A sensitive amperometric detection of neurotransmitter acetylcholine using carbon dot-modified carbon paste electrode

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Abstract

Acetylcholine is a neurotransmitter, which is located at the intersections of the nerve and muscles in the lymph nodes of the internal organs motor systems and in various parts of the central nervous system. A decrease of acetylcholine in brain is associated with Alzheimer's disease. That is why it is an important agent for this disease. In this study, a bienzymatic biosensor system with acetylcholine esterase and choline oxidase was prepared with carbon paste electrode modified with carbon nano Dot-(3-Aminopropyl) triethoxysilane (CDs-APTES) for determination of the amount of acetylcholine. Acetylcholine esterase and choline oxidase enzymes were immobilized onto a modified carbon paste electrode by cross-linking with glutaraldehyde. Determination of acetylcholine was carried out by the oxidation of enzymatically produced H_2O_2 at 0.4 V versus Ag/AgCl. The effect of temperature, pH, and substrate concentration on the acetylcholine response of the prepared biosensor was investigated. In addition, the optimum CDs-APTES amount, the linear operating range of the biosensor, and the interference effect were also investigated. Volume 00, Number 0, Pages 1–10, 2020

Keywords: *acetylcholine esterase, biosensor, choline oxidase*

1. Introduction

Alzheimer's disease is a common brain disorder in which behavioral disorders can be seen, and daily practices, such as memory, speech, direction finding, problem solving, and getting to know people, are weakened as a result of the gradual disappearance of cells in some parts of the brain [1].

Biosensors are highly selective and require high detection sensitivity and a wide range of analytical devices that enable the detection of various substances in living beings. Because of their low cost and fast response time, continuous signal detection makes the use of biosensors attractive [2].

Although there are many causes of Alzheimer's disease, these are summarized by the cholinergic hypothesis. According to this hypothesis, acetylcholine (ACh), which is a neurotransmitter, enables the transmission of messages between nerve cells in the brain and is stable in a healthy brain [1, 3]. As a result of the researches, in most of the patients with Alzheimer's disease, decreased amounts of ACh in their brains were determined and it was concluded that the memory was deteriorated with the loss of this important chemical substance. Studies on Alzheimer's disease patients also showed that ACh synthesis decreased with a decrease in choline acetyltransferase activity. Research on the ways of eliminating ACh deficiency in the brains of people with Alzheimer's disease and increasing the amount of ACh and determining the level is very important [3].

ACh loses its activity by being hydrolyzed by AChE enzyme. Two molecules are formed as choline and acetic acid as a result of hydrolysis. Choline converts to betaine and hydrogen peroxide in the presence of choline oxidase (ChO) (E.C. 1.1.3.17) with oxygen and water. By combining these two enzymes with the biosensor system, biosensors are being developed for use in the diagnosis of Alzheimer's disease [4-6].

Abbreviations: *ACh, Acetylcholine; AChE, Acetylcholine esterase; BSA, Bovine serum albumine; CDs, Carbon nanodot; CDs-APTES, Carbon nanodot-(3-Aminopropyl)triethoxysilane; ChAT, Choline acetyltransferase; ChO, Choline oxidase; CPE, Carbon paste electrode; MCPE, Modified carbon paste electrode; GA, Glutaraldehyde; LOD, Limit of dedection.*

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Received 23 October 2019; accepted 11 January 2020

DOI: 10.1002/bab.1886

Published online in Wiley Online Library (wileyonlinelibrary.com)

ACh could be detected by using various analytical methods like HPLC, GC-MS, chemiluminescence, etc., but these methods are expensive, time-consuming, and require a specialist to comment on the results. Biosensors are a kind of instrument that enables cheaper and rapid detection of analyte with low detection limit [4-6]. This situation makes biosensors preferable sensors.

In this study, a new amperometric ACh biosensor based on carbon paste including carbon nanodots-(3-aminopropyl)triethoxysilane (CDs-APTES) was prepared. For this purpose, first CDs-APTES were synthesized. Subsequently, the carbon paste electrode (CPE) was modified with CDs-APTES. AChE and ChO enzymes were immobilized on modified carbon paste electrode (MCPE) using cross-linking agents such as glutaraldehyde and bovine serum albumin (BSA). The amount of ACh was determined by measuring the anodic currents of H_2O_2 released by the enzymatic reaction. The optimum working conditions (temperature, pH, glutaraldehyde concentration, and substrate concentration) of the prepared ACh biosensor were investigated. The linear range and limit of detection of the biosensor were determined. Then, the factors affecting the performance of the biosensor (repeatability and interference effect) were examined.

Highlights

1. Alzheimer is a common brain disorder, and acetylcholine, a neurotransmitter, is an important marker for detection of Alzheimer's disease.
2. Biosensors are highly selective and require high detection sensitivity and a wide range of analytical devices.
3. The amount of acetylcholine can be determined by using biosensors for early diagnosis of Alzheimer's disease.

2. Materials and Method

Acetylcholinesterase (EC 3.1.1.7, purified from the *Electrophorus electricus* (electric eel) type V-S activity of 200.0 unit/mL) and choline oxidase (EC 1.1.3.17, purified from the *Alcaligenes* sp., activity of 10.0 unit/mL) were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Acetylcholine chloride, BSA, and Nujol were also purchased from Sigma Aldrich (St. Louis, Missouri, USA). Electrochemical measurements were performed by using the three-electrode system using the CHI 1230B instrument. All electrochemical experiments were performed on a CHI 1230B electrochemical workstation (CH Instrument, BASi, Kent avenue, West Lafayette, USA). The three-electrode system was used that consists of carbon paste electrode ,which has a paste area of 0.6 mm deep and 0.3 cm diameter (specially made of Teflon) as the working electrode (Fig. 1), a platinum wire as the counter electrode, and Ag/AgCl as the reference electrode. An Orion 720A pH/ion meter (Thermo Fisher Scientific, Waltham, MA, USA) was used for pH measurements. The solution temperature was controlled using a Grant W14 thermostat (Grant Instruments, Cambridge, UK).

2.1. Extraction and characterization of carbon dots

Carbon dots were simply extracted from sugar beet molasses supplied by Konya Sugar Factory. Briefly, approximately 5 g of sugar beet molasses was dissolved in 10 mL of deionized water and mixed until to obtain a homogeneous solution. Purification was made using centrifugation for 5 min at 2,800 g, and supernatant-containing CDs was diluted with water (1:80) (4.3 mg/mL) [7].

Morphologies of molasses CDs were characterized by using a transmission electron microscope (TEM), selected area electron diffraction (SAED) ,and X-ray diffraction (XRD) analysis [8].

2.2. Silica coating of CDs with APTES

CDs were coated with APTES according to Lin et al.'s method [9]. First, CDs were dispersed into PBS 7.4 solution. Then, CDs (0.9 mg mL^{-1}) and APTES (3.33%) were vigorously stirred for 24 h at room temperature (Fig. 2).

2.3. Nanodots-modified carbon paste electrode preparation

Graphite powder (0.0650 g) was weighed precisely to prepare the carbon paste electrode, and 100 μL (2.21 mg/mL) of CDs-APTES and 30 μL of Nujol were added. The homogeneously stirred mixture was filled into the electrode chamber so that no space was left in between, and the surface became smooth.

2.4. Enzyme electrode preparation and amperometric measurements

A mixture was prepared consisting of 1 mg of BSA , 60 μL phosphate buffer solution (pH 7.0), 10 μL of AChE (200.0 units/mL), 200 μL of ChO (10.0 units/mL), and 20 μL of glutaraldehyde (2.5%). The ratio of enzymes was used as 1:1 [6]. This mixture was dripped in equal amounts to the surface of the nanodots-modified carbon paste electrode and dried at room temperature. The prepared enzyme electrode was first washed with distilled water and then with buffer solution and stored in the refrigerator at +4 °C in the buffer solution when not in use.

The amount of acetylcholine was determined by electrochemical measurement of hydrogen peroxide released by enzymatic reactions:

$$\text{Acetylcholine} + H_2O \rightarrow \text{Acetate} + \text{Choline} \quad (\text{AChE})$$

$$\text{Choline} + 2O_2 + H_2O \rightarrow \text{Betaine} + 2H_2O_2 \quad (\text{ChO})$$

$$H_2O_2{}^{-} \rightarrow O_2 + 2H^+ + 2e^- \quad (\text{Electrode})$$

The enzyme electrode was immersed in pH 7.0 (0.1 M) phosphate buffer solution. The solution contains 0.1 M sodium chloride as a support electrolyte. The electrode was equilibrated at +0.4 V (Ag/AgCl vs. electrode (3.0 M KCl)) until a constant current was obtained. The equilibrium current (i_a)

FIG. 1 *Teflon working electrode.*

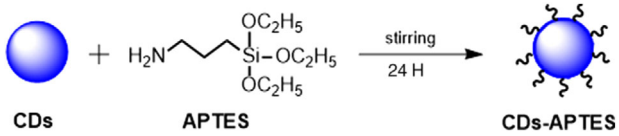

FIG. 2 *Synthesis of CDs-APTES.*

was recorded, the ACh solution was added from the stock solution to the cell, and the system was stirred. At the end of the reaction, the final current (i_b) was recorded. The ACh concentration was plotted against the obtained current difference (Fig. 3).

3. Results and Discussion

In this study, a new amperometric ACh biosensor was prepared based on carbon paste modified with CDs-APTES. For this purpose, CDs-APTES was first synthesized. Subsequently, the carbon paste electrode was modified with CDs-APTES. A double enzyme modified electrode was prepared by immobilizing AChE and ChO enzymes on MCPE.

The scheme for the determination of acetylcholine is shown in Fig. 4. Biochemical reactions occur between AChE and ChO enzymes immobilized on the modified carbon paste electrode surface with ACh. First, ACh is hydrolyzed to Ch and acetic acid with the help of AChE where Ch is oxidized to betaine with ChO. FAD, the prosthetic group of ChO, is reduced to $FADH_2$ by taking electrons. $FADH_2$ in the enzyme is oxidized by giving electrons to the oxygen in the solution to make the reaction reversible. Thus, the enzyme returns to its initial form. Oxygen is reduced to H_2O_2 by taking electrons. The H_2O_2 produced by these reactions is oxidized onto the surface of the MCPE. Thus, ACh determination can be made by measuring the anodic current of H_2O_2 on the electrode surface.

The optimum conditions of the prepared ACh biosensor and the factors affecting the performance of the biosensor were examined.

3.1. Silica-coated molasses CDs

As mentioned previously, CDs naturally found in sugar beet molasses were extracted from molasses following the green, simple, and low-cost extraction method [7]. Molasses CDs gave blue fluorescent and had hydrophilic surface functional groups of O-–H, C–H, C=C, C–C, and C–O.

As shown in Fig. 5, molasses CDs were nearly spherical in shape and monodispersed [20]. Average particle sizes of molasses CDs were calculated as 2.3 nm (Fig. 5B). According to TEM-SAED analysis (Fig. 5A), molasses CDs had a halo-like view, which shows a typical amorphous structure of CDs. However, bright diffraction spots as shown in the figure were attributed to some crystallinity in molasses CDs due to the presence of other larger-sized particles. According to XRD patterns of CDs in Fig. 6, CDs gave a broad peak centered at ca. 20° (2θ), which indicates the amorphous structure of CDs [8].

Surface functionalization improves stability of nanoparticles including CDs and supplies different functional groups of most popular functionalization methods; silica coating become prominent due to easiness and high stability etc. [10]. In this study, first, molasses CDs are capped with silica using APTES, which is commonly used as an economic and efficient surface modification organosilane agent [11]. The broad characteristic peak around 3400 cm^{-1} can be assigned to the stretching vibration of O–H. The peaks at 2930, 1410, and 1633 cm^{-1} demonstrated the presence of $–CH_2–$ and C=O, respectively. The stretching vibration of Si–O around 1100 cm^{-1} is due to the silane bonding on the surface of the CDs (Fig. 7).

3.2. Determination of sensitivity and working potential of CPE and MCPE to H_2O_2

To determine the working potential, the sensitivity of MCPE to H_2O_2 was investigated at potentials ranging from + 0.1 to +0.7 V. When Fig. 8 is examined, it is seen that as potential increases anodic currents of H_2O_2 also increase. However, it is known that the interference effect of some species (ascorbic acid, uric acid, etc.) found in body fluid is quite high at high potentials [12]. Therefore, +0.4 V, which provides high response current and linearity, was determined as the most suitable working potential [5, 13].

Standard H_2O_2 solutions were used to compare the sensitivity of CPE and MCPE to H_2O_2. After the CPE and MCPE were brought to constant current at +0.4 V potential, the equilibrium current was recorded and in additions were made in such a way that the concentration of H_2O_2 in the electrochemical cell was in the range of 1.0×10^{-8} to 1.0×10^{-3} M. Current differences after H_2O_2 addition at increasing concentrations were recorded and plotted (Fig. 9). Figure 9 shows that MCPE is approximately 100 times more sensitive to H_2O_2 than CPE.

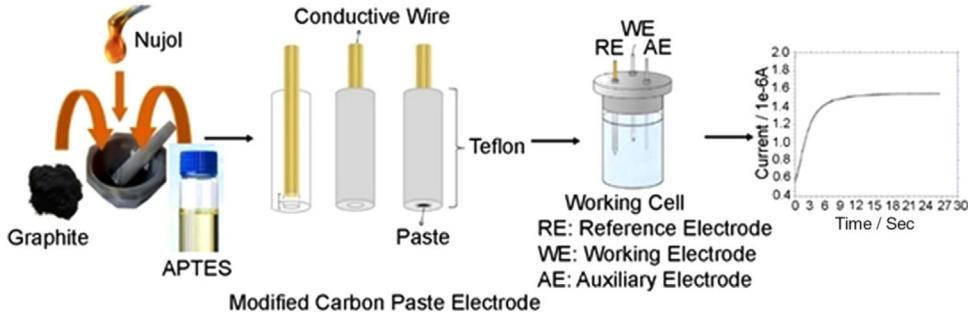

FIG. 3 *MCPE preparation and working cell diagram.*

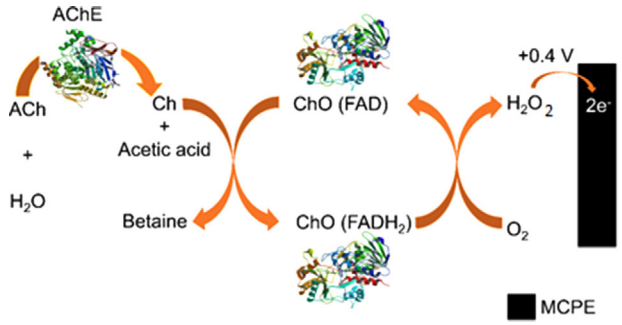

FIG. 4 *Scheme of ACh determination reaction.*

3.3. Effect of CDs-APTES amount on H_2O_2 sensitivity of MCPE

To test the amount of CDs-APTES in MCPE in the sensitivity of H_2O_2, carbon nanodots-APTES in varying proportions were added to the prepared paste and working electrode was prepared. Anodic currents of H_2O_2 were measured with each electrode (Fig. 10). Mechanical stability of the MCP electrode prepared with 200 μL CDs-APTES was not good. CDs-APTES flowed from the electrode surface. The electrode prepared with 100 μL CDs-APTES (2.21 mg/mL) showed the best linearity and the highest response current.

3.4. Effect of pH on amperometric response of biosensors

Buffer solutions at different pH (5.0, 6.0, 7.0, 8.0, and 9.0) were used to determine the effect of pH on the amperometric response of the biosensor. The buffer solution of pH 5.0 was prepared from acetic acid/sodium acetate solutions, and the buffer solutions of pH 6.0, 7.0, and 8.0 were prepared from sodium dihydrogen phosphate/sodium monohydrogen phosphate solutions. When the Δi response current values obtained at constant ACh (1.0×10^{-4} M) concentration were plotted against pH, the highest current was obtained from pH 7.0 phosphate buffer (Fig. 11). AChE biosensor studies with different materials show optimum pH values comparable with this study. For example, pH has been found as 7.4 and 7.5, respectively [14-17]. However, there are also different pH values as 9.0 [6], 9.5 [18], and 8.0-9.0 [19]. This change in pH values may be due to the different immobilized material and the type of immobilization, because changes in the environment around the enzyme active site can lead to different interactions between the enzyme and the immobilization material [20]. In addition, immobilized enzymes can form a more stable substrate–enzyme complex compared to their free forms and show high activity at different pH values.

3.5. Effect of temperature

Temperature is another important factor that affects enzyme activity. It is therefore important to investigate the temperature-dependent response current of the enzyme electrode. 1.0×10^{-4} M of ACh solution was used at temperatures ranging from 20 to 70 °C (Fig. 12). When Fig. 12 is examined, it is seen that the current difference values increase up to 60 °C and decrease after 60 °C. Working conditions for biosensors should be practical, so studies were performed at room temperature. In the literature, there are studies of ACh biosensor with optimum temperature value different from 60 °C, for example, 35 °C [18] and 55 °C [4]. These different values may be due to the type of polymer and immobilization used in the process. In addition, it was found that the immobilized enzymes showed higher activity at temperatures different from physiological temperature compared to their free forms. It can be said that the CDs-APTES, which we studied, increased the thermal stability of AChE and ChO enzymes.

3.6. Substrate concentration and calibration curves

The effect of substrate concentration on the reaction rates catalyzed by immobilized AChE and ChO enzymes was examined in the range of 1.0×10^{-8} M to 1.0×10^{-4} M in the cell (Fig. 13). Two linear regions were determined between 1.0×10^{-8} to 1.0×10^{-7} M and 1.0×10^{-7} to 1.0×10^{-5} M intervals (Figs. 14 and 15). Both graphs show good linearity and can be used for quantitative determination of ACh. There are also biosensors with different linear working ranges (2.0×10^{-4} to 1.3×10^{-3} M and 1.2×10^{-4} to 1.0×10^{-2} M) in the literature [17, 21]. For the ACh biosensor, limit of detection was calculated according to $3s/m$ (where s is standard deviation of measurements and m is slope of the curve) and was found as 5.0×10^{-9} M) and

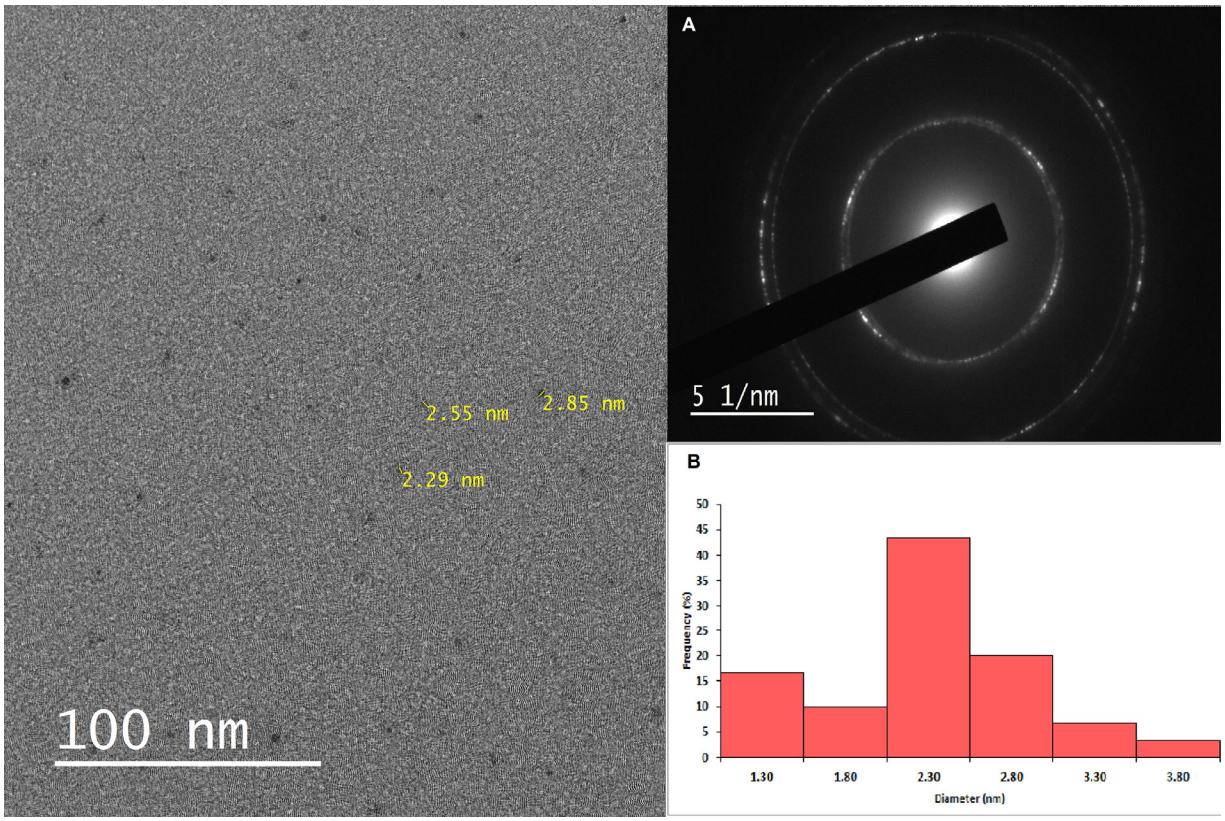

FIG. 5 *TEM image of molasses CDs. (A) SAED pattern of molasses CDs. (B) The size distribution histogram of CDs [8].*

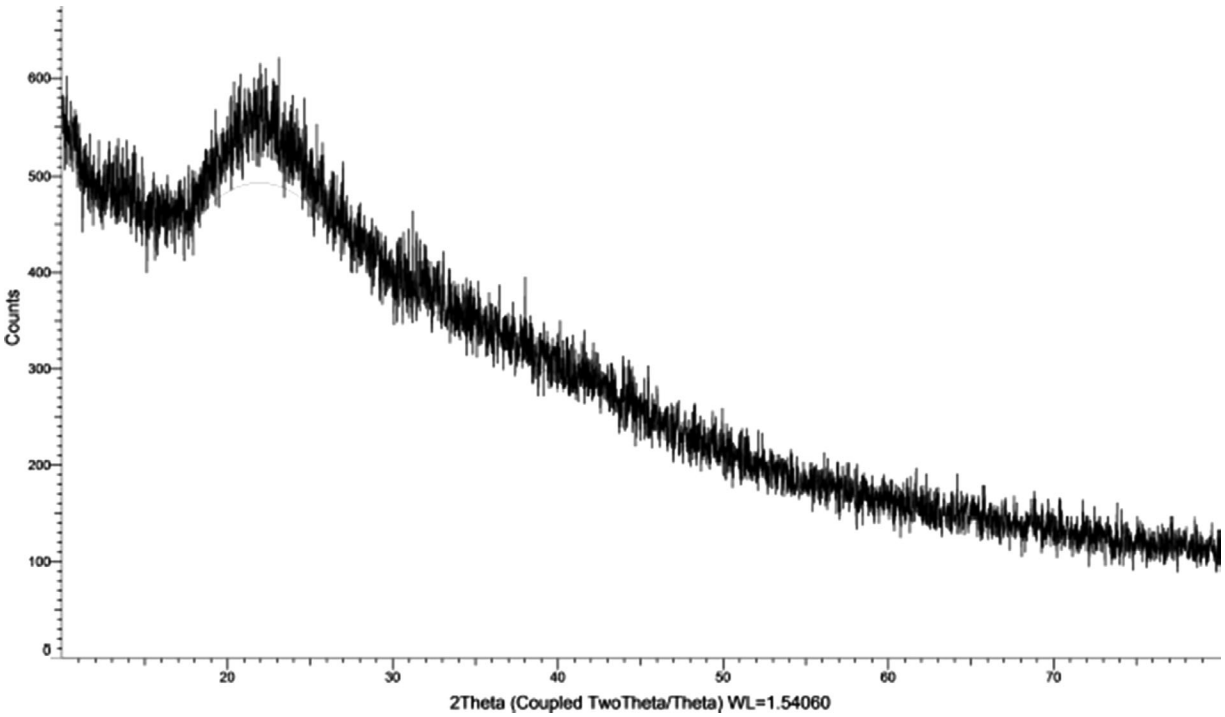

FIG. 6 *XRD pattern of CDs [8].*

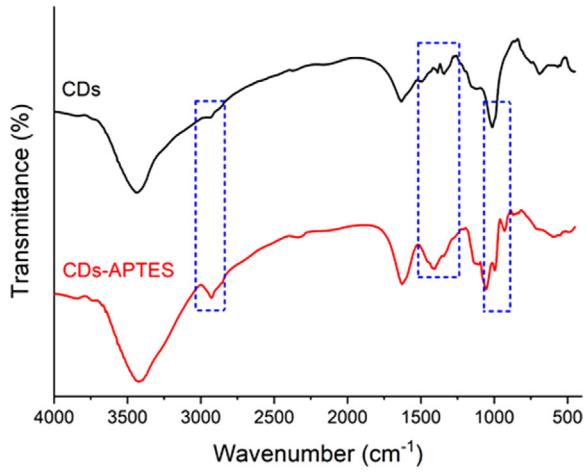

FIG. 7 *FT-IR spectra of CDs and silica coated CDs.*

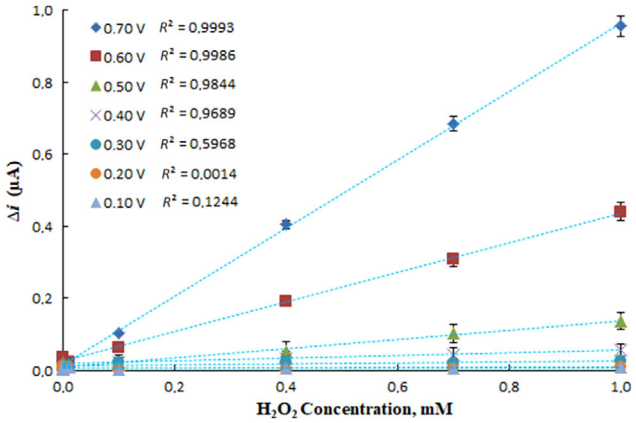

FIG. 8 *Effect of working potential on H_2O_2 sensitivity of MCPE.*

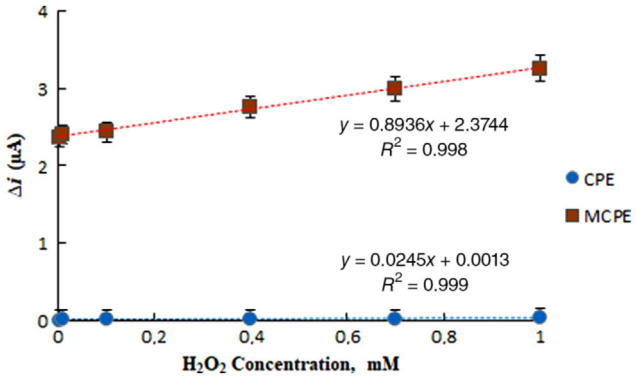

FIG. 9 *Amperometric responses of CPE and MCPE to different concentrations of H_2O_2.*

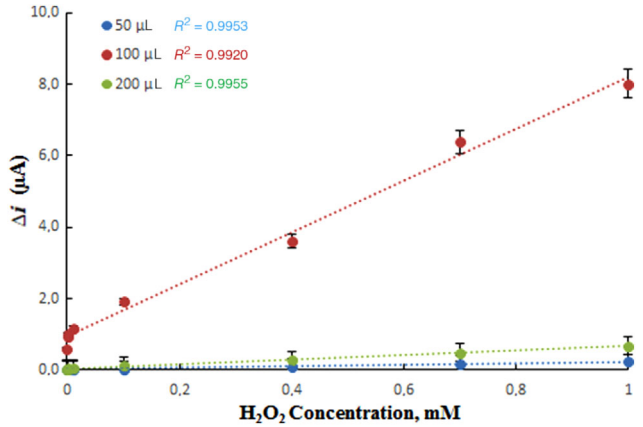

FIG. 10 *Effect of CDs-APTES amount on H_2O_2 sensitivity of MCPE.*

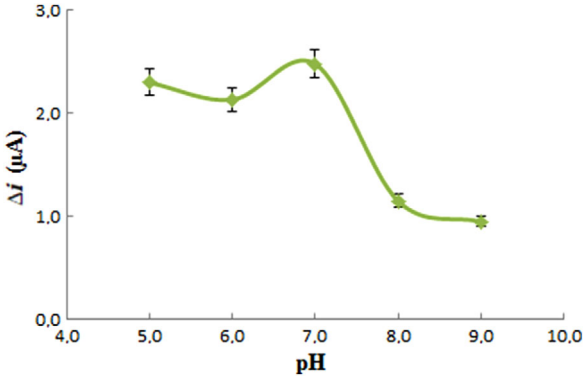

FIG. 11 *Effect of pH on the response of the biosensor (at 25 °C, 1.0 × 10^{-4} M ACh, at +0.4 V operating potential, n = 3).*

response time was determined as 200 sec. The limit of detection of ACh biosensors in the literature was found to be lower than the limit of detection values [14]. In Table 1, important analytical parameters are compared with other ACh sensors including carbon material. Owing to its wide working range and low detection limit, the ACh biosensor prepared in this study makes it possible to determine ACh at low concentrations. To find the K_m constant, ACh concentration versus current change data was used and 1/[ACh] – 1/Δi graph (Lineweaver–Burk curve; Fig. 16) was plotted. K_m (observed) and I_{max} (observed) values were calculated as 0.0012 μM and 3.12 μA, respectively. The K_m (observed) value of the prepared biosensor indicating ACh affinity is lower than the K_m (observed) values given in the literature [4, 18]. This result shows that the enzyme has a high affinity for the substrate.

3.7. Operational stability of the ACh biosensor

To investigate the reusability factor, the current changes obtained as a result of successive measurements at a constant ACh concentration (1.0 × 10^{-6} M) were plotted against the

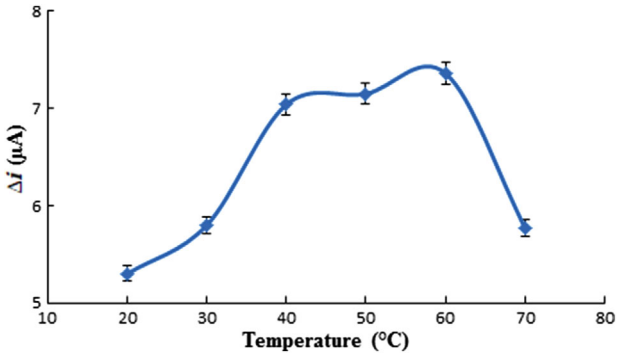

FIG. 12 *Effect of temperature on the response of the biosensor (1.0 × 10^{-4} M ACh, at +0.4 V operating potential,* n = *3).*

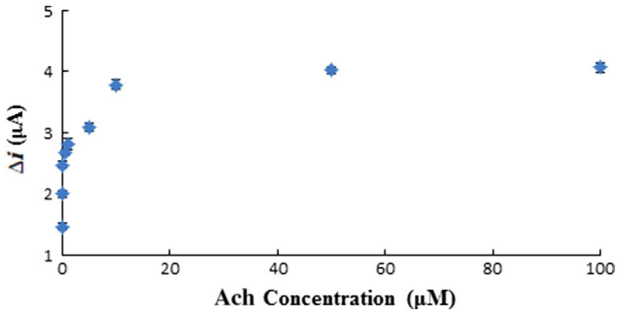

FIG. 13 *Effect of ACh concentration upon the amperometric response of the biosensor.*

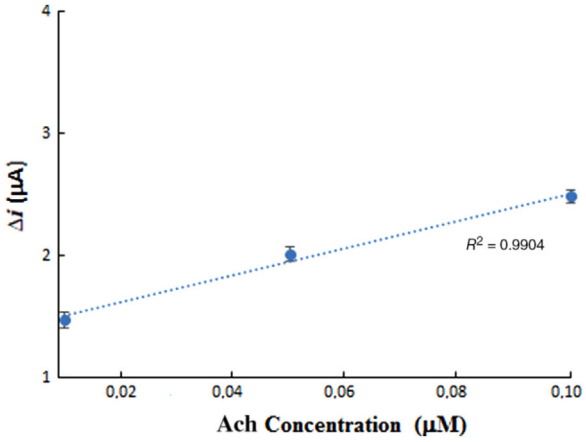

FIG. 14 *The calibration curve of the ACh biosensor 1 (0.1 M, pH 7.0 PBS, 25 °C, +0.4 V).*

number of measurements (Fig. 17). The relative standard deviation calculated from the current changes obtained as a result of 20 measurements was found to be 2.7%. It was observed that the biosensor lost 5.1% of initial activity after 20 measurements. It is stated that the electrode lost its activity at the end of 18 measurements by 16.9% compared to the

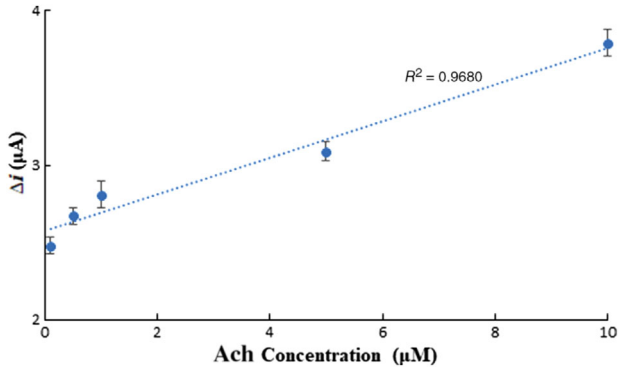

FIG. 15 *The calibration curve of the ACh biosensor 2 (0.1 M, pH 7.0 PBS, 25 °C, +0.4 V).*

beginning [6]. The reusability of the electrode used in this study was found to be quite good.

3.8. Storage stabilization of the enzyme electrode

The amperometric response current of the biosensor was measured at 1.0 × 10^{-6} M ACh concentration at certain intervals (1–3–10–15–20. days) for 20 days by resting the enzyme electrode in a pH 7.0 phosphate buffer in the refrigerator (+4 °C). A graph showing the response current change values versus the storage time was plotted (Fig. 18). When Fig. 18 was examined, it was found that there was no change during the first 3 days, and that the current decrease was observed between 3 and 10 days and the decrease continued after the 10th day. Biosensor retained 30% of initial activity after 20 days.

3.9. Interference effects

The effect of some substances in blood serum that may have the interference effect on ACh determination was investigated. These species and their concentrations are ascorbic acid (1.0×10^{-4} M), uric acid (3.0×10^{-4} M), paracetamol (1.0×10^{-4} M), and glucose (5.0×10^{-3} M). The ACh concentration was kept constant at 5.0 × 10^{-6} M. The interference effect of these substances after 300-fold dilution was examined. The interference effect is one of the most important problems faced in the real sample analysis during amperometric biosensor studies. Dilution is a commonly used method for preventing or reducing interference [22, 23]. The elimination of the interference or its reduction to the minimum percentage is a crucial factor in the detection and accuracy of real sample analyses. The interference values determined as a result of the studies are given in Table 2.

3.10. ACh determination in a synthetic blood sample

A synthetic blood solution containing interferes such as ascorbic acid (1.0 × 10^{-4} M), uric acid (3.0 × 10^{-4} M), ethanol (0.0109 M), methanol (5.0 × 10^{-5} M), paracetamol (1.0 × 10^{-4} M), cystine (6.5 × 10^{-5} M), cysteine (1.0 × 10^{-4} M), and glucose (5.0 × 10^{-3} M) was prepared, and the amount of ACh in this solution was kept constant at 5.0 × 10^{-6} M. Amperometric

TABLE 1 *Comparison of analytical characteristics of the different amperometric ACh biosensors*

Immobilization matrix	*Working potential (V)*	*Linearity*	*LOD*	*Immobilization technique*	K_m	*Optimum pH*	*Optimum temperature (°C)*	*Repeatability*	*Long-term stability*	*Reference*
Pt/PVF$^+$ClO4$^-$/AChE-ChOx	+0.7	0–12.0 mmol L^{-1}	1.0 μmol L^{-1}	Adsorption	1.74 mM	7.4	55	nr	The current response decreased about 50% after 30 days	[4]
Pt/PPy-PVS	+0.3	0.01–1.0 μmol L^{-1}	5.2 nmol L^{-1}	Cross-linking	0,0332 μM	9.0	65	RSD: 4.4% ($n = 18$)	The response on the 35th day was 31% of the initial value	[6]
GCE/CS-MWCNTs-Fe_3O_4NPs/AChE-ChOx	+0.4	0.02–0.111 μmol L^{-1} and 0.111–1.87 μmol L^{-1}	0.61 nmol L^{-1}	Covalent binding	nr	7.5	60	RSD: 3.51% ($n = 5$)	After 30 days, the modified electrode remained 60% of its initial response	[15]
Pt/MWCNT-AuNP-PDDA-AChE	+0.35	0.005–0.4 mmol L^{-1}	1.0 μmol L^{-1}	More than one technique	nr	8.0	nr	RSD: 5% (reproducibility) ($n = 5$)	The electrode remained 80% of its initial current response after 2 months	[14]
Poly(vinyl alcohol) cryogel membrane	+0.65	5.0–100 μmol L^{-1}	2.0 μmol L^{-1}	Adsorption	0.4 mM	9.5	35	RSD: 4% ($n = 5$)	30 days	[17]
Hybrid mesoporous membranes	+0.6	6.0–800 μmol L^{-1}	5.2 μmol L^{-1}	Adsorption	nr	8–9	nr	RSD: 5.7 (n =10)	The amperometric response after 80 days is 80%	[18]
CDs-APTES modified carbon paste electrode	+0.4	0.01–10 μmol L^{-1}	5.0 nmol L^{-1}	Cross-linking	0,0012 μM	7.0	60	RSD: 2.7% (n =20)	The amperometric response after 20 days is 30%	This study

LOD, limit of detection; RSD, relative standard deviation; nr, not remarked.

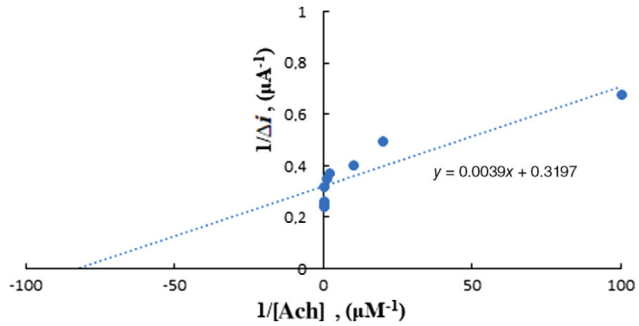

FIG. 16 *Lineweaver–Burk plot (0.1 M, pH 7.0 PBS, 25 °C, +0.4 V).*

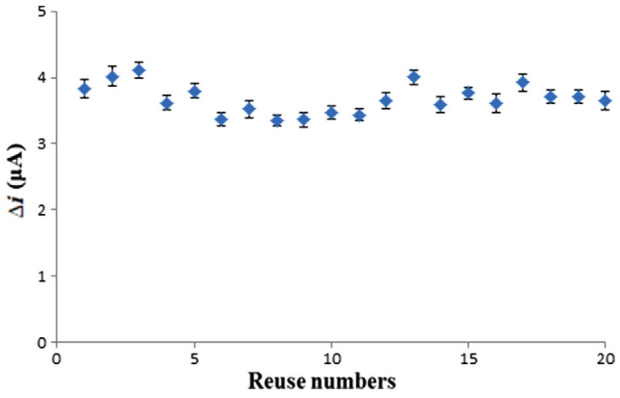

FIG. 17 *Operational stability of the biosensor at pH 7.0 PBS at a +0.4 V operating potential at 25 °C.*

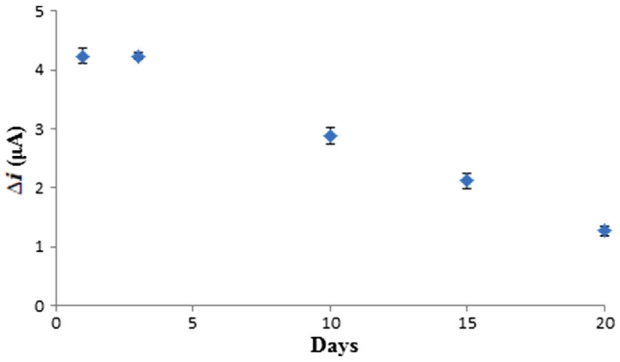

FIG. 18 *Storage stability of the biosensor.*

response currents were measured by adding 300-fold dilution to the working cell from the synthetic sample. After three experiments, the ACh concentration was calculated as $(5.45 \pm 0.22) \times 10^{-6}$ M using calibration graph.

TABLE 2 *Effects of interferants (%)*

Interfere agent	*Concentration (M)*	*ACh concentration (M)*	*Interfere (%)*
Glucose	5.0×10^{-3}	5.0×10^{-6}	1.7
Uric acid	3.0×10^{-4}	5.0×10^{-6}	5.0
Ascorbic acid	1.0×10^{-4}	5.0×10^{-6}	2.9
Paracetamol	1.0×10^{-4}	5.0×10^{-6}	4.6

4. Conclusion

In this study, we designed a new ACh biosensor with using APTES-modified CDs. This modified CD was first synthesized for this study. The biosensor has acceptable response time of 200 sec, and it has very low detection limit (5.0×10^{-9} M). The biosensor has a wide working range (1.0×10^{-8} to 1.0×10^{-5} M). In the literature, the amount of acetylcholine in the blood is indicated as 0.49 μM [24]. We can measure the amount of ACh less than 0.49 μM with the prepared ACh biosensor. Owing to its wide working range and low detection limit, the ACh biosensor prepared in this study makes it possible to determine ACh at low concentrations. The ACh biosensor shows perfect reproducible results. Thus, ACh determination can be carried out with our biosensor for many times. The ACh biosensor prepared in this study is easy to produce in the laboratory.

5. Acknowledgement

The authors would like to thank *Applied Physics A Materials Science & Processing* Journal for permitting to reuse Figs. 5 and 6.

6. References

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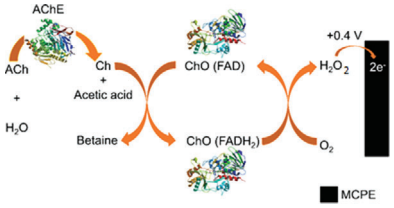

A Sensitive Amperometric Detection of Neurotransmitter Acetylcholine Using Carbon Dot-Modified Carbon Paste Electrode

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A sensitive amperometric detection of neurotransmitter acetylcholine using carbon dot-modified carbon paste electrode