Conducting polymer-based electrochemical biosensor for the detection of acetylthiocholine and pesticide via acetylcholinesterase

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Abstract

A voltammetric biosensor for acetylthiocholine (ATCh) and paraoxon detection was successfully developed. To achieve this goal, polypyrrole (PPy) was synthesized onto the platinum (Pt) electrode surface in 0.30 M oxalic acid solution containing 25 mM pyrrole. PPy-coated Pt (Pt/PPy) electrode surface was covered with chitosan (Chi) (Pt/PPy/Chi). The acetylcholinesterase (AChE) enzyme was immobilized on the Pt/PPy/Chi electrode surface to build a voltammetric biosensor (Pt/PPy/Chi/AChE). The storage stability of the biosensor was determined to be 72% even after 60 days. The operational stability was determined to be 94% after 20 consecutive measurements. For the biosensor, the linear range was determined to be 30–50 μM for ATCh and 0.46–1.84 nM for paraoxon. The limit of detection (LOD) was determined to be 0.45 μM for ATCh and 0.17 nM for paraoxon Volume 68, Number 6, Pages 1113–1119, 2021

Keywords: *biosensors, enzyme, immobilization, pesticide, polypyrrole*

1. Introduction

Biosensors are devices that can be commonly used to detect and quantify target molecules. Due to their advantages such as high sensitivity, fast response time, low cost, and selectivity, the usage of biosensors is getting very popular in clinical, environment, food, and pharmaceutical applications [1–4]. There have been many different developments conducted on enzyme-based sensors. Among these sensors, cholinesterase-based biosensor has a special place as it has been used to detect bacterial toxins, acetylcholine, pesticides, nerve agents, and choline [5]. Acetylcholine (ACh), which affects learning, memory, and muscle tones, is a neurotransmitter that has an important function in the cholinergic system and is effective in nerve conduction in cholinergic synapses. They actively take role in the neuromuscular junctions, motor neurons, preganglionic, spinal cord, and central nervous system. Various neurodegenerative diseases such as Alzheimer's, Parkinson's, and schizophrenia may occur due to not only excessive accumulation in nerve tissue when acetylcholine is not metabolized but also the decrease in the amount of acetylcholine in the perisynaptic region due to being exposed to an excessive hydrolyzed process [6]. In the context of these facts, the determination of ACh, as *in vitro* or *in vivo* studies, is crucial for analytical methods [7].

The acetylcholinesterase (AChE) enzyme, which plays a role in the cholinergic system, regulates the level of neurotransmitter ACh. Organophosphates (OP) are widely used

Abbreviations: *ACh, acetylcholine; ATCh, acetylthiocholine; AChE, acetylcholinesterase; Chi, chitosan; DPV, differential pulse voltammetry; EDX, energy dispersive X-ray; LOD, limit of detection; PPy, polypyrrole; Pt, platinum; SEM, scanning electron microscopy.*

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synthetic pesticides [8]. It is well known that a small amount of OP such as paraoxon inhibits the catalytic activity of AChE [9]. Investigation of OP pesticides has been conducted via different techniques such as mass spectrometry (MS), gas chromatography (GC), high-performance liquid chromatography, capillary electrophoresis, and GC–MS. However; these techniques have drawbacks including but not limited to being time consuming and offering expensive instrumentation. In addition, they require sample preprocessing, trained personnel, and cannot be operated outside laboratories. These challenges created a high demand for portable devices that can enable simple, reliable, selective, rapid, low cost, and highly sensitive OP detection [10]. Enzyme-based electrochemical biosensors have such desired properties. The working principle of the enzyme-based electrochemical biosensor is simply detecting and interpreting the change in the electrical signal. This signal is either the result of production or the reduction process of an electroactive species through an enzymatic reaction. These types of biosensors not only eliminate complicated instrumentation but also offer a relatively low-cost solution [11]. That is why they have been used in countless of point-of-care applications with a wide range of analytes [11] and draw high demands with new application fields [12].

Chitosan (Chi), which is a biodegradable material, is widely used in the immobilization of enzymes. In the enzyme immobilization, chitosan is one of the most popular polymers due to its biocompatible, inexpensive, and nontoxic properties [13,14].

Conductive polymers have become more popular among the frequently studied materials especially after the discovery of their conductivity properties. Batteries, artificial muscles, membranes, and corrosion technology can be given as examples for the application of the conductive polymers [15]. Conductive polymers can be synthesized via chemical [16,17] or electrochemical methods [18–20]. Their easy preparation, high conductivity, and stability enabled them to be used in enzyme immobilization [21]. Polyaniline [22,23], polypyrrole (PPy) [24,25], and their derivatives are conductive polymers that are widely used in enzyme immobilization. PPy can be used in electrochemical sensor materials because it is easy to synthesize and having good physical, chemical, and electronic properties.

In this study, it was aimed to detect acetylthiocholine (ATCh) and pesticide. For this purpose, the conducting polymer/enzyme-based electrochemical biosensor was designed with AChE immobilization on the Pt/PPy/Chi electrode. The linear range, the limit of detection (LOD), operational and storage stability of designed biosensor (Pt/PPy/Chi/AChE) were investigated by the electrochemical method.

Highlights

- An electrochemical biosensor was proposed by using polypyrrole/acetylcholinesterase for acetylthiocholine and pesticide detection.
- The biosensor exhibited good operational and storage stability.
- The biosensor has a low detection limit.

2. Material and Methods

2.1. Materials

All the chemicals were obtained from commercial suppliers (Sigma-Aldrich, Merck).

2.2. Preparation of chitosan solution

Chitosan (0.3 g) was slowly added to 10 mL of 1% acetic acid solution at room temperature and then dissolved by stirring in a magnetic stirrer (150 rpm) for 3 h. To prepare the coagulation liquid, 2 g of sodium hydroxide (1 N NaOH) was added to 50 mL of distilled water containing 26% ethanol [26, 27].

2.3. Synthesis of PPy Film

The PPy film was synthesized onto a platinum (Pt) electrode with a cyclic voltammetry technique in 0.30 M oxalic acid solution containing 25 mM pyrrole monomer. The cyclic voltammetry technique was applied at a potential between 0 and 1 V by 50 mV s^{-1} scan rate.

2.4. Preparation of enzyme electrode

An enzyme-based electrode was prepared in the following steps: First, a PPy homopolymer film was synthesized onto a platinum (Pt) electrode (Pt/PPy). Second, chitosan solution was dropped onto the Pt/PPy electrode (Pt/PPy/Chi). The Pt/PPy/Chi electrode was immersed in a coagulation solution for 20 min. The Pt/PPy/Chi electrode was washed until its pH was neutral. Third, the Pt/PPy/Chi electrode was incubated in 50 mM phosphate buffer (pH 7.0) containing 5% glutaraldehyde solution for cross-linking for 2 h, and then the excess of glutaraldehyde was removed with distilled water. Lastly, the AChE enzyme (5×10^{-3} EU) was immobilized onto the Pt/PPy/Chi electrode by immersing it in the AChE solution for 24 h at 4 °C, and then the excess of AChE was removed with distilled water. Electrodes were kept in the Tris/HCl buffer (20 mM, pH 7.5) at 4 °C, when they were not in use.

2.5. Electrochemical measurements

All the electrochemical experiments were performed in a single compartment cell with three electrode configuration. The reference electrode was an Ag/AgCl (3 M KCl) electrode, and the counterelectrode was a platinum sheet. The working electrode was a platinum sheet with a surface area of 0.18 cm^2. A CHI 660E (TX, USA) model digitally controlled electrochemical analyzer was used in the electrochemical experiments. The biosensor response was monitored by the differential pulse voltammetry (DPV) technique. The parameters of DPV were as fallows: increment potential, 4 mV; pulse amplitude, 50 mV; pulse width, 10 ms; pulse period was 40 ms. The DPV measurements were performed at room temperature in Tris/HCl

buffer (20 mM, pH 7.5) solution containing 5,5-dithiobis- (2-nitrobenzoic acid) for ATCh. The current responses and linear ranges of AChE immobilized on Pt/PPy/Chi (Pt/PPy/Chi/AChE) was investigated using a series of different concentrations of acetylthiocholine iodide (ATChI) and paraoxon.

2.6. Storage and operational stability

The operational and storage stability of the Pt/PPy/Chi/AChE electrode were presented in a normalized form, where the highest value of each set was assigned to the value of 100% activity. Storage and operational stability were determined in the Tris/HCl buffer (20 mM, pH 7.5) solution containing 50 mM ATCh by DPV.

2.7. SEM-EDX analysis

Scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX) of the Pt/PPy/Chi electrode surface before and after AChE immobilization was used to understand the differences in morphology and elemental composition. The device was operated at a typical acceleration voltage of 10.000 kV.

3. Results and Discussion

The use of enzyme immobilization in different biotechnological applications has many advantages, such as the stabilization of the catalytic activities of the enzyme, its stability, reusability, the ability to obtain products with pure ease, high stability against environmental effects, continuity of production, and cost reduction [28,29]. AChE-based biosensors are used in both determination of pesticides, such as OP and carbamate in tap and river waters and soil extracts in food and environmental fields [30].

Electrochemical sensors convert chemical reactions of target species on electrodes into electrical signals where measure the changes in current. Electrochemical techniques enable high selectivity with a low detection limit in a relatively small volume of samples via interpreting the change in the electrical signal [31]. These features have increased the interest in electrochemical biosensors and have led to intensification of studies in this field.

3.1. Synthesis of PPy

Figure 1 shows the first cyclic voltammograms obtained in the solution of 0.30 M oxalic acid and 0.30 M oxalic acid with 25 mM pyrrole by using the platinum electrode. Measurements were obtained between 0 and 1 V at a scan rate of 50 mV s^{-1}. The current values remained constant up to about 0.70 V (Fig. 1) during anodic scanning in 0.30 M oxalic acid solution. The current increase above 0.70 V represents the oxygen gas. It was observed that there was no change in the current values up to approximately 0.60 V in 0.30 M oxalic acid + 25 mM pyrrole solution. However, above 0.60 V, it was observed that the rate of increase in the current was higher compared to the environment without the monomer. This corresponds to the oxidation of the pyrrole monomer.

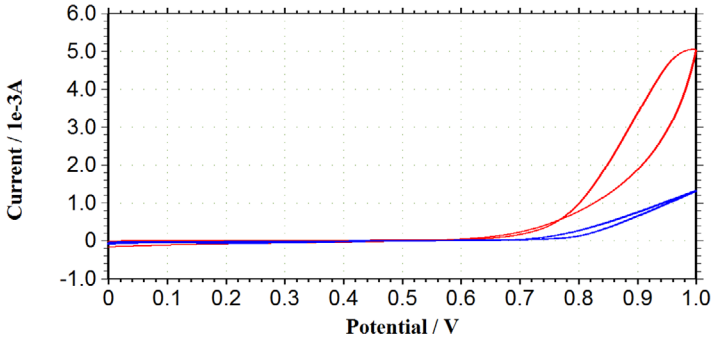

FIG. 1 *The first cyclic voltammograms recorded for the Pt electrode in 0.30 M oxalic acid and 0.30 M oxalic acid + 25 mM pyrrole.*

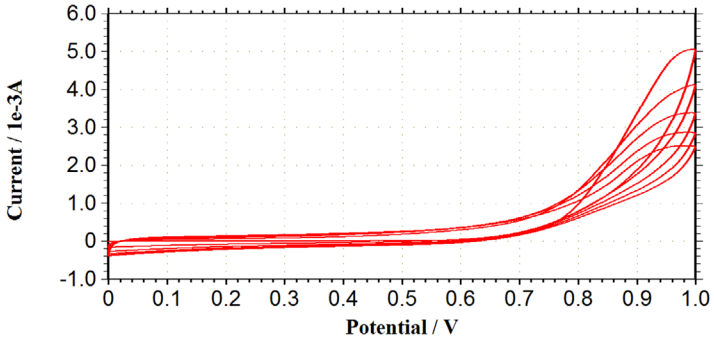

FIG. 2 *The film growth curves recorded for the Pt electrode in 0.30 M oxalic acid + 25 mM pyrrole.*

Film growth curves obtained in 0.30 M oxalic acid + 25 mM pyrrole solution are given in Fig. 2. Five cycles were taken for the film development. The first segment in these curves has the highest current values, and the current values of each segment are lower in comparison to the previous segment. This indicates that in each segment the electrode surface is slightly more coated with the PPy film in comparison to the previous segment.

3.2. Characterization of Pt/PPy/Chi/AChE

Cyclic voltammetry curves obtained from the Tris/HCl buffer (20 mM, pH 7.5) solution of Pt/PPy and Pt/PPy/Chi/AChE electrodes are given in Fig. 3. In these curves, it is observed that the current values of the Pt/PPy/Chi/AChE electrode decreases considerably compared to the Pt/PPy electrode. This is due to the fact that the chitosan/enzyme was immobilized on the Pt/PPy/Chi/AChE electrode surface.

A scanning electron microscopy (SEM) image and corresponding energy dispersive X-ray (EDX) diagram of Pt/PPy/Chi/AChE electrode are given in Fig. 4. As can be seen in Fig. 4a and 4b, the images of Pt and Pt/PPy/Chi/AChE electrodes are different from each other. The uncoated Pt electrode surface appears to have a smooth structure, whereas the Pt/PPy/Chi/AChE electrode surface has a wavy structure.

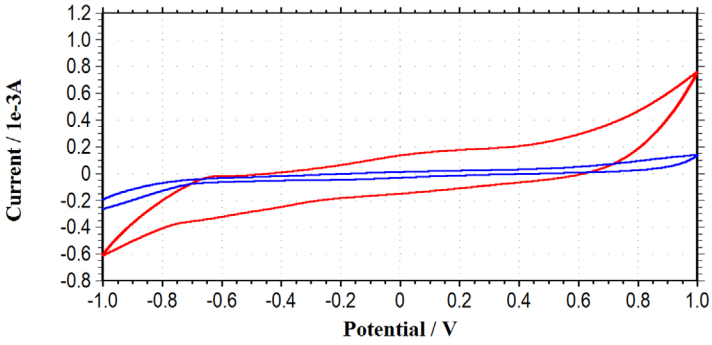

FIG. 3 *The cyclic voltammograms for Pt/PPy and Pt/PPy/Chi/AChE electrodes in the Tris/HCl buffer (20 mM, pH 7.5) solution.*

This is due to the presence of PPy/Chi/AChE on the Pt electrode surface. The appearance of the sulfur element (0.06 wt%) in the EDX diagram in Fig. 4c shows that AChE is immobilized to the Pt/PPy/Chi/AChE electrode surface, because methionine, containing sulfur, is an amino acid and is found in the structure of enzymes.

3.3. Substrate detection

DPV curves at different substrate concentrations of the Pt/PPy/Chi/AChE electrode are given Fig. 5a. The current values in the solutions containing the substrate appear to be higher than the medium without the substrate. The current values increase as the substrate concentration increases in a media containing substrate. These findings are also compatible with the literature. For example, Somerset et al. [32] immobilized polyaniline–AChE onto the gold electrode surface. They stated that amperometric biosensor current signals increased with the increase in the concentration of the acetylcholinechloride. Parsajo and Kauffmann [33] found that the current values of AChE-immobilized electrodes increase in a direct proportion to the ACh concentration. Thelinear range of the Pt/PPy/Chi/AChE electrode was determined to be 30–50 µM for ATCh. The linear regression equation is y (µA) = 0.305x (µM) − 0.1067 with a correlation coefficient of 0.9921. The limit of detection (LOD) was calculated to be 0.45 µM (the LOD is calculated based on $3S_b/m$, where S_b and m are the standard deviation of the blank and the slope of the calibration graph, respectively). This LOD value is low compared to some other electrochemical biosensors [33–36].

3.4. Pesticide detection

Figure 6 shows the DPV curves of the Pt/PPy/Chi/AChE electrode in solutions containing paraoxon at different concentrations. It can be seen that the current values in solutions containing high concentrations of paraoxon are lower in comparison to solutions with low concentrations of paraoxon. This is due to the irreversible binding of paraoxon to the active site of the AChE enzyme. These findings are also compatible with studies identified pesticides. For instance, Zheng et al. [37] performed the amperometric detection of paraoxon with the nanosilver electrode that they immobilized with AChE/Chi. They observed that current values decreased with the increase in the paraoxon concentration. Rodrigues et al. [38] modified the screen-printed electrode with AChE/Chi/Fe_3O_4 to detect malathion. They stated that the concentration of malathion is inversely proportional to the current values. The linear range of the Pt/PPy/Chi/AChE electrode was determined to be 0.46–1.84 nM for paraoxon, where the linear regression equation is y (μA) = 2.4326x (nM) + 0.480 with a correlation coefficient of 0.9939. The LOD was calculated to be 0.17 nM ($3S_b/m$), and this LOD value is low compared to some other electrochemical biosensors [39–42].

3.5. Stability

Immobilization is an important factor that increases the stability of enzymes. In a study conducted by Işık, the storage and operational stability of AChE, immobilized to the chitosan surface, was reported to be quite high compared to the free enzyme [43]. The immobilized AChE activity was found to be 75% more stable than the free enzyme in a study conducted by Gabrovska et al. [44], where AchE was immobilized on acrylonitrile copolymer membranes. In another study on the immobilization of AChE on porous silicon, immobilized AChE maintained its activity 50% longer than the free enzyme [45].

In our study, the operational stability of the Pt/PPy/Chi/AChE electrode was examined in a buffer solution containing 50 mM ATCh. Twenty measurements were taken for this purpose. To determine relative activity of the Pt/PPy/Chi/AChE electrode, the currents obtained from all separate measurements were calculated as the percentage of the initial current. The results obtained in this way are plotted in Fig. 7. As can be seen in Fig. 7, the current values keep on decreasing till 17th measurement and show very little change after the 17th measurement. 94.13% of the initial current was obtained at the end of the 20th measurement. This value shows that operational stability is very good. Aynaci et al. [5] took 18 measurements from the ATCh solution to test the operational stability of the AChE-choline oxidase enzyme electrode that was immobilized to the PPy-polyvinylsulfonate film. They found that the activity at the end of the 18th measurement was 83.1% of the initial activity. Moradzadegan et al. [46] stated that AChE, which they immobilized to polyvinylalcohol nanofibers, had 70% of the initial activity after 10 measurements.

While free enzymes cannot maintain their long-term activities under certain conditions, immobilized enzymes can maintain their activities for a longer time. Increased storage stability of enzymes shows that these activities are preserved for a longer period of time. In our study, immobilized AChE enzymes on the Pt/PPy/Chi electrode surface were stored at 4 °C for 60 days. As seen in Fig. 8, the immobilized AChE maintained approximately 72% of the initial activity after 60 days at 4 °C. This value shows that storage stability is very good. An ACh biosensor prepared by Aynaci et al. [5] retained 31% of its initial activity after 35 days. Marinov et al. [47] found that the sensor maintained 75% of initial activity after 20 days of storage

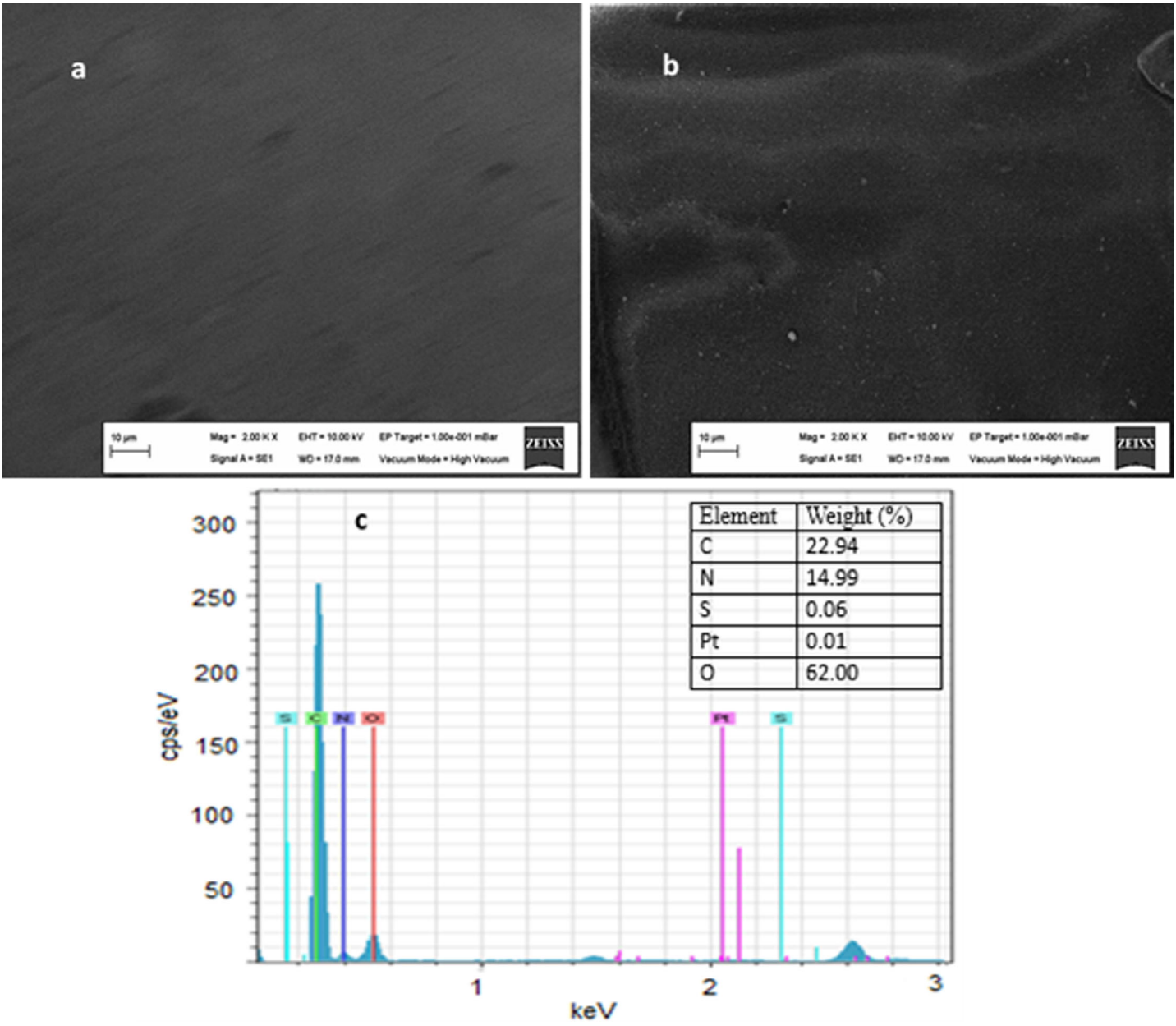

FIG. 4 *SEM images of Pt (a) and Pt/PPy/Chi/AChE (b) and corresponding EDX diagram of Pt/PPy/Chi/AChE (c).*

and 25% after 30 days, where they immobilized AChE on poly(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membranes. In this study, the results show that the immobilized enzyme has significant advantages for many reasons such as longer shelf life, proper use, improved stability, and prevention from contamination with enzyme products

3.6. Kinetic parameters

In our study, the kinetic parameters of the immobilized AChE were determined using ATCh as a substrate at different concentrations. The K_m and I_{max} values for immobilized AChE were calculated from the Michaelis–Menten graph (Fig. 9). K_m(app) and I_{max} were found to be 2.20 μM and 87.84 μA, respectively. These kinetic parameters show the change in the affinity of the substrate to the active site of the enzyme. The K_m value that shows the affinity of the biosensor was found to be 2.20 μM in comparison to 1.74 mM [48] and 0.4 mM [49]. The high affinity of the AChE enzyme immobilized onto the Pt/PPy/Chi electrode used in this study can be understood from the low K_m value.

4. Conclusions

AChE was successfully immobilized onto the platinum electrode surface coated with PPy and Chi. The operational and the storage stability of the Pt/PPy/Chi/AChE electrode was found

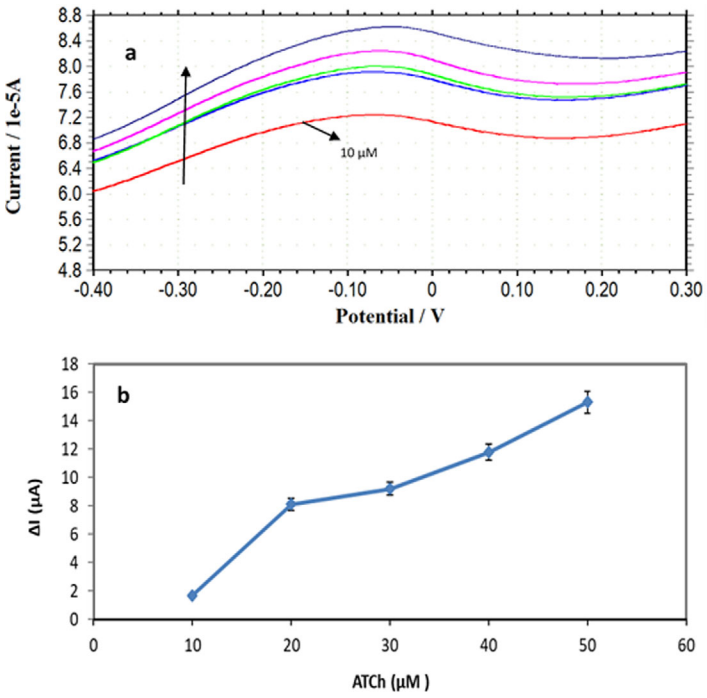

FIG. 5 *Differential pulse voltammograms (a) and calibration plot (b) of the Pt/PPy/Chi/AChE electrode in the Tris/HCl buffer (20 mM, pH 7.5) solution containing 10, 20, 30, 40, and 50 μM ATCh.*

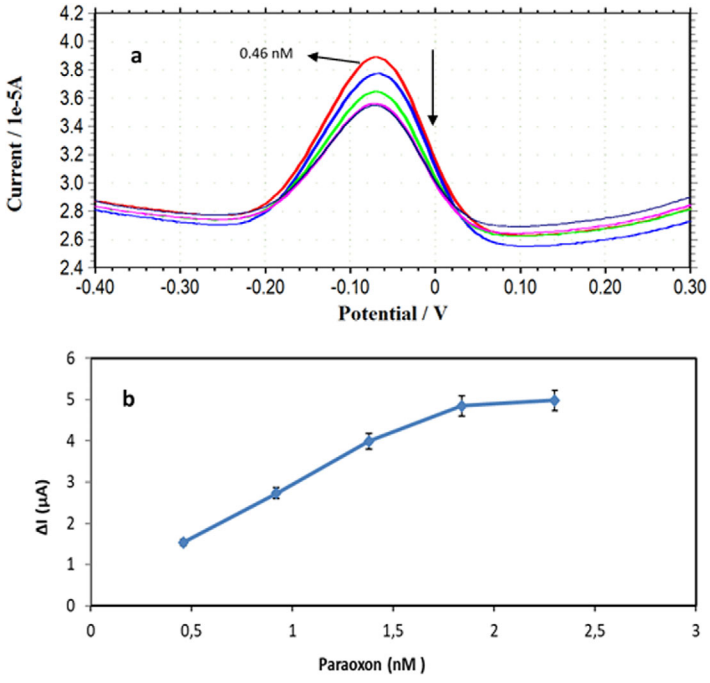

FIG. 6 *Differential pulse voltammograms (a) and calibration plot (b) of the Pt/PPy/Chi/AChE electrode in the Tris/HCl buffer (20 mM, pH 7.5) solution containing 0.46, 0.92, 1.38, 1.84, and 2.30 nM paraoxon.*

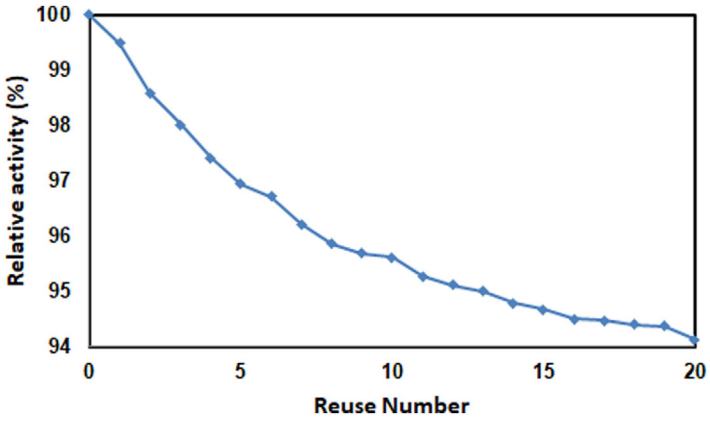

FIG. 7 *Operational stability of the Pt/PPy/Chi/AChE electrode.*

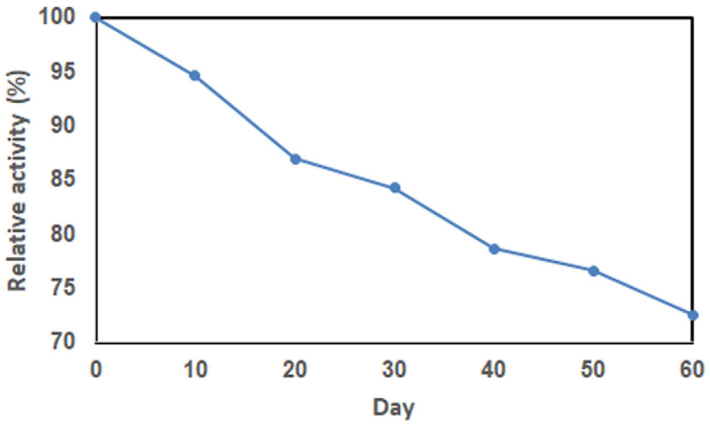

FIG. 8 *Storage stability of immobilized AChE on the Pt/PPy/Chi electrode.*

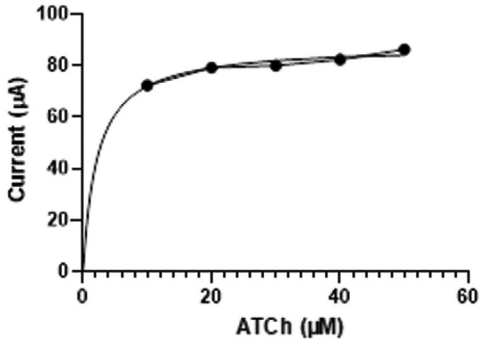

FIG. 9 *Michaelis–Menten graph of the Pt/PPy/Chi/AChE electrode*

to be quite good. After 20 consecutive measurements, the operational stability was determined as 94.13%. In addition, the storage stability was found as 70% even after 60 days. The linear range was found to be between 0.46 and 1.84 nM for paraoxon and 30–50 μM for ATCh according to the results. LOD was determined to be 0.45 μM for ATCh and 0.17 nM for paraoxon. It was found that the biosensor with these properties is quite suitable for detecting ATCh and paraoxon.

5. Conflict of Interest

All authors declare that they have no conflicts of interest.

6. References

[1] Andreescu, S., and Marty, J. L. (2006) Biomol. Eng. 23, 1–15.
[2] Amine, A., Mohammadi, H., Bourais, I., and Palleschi, G. (2006) Biosens. Bioelectron. 21, 1405–1423.
[3] Huang, B., Xiao, L., Dong, H., Zhang, X., Gan, W., Mahboob, S., Al-Ghanim, K. A., Yuan, Q., and Li, Y. (2017) Talanta 164, 601–607.
[4] Wang, D., Wang, J., Zhang, J., Li, Y., Zhang, Y., Li, Y., and Ye, B. (2019) Talanta 196, 479–485.
[5] Aynaci, E., Yaşar, A., and Arslan, F. (2014) Sensors Actuators, B Chem. 202, 1028–1036.
[6] Sattarahmady, N., Heli, H., and Moosavi-Movahedi, A. A. (2010) Biosens. Bioelectron. 25, 2329–2335.
[7] Pepeu, G., and Giovannini, M. G. (2004) Learn. Mem. 11, 21–27.
[8] Joshi, S. C., Mathur, R., and Gulati, N. (2007) Toxicol. Ind. Health 23, 439–44.
[9] Jaffrezic-Renault, N. (2001) Sensors 1, 60–74.
[10] Songa, E. A., and Okonkwo, J. O. (2016) Talanta 155, 289–304.
[11] Putzbach, W., and Ronkainen, N. (2013) Sensors 13, 4811–4840.
[12] Zhao, Z., Lei, W., Zhang, X., Wang, B., and Jiang, H. (2010) Sensors 10, 1216–1231.
[13] Tukel, S. S., and Alptekin, O. (2004) Process Biochem. 39, 2149–2155.
[14] Çetinus, Ş. A., Şahin, E., and Saraydin, D. (2009) Food Chem. 114, 962–969.
[15] Bazzaoui, M., Bazzaoui, E. A., Martins, L., and Martins, J. I. (2002) Synth. Met. 130, 73–83.
[16] Sharma, A. L., Saxena, V., Annapoorni, S., and Malhotra, B. D. (2001) J. Appl. Polym. Sci. 81, 1460–1466.
[17] Müller, D., Rambo, C. R., Recouvreux, D. O. S., Porto, L. M., and Barra, G. M. O. (2011) Synth. Met. 161, 106–111.
[18] Ozyilmaz, A. T., and Akdag, A. (2013) Trans. Inst. Met. Finish. 91, 44–51.
[19] Akdag, A., and Ozyilmaz, A. T. (2017) Acta Chim. Slov. 64, 312–318.
[20] Akdag, A. (2020) ChemistrySelect 5, 2496–2500.
[21] Ozyilmaz, G., Ozyilmaz, A. T., and Can, F. (2011) Appl. Biochem. Microbiol. 47, 196–205.
[22] Hundiwale, D. G., Borole, D. D., Kapadi, U. R., and Mahulikar, P. P. (2007) Journal of Materials Science, 42, 4947–4953.
[23] Thakur, B., and Sawant, S. N. (2013) ChemPlusChem. 78, 166–174.
[24] Shirsat M. D., Too C. O., and Wallace G. G. (2008) Electroanalysis. 20, 150–156.
[25] Ozyilmaz, G., Ozyilmaz, A. T., and Bayram, E. I. (2019) Acta Chim. Slov. 66, 950–957.
[26] Chiu, S. H., Chung, T. W., Giridhar, R., and Wu, W. T. (2004) Food Res. Int. 37, 217–223.
[27] Kaushal, J., Seema, G. S, and Arya, S. K. (2018) Biotechnol. Rep. 18, e00258.
[28] Brady, D., and Jordaan, J. (2009) Biotechnol. Lett. 31, 1639–1650.
[29] Moehlenbrock, M. J., and Minteer, S. D. (2017) in Methods in Molecular Biology, Humana Press, New York, pp. 1–7.
[30] Ion, A. C., Ion, I., Culetu, A., Gherase, D., Moldovan, C. A., Iosub, R., and Dinescu, A. (2010) Mater. Sci. Eng. C 30, 817–821.
[31] Naveen, M. H., Gurudatt, N. G., and Shim, Y. B. (2017) Appl. Mater. Today 9, 419–433.
[32] Somerset, V. S., Klink, M. J., Baker, P. G. L., and Iwuoha, E. I. (2007) J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 42, 297–304.
[33] Parsajoo, C., and Kauffmann, J. (2013) Talanta 109, 116–120.
[34] Yang, M., Yang, Y., Yang, Y., Shen, G., and Yu, R. (2005) Anal. Chim. Acta 530, 205–211.
[35] Shimomura, T., Itoh, T., Sumiya, T., Mizukami, F., and Ono, M. (2009) Enzyme Microb. Technol. 45, 443–448.
[36] Hou, S., Ou, Z., Chen, Q., and Wu, B. (2012) Biosens. Bioelectron. 33, 44–49.
[37] Zheng, Q., Yu, Y., Fan, K., Ji, F., Wu, J., and Ying, Y. (2016) Anal. Bioanal. Chem. 408, 5819–5827.
[38] Rodrigues, H. Y. N. F. M., Neto, S. Y., Luz, R. C. S., and F. S. D. (2018) Bosensors 8, 1–12.
[39] Sinha, R., Ganesana, M., Andreescu, S., and Stanciu, L. (2010) Anal. Chim. Acta 661, 195–199.
[40] Di Tuoro, D., Portaccio, M., Lepore, M., Arduini, F., Moscone, D., Bencivenga, U., and Mita, D. G. (2011) N. Biotechnol. 29, 132–138.
[41] Tuteja, S. K., Kukkar, M., Kumar, P., Paul, A. K., Deep, A. (2014) Bionanoscience 4, 149–156.
[42] Zheng, Q., Yu, Y., Fan, K., Ji, F., Wu, J., and Ying, Y. (2016) Anal. Bioanal. Chem. 408, 5819–5827.
[43] Işık, M. (2020) ChemistrySelect 5, 4623–4627.
[44] Gabrovska, K., Nedelcheva, T., Godjevargova, T., Stoilova, O., Manolova, N., and Rashkov, I. (2008) J. Mol. Catal. B Enzyme 55, 169–176.
[45] Saleem, M., Rafiq, M., Seo, S. Y., and Lee, K. H. (2016) Biosci. Rep. 36, e00311.
[46] Moradzadegan, A., Ranaei-Siadat, S. - O., Ebrahim-Habibi, A., Barshan, M., Tashnizi, R. J, Torabi, S. - F., and Khajeh, K. (2010) Eng. Life Sci. 10, 57–64.
[47] Marinov, I., Ivanov, Y., Gabrovska, K., and Godjevargova, T. (2010) J. Mol. Catal. B Enzyme 62, 66–74.
[48] Şen, S., Gülce, A., and Gülce, H. (2004) Biosens. Bioelectron. 19, 1261–1268.
[49] Doretti, L., Ferrara, D., Lora, S., Schiavon, F., and Veronese, F. M. (2000) Enzyme Microb. Technol. 27, 279–285.