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ARTICLES

Determination of Glucose and Lactose in Food Products with the Use of Biosensors Based on Berlin Blue

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Abstract—The immobilization of enzymes into polyelectrolyte membranes with the use of organic solvents was applied to the development of the biosensing elements of biosensors. The following domestically produced preparations were used: the enzymes glucose oxidase and β-galactosidase and a perfluorosulfonated polymer. The compositions of mono- and bienzymic polyelectrolyte membranes were optimized. The glucose and lactose biosensors based on Berlin blue (as a signal transducer) and polyelectrolyte membranes exhibited high sensitivity, low detection limits, and fast response. The results of the analysis of milk whey in a flow-injection system that included biosensors completely correlated with measurement data obtained by a standard chromatographic technique.

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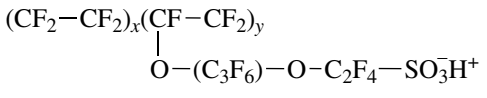
According to IUPAC recommendations, only a device in which a biological recognition element is arranged in direct spatial contact with a signal transducer can be referred to as a biosensor [1]. In the case of enzyme electrodes, this means that enzymes should be immobilized immediately on the surface of a modified electrode. Therefore, the biosensor sensitivity, the simplicity and reproducibility of the procedure, and the minimization of reagent consumption can serve as important characteristics of the efficiency of an immobilization procedure.

Ion-exchange polyelectrolytes, in particular, Nafion®, a copolymer based on tetrafluoroethylene and perfluoroalkyl vinyl ether from Sigma-Aldrich, are widely used for the development of enzyme membranes. The main advantages of this polymer consist in its compatibility with biological tissues (i.e., applicability to implanted sensors), low resistivity, and good adhesion to electrode materials [2, 3].



Nafion®

Because Nafion® is supplied as a solution in a mixture of alcohols, the membrane preparation procedure is reduced to applying a drop of an enzyme and polyelectrolyte mixture to an electrode followed by drying in air until the solvent is evaporated. However, previously [4], an excessive dilution of a Nafion® preparation with water or a buffer solution in order to prevent enzyme inactivation under the action of an organic solvent resulted in the supported membranes beng inhomogeneous and, as a consequence, unstable.

We proposed a new procedure for the development of membranes based on polyelectrolytes with the use of aqueous organic mixtures with high concentrations of an organic solvent [5]. It was found that enzymes, among them glucose oxidase from *Aspergillus niger,* retained their activity after incubation in 85–95% organic solvents. Moreover, Nafion® can stabilize the enzyme in these media by the formation of enzyme–polyelectrolyte complexes [6]. Thus, highly active and stable polyelectrolyte membranes were prepared. The high stability of enzyme and Nafion mixtures in an organic medium in storage was demonstrated: after the storage of a mixture for five days, the activity of the membrane was not different from the activity of a freshly prepared membrane. This allowed us to decrease the consumption of expensive enzymes and to increase reproducibility in the commercial manufacture of sensors.

A glucose biosensor based on Berlin blue can serve as an example of the use of the immobilization procedure developed [6]. Hydrogen peroxide, which is formed in the course of enzymatic reaction (1), is reduced at the surface of Berlin blue (reaction (2)):

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} \text{Gluconolactone} + H_2O_2, \quad (1)$$

$$H_2O_2 + 2\bar{e} \longrightarrow 2OH^-. \quad (2)$$

In terms of sensitivity (0.05 A M^{-1} cm^{-2}), the sensor prepared in accordance with the new procedure outperforms a previously developed analogous sensor without enzyme stabilization by a polyelectrolyte by a factor of 20. The limit of detection was decreased by a factor of 5 to be as low as 1×10^{-7} M. As compared with other biosensors of the first generation based on hexacyanof-

errates, the sensitivity of the determination of glucose was increased by a factor of 100 [6].

It is of interest to develop multienzyme membranes based on polyelectrolytes. Many analytically important compounds can be determined with the use of consecutive enzymatic reactions, for example, in the decomposition of lactose under the action of β-galactosidase

$$\text{lactose} \xrightarrow{\beta\text{-galactosidase}} \text{galactose} + \text{glucose}, \qquad (3)$$

followed by reactions (2) and (3) analogously to the determination of glucose.

Lactose is the main carbohydrate present in milk (4–5%) and dairy products. Its concentration in food products is indicative of the amount of powdered milk added to these products. Lactose is routinely determined in dairy products in the milk industry in order to provide process efficiency and product quality control. Liu et al. [7] described an amperometric biosensor based on the immobilization of glucose oxidase, mutarotase, and ferrocene into a cyclodextrin polymer on the surface of a glassy carbon electrode. Ferrocene served as a mediator between glucose oxidase and the electrode. The response time was 40 s. The linear analytical range was 5×10^{-5}–1.2×10^{-2} M. The sensitivity was 0.003 A M^{-1} cm^{-2}. Liu et al. [8] proposed an amperometric biosensor based on the incorporation of *N*-methylphenazine methosulfate into a Nafion film as a mediator between peroxidase and the electrode. With the use of glutaraldehyde, peroxidase, glucose oxidase, and β-galactosidase were immobilized on the surface of a glassy carbon electrode modified with Nafion–*N*-methylphenazine methosulfate. The linear analytical range of lactose concentrations was 5×10^{-6}–7×10^{-3} M; the sensitivity was 0.006 A M^{-1} cm^{-2}. The incorporation of additional enzymes (peroxidase and mutarotase) into mediator-type biosensors requires the combined immobilization of several enzymes catalyzing consecutive reactions; this deteriorates the stability and reproducibility of sensors.

Previously, we used enzyme preparations and a Nafion polyelectrolyte from Sigma-Aldrich in the development of biosensors. The aim of this work was to use new preparations for the development of the biosensing elements of sensors. For this purpose, we used glucose oxidase and β-galactosidase enzyme preparations, which were isolated in the course of cooperative studies performed at the Institute of the Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino) and the Chair of Chemical Enzymology, Department of Chemistry, Moscow State University, and a perfluorosulfonated polyelectrolyte (Plastpolimer, St. Petersburg). The use of new domestic preparations will allow us to study the capabilities and limitations of the method developed previously and to reduce significantly expenditures in the commercial manufacture of sensors.

EXPERIMENTAL

Materials. All of the experiments were performed in distilled water. The inorganic salts and acids (of analytical grade), organic solvents (chemically pure), lactose, and glucose were purchased from Russian and foreign companies. A 25% ammonia (NH_3) solution (Ferrein, Russia) was used for the neutralization of perfluorosulfonated polymer solutions. A perfluorosulfonated polymer (PFS) (M_e 1000; 14% solution in isopropanol) from Plastpolimer (Russia) was used for the immobilization of enzymes.

Glucose oxidase (EC 1.1.3.4) from *Penicillium vitale* as an enzyme solution purified to a homogeneous state with a concentration of 15 mg/mL had an activity of 73 MU/mg. The enzyme preparation *Penicillium canescence* no. 579.1 with a multicopied gene of β-galactosidase was obtained from the Institute of the Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino). The preparation was purified using a Pharmacia fast protein liquid chromatography (FPLC) system (Sweden) on a Mono Q column. Thereafter, the enzyme concentration in solution was 46.4 mg/mL; the activity with respect to lactose was 3510 MU/mL. The milk whey was prepared from 1.5% fat milk (Russia) by milk fermentation at pH 4.

The following solutions were used in this work: supporting electrolyte (0.1 M KCl; 0.1 M HCl), buffer 2 (0.1 M KCl; 0.05 M KH_2PO_4; pH 6.0), and buffer 3 (0.05 M KH_2PO_4; pH 5.5).

Instrumentation. Type 2500 glassy carbon disk electrodes (d = 2 mm) from NII Grafit (Russia) were used. The electrodes were pretreated using aluminum oxide powders (Buehler, the United States) with grain diameters of 3 and 0.5 μm. The Clark oxygen electrode had working cells of volume 1.2–1.5 mL. The electrochemical measurements were performed using a Model 1286 potentiostat (Solartron, the United Kingdom). A three-electrode cell attached to the potentiostat contained a working electrode, a silver–silver chloride reference electrode (in 1 M KCl), and a platinum auxiliary electrode.

The flow-injection system consisted of a Macroflex peristaltic pump (LKB Bromma, Sweden) and an injector with a sample volume of 200 μL, which were coupled to an amperometric flow cell. The inlet opening of the cell was equipped with a silver–silver chloride reference electrode in 0.1 M KCl. The auxiliary electrode was a stainless tube, which served as the outlet opening of the cell. The distance between the working electrode and the nozzle of the cell was about 2 mm. For testing the sensor, the cell was connected to a 641 VA-Detector potentiostat (Metrohm, Switzerland). The flow rate of the buffer solution was 0.25 mL/min.

Methods. The rate of glucose oxidation under the action of glucose oxidase in water–organic solvent mixtures and polyelectrolyte membranes was measured

using a Clark oxygen electrode: the change of current, which was proportional to the rate of oxygen consumption in the system in the course of enzyme-catalyzed reaction, was measured. The base current at –0.6 V with reference to a silver–silver chloride electrode was proportional to the solubility of molecular oxygen in a buffer solution with constant stirring at 25°C, that is, 2.5×10^{-4} M (recalculated from [9]). The concentration of glucose in buffer 2 was 50 mM. To prepare enzyme suspensions in aqueous organic mixtures, a solvent was mixed with water; next, the mixture was added to an initial aqueous solution of the enzyme, and mixtures with water concentrations of 5–30% and an enzyme concentration of 0.75 mg/mL were obtained. The suspensions were allowed to stand for 0.5 h before measuring the residual activity.

Glucose oxidase immobilization in a PFS membrane involved the following stages: the dilution of a commercial PFS preparation with isopropanol to a concentration of 1%; the neutralization of the resulting PFS solution with an aqueous 25% ammonia solution to reach pH 5.5 in the polyelectrolyte solution; the mixing of an aqueous enzyme solution with the neutralized PFS solution; and the application of an aliquot portion of the enzyme–polyelectrolyte mixture to a substrate. The concentrations of glucose oxidase and PFS and the water content of the mixture were varied. The enzyme-containing membranes were prepared by applying 25 μL of a mixture of the polyelectrolyte with the enzyme to the bottom of a Clark electrode cell followed by drying the drop for 1 h.

The coimmobilization of the glucose oxidase–β-galactosidase bienzymic system in a PFS membrane involved mixing an aqueous solution of the mixture of the enzymes with the neutralized PFS solution and supporting an aliquot portion of the enzyme–polyelectrolyte mixture onto a substrate. The initial glucose oxidase and β-galactosidase preparations were diluted with water to concentration ratios of 0.5 : 1.5, 1 : 1, and 1.5 : 0.5 mg/mL. An alcoholic 0.3–0.5% PFS solution was added to the resulting enzyme mixture, and the contents were stirred. The water content of the resulting system was 12%. The enzyme-containing membranes were prepared by applying 25 μL of the mixture of the polyelectrolyte with the enzymes to the bottom of a Clark electrode cell and drying the drop of the mixture in air for 1 h. The reaction rate of lactose conversion under the action of coimmobilized β-galactosidase and glucose oxidase was measured by injecting a 0.02–0.04 M lactose solution in buffer 3. The rate of reaction was determined as the rate of oxygen consumption in the course of the oxidation of intermediately formed glucose (μM/min).

Modification of the working electrode with Berlin blue. The electrochemical growth of Berlin blue on the surface of a glassy carbon electrode was performed from a growth solution containing 4 mM $K_3[Fe(CN)_6]$ and 4 mM $FeCl_3$ in buffer 1 in accordance with a well-known procedure [6]. The growth of Berlin blue on the surface of the working electrode was performed potentiodynamically with cycling the working electrode potential from +0.4 to +0.75 V (silver–silver chloride electrode) at a rate of 40 mV/s (20 cycles). The Berlin blue coatings were activated by cycling the potential of the modified electrode from –0.05 to +0.35 V in solution 1. Next, the electrodes were heated at 100°C for 1 h to remove any excess of water and activated in buffer 2 under cyclic changes in the working electrode potential from –0.05 to +0.35 V at a potential sweep rate of 40 mV/s in the course of ten cycles.

Preparation and testing of a glucose biosensor based on Berlin blue. A drop (5 μL) of an enzyme–polyelectrolyte mixture of glucose oxidase and PFS was applied to the surface of an electrode modified with Berlin blue and dried for 10 min. The concentration of the PFS solution was varied over the range 0.2–0.6%, whereas the concentration of the enzyme was varied over the range 0.5–1.5 mg/mL. The water content of the enzyme–polyelectrolyte complex was 12%. For the determination of glucose by flow-injection analysis (FIA), buffer 2 was passed through a cell containing the biosensor until the establishment of a base current (10–15 min). The potential of the working electrode was 0 V. To construct a calibration graph of the biosensor, 200 μL of a model solution (1×10^{-7}–1×10^{-2} M) of glucose in buffer 2 was injected.

Preparation and testing of a lactose biosensor based on Berlin blue. A drop (5 μL) of an enzyme–polyelectrolyte mixture of glucose oxidase, β-galactosidase, and PFS was applied to the surface of an electrode modified with Berlin blue and dried. In this case, the concentration of the PFS solution was 0.3 or 0.5%, whereas the water content of the enzyme–polyelectrolyte complex was 12%. The enzymes were taken in glucose oxidase : β-galactosidase ratios of 0.5 : 0.5, 0.75 : 0.75, 1 : 1, and 0.5 : 1.5 mg/mL. The FIA of lactose with a lactose biosensor was performed analogously to the analysis of glucose with the use of buffer 3 and lactose concentrations from 1×10^{-6} to 1×10^{-2} M.

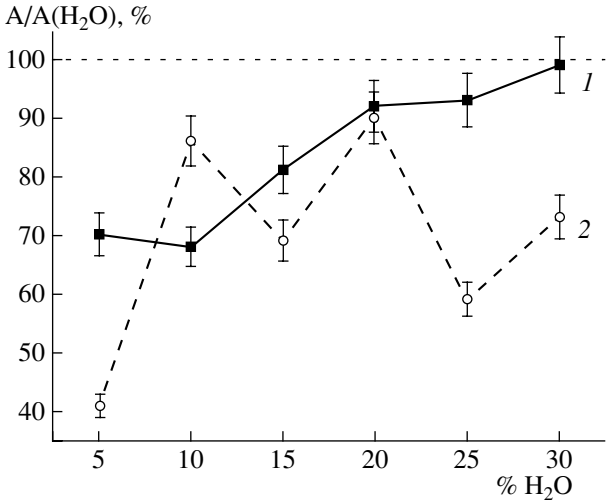
RESULTS AND DISCUSSION

Residual activity of glucose oxidase after incubation in aqueous organic mixtures. In accordance with the previously developed procedure for enzyme immobilization into polyelectrolyte membranes [5, 6], an aqueous enzyme solution is mixed with a polyelectrolyte solution in an organic solvent; the resulting mixture is applied to the surface of an electrode and dried. Consequently, the enzyme contacts with an organic medium for a short time, which is required for solvent evaporation.

Nafion® is commercially available as a solution in a mixture of light alcohols and 10–20% water (preparations from Sigma-Aldrich); the perfluorosulfonated polyelectrolyte (PFS) from Plastpolimer is a solution in

86% isopropanol. Polymers of this kind also form true solutions in acetonitrile. Therefore, at the first stage of this work, we studied the tolerance of the new glucose oxidase preparation from *Penicillium vitale* to these solvents.

The figure shows the residual activity of glucose oxidase after 30-min incubation in aqueous organic mixtures containing from 5 to 30% water. The percentage ratio of the activity of glucose oxidase, which was incubated in an aqueous organic mixture, to the enzyme activity from a control solution was taken as the percentage of retained glucose oxidase activity after incubation in the aqueous organic mixture. Note that the water contents of Nafion® and PFS preparations were 10–20%. It can be seen in the figure that the enzyme activity was retained at a 70–100% level of the initial activity over this interval. Consequently, only a small portion of glucose oxidase (most likely, molecules arranged at the surface of suspension particles) was irreversibly inactivated in contact with an organic medium. In this work, we used a PFS preparation in isopropanol; therefore, the subsequent experiments were performed with this solvent.



Residual activity of glucose oxidase after incubation in (*1*) water–isopropanol and (*2*) water–acetonitrile mixtures.

Optimization of Nafion–glucose oxidase and Nafion–glucose oxidase–β-galactosidase membranes. A published procedure [6] was used for the immobilization of the enzymes. An aqueous solution of enzymes was mixed with an aqueous isopropanol solution of PFS containing from 5 to 30% water. The dependence of the activity and operational stability of polyelectrolyte membranes containing glucose oxidase or coimmobilized glucose oxidase and β-galactosidase on immobilization parameters (concentrations of both of the enzymes and PFS and the water content of the mixture) was studied in detail. Table 1 summarizes the data obtained. Each value is the average of seven measurements. It can be seen that, at a 12% concentration of water in the mixture, the activities of membranes exhibited maximums; this is consistent with our previous data on the immobilization of glucose oxidase from *Aspergillus niger* into Nafion® membranes with the use of a water–ethanol mixture as a solvent [5]. At an enzyme concentration higher than 1 mg/mL, the activity of the membrane decreased. This can be due to the fact that an excess of an enzyme is poorly retained in the membrane and the coarse conglomerates of enzyme molecules cause diffusion hindrances in the membrane.

Membranes prepared with the use of 0.5% PFS exhibited higher activities than that of the membranes of 0.3% PFS at any water content. Thus, the membranes prepared from a mixture of glucose oxidase (1 mg/mL) and 0.5% PFS in 88% isopropanol exhibited the most optimal characteristics.

For the coimmobilization of glucose oxidase and β-galactosidase, an aqueous solution of the mixture of the two enzymes was added to a solution of PFS in 88% isopropanol. The membranes prepared at the glucose oxidase : β-galactosidase enzyme ratio equal to 1 : 3 (by weight) exhibited a maximum activity. Taking into account the activities of the initial enzyme preparations and their molecular weights, we can conclude that an approximately tenfold excess of β-galactosidase was present in the membrane. It is believed that β-galactosidase loses a considerable portion of its activity upon immobilization or not all of the glucose molecules formed in the decomposition of lactose under the action of β-galactosidase can diffuse to the active centers of glucose oxidase in the membrane. However, a final conclusion can be made only in the course of the direct

Table 1. Effect of the component ratio in water–isopropanol mixtures on the activity of glucose oxidase–containing membranes based on PFS (specific activities of the membranes are given in units of (μmol O_2) min^{-1} (mg glucose oxidase)$^{-1}$; $n = 5$; $P = 0.95$)

$c_{glucose\ oxidase}$, mg/mL	c_{PFS}, %	% H_2O				
		6.67	10	12	15	20
1	0.3	0.9 ± 0.1	0.1 ± 0.1	1.9 ± 0.1	1.5 ± 0.2	1.4 ± 0.1
1.5	0.3	–	0.7 ± 0.1	–	0.3 ± 0.1	0.5 ± 0.1
1	0.5	1.4 ± 0.2	2.1 ± 0.1	2.3 ± 0.2	1.9 ± 0.1	2.1 ± 0.1
1.5	0.5	–	1.1 ± 0.1	–	0.4 ± 0.1	0.7 ± 0.1

Table 2. Effect of the concentration of PFS on the analytical characteristics of the glucose biosensor

Characteristic	Concentration of PFS, %				
	0.2	0.3	0.4	0.5	0.6
Sensitivity, A M^{-1} cm^{-2}	0.012	0.034	0.006	0.016	0.006
Detection limit, M	1×10^{-7}	1×10^{-7}	1×10^{-6}	1×10^{-7}	1×10^{-6}

measurement of the activity of β-galactosidase in the membrane.

The operational stability of mono- and bienzymic membranes was determined in repeated activity measurements with the use of a Clark electrode. The membranes prepared at optimum ratios between the concentrations of all of the components retained 100% initial activity after ten repeated measurements.

Thus, the use of new glucose oxidase and PFS preparations and 88% isopropanol as a medium for the production of an enzyme–polyelectrolyte mixture allowed us to obtain enzyme-containing membranes whose activity and stability are comparable to those of previously developed membranes. Bienzymic membranes containing glucose oxidase and β-galactosidase were also prepared.

Glucose and lactose biosensors based on Berlin blue and PFS. Polyelectrolyte membranes containing enzymes were applied to electrodes modified with Berlin blue in order to develop biosensors. The above optimum ratios between components in the membranes were used for the preparation of enzyme electrodes. However, for the use of the resulting biosensors under flow-injection conditions, the membranes should be further optimized in order to improve the analytical characteristics of the sensor as a whole.

At the first stage, the sensitivity of the biosensor to glucose was optimized. This value was calculated from the slope of the linear portion of calibration graphs plotted based on biosensor responses to glucose concentrations from 1×10^{-7} to 1×10^{-3} M. The measurement error was no higher than 7–9%. We found that the optimum concentrations of glucose oxidase and water in the mixture applied to the electrode remained unchanged and were equal to 1 mg/mL and 12%, respectively. However, as the concentration of PFS in the mixture was decreased from 0.5 to 0.3%, the sensitivity increased by a factor of 2 (Table 2). Because the operational stability decreased as the polyelectrolyte concentration was further decreased and the limit of detection increased as the concentration was increased, a PFS concentration of 0.3% was chosen optimal.

Table 3. Analytical characteristics of optimized biosensors in a flow-injection system

	Glucose biosensor	Lactose biosensor
Sensitivity, A M^{-1} cm^{-2}	0.034 ± 0.002	0.0015 ± 0.0001
Detection limit, M	1×10^{-7}	1×10^{-5}
Response linearity range, M	1×10^{-6}–1×10^{-3}	1×10^{-5}–1×10^{-2}
Analysis time, min	1	2–3

Table 3 summarizes the analytical characteristics of a glucose biosensor in a flow-injection system. The biosensor response was linear over the concentration range 1×10^{-6}–1×10^{-3} M. The calibration equation was $y = 0.0024 + 0.0340x$ (RSD = 0.16%; $n = 5$). A comparison of these results with previously obtained data demonstrates that the new domestic glucose oxidase preparations from *Penicillium vitale* and PFS allow us to obtain a glucose biosensor, which is highly competitive with previously developed analogs based on preparations from Sigma-Aldrich [6] in terms of sensitivity, measurement times, and detection limits.

In the development of a lactose biosensor, we optimized both the ratio of enzyme concentrations and the PFS content of the membrane. We found that the sensitivity of the resulting biosensor reached a maximum of 0.0015 μA M^{-1} cm^{-2} at glucose oxidase and β-galactosidase concentrations of 0.5 mg/mL in the applied mixture. As demonstrated above, at the total enzyme concentration of 1 mg/mL, an excessive loading of the membrane with protein does not occur in glucose oxidase–PFS membranes; this excessive loading would adversely affect the response time and operational stability of the sensor as a whole.

A PFS concentration of 0.3%, at which sensitivity was higher than that with the use of a 0.5% PFS solution by a factor of about 2, was also optimal.

Table 3 summarizes the analytical characteristics of a lactose biosensor. The biosensor response was linear over the concentration range 1×10^{-5}–1×10^{-2} M. The calibration equation was $y = 0.0004 + 0.0015x$ (RSD = 0.02%; $n = 5$). A comparison of the two developed sensors demonstrates that the lactose biosensor ranks below the glucose biosensor in all parameters. This can be explained by the occurrence of consecutive enzymatic reactions in the case of the determination of lactose. The intermediate product—glucose—is formed in the decomposition of lactose; it diffuses to the active center of glucose oxidase within the membrane. In this case, a portion of glucose can diffuse into solution to decrease the sensor response.

An amperometric biosensor developed based on membranes with jointly immobilized β-galactosidase

Table 4. Comparison between the analytical characteristics of the lactose biosensor and a chromatographic method for the determination of lactose concentration

Method	Analysis time, min	Detection limit, M	Analytical range, M
Lactose biosensor	2–3	1×10^{-5}	1×10^{-5}–1×10^{-2}
Chromatographic method	3.5–5	1.5×10^{-4}	1.5×10^{-4}–4.4×10^{-2}

and glucose oxidase is well known; the operation of this sensor is based on the detection of hydrogen peroxide at a platinum electrode. The analytical range was 2×10^{-3}–1×10^{-2} M; the limit of detection was 1×10^{-3} M [10].

From an analysis of the literature, we can conclude that a higher sensor sensitivity to lactose can be reached with the use of mediators [7, 8]. However, in this case, not only a mediator but also three enzymes, which catalyze consecutive reactions, should be immobilized. This noticeably complicates the procedure of electrode preparation, increases the consumption of expensive reagents, and decreases the reproducibility of sensor making.

Determination of lactose in milk whey with the use of biosensors based on Berlin blue and PFS. The successful tests of lactose and glucose biosensors based on Berlin blue and a PFS membrane in model systems allowed us to analyze a real material—milk whey. As mentioned above, data on the concentration of lactose can serve as a quality assessment of dairy products.

In the FIA of whey with the use of the developed biosensor, the effect of glucose on the response of the enzyme electrode should be tested. Initially, we measured the response of the lactose biosensor to model glucose solutions (1×10^{-5}–1×10^{-3} M). Next, the glucose content of the whey sample was determined from a calibration graph with the use of the glucose biosensor; this value was equal to 6×10^{-3} M.

Three samples were prepared for the analysis of milk whey by the addition technique. Aliquot portions of standard lactose solutions in buffer 3 (0.2–1×10^{-2} M) were added to a whey solution diluted with buffer 3 by a factor of 100 or 1000. To take into account the glucose content of the whey sample, the concentration of glucose found with the use of a glucose biosensor was subtracted from the concentration of lactose found.

The analyzed sample of whey from 1.5% fat milk contained 0.13 M lactose, which is consistent with data obtained by a standard chromatographic method [11]. Table 4 compares the characteristics of the two methods. It can be seen that the results of the determination of lactose in milk whey with the use of the developed biosensor correlate with the chromatographic data. The use of the lactose sensor has certain advantages over chromatography: a shorter response time, a lower detection limit of lactose, and a lower labor intensity in combination with the use of less expensive instrumentation.

The method developed previously for the immobilization of enzymes into polyelectrolyte membranes from aqueous organic media was successfully applied to the creation of biosensors for the determination of glucose and lactose with the use of domestic preparations. It was found that the advantages demonstrated previously for the Nafion® polyelectrolyte (i.e., the simplicity of membrane preparation, good adhesion to electrode materials, and the possibility of varying component concentrations in the membrane and producing multienzyme membranes) are also characteristic of the perfluorosulfonic polymer from Plastpolimer. Taking into consideration the fact that new enzyme preparations (glucose oxidase and β-galactosidase) are tolerant of some organic solvents, the range of polymers used for the development of biosensors can be extended.

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