Novel simple enzymatic potentiometric approach for toxicological assessment of anticholinesterase and Alzheimer's drugs Enzymatic approach toxicological assessment

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Background and objectives
A simple and sensitive enzymatic potentiometric biosensor has been developed for toxicological studies of anticholinesterase drugs based on their inhibitory effect on cholinesterase activity. To verify the applicability, eight pharmaceutical formulations for Alzheimer's treatment were assayed, namely rivastigmine, pyridostigmine, cyclopentolate, memantine, meclofenoxate, carbamazepine, oxfendazole, and methotrexate.

Materials and methods
Disposable screen-printed potentiometric sensors were utilized for monitoring the cholinesterase activity. The reaction conditions including the optimal enzyme substrate, incubation periods, and the linearity range for each drug were optimized for each drug.

Results and conclusion
Different sensitivities within subnanogram levels were reported based on the inhibitory effect of the aforementioned pharmaceutical compounds and their LD50 value. The proposed method showed improved sensitivity for the investigated compounds compared with their reported electroanalytical approaches. The introduced analysis protocol was successfully utilized for assaying the cited drugs in their pharmaceutical and environmental samples using a portable measuring system. Moreover, the toxicity of the pharmaceutical compounds against in-vitro cholinesterase enzymes studies can be performed with simple instrumentation requirements.

Keywords:
Cholinesterase inhibitors, environmental samples, enzymatic potentiometric biosensor, pharmaceutical analysis, toxicological assessment

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Introduction

Alzheimer's disease (AD) is reported to be one of the most common cases of dementia worldwide [1]. AD is a chronic syndrome of the central nervous system causing the decline in cognitive function, memory, language ability, and learning [2]. Based on the cholinergic hypothesis [3], a reduction in the acetylcholine (ACh) level is the main cause of AD.

Cholinesterase enzymes (ChE) are responsible for breaking down of the alkylcholine esters into their corresponding parts: choline and aliphatic acid. ChE possess two main active subsites, namely, the esteratic subsite and the anion-binding site. The anionic site binds to the positively charged quaternary amine of the alkylcholine, in addition to other competitive cationic substrates and inhibitors [4,5]. On the other hand, the esteratic site contains a catalytic cage consisting of Ser 200, Glu 327, and His 440 amino acid residues, which acts through hydrolysis of choline esters by the proton transfer mechanism [4–6].

Biologically, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are the two ChE that are present in the body. The former primarily presents in the neural synapses and blood, while BuChE is present mainly in the liver [7]. AChE hydrolyzes ACh more quickly compared with butyrylcholine (BuCh), while BuChE hydrolyzes BuCh more quickly than ACh. Many of the available cholinesterase (ChE) inhibitors for the treatment of AD targeted both AChE and/or BuChE with different efficiencies and selectivities. Inhibition of the biological activity of cholinesterases is the main potential therapeutic strategy to improve ACh levels in the brain [8].

Contrary, the irreversible or prolonged inhibition of ChE leads to the accumulation of ACh causing severe

central and peripheral effects which fall under the cholinergic syndrome spectra [9]. Nowadays, inhibition of AChE and BuChE have been a very crucial issue for pharmacological treatments of several disasters.

Cholinesterases are the target of several inhibitors or toxins such as insecticides, nerve agents, and nerve gases. Besides toxins, many pharmaceuticals administrated for the treatment of AD and myasthenia gravis are known as ChE inhibitors [10–15]. Based on the sensitivity of cholinesterase toward various inhibitors and toxins, cholinesterase biosensors can be utilized as fast and sensitive efficient tools for toxicity assessment of several natural toxins and drugs, food quality control, and environmental monitoring [16–19].

ChE inhibitors used for the treatment of AD were commercially available in the market where donepezil, galantamine, stigmine family, and memantine are the most common [20–24]. For pharmaceutical analysis, spectrophotometric approaches lack selectivity and sensitivity, especially for biological samples and complex matrices. Operation of chromatographic instrumentation requires expert personnel for handling and pretreatment of the biological sample under tedious and sophisticated protocol. These requirements impede chromatographic techniques for large-scale sample analysis and field onsite measurement. Thus, development and validation of novel, simple, sensitive, and economic analytical methods for pharmaceutical compounds is welcomed. Electroanalytical approaches based on electrochemical cholinesterase biosensors were reported as simple and sensitive tools for assaying of cholinesterase inhibitors in pharmaceutical, food, and environmental samples with the advantages of adequate performance and inexpensive instrumentation equipment [18,20,24–28].

Therefore, the present study aimed to utilize a simple cholinesterase potentiometric biosensor for assaying of eight different cholinesterase inhibitors and AD drugs. The recommended procedures were based on incubation of the free AChE with the target inhibitor followed by measuring of the residual AChE activity utilizing a simple disposable potentiometric biosensor. Improved performances were recorded compared with the direct potentiometric measurements and potentiometric titration modes applying the corresponding ion-selective electrode. Moreover, the proposed analysis protocol is attractive for their simple use in the direct screening and monitoring of detoxification processes.

Materials and methods

Chemicals and reagents

Analytical grade reagents were used throughout the experiments. Acetylcholine bromide (ACh), acetyl thiocholine chloride (AtCh), acetylmethylcholine chloride (AmCh), and butylcholine chloride (BuCh) were purchased from Sigma. Alpha cyclodextrin (Sigma-Aldrich, USA), potassium tetrachloro tetraphenylborate(Fluka, USA), 360 mg fluoro nitrophenyl ether(Sigma), polyvinyl chloride (relative high-molecular weight; Sigma-Aldrich), and multiwall carbon nanotubes (Sigma-Aldrich) were applied for construction of the sensing membrane. Aqueous solutions of alkylcholine esters were prepared in phosphate buffer solution (pH 7.0). One vial of AChE enzyme (2000 IU; Sigma) was dissolved in 10 ml of phosphate buffer solution (pH 7.0) as the stock enzyme solution and the specific enzyme activity was assayed following Ellman's photometric method [29].

Authentic sample

Authentic meclofenoxate hydrochloride ($C_{12}H_{17}Cl_2NO_3$, 294.17 g/mol), memantine hydrochloride ($C_{12}H_{22}ClN$, 215.76 g/mol), cyclopentolate hydrochloride ($C_{17}H_{26}ClNO_3$, 327.8 g/mol), carbamazepine ($C_{15}H_{12}N_2O$, 236.27 g/mol), methotrexate ($C_{20}H_{22}N_8O_5$, 454.447 g/mol), oxfendazole ($C_{15}H_{13}N_3O_3S$, 315.35 g/mol), rivastigmine ($C_{14}H_{22}N_2O_2$, 250.342 g/mol), and pyridostigmine ($C_9H_{13}N_2O_2$, 181.215 g/mol) were achieved from the National Organization for Drug Control and Research, Egypt.

Pharmaceutical samples

Exelon (rivastigmine hydrogen tartrate, 1.5 mg/capsule; Novartis, Cairo, Switzerland) were obtained from local drug stores. Content of four capsules was dissolved in 24 ml bidistilled water. One Pystinon tablets (pyridostigmine bromide, 60 mg/tablets; Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) was ground and dissolved in 229 ml bidistilled water.

Memantine pharmaceutical formulation (Alzmenda, 10 mg/tablet of memantine hydrochloride; Marcyrl Pharmaceutical Industries, Cairo, Egypt) was dissolved in 46.3 ml bidistilled water. Lucidril tablets (250 mg/tablet of meclofenoxate hydrochloride; Minapharm Pharmaceuticals, Cairo, Egypt) was ground and dissolved in 85 ml bidistilled water. One Tegretol tablet (carbamazepine, 200 mg/tablet; Novartis Pharmaceuticals, UK) was dissolved in 84.7 ml bidistilled water. Cyclopentolate commercial

sample, Pentolate, eye drops was assigned to contain 1% cyclopentolate, Amoun (Cairo, Egypt). The tested oxfendazole sample, Unifendazole suspension (22.5% w/v oxfendazole; Unipharma Co., Cairo, Egypt) was diluted to the appropriate concentration and assayed according to the recommended procedures. Unitrexate (50 mg/2 ml of methotrexate; Hikma Pharmaceutical Industries, Beni Sweef, Egypt) was purchased from local drug stores.

Water samples

Water samples (River Nile, Giza Governorate) was sterilized under aseptic conditions and adjusted to pH 5.0 with acetate buffer. Samples were spiked with authentic drug solutions and analyzed according to the proposed solution in comparison with the official pharmacopoeial protocol.

Apparatus

Radio Shack Digital multimeter with PC interface was utilized to monitor the cell potential with read output mV/s. The bielectrode potentiometric strips were through printing of the working and reference electrodes (each 5×35 mm) on the polyvinyl chloride sheet as described in detail elsewhere [26]. The sensing membrane layer was printed over the carbon track layer using sensing cocktail composed of 0.75 mg alpha cyclodextrin as recognition element, 0.5 mg potassium tetrachloro tetraphenylborate as ionic sites, 360 mg fluoro nitrophenyl ether, 6 ml THF, 30 mg multiwall carbon nanotubes as transducers, and 240 mg polyvinyl chloride. Prior to measurement, the printed sensors were preconditioned in 10^{-3} mol/l of the corresponding alkylcholine ester for 30 min.

Relative inhibition measurements

Aliquots of the selected drug solution were incubated with 100 µl of AChE for the appropriate incubation period followed by measuring the residual enzymatic activity in a reaction mixture containing 3 ml of 10^{-3} mol/l ACh solution and 7 ml of phosphate buffer (pH 7.0) at 25°C. The electrode potential was monitored to estimate the residual AChE activity applying the initial rate kinetic method (slope of the tangent for the first linear part of potential-time curves). Real samples were analyzed after suitable dilution applying the proposed enzymatic procedures in comparison to the official method and Ellman's method [29].

Results and discussion

Cholinesterase inhibitors

Cholinesterase inhibitors, or anticholinesterase, showed a significant role in the biochemical processes of the human body. As a result of their inhibitory action on ChEs activity, the extent and duration of the neurotransmitter action were improved. Based on the mode of their action, anticholinesterase can be categorized into two main groups: reversible and irreversible ones. Irreversible inhibitors showed the ability to form a covalent bond to the esteratic or serine active site with acute toxic effects on the enzyme activity. This category includes organophosphorous pesticides, nerve gases, and some common drugs administrated for Alzheimer's treatment such as donepezil, galantamine, memantine, rivastigmine, and pyridostigmine.

Challenges to the development of a new drug for Alzheimer's treatment include its safety, side effects, and potency as well as comparison to current ChE inhibitors. It is noteworthy to mention that many of the administrated developed medications have no human or animal data. Therefore, their safety, toxicity, and efficacy have to be established. Based our previous work [28], the research team established a simple analytical approach for eight cholinesterase inhibitors, namely rivastigmine, pyridostigmine, cyclopentolate, memantine, meclofenoxate, carbamazepine, oxfendazole, and methotrexate utilizing a simple potentiometric biosensor.

Effect of the enzyme substrate

Among CEs, ACh was the first identified neuromodulator as the only one in the somatic nervous system of vertebrates and insects. Other alkylcholine esters were synthetic including BCh, AmCh, and AtCh and not present naturally in the body. ChE catalyze the hydrolysis of alkylcholine esters into their inactive metabolite choline (Ch) and the corresponding carboxylic acid. ChE showed relatively high specific catalytic activity; one molecule of AChE has the ability to degrade about 25 000 molecules of ACh per minute [30,31]. AChE showed substrate relative reaction rate values equal to 1.0 for ACh, 0.5 for AtCh, and 0.4 for both BuCh and AmCh [32–34]. In our previous work, feasible, sensitive disposable sensors were fabricated for sensitive and selective monitoring of different choline ester derivatives [25,26,35]. As the developed sensors were more selective to CEs compared with their inactive metabolite choline depending on the aliphatic substitution of the side chain and replacing of the oxygen atom with sulfur, resulting in more favorable host–guest interactions with the cyclodextrin recognition element.

After successful application of the developed sensors for potentiometric determination of CEs, the

aforementioned sensors were utilized as an indicator electrode for monitoring the ChE enzymatic activity. Based on the enzymatic activity, the CE concentration in the membrane–solution interface was diminished, and the corresponding electrode potential decreases. The minimum steady-state electrode potential can be reached when the choline esters at the sample–membrane interface are consumed and converted to the reaction product (Ch), which governs the electrode response [36].

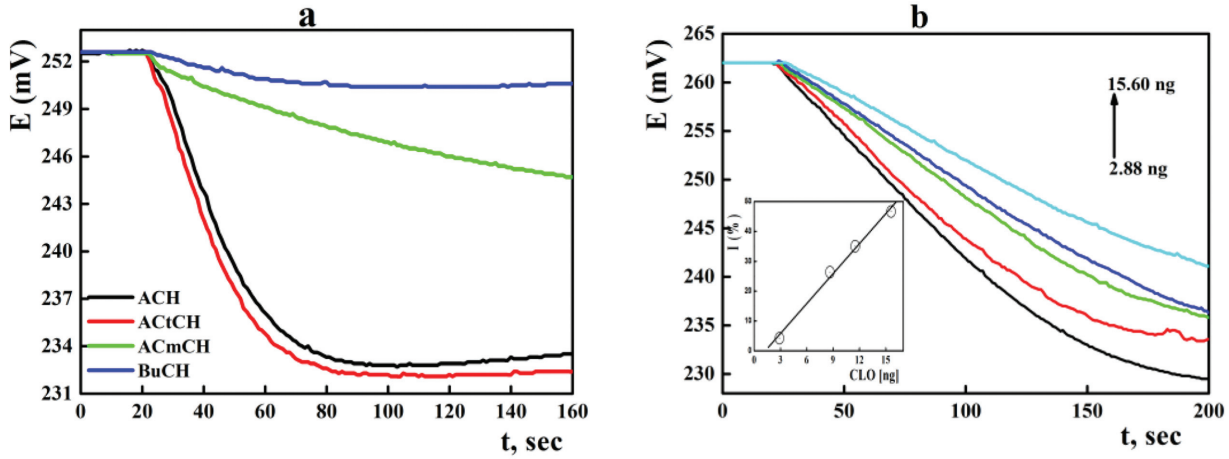
The influence of the applied enzyme substrate on the enzymatic reaction rate monitored with the fabricated sensors is illustrated in Fig. 1 a. The highest enzymatic activity indicated by the highest ($\Delta E/\Delta t$) value was achieved utilizing ACh and AtCh as enzyme substrates, which may be attributed to the enzyme/substrate specificity and the sensitivity of the sensor toward the AtCh.

Moreover, the enzymatic reaction was performed at different ACh concentrations aiming at selection of the most appropriate condition. Low enzyme activities were monitored at a lower substrate concentration with a total potential jump of about 13 and 20 mV, for 50 and 100 µmol/l. Higher ACh was selected with higher enzymatic activity and total potential change of about 30 mV (Fig. 1b).

Potentiometric determination of cholinesterase inhibitors

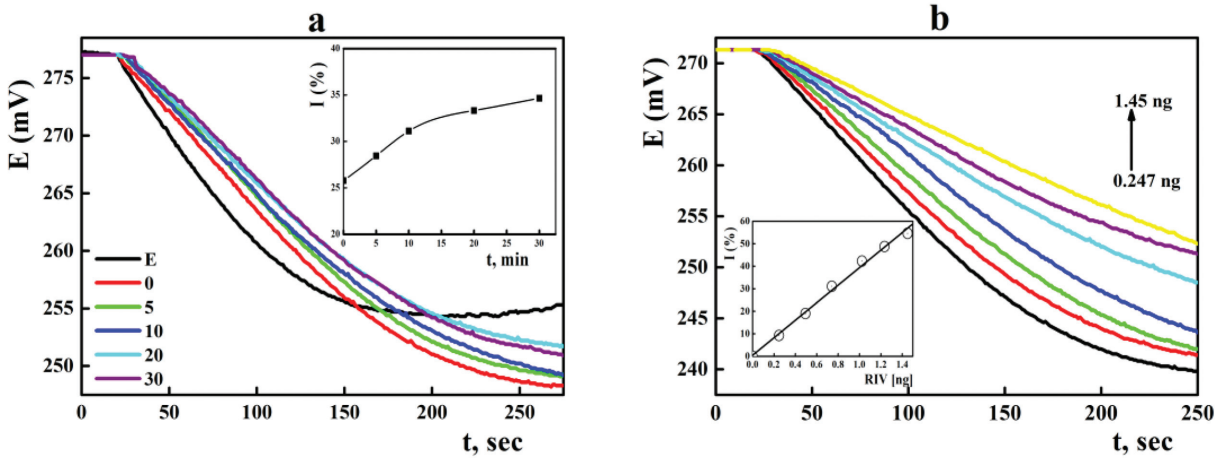
Aiming at achieving the highest sensitivity of the proposed analysis protocol, each investigated drug

Figure 1



Acetylcholinesterase activity recorded: (a) utilizing different choline esters at 100 µmol/l substrate concentration and (b) at different ACh concentrations. Experimental conditions: 7.0 ml phosphate buffer of pH 7.0 utilizing ACh/SPE sensors. ACh, acetylcholine.

Figure 2

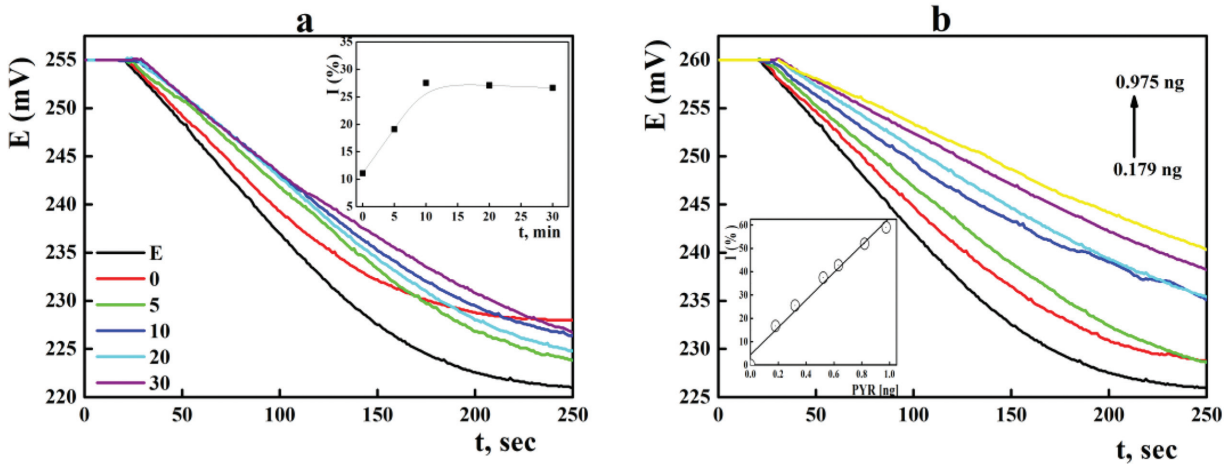


(a) Relative inhibitory activity of AChE enzyme after incubation with RIV: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 0.741 ng RIV incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by RIV was conducted for 20 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; RIV, rivastigmine.

was incubated with AChEs for different incubation periods, after which the residual enzymatic activity was assayed at the recommended experimental conditions. Incubation of 0.741 ng of rivastigmine with AChE showed instant inhibition value 25.77% of the original enzymatic activity which increased to 33.3% after a 20 min incubation period(Fig. 2a). Calibration curves were linear within the rivastigmine concentration ranging between 0.247 and 1.45 ng/ml with a limit of detection (LOD) value of 0.097 ng of rivastigmine (Fig. 2b).

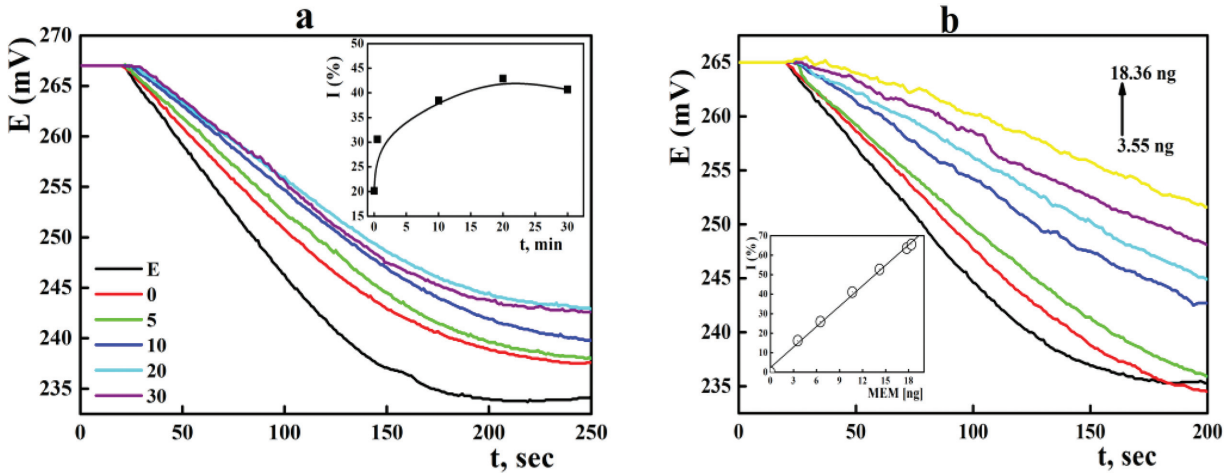
Administration of pyridostigmine, the drug for the treatment of Alzheimer's, showed a potent inhibitory effect on AChE activity. For 0.32 ng of pyridostigmine, 11.11% relative inhibition was recorded at zero incubation time, which was improved to reach its maximum value (27.56%) after 10 min of incubation (Fig. 3a). Performing the inhibition of AChE at different pyridostigmine concentrations showed linear calibration curve [I%=4.429+58.698 pyridostigmine (ng), r=0.9917] within the pyridostigmine concentration that ranged from 0.179 to 0.975 ng and LOD value of 0.113 ng pyridostigmine (Fig. 3b). The method was highly reproducible with average recovery of 99.85±2.78% for 0.523 ng of pyridostigmine.

Figure 3



(a) Relative inhibitory activity of AChE enzyme after incubation with PYR: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 0.325 ng PYR incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by PYR was conducted for 10 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; PYR, pyridostigmine.

Figure 4



(a) Relative inhibitory activity of AChE enzyme after incubation with MEM: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 8.77 ng MEM incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by MEM was conducted for 20 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; MEM, memantine.

Memantine, a well-known Alzheimer's drug, was also assayed according to the presented procedures. Incubation of 8.877 ng of memantine showed a 20.15% inhibition value after instant mixing with the enzyme, which was improved to 43.0% when the incubation period was prolonged to 20 min (Fig. 4a).

A linear relationship [I%=2.402+3.496 memantine (ng), r=0.9983] was constructed within the memantine concentration range from 3.55 to 18.36 ng with an LOD value of 1.056 ng of memantine (Fig. 4b).

Meclofenoxate was administrated as one of the oldest nootropic drugs used to treat the symptoms of senile dementia and AD [37]. Similarly, this compound can be assayed based on its inhibitory effect on AChE enzyme. With less potency and the requirement for longer incubation periods, incubation of 1275 ng of meclofenoxate revealed the maximum inhibitory effect after incubation for 30 min (Fig. 5b). Calibration curves were linear within the meclofenoxate concentration that ranged from 375 to 1725 ng with an LOD value of 30.924 ng/ml.

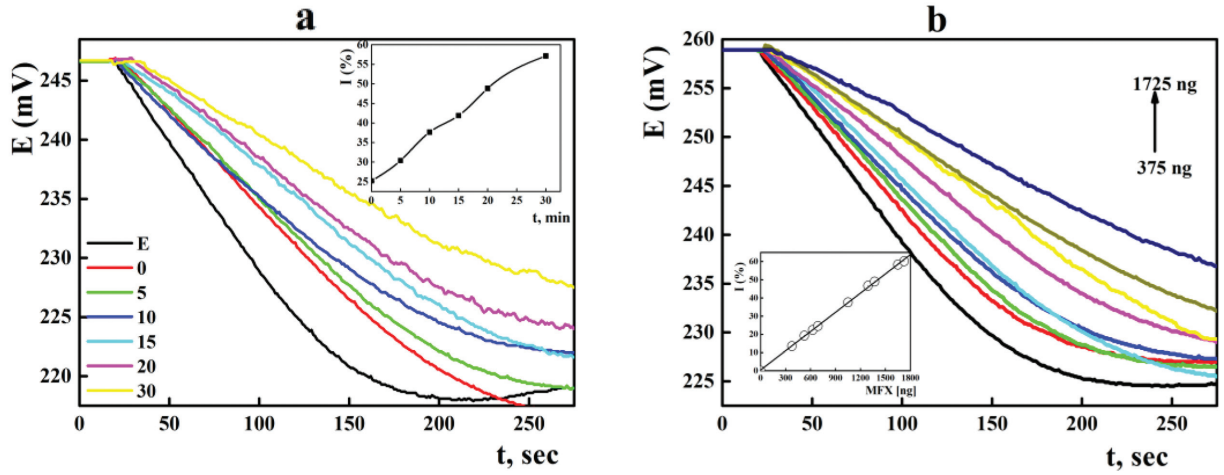
Cyclopentolate is an antineoplastic compound that acts close to atropine causing ciliary muscle paralysis and mydriasis (cycloplegia), which resulted in failure of the lens accommodation. Cyclopentolate belongs to a class of drugs known as anticholinergics [38,39] through blocking of the ACh receptor in the sphincter muscle of the iris and the ciliary muscle, thus the contraction and dilation of the pupil was prevented. Based on its anticholinesterase effect, it can be assayed according to the proposed method.

Incubation of 14.4 ng of cyclopentolate showed inhibition value 17.7% at zero time which was enhanced to 29 and 42% after 10 and 20 min, respectively (Fig. 6). Performing the assaying after 20 min incubation, the relative inhibition was linear against the cyclopentolate concentration that ranged from 2.88 to 15.6 ng with a correlation coefficient of 0.9966 and an LOD value of 0.947 ng (Fig. 6).

Oxfendazole is a broad-spectrum benzimidazole anthelmintic. It is the sulfoxide metabolite of fenbendazole and administrated for protecting different livestock against strongyles, round worms, and pinworms [40]. Oxfendazole was reported as one of the cholinesterase inhibitors [41]. Incubation of 6.24 ng of oxfendazole showed a relative inhibitory effect of 12.92%, which was improved to 28% after a 20 min incubation period (Fig. 7). Calibration curves constructed at the optimized conditions were rectilinear [I%=12.481+1.457 oxfendazole (ng), r=0.9992] within the oxfendazole concentration that ranged from 3.12 to 34.32 ng with an LOD value of 1.434 ng (Fig. 7).

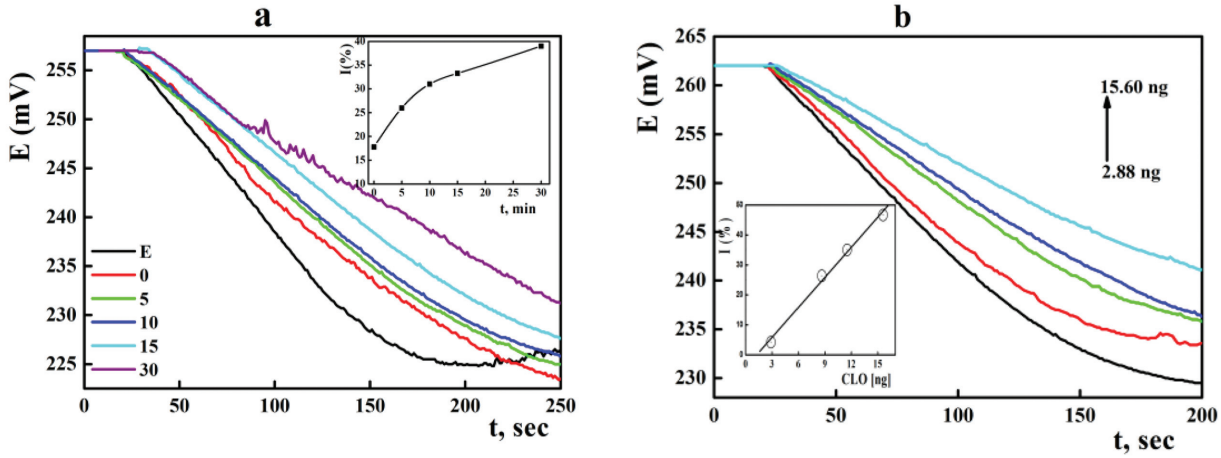
Methotrexate is a folate antagonist drug suggested for the treatment of cancers, rheumatoid arthritis, various malignancies, and inflammatory bowel disease. The molecular interaction of methotrexate with human brain cholinesterase was reported [42]. Herein, the relative inhibitory effect for 9.0 ng methotrexate on

Figure 5



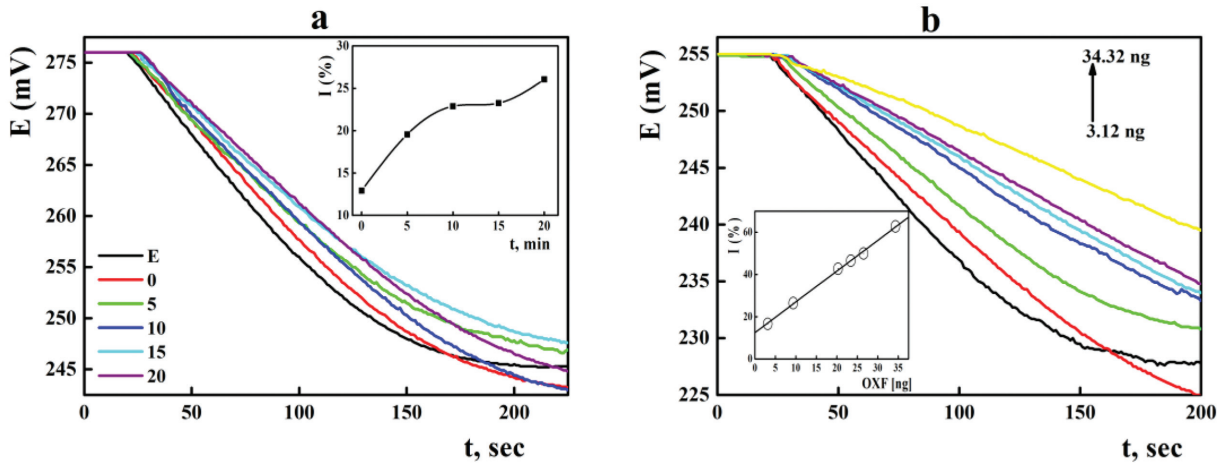
(a) Relative inhibitory activity of AChE enzyme after incubation with MFX: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 1275 ng MFX incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by MFX was conducted for 30 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; MFX, meclofenoxate.

Figure 6



(a) Relative inhibitory activity of AChE enzyme after incubation with CLO: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 14.4 ng CLO incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by CLO was conducted for 20 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; CLO, cyclopentolate.

Figure 7



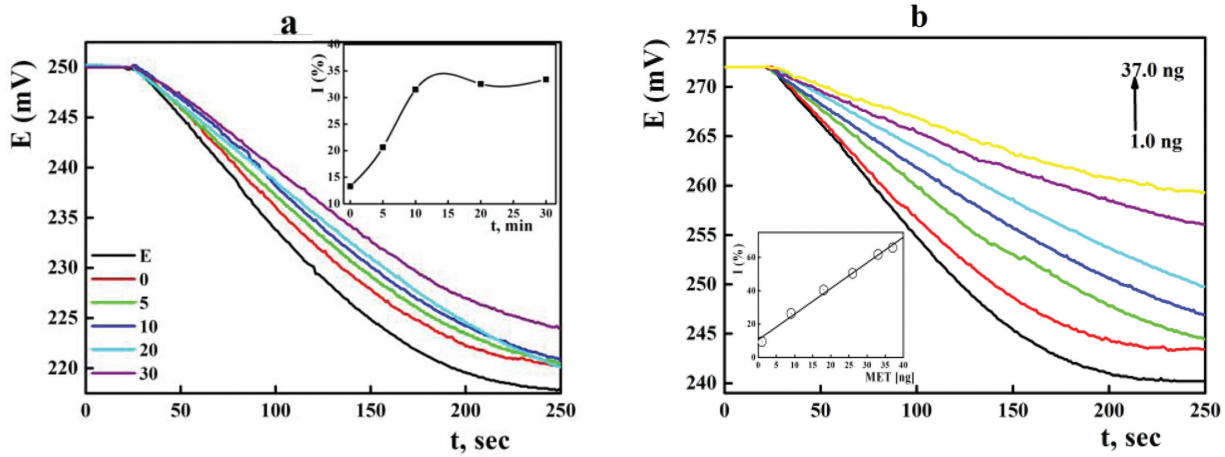
(a) Relative inhibitory activity of AChE enzyme after incubation with OXF: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 6.24 ng OXF incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by OXF was conducted for 20 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; OXF, oxfendazole.

AChE increased from 13.3% at zero time to reach 30.73% after incubation for 10 min with minor improvement after 30 min (Fig. 8). Calibration curves showed enhanced linearity in the methotrexate concentration that ranged from 1.0 to 37.0 ng with an LOD value of 3.557 ng (Fig. 8).

Carbamazepine is a tricyclic compound that acts as an antiepileptic, anticonvulsant, and also as a mood-stabilizing drug, which is widely prescribed drug for the treatment of seizure disorder, mental depression, neuralgia, and posttraumatic stress [43]. The inhibitory effect of carbamazepine cholinesterase enzymes was studied applying molecular docking models [44]. Herein, the inhibitory effect of 4.68 ng of carbamazepine on AChE was performed after different incubation periods. For zero incubation time, 16.41% relative inhibition was recorded which reach its maximum value after 15 min (Fig. 9). The carbamazepine concentration ranged from 2.344 to 9.50 ng; calibration curves were linear (r=0.9972) with an LOD value of 0.590 ng (Fig. 9).

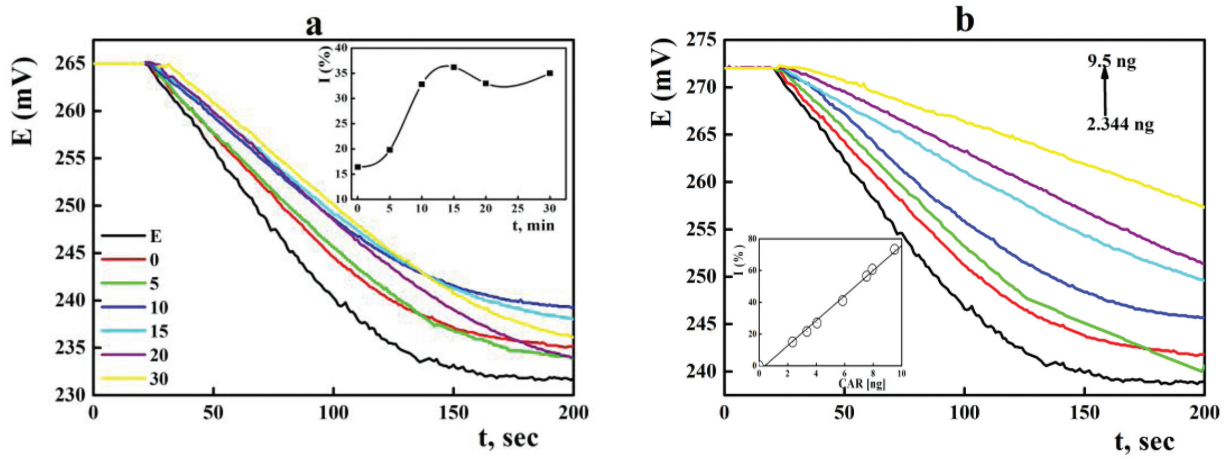
Collectively, the linearity and sensitivity parameters for the aforementioned different investigated pharmaceutical compounds are tabulated in Table 1. It can be clearly noticed that the sensitivity, expressed

Figure 8



(a) Relative inhibitory activity of AChE enzyme after incubation with MET: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 9.0 ng MET incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by MET was conducted for 10 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; MET, methotrexate.

Figure 9



(a) Relative inhibitory activity of AChE enzyme after incubation with CAR: measuring cell contains 10 ml of 3×10^{-4} mol/l ACh solution and 4.68 ng CAR incubated with 0.4 U enzyme and (b) inhibition of AChE enzymes by CAR was conducted for 15 min at 25°C. ACh, acetylcholine; AChE, acetylcholinesterase; CAR, carbamazepine.

as the slope of the calibration graphs, of the LOD and limit of quantification (LOQ) values was correlated with the amount of an ingested substance that kills 50 percent of a test sample (LD50) of the reported drugs. Compounds with a low LD50 value such as rivastigmine and pyridostigmine showed the lowest LOD values, and therefore were widely applied in the treatment of AD.

Sample analysis

To demonstrate the applicability of the introduced analytical approach, the investigated drugs in their corresponding pharmaceutical preparations and environmental samples were assayed. The reported recovery values were acceptable in comparison with the official Ellman's spectrophotometric method for AChE activity with the advantages of simple, inexpensive analysis equipment, which can be introduced for field measurement with portable potentiometric systems (Tables 2 and 3).

Conclusion

The present study introduced a novel disposable potentiometric biosensor as an efficient tool for toxicological assessment of different anticholinesterase drugs. Based on the relative inhibition degree, different sensitivities, linearity ranges, and LOD values were recorded for the drugs under investigation. Generally, rivastigmine and

Table 1 Enzymatic potentiometric determinations of Alzheimer and anticholinesterase drugs using disposable acetylcholine potentiometric sensors

Drug	Rivastigmine	Pyridostigmine	Memantine	Meclofenoxate	Methotrexate	Cyclopentolate	Oxfendazole	Carbamazepine
Linear range (ng/ml)	0.247–1.45	0.179–0.975	3.55–18.36	375–1725	1.0–37.0	2.88–15.6	3.12–34.32	2.344–9.50
Incubation time (min)	20	10	20	30	10	20	20	15
Slope (I % ml/ng)	38.929	58.698	3.496	0.035	1.540	3.341	1.457	7.875
S slope (I % ml/ng)	1.28606	3.404	0.093	0.0003	0.068	0.195	0.029	0.239
Intercept (I %)	0.44486	4.429	2.402	0.4473	10.590	–4.183	12.481	–3.043
SD intercept	1.14265	2.006	1.119	0.32986	1.660	1.648	0.633	1.409
R	0.9973	0.9917	0.9983	0.9997	0.9961	0.9966	0.9992	0.99724
LOD (3.3× SD/S, ng/ml)	0.097	0.113	1.056	30.924	3.557	0.947	1.434	0.590
LOQ (10×SD/S, ng/ml)	0.294	0.342	3.201	93.710	10.779	4.933	4.345	1.789
Average recovery (%)	101.25	99.85	100.45	97.55	98.64	96.65	99.20	97.55
LD50 (mg/kg)	5.6	16.0	437.0	1750.0	40.0	960.0	6400.0	1957.0

LOD, limit of detection.

Table 2 Potentiometric determination of anticholinesterase drugs in their pharmaceutical formulations

Samples	Taken (ng)	Recovery (%) Present biosensor	Gorun method
Exelon capsules	1.0	99.85±2.30	95.55±4.54
Pystinon	0.5	100.55±4.35	94.60±5.55
Alzmenda	10.0	98.46±4.20	92.70±6.40
Lucidril	1000.0	102.03±3.15	96.34±4.50
Tegretol	5.0	97.30±3.20	93.00±4.10
Pentolate	10.0	96.25±3.75	91.55±5.10
Unifendazole	20.0	95.15±3.60	90.45±4.12
Unitrexate	20.0	99.60±4.52	95.51±5.15

Table 3 Potentiometric determination of anticholinesterase drugs in water samples

Samples	Taken (ng)	Recovery (%)[a] Present biosensor	Gorun method[b]
Rivastigmine	1.0	101.58±1.25	99.15±2.55
Pyridostigmine	0.5	99.43±2.53	95.06±3.65
Memantine	10.0	100.60±3.35	95.60±5.55
Meclofenoxate	1000.0	99.30±3.75	94.44±5.60
Carbamazepine	5.0	98.50±3.80	94.00±5.25
Cyclopentolate	10.0	97.33±3.12	92.00±5.75
Oxfendazole	20.0	92.50±4.00	89.15±6.00
Methotrexate	20.0	97.45±3.25	93.55±5.00

[a]Mean recovery and relative standard deviations of five determinations. [b]Recovery was calculated according to spectrophotometric assay of cholinesterase activity reference [28].

pyridostigmine were the most potent inhibitors compared with other investigated compounds. The reported sensitivities and lower limits of detections within the subnanogram range illustrate the superiority of the proposed analysis protocol compared with the reported electrometric method for the cited drug compounds.

Moreover, the disposable biosensors are attractive for their potential use as simple devices for monitoring detoxification processes, which are suitable for unskilled people with significant reduction in cost per analysis that complement or replace the classical analytical methods for field measurements with a portable system.

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Conflicts of interest

There are no conflicts of interest.

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