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AMPEROMETRIC BIOSENSORS FOR DETERMINING ASCORBIC ACID IN FOOD PRODUCTS AND PHARMACEUTICAL PREPARATIONS

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The use of the unique properties of enzymes and their various modifications has recently become particularly promising for the creation of "reagent-free" amperometric biosensors [1]. A particularly important position is occupied by immobilized enzymes used as industrial catalysts [2] and by specific analytical reagents permitting the selective determination of microcomponents in biological materials [3]. The activity and selectivity of enzymes in combination with the mild conditions of performing the reactions has permitted the development of a new direction of the analytical chemistry of reagent-free methods of analysis and the creation of biochemical sensors [4].

In the development of enzyme electrodes, particular interest is presented not only by preparations of the enzymes themselves but also by their content of materials, tissue media, that in the biosensors fulfill the function of sources of catalytic activity.

In the present paper we consider the reagent-free amperometric determination of ascorbic acid in biological materials and pharmaceutical preparations. We have developed an enzyme sensor consisting of a platinum (gold) microelectrode and a plate (4 × 5 × 6 mm) of watermelon (melon, onion, cucumber) skin serving as a source of ascorbic acid oxidase. The determination is performed at a potential difference of 0.75-0.80 V in universal buffer solution containing 100 ml of 0.04 M CH_3COOH, H_3PO_4, and H_3BO_3 and 47.5 ml of 0.2 M NaOH at pH 6.0-7.0 with 0.5 mM of EDTA. We have established that the activity of the enzyme is sufficient for 60-70 determinations. The bioplates prepared in this way can be stored in a mixture with 40% of glycerol and 20% of ethylene glycol without loss of electrochemical activity (working life) for 8-10 months. Experiments have shown that the determination is not affected by phenols, various acids, and vitamin B.

TABLE 1. Result of the Amperometric Determination of Ascorbic Acid (AA) in Plant Materials (control method — electrochemical according to GOST 26932–86, GOST 26927–86, GOST 26928–86, and GOST 26933–86)

Material analyzed	GOST-certified AA content, mg-%	AA found (mg-%) by the given method (P=0.95; $\bar{x} \pm \Delta X$)		n	S	$S_r \cdot 10^2$
		control	newly developed			
Dog rose concentrate	17.46	17.09	17.13±0.45	3	0.18	1.0
Unripe walnuts	12.23	11.90	11.96±0.41	4	0.26	2.2
Blackcurrant concentrate	10.34	10.15	10.21±0.38	4	0.20	2.0
Orange and lemon concentrate	9.02	8.69	8.74±0.37	3	0.15	1.7

Note. n) number of parallel determinations — measurements; S) standard deviation — the scatter of the results obtained; S_r) relative standard deviation — error; P) confidence level; $\bar{x}$) mean found; X) confidence interval.

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Ascorbic acid has been determined in juices and food products, and also in pharmaceutical preparations, by means of the modified electrode and the biosensor created The relative standard deviation did not exceed 0.025. The validity of the procedures developed was established by the added-found method, by comparison other known methods, and by certified results of GOSTs [State Standards] in force for the ascorbic acid contents of food products and pharmaceutical preparations. The time for a single determination is 15-20 min.

Some of the results obtained in the analysis of various plant products are given in Table 1, from which it can be seen that the amounts of ascorbic acid found are in full agreement with its GOST and certified levels, which shows the significance and reliability of the results obtained and that the method itself is distinguished by high accuracy, rapidity, and reproducibility.

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