COLORIMETRIC DETERMINATION OF QAC BASED ON ITS INHIBITORY EFFECT ON HOLINESTERASE

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Introduction. Increasing public concern about pesticide pollution of food and the environment has increased the demand for wider and rigorous monitoring of pesticides. However, common laboratory techniques (such as gas or liquid chromatography) often require a lot of time, labor and financial costs. They are bad for analyzing large volumes of samples.

However, analyzes using colorimetric strips or papers to detect cholinesterase inhibitors by the appearance of a color that is visible to the naked eye provide a qualitative and semi-quantitative detection of pesticides in non-laboratory conditions (for example, in the field). Test devices, the work of which is based on biochemical reactions, as a rule, easy to manufacture and operate, are economically sufficient in serial production and are the means of primary control (detection) of a toxicant in the analyzed sample.

In the present work, the general principles of the work of biosensors based on the use of cholinesterase enzyme for the determination of quaternary ammonium compounds (QAC) on the example of «Diquat» herbicide for the inhibitory action in the reaction of hydrolysis of the substrate of the biochemical reaction of acetylcholine are considered, as well as a method for determining the said toxicants in water and washings from fruits using the newly developed visual optical biosensor.

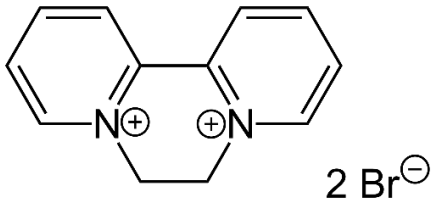

Fig. 1. Diquat
1,1′-Ethylene-2,2′-bipyridyldiylium dibromide – $C_{12}H_{12}Br_2N_2$, M_r = 360,1

Aim. Development of a new test system (optical biochemical sensor with visual indication) to detect the microhardness of anticholinesterase compounds, namely, QAC, in water and fruit flushes.

Materials and methods. The method is based on extracting (flushing) a toxicant (QAC) from the test sample with water and determining it by the effect of inhibition of the enzymatic hydrolysis of acetylcholine.

Fig. 2. Enzymatic hydrolysis of acetylcholine scheme

Reagents:

1. Phosphate buffer solution (B) 0.2 M with pH 8.2. Transfer 3,575 g of aqueous disodium hydrogen phenate ($Na_2HPO_4 \cdot 12H_2O$) into a volumetric flask of 50 ml capacity, dissolve it in approximately 40 ml of water, bring the pH to pH 8.2 using a standard titer of 0,1 M HCl solution and add to distilled water to marks. The resulting reagent is poured into a 50 ml bottle and poured into a bottle.
2. Acetylcholine chloride solution – ampoule content (0.02 g) was dissolved in 4 ml of phosphate buffer solution.
3. Cholinesterase solution – the contents of the vial (class VI, activity 25 AO/mg, mass 80 mg) was dissolved in 16 ml of phosphate buffer solution.
4. Hydrogen peroxide solution 10% (w/w), obtained by a five-fold dilution of commercial 50% (w/w) solution H_2O_2.
5. Test strips with tetramethylbenzidine.

6. The device DYM-108, which allows thermostat solutions at +38°C.

Results and discussion. A new test system (optical biochemical sensor with visual indication) for the detection of the microhardness of anticholinesterase (in particular quaternary ammonium compound «Diquat») in water and fauces of fruit is proposed for practical application. Its advantages include increasing selectivity, shortening the duration of analysis and reducing the volume of the sample, simplifying the procedure and interpreting the results that give grounds to recommend the proposed sensor for use in field and laboratory conditions as a means of primary express control of pollution of environmental objects toxicants anticholinesterase. The analysis of the manufactured model solutions containing different amounts of «Diquat» showed that the processed method detects micrograms, an hourly (0,005 μg in 0.5 ml).

On model solutions of «Diquat» (water washings) it is shown that the concomitant components at concentrations in which they may be present in aqueous washes, did not significantly affect the course of the enzyme hydrolysis process and the inhibitory effect of the time. The analysis of oranges, artificially infected with time, showed that the standard preparation of samples using distilled water provides not only the detection, but also semi-quantitative determination of the toxicant «Diquat».

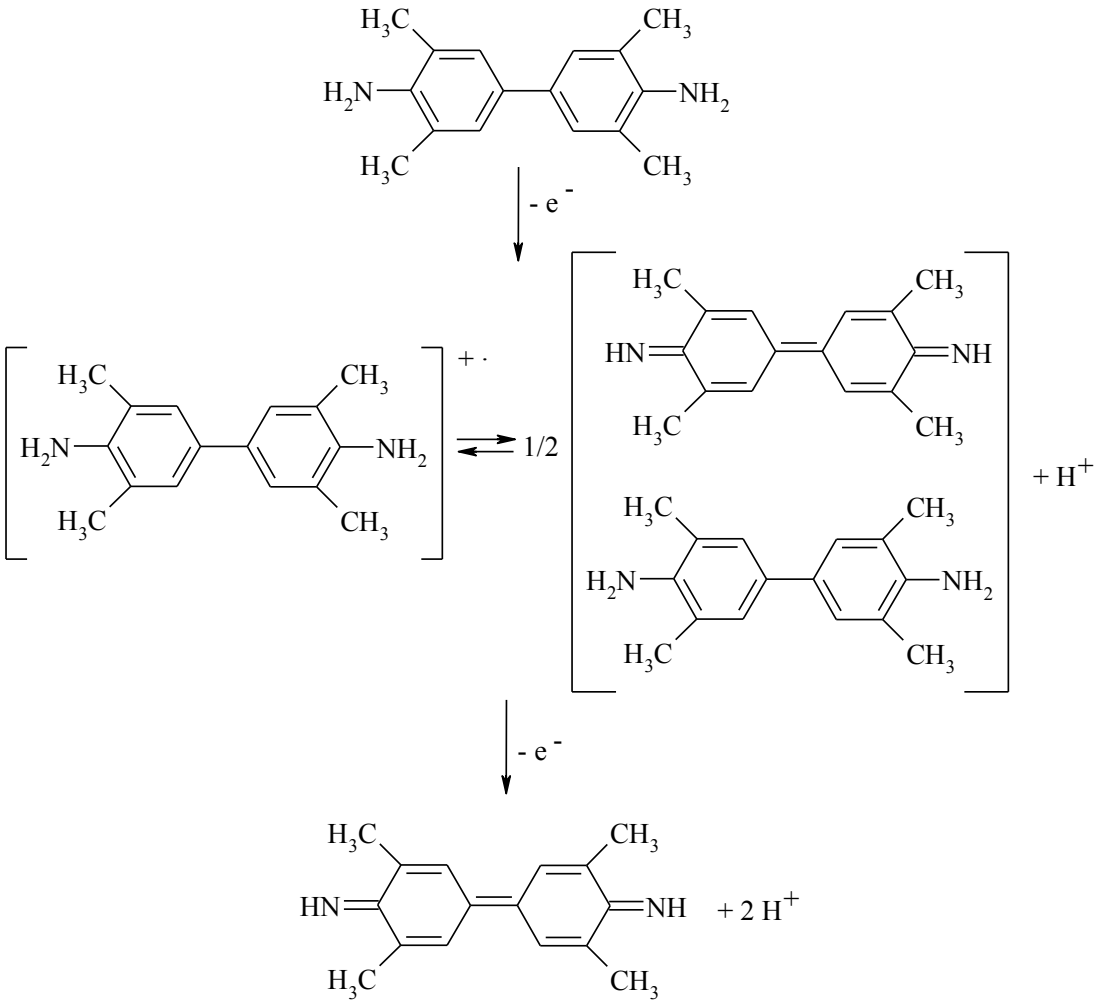

Fig. 3. Shows the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) to 3,3',5,5'-tetramethylbenzidine diamine

Conclusions.

1. An analytical review of literary sources on the use of enzymes in the chemical analysis of toxicants (in particular, QAC) has been made.
2. A visual biosensor (test system) has been developed for inhibitors of cholinesterase – QAC, which allows them to be detected in the fauces of fruits and water.
3. The tried-and-tested test method has been tested when detecting the trace amounts of «Diquat» in aqueous solution (dilution 1:100,000,000).

4. The analysis of model solutions showed that the proposed method is capable of detecting hundreds of particles of micrograms of inhibitors to 1 ml.

DEVELOPMENT OF UV-SPECTROPHOTOMETRIC METHOD OF QUANTITATIVE DETERMINATION OF ANTIDEPRESSANT MOCLOBEMIDE

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Introduction. Moclobemide (4-chloro-*N*-[2-(4 morpholinyl)ethyl]benzamide) is a bicyclic antidepressant related to a reversible inhibitor of monoamine oxidase. The drug primarily used to treat depression and social anxiety. Some cases of acute and lethal intoxications caused by moclobemide overdose were reported. Postmortem fluid and tissue distribution of moclobemide were within the range for various cases: blood – 5.62 and 137 mg/L, urine – 204 mg/L, liver – 432 mg/kg. The most of bioanalytical methods for moclobemide determination are based on using HPLC and GLC.

Aim. To develop the simple and sensitive method for moclobemide quantitative determination using UV-spectrophotometry suitable for the chemical and toxicological analysis.

Materials and methods. The UV-spectrum of moclobemide in 0.1 M hydrochloric acid solution was measured over 215–380 nm wavelength range, 10 mm light pathway cuvette was used. The reference solution was 0.1 M hydrochloric acid. Absorption maximum was detected at 238 nm. Stock solution (SS) (20 μg/mL) and 8 working standard solutions (WSS) (2.0; 4.0; 6.0; 8.0; 10.0; 12.0; 16.0 and 18.0 μg/mL) of the drug were prepared.

Results and discussion. The absorption values obtained for the SS and 8 WSS were processed by linear regression method, its general form is described by the following equation: y=bx+a. The equation of the regression line was the following: y=(0.0534±0.0009)x-(0.03±0.01)·(r=0.9993), LOD and LOQ values were of 0.3 μg/mL and 1.1 μg/mL, respectively. They were calculated from the standard deviation of the intercept of the regression (Sa) accordance with the relevant equations: LOD=3.3Sa/b and LOQ=10Sa/b. The linearity of the calibration curve was within the range of moclobemide concentrations from 2.0 to 20 μg/mL. The "intra day" and "inter day" accuracy and precision were respectively 101.54 and 101.47% (RSD 2.19 and 2.18%) at the low concentration level of the analyte, 100.30 and 100.04% (RSD 1.47 and 1.23%) at the middle concentration level, 99.62 and 99.87% (RSD 1.47 and 1.46%) at the high concentration level.

Conclusions. Thus, the UV-spectrophotometric method developed satisfies the requirements of the chemical and toxicological analysis by the sensitivity and can be used in toxicological study of the biological samples for presence of moclobemide.

DEVELOPMENT OF THE METHODS OF AMISULPRIDE DETECTION AND QUANTITATIVE DETERMINATION SUITABLE FOR THE CHEMICOTOXICOLOGICAL ANALYSIS

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Introduction. Amisulpride (4-amino-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide) is an antipsychotic drug. Numerous cases of lethal intoxications caused by amisulpride overdose were reported in the literature.

Aim. To develop sensitive and accessible methods for amisulpride detection and quantitative determination with help of colour reactions, thin layer chromatography (TLC) and UV-spectrophotometry.