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STUDY SPECTROPHOTOMETRICALLY ACTIVE ENZYME, CHEMICALLY INCORPORATED INTO THE STRUCTURE OF BIOPOLYMERS

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Abstract. In this article the feasibility study spectrophotometrical methods specific activity of the immobilized enzymes by chemical methods in the structure of biopolymers.

Key words: quantitative determination of specific activity, biopolymers, enzymes immobilization.

Introduction.

Chemical methods of enzyme immobilization are now the dominant method for producing heterogeneous biocatalysts.

Literature review.

When choosing conditions for immobilisation of the drug Kovaleva T.A. and co-authors found that the most suitable carrier is an anion exchange resin AV-17-2 P. Optimal immobilization method is a modified glutaraldehyde method of covalently binding an enzyme to a carrier, the process comprising binding capacity link between the enzyme and the anion exchanger with a number of organic reagent treated [1].

Mirzarahmetova D.T. and co-authors et carried yeast invertase covalent immobilization using glutaraldehyde activated carbon previously modified by treatment with urea and dimethylformamide. We investigated some physicochemical properties of the enzyme immobilized and soluble in aqueous and aqueous-organic media. When immobilizing me as hydrolytic and transferase enzyme properties. Optimal conditions for manifestation hydrolytic and transferase activities immobilized invertase - 6.0 and pH 7.0, respectively [2].

Kumarev V.P. discloses a method for immobilizing enzymes by modifying the surface of the inorganic carrier of magnesium-organic compounds in absolute ether followed by the chemical bonding of the enzyme. However, this method has a drawback - multistage process and the complexity of processing inorganic carrier organomagnesium compounds.

Morozova O.V. and co-authors studied the adsorption of some proteolytic enzymes (L-bovine chymotrypsin, and porcine pepsin subtimezina) on inorganic carriers (silohrom C-80 and C-120 macroporous glass, Celite 535). The ability of enzymes adsorbed on the carrier surface dependent on the specific properties of the carrier and of the enzyme. Best observed for cation adsorption enzyme L-chymotrypsin (pH 8.2) 270 mg (9.6 mmol) of enzyme per 1 g silochrome. For subtilisin (pH 8.15), the maximum adsorption capacity silochrome C-80 was 100 mg (3.5 mmol) of 1 g silochrome [3].

Input data and methods.

A process for preparing compositions undergoing biodegradation is presented in co-operation Avanesyan S.S. and co-authors [5]. Prepared 3.5% methylcellulose

solution. To prevent the formation of air bubbles resulting solution was maintained at a temperature 8÷10 oC for 12-15 hours. Reagent was then introduced for modification of the rheological characteristics (gelatin at a concentration of 3 to 8% by weight), the plasticizer (glycerin at a concentration of 0.5 to 1% by weight). gives the product flexibility, and stir until smooth. The resulting composition was coated on a smooth glass surface of a desired shape to a thickness of 1 mm 3 and allowed to air at a temperature 20÷22 ° C for 2-3 days until dry (№ Patent 2,395,540).

Protein was quantified in biopolymers obtained by the method of O. Warburg and W. Christian comparing protein absorption at 280 and 260 nm on a spectrophotometer SF-46 [4]. Calculations were performed using the formula:

$$C = 1{,}55A_{280} - 0{,}76A_{260}$$, where

A_{260} и A_{280} – absorption coefficients at a wavelength λ;

1,55 и 0,76 – constant coefficients.

To confirm the reproducibility and reliability of the results obtained in the study, statistical methods were used. The mathematical processing of the results of experiments performed on the computer (EXCEL software, STSTISTICA 6.0).

Results. Discussion and Analysis**.**

To determine the specific activity of the immobilized enzyme trypsin method was developed where casein was used as substrate. 0.05 -0.1 g film containing okolo1,63 mg of enzyme dissolved in 20 ml of phosphate buffer solution pH = 8.15. To determine the activity of an aliquot taken from the film of the solution volume of 0.01 ml -1.

The specific enzyme activity of trypsin was determined spectrophotometrically according to the method presented in Section 2.3.1, which is based on quantification of tyrosine digestion products of casein.

1% - th casein protein solution was prepared by dissolving the sample in 0.05 M sodium acetate. To 1 ml of the casein solution were added 1.5 ml of phosphate buffer solution (pH = 8,15); 0.5 ml of trypsin solution (10 mg trypsin in 100 ml of 0.005 M HCl). The sample was incubated at 37? C for 20 minutes. Then it was added trichloroacetic acid (TCA). As a control sample, similar trial, but TCA was added to the incubation. The absorbance was measured against a control sample in a cuvette with layer thickness of 1 cm at a wavelength of 260 nm and 280 nm. By the number of tyrosine in solution specific activity of the enzyme was calculated (Fig. 1).

Summary and Conclusions.

For film materials the samples obtained were examined by the enzyme-immobilized film trypsin different hydrophilicity, flexibility, transparency, and ability to degradation by basic hydrolysis of macromolecules bonds basis for interaction with a physiological environment. Absorption spectra in the ultraviolet region. The additional absorption peak at 280 nm due to the presence of the enzyme trypsin into the film structure. This fact can be used to determine the enzyme content in the film structure [5].

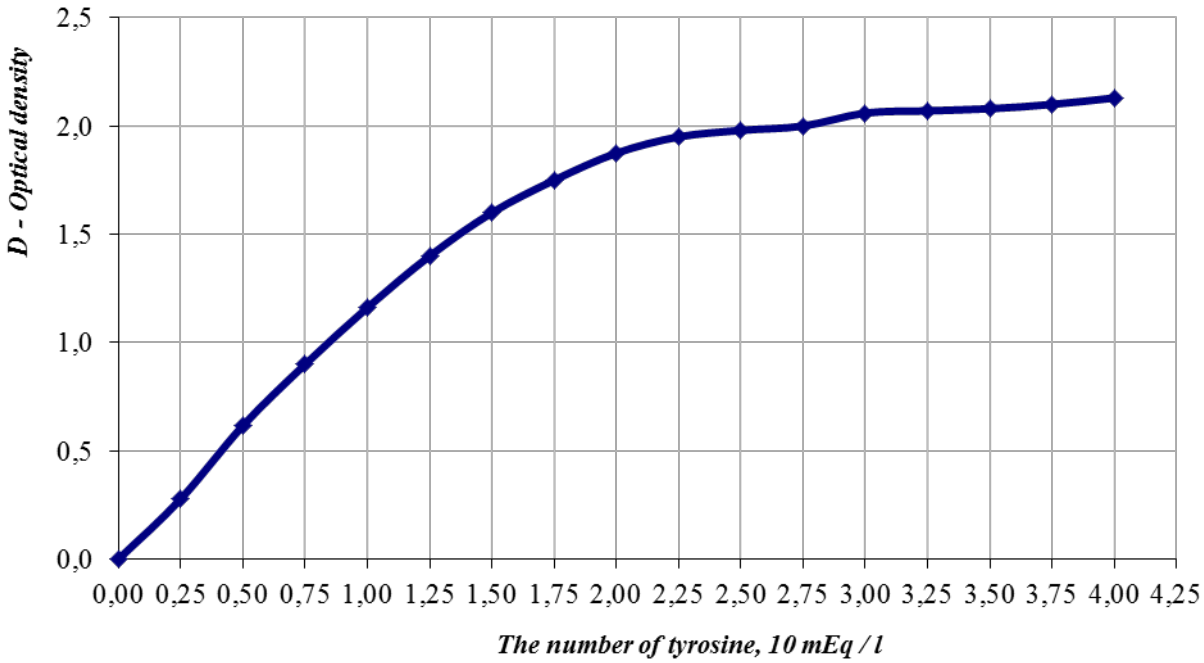

Fig. 1 - Spectrophotometric determination of tyrosine solution

References:

1. Kovaleva T.A., Kozhokina O.M., Bagno O.P, Trofimova O.D., Belenova A.S. Immobilization of hydrolytic enzymes on anion exchangers // Sorption and chromatographic processes. - 2008. - T. 8. number 6. - P. 1035-1041.

2. Mirzarahmetova D.T., Dehkonov D.B., Rakhimov M.M. Properties invertase immobilized covalently on charcoal // Applied Biochemistry and Microbiology. - 2009. - T. 45. № 3. - P. 287-291.

3. Morozova O.V., Voyushina T.L., Stepanov V.M. On interaction with native proteolytic enzymes used for the enzymatic synthesis of peptides in organic solvents // Applied Biochemistry and Microbiology. - 1994. - T. 30. № 6. - P. 786-793.

4. Darbre A. Practical Protein Chemistry: Per. from English. M: Mir, 1989. - P. 22-23.

5. Volosova E.V. Stabilization of biologically active compounds by incorporating them into the structure of the natural biodegradable polymer materials: Dis. Ph.D. - Stavropol, 2011. - P. 134.

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