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NOVEL TYROSINASE-BASED BISPHENOL-A BIOSENSOR FOR THE DETERMINATION OF BISPHENOL-A IN BRACKET ADHESIVES IN ORTHODONTICS

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A novel biosensor for the determination of Bisphenol-A has been developed in this study. For this purpose, a carbon paste electrode modified with poly(amidoamine)-salicylidenimine platinum(II) (PAMAM-Sal-Pt(II)) and the tyrosinase enzyme was prepared. BPA determination was based on the electrochemical reduction of an enzymatically produced quinone compound at –0.15 V. Optimum working conditions for the prepared biosensor were investigated. The linear working range and detection limit were found to be 0.01–1.0 μM and 1 nM, respectively. The optimum pH value and working temperature were defined as 7.0 and 40 °C, respectively. The reproducibility of the biosensor is very good. It was found that phenol, nitrophenol, urea, potassium nitrate, hexane, acetonitrile, and ethyl acetate do not interfere with the BPA determination. The prepared biosensor was used for the first time in dentistry for the determination of BPA in a resin-based composite material used as a bracket adhesive in orthodontics.

Keywords: amperometry; biosensor; bisphenol-A; dendritic structure; tyrosinase

НОВ БИОСЕНЗОР ЗА ОПРЕДЕЛУВАЊЕ БИСФЕНОЛ-А БАЗИРАН НА ТИРОЗИНАЗА ВО СРЕДСТВАТА ЗА ЛЕПЕЊЕ ВО ОРТОДОНЦИЈАТА

Во рамките на оваа студија е развиен нов тип биосензор наменет за определување на бисфенол-А (BPA). За таа намена е дизајнирана електрода од јаглеродна паста која е модифицирана со поли(амидоамин)-салицилденимин платина(II) (PAMAM-Sal-Pt(II)) и со ензимот тирозиназа. Определувањето на BPA е базирано на електрохемиската редукција на ензимско генерираниот кинонски продукт на потенцијал од –0.15 V. Во рамките на студијата беа оптимизирани условите за функционирање на биосензорот. Регионот на линеарност и границата на детекција за определување на BPA изнесуваа соодветно 0,01–1,0 μM и 1 nM. Оптималната вредност на pH и на работната температура изнесуваа соодветно 7,0 и 40 °C. Дизајнираниот сензор покажа доста добра репродуцибилност. Беше најдено дека соединенијата фенол, нитрофенол, уреа, калиум нитрат, хексан, ацетонитрил и етил ацетат не покажуваат интерференции при определувањето на BPA. Дизајнираниот биосензор беше употребен за прв пат во денталната медицина за определување на BPA во композитен на смоли базиран материјал што се употребува како атхезивен материјал во ортодонцијата.

Клучни зборови: амперометрија; биосензор; бисфенол-А; дендритска структура; тирозиназа

1. INTRODUCTION

Along with industrialization and technological developments, different chemicals have entered human life in recent centuries. It has been mentioned that some of these chemicals may have disruptive effects on the function of the endocrine system,[1] and today, studies about the harmful effects of these substances,[2,3] which form the structure of many objects that are used in daily life, are increasing.

Bisphenol-A (BPA, 2,2'-bis (4-hydroxyphenyl) propane) is one of the endocrine-disrupting chemicals mainly used for polycarbonate plastics and the production of epoxy resins. It is used in the production of packaging materials for foods and beverages,[4,5] flame retardants, adhesives, construction materials, electronic components, and some dental materials.[6,7] BPA contamination in food usually occurs as the chemical migrates from the packages that contain it. Some studies mentioned that BPA has an estrogenic effect on human metabolism and may affect the development of the reproductive system, leading to various disorders.[8,9] It is also known that this chemical may be linked with obesity, neural system disorders, and thyroid hormone problems.[10–12]

Some studies mention that the daily exposure of humans to BPA is below the tolerable daily intake level, which is limited to 50 μg/kg bw/day.[13–16] However, the disruptive effects of this chemical even at low doses is also a discussion point.[17,18] Various chemical methods are available in the literature for the determination of BPA, such as gas chromatography and high-performance liquid chromatography.[19,20] However, the devices used in these techniques are quite expensive. All these reasons make BPA a significant chemical that needs to be researched more and detected with easy-to-apply and cost-effective methods. Therefore, it is important to design a cheaper, responsive, accurate, and selective biosensor for BPA determination. Several biosensor studies for this topic can be found in the literature, and various macromolecular structures are used in these studies.[21–26] Dendrimers are one of these macromolecular structures and are popular in the preparation of electrochemical biosensors.

Dendrimers have a wide application area thanks to their high molecular mass, symmetrical structure, controlled chemical reactivity, change of functional groups on the surface, and interior spaces.[27] They are important molecules for use as biosensors because of their three-dimensional structure and large number of outer surface groups.[28] PAMAM dendrimers have the advantage of being nontoxic and efficient in transporting bioactive agents. Furthermore, PAMAM dendrimers shorten the reaction times due to the large number of outer surface groups. However, they reduce the conductivity of the electrode surface. This feature limits their application as biosensors. To overcome this situation, various materials including Au or Pt nanoparticles, multi-walled carbon nanotubes, and Schiff bases have been used on the surface of the dendrimer.[29–34]

The present study aimed to prepare an amperometric BPA biosensor using PAMAM-Sal-Pt(II) (Poly(amidoamine)-salicylidenimine platinum(II)) and a carbon paste electrode modified with the tyrosinase enzyme. The Pt(II) ion was used to increase the conductivity of PAMAM-Sal containing the Schiff base. Based on this, PAMAM-Sal-Pt(II) used in the preparation of the biosensor was synthesized and characterized for the first time in this study. The BPA biosensor prepared in this study differs from the literature in this aspect.

In order to determine the contribution of the PAMAM-Sal-Pt(II) biosensor to BPA determination, firstly, amperometric response currents of the carbon paste electrode (CPE) and modified carbon paste electrode (MCPE) and biosensor to BPA were compared. It was observed that the amperometric response currents of MCPE to BPA were approximately 2 times higher than that of CPE. It was thought that PAMAM-Sal-Pt(II) could be a good biosensor candidate for BPA determination by increasing the conductivity of MCPE. In subsequent studies, the preparation of the biosensor, optimum operating conditions, and the factors affecting its performance were investigated. The prepared biosensor was used for the first time in dentistry for the determination of BPA in a resin-based composite material used as a bracket adhesive in orthodontics.

2. MATERIALS AND METHODS

2.1. *Materials*

All electrochemical experiments were carried out using a computer-connected CHI firm's 1230-B model electrochemical analyzer. Amperometric measurements were made in a cell system that contains three electrodes. The working, reference, and counter electrodes were a 0.5 cm diameter carbon paste electrode (specially made of teflon), BAS RE-5B Ag/AgCl and a platinum wire MW-1032, respectively. The working electrode

was kept at +4 °C in a phosphate buffer solution when not in use. Tyrosinase (purified from fungus) with an activity of 8503 U/mg was obtained from Fluka. BPA was dissolved in 60 % methanol, and the solution was prepared daily with a phosphate buffer at pH 7.0. BPA was supplied from Sigma. The PAMAM dendrimer ([$NH_2(CH_2)_2NH_2$] core, 20 wt.% in methanol; (G = 1); PAMAM(NH_2)$_8$), salicylaldehyde, and potassium tetrachloroplatinum(II) were provided by Aldrich. All other chemical materials were provided by Sigma. Distilled water was used in all studies. Infrared spectra were read at 400–4000 cm^{-1} utilizing a Perkin Elmer Spectrum 100 FT-IR spectrometer. Electronic spectra were obtained utilizing a UV-1800 ENG240V spectrophotometer in DMSO. Elemental analyses were performed using a LECO-932 CHNS analyzer.

2.2. *Methods*

2.2.1. *Synthesis of poly(amidoamine) salicylidenimine platinium(II) [PAMAM-Sal-Pt(II)]*

The novel dendritic structure containing Pt(II) was synthesized by the template method. Salicylaldehyde (0.8 mmol) was supplemented to the PAMAM dendrimer solution drop by drop (0.1 mmol/g PAMAM dendrimer dissolved in ethanol 15 ml).).

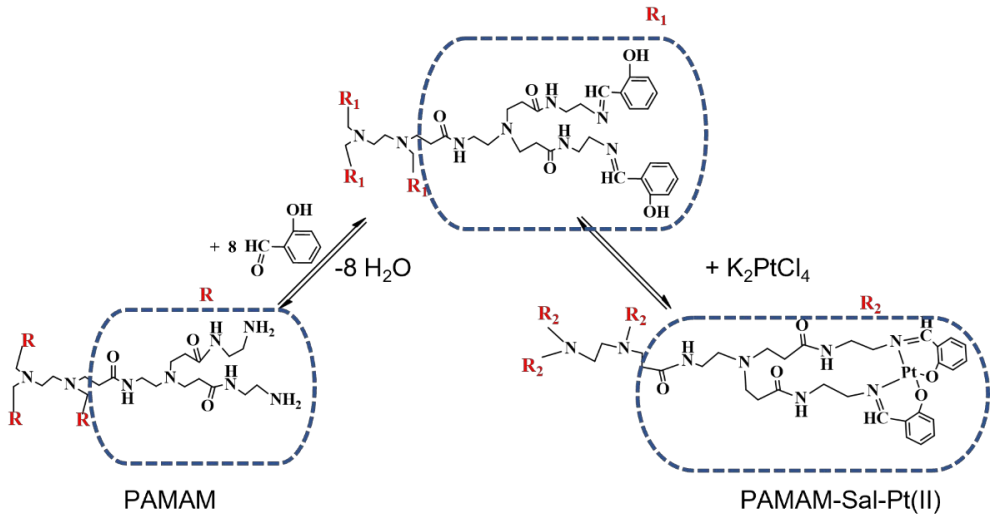


Fig. 1. Synthesis schematic of PAMAM-Sal-Pt(II)

T a b l e 1

Analytical, UV-Vis (nm), and IR (cm^{-1}) spectral data of the PAMAM-Sal and PAMAM-Sal-Pt(II)

Compound Formula (Mw) Color	Found (Calcd) %				ν-O-H νNH(sm,as) ν-M-O M-N	ν-CH=N νCH(arm/alp) ν-C-H(op) δ-C-C(as)	π→π*(C=C) n→π*(C=O) n→π*(N-N) d-d
	C	H	N	Pt			
PAMAM-Sal	65.17	7.65	15.70	–	3274	1629	260; 286
$C_{118}H_{160}N_{26}O_{20}$	(62.64)	(7.13)	(16.09)		3062,2937	2833	317; –
(2261.23)					–	849	
Yellow					–	1495	
PAMAM-Sal-Pt(II)	47.09	4.98	11.89	25.45	3492	1649	258; 291
$C_{118}H_{152}N_{26}O_{20}Pt_4$	(46.70)	(5.05)	(12.00)	(25.71)	3206,3089	2886	316; 451
(3033.03)					578	867	
Green					475	1434	

sm: symmetric, as: asymmetric stretch, aro: aromatic, alp: aliphatic

The boiling and stirring process was continued for 24 h under reflux. 0.8 mmol potassium tetrachloroplatinum(II) ($K_2[PtCl_4]$: 0.3325 g) dissolved in ethanol : water (1:1) (10 ml) was supplemented drop by drop to the mixture solution. The mixture was magnetically stirred for 12 h and heated to 85 °C under reflux. Then, the mixture was cooled to room temperature and precipitated

by adding acetone. The resulting solid was filtered and dried in the oven for one day, and the yield of PAMAM-Sal-Pt(II) was calculated to be 0.7421 g (57 %). The physical characterization, the analytical data of the PAMAM-Sal-Pt(II), and the synthesis schematic of PAMAM-Sal-Pt(II) are given in Table 1 and Figure 1, respectively.

2.2.2. *Preparation of the working electrode: Carbon paste and modified carbon paste electrode*

CPE, which was made of teflon, was cleaned with distilled water. 0.142 g of graphite powder was weighed and placed on the watch glass. 95 µl of nujol was added. These two ingredients were mixed and turned into a paste consistency. Then, the space in the CPE was filled tightly with the paste. A special pad was used to make the CPE surface smooth and flat. Finally, it was washed with pure water to make it ready for use. In the process of modifying the CPE, 1.0 mg of the PAMAM-Sal-Pt(II) polymer was added to the paste (graphite powder + nujol) prepared differently from the preparation method mentioned above, and the mixture was turned into a paste consistency and added into the electrode (Fig. 2).

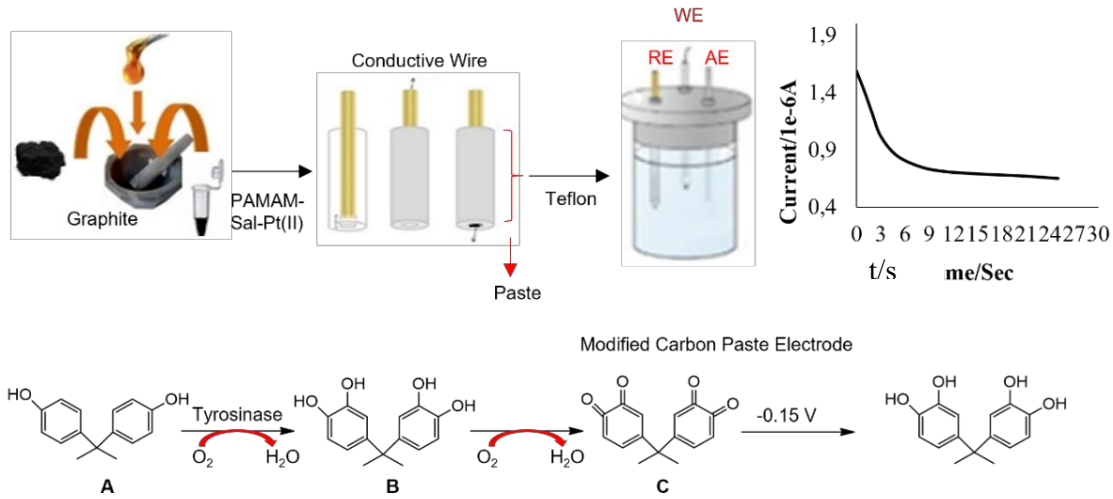


Fig. 2. Preparation of the MCPE and working cell scheme (A: 4,4′-(propan-2,2dyil)diphenol; BPA, B: 4,4′-(propane-2,2dyil)bisbenzo-1,2-diol, C: 4,4′-(propane-2,2dyil)biscyclohexane-3,5-diene-1,2-dione))

2.2.3. *Immobilization of the tyrosinase enzyme to MCPE*

A mixture of 2.0 mg bovine serum albumin (BSA), 50 µl phosphate buffer (pH 7.0), 30 µl glutaraldehyde (GL), and 100 µl tyrosinase (5000 units/ml) enzyme was supplemented drop by drop to the prepared MCPE surface, and the electrode was allowed to dry at room temperature. After it was completely dried, it was washed with distilled water so that the enzyme that could not adhere to the surface was removed from the electrode. When not in use, it was kept in a phosphate buffer at +4 °C in the refrigerator.

2.2.4. *Electrochemical measurements*

The tyrosinase enzyme creates a quinone compound by catalyzing the oxidation of BPA in two steps in an oxygenated environment. In the first step, 4,4'-(propane-2,2diyl)bisbenzo-1,2-diol and in the second step, 4,4'-(propane-2,2-diyl)bis(cyclohexane-3,5-diene-1,2-dione) which is a quinone compound, is formed (Fig. 2). The determination of BPA was made based on the measurement of the reduction currents at –0.15 V of this quinone compound formed at the end of the enzymatic reaction (Fig. 2). The prepared MCPE was kept at –0.15 V in the cell containing 1 ml sodium chloride (1 M) (supporting electrolyte) and 9 ml phosphate buffer (pH 7.0) at room temperature until it reached equilibrium. Then, the BPA solution was added, the solution was mixed, and after 200 s, the measurement was made.[32] The current difference (Δi) value was calculated by subtracting this measured current value from the equilibrium current.

3. RESULTS AND DISCUSSION

In this study, a new BPA-sensitive biosensor was prepared by modifying it with PAMAM-Sal-Pt(II). PAMAM-Sal-Pt(II) was synthesized for

the first time for this biosensor. The best working conditions and the factors affecting the performance of the developed biosensor were examined. The prepared biosensor was used in the determination of BPA in the orthodontic material used as a bracket adhesive in dentistry. The determination of BPA was made by reducing the compound of quinone (4,4'-(propane-2,2-diyl)bis(cyclohexane-3,5-diene-1,2-dione)) formed as a result of the enzymatic reaction at –0.15 V. The working scheme of the BPA biosensor is shown in Figure 3.

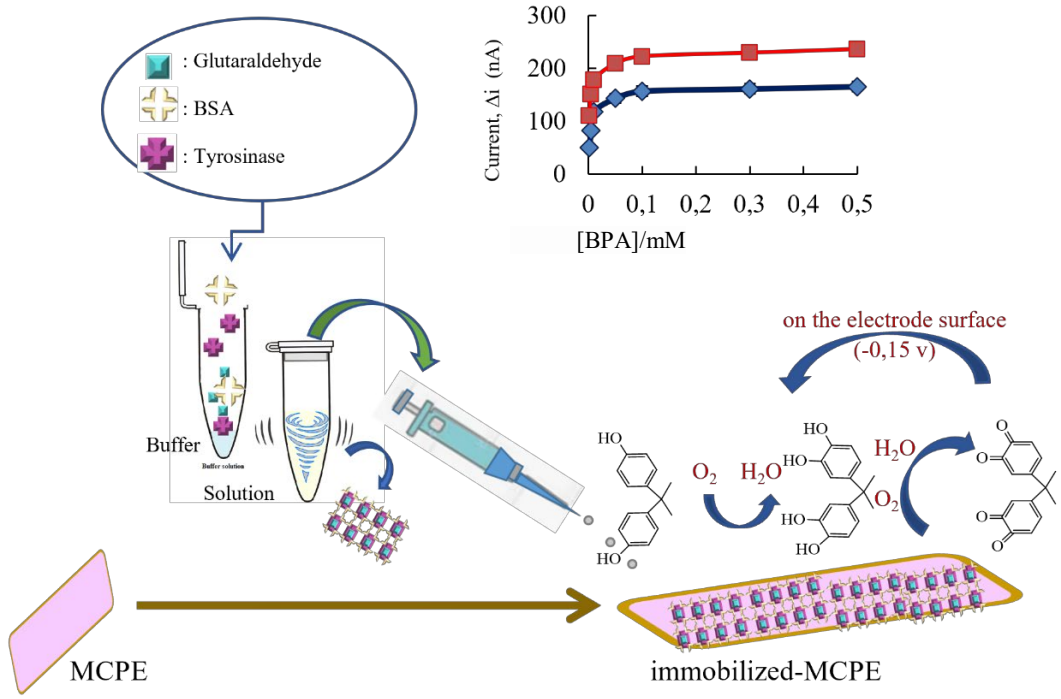


Fig. 3. Operation scheme of the BPA biosensor

3.1. *Characterization of the dendritic structure PAMAM-Sal-Pt(II)*

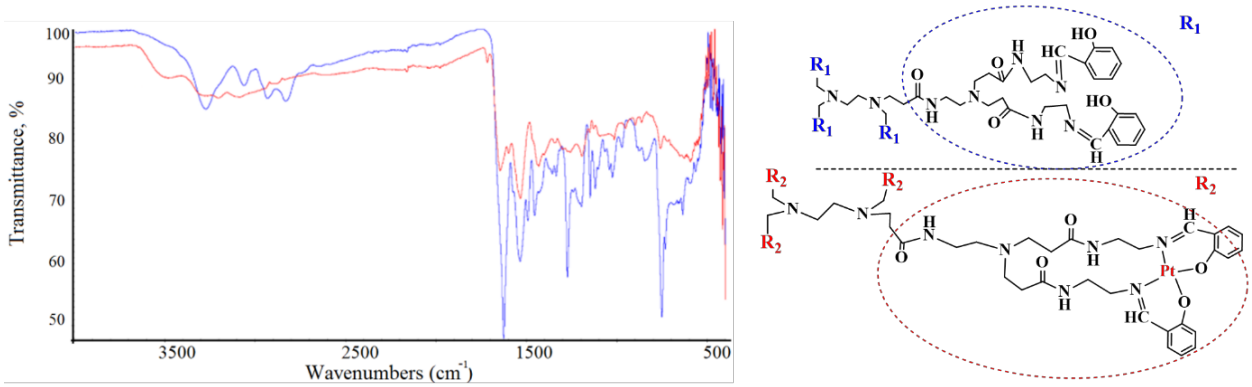
The synthesis and the characterization of the novel dendritic structure containing Pt(II) were carried out by adopting different physicochemical methods, including molar conductivity, magnetic susceptibility measurements, and spectroscopic techniques. The PAMAM-Sal-Pt(II) is only soluble in DMF and DMSO and is insoluble in apolar solvents such as CCl_4 and benzene. The molar conductance value of PAMAM-Sal-Pt(II) is found to be 42.1 Ω^{-1} cm^2 mol^{-1} in a 10^{-4} M DMSO solution (Table 1). According to this result, PAMAM-Sal-Pt(II) is a nonelectrolyte.[31] The magnetic moment value of the dendritic structure indicates that this platinum complex is diamagnetic.[32]

The FT-IR spectrum of the synthesized PAMAM-Sal-Pt(II) is given in Figure 4 in an overlapping manner with PAMAM-Sal. The PAMAM-Sal spectrum shows medium broad bands in the region 3274, 3062, 2937 cm^{-1} and 1629 cm^{-1} assigned to the υNH (sym, asym) and υCH=N, respectively.[35,36] The appearance of bands in the 1495 cm^{-1} and 1276 cm^{-1} regions was due to δ-C-C(as) and δ-C-C-H(bv), respectively.[37,38] The azomethine stretching band υ(CH=N) was observed at 1629 cm^{-1}. The IR spectrum showed the disappearance of the NH_2 bands together with the presence of the CH=N band, which took place with the reaction of salicylaldehyde with an amine. When the spectrum of PAMAM-Sal-Pt(II) is examined, the amide-I, II, III bands observed in the spectrum of the PAMAM-Sal were shifted to the high wavenumber in the range of 3400–3100 cm^{-1}. Then, the υC=N stretching vibration of the azomethine group was observed at a high wavenumber. These results showed that the nitrogen atom of the azomethine group is coordinated with the metal ion (Fig. 1). The appearance of weak new bands in the 578 and 475 cm^{-1} region was due to ν (M-O) and ν (M-N), respectively.[37,39] The IR spectral results suggested that the anion of the dendrons was coordinated with the metal ion as a bidentate ligand.

The electronic spectra of the dendrimers in DMSO show that bands at *ca.* 210 nm are attributed to the azomethine n→π*(-C=N) transition. The bands at higher energies (260, 286, 317 nm) are

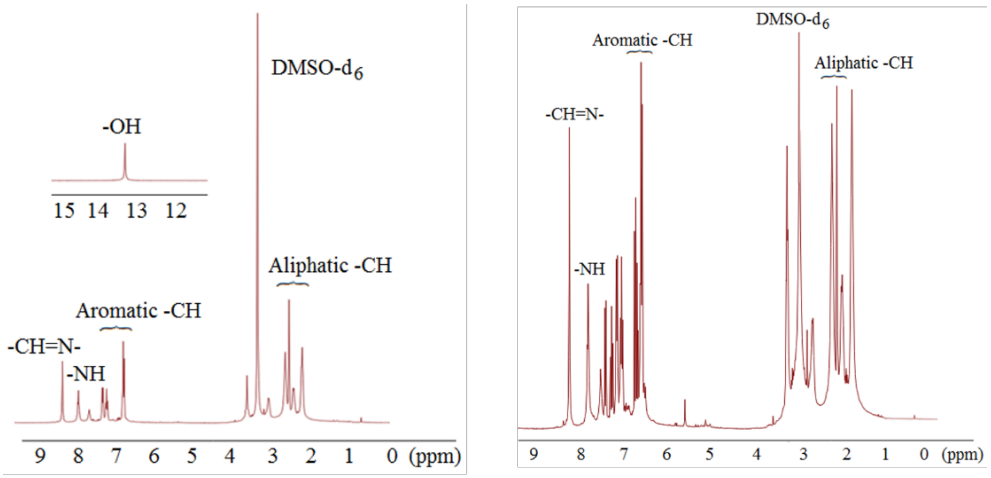
associated with the C=C (π→π*), C=O (n→ π*), and N-N (n→π*) transition, respectively. The d-d bands from the spectra of the Pt(II) complex having low intensities appeared at 570 nm and were assigned to the transition in the square planar environment, while the band centered at *ca.* 450 nm is assigned to the metal to ligand charge-transfer.[34,39]

The ^{1}H-NMR data of the PAMAM-Sal and PAMAM-Sal-Pt(II) are collected in Table 2. As seen in Figure 5, in general, the multiplets observed at 6.82–7.49 ppm and the singlets at 8.10–8.46 ppm are assigned to -CH$_{(aromatic)}$ protons and imine protons, respectively. The -CH$_{(aliph)}$, -NH, and -OH protons of the PAMAM-Sal and PAMAM-Sal-Pt(II) were also observed, as expected. The ^{13}C NMR spectral data of the PAMAM-Sal and PAMAM-Sal-Pt(II could not be obtained due to poor resolution.



(a) (b)

Fig. 4. (a) FT-IR spectra and (b) proposed of structures [PAMAM-Sal] and [PAMAM-Sal-Pt(II)]



(a) (b)

Fig. 5. ^{1}H-NMR spectrum of (a) PAMAM-Sal and (b) PAMAM-Sal-Pt(II)

T a b l e 2

^{1}H-NMR chemical shift (ppm) of PAMAM-Sal and PAMAM-Sal-Pt(II)

Compound	-OH; -CH=N	-NH	-CH(aromatic)	-CH(aliph)
PAMAM-Sal	13.33(s) 8.46(s)	7.97(dd)	7.51(d), 7.49(m) 7.34(m), 6.92(m)	3.16(s), 3.06(s) 2.85(d), 2.39(d)
PAMAM-Sal-Pt(II)	– 8.1(s)	7.97(dd)	7.43(d), 7.41(m) 7.16(m), 6.82(m)	3.16(s), 3.06(s) 2.83(d), 2.37(d)

3.2. *Amperometric response of CP and MCP electrodes to BPA*

The prepared CPE and MCPE were placed in the cell containing 0.1 M NaCl, phosphate buffer, and 100 µl tyrosinase (5000 U/ml) enzyme. The amperometric response currents of the CPE and MCPE to BPA were determined at –0.2 V. Additions were made to the cell between the range of $1.0 \cdot 10^{-6}$ – $5.0 \cdot 10^{-4}$ M BPA concentration, and the response currents obtained against the BPA concentration were plotted (Fig. 6a). Examining Figure 6a, it was observed that the currents in MCPE were nearly two times more of those in CPE. It was considered that PAMAM-Sal-Pt(II) in MCPE increased the currents by increasing the conductivity.

3.3. *Determination of the working potential*

To determine the working potential, at –0.10 V, –0.15 V, and –0.25 V potentials, MCPE was placed in a cell containing 0.1 M NaCl, phosphate buffer, and 100 µl tyrosinase (5000 U/ml) enzyme. BPA was added to the cell in the range of $1.0 \cdot 10^{-6}$ – $5.0 \cdot 10^{-4}$ M, and reply currents read against the BPA concentration were plotted. Examining Figure 6b, it was found that the quinone compound formed as a result of the enzymatic reaction had the highest reduction current at –0.15 V, and the working potential was determined to be –0.15 V.

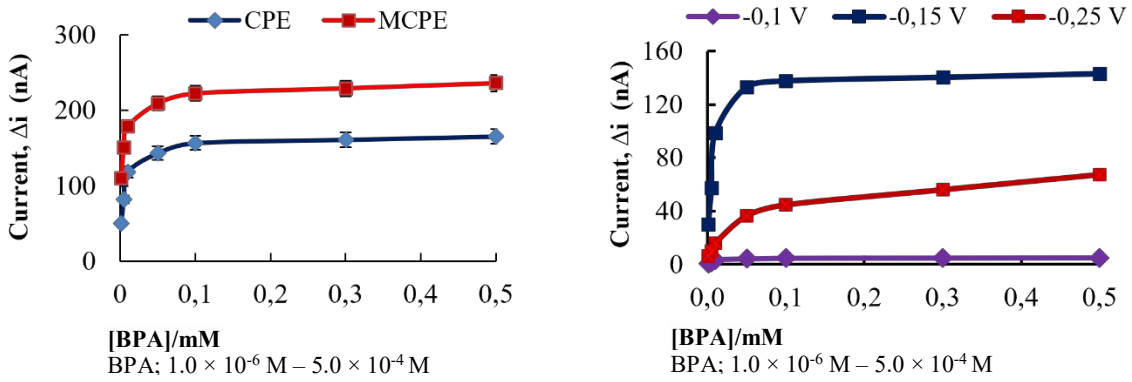


Fig. 6. (a) Amperometric response of CPE and MCPE to BPA. (b) Effect of working potential on the BPA sensitivity of MCPE (0.1 M, pH 7.0 phoshate buffer, 25 °C)

3.4. *Determination of the PAMAM-Sal-Pt(II) amount*

MCPE was prepared by using 0.5 mg, 1.0 mg, 1.5 mg, and 2.0 mg of polymer to examine the effect of the amount of polymer on the amperometric response of BPA. MCPE was placed in the cell containing 0.1 M NaCl, phosphate buffer, and 100 µl tyrosinase (5000U/ml). BPA was added to the cell so that the cell concentration was $1.0 \cdot 10^{-6}$ – $5.0 \cdot 10^{-4}$. The currents read against the BPA concentration were plotted (Fig. 7a). When Fig. 7a is analyzed, it is observed that the current differences increase as the amount of polymer increases. However, even though MCPE prepared in 2.0 mg of polymer gives a high current, the carbon paste in the electrode swelled and dispersed at the end of the experiment. Its mechanical stability was not good. It was observed that the most useful and highest current was in the electrode prepared by using 1.0 mg of polymer. At the end of this study, the amount of polymer was determined to be 1.0 mg.

3.5. *Determination of the amount of glutaraldehyde*

Glutaraldehyde (CHO-$CH_2CH_2CH_2$-CHO) is a cross-linking reagent commonly used in anologue studies. Glutaraldehyde is reacted with the amine groups in the enzyme's structure. Otherwise, because its molecular size is small, glutaraldehyde does not only interact with the amine groups on the enzyme surface but also diffuses into the interior of the enzyme and interacts with the internal amine groups. This reaction reduces the catalytic activity of the enzyme[40]. Therefore, the effect of the amount of glutaraldehyde to be used on the activity should be examined.

Biosensors prepared with 20.0 µl, 30.0 µl, and 40.0 µl glutaraldehyde (2.5 %) were used for the BPA determination to determine the amount of glutaraldehyde used in the prepared enzyme electrode. The currents read against the BPA concentrations were plotted (Fig. 7b). As seen in Figure 7b, the response currents increased when the

amount of glutaraldehyde increased from 20.0 µl to 30.0 µl. It was observed that the response currents decreased when the amount of glutaraldehyde increased to 40.0 µl. The results obtained were interpreted as follows; it was thought that 20.0 µl of glutaraldehyde was not enough to immobilize the enzyme and could not hold the structure together, since 40.0 µl of glutaraldehyde became too much and lost the enzyme's activity due to excessive binding.[39] When the graph obtained (Fig. 7b) was examined, it was found that the highest current value was in the electrode using 30 µl of glutaraldehyde.

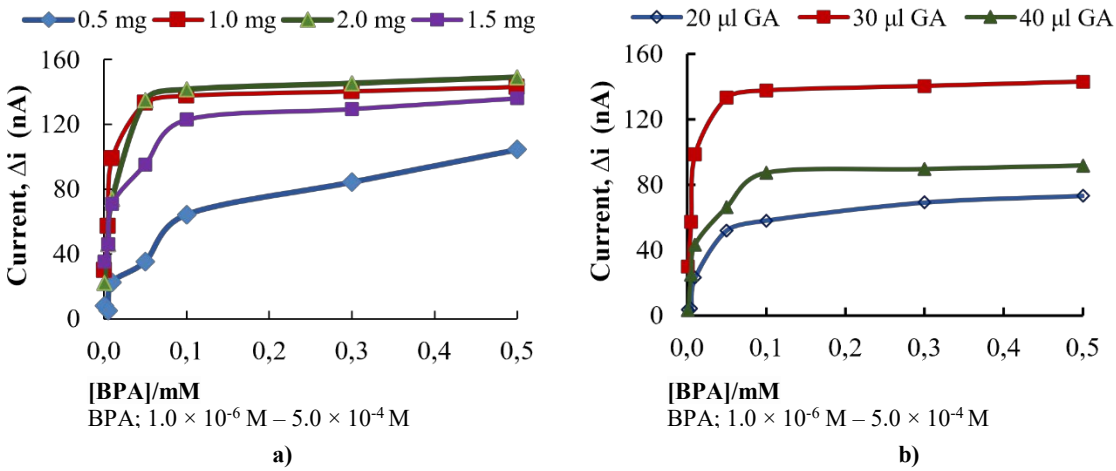


Fig. 7. (a) Effect of the amount of PAMAM-Sal-Pt(II) on the BPA sensitivity of MCPE
(b) Effect of the amount of glutaraldehyde (2.5% glutaraldehyde solution was used)

3.6. *Effect of pH and temperature*

pH is a significant parameter that influences the activity of enzymes. To investigate the influence of pH on the amperometric response of the prepared biosensor to BPA, 0.1 M pH 5.0 acetic acid/sodium acetate buffer, 0.1 M pH 6.0, 7.0, and 8.0 phosphate buffer, and 0.1 M pH 9 tris/glycine buffer were used. The BPA solution was added so that the cell concentration was $1.0 \cdot 10^{-4}$ M. Current differences versus BPA concentration were plotted (Fig. 8a). Examining Figure 8a, it was seen that the highest current was at pH 7.0. In the next studies, 0.1 M phosphate buffer solution with a pH of 7.0 was used. Although there is a pH value compatible with our study in biosensor studies prepared with different support materials and tyrosinase enzymes in the literature, there are also different pH values (e.g., pH 7.0[42,43], pH 6.5[44], pH 6.0[45], pH 7.4[46], pH 6.2[47]). This change in pH values may be due to the dissimilar immobilization type and the material that is immobilized.

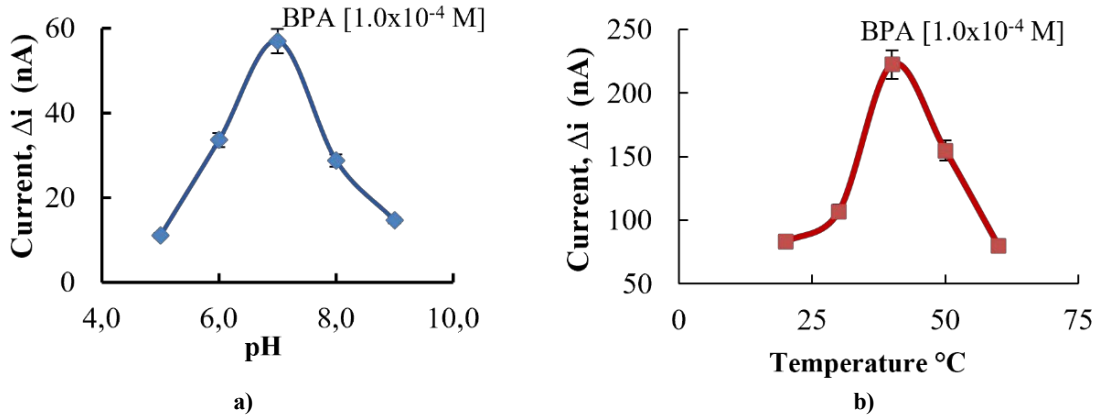


Fig. 8. (a) Effect of pH on the response of the biosensor (25 °C, $1.0 \cdot 10^{-4}$ M BPA)
(b) Effect of temperature on the response of the biosensor (pH 7.0 PBS, $1.0 \cdot 10^{-4}$ M BPA)

Because enzymes have a protein structure, they are affected by temperature changes. There are optimum temperature values at which enzymes show their maximum activity. The effect of temperature on the amperometric response to BPA of the biosensor prepared at temperatures of 20 °C, 30 °C, 40 °C, 50 °C, and 60 °C was investigated. The BPA solution was added so that the cell concentration was $1.0 \cdot 10^{-4}$ M. The current differences against BPA concentrations were plotted (Fig. 8b). Examining Figure 8b, it was observed that the highest current was at 40 °C. However, room temperature was used to make the measurements more practical and easy in future studies.

When biosensor studies prepared with tyrosinase enzyme combined with different materials[40–42,45] are examined, various optimum temperature values can be seen. The reason for the different temperature values may be due to the difference in the immobilization type and material that is immobilized.

3.7. *Effect of Bisphenol-A concentration*

To examine the effect of the substrate concentration on the amperometric response of the biosensor to BPA, the reduction currents of the quinone compound formed as a result of the enzymatic reaction at –0.15 V with increasing BPA concentrations ($1.0 \cdot 10^{-9}$–$1.0 \cdot 10^{-3}$ M) were recorded. The currents obtained and the current differences versus BPA concentration were plotted (Fig. 9a). Examining Figure 9a, it is observed that as the concentration of BPA increases, the current differences first increase linearly and then deviate from linearity in a hyperbolic manner. After the BPA concentration become $1.0 \cdot 10^{-4}$ M, it can be seen that the increase in the current differences is very small. On that concentration, the tyrosinase enzyme became saturated to BPA, and therefore, the change in the current differences was very small.

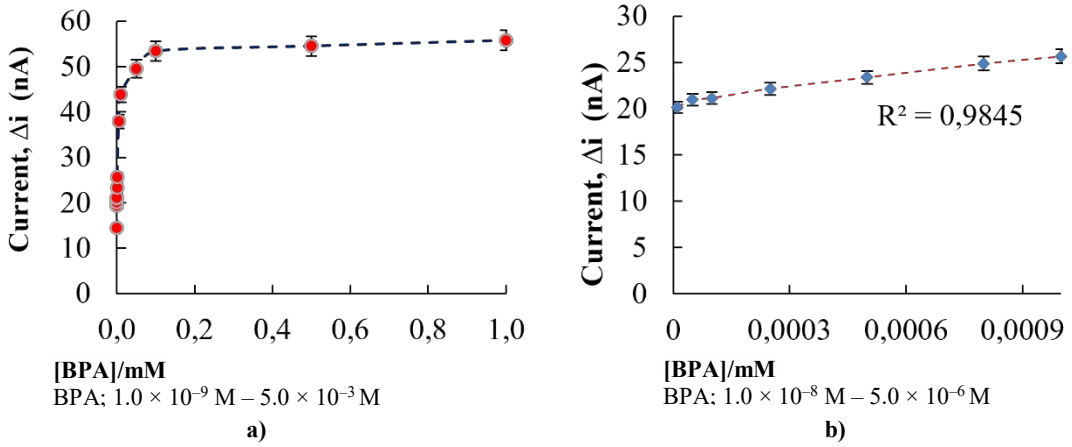


Fig. 9. (a) The effect of BPA concentration on the response of the biosensor (25 °C, $1.0 \cdot 10^{-9}$–$1.0 \cdot 10^{-3}$ M BPA, 0.1 M pH 7.0 PBS) (b) The calibration curve of the BPA biosensor (25 °C, 0.1 M pH 7.0 PBS).

A calibration graph for BPA determination (Fig. 9b) was obtained by graphing the data in the region where Figure 9a is linear. From Figure 9b, it was determined that the linear working range was between 0.01–1.0 μM ($R^2 = 0.9845$), and the detection limit was 1 nM.

When the literature is examined, it can be seen that there are biosensors with different linear working range (LWR) and detection limit (DL) values such as LWR of $2.5 \cdot 10^{-3}$ – 3.0 μM, DL of 1 nM[24], LWR of 5–40 μM, DL of 0.082 μM[48], LWR of $1.0 \cdot 10^{-6}$ – $5.0 \cdot 10^{-5}$ M, DL of $5.0 \cdot 10^{-7}$ M[32], LWR of 0.28–45.05 μM, and DL of 0.066 μM[45]. When compared with the literature, it can be said that the linear working range of the BPA biosensor prepared in this study is quite wide and the detection limit is low. This situation can be explained by the PAMAM dendrimers used in the preparation of the biosensor. In the literature, it is stated that PAMAM dendrimers increase the detection sensitivity of various biomolecules such as antibodies and enzymes with the help of their large number of terminal functional groups[28]. It is known that a wide operating range and low detection limit in electrochemical biosensors increase the value of the biosensor.

It was determined that the *Km* (*app*) value of the biosensor was 0.00125 μM and the Imax (*app*) value was 0.00312 μA. The *Km* (*app*) value showing the affinity of the prepared biosensor to BPA is

lower than the *Km* (app) values given in the literature; 3.26 μM[48], $1.2 \cdot 10^{-5}$ M[49], 0.34 mM[50], and $1.04 \cdot 10^{-3}$ mM[32]. The Km value of the prepared biosensor is lower than those in the literature. A low Km value indicates that the enzyme has a high affinity for the substrate. The PAMAM-Sal-Pt(II) used in the preparation of the biosensor increased the affinity of the enzyme to the substrate by facilitating the electron transfer between the enzyme and the substrate.

3.8. *Reproducibility and storage stability of the biosensor*

To determine the reproducibility of the biosensor brought into equilibrium under optimum conditions, the measurements were taken by adding a $2.0 \cdot 10^{-5}$ M BPA solution to the cell. This process was repeated consecutively 16 times. The relative standard deviation calculated from the current changes obtained as a result of 17 measurements was found to be 3.49 %. At the end of 17 measurements, it was observed that the biosensor preserved 99 % of its initial activity. From these results, it can be said that the reproducibility of the prepared biosensor is very good.

To measure the change in the performance of the prepared BPA biosensor when it is not used, measurements were taken from the biosensor at certain intervals for 25 days. The reduction currents of the quinone compound formed as a result of the enzymatic reaction were measured by adding a BPA solution with an intracellular concentration of $2.0 \cdot 10^{-5}$ M to the equilibrated biosensor under optimum conditions. The amperometric response of the biosensor to BPA decreased over time. It was observed that the biosensor preserved 24.05 % of its initial activity at the end of 25 days. According to this result, it can be said that the storage stability of the prepared BPA biosensor is short.

As a result, it is thought that the prepared biosensor is more suitable for multiple uses in the same day.

3.9. *Investigation of the effect of species that interfere with the Bisphenol-A determination*

Interference effects of phenol, nitrophenol, urea, potassium nitrate, hexane, acetonitrile, and ethyl acetate, which are substances that may interfere with BPA determination, were examined. The concentration of Bisphenol-A in the cell was $2.0 \cdot 10^{-8}$ M, and the concentration of interfering substances was $2.0 \cdot 10^{-9}$ M.[45–48] Eliminating the interference or reducing it to the lowest possible percentage is a very important factor in the detection and accuracy of a real sample analysis. It was found that none of these substances interfere with BPA determination.

3.10. *Application of the biosensor to real samples*

Orthodontic bracket adhesive is a chemical product that is used in dentistry to attach the bracket to the tooth surface, and the use of this material is by means of a light-assisted polymerization reaction. It includes BPA derivates such as bisphenol a diglycidyl ether dimethacrylate (BIS-GMA), bisphenol a dimethacrylate. Previous studies showed that BPA molecules may leach after the polymerization of this material during bracket bonding[51–53]. Because of this reason, an orthodontic adhesive (Transbond XT Light Curing Adhesive; 3M Unitek, Monrovia CA, USA) was used for testing the biosensor on real samples. An adhesive sample was prepared in a metal mold (2 mm in diameter and 2 mm in thickness), and a LED light source (VALO Ortho; Ultradent Products, South Jordan, Utah) was used for activation of the photopolymerization. All polymerization processes were made according to the products' manufacturer rules. The polymerized orthodontic adhesive was put into a glass tube containing 5 ml of distilled water and kept for 2 h. After that, the water in the tube was taken to another tube. 100 μl of the sample was added to the cell, and a current value was read. This value was placed on the calibration graph (Fig. 9b), and 0.167 μg/l BPA was detected in 100 μl of water. Considering that the composite was kept in 5 ml of water, it was calculated that there was 8.35 μg/l BPA in 5 ml of water. As a result, the efficiency of the biosensor on real samples was observed.

4. CONCLUSIONS

In this study, a new biosensor based on the tyrosinase enzyme was prepared by using a carbon paste electrode modified with PAMAM-Sal-Pt(II) for BPA determination. PAMAM-Sal-Pt(II) was synthesized and characterized for the first time for this biosensor system. In the biosensor design, the tyrosinase enzyme was successfully immobilized on the MCPE surface by cross-linking with glutaraldehyde. The experimental results explain that the prepared biosensor system performs well for the determination of BPA. The PAMAM-Sal-Pt(II) nanoparticle, which we used in the MCPE preparation, increased the sensitivity of the biosensor to BPA by increasing the conductivity and facilitating

electron transfer in the enzymatic reaction. The biosensor has a wide working range (0.01–1.0 μM (R^2 = 0.9845)) and a very low detection limit (1 nM). The wide operating range and low detection limit are very important advantages for a biosensor system. Therefore, it is seen that low concentrations of BPA can be determined with the prepared biosensor. The reproducibility of the biosensor is very good. Considering the shelf life of the biosensor system, it was observed that the used enzyme lost its activity over time. Therefore, the prepared biosensor is considered to be more suitable for multiple uses in the same day. It was observed that no substances interfered in the BPA determination in the biosensor system, which is crucial to the accuracy of the determination. Therefore, the prepared BPA biosensor can be used for BPA determination in different real samples, and it can be prepared easily and cost-effectively.

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***Author contributions*:** F. Arslan and N. Sarı conceived and planned the experiments and contributed to the interpretation of the results. H. Koçak and O. C. Bodur carried out the experiments about biosensor. E. Hasanoğlu contributed to the synthesis preparation. B. Arslan carried out the real sample experiments. All authors helped designing of the research, analysis, and manuscript.

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