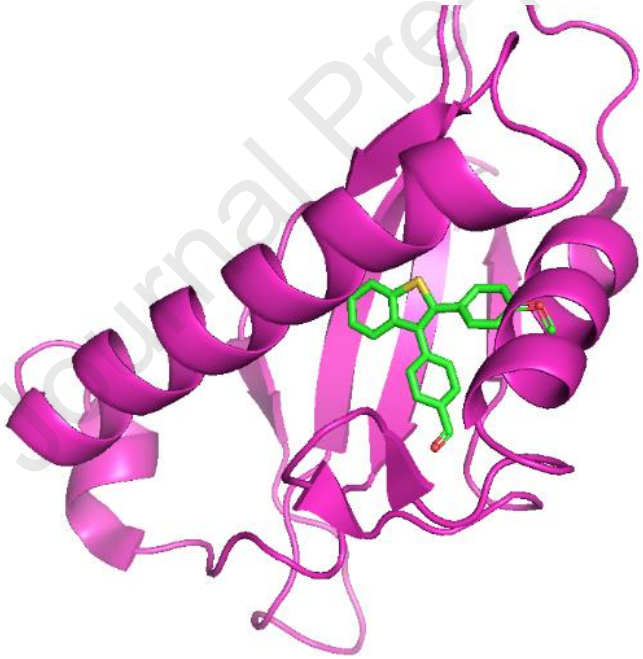


Figure 11: Amino acid residues involved in benzothiophene **6** and target complexes with binding affinity for the best docking positions.

4. Conclusions

In the present study, **MPBB** benzothiophene derivative was synthesized via by using Pd-catalyst coupling reactions and electrophilic cyclization reactions. **MPBB** was employed as a

chemical antibody which could be an alternative to currently-marketed CA125 immunoassays showing acceptable performance though discrepancies between assay results were observed for some samples. To detect CA125 (MUC-16) level via electrochemical methods against **MPBB** antibody, electrochemical methods such as CV, DPV, and EIS were performed. To construct an electrode from **MPBB** antibody, firstly, this new **MPBB** structure was dispersed in Nafion solution and CA125 antigen was incubated on this electrode. Electrochemical parameters affecting the sensitivity of the sensor such as CA125 antigen concentration, **MPBB** loading, incubation time, and scan rate were examined. Results revealed that optimum conditions were found as 3 μL **MPBB** loading, 30 ng/mL CA125 antigen concentration, and 90 min incubation time. By employing the DPV technique, two distinct linear ranges of the electrodes prepared under optimum conditions were determined as 5-50 ng/mL and 100-500 ng/mL. Interference and artificial serum results reveal that this electrode is a promising electrode for CA125 antigen determination for the OC. According to the electrochemical results, **MPBB** antibody is a promising antibody and good sensitivity, LOD, and LOQ values are better than the literature values, ascribed to the fact that **MPBB** antibody and CA125 antigen are bonded like antibody and antigen pairs. To understand how CA125 antigen and **MPBB** antibody bind, molecular docking results also illustrated that MPBB had more potent activity for CA125 with the binding energy of -6.16 kcal/mol. The estimated inhibition constant (Ki) was 30.28 μM (micromolar) at 298.15 K from the HIS13 (histidine) amino acid part. In conclusion, it is clear that **MPBB** is a promising antibody to detect CA125 (MUC-16) levels via electrochemical methods.

Author Contributions

We have no conflict of interest to declare.

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Abbreviations

MPBB, 4-(2-(4-methoxyphenyl)benzo[*b*]thiophen-3-yl)benzaldehyde; ELISA, Enzyme-linked Immunosorbent Assay; DPV, differential pulse voltammetry; OC, Ovarian cancer; CA125, Cancer antigen 125; LOD, A limit of detection; HRMS, High Resolution Mass Spectrometry; EIS, electrochemical impedance spectroscopy; GCE, Glassy carbon electrode.

References

(1) Duffy, M. J. Clinical uses of tumor markers: A critical review. *Critical Reviews in Clinical Laboratory Sciences* **2001,** *38* (3), 225-262, DOI: 10.1080/20014091084218.

(2) Han, S. N.; Lotgerink, A.; Gziri, M. M.; Van Calsteren, K.; Hanssens, M.; Amant, F. Physiologic variations of serum tumor markers in gynecological malignancies during pregnancy: a systematic review. *Bmc Medicine* **2012,** *10*, 86, DOI: 10.1186/1741-7015-10-86.

(3) Aras, A.; Kiziltan, R.; Batur, A.; Celik, S.; Yilmaz, O.; Kotan, C. Colon cancer presented with sigmoid volvulus: A case report. *International Journal of Surgery Case Reports* **2015,** *17*, 16-18, DOI: 10.1016/j.ijscr.2015.10.021.

(4) Celik, S.; Yjlmaz, E. M.; Ozden, F.; Kotan, C.; Okut, H. The Relationship between Eating and Lifestyle Habits and Cancer in Van Lake Region: Another Endemic Region for Esophageal and Gastric Cancers. *Journal of Cancer Epidemiology* **2015,** *2015*, 7, DOI: 10.1155/2015/254823.

(5) Schwartz, M. K. A pragmatic review of tumor markers risk assessment analysis (screening). *Journal of Clinical Ligand Assay* **1997,** *20* (1), 152-154, WOS: A1997WN39100030.

(6) Duffy, M. J. Role of tumor markers in patients with solid cancers: A critical review. *European Journal of Internal Medicine* **2007,** *18* (3), 175-184, DOI: 10.1016/j.ejim.2006.12.001.

(7) Husseinzadeh, N. Status of tumor markers in epithelial ovarian cancer has there been any progress? A review. *Gynecologic Oncology* **2011,** *120* (1), 152-157, DOI: 10.1016/j.ygyno.2010.09.002.

(8) Duffy, M. J. Tumor Markers in Clinical Practice: A Review Focusing on Common Solid Cancers. *Medical Principles and Practice* **2013,** *22* (1), 4-11, DOI: 10.1159/000338393.

(9) Li, H. J.; Qin, J. Q.; Li, M. J.; Li, C. P.; Xu, S.; Qian, L. R.; Yang, B. H. Gold-nanoparticle-decorated boron-doped graphene/BDD electrode for tumor marker sensor. *Sensors and Actuators B-Chemical* **2020,** *302*, 127209, DOI: 10.1016/j.snb.2019.127209.

(10) Faggiano, P.; D'Aloia, A.; Antonini-Canterin, F.; Vizzardi, E.; Nicolosi, G. L.; Cas, L. D. Tumour markers in chronic heart failure. Review of the literature and clinical implications. *Journal of Cardiovascular Medicine* **2006,** *7* (8), 573-579, DOI: 10.2459/01.jcm.0000237903.95882.06.

(11) Fechner, E.; Smeets, E.; Schrauwen, P.; Mensink, R. P. The Effects of Different Degrees of Carbohydrate Restriction and Carbohydrate Replacement on Cardiometabolic Risk Markers in Humans-A Systematic Review and Meta-Analysis. *Nutrients* **2020,** *12* (4), 991, DOI: 10.3390/nu12040991.

(12) McCullough, D.; Harrison, T.; Lane, K.; Boddy, L.; Amirabdollahian, F.; Schmidt, M.; Enright, K.; Stewart, C.; Davies, I. The effect of a low carbohydrate high fat diet on emerging biochemical markers of cardiometabolic risk. *Proceedings of the Nutrition Society* **2020,** *79* (OCE2), E530-E530, DOI: 10.1017/s0029665120004796.

(13) Xu, X.; Ji, J. R.; Chen, P. P.; Wu, J. F.; Jin, Y.; Zhang, L. Y.; Du, S. H. 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* **2020,** *1125*, 41-49, DOI: 10.1016/j.aca.2020.05.027.

(14) Gadducci, A.; Tana, R.; Cosio, S.; Genazzani, A. R. The serum assay of tumour markers in the prognostic evaluation, treatment monitoring and follow-up of patients with cervical cancer: A review of the literature. *Critical Reviews in Oncology Hematology* **2008,** *66* (1), 10-20, DOI: 10.1016/j.critrevonc.2007.09.002.

(15) Majd, S. M.; Salimi, A. Ultrasensitive flexible FET-type aptasensor for CA 125 cancer marker detection based on carboxylated multiwalled carbon nanotubes immobilized onto reduced graphene oxide film. *Analytica Chimica Acta* **2018,** *1000*, 273-282, DOI: 10.1016/j.aca.2017.11.008.

(16) Maillard, F.; Eikerling, M.; Cherstiouk, O. V.; Schreier, S.; Savinova, E.; Stimming, U. Size effects on reactivity of Pt nanoparticles in CO monolayer oxidation: The role of surface mobility. *Faraday Discussions* **2004,** *125*, 357-377, DOI: 10.1039/b303911k.

(17) Wu, L. N.; Chen, J.; Du, D.; Ju, H. X. Electrochemical immunoassay for CA 125 based on cellulose acetate stabilized antigen/colloidal gold nanoparticles membrane. *Electrochimica Acta* **2006,** *51* (7), 1208-1214, DOI: 10.1016/j.electacta.2005.06.011.

(18) Wei, S. L.; Wu, D. C.; Shang, X. L.; Fu, R. W. Studies on the Structure and Electrochemical Performance of Pt/Carbon Aerogel Catalyst for Direct Methanol Fuel Cells. *Energy & Fuels* **2009,** *23* (1), 908-911, DOI: 10.1021/ef8006432.

(19) Gasparotto, G.; Costa, J. P. C.; Costa, P. I.; Zaghete, M. A.; Mazon, T. Electrochemical immunosensor based on ZnO nanorods-Au nanoparticles nanohybrids for ovarian cancer antigen CA-125 detection. *Materials Science & Engineering C-Materials for Biological Applications* **2017,** *76*, 1240-1247, DOI: 10.1016/j.msec.2017.02.031.

(20) Liang, X. Y.; Han, H. L.; Ma, Z. F. pH responsive amperometric immunoassay for carcinoma antigen 125 based on hollow polydopamine encapsulating methylene blue. *Sensors and Actuators B-Chemical* **2019,** *290*, 625-630, DOI: 10.1016/j.snb.2019.04.030.

(21) Rebelo, T.; Costa, R.; Brandao, A.; Silva, A. F.; Sales, M. G. E.; Pereira, C. M. Molecularly imprinted polymer SPE sensor for analysis of CA-125 on serum. *Analytica Chimica Acta* **2019,** *1082*, 126-135, DOI: 10.1016/j.aca.2019.07.050.

(22) Senocak, A.; Khataee, A.; Demirbas, E.; Doustkhah, E. Ultrasensitive detection of rutin antioxidant through a magnetic micro-mesoporous graphitized carbon wrapped Co nanoarchitecture. *Sensors and Actuators B-Chemical* **2020,** *312*, 127939, DOI: 10.1016/j.snb.2020.127939.

(23) Kivrak, H.; Alal, O.; Atbas, D. Efficient and rapid microwave-assisted route to synthesize Pt-MnOx hydrogen peroxide sensor. *Electrochimica Acta* **2015,** *176*, 497-503, DOI: 10.1016/j.electacta.2015.06.151.

(24) Kazici, H. C.; Salman, F.; Kivrak, H. D. Synthesis of Pd-Ni/C bimetallic materials and their application in non-enzymatic hydrogen peroxide detection. *Materials Science-Poland* **2017,** *35* (3), 660-666, DOI: 10.1515/msp-2017-0072.

(25) Kazici, H. C.; Caglar, A.; Aydogmus, T.; Aktas, N.; Kivrak, H. Microstructured prealloyed Titanium-Nickel powder as a novel nonenzymatic hydrogen peroxide sensor. *Journal of Colloid and Interface Science* **2018,** *530*, 353-360, DOI: 10.1016/j.jcis.2018.06.079.

(26) Alal, O.; Caglar, A.; Kivrak, H.; Sahin, O. Dendrimer Templated Synthesis of Carbon Nanotube Supported PdAu Catalyst and its Application as Hydrogen Peroxide Sensor. *Electroanalysis* **2019,** *31* (9), 1646-1655, DOI: 10.1002/elan.201900140.

(27) Caglar, A.; Kazici, H. C.; Alpaslan, D.; Yilmaz, Y.; Kivrak, H.; Aktas, N. 3-Acrylamidopropyl-trimethylammoniumchloride cationic hydrogel modified graphite electrode and its superior sensitivity to hydrogen peroxide. *Fullerenes Nanotubes and Carbon Nanostructures* **2019,** *27* (9), 736-745, DOI: 10.1080/1536383x.2019.1634056.

(28) Kazici, H. C.; Yayla, M.; Ulas, B.; Aktas, N.; Kivrak, H. Development of Nonenzymatic Benzoic Acid Detection on PdSn/GCE/Vulcan XC-72R Prepared via Polyol Method. *Electroanalysis* **2019,** *31* (6), 1118-1124, DOI: 10.1002/elan.201900088.

(29) Kivrak, H. D.; Aktas, N.; Caglar, A. Electrochemical production of Graphene Oxide and its application as a novel Hydrogen Peroxide sensor. *International Journal of Nano Dimension* **2019,** *10* (3), 252-259, DOI: 20.1001.1.20088868.2019.10.3.3.8.

(30) Ertas, N. A.; Kavak, E.; Salman, F.; Kazici, H. C.; Kivrak, H.; Kivrak, A. Synthesis of Ferrocene Based Naphthoquinones and its Application as Novel Non-enzymatic Hydrogen Peroxide. *Electroanalysis* **2020,** *32* (6), 1178-1185, DOI: 10.1002/elan.201900715.

(31) Sha, R.; Badhulika, S. Recent advancements in fabrication of nanomaterial based biosensors for diagnosis of ovarian cancer: a comprehensive review. *Microchimica Acta* **2020,** *187* (3), 181, DOI: 10.1007/s00604-020-4152-8.

(32) Gazze, A.; Ademefun, R.; Conlan, R.S.; Teixeira, S.R. Electrochemical impedence spectroscopy enabled CA125 detection; toward early ovarian cancer diagnosis using graphene biosensors. *Journal of Interdisciplinary Nanomedicine* **2018**, 3, 82-88, DOI: https://doi.org/10.1002/jin2.40.

(33) Ren, X.; Wang, H.; Wu, D.; Fan, D. W.; Zhang, Y.; Du, B.; Wei, Q. Ultrasensitive immunoassay for CA125 detection using acid site compound as signal and enhancer. *Talanta* **2015,** *144*, 535-541, DOI: 10.1016/j.talanta.2015.06.086.

(34) Wang, X.; Deng, W. P.; Shen, L.; Yan, M.; Yu, J. H. A 3D electrochemical immunodevice based on an Au paper electrode and using Au nanoflowers for amplification. *New Journal of Chemistry* **2016,** *40* (3), 2835-2842, DOI: 10.1039/c5nj03222a.

(35) Wu, Y. F.; Xue, P.; Hui, K. M.; Rang, Y. J. A paper-based microfluidic electrochemical immunodevice integrated with amplification-by-polymerization for the ultrasensitive multiplexed detection of cancer biomarkers. *Biosensors & Bioelectronics* **2014,** *52*, 180-187, DOI: 10.1016/j.bios.2013.08.039.

(36) Rafique, S.; Tabassum, S.; Akram, R. Sensitive competitive label-free electrochemical immunosensor for primal detection of ovarian cancer. *Chemical Papers* **2020,** *74* (8), 2591-2603, DOI: 10.1007/s11696-020-01100-w.

(37) Wang, M.; Hu, M.; Li, Z.; He, L.; Song, Y.; Jia, Q.; Zhang, Z.; Du, M. Construction of Tb-MOF-on-Fe-MOF conjugate as a novel platform for ultrasensitive detection of carbohydrate antigen 125 and living cancer cells. *Biosensors and Bioelectronics* ***2019***, 142, 111536, DOI: https://doi.org/10.1016/j.bios.2019.111536.

(38) K. Nustad, R. Bast Jr, T. O'brien, O. Nilsson, P. Seguin, M. Suresh, T. Saga, S. Nozawa, O. Børmer, H. De Bruijn, Specificity and affinity of 26 monoclonal antibodies against the CA 125 antigen: first report from the ISOBM TD-1 workshop. *Tumor Biology* **1996**, 17, 196-219. DOI: https://doi.org/10.1159/000217982.

(39) Zora, M.; Kivrak, A.; Yazici, C. Synthesis of Pyrazoles via Electrophilic Cyclization. *Journal of Organic Chemistry* **2011,** *76* (16), 6726-6742, DOI: 10.1021/jo201119e.

(40) Kivrak, A.; Larock, R. C. Synthesis of Dihydrobenzisoxazoles by the 3+2 Cycloaddition of Arynes and Oxaziridines. *Journal of Organic Chemistry* **2010,** *75* (21), 7381-7387, DOI: 10.1021/jo101656c.

(41) Kivrak, A.; Zora, M. A novel synthesis of 1,2,4-oxadiazoles and isoxazoles. *Tetrahedron* **2014,** *70* (4), 817-831, DOI: 10.1016/j.tet.2013.12.043.

Highlights

- MPBB, which is a benzaldehyde derivative, is employed as antibody to detect CA125 antigen in serum medium.
- Electrochemical results show that MPBB is a promising antibody for the CA-125 antigen.
- Sensor have two distinct linear ranges as 5-50 ng/ml and 100-500 ng/ml with 0.03385 ng/mL LOD and 0.10155 ng/mL LOQ.

Declaration of Competing Interest

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.