Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *88* (5) 521–536 (2023)
JSCS–5643
Original scientific paper
Published 21 May 2023

Designing an electrochemical biosensor based on tyrosinase for highly sensitive and rapid detection of bisphenol A and its derivatives

ONUR CAN BODUR[1], MERVE KESKIN[2]*, BAŞAK ARSLAN AVAN[3] and HALIT ARSLAN[1]

[1]*Department of Chemistry, Faculty of Science, Gazi University, Ankara, Republic of Türkiye,* [2]*Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Republic of Türkiye and* [3]*Department of Orthodontics, Faculty of Dentistry, Gazi University, Ankara, Republic of Türkiye*

(Received 22 November 2022, revised 13 February, accepted 21 March 2023)

Abstract: Bisphenol A (BPA) is a monomer commonly used in the production of epoxy resins, plastic bottles and dental filling materials. Due to its chemical structure, BPA and its derivates show activity similar to the endocrine hormones. It can bind to estrogen receptors and cause neurological disturbances, even at low doses. Therefore, it is important to determine BPA and its derivatives quickly and sensitively at low concentrations. In this study, a single amperometric tyrosinase enzyme biosensor was designed for the determination of the amount of BPA, bisphenol F (BPF) and bisphenol S (BPS) monomers. Tyrosinase was immobilized onto a modified carbon paste electrode by cross-linking with glutaraldehyde. The amount of BPA (BPS and BPF) was determined directly on the reduction of quinone compound released as a result of the enzymatic reaction at –0.15V. $K_{m(app)}$ value of the designed biosensor for BPA was found 0.00067 μM, the linear operating range was 0.001–0.005 μM (a) and 0.03–0.1 μM (b) and the lower detection limit was found 1 nM for each monomer. It is clear that designed biosensor enable the fast, efficient and precise determination of BPA and its derivatives released from materials used in dental materials.

Keywords: BPA; BPF; BPS; tyrosinase enzyme; biosensor; estrogen.

INTRODUCTION

Bisphenol A (2,2-bis(4-hydroxyphenyl) propane, 4,4′-isopropylidenediphenol, BPA) is an important industrial chemical used as a basic component in the production of epoxy resins and polycarbonate plastics, which have wide applications in industry. It is frequently used in plastic products in our daily lives such

* Corresponding author. E-mail: merveozdemirkeskin@gmail.com
https://doi.org/10.2298/JSC221122013B

as large returnable, refillable water bottles, food service products, feeding bottles, jugs, glasses, household food containers. In addition, BPA is an important component of bisphenol A glycidylmethacrylate (Bis-GMA), a molecule known to be the basis of composite resins used in dentistry.[1,2] In the international dentistry literature, it is stated that BPA and/or its derivatives can be released into the oral cavity from composite fillings and fissure sealants at doses that can produce active substance.

BPA shows activity similar to endocrine hormones due to its chemical structure. In other words, the emphasis that estradiol and diethylstilbestrol, due to the presence of phenol groups in their structures, BPA and its derivatives have similar effects by binding to estrogen receptors, and that they can cause neurological disorders even at low doses worries researchers.[3] For this reason, it is important to determine BPA and its derivatives (bisphenol S, BPS, bisphenol F, BPF, *etc.*) quickly and sensitively at low concentrations.

Due to the negative effects of BPA and its derivatives on human health, a number of methods have been developed to determine its amount in the liquids which comes into contact with BPA containing products.[4] The BPA derivates BPF (4,4′-dihydroxydiphenylmethane) and BPS (4,4′-sulfonyldiphenol) also have estrogenic, progesteronic and anti-androgenic effects and are used in production of daily life products such as food cans, plastic bottles, *etc*.

However, most of methods to determine BPA and its derivates are based on spectrophotometry and/or chromatography, requiring time-consuming sample preparation and pre-analysis processes. In addition, these methods depend on large capital investment causing high operating costs. Moreover, the analysis with these methods require qualified person and interpretation of analysis results is time-consuming.[5,6] For this reason, many research groups are working on developing fast, sensitive, innovative and relatively cheaper methods for the analysis of BPA and its derivatives. Biosensor could be one of the innovative methods used in BPA determination. Biosensor is defined as a device combined with a biological agent and a physicochemical converter.

In this study, a new biosensor modified with Fe_3O_4 nanoparticle was designed to determine the released amounts of BPA and its derivatives contained in some orthodontic materials. For this purpose, carbon paste electrode was modified by using commercial Fe_3O_4 nanoparticle. Tyrosinase enzyme was immobilized onto modified electrode by cross-linking with bovine serum albumin (BSA) and glutaraldehyde. Optimum operating conditions of the biosensor as temperature, pH, glutaraldehyde amount and substrate concentration were studied. Linear operating range and detection limit were defined. Released BPA concentration from different dental filling materials as real sample was determined under determined optimum operating conditions of the biosensor. Although there are biosensor studies for BPA determination in the literature, it was seen that there are

almost no biosensor studies for the determination of BPA derivate such as BPS and BPF. In this study, a fast, high sensitivity and sensitivity low detection limit as well as low cost new biosensor has been developed for the determination of both BPA and BPS and BPF.

EXPERIMENTAL

Tyrosinase enzyme (purified from fungus and as 2500 units) was purchased from Sigma-Aldrich. BPA, BPS, BPF, mineral oil, glutaraldehyde, carbon powder, disodium hydrogen phosphate and monosodium hydrogen phosphate were obtained from Merck.

All electrochemical experiments were performed using a computer-connected CHI1230A model electrochemical analyzer of CHI Company (CH Instrument, BASi, Kent Avenue, West Lafayette, IN, USA). Amperometric measurements were performed in a three-electrode cell system. A carbon paste electrode with a surface area of 0.6 cm^2 was used as the working electrode, Ag/AgCI (BAS RE-5B) was used as the reference electrode and platinum wire (MW--1032) was used as the counter electrode. After each electrochemical study, the working electrode was stored at 4 °C in distilled water or in the buffer solution.

Modification of carbon paste electrode

A known amount of graphite powder (0.0650 g) was weighed precisely, 30 µl of nujol and 40 mg of Fe_3O_4 nanoparticles were added to it and mixed homogeneously. The resulting mixture was filled into the electrode chamber so that there was no space left. Finally, the surface was cleaned and polished.[7]

Enzyme electrode preparation and amperometric measurements

2.0 mg BSA (bovine serum albumin), 50.0 µL phosphate buffer solution, 100.0 µL tyrosinase (50.0 units/mL) and 40.0 µL glutaraldehyde (2.5%) was mixed homogeneously. This mixture (190 µL totally) was dropped onto the surface of modified carbon paste electrode (MCPE) and dried at room temperature. The prepared enzyme electrode was washed first with distilled water and then with buffer solution. It was stored in the buffer solution at 4 °C when not in use (Fig. 1 a).[8]

BPA, BPF and BPS were used as different substrates of tyrosinase separately. Determination of the amount of BPA is based on the reduction of quinone compound released as a result of the enzymatic reaction (Fig. 1b). For this purpose, 0.1 M supporting electrolyte (NaCl) was added to the measurement medium (pH 7.0 phosphate buffer). The electrode was equilibrated at varying potentials (*versus* Ag/AgCl electrode (3.0 M KCl)) until a constant current value was obtained. BPA solution was added to the cell after equilibrium current (i_a) was recorded, and the system was stirred. At the end of the reaction, the final current (i_b) was recorded. The BPA concentration was plotted against the obtained current difference.[9]

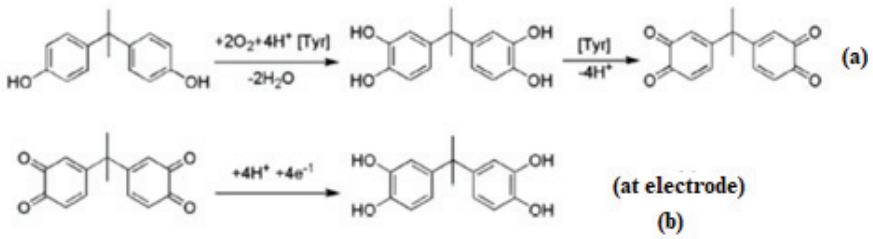


Fig. 1. Possible oxidation mechanism of BPA by tyrosinase.[4]

After determining the working potential, the optimum conditions (substrate concentration, pH, temperature) and the factors affecting its performance (operational stability, storage stabilization, interference effects) of the designed biosensor were determined. Finally, the amounts of BPA released from different dental filling materials were determined.

RESULTS AND DISCUSSION

BPA, a monomer of resins and plastics in the structure of many products used in daily life, released over time, increases the amount of intake into the human body and creates a toxic effect. Released BPA should be below the tolerable daily intake level (0.05 mg per kg of body mass).[6] Due to its chemical structure, BPA can imitate estrogen and exhibit similar activity. It is stated in studies that BPA has negative effects on human health, especially on the endocrine system.[3,11] For this reason, it is important to determine BPA quickly and with high sensitivity.[11]

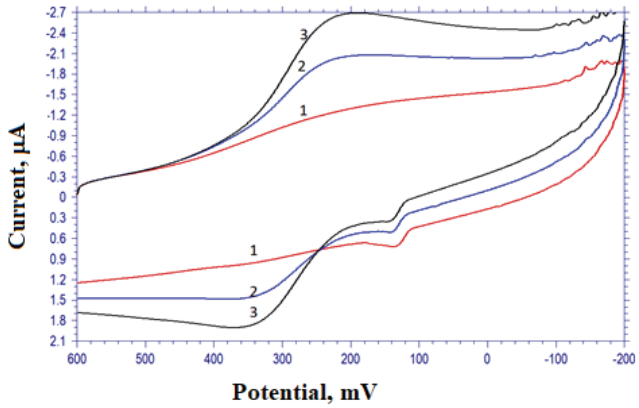
Electrochemical characterization and determination of the response and working potential of CPE and MCPE electrodes to BPA

The electrochemical performance of the CPE and MCPE were studied using cyclic voltammetry and $K_3Fe(CN)_6$ as a redox probe.[12] The cyclic voltammograms of the electrodes are presented in Fig. 2. 0.05 mM $K_3Fe(CN)_6$ solution in 0.10 M H_2SO_4 supporting electrolyte with a scan rate of 100 mV s^{-1} were documented. It was clear that the reduction and oxidation peaks of 0.05 mM $K_3Fe(CN)_6$ are seen quite clearly in the alternating voltammograms of the bare carbon paste electrode, while the 0.05 mM $K_3Fe(CN)_6$ obtained in the modified carbon paste electrode under the same conditions.

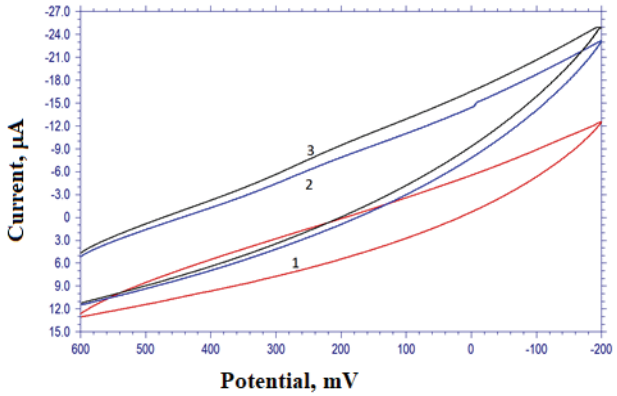
The voltammograms of CPE was shown in Fig. 2a (2). The reduction peak at 0.22 V and the oxidation peak at 0.34 V were obtained. When 0.05 mM $K_3Fe(CN)_6$ solution was added in cell (voltammogram 3), the reduction peaks at 0.22 V and oxidation peaks at 0.34 V increased. This increasing of the peaks were related with increased amount of $K_3Fe(CN)_6$. The same process was performed with the MCPE under the same conditions (Fig. 2b). As examined from the voltammograms 2 and 3 in Fig. 2b, no visible reduction or oxidation peak was observed with the addition of $K_3Fe(CN)_6$. According to this results it could be concluded that the surface of the carbon paste electrode has changed by modification of Fe_3O_4 nanoparticles. The decrease in the peaks indicated the modification of the carbon paste electrode (Fig. 2).

To determine the response of carbon paste electrode (CPE) and MCPE to BPA, the electrodes established equilibrium at –0.20 V potential separately.[13,14] Then, tyrosinase enzyme was added into the cell and was kept for a while until equilibrium was reached, and the equilibrium current recorded. The current differences (Δi) were recorded by adding bisphenol A solution at increasing cell concentrations between 1–500 μM. The current differences against increasing

bisphenol A concentrations were plotted (Fig. 3a). As seen in Fig. 3a, the bisphenol A response of the prepared modified carbon paste electrode is approximately two times higher than that of the carbon paste electrode.



(a)



(b)

Fig. 2. Cyclic voltammograms of $K_3Fe(CN)_6$ in 0.10 M H_2SO_4 supporting electrolyte (scan rate of 100 mV s^{-1}); a) bare CPE; b) MCPE. 1. 10.0 mL 0.10 M supporting electrolyte; 2. 1 + 0.05 mM $K_3Fe(CN)_6$; 3. 2 + 0.05 mM $K_3Fe(CN)_6$.

The working potential of the MCPE was determined after the working electrode reached the equilibrium at potentials ranging from –0.1 to –0.25V. BPA solution was added into the cell at increasing concentrations (1–500 µM) and the current values obtained at the end of the reaction were recorded. Current differences *versus* bisphenol A concentration were plotted (Fig. 3b). With the data obtained, the best reduction potential of the quinone compound formed at the end of the reaction was determined as –0.15 V.

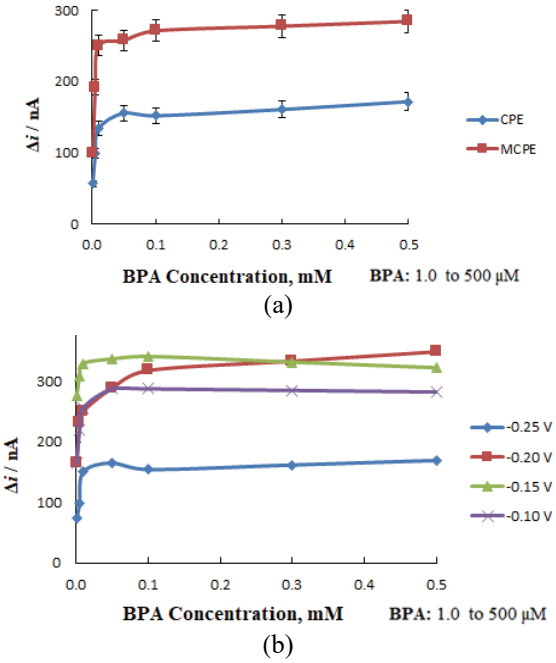


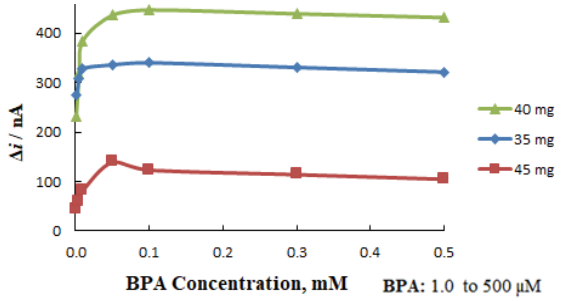
Fig. 3. a) Amperometric responses of CPE and MCPE to different concentrations of BPA; b) effect of working potential on BPA response of MCPE (at 25 °C, in 0.1 M, pH 7.0, phosphate buffer).

Effects of Fe_3O_4 nanoparticles amount on amperometric response of BPA effect of the amount of glutaraldehyde

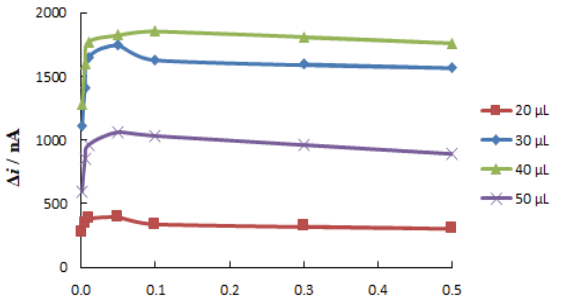
In order to find out the optimum quantity of Fe_3O_4 nanoparticles in MCPE on response of quinone compound, working electrodes were prepared by adding 35.0, 40.0 and 45.0 mg Fe_3O_4 separately. The reduction currents of quinone compound were measured with each electrode at –0.15 V. The BPA concentration was plotted against Δi results and 40.0 mg Fe_3O_4 showed the best linearity and the highest response current (Fig. 4a).

In many studies, glutaraldehyde ($CHO–CH_2CH_2CH_2–CHO$) is preferred as a cross linker.[7,15] Since glutaraldehyde is a small molecule, it can interact with amine groups both on the enzyme surface and interior and as a result the active site of the enzyme may be blocked by excessive cross-linking. The three-dimensional structure of the enzyme may change and activity losses may occur.[16] The amount of glutaraldehyde used should be investigated, for being sure that the active site of the enzyme is not affected, to avoid loss of activity. Best glutaraldehyde amount was defined by using prepared electrodes with the addition of 20, 30, 40 and 50 μL (2.5 %) glutaraldehyde solution separately for the determination of bisphenol A. Equilibrium current was recorded and current differences

were plotted. An increase in currents was observed when the amount of glutaraldehyde was increased from 20 to 30 μL and from 30 to 40 μL. There was a loss in activity detected by decreased currents with the electrode prepared by the addition of 50 μL glutaraldehyde. It was clearly seen from the graph that the highest currents recorded with the electrode designed by using 40 μL glutaraldehyde (Fig. 5b). The findings could be explained as 30 μL glutaraldehyde was insufficient to immobilize the enzyme and had difficulty in holding the structure together, and 50 μL glutaraldehyde caused loss of activity in the enzyme due to excessive binding.



(a)



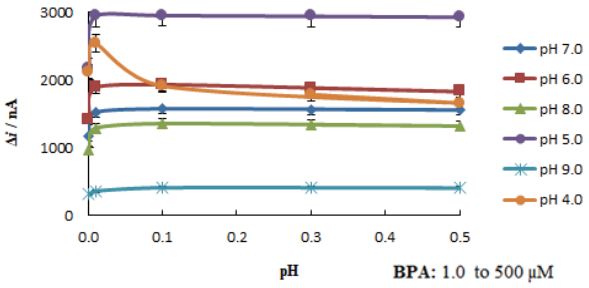
(b)

Fig. 4. Effect of Fe_3O_4 (a) and glutaraldehyde (b) amount on BPA response of MCPE (at 25 °C, in 0.1 M, pH 7.0, phosphate buffer).

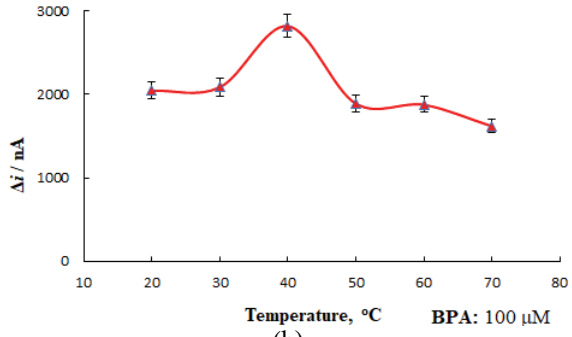
Effects of pH and temperature

One of the most important factors for enzymes, to show their activities with maximum velocity and low K_m value, is pH. Since there could be different ionizations on the active site or side groups of the enzyme at different pH values, the optimum pH value at which the enzyme shows the best activity should be determined. For this purpose, different buffer solutions ranged between 4.0 and 9.0 were used to determine the effect of pH on amperometric response of designed electrode. An acetate (acetic acid/sodium acetate) buffer solutions of pH 4.0 and

5.0, phosphate (Na_2HPO_4/NaH_2PO_4) buffer solutions of pH 6.0, 7.0 and 8.0 and glycine/sodium hydroxide buffer of pH 9.0 were used. When the Δi response current values at increasing concentrations (1–500 μM) were plotted against pH, the highest response current values were at pH 5.0 representing the optimum pH of the designed biosensor (Fig. 5a).



(a)



(b)

Fig. 5. Effect of pH (a) and temperature (b) on BPA response of MCPE.

In literature, it was stated that BPA biosensors prepared with different materials had optimum operating performance at different pH values varied between 3.0 and 7.0.[11,17–19] The reasons for this difference in pH values could be explained by the different modification materials and immobilization process.

Temperature is an important parameter for enzyme activity. Enzymes degrade at high temperature. To examine the effect of temperature on the current response of enzyme electrode, the temperature of the solution in the thermostatic cell was adjusted to 20 °C using a thermostatic circulating water bath. Then, BPA solution was added with an intracellular concentration of 1.0×10^{-4} M, and at the end of the reaction Δi value for 20 °C was calculated. The same process was carried out for the temperatures between 30 and 70 °C with 10 °C step. These amperometric response currents were measured at different temperatures in triplicate and plotted (Fig 5b). It is clear that the enzyme electrode showed the best

activity at 40 °C. Nevertheless, in terms of the practicality of the biosensor, all studies were carried out at room temperature.[7,11,15]

Substrate concentration and calibration curves BPA and its derivates

In order to examine the effect of the substrate concentration on the prepared biosensor, the amperometric currents of the increasing bisphenol A concentration (1–500 nM) at –0.15 V were recorded. Δ*i* current obtained by increasing bisphenol A concentration results were used for preparing Michaelis–Menten curve. It was observed that the current differences increased linearly as the bisphenol A concentration increased, then deviated from linearity and increased hyperbolically at increasing concentrations. The same process was also carried out separately for BPS and BPF substrates. Lineweaver–Burk curve was prepared to calculate the K_m constant by plotting the data as (1/([BPA]–1) Δi^{-1}. The observed value of $K_{m(app)}$ provides important information about the catalytic activity and affinity between enzyme and substrates. A low $K_{m(app)}$ value indicates a high affinity and kinetic activity between enzyme and substrate. Increasing concentrations of bisphenol A, BPS and BPF separately resulted in a quite small current differences after a certain concentration.

When the data of Fig. 6a in linear regions were plotted, it was seen that there were two calibration charts that could be used for the determination of bisphenol A. When the calibration graphs were examined, it was determined that there were linear operating ranges between the concentrations 1–5 nM and 30–100 nM. This situation allows to determine BPA and its derivatives in wide range. The limit of detection (*LOD*), was calculated according to *S*/*N* = 3 criterion and calculated as 1 nM.

The $K_{m(app)}$ value as 0.00067 μM and the $I_{max(app)}$ value as 3.34 μA was clearly seen in Fig. 6a. Different $K_{m(app)}$ values as 0.00815 M;[20] 3.26 μM;[21] 12 μM;[22] 0.34 mM[23] were reported earlier. It is obvious that the $K_{m(app)}$ value of designed biosensor, in the present study, is quite lower than the $K_{m(app)}$ values reported in literature. One of the biggest advantages of the designed biosensor is its ability to measure even at very low concentrations.

When the data of Fig. 6b in linear regions were plotted, two calibration charts were seen that could be used for the bisfenol S determination. When the calibration graphs were examined, it was observed that there were linear operating ranges between 1–5 nM and 10–100 nM concentrations and the lower detection limit was 1 nM. It is seen that the $K_{m(app)}$ value is 0.00075 μM and the $I_{max(app)}$ value is 2.50 μA (Fig. 6b).

When the data of Fig. 7 in linear regions were plotted, it was noticed that there were two calibration charts that could be used for the determination of bisphenol F. When the calibration graphs were examined, it was determined that there were linear operating ranges between 3–10 nM and 10–50 nM concentrate-

ions and the lower detection limit was 1 nM. It is seen that the $K_{m(app)}$ value is 0.00075 μM and the $I_{max(app)}$ value is 2.50 μA (Fig. 7).

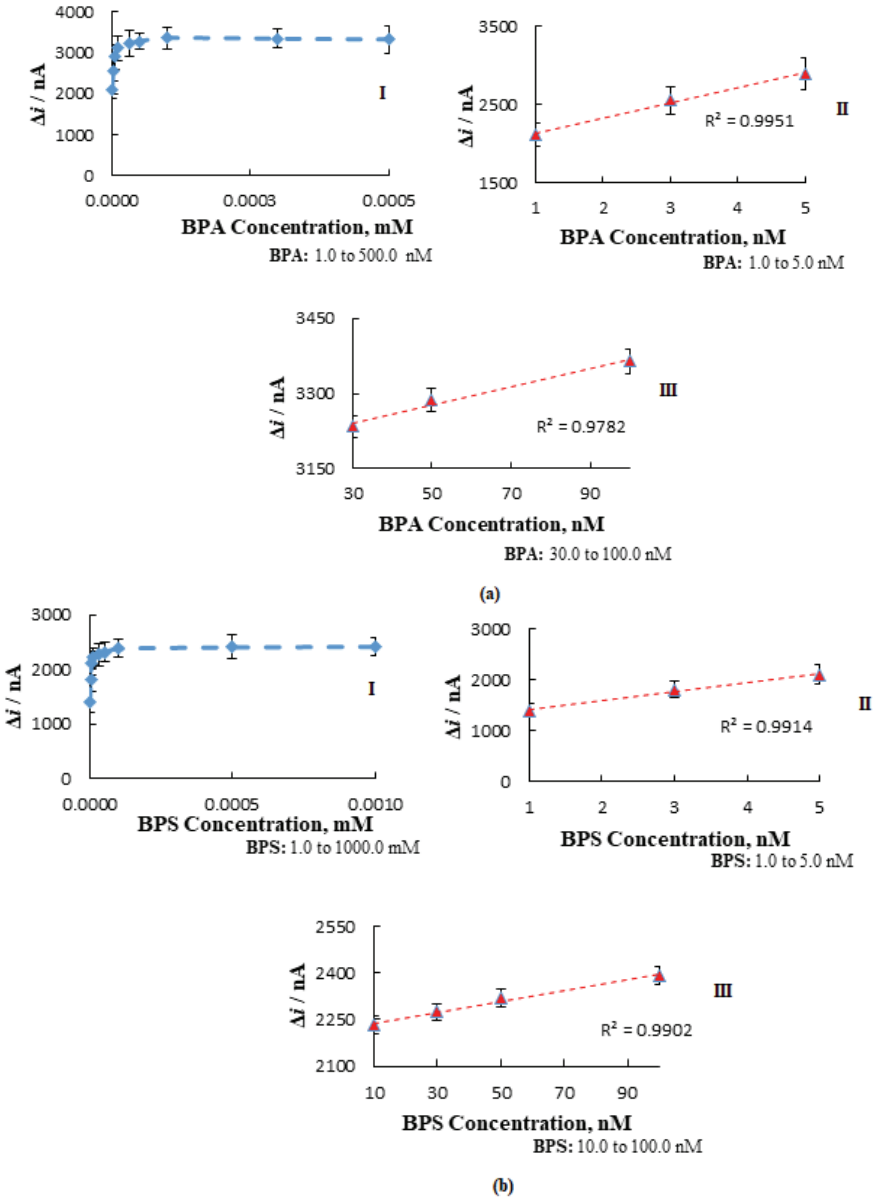


Fig. 6. Effect of: a) bisphenol-A (BPA) and b) bisphenol-S (BPS) concentration on the response of biosensor; I) Michael–Menten curve, II) calibration curve 1 and III) calibration curve 2 (–0.15 V operating potential, at 25 °C, in 0.1 M, pH 5.0, acetate buffer).

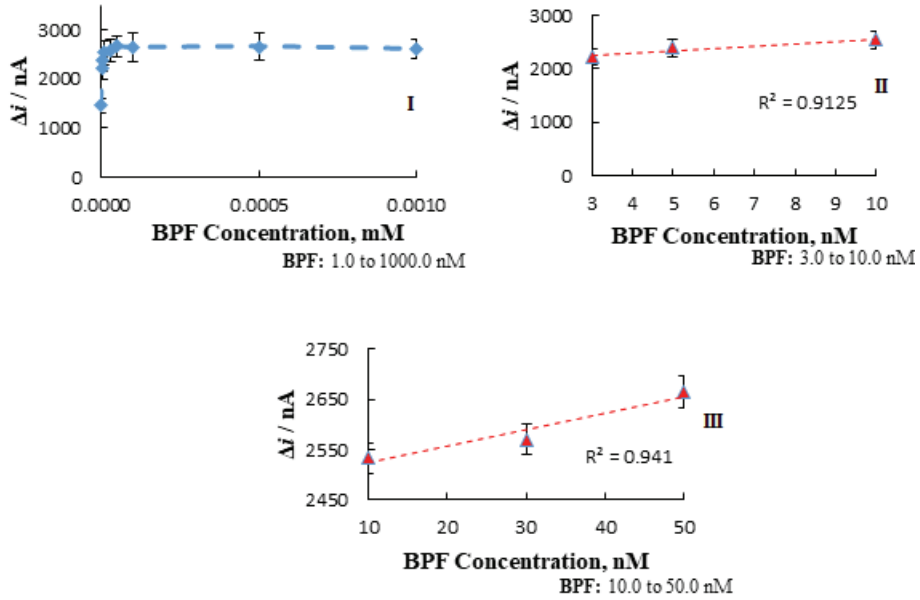


Fig. 7. Effect of bisphenol F (BPF) concentration on the response of biosensor; I) Michael–Menten curve, II) calibration curve 1 and III) calibration curve 2 (–0.15 V operating potential, at 25 °C, in 0.1 M, pH 5.0, acetate buffer).

It is clear that the designed biosensor has low $K_{m(app)}$, *LOD* and *LOQ* values for all three substrates. This shows that the tyrosinase enzyme has a high affinity for all three substrates (BPA, BPF and BPS) individually.

In Table I, characteristic analytical parameters of the different BPA biosensors were compared. It is clear that the designed biosensor has a low detection limit and wide calibration range for each substrate, and that BPA and its derivatives can be determined with high sensitivity in complex analysis environments.

TABLE I. Comparison of analytical characteristics of the different BPA biosensors

Immobilization matrix	Working *E* / V	Linearity of biosensor μmol L^{-1}	*LOD* nmol L^{-1}	K_m μM	Optimum pH	*RSD* %	Long-term stability	Ref.
Tyr/Au@PDA-rGO-CS/GCE	–0.20	0.012–3.68	0.01	nr	7.0	1.69	After 30 days, the modified electrode remained 92 % of its initial response	14
CYP2C9-PAM/GCE	–0.36	1.25–10.0	0.58	3.90	7.0	nr	The amperometric response after 10 days is 92 %	24

TABLE I. Continued

Immobilization matrix	Working E / V	Linearity of bio-sensor μmol L^{-1}	*LOD* nmol L^{-1}	K_m μM	Optimum pH	*RSD* %	Long-term stability	Ref.
Tyr/SWCNT-PolyLys/GCE	–0.10	4.00–11.5	0.97	nr	6.0	2.1 (n = 5)	The amperometric response after 30 days is 94.2 %	25
Tyr/TiO_2-MWCNT-PDDA-Nafion/GCE	–0.50	0.28–45.05	0.066	nr	6.0	5.1 (n = 5)	The response on the 14th day was 25 % of the initial value	26
Au-rGO paste based 3D-printed platform	–	100 nM L^{-1} to 10 mM L^{-1}	3.52	–	7.4	5.60 (n = 5)	–	27
MD/Graphene	–	10 nM L^{-1} to 0.10 mM L^{-1}	3.05	–	–	–	–	28
Try/Fe_3O_4 NPs modified carbon paste electrode	–0.15	0.001–0.005 and 0.03–0.1	1.0	0.00067	5.0	4.91 (n = 18)	The amperometric response after 20 days is 57.11 %	This study

Operational stability of BPA biosensor and storage stabilization of the enzyme electrode

To investigate the reusability of the electrode, the current changes were determined after successive measurements at a constant substrate concentration of 10 nM. The Δi values were plotted against the number of measurements (Fig. 8a). The relative standard deviation (*RSD*) calculated from the current changes obtained as a result of 18 measurements was found 4.91 % and it was observed that the electrode lost 5.43 % of its initial activity. The reusability of the electrode prepared in this study was found to be quite good.

In order to determine the storage stabilization of the electrode, amperometric response current was measured using 10 nM BPA concentration at certain intervals (1–3–10–15–20. days) for 20 days by resting the enzyme electrode in the buffer solution (pH 5.0) at 4 °C. At the end of the 20th day, the electrode retained 57 % of its initial activity (Fig. 8b).

Interference effects

Acetonitrile, ethyl acetate, hexane, phenol, nitrophenol, urea and potassium nitrate were chosen as interfering agents. The effect of interfering agents on bisphenol A determination was examined at concentration of 10 nM both for substrate and interfering agents. Minimizing or eliminating the interferences that may occur is important for the detection and accuracy of real sample analysis. It

was determined that none of these interfering agents caused an interference in bisphenol A determination.

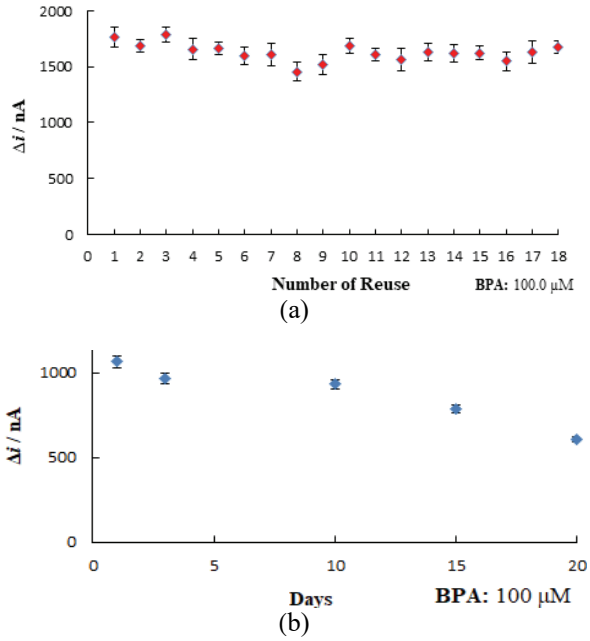


Fig. 8. a) Operational stability of the biosensor; b) storage stability of the biosensor.

BPA determination in different dental materials

Three different orthodontic materials were examined to determine the effectiveness of the biosensor on real samples. In the first sample, the orthodontic adhesive (Transbond XT, 3M Unitek, CA, USA) used to bond the brackets to the tooth surface was prepared in a metal mold with a height of 2 mm and a diameter of 4 mm, and the LED light source (VALO LED, Ultradent, South Jordan, UT, USA) It was polymerized by applying it for 3 s on both the front and back sides. The obtained sample was placed in a glass tube containing 5 mL of distilled water.[9]

In the second sample, a 1 mm thick 1 cm×1 cm piece of thermoplastic plaques (Simona Ag, Kirn, Germany) used in the reinforcing treatment was placed in a glass tube containing 5 mL of distilled water in order to preserve the new position of the teeth after orthodontic treatment.

In the third sample, during the fixed orthodontic treatment, the braces remain in the slots on the brackets without moving, thus transmitting force towards the tooth; 10 pieces of elastic ligature (Dentsply GAC, Islandia, NY, USA) were placed in a glass tube containing 5 ml of distilled water, considering that they

would be placed on the brackets of the incisors, canines and premolars on a single jaw.

Measurements were made after the samples were kept in glass tubes for 1 h. Care was taken not to use plastic materials during the preparation and experimentation of the samples. 100 μL of the obtained sample was added to the measurement medium, and after 20 min of mixing, the measurement was taken in 200 s. These studies were repeated three times and averaged, Δi mean value was calculated and plotted against bisphenol A concentration. The average of the current difference (Δi) for 3 different samples was calculated to determine the amount of BPA of the samples. It was determined that $(0.0002196\pm8.18)\times10^{-7}$, $(0.0000909\pm1.02)\times10^{-6}$ and $(0.000275\pm4.08)\times10^{-6}$ mg/kg BPA from 1 mg of composite to 1 mL of water was released for samples I, II and III, respectively.

CONCLUSION

In this study, a new tyrosinase enzyme-based biosensor was designed by modifying the carbon paste electrode using Fe_3O_4 nanoparticles. The designed biosensor has a response time of 200 s, two linear detection intervals and a very low detection limit (1 nM) for each substrate (BPA, BPS and BPF). Released BPA should be below the tolerable daily intake level (0.05 mg per kg of body mass).[6] With this designed biosensor, measurements can be made at concentrations lower than 0.05 mg/kg.

With this newly designed biosensor, it is possible to detect three different species (BPA, BPS and BPF) separately with a single biosensor, at low concentrations, with high sensitivity, and with a wide detection range. Considering the reproducibility, shelf life and interference effects on the biosensor, the designed biosensor is capable of providing fast, high-sensitivity and economical BPA and its derivatives determination.

Acknowledgement. This study was supported by Gazi University with project number 05/2020-11.

ИЗВОД

ДИЗАЈНИРАЊЕ ЕЛЕКТРОХЕМИЈСКОГ БИОСЕНЗОРА БАЗИРАНОГ НА ТИРОЗИНАЗИ ЗА ВИСОКО ОСЕТЉИВУ И БРЗУ ДЕТЕКЦИЈУ БИСФЕНОЛА А И ЊЕГОВИХ ДЕРИВАТА

ONUR CAN BODUR[1], MERVE KESKIN[2], BAŞAK ARSLAN AVAN[3] и HALIT ARSLAN[1]

[1]Department of Chemistry, Faculty of Science, Gazi University, Ankara, Republic of Türkiye, [2]Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Republic of Türkiye и [3]Department of Orthodontics, Faculty of Dentistry, Gazi University, Ankara, Republic of Türkiye

Бисфенол А (BPA) је мономер који се обично користи у производњи епокси смола, пластичних боца и материјала за зубне пломбе. Због своје хемијске структуре, BPA и његови деривати показују активност сличну ендокриним хормонима. Он се може везати за естрогенске рецепторе и изазвати неуролошке поремећаје, чак и у малим дозама. Стога је важна брза и осетљива детекција BPA и његових деривата у малим концентра-

цијама. У овом истраживању је дизајниран амперометријски биосензор на бази ензима тирозиназе за квантитативно одређивање мономера BPA, бисфенол F (BPF) и бисфенол S (BPS). Тирозиназа је имобилисана на електроди од угљеничне пасте модификованој умрежавањем са глутаралдехидом. Количина BPA (BPS и BPF) је одређена директно редукцијом хинонског једињења које је ослобођено у ензимској реакцији на –0,15 V. Одређено је да је вредност $K_{m(app)}$ дизајнираног биосензора за BPA $6{,}7\times10^{-4}$ μM, линеарни радни опсег је 0,001–0,005 μM и 0,03–0,1 μM и да доња граница детекције износи 1 nM за сваки од мономера. Јасно је показано да дизајнирани биосензор омогућава брзо, ефикасно и прецизно одређивање BPA и његових деривата ослобођених из денталних материјала.

(Примљено 22. новембра 2022, ревидирано 13. фебруара, прихваћено 21. марта 2023)

REFERENCES

1. F. Fleisch, P. E. Sheffield, C. Chinn, B. L. Edelstein, P. J. Landrigan, *Pediatrics* **126** (2010) 760 (https://doi.org/10.1542/peds.2009-2693)
2. L. A. D. Gugoasa, *J. Electrochem. Soc.* **167** (2020) 037506 (https://doi.org/10.1149/2.0062003JES)
3. K. V. Ragavan, N. K. Rastogi, M. S. Thakur, *TrAC - Trends Anal. Chem.* **52** (2013) 248 (http://doi.org/10.1016/j.trac.2013.09.006)
4. EFSA, *EFSA J.* **428** (2006) (https://doi.org/10.2903/j.efsa.2004.86)
5. X. Lu, X. Wang, L. Wu, L. Wu, L. Fu, Y. Gao, J. Chen*, ACS Appl. Mater. Interf.* **8** (2016) 16533 (https://doi.org/10.1021/acsami.6b05008)
6. H. Yin, L. Cui, Q. Chen, W. Shi, S. Ai, L. Zhu, L. Lu, *Food Chem.* **125** (2011) 1097 (https://doi.org/10.1016/j.foodchem.2010.09.098)
7. O. C. Bodur, S. Dinç, M. Özmen, F. Arslan, *Biotechnol. Appl. Biochem*. **68** (2021) 20 (https://doi.org/10.1002/bab.1886)
8. S. Donmez, F. Arslan, N. Sarı, E. Hasanoğlu Özkan, H. Arslan, *Biotechnol. Appl. Biochem.* **64** (2017) 745 (https://doi.org/10.1002/bab.1533)
9. F. Arslan, *Sensors* **8** (2008) 5492 (https://doi.org/10.3390/s8095492)
10. P. C. Pwavodi, V. H. Ozyurt, S. Asir, M. Ozsoz, *Micromachines* **12** (2021) 312 (https://doi.org/10.3390/mi12030312)
11. P. Deng, Z. Xu, Y. Kuang, *Food Chem.* **157** (2014) 490 (https://doi.org/10.1016/j.foodchem.2014.02.074)
12. S. S., Shankar, R. M., Shereema, V., Ramachandran, T. V., Sruthi, V. S., Kumar, R. B. Rakhi, *ACS Omega* **4** (2019) 7903 (https://doi.org/10.1021/acsomega.9b00230)
13. S. Wang, Y. Tan, D. Zhao, G. Liu, *Biosens. Bioelectron.* **23** (2008) 1781 (https://doi.org/10.1016/j.bios.2008.02.014)
14. X. Xu, Q. Zheng, G. Bai, L. Song, Y. Yao, X. Cao, C. Yao, *Electrochim. Acta* **242** (2017) 56 (https://doi.org/10.1016/j.electacta.2017.05.007)
15. E. Aynacı, A. Yaşar, F. Arslan, *Sensors Actuators, B* **202** (2014) 1028 (https://doi.org/10.1016/j.snb.2014.06.049)
16. Q. Xin, R. M. Wightman, *Brain Res.* **776** (1997) 126 (https://doi.org/10.1016/S0006-8993(97)00996-7)
17. L. Wu, X. Lu, K. Niu, Dhanjai, J. Chen, *Biosens. Bioelectron*. **165** (2020) 112407 (https://doi.org/10.1016/j.bios.2020.112407)

18. M. Sýs, M. Obluková, V. Kolivoška, R. Sokolová, L. Korecká, T. Mikysek, *J. Electroanal. Chem.* **864** (2020) 114066 (https://doi.org/10.1016/j.jelechem.2020.114066)
19. Erkmen, S. Kurbanoglu, B. Uslu, *Sensors Actuators, B* **316** (2020) 128121 (https://doi.org/10.1016/j.snb.2020.128121)
20. M. Najib, M. E. Ghicad, C. Dridia, B. M. Alia, M. A. Christopher, *Talanta* **184** (2018) 388 (https://doi.org/10.1016/j.talanta.2018.03.031)
21. F. A. A. Manan, W. W. Hong, J. Abdullah, N. A. Yusof, I. Ahmad, *Mater. Sci. Eng., C* **99** (2019) 37 (https://doi.org/10.1016/j.msec.2019.01.082)
22. Wong, A. Santos, O. Fatibello Filho, M. Sotomayor, *Electroanalysis* **33** (2020) 431 (https://doi.org/10.1002/elan.202060084)
23. L. A. Mercante, L. E. Iwaki, V. P. Scagion, O. N. Oliveira, L. H. Mattoso, D. S. Correa, *Electrochem*. **2** (2021) 41 (https://doi.org/10.3390/electrochem2010004)
24. P. Sun, Y. Wu, *Sensors Actuators, B* **178** (2013) 113 (https://doi.org/10.1016/j.snb.2012.12.055)
25. M. Han, Y. Qu, S. Chen, Y. Wang, Z. Zhang, M. Ma, Z. Wang, G. Zhan, C. Li, *Microchim. Acta* **180** (2013) 989 (https://doi.org/10.1007/s00604-013-1018-3)
26. J. Kochana, K. Wapiennik, J. Kozak, P. Knihnicki, A. Pollap, M. Woźniakiewicz, P. Kościelniak, *Talanta* **144** (2015) 163 (https://doi.org/10.1016/j.talanta.2015.05.078)
27. L.A. Gugoasa, R.I. Stefan-van Staden, J.F. van Staden, M. Coroş, S. Pruneanu, *Anal. Lett.* **52** (2019) 2583 (https://doi.org/10.1080/00032719.2019.1620262)
28. R.I. Stefan-van Staden, L.A. Gugoaşă, B. Calenic, J.F. van Staden, J. Legler, *Anal. Chem. Res.* **1** (2014) 1 (https://doi.org/10.1016/j.ancr.2014.06.001)
29. B. Arslan, E. Yıldırım, C. O. Bodur, B. B. Tuncer, Ç. M. Ulusoy, C. Tuncer, *Turk. J. Orthod.* **35** (2022) 27 (https://doi.org/10.5152/TurkJOrthod.2021.21176).