Amperometric biosensor based on glycerol oxidase for glycerol determination


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Abstract

A comparative analysis of the efficiency of using glycerol oxidase preparations, which differ in their characteristics and methods of production, in the development of an amperometric biosensor for glycerol determination, was performed. The enzyme preparation which, being immobilized on the transducer surface, ensured the best working characteristics of the sensor, was selected. Electrochemical polymerization of the selected enzyme preparation in polymer poly(3,4-ethylenedioxythiophene) was chosen as the most effective method of glycerol oxidase immobilization on the surface of amperometric biosensor. pH optimum of the developed amperometric biosensor was determined to be 7.2. Buffer capacity and background electrolyte concentration in buffer solution were shown to have no effect on the work of glycerol biosensor. Glycerol concentration in wine sample solutions was measured using the developed amperometric biosensor based on glycerol oxidase.

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1. Introduction

Glycerol, one of the metabolites of alcoholic fermentation, is widely used in industry and pharmacology. As far back as in 1857 Louis Pasteur in his classic studies on alcohol fermentation showed that along with basic products of fermentation, alcohol and carbonic acid, some by-products appear including glycerol (2.5–3.6 g per 100 g digested sugar) [1]. Large amount of glycerol is generated at must fermentation in the presence of natrium bisulphite. Whereas regularly acetaldehyde is a hydrogen acceptor, in this case it is bound by bisulphite which is replaced in the transformation chain by dioxiacetophosphate and 3-phosphoglycerol aldehyde. These substituents receive hydrogen from reduced NADH and generate 1-glycerophosphate, a hydrogen acceptor, in this case it is bound by bisulphite which is replaced in the transformation chain by dioxiacetophosphate and 3-phosphoglycerol aldehyde. These substituents receive hydrogen from reduced NADH and generate 1-glycerophosphate, the dephosphorylation of which results in glycerol formation. This kind of fermentation, called glyceropyruvate, is most intensive during the first stages of fermentation and plays an important role in synthesis of numerous secondary products having effect on wine quality [1]. Besides, glycerol appears at must fermentation in alkaline medium.

Determination of glycerol concentration is of essential importance in wine production because glycerol is second to ethyl alcohol among all components of wine. The total value of glycerol generated at must fermentation is 1/10 to 1/15 of alcohol end concentration, which is 1–10 g/l [2]. Glycerol considerably influences wine flavor imparting oiliness and mildness [2–4]. Therefore, the data on the glycerol presence and its volume in alcohol beverages are significant characteristics of their quality.

Glycerol determination is also important in clinic diagnostics for control of the triacylglycerides level in blood since the monitoring of these components has prognostic value to drop the risks of diseases of the cardiovascular system [5].

The standard methods of glycerol determination, such as liquid chromatography and spectrophotometric approach based on chemical and enzymatic reactions, in particular, oxidase–peroxidase method [6,7], are disadvantageous for the analysis since highly qualified personnel and expensive apparatus are required, besides, complex sample pretreatment is necessary.

The test kits for enzymatic analysis of glycerol proposed by known firms Boehringer Mannheim and Sigma are very expensive because they use some enzymes and coenzymes or cosubstrates. For example, multienzymatic system [8] involves three enzymes: glycerol kinase, pyruvate kinase and lactate dehydrogenase, and requires the presence of coenzyme NADH and two cosubstrates, namely, ATP and phosphoenolpyruvate:

\[
glycerol + ATP \xrightarrow{\text{glycerol kinase}} \text{glycerol phosphate} + ADP
\]
phosphoenolpyruvate + ADP $\rightarrow$ pyruvate + ATP

\[
\text{glycerol kinase} + \text{glycerol} \rightarrow \text{glycerol-3-phosphate} + \text{ADP}
\]

In this method, glycerol concentration is determined by decrease in consumed NADH registered photometrically at wavelength of 340 nm.

In two-enzyme variant, glycerol kinase and glycerol-3-phosphate dehydrogenase are used, the reaction is monitored at 340 nm by the generation of NADH (H+).

In another variant of two-enzyme method, glycerol-3-phosphate oxidase reaction is used instead of glycerol-3-phosphate dehydrogenase one, this variant was applied as a basis of diagnostic kit for glycerol determination.

\[
\text{glycerol-3-phosphate} \rightarrow \text{dihydroxyacetone phosphate} + \text{NADH (H+)}
\]

The third method, dehydrogenase, is based on the determination of value of NADH (H+) generated as a result of the enzymatic reaction catalyzed by glycerol dehydrogenase:

\[
\text{glycerol + NAD$^+$} \rightarrow \text{glyceraldehyde + NADH (H$^+$)}
\]

Application of this method in analytical practice is problematic since glycerol oxidation by glycerol dehydrogenase is a reversible reaction, and selectivity of the enzyme is insufficient.

Traditional enzymatic approaches for glycerol determination are disadvantageous either in low selectivity like in case of dehydrogenase reactions [9] or in high cost of their everyday laboratory application [8]. Therefore, creation of opportune, accurate, selective, quick and cheap method for glycerol analysis in diverse foodstuffs is considered as an actual challenge.

Development of enzyme biosensors for glycerol measurement can be a way to solve the problem and meet the requirements mentioned. Their basic advantages are high specificity, fast results, facility of data processing, low analysis prime cost.

To date, a number of biosensors for glycerol determination are designed and investigated. They are based on enzymes of glycerol dehydrogenase [4,10] and glycerol kinase co-immobilized with glycerol-3-phosphate oxidase [2]. The latter was short of stability: after three-day storage in a working buffer only 10% enzymes remained active. As to glycerol dehydrogenase, its key disadvantage is that there is no cofactor (NAD) in the enzyme composition, so it is necessary to add NAD to the system which essentially raises the price of the analysis. Besides, NAD can easily diffuse out of the enzyme membrane causing remarkable drop in biosensor sensitivity [10].

An amperometric glycerol biosensor based on glycerol oxidase (GO) is considered to be an attractive alternative to the above-mentioned biosensors since GO has a native cofactor FAD in its composition.

The problem is that now GO is absent at the enzyme market, therefore search of GO producers and elaboration of methods of GO purification are important. GO is revealed in numerous mycelial fungi of the species Aspergillus, Penicillium [11], Neurospora [12], Botrytis [13], and also in actinomycetes, while a highly purified form of the enzyme was isolated only from Aspergillus japonicus [5], Penicillium sp. [14] and Botrytis allii [15]. In our previous study, mycelia fungi strains were screened as the potential GO producers; fungus B. allii 100(5) was chosen as a source of this enzyme; conditions of the producer cells cultivation were optimized with reference to obtaining maximum GO synthesis [16]; methods of production of cell-free extracts and schemes of the enzyme extraction and purification were elaborated; methods of stabilization of GO preparations were proposed; a possibility of bioanalytical usage of the enzyme was shown [17].

This study was aimed at development of an amperometric biosensor based on platinum printed electrode SensLab (SensLab GmbH, Leipzig, Germany) with immobilized GO and at optimization of its operational characteristics.

2. Materials and methods

2.1. Materials

The GO preparations obtained from mycelial fungi B. allii (culture 100(5)) [16] were used. The cells were grown during 3 days at 28°C in 500 ml retorts on a shaker at permanent aeration (200 rpm) in the medium, pH 3.0–3.5, of the composition, g/l: KNO$_3$—3.5, KH$_2$PO$_4$—3, NaCl—0.5, MgSO$_4$·7H$_2$O—0.5, CaCl$_2$—0.03, CuSO$_4$·5H$_2$O—0.001, glycerol—60, molasses—2 (components were dissolved in tap water). The fungi cells were washed with water, re-suspended in 50 mM borate buffer, pH 9.8 (BB), frozen and stored at −20°C. To obtain cell-free extract (CFE), the fungi mycelium was destroyed by mechanical grinding in a mortar at air cooling in the presence of protease inhibitors—2 mM EDTA, 0.5 mM phenyl methyl sulfonyl fluoride, 0.1 mM m-aminophenyl boric acid, and 10 μM leupeptine. CFE was separated from cell fragments by centrifugation (15,000 rpm, $\bar{r}_{ave} = 8$ cm, 30 min, 4°C), the activity was detected [16], the protein concentration determined according to Lowry.

Primary GO purification from CFE was performed according to the schemes A or K.

Scheme A—fractionating by acetone. CFE (GO specific activity—0.16–0.2 μmol min$^{-1}$ mg$^{-1}$ of protein) with added to 10% cooled (−25°C) acetone was incubated for 30 min at −10°C and centrifuged for 30 min (15,000 rpm, $\bar{r}_{ave} = 8$ cm, −10°C). The sediment was thrown away, a portion of acetone (1:1) was added to the supernatant to 55%, the incubation for 30 min at −10°C was carried out, the sediment was gathered by centrifugation and suspended in BB. The suspension was cleared by centrifugation, the sediment was thrown away. The extract of 55% sediment contained active GO.

Scheme K—column chromatography. CFE was deposited on the column with bacitracin–silochrom balanced by BB with protease inhibitors; the sorbent was washed with threefold volume of BB. The unabsorbed proteins contained GO activity.

Chromographic enzyme purification on anion-exchange sorbent. The solutions containing primarily purified GO preparations with specific activity of 0.25–0.5 μmol min$^{-1}$ mg$^{-1}$ of protein were deposited on the column with anionite sorbent of cellulose nature—DEAE-Toyopearl 650 M (TSK-Gel, Japan) balanced by BB. GO was eluted with 0.2–1 M NaCl or 20% (regarding saturation, at 0°C)
sulfate ammonium solution in output buffer BB, the GO specific activity was determined in each fraction. The fractions of column eluent with GO activity above 1.5 μmol min⁻¹ mg⁻¹ of protein were collected. The enzyme was concentrated and purified additionally by precipitation with ammonium sulfate to 70% (regarding saturation, at 0°C). The sediment collected by centrifugation was dissolved in minimum BB volume, the GO and catalase activities were determined [16]. GO preparations with different stabilizing additions were stored at 0°C.

The monomer 3,4-ethylenedioxythiophene (EDT) produced by Baytron M and poly(ethylene glycol) MM=1450 of Sigma (Switzerland) were used as a polymer matrix for the enzyme electrochemical polymerization.

Commercial resin Resydrol (Resydrol AY 498 w/35WA (Slutia GmbH, Austria)) and glutaraldehyde produced by “Fluka” (Switzerland) were also used as a polymer matrix for the enzyme deposition.

The reagents Na₂HPO₄·7H₂O, KH₂PO₄, KCl, NaOH and glycerol were of Ukrainian production. All reagents, both domestically manufactured and imported, were of analytical reagent grade and used as received without additional purification.

2.2. Methods of enzyme immobilization on the surface of SensLab printed platinum electrode

2.2.1. GO immobilization by electrochemical polymerization in polymer EDT

Electrochemical polymerization is of remarkable interest due to its technological facilities. It enables to select and maintain dimensions, shape and thickness of the matrix and to provide exact control over precipitation [18,19].

Poly(3,4-ethylenedioxythiophene) (PEDT) is one of polythiophenes, conductive polymers with novel promising properties. Previous investigations [20] demonstrated low conductivity of PEDT, small charge change, increased stability and high capability to film formation. Monomer 3,4-ethylenedioxythiophene, commercially manufactured by Baytron, recently appeared in the market from Bayer AG (Germany).

A homogenous film PEDT is fixed on the surface of working electrode polymerized electrochemically by EDT at neutral pH and room temperature. Film formation is enhanced in aqueous and, possibly, in hydrophilic polymers of the polyanion matrix [23]. GO solution, was prepared in 20 mM phosphate buffer, pH 6.2.

EDT was polymerized by application of the potential from +0.2 V to +1.5 V at a current of 0.1 V/s during 15 cycles using potentiostate PI-50-11 (Ukraine).

2.2.2. GO immobilization by electrochemical deposition in the polymer Resydrol

For GO immobilization, electrochemical deposition of enzyme in the polymeric Resydrol film was used. It is a new technology of proteins immobilization based on electrochemical precipitation into the polyionic polymer matrix owing to its solubility change during pH-modulation. Commercial resin Resydrol is a mixture of polyethylene glycol, protonisation and solubility of which depend on pH value [22,23].

The electrode is placed in the electrolyte solution containing polymer suspension of Resydrol and an enzyme. During redox polymerization, the pulse-profile electric potential is applied to the electrode, invoking water oxidation with the liberation of H⁺-ions and corresponding local change in pH value near the electrode surface. This local acidification leads to the changes in solubility of micellar negatively charged poly(acrylic carboxylic acid) resin. The latter precipitates on the electrode surface, concomitantly entrapping the enzyme molecules.

The mixture of 50 μl polymer solution (300 μl of Resydrol dissolved in 1 ml of water) and 10 μl GO solution was used for electrochemical deposition of polymeric matrix on the surface of working electrode.

Resydrol was polymerized in potentiostatic mode: pulsating tension profile with 20 successive impulses. Primary tension (+1900 mV during 1 s) initiated water oxidation. Further tension impulses (−300 mV during 5 s) caused transient pH-modulation and subsequent binding of enzyme molecules in the Resydrol polyionic matrix [23].

Potentiostate PI-50-11 (Ukraine) was used for electrochemical deposition of Resydrol.

After sensitive membrane formation electrodes were washed by 100 mM phosphate buffer, pH 7.2.

2.2.3. GO immobilization in glutaraldehyde vapour

Glutaraldehyde is a polyfunctional agent which forms covalent bonds between biocatalytic particles or proteins. Therefore enzyme immobilization with glutaraldehyde is often used for enzyme biosensor development.

This immobilization method produced a three-dimensional matrix in which the enzyme is closely trapped with the electrode material, thus improving both retention of the biomolecule on the electrode surface and its electrical communication [24].

For formation of the glutaraldehyde-based bioselective membrane, a drop of GO solution was put on the surface of working electrode. Then sensors were placed into glutaraldehyde vapour atmosphere and after this dried in the air during 10 min.

2.3. Measurements

All electrochemical experiments were performed using the traditional three-electrode system in which the SensLab printed electrode (SensLab GmbH, Leipzig, Germany) itself involves all three electrodes—platinum working, auxiliary and reference [23].

Platinum printed electrodes SensLab were tested towards reproducibility and efficiency within the range of 0–300 mV (the rate of potential evolution 20 mV/s). The cyclic voltamperometry was performed on the potentiostate PI-50-11 (Ukraine).

Amperometric measurement at a constant potential was carried out in the electrochemical cell of 5 ml volume by means of the potentiostate PI-50-11 and programmer PR-8 (Ukraine).

Measurements of glycerol content were performed in 100 mM K, Na–phosphate buffer solution, pH 7.2, at room temperature in an open bulk at intensive stirring. The substrate concentration was varied by the addition of certain aliquots of concentrated solutions. After each measurement the sensor was washed with buffer solution to stabilize the basic signal.

3. Results and discussion

Glycerol determination by amperometric system is based on the enzymatic reaction

\[ \text{glycerol + O}_2 \xrightarrow{\text{glycerol oxidase}} \text{glyceraldehyde + H}_2\text{O} \]

The process of glycerol enzymatic transformation results in generating electrochemically active substance, hydrogen peroxide, oxidation of which causes formation of electrons measurable by
The absence of commercial GO preparations provoked us to isolate the enzyme from the chosen strain-producer, i.e. B. allii (strain 100(5)) [16]. The offered systems of enzyme isolation and purification from cell-free fungus extract included the stages of primary GO purification according to above described schemes A (fractioning by acetone) or K (column chromatography) with subsequent chromatographic enzyme purification on the anion-exchange sorbent. They ensured obtaining GO preparations with specific activity to 5.7 μmol min⁻¹ mg⁻¹ of protein, i.e. 30-fold enzyme purification [17]. The stabilizing effect of ions of Mn²⁺ (5–10 mM), Ca²⁺ (1–2 mM), 1 mM EDTA, sucrose (50%) and poly(ethyleneimine) (PEI, up to 0.05%) on the GO activity in solution was proved, therefore these compounds were added to the purified GO preparations used for immobilization on the electrodes in the construction of glycerol selective biosensor (Table 1).

At the first stage of biosensor development, all GO preparations obtained were immobilized on the surface of amperometric transducer by the method of electrochemical polymerization in PEDT. The operational range of designed biosensors, the detection limit of glycerol concentration, and their stability at storage were under comparative investigation (Table 2). We did not obtain any responses for GO preparations No. 2 (previous purification at scheme K, specific activity 5.7 U/mg, stabilizing additions—70% ammonium sulfate; 1 mM CaCl₂; 1 mM EDTA; 0.05% poly(ethyleneimine) in 50 mM borate buffer, pH 9.18), No. 5 (previous purification at scheme K, specific activity 2.0 U/mg, stabilizing additions—0.3 M NaCl; 1 mM CaCl₂; 0.05% poly(ethyleneimine) in 50 mM borate buffer, pH 9.18), and No. 7 (previous purification at scheme A, specific activity 2.5 U/mg, stabilizing additions—70% ammonium sulfate; 1 mM CaCl₂; 0.05% poly(ethyleneimine)) in 50 mM borate buffer, pH 9.18). The calibration curves of the laboratory prototypes of amperometric biosensors based on other GO preparations are shown in Fig. 1.

Due to the results obtained, the GO preparation No. 1 (previous purification at scheme A, specific activity 5.0 U/mg, stabilizing additions—70% ammonium sulfate; 5 mM CaCl₂; 1 mM EDTA; 0.05% poly(ethyleneimine) in 50 mM borate buffer, pH 9.18) was chosen for further experiments owing to its higher activity and stability after immobilization in comparison with other preparations.

At the next stage, different methods of glycerol oxidase immobilization on the surface of SensLab printed platinum electrode were compared. We analyzed three methods of GO preparation No. 1 immobilization: electrochemical polymerization in polymer PEDT, electrochemical deposition in the Resydrol polymer, and immobilization in glutaraldehyde vapour.

Table 1

<table>
<thead>
<tr>
<th>Number of GO preparations</th>
<th>Scheme of previous purification</th>
<th>Stabilizing additions into 50 mM BB, pH 9.18</th>
<th>Specific activity (U/mg)</th>
<th>GO content in biomembrane (U × 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>70% SA; 5 mM MnCl₂; 1 mM EDTA; 0.05% PEI</td>
<td>5.0</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>70% SA; 1 mM CaCl₂; 1 mM EDTA; 0.05% PEI</td>
<td>5.7</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>70% SA; 1 mM CaCl₂; 0.05% PEI</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>K</td>
<td>1 M NaCl; 1 mM MnCl₂</td>
<td>1.7</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>K</td>
<td>0.3 M NaCl; 1 mM CaCl₂; 0.05% PEI</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>1 M NaCl; 1 mM MnCl₂; 50% saccarose</td>
<td>3.0</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>K</td>
<td>70% SA; 5 mM CaCl₂; 0.05% PEI</td>
<td>2.5</td>
<td>4.9</td>
</tr>
<tr>
<td>8</td>
<td>K</td>
<td>70% SA; 5 mM MnCl₂; 0.05% PEI</td>
<td>1.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

The calibration curves of amperometric biosensors based on glycerol oxidase (preparation No. 1) immobilized by different methods are shown in Fig. 2. The results of comparative analysis of developed biosensors are presented in Table 2.

The results of our experiments demonstrate that biosensors with the glycerol oxidase preparation No. 1 immobilized both in the Resydrol polymer and in the glutaraldehyde vapours are characterized by a narrow dynamic range and lesser response value, as compared to the biosensor with glycerol oxidase immobilized in PEDT by the method of electrochemical polymerization. The latter also has higher stability at storage. The minimum detection limits of glycerol concentration for all these biosensors are the same.

Therefore, as a result of this comparative analysis, electrochemical polymerization in PEDT was chosen as the most effective method of glycerol oxidase immobilization on the surface of amperometric biosensor. The developed glycerol biosensor based on this immobilization method is characterized by the linear response dependence on glycerol concentration in the range of 0.05–25.6 mM and minimum detection limit of 0.05 mM glycerol (Fig. 3). An examination of its storage stability showed fast response decrease in 2 days, relatively stable signal at 75% activity level in 15 days after immobilization, subsequent response decrease by 3% daily, and almost no response was obtained after 50-day storage (Fig. 4).

At the next stage, pH optimum of the amperometric biosensor with GO No. 1 immobilized in polymer PEDT was determined to be 7.2 (Fig. 5). The results are in good correlation with the pub-
Table 2
Comparative analysis of laboratory prototypes of amperometric biosensors based on different glycerol oxidase preparations immobilized in PEDT by the method of electrochemical polymerization.

<table>
<thead>
<tr>
<th>Number of GO preparation</th>
<th>Minimum detected glycerol concentration (mM)</th>
<th>Linear range of sensor operation (mM)</th>
<th>Residual sensor activity at storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>0.05–25.6</td>
<td>75% in 15 days, 14%—in 40 days</td>
</tr>
<tr>
<td>3</td>
<td>0.002</td>
<td>0.002–6.4</td>
<td>30% in 4 days</td>
</tr>
<tr>
<td>4</td>
<td>0.002</td>
<td>0.002–0.05</td>
<td>10% in 3 days</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td>0.2–25.6</td>
<td>10% in 1 day</td>
</tr>
</tbody>
</table>

Table 3
Comparative analysis of laboratory prototypes of amperometric biosensors based on different methods of glycerol oxidase immobilization.

<table>
<thead>
<tr>
<th>Methods of immobilization</th>
<th>Minimum detection limit (mM)</th>
<th>Linear range (mM)</th>
<th>Maximum response value (nA)</th>
<th>Residual sensor activity at storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical polymerization in the PEDT</td>
<td>0.05</td>
<td>0.05–25.6</td>
<td>1405</td>
<td>75% in 15 days, 14%—in 40 days</td>
</tr>
<tr>
<td>Electrochemical deposition in the Resydrol polymer</td>
<td>0.05</td>
<td>0.05–0.4</td>
<td>400</td>
<td>38% in 2 weeks, 13%—in 40 days</td>
</tr>
<tr>
<td>Glutaraldehyde vapour</td>
<td>0.05</td>
<td>0.05–0.2</td>
<td>130</td>
<td>10% in 1 day</td>
</tr>
</tbody>
</table>

Fig. 2. The calibration curves of amperometric biosensors based on immobilized glycerol oxidase (preparation No. 1) in PEDT by the electrochemical polymerization (1), in Resydrol polymer by electrochemical deposition (2), and in the glutaraldehyde vapour (3). Measuring conditions: 100 mM phosphate buffer, pH 7.2, at potential of +300 mV versus intrinsic reference electrode.

Fig. 3. The calibration curve of amperometric biosensor based on immobilized glycerol oxidase (preparation No. 1) in PEDT by the electrochemical polymerization. Measuring conditions: 100 mM phosphate buffer, pH 7.2, at potential of +300 mV versus intrinsic reference electrode.

Fig. 4. Stability of the amperometric biosensor based on immobilized glycerol oxidase (preparation No. 1) in PEDT by the electrochemical polymerization. Measuring conditions: 100 mM phosphate buffer, pH 7.2, at potential of +300 mV versus intrinsic reference electrode.

Fig. 5. Dependence of response of amperometric biosensor based on GO immobilized by electrochemical deposition in PEDT on pH of solution. Measuring conditions: 100 mM phosphate buffer, pH 7.2, at potential of +300 mV versus intrinsic reference electrode. Glycerol concentration in vessel: 3.2 mM (1); 1.6 mM (2); 0.8 mM (3); 0.4 mM (4).
lished data [14,25] according to which pH optimum of free enzyme preparation No. 1 immobilized in polymer PEDT (Figs. 6 and 7). Concerning other immobilization methods, there was also no effect of these parameters on the work of glycerol biosensor. The absence of influence of buffer capacity and background electrolyte concentration is typical for most enzyme amperometric biosensors.

Glycerol concentration in real wine samples from Magarach Institute of grapes and wine (Crimea, Ukraine) was measured by the developed amperometric biosensor based on GO preparation No. 1 immobilized in polymer PEDT. The results show a good agreement with those obtained by conventional methods.

4. Conclusion

A comparative analysis of the efficiency of using glycerol oxidase preparations, which differ in their characteristics and methods of production, for development of an amperometric biosensor for glycerol determination has been performed. The biosensor based on GO preparation No. 1 (previous purification at scheme A, specific activity 5 U/mg, stabilizing additions—70% ammonium sulfate; 5 mM MnCl₂; 1 mM EDTA; 0.05% poly(ethyleneimine) in 50 mM borate buffer, pH 9.18) has been stated to have the best working properties.

Three methods of glycerol oxidase preparation No. 1 immobilization on the surface of SensLab printed platinum electrode have been compared. Electrochemical polymerization in polymer poly(3,4-ethylenedioxythiophene) has been chosen as the most effective method of glycerol oxidase immobilization on the surface of amperometric biosensor.

The glycerol concentration in model solutions has been measured by the developed amperometric biosensor based on immobilized GO. The developed glycerol biosensor is characterized by linear range of 0.05–25.6 mM and minimum detection limit of 0.05 mM glycerol, storage stability at the 75% activity level in 15 days after immobilization into sensitive membrane.

The developed glycerol amperometric biosensor could be recommended in food industry to monitor and optimize the fermentation process and to control wine quality.

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References

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Biographies

Tatiana Goriushkina was born in 1984 in Orlovskaya region, Russia. She received her MS degree in biochemistry from Taras Shevchenko Kiev University (Ukraine) in 2007. Since 2007 she is PhD-student in biotechnology of Taras Shevchenko Kiev University (Ukraine) and leader engineer in the Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine. Her field of interests is amperometric enzyme biosensors for wine analysis.

Sergei Dzyadevych was born in 1967 in Crimea, Ukraine. He received his MS degree in the radiophysics and electronics from Taras Shevchenko Kiev University (Ukraine) in 1992, and his PhD degree in biotechnology in 1995 from Institute of Biochemistry (Kiev), and Doctor Science degree in biotechnology in 2005 from Institute of Molecular Biology and Genetics (Kiev). Since 1995 he worked as research, senior and leader researcher in the Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine. During 1996–2005 he worked as an invited scientist at Institute of Chemo- and Biosensors (Muenster, Germany), Enzyme Biotechnology Group, Leeds University (UK), Claude Bernard University Lyon 1 (France), Ecole Centrale de Lyon (France). His fields of interests are conductometric biosensors, ENFET, amperometric biosensors.