Egypt. J. Chem. **Vol. 62**, No. 3 pp. 441 - 449 (2019)

Highly Sensitive Method for Analysis Anticholinesterase Drugs

Elmorsy Khaled[1*], Hassan N.A. Hassan[1], Mona A. Ahmed[2], Rehab O. El-Attar[1]
[1]Microanalysis Lab, Applied Organic Chemistry Department, National Research Centre (NRC), El Bohouth st., Dokki, 12622-Giza, Egypt.
[2]Analytical Chemistry Dept., Girls College, Ain Shams University, Cairo, Egypt.

THE PRESENT study described a novel application of simple potentiometric biosensors for analysis of anti-cholinesterase (Alzheimer) drugs in vitro. The proposed method was based on inhibition of acetylcholineesterase enzyme (AChE) by Alzheimer drugs namely; donepezil (DOP) and galantamine (GAL) in addition to biperdien (BP) and ipratropium (IPBr). Based on the relative inhibition action of drug on AChE activity, different sensitivities were recorded ranging between 0 to 18.0 ng, 0 to 90.0 ng, 0 to 4.69 µg and 0 to 9.96 µg $mL^{-1}$for the aforementioned drugs in the same order depending on the nature of drugs and their corresponding LD50 values. The proposed procedures were successfully applied for determination of drugs in pharmaceutical formulation and biological samples with sensitivity and accuracy comparable with the official method. The presented approach can be suggested for testing of pharmaceutical preparation toxicity against cholinesterase enzymes in vitro.

Keywords: Alzheimer drug, Cholinesterase, Disposable biosensor, Pharmaceutical analysis.

Introduction

Cholinesterases are a family of enzymes present in vertebrates and insects that catalyze the hydrolysis of neurotransmitter acetylcholine into choline and acetic acid and an essential process allowing for the restoration of the cholinergic neuron [1]. In the body, only two cholinesterases are known: acetylcholinesterase (AChE; EC3.1.1.7.) and butyrylcholinesterase (BChE; EC 3.1.1.8). Despite high structural similarities, the enzymes are expressed in various tissues and they have different roles in the body. AChE is an enzyme participating in cholinergic neurotransmission but the role of BChE cannot be recognized so easily [2,3].

Some compounds can inhibit cholinesterase enzymes [2-8]. Based on this inhibition, cholinesterases are widely used for analytical purposes [9-14]. AChE has been used extensively for the enzymatic detection of organophosphates (Ops) as well as carbamate pesticides, nerve agents, several natural toxins, and some drugs [5]. Hence, AChE is widely used as a potent recognition element for the construction of biosensors for pesticide detection [9, 10]. Biosensors based on AChE as well as butyrylcholinesterase were first reported during the 1980s. Since then, there has been a continuous improvement of cholinesterase-based biosensors due to the gradual improvement of transducer devices and the availability of pure enzymes [10]. Most of the biosensors constructed for the determination of OPs are based on an electrochemical transducer, which is usually incorporated in a single unit with the biological recognition element. Both amperometric and potentiometric cholinesterase biosensors were developed and demonstrated comparable analytical parameters. It was pointed out previously [15, 16] that potentiometric biosensors are preferable for inhibitor measurement than amperometric ones, due to their higher inhibitor sensitivity and in general their much easier preparation technique and measurement procedure. Furthermore, the potentiometric working principle fits better to in-field measurements. Many cholinesterase biosensors configurations can be found in literature [14].

Though the structure of BChE closely resembles the structure of AChE, the enzymes have unequal sensitivity to inhibitors. Inhibitors of ChEs do not have one target site. The inhibitors can bind into esteratic part of active site, anionic part of active site (α anionic site), aromatic gorge,

*Corresponding author e-mail: elmorsykhaled@yahoo.com
DOI: 10.21608/EJCHEM.2018.4591.1409

and peripheral (or β in some sources) anionic site [17, 18]. Inhibitors binding into aromatic gorge are quite rare. The active sites are composed of glutamate, histidine, and serine in case of esteratic site. For the α-anionic site, tryptophan, tyrosine and phenylalanine are the crucial amino acid residues allowing interaction cation-π with electrons of the aromatic amino acids. Reversible inhibitors of ChEs bind to anionic part of active site. It is a common phenomenon, that reversible inhibitors have unequal affinity to AChE and BChE. The anionic part of the active site can interact with compounds having quaternary ammonium or another structural similarity to choline. Alzheimer disease current and past drugs donepezil [19], huperzine [20], tacrine (Cognex) and its derivatives [21] and galantamine [22] are examples. The aforementioned compounds can be involved in inhibition of AChE via the peripheral anionic site as well.

The widespread dosefication and/or adulteration of commercially available pharmaceutical preparations demands simple, sensitive, selective and rapid methods for drug quality control. Chromatographic and spectrophotometric techniques are the most popular techniques. Nevertheless, most of these methods require expensive apparatus or involve several manipulation steps before the final result of analysis. Chemical sensors and biosensors are shown to offer alternative solutions, capable of satisfying the increasing demand for precise analytical information at lower cost through devices that require relatively simple instrumentation. Ideally, a sensor-based system needs only little, if any, pre-treatment of the sample and possible interfacing with FIA systems [23–28].

The principle aim of the present work is the determination of some anticholinesterase drugs using a simple potentiometric biosensor. As there is an increasing end-user demand for the use of rapid, reliable and low-cost field-based methods for the determination of pharmaceutical formulations, a fast response, disposable, robust, potentiometric AChE biosensor was fabricated. The free enzyme in solution was incubated with the drug sample where the residual AChEs activity was estimated to measure the inhabitation degree by the drug.

Experimental

Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Acetylcholine chloride (ACh) was purchased from Fluka and used without further purification. Aqueous 10^{-2} mol L^{-1} solution of ACh was prepared in phosphate buffer solution (pH 7.0). Stock solutions of acetylcholinesterase enzyme (Sigma) was prepared by dissolving the vial in phosphate buffer solution (pH 7.0) and the specific enzyme activity was verified using Ellman's photometric method as modified by Gorun et al., [29].

Authentic Samples

Authentic biperiden hydrochloride ($C_{21}H_{29}NO.HCl$, molar weight 346.46 g·mol^{-1}) and Ipratropium bromide authentic sample ($C_{20}H_{30}NO_3Br$, 412.37 g mol^{-1}) samples were supplied by the Arab Drug Company, ADCo, Egypt. Dopenzile hydrochloride ($C_{24}H_{30}NO_3Cl$, molar weight 379.492 gmol^{-1}) and galantamine ($C_{17}H_{21}NO_3$, molar weight 287.354 gmol^{-1}) authentic sample were kindly provided fromNational Organization for Drug Control and Research, Giza, Egypt. Stock drug solutions (10^{-2} mol L^{-1}) were prepared by dissolving the appropriate amount of the active ingredient distilled water and kept at 4 ^{O}C.

Pharmaceutical preparations

Atrovent® unit dose vials (Arab Drug Company, ADCo, Egypt, 250 µg IPBr in 2 mL solution) were obtained from local Pharmacy. Two ampoules were transferred into a beaker and completed to 10 mL with bidistilled water prior analysis. The purity of the samples was estimated according to the European Pharmacopoeia [30] by potentiometric titration with silver nitrate solution.

Akineton tablets (Arab Drug Company, ADCo, Egypt, 2 mg BP per tablet) were purchased from local drug stores. Ten tablets were ground and dissolved in 50 mL of bidistilled water. BP content was assayed according to the proposed potentiometric method and colorimetric method using phosphate buffer–bromocresol purple solution and measuring the absorbance of the produced color at 408 nm [31].

Reminyl PR (Galantamine hydrobromide 16 mg, Janssen, Cairo, Egypt) and Donepezil (Aricept10 mg, Pfizer, Cairo, Egypt) were purchased from local drug stores and analyzed according to their official pharamcopial procedures [32].

Apparatus

All potentiometric measurements were carried

out using Radio Shack Digital multimeter with PC interface. The pH measurements were performed using Metrohm 692-pH meter with combined pH glass electrode (6.0202.100). The potentiometric bielectrode strips were printed on a PVC support (dimensions 5 × 35 mm) using silver- and graphite-based inks for reference and working electrodes, as described elsewhere by the research team [33] and directly used in measurements.

Measurement of inhibition by drug

Drug solutions were incubated with AChE for appropriate incubation time and the residual enzymatic activity was measured. The enzymatic activity was estimated by monitoring the change of electrode potential within the reaction time. Calibration curves were constructed by plotting the initial reaction rate against enzyme concentration. For each concentration, 5 replicates were measured and the mean value of the inhibition degree (I%, calculated from the residual enzyme activity) was represented against the drug's concentration.

For comparison the obtained potentiometric results were compared with the official spectrophotometric method for cholinesterase enzyme [29] and official method for the investigated drugs according to their pharmacopeia [30-32].

Results and Discussion

Pharmacological neuromodulation has become one of the suitable tools for influencing the whole system of the human body. However, the interest in this is undermined by the fact that drugs specifically implicated in neuromodulation will be more potent than the ones influencing the periphery due to their effect amplification. Nevertheless, the current attention is aimed at the treatment of illnesses associated with neuropathology e.g., schizophrenia, Alzheimer's and Parkinson disease.

AChE inhibitors play a significant role in the biochemical processes of the human body due to the physiological importance of AChE. Cholinesterase inhibitors binding to the α-anionic site are a group of chemical compounds containing certain common motives. Firstly, these compounds typically contain condensed aromatic cores. Secondly, there should be quarternary ammonium or nitrogen included as a heteroatom. Acrdines and tetrahydroacridines can be mentioned as examples. Quinolines and isoquinolines are other common structures interacting with the α-anionicsite of cholinesterases. Galantamine (Nivalin) is another well-known drug interacting with the α-anionic site of cholinesterase enzymes. Beside the α-anionic site, galantaminealso binds at another important part of the AChE active site including aromatic gorge [34].

The peripheral anionic site is a target of newly synthesized drugs for Alzheimer's disease treatment. Inhibition of the peripheral anionic site can be considered the most promising for Alzheimer's disease treatment. Drugs that bind at the peripheral (e.g. donepezil, huperzine) as well as at the α-anionic site (e.g. tacrine, galantamine) cause elevated expression of AChE. Donepezil is another drug suitable for Alzheimer's disease treatment with good penetration through the blood brain barrier and slow excretion.

The widespread dosefication and/or adulteration of Alzheimer commercially available pharmaceutical preparations demand reliable method for quality control that are preferably selective, rapid and can be undertaken with simple equipment. Electrometric methods using sensors and biosensors, which is now a well established method, with advantage of simplicity, short measurement time, adequate precision and accuracy, and the ability to measure the activity of the target species in colored or cloudy samples. Although sensors had found wide applications for drug quality control [23-28], to the best of our knowledge, few galantamine potentiometric sensors were found in literature. Abdel-Haleem *et al.*, recently published the fabrication of PVC membrane, coated-wire, and carbon-paste sensors for potentiometric determination of galantamine hydrobromide [35]. A new kinetic-potentiometric method for the characterization and analytical determination of competitive reversible enzyme inhibitors was developed [36]. Regarding other tested pharmaceutical compounds, only polyvinylchloride (PVC) sensors were found for donepezil [37], ipratropium [38, 39] or biperdien [40, 41]. The sited methods were linear in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} of the corresponding drugs.

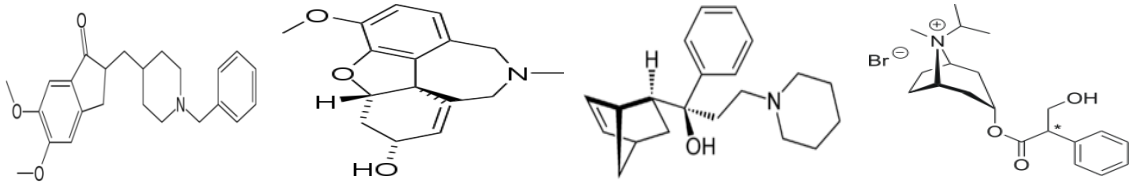
The present work was motivated by the highly sensitive cholinesterase biosensors for analysis of pesticide as cholinesterase inhibitor [33, 42, 43] for detection of other inhibitors such as Alzheimer drugs with improved sensitivity compared with the reported method. The present study includes four drugs namely; donepezil (DP)

and galantamine (GAL) in addition to biperdien (BP) and ipratropium (IPBr) (Fig.1).

Determination of donepezil

The inhibition effect of donepezil on AChE was investigated by incubation of 9.5ng of the drug with 0.4U enzyme (Fig. 2a). The investigated drug showed strong inhibition effect of the enzyme, even at zero time. The relative inhibition degree (I %) was 18.6% at zero and increased to 29.35% after 5 min. Slight increase at 10 min (31.17%) then the inhibition curve tends to a stable value with longer incubation time. Incubation for 10 min was selected to compromise between the sensitivity and analysis time.

Upon construction, the fabricated sensors were used for measuring the remained cholinesterase activities (either BuChE or AChE using their corresponding substrates) after incubation with different donepezil concentrations. The relative inhibition of AChE was much higher than that of BuChE (about 100 fold) which agreed with that reported in literature [44].



DP GAL BP IPBr

Fig.1. Chemical structure of drugs

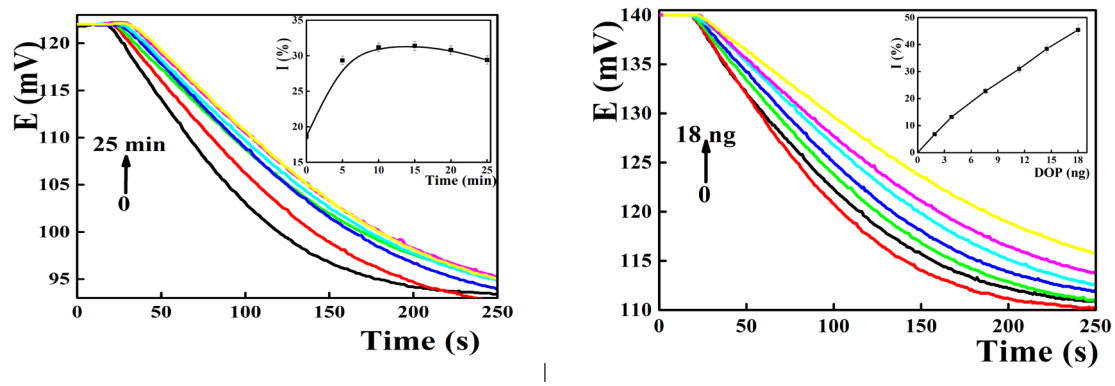


Fig. 2. a) Effect of incubation time on the inhibitory action of DOP on AChE enzyme: measuring cell contains 10 mL of 10^{-4} mol L^{-1}ACh solution and 9.5 ng DOP incubated with 0.4 U enzyme; b) inhibition of AChE enzymes by DOP was conducted for 10 min at 25 °C.

Following the optimal incubation time, the relative inhibition degree of AChE was proportional to dopenzel in the concentration range from 0 to 18 ng with regression equation: I % =2.2380+2.4818 donepezil [ng] and detection limit of1.0 ng. For the developed sensors, five runs (at fixed AChE and 11.4 ng DOP) were performed on 4 different days, in order to evaluate the reproducibility of the results. Average recoveries were101.69±4.78% (Table 1).

Determination of galantamine

Similar to donepezil, galantamine showed potent inhibition of cholinesterase enzymes. By incubation of 66.9 ng of GAL with 0.4 U enzyme, the relative inhibition degree increased from 17.84% at zero time to 19.9 % after 5 min, and to about 20.06 % after 10 min. Further incubation did not improve the inhibition of enzyme (Fig. 3a).

Constructing the calibration curve, different GAL concentrations were incubated with AChE enzyme for 10 min and the residual enzymatic activity was measured. Linear calibration curve was obtained in the concentration range from 0 to 90 ng of galantamine (I % = 0.69358+ 0.28452 [µg mL^{-1}] with detection limit 15 ng. The average recovery for 66 ng was 102.4±3.5% (Table 1).

TABLE 1. Coefficients of calibration curves (I % = a + b × [Drug]), detection limits and working concentration range determined with acetylcholinestrase screen printed biosensor

Drug	DOP	GAL	BP	IPBr
Linear range	0-18 ng	0-90 ng	0-4.69 µg	0-9.96
Slope (b)	2.4818±0.0957	0.28452±0.010	13.4191±0.5442	4.8421±0.0973
Intercept (a)	2.2380±0.9835	0.69358±0.0589	2.3659±0.4581	1.5643±0.6745
R	0.9963	0.9980	0.9967	0.9988
Detection limit	1.0 ng	15.0 ng	0.6 µg	1.0 µg
Average recovery	101.69±4.78% (11.4 ng)	102.40±3.50% (66 ng)	93.35±4.66%. (1.875 µg)	106.98±4.86% (6.64 µg)

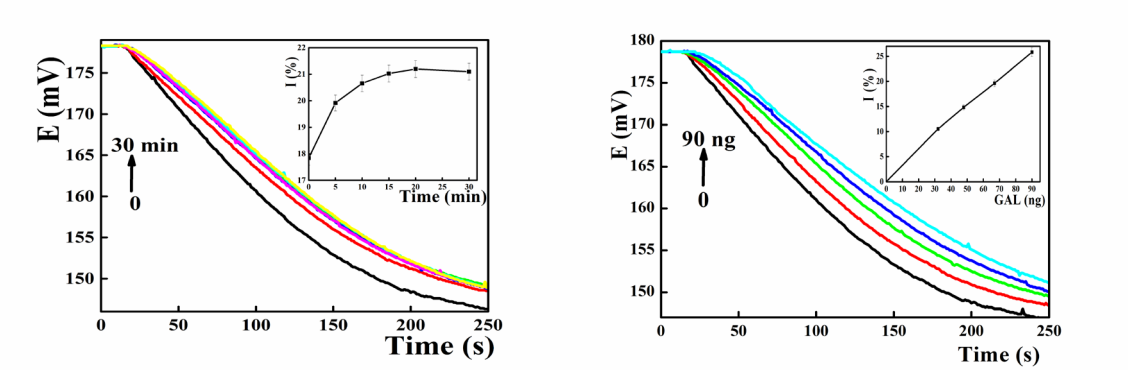


Fig. 3. a) Effect of incubation time on the inhibitory action of GAL on AChE enzyme: measuring cell contains 10 mL of 10-4mol L-1ACh solution and 66.9 ng GAL incubated with 0.4 U enzyme; b) inhibition of AChE enzymes by GAL after incubation period for 10 min at 25 °C.

Determination of biperiden

Not only Alzheimer drug can inhibit cholinesterase, biperiden hydrochloride (1-(5-bicyclok [2.2.1] hept-2-enyl)- 1-phenyl-3-(1-piperidinyl)propan-1-ol hydrochloride), anti-Parkinson that is used in treatment of Parkinsonism [45] can inhibit cholinesterase even to lower extent. By incubation of 1.875µg of BP with 0.4 U enzyme, the relative inhibition degree increased from 0% at zero time to 26 % after 5 min, and to about 46.8 % after 20 min. Further incubation did not improve the inhibition of enzyme (Fig. 4a).

Linear relationship between the BP concentration and the relative inhibition degree of acetylcholinesterase in the concentration range from 0 to 4.69µg of BP (I % = 2.36594+ 13.4191 BP [µg] with detection limit 0.6µg. The average recovery for 1.875 µg was 93.35±4.66% (Table 1).

Determination of ipratropium

Ipratropium bromide (IPBr, 8- azoniabicyclo-octane-3-(3-hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methylethyl) bromide), is a synthetic quaternary ammonium antimuscarinic agent with peripheral effects similar to those of atropine [46, 47]. Ipratropium exhibits broncholytic action by reducing cholinergic influence on the bronchial musculature. It blocks muscarinic acetylcholine receptors, without specificity for subtypes, and therefore promotes the degradation of cyclic guanosine monophosphate (cGMP), resulting in a decreased intracellular concentration of cGMP. Based on its inhibitory effect on AChE, a new analytical approach can be suggested.

The inhibitory effect of IPBr on AChE was time dependent as it increases 0% at zero time to 16.6 % after 5 min, and to about 19.9 % after 20 min. Further incubation did not improve the inhibition of enzyme (Fig. 5a). Calibration curve (Fig. 5) showed working concentration range from 0 to 9.96 µg of IPBr (I % = 1.56425+ 4.84205

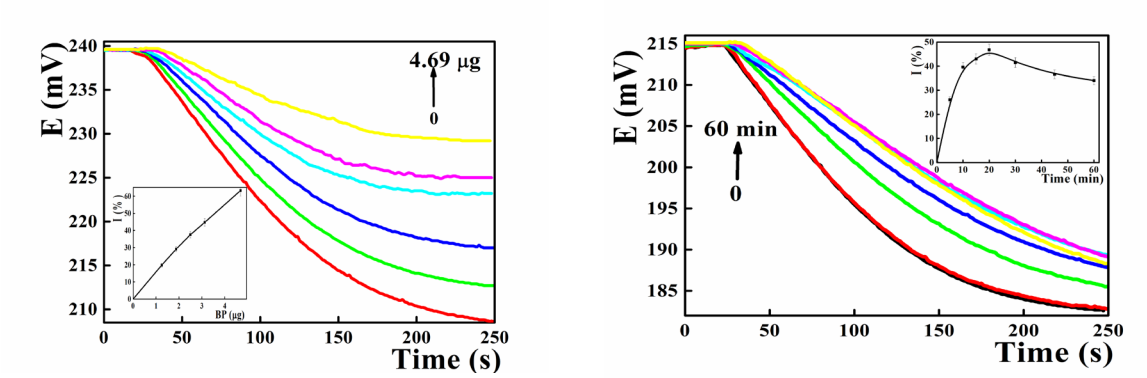


Fig. 4. a) Effect of incubation time on the inhibitory action of BP on AChE enzyme: measuring cell contains 10 mL of 10-4 mol L-1ACh solution and 1.875 µg BP incubated with 0.4 U enzyme; b) inhibition of AChE enzymes by BP after incubation period for 20 min at 25°C.

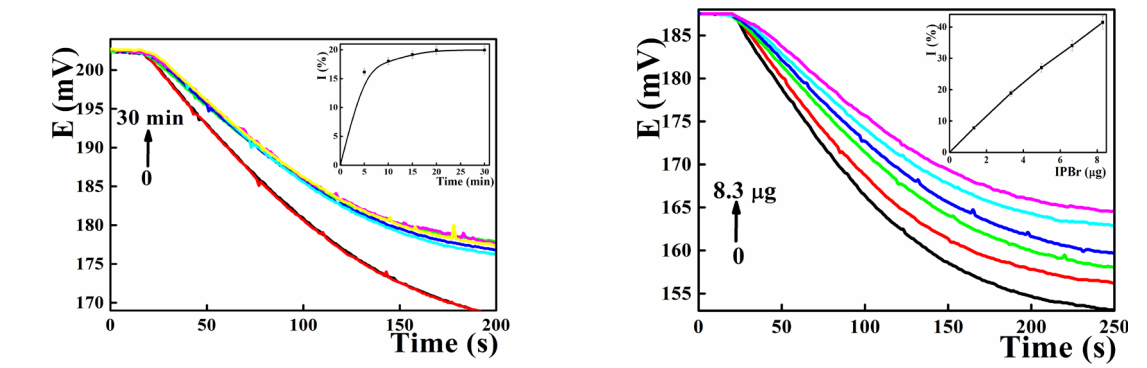


Fig. 5. a) Effect of incubation time on the inhibitory action of IPBr on AChE enzyme: measuring cell contains 10 mL of 10-4 mol L-1ACh solution and 3.32 µg IPBr incubated with 0.4 U enzyme; b) inhibition of AChE enzymes by IPBr after incubation period for 20 min at 25°C.

[µg] with detection limit 1.0 µg. Reproducibility was performed on 4 different days using 6.64 µIPBr and showed average recoveries were 106.98±4.86% (Table 1).

Sample analysis

To further demonstrate the practicality of the proposed method, the investigated drugs in their pharmaceutical were analyzed. The obtained results (Table 2) were in agreement with the official methods corresponding for each drug [30-32].

In addition, the average recoveries of the proposed potentiometric method were compared with the official spectrophotometric Ellman's method for acetylcholinesterase [29] activity. Absorbance was measured using standard spectrophotometer or microplate reader adjusted up wavelength 412nm. Cuvette was consequently filled with: 400 µl of 5,5-dithiobis-(2-nitrobenzoic) acid 1mmol/l,100 µl of Ach E solution, 100 µl of tested compound or phosphate buffered saline and 300 µl of phosphate buffered saline. Reaction was started by adding100 µl of 10mmol/lbutyrylthiocholine and absorbance was measured after 15 s and then after 315 s. Difference of the absorbances was used further.

Conclusion

The present study suggests the application of disposable potentiometric biosensor for analysis of anticholinesterase drugs. Based on relative degree of cholinesterase inhibition, different sensitivities were recorded. Generally, donepezil and galantamine were more potent inhibitors inhibition than biperdien hydrochloride and ipratropium bromide (Ld 50 were 45.2, 10, 545 and 1001 mh kg $^{-1}$, for the aforementioned drugs in the same order). The achieved sensitivities reached the nanograme range which is more sensitive than any reported electrometric method

TABLE 2. Potentiometric determination of drug in their pharmaceutical preparations.

Sample	Taken	Recovery (%) [a]	
		Present biosensor	Gorun method [b]
Aricept[b]	10.0 ng[c]	100.80±4.30	94.65±6.45
Reminyl PR[b]	50.0 ng[c]	101.14±3.355	96.80±4.55%
Akineton[c]	2.0µg[d]	94.66±5.20	91.20±7.45%
Atrovent[d]	5.0µg[e]	104.33±4.25	98.44±5.50%

[a]Mean recovery and relative standard deviations of five determinations

[b] Recovery was calculated according to spectrophotometric assay of cholinesterase activity reference 29

[c]Recovery was calculated according to reference 32.

[d] Recovery was calculated according to reference 31.

for the investigated drugs.

Moreover, the fabricated biosensors are attractive for their potential use as simple and direct screening devices for monitoring detoxification processes available to unskilled users with significant decrease in cost per analysis to complement or replace the classical analytical methods.

Acknowledgment

This work was supported by the National Research Center project (10140002 NRC).

References

1. Barry W.W, Cholinesterases In *Hayes' Handbook of Pesticide Toxicology* (Third Edition) Edited by Robert Krieger (2010).
2. Colovic M.B., Krstic D.Z., Lazarevic-Pasti T.D., Aleksandra M.B and Vasic V.M., Acetylcholinesterase Inhibitors: Pharmacology and Toxicology, *Current Neuropharmacology*, **11**, 315-335 (2013).
3. Pohanka M., Cholinesterases, a target of pharmacology and toxicology. *Biomedical Papers and Medical Faculty for University of Palacky Olomouc Czech Repub,***155**, 219-29 (2011).
4. Pohanka, M., Acetylcholinesterase inhibitors: a patent review (2008 - present). *Expert Opinion on Therapeutic Patents*, **22**, 871-86 (2012).
5. Pepeu G and Giovannini M.G., Cholinesterase inhibitors and beyond. *Current Alzheimer Research,* **1359**(6), 86–96 (2009).
6. Pohanka M., Inhibitors of Acetylcholinesterase and Butyrylcholinesterase Meet Immunity. *International Journal of Molecular Sciences,* **15**, 9809-9825 (2014).
7. De los Rios C., Cholinesterase inhibitors: a patent review (2007-2011). *Expert Opinion on Therapeutic Patents,***22**, 853-869 (2012).
8. Ahmed M., Rocha J. B., Correa, M., Mazzanti C.M., Zanin R. F., Morsch A.L., Morsch V.M and Schetinger M.R., Inhibition of two different cholinesterases by tacrine. *Chemistry and Biological Interactions*, **162**, 165-171 (2006).
9. Pohanka M., Musilek K and Kuca K., Progress of biosensors based on cholinesterase inhibition. *Current Medical Chemistry,* **16**, 790–1798 (2009).
10. Andreescu S and Marty J.L., Twenty years research in cholinesterase biosensors: from basic research to practical applications. *Biomolecular Anger*, **23**,1–15 (2006).
11. Budnikov G.K and Evtyugin G.A., Electrochemical biosensors for inhibitor determination: selectivity and sensitivity control. *Electroanalysis*, **8**, 817–820 (1996).
12. Pohanka M., Cholinesterases in biorecognition and biosensors construction, a review, *Analytical letters,***46,** 1849-1863 (2013).
13. Amine A., Arduini F., Moscone D. and Palleschi G., Recent advances in biosensors based on enzyme inhibition, *Biosensing and Bioelectronics*,**76,** 180–194 (2016).
14. Khaled E and Aboul-Enein H.Y., Pesticides. In: Moretto L., Kalcher K., (Eds) *Environmental Analysis by Electrochemical Sensors and Biosensors,* vol 2, Springer (2014).
15. Evtugyn G.A., Budnikov H.C., Nikolskaya

E.B., Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination. *Talanta*, **46**, 465–484 (1998).

16. Budnikov G.K and Evtyugin G.A., Electrochemical biosensors for inhibitor determination: selectivity and sensitivity control. *Electroanalysis,* **8**, 817–820 (1996).

17. Weiner L., Shnyrov V.L., Konstantinovskii L., Roth E., Ashani Y and Silman I., Stabilization of Torpedo californica Acetylcholinesterase by Reversible Inhibitors. *Biochemistry,* **48**, 563-574 (2009).

18. Macdonald I.R., Martin E., Rosenberry T.L and Darvesh S., Probing the peripheral site of human butyrylcholinesterase. *Biochemistry,* **51**, 7046-7053 (2012).

19. Berg L., Andersson C.D., Artursson E., Hornberg A., Tunemalm A.K., Linusson A and Ekstrom F., Targeting acetylcholinesterase: Identification of chemical leads by high throughput screening, structure determination and molecular modeling. *Plos One* **6**, e26039 (2011).

20. Rampa A., Belluti F., Gobbi S. and Bisi A., Hybrid-based multi-target ligands for the treatment of Alzheimer's disease. *Current Tropical Medical and Chemistry*, **11**, 2716-2730. (2011).

21. Luo W., Li Y.P., HeY., Huang S.L., Li D., Gu L.Q and Huang Z.S., Synthesis and evaluation of heterobivalent tacrine derivatives as potential multi-functional anti- Alzheimer agents. *European Journal of Medicinal Chemistry*, **46**, 2609-2616 (2011).

22. Da Silva V.B., De Andrade P., Kawano D.F., Morais P.A.B., De Almeida J.R., Carvalho I., Taft C.A and Da Silva C., In silico design and search for acetylcholinesterase inhibitors in Alzheimer's disease with a suitable pharmacokinetic profile and low toxicity. *Future Medicinal Chemistry*, **3**, 947-960 (2011).

23. Stefan R.I., Baiulescu G.E and Aboul-Enein H.Y., Ion-selective membrane electrodes in pharmaceutical analysis. *Critical Reviews in Analytical Chemistry,* **27**, 307–321 (1997).

24. Angnes D., Pharmaceutical and Personal Care Products. In: Moretto L., Kalcher K., (Eds) *Environmental Analysis by Electrochemical Sensors and Biosensors* vol 2, Springer (2014).

25. Gupta V.K., Nayak A., Agarwal S and Singhal B., Recent advances on potentiometric membrane sensors for pharmaceutical analysis, *Combinatorial Chemistry High and Throughput Screening,* **14**,284 (2011).

26. Ozkan S.A., Kauffmann J.M and Zuman P., *Electroanalysis in Biomedical and Pharmaceutical Sciences,* Springer(2016).

27. Mohamed H.M., Screen-printed disposable electrodes: Pharmaceutical applications and recent developments, *Trends in Analytical Chemistry,* **82**, 1–11 (2016).

28. Couto R.A.S., LimaJ.L.F.C and Quinaz M.B., Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis. *Talanta,* **146**801–814 (2016).

29. Gorun V., Proinov L., Baltescu V., Balaban G and Barzu G., Modified Ellman procedure for assay of cholinesterase in crude enzymatic preparations. *Analytical Biochemistry,* **86**, 324-360 (1978).

30. European Pharmacopoeia, 3rd edn. Supplement 13, Council of Europe, Strasbourg, (2001).

31. USP 30, NF 25, United States Pharmacopeial Convention 12601 Twinbrook, Parkway, Rockville, MD 20852 (2008).

32. United States Pharmacopeia 28th ed., Rockville, MD, The United States Pharmacopoeial Convention Incorporated pp.2749-2751 (2005).

33. Khaled E., Kamel M.S., Hassan H.N.A., Abdel-Gawad H and Aboul-Enein H.Y., Performance of a portable biosensor for analysis of ethion residue. *Talanta,* **117** 467 (2014).

34. Pohanka M., Cholinesterases, a target of pharmacology and toxicology. *Biomedical Papers and Medical Faculty for University of Palacky Olomouc Czech Repub.,* **155**, 219-29 (2011).

35. Abdel-Haleem F.M., Saad M., Barhoum A., Bechelany M., Rizk M.S., PVC membrane, coated-wire, and carbon-paste ion-selective electrodes for potentiometric determination of galantamine hydrobromide in physiological fluids. *Materials Science & Engineering C*, **89,** 140–148 (2018).

36. Cuartero M., García M.S, García-Cánovas F, Ortuño, J.A, New approach for the potentiometric-enzymatic assay of reversible-competitive enzyme inhibitors. Application to acetylcholinesterase inhibitor galantamine and its determination in pharmaceuticals and human urine*, Talanta*, **110,** 8-14 (2013).

37. Mostafa G.A., Hefnawy Mand Al-Majed A., Membrane sensors for the selective determination of donepezil hydrochloride. *Journal of Association of Official Agriculture Chemists International.* **93**, 549-55 (2010).

38. Hassouna M.E and Elsuccary S.A., PVC membrane electrode for the potentiometric determination of ipratropium bromide using batch and flow injection techniques. *Talanta,* **75**, 1175-1183 (2008).

39. Khaled E., Hassan H.N.A., Ahmed M.A., El-Attar R.O., Novel Ipratropium bromide Nanomaterial Based Screen-Printed Sensors, Anal. *Methods,* **9,** 304-311 (2017).

40. Khaled E., El-Sabbagh I.A., Husseny N.G. and Abdel Ghahni E.Y., Novel PVC Electrode for Flow Injection Potentiometeric Determination of Biperiden in its Pharmaceutical Preparations, *Talanta*, **87**, 40-45 (2011).

41. Khaled E., Hassan H.N.A., Ahmed M.A., El-Attar R.O., Crown Ether/Carbon Nanotubes Based Biperiden Disposable Potentiometric Sensor, *Electroanal.* **29**, 978-982 (2017).

42. Khaled E., Hassan H.N.A., Mohamed G.G., Ragab F.A and Seleim A.A., Disposable Potentiometric Sensors for Monitoring Cholinesterase Activity. *Talanta*, **83,** 357 (2010).

43. Khaled E., Kamel M.S., Hassan H.N.A., Malhat F.M and Abdel-Gawad H., Rapid detection of methomyl and organophosphorous pesticides with portable potentiometric biosensor. *Analytical Chemistry Letters*, **5,** 117-126 (2015).

44. Darvesh S., Walsh R., Kumar R., Caines A., Roberts S., Magee D., Rockwood Kand Martin E., Inhibition of human cholinesterases by drugs used to treat Alzheimer disease. *Alzheimer Disease and Associated Disorders,* **17** (2), 117-126 (2003).

45. British Pharmacopeia, The Stationery Office, London (2008).

46. Rominger K.L., Chemistry and Pharmacokinetics of Ipratropium Bromide. *Scandinavian Journal of Respiratory Diseases Supplementum.,* **103**, 29-116(1979)

47. Martindale X., The Complete Drug Reference, 35th ed., Pharmaceutical Press, London (2007).

(Received 31/7/2018;
accepted 3/10/2018)

اتجاهات انزيميه جهديه عاليه الحساسية لأدوية مضادات الكولين استريز

المرسى خالد[١]، حسن نجيب أحمد حسن[١]، منى عبد العزيز أحمد[٢]، رحاب السيد عمر العطار[١]
[١]معمل التحليلات الدقيقه - قسم الكيمياء العضويه التطبيقيه - شعبه بحوث الصناعات الكيميائيه - المركز القومى للبحوث - الجيزة - مصر.
[٢]قسم الكيمياء - كلية البنات للأداب والعلوم والتربية - جامعة عين شمس - القاهرة - مصر.

تصف الطريقه الحاليه تطبيق حديث لمحسات حيويه جهديه حديثه لتحليل لأدويه مثبطات انزيم الكولين استيريز (الزهايمر). تعتمد الطريقه المقترحه تثبيط انزيم الكولين استيريز بواسطه أدويه الزهايمر وهى الدونيبيزل، الجالانتامين، الابراتروبيوم والبابيريدين. اعتمادا على معدل التثبيط النسبى للأدويه على نشاط انزيم الكولين استيريز تم الوصول الى مدى حساسيه مختلف يتراوح بين ١٠الى ١٨ نانوجرام، ٠ الى ٩٠ نانوجرام، ٠ الى ٤,٩٦ ميكروجرام و ٠ الى ٨,٣ ميكروجرام من الادويه المذكوره على الترتيب اعتمادا على قيم تركيز ٥٠٪ المميت منها. تم تطبيق الطريقه بنجاح لقياس الادويه فى مستحضراتها الدوائيه بحساسيه تماثل الطرق القياسيه. يمكن اقتراح هذه الطريقه لقياس مدى سميه الادويه تجاه انزيم الكولين استيريز.