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ХІМІЧНИЙ ФАКУЛЬТЕТ

ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ
УНІВЕРСИТЕТ імені В. Н. КАРАЗІНА

A NEW METHOD FOR KINETIC PHOTOMETRIC DETERMINATION OF THE ACTIVITY OF ACETYLCHOLINESTERASE AND ITS INHIBITORS USING THE ACETYLCHOLINE-CATALYZED OXIDATION OF P-PHENETHIDINE BY HYDROGEN PEROXIDE

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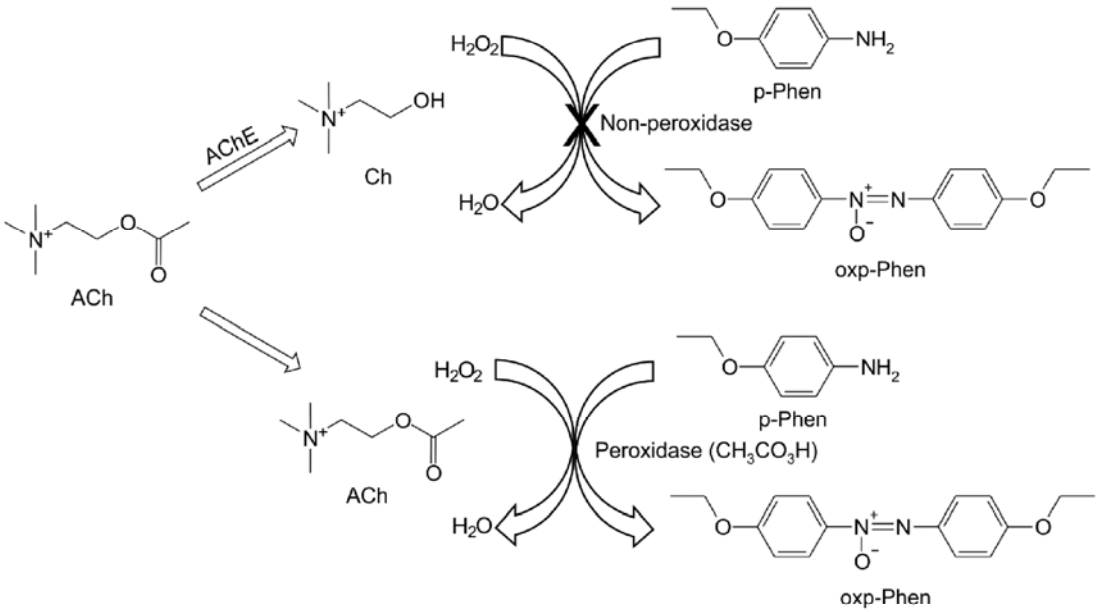
Activity of Acetylcholinesterase (AChE) is frequently measured because of new drugs testing or in an assay of neurotoxic compounds in food or environment. When activity of AChE assessed, Ellman's method is typically preferred as a standard protocol [1]. The method was discovered by Ellman et al. and used without particular changes up today. The method consists of two steps. In the first step, substrate biochemical reaction acetylthiocholine (or butyrylthiocholine when BChE assayed) is split by AChE into thiocholine and acetic acid. In the next step, thiocholine reacts with 5,5′-dithiobis(2-nitrobenzoic acid) providing yellow colored 5-thio-2-nitrobenzoic acid. The method has some drawbacks such as low stability of 5,5′-dithiobis(2-nitrobenzoic acid) and interference of hemoglobin, thiol moiety containing compounds such as cysteine, reduced glutathione or oximes. Because of the aforementioned disadvantages of the assay, there is a demand for a new, more reliable, assay suitable for a routine determination of cholinesterases activity. The aim is development a new enzymatic kinetic photometric procedure for determination of acetylcholinesterase activity and its inhibitors for example quaternary ammonium compounds (QAC) dequalinium chloride.

We describe a sensitive and selective colorimetric method for the determination of the activity of the enzyme acetylcholinesterase (AChE) and its inhibitors. Detection is based on the fact that acetylcholine (ACh) catalyzes (due to formation of peracetic acid in the previous reaction of perhydrolysis with excess hydrogen peroxide) the oxidation of the substrate *p*-Phenethidine (*p*-Phen) by H_2O_2 into 4.4'-azoxyphenetole with an absorption peak at 358 nm, but this oxidation is suppressed if ACh is pre-hydrolyzed by AChE to form acetic acid, which does not catalyze the formation of 4.4'-azoxyphenetole [1]. The residual quantities of dequalinium chloride on the surface of the pharmaceutical equipment were determined by the degree of inhibition of the enzymatic reaction assessed by the unreacted residue of the acetylcholine. The residual quantities of acetylcholine in the reaction mixture was determined by the kinetic photometric method by the indicator reaction of *p*-Phen oxidation with peracetic acid (formed during the perhydrolysis reaction when adding an excess of hydrogen peroxide to the reaction mixture) by of registering the light absorbance of the resulting reaction product – azoxyphenetole (λmax = 358 nm) for a definite period of time.



Scheme 1. Proposed method for determination of cholinesterases activity using *p*-Phen as a chromogenic reagent.

The method has been extended to the analysis of some Cationic active compounds which act as anticholinesterase compounds where the decrease in rate of the Cholinesterase - Acetylcholine ester hydrolysis is linearly related to concentration of the QAC. Quaternary ammonium compounds mainly represent cationic surfactants. They are the most used antiseptics and disinfectants. The optimal conditions for the enzymatic reaction course were determined – the order of mixing and the concentration of acetylcholine (0.05 mg/mL), cholinesterase (0.4 mg/mL), hydrogen peroxide (10 %) and *p*-phenetedine (1 %), the time of the reaction mixture maintaining (20 min), pH (8.35), the effect of the nature of the buffer solution.

The enzymatic kinetic photometric procedure has been developed to determine of acetylcholinesterase activity and the residual quantities of QACs for example dequalinium chloride on the surface of the pharmaceutical equipment after its cleaning. The procedure developed has been validated by such parameters as linearity, accuracy, precision and the limit of quantification.

[1] Патент на корисну модель 117829 Україна, МПК G01N 33/68 (2006.01), G01 N 21/79(2006.01). Спосіб визначення активності холінестерази крові / Блажеєвський М.Є., Дядченко В.В., Ковальська О.В. – № u 2017 00717; заявл. 26.01.17; опубл. 10.07.2017, Бюл. №13. – 4 с.