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Enzyme electrodes constructed on the basis of oxygen electrode with oxidases immobilised by sol-gel technique*

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The results of immobilisation of enzymes in silica gel on oxygen electrode are reported. As the model, enzyme glucose oxidase was used. The influence of the composition of the casting solution (gel precursor, pH of the enzyme solution, sol to buffer ratio) on the electrode response was investigated. Also, the addition of γ-aminopropyltriethoxysilane to the casting solution was checked. The best electrode with stable signal was obtained, when the formed gel was not very rigid (buffer, pH 6 or 7 and high buffer to sol ratio). For the optimal composition of the casting solution, some properties of the glucose electrode were investigated (stability, pH profile and influence of temperature). The method of sol-gel entrappment was also used to obtain the electrodes sensitive for disaccharides by co-immobilisation of invertase, lactase and maltase with glucose oxidase. In addition, preliminary results for other oxidases immobilised by this method are presented.

Key words: *enzyme electrode, glucose electrode, polyphenol biosensor, sol-gel technique, enzyme immobilisation*

1. Introduction

The method of sol-gel entrapment of biomolecules is a very promising technique of immobilisation for biosensors construction, because of its simplicity, low temperature of the process, large amount and low leakage of entrapped material. Since 1990, when Braun et al. [1] reported for the first time entrapment of proteins in silica gel, the sol-gel process has become an attractive way of immobilisation of biological material in biosensor construction and has been reviewed few times [2–4].

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Sol-gel technique provides a simple method to obtain glass materials by hydrolysis (acidic or basic) and condensation of metal alkoxides. Typically, the gels are obtained from tetramethoxysilane [5] or tetraethoxysilane [6–8]. After hydrolysis (typically acid catalysed) the gel is obtained by changing the pH. Other precursors and gels are also used like alumina [9] or vanadia [10]. For the purpose of the immobilisation of biomolecules, the conditions of precursor hydrolysis and condensation like pH and organic solvent content have to be controlled to avoid denaturation of proteins. When the sol-gel technique is used to construct the optical biosensors, the conditions of the process must allow obtaining transparent gels. The properties of silica gels can be modified by hydrolysis and condensation of organosilicon derivatives like 3-aminopropyltriethoxysilane, 3-glycidoxypropyltrimethoxysilane, 2-(3,4-epoxycyclohexyl) -ethyltrimethoxysilane [11, 12] or others. The resulting gel has better structure, porosity and regular distribution of immobilised biomaterial as compared with conventional one [12]. The use of organosilicon derivatives allows also incorporating ionogenic or redox-active groups into the gel structure [3]. Very promising composite materials for biosensor construction can be obtained by mixing silica sol with redox polymers [13], colloidal gold or graphite powder [3]. Such composites have good electrical properties [3] and can be used for screen-printing approach [14].

The sol-gel technique has been applied in construction of biosensors of different types: conductometric [5], amperometric [6, 8–14], spectrophotometric [6, 8] and fluorometric [7]. A simple enzyme electrode can be obtained by immobilisation of oxidase on the surface of oxygen electrode. The oxygen concentration depletion is proportional to the concentration of oxidase substrate. Local changes of oxygen concentration caused by enzymatic reaction are measured by oxygen electrode. Only once sol-gel process was used by Tatsu et al. to immobilise glucose oxidase on the tip of Clark oxygen electrode and the resulting biosensor was used in flow injection analyser [15].

In this work, the preliminary results of immobilisation of selected enzymes in silica gel obtained by sol-gel process on an oxygen electrode are reported. The aim of this work was to optimise the conditions of immobilisation of the model enzyme (glucose oxidase) in silica hydro gel on the oxygen electrode, to evaluate some of the properties of the obtained glucose electrode and to check the applicability of the sol-gel process to immobilisation of other enzymes.

2. Experimental

2.1. Reagents

As gel precursors tetramethoxysilane (TMOS) (99+%, Aldrich Chemie GmbH, Germany) or tetraethoxysilane (TEOS) (99+%, Aldrich Chemie GmbH, Germany) were used. To some gels 3-aminopropyltriethoxysilane (APTES) (Merck, Germany) was added. As the model enzyme glucose oxidase (GOD) from *Aspergillus niger* (solution, 5370 U/ml, Serva) was used. Other enzymes used in experiments were: catalase

from bovine liver (solution, 155 000 U/ml, Serva), invertase from bakers yeast (solid, 500 U/mg, Sigma), β-galactosidase (lactase) from *Escherichia coli* (solid, 388 U/mg, Sigma), β-galactosidase (lactase) from *Aspergillus oryzae* (solid, 3.8 U/mg, Sigma), α-glucosidase (maltase) from bakers yeast (solid, 3.5 U/mg, Sigma), β-glucosidase from almonds (solid, 6.6 U/mg, Sigma), mutarotase from porcine kidney (suspension, 25000 U/ml, Sigma), ascorbate oxidase from cucurbita species (solid, 146 U/mg, Sigma), phenolase from potato (solid, 570 U/mg, Sigma), laccase from *Rhus vernicifera* (solid, 180 U/mg, Sigma), tyrosinase from mushroom (solid, 3 000 U/mg, Sigma), alcohol oxidase from *Candida boidinii* (solid, 0.4 U/mg, Sigma), galactose oxidase from *Dactylium dendroides* (solid, 16 U/mg, Sigma), choline oxidase from *Alcaligenes* species (solid, 14 U/mg, Sigma), cholinesterase, butyryl (pseudocholinesterase) from horse serum (solid, 8.7 U/mg, Sigma). The enzymes were used with no further purification; if solid they were dissolved in 0.05 M phosphate buffer, pH 6. All other reagents were of analytical grade. Double-distilled water was used throughout. At pH 5.5 and lower 0.05 M acetate buffers were used, for higher pH 0.05 M phosphate ones were used.

2.2. Apparatus

The measurements of dissolved oxygen were done using galvanic silver-zinc oxygen electrode CTN-920.S (MES-EKO, Wrocław, Poland). This electrode consists of silver cathode and zinc anode and is covered by Teflon® membrane. The operating principle of this electrode is the same as the Clark one [16]. The electrode was connected with microcomputer oxygen meter CO-551 (Elmetron, Zabrze, Poland). The oxygen meter measures the current caused by reduction of oxygen on silver cathode after diffusion through the membrane. The results of measurements are expressed as the percentage of oxygen concentration in saturated solution from air at given conditions (pressure and temperature).

The temperature was maintained by the thermostat U1 (MLW, Medingen, Germany). The constant stirring rate of the solution was maintained by magnetic stirrer BMM 21 (DHN Wigo, Piastów, Poland).

2.3. Immobilisation of the enzymes and electrode preparation

The stock solutions of sol were prepared as follows: SOL I – 4.5 ml TEOS, 1.4 ml H_2O and 0.1 ml 0.1 M HCl [6]; SOL II – 5 ml of TMOS, 1.0 ml H_2O and 0.05 ml 0.1 M HCl [5] were stirred vigorously at room temperature until the transparent homogeneous solution was obtained (SOL I – 3 h, SOL II – 15 min) and stored in refrigerator. SOL I was stable for 1 week, SOL II – for 6 weeks.

The casting solution was prepared by mixing 200 μl of the sol solution with enzyme solution and buffer of pH ranging from 5 to 9 (the total volume of enzyme and buffer ranging from 200 to 800 μl). 20 μl of this mixture was dropped immediately

after mixing on the surface of oxygen electrode covered with nylon mesh. The mesh was fixed on the electrode by rubber O-ring and was used as a mechanical holder of gel layer [15] because of the poor adhesion to the Teflon® membrane of oxygen electrode. To some casting solutions APTES was added to check if the introduction of amine groups would change the properties of the resulting enzyme electrode. The compositions of casting solutions and gelation times are given in Table 1. The reported gelation time is the time from mixing sol with buffer containing dissolved enzyme to the moment at which bulk gelation occurs. When the gel was formed, the resulted enzyme electrode was dipped in 100 ml of phosphate buffer, pH 7, at 25 °C.

Table 1. Dependence of gelling time on composition

Sol	pH of the buffer	Sol:buffer ratio	Volume of added APTES	Gelling time/min
TMOS	5	1: 1	none	3
	6			4
	7			1
	8			0.5
	9			0.25
	5	1: 4	none	70
	6			30
	7			1.5
	8			8
	9			7
	6	1: 1	2.5 µl	> 0.1
TEOS	5	1: 1	none	9
	6			17
	7			4
	8			1.5
	9			1
	7	1: 1.5	none	2.5
		1: 2		3
		1: 3		3
	5	1: 4	none	30
	6			40
	7			4
	8			1.5
	9			2
	6	1: 1	2.5 µl	1
			5 µl	0.5
			10 µl	0.5
			20 µl	0.5
	7	1: 1	5 µl	0.5

2.4. Measurement procedure

The measurement procedure was similar to that reported earlier [17]. The response of the electrode was measured using oxygen meter and expressed as per cent of oxygen concentration at saturation from air [18].

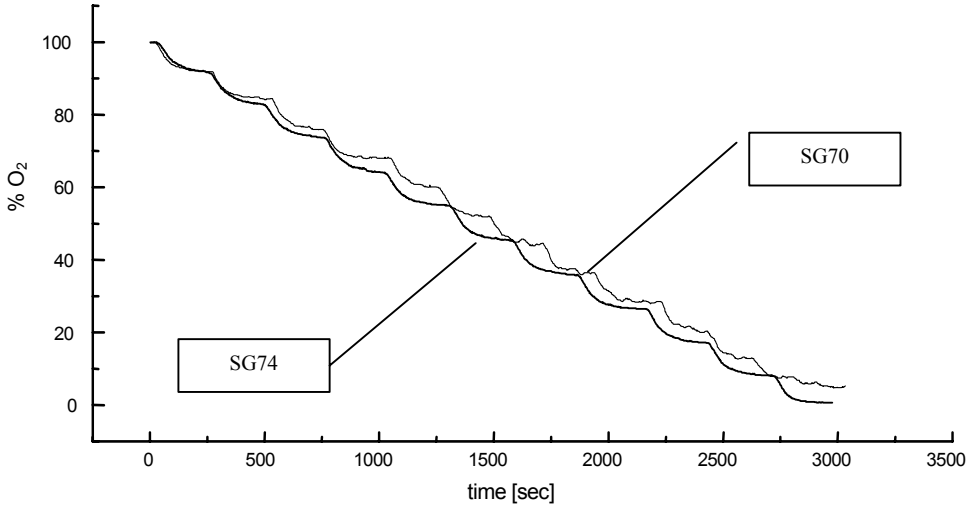

Fig. 1. Examples of response of glucose electrodes: SG70 – 200 µl ZOL I + 180 µl buffer, pH 7 + 20 µl GOD, SG74 – 200 µl ZOL I + 750 µl buffer, pH 7 + 50 µl GOD

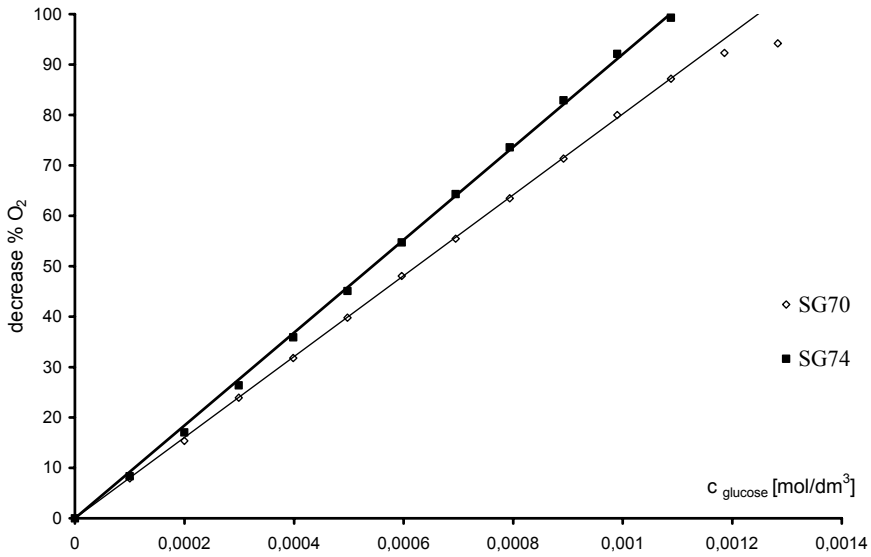

Fig. 2. Examples of the calibration curves of glucose electrode: SG70 – 200 µl ZOL I + 180 µl buffer, pH 7 + 20 µl GOD, SG74 – 200 µl ZOL I + 750 µl buffer, pH 7 + 50 µl GOD

The measurements were done in phosphate buffer, pH 7, at 25 °C in stirred solution (stirring rate 500 rpm). When the electrode response become stable, 0.1 ml of 0.1 M substrate solution (if not stated otherwise) was added.

The solutions of saccharides in water (glucose, galactose, lactose, maltose, saccharose, cellobiose) were made one day before use to allow the mutarotation. All other substrate solutions were made freshly every day. Subsequent addition of substrate was made when the response was time independent. A typical response of glucose electrode is given in Fig. 1. As the result, the calibration curves were obtained (the dependence of decrease of O_2 percentage against concentration of substrate) and the slopes of their linear part (sensitivity) were calculated [19]. The examples of calibration curve are given in Fig. 2.

3. Results

3.1. Glucose electrode

As a model enzyme glucose oxidase was used because of its high activity, selectivity, stability and very well known properties. This enzyme catalyses the reaction:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \qquad (1)$$

The depletion of oxygen concentration is thus the measure of glucose concentration.

In preliminary investigations, the response of glucose electrode and its sensitivity was measured for gels prepared from different precursors, with different pH of the buffer and different sol to buffer ratio. For some compositions of the casting solution, also the influence of APTES addition was tested. The details of tested compositions are given in Table 1. The casting solutions with gelation times longer than 30 min and shorter than 0.25 min cannot be used to prepare the glucose electrode because of technical problems. For very long gelation times the sol, when dropped onto the tip of the electrode, was drying before the gel was formed and the resulting layer was cracking. For very short times, it is impossible to take the desired volume from the casting solution and drop it on the electrode because the gel is formed in the pipette. When TMOS was used as a precursor, some precipitation of silica could be observed for sol to buffer ratio 1:4 before gelation, when sol and buffer were mixed together. The less pH of the buffer, the more silica was precipitated.

For some tested compositions of the casting solution the response of the electrode is not stable but varies about some value ("noisy" response) – for example the line SG70 in Fig. 1. The electrodes with noisy response are not suitable to measure the glucose content in solution. Probably, such unstable response is caused by the poor adhesion of gel layer to Teflon® membrane. The adhesion could be improved by addi-

tion of surfactant to the casting solution [20]. Thus, to some casting solutions, surfactant Triton X-100 was added but no improvement of the electrode response was observed.

The typical calibration curves for glucose were linear with some deviations from linearity for higher concentrations of glucose (Fig. 2) caused by the lack of oxygen in diffusion layer near the electrode surface. The reciprocal plots (not shown) of the data indicate that the response of electrode is controlled by diffusion of substrates to electrode surface [21]. In Table 2, analytical properties of glucose electrodes are summarised.

Table 2. Analytical parameters of glucose electrodes with stable response

Sol	Buffer pH	Sol:buffer ratio	Volume of added APTES	Linear range /mol/dm^3	Sensitivity /% of O_2/(mol·dm^{-3})	*r*
TMOS	7	1: 4	none	0–0.0011	79220	0.999
	8			0–0.0007	64520	0.997
	9			0–0.0009	102960	0.999
TEOS	5	1: 1	none	0–0.0008	88330	0.998
	6			0–0.0011	86190	0.999
	7	1: 1.5	none	0–0.001	95080	0.999
		1: 2		0–0.001	86020	0.997
		1: 3		0–0.001	87000	0.999
	5	1: 4	none	0–0.001	92760	0.999
	6			0–0.001	90880	0.999
	7			0–0.001	92260	0.999
	8			0–0.0009	99840	0.999
	9			0–0.001	100110	0.999
	6	1: 1	2.5 µl	0–0.0009	103500	0.997
			5 µl	0–0.001	86440	0.999

A typical response time of the electrodes (time interval from addition of substrate until the signal become stable) varies from 3 to 5 min with some tendency to be lower for higher water content in gel.

The results of investigations of the influence of the composition of the casting solution on gelling time and glucose electrode response indicate that TEOS is better gel precursor than TMOS, because for the latter the response of the electrodes is noisy and there are some problems with gel formation (precipitation of silica for high buffer to sol ratio). For TEOS better electrodes with higher sensitivity are obtained with high buffer to sol ratio (Table 2, Fig. 3) especially for basic pH. A lower response of electrodes with low sol to buffer ratio is probably caused by greater diffusional resistance of the gel, which is denser in this case [21]. The addition of APTES caused the shortening of the gelation time but does not improve the properties of the electrodes. In

contrary, when the high amount of APTES is added, the response of the electrode becomes unstable.

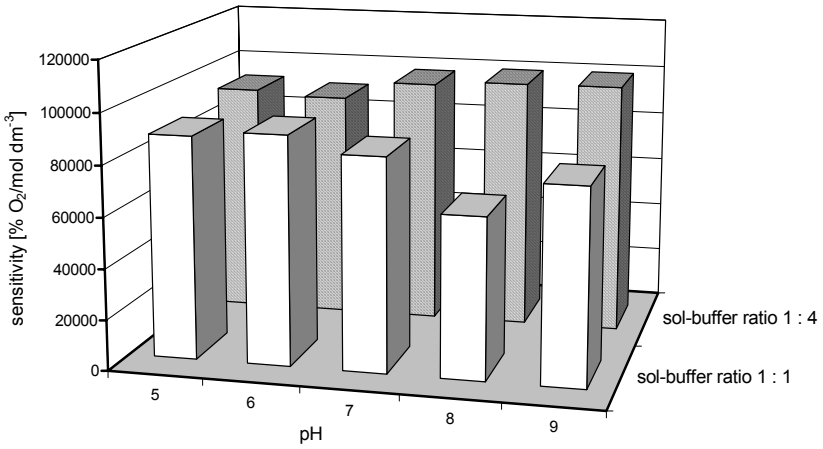

Fig. 3. The glucose electrode sensitivity for different compositions of casting solution (sol precursor TEOS)

For further experiments with glucose oxidase the gels of following composition were chosen: SG60 – buffer pH 6, sol to buffer ratio 1:1, SG61 – buffer pH 6, sol to buffer ratio 1:1 + 5 μl APTES added, SG74 buffer pH 7, sol to buffer ratio 1:4. For these gels, the dependence of electrode response on pH (Fig. 4) and temperature (Fig. 5) was investigated. For all gels tested, the response of the electrode shows the maximum at pH = 7, with sharp loss of the sensitivity for greater values. Similar results were obtained by other authors [23]. With increasing temperature the measured signal of the electrode (decrease of oxygen concentration) is growing up to about 35 °C; for the temperatures higher than 30 °C, the calibration curves become non-linear and because of that the maximum response is obtained for different temperatures for low and high glucose concentration (Fig. 5). The glucose oxidase is known as a very stable enzyme [24], but the results obtained indicate that in silica environment its stability is poor. Also, the stability of the electrodes was tested. For this purpose, they were stored for 10 days in 50 ml of buffer, pH 7, in refrigerator (4 °C). After this storage, the response of electrodes decreases (for SG60 only 7.7% of the initial sensitivity) and unstable. Similar results for GOD immobilised in silica gel were obtained by other authors [23]. In storage buffer, the traces of GOD activity could be found. The results indicate that there is leakage of enzyme from the gel. Also the gel by itself is not stable and some cracking could be observed.

Glucose oxidase was co-immobilised with catalase to broaden the calibration range of the electrode. The upper limit of glucose concentration that can be assayed by glucose electrode is limited by the solubility of oxygen in solution. By addition of

catalase the upper limit could be extended twice, because half of the consumed oxygen (Eq. (1)) is in a half recovered in reaction:

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \frac{1}{2}O_2 \tag{2}$$

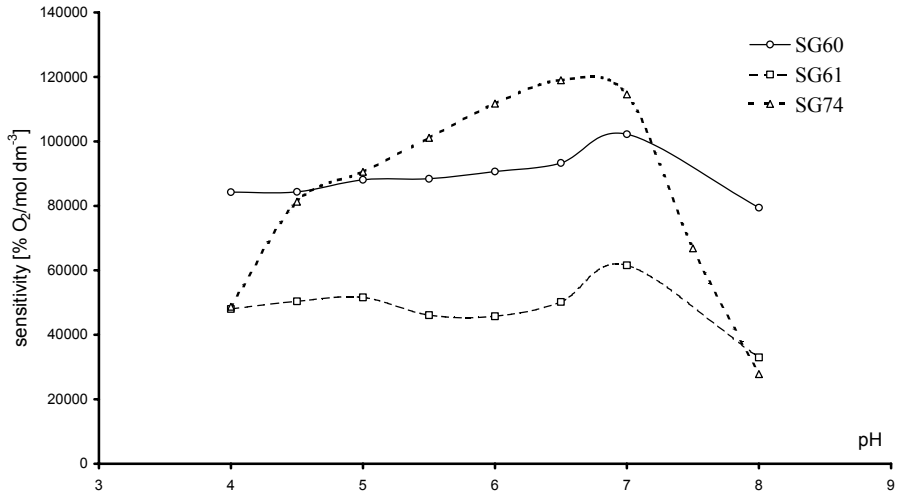

Fig. 4. The dependence of glucose electrode sensitivity on pH (description of legend key in text)

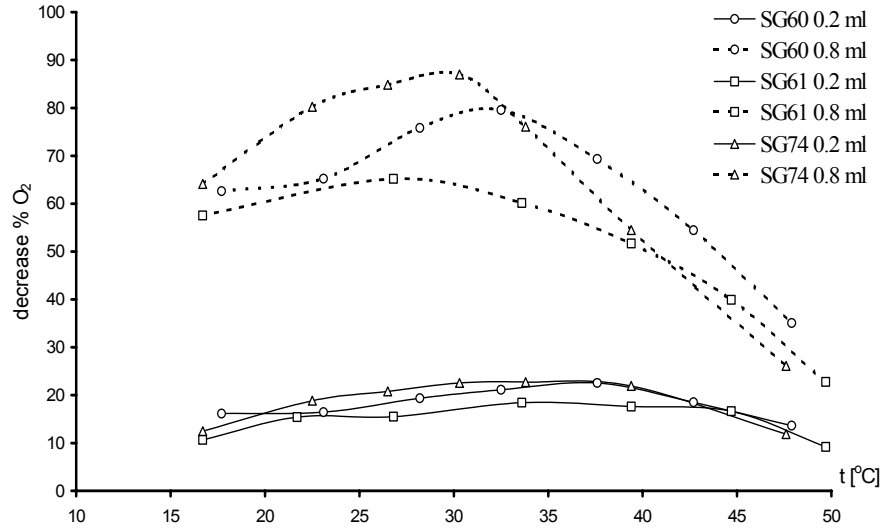

Fig. 5. The dependence of glucose electrode response on temperature for 0.2 ml and 0.8 ml of added 0.1 M glucose solution (description of legend key in text)

To casting solutions SG60 and SG74 catalase was added and the ratio of activities, catalase to GOD was 14.4:1. As the result, the linear parts of the calibration curves were extended to 0.0017 mol/dm^3 for SG60 and 0.0013 mol/dm^3 for SG74. The sensitivity decreased by the factor of 0.5 for SG60 and 0.6 for SG74.

3.2. Electrodes for disaccharides

By co-immobilisation of GOD with suitable hydrolases (and mutarotase optionally) the electrodes sensitive for disaccharides (maltose, lactose, saccharose and cellobiose) could be obtained. The hydrolase hydrolysis disaccharide to glucose and other monosaccharide in reactions:

$$\text{maltose} + H_2O \xrightarrow{\text{maltase}} 2\ \text{glucose} \tag{3}$$

$$\text{lactose} + H_2O \xrightarrow{\text{lactase}} \text{galactose} + \text{glucose} \tag{4}$$

$$\text{saccharose} + H_2O \xrightarrow{\text{invertase}} \text{fructose} + \text{glucose} \tag{5}$$

$$\text{cellobiose} + H_2O \xrightarrow{\beta\text{-glucosidase}} 2\ \text{glucose} \tag{6}$$

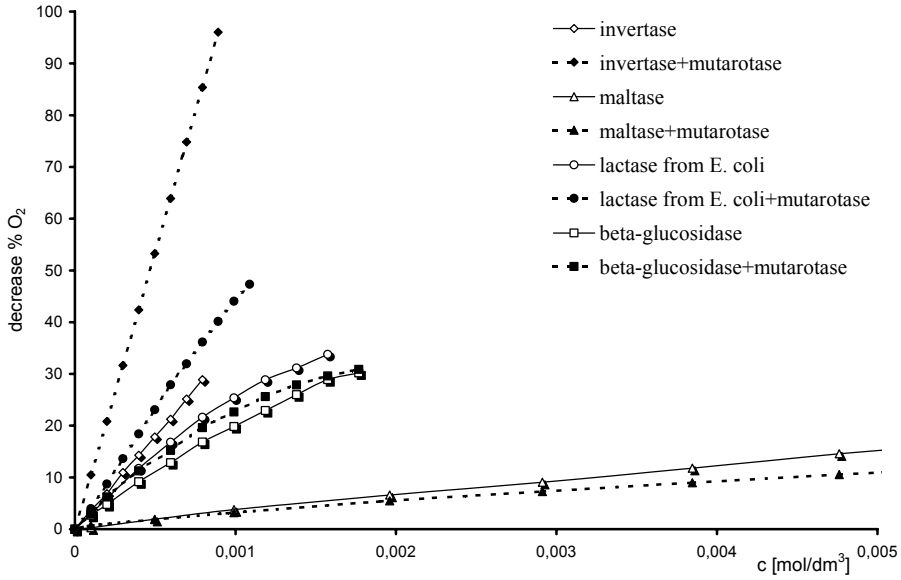

Fig. 6. Examples of calibration curves for disaccharides with casting solution composition SG74 (details about added activities of enzymes in Table 3) at pH 7

Glucose produced in reactions (3)–(6) is then consumed by GOD. Because GOD oxidases only β-D-glucose and during hydrolysis of some disaccharides (e.g., saccha-

rose, maltose) α-D-glucose is produced, mutarotase is added [18]. Mutarotase increases the rate of reaction of mutarotation of glucose:

$$\alpha\text{-D-glucose} \xrightarrow{\text{mutarotase}} \beta\text{-D-glucose} \tag{7}$$

and thus, the response of the electrode for disaccharide is increasing.

For hydrolases (not for all) only casting solutions SG60 and SG74 were tested (not for all enzymes, Table 3), because the addition of APTES (SG61) does not change the properties of electrode significantly. Typically, the slopes of the calibration curves were less for sol to buffer ratio 1:1 or in the case of maltase, there was no response. The reason is that the gel is denser and denaturation of the enzymes during the gelling process occurs by high concentration of ethanol, which is produced during hydrolysis of TEOS. Some results (calibration curves) for different disaccharides are presented in Fig. 6 and some analytical parameters are collected in Table 3.

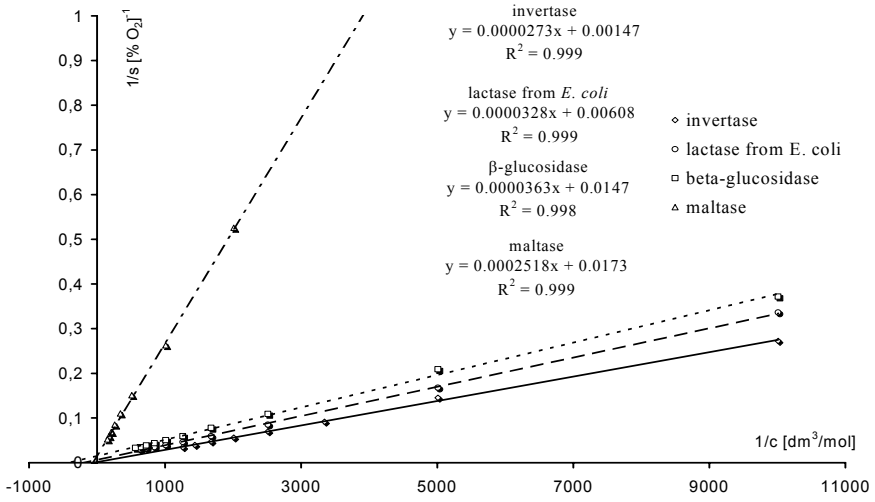

Fig. 7. The double reciprocal plots for electrodes with disaccharide hydrolases without added mutarotase (data from Fig. 6)

Except of maltase, the improvement of the electrode response by addition of mutarotase could be seen especially for invertase and lactase from *Escherichia coli*. For lactase from *Aspergillus oryzae* this effect was not observed but for this enzyme the calibration was made at pH very different from its optimum pH, which is 4.5 and due to that its activity was low. Also, some results reported for electrodes with β-galactosidase applied in flow injection analysis indicate that the addition of mutarotase did not improve the characteristics of the electrode [25]. The calibration curves for disaccharides are not linear. The double reciprocal plot of the data (Fig. 7) indicates that the electrode response is controlled by the kinetics of the enzymatic reaction [21]. From

these plots, the apparent Michaelis constants for enzymes could be calculated using Lineweaver–Burk equation [22]. They are 18.6, 5.39, 2.47 and 16.6 mM for invertase, lactase from *Escherichia coli*, β-glucosidase and maltase, respectively.

Table 3. Analytical parameters of electrodes for disaccharides at pH 7

Composition	Enzyme*	Activity/U**	Substrate	Sensitivity /% of O_2/mol·dm^{-3}	Linear range /mol/dm^3	*r*
SG60	invertase	50	saccharose	50346	0–0.0005	0.999
	invertase + mutarotase	50 + 12.5		61676	0–0.001	0.999
SG74	invertase	50		36074	0–0.0008	0.999
	invertase + mutarotase	50 + 12.5		107308	0–0.0009	0.999
SG60	maltase	4	maltose	no response		
	maltase + mutarotase	4 + 12.5				
SG74	maltase	4		3017	0–0.007	0.998
	maltase + mutarotase	4 + 12.5		2369	0–0.005	0.987
SG74	lactase from *E. coli*	13.5	lactose***	27907	0–0.0008	0.999
	lactase from *E. coli* + mutarotase	13.5 + 1.5		45381	0–0.001	0.999
SG74	lactase from *A. Oryzae*	1.9	lactose***	22836	0–0.0008	0.999
	lactase from *A. Oryzae* + mutarotase	1.9 + 12.5		21981	0–0.0008	0.999
SG74	β-glucosidase	2.64	cellobiose***	21686	0–0.0008	0.998
	β-glucosidase + mutarotase	2.64 + 12.5		25687	0–0.0008	0.995

*In each case the activity of added GOD was 5.4 U per electrode.
**Activity of enzyme immobilised on electrode.
***For this saccharides the compositions SG60 was not tested.

The sensitivities of the tested electrodes sensitive for disaccharides except that for saccharose are too low to find the practical application for assay of disacharides. But the improvement of their properties could be achieved by increasing the amount of added hydrolase and assay at optimal pH.

3.3. Electrodes with other oxidases

The enzymes tested were: galactose oxidase, ascorbate oxidase, alcohol oxidase, three types of polyphenolase (laccase, tyrosinase from mushroom and phenolase from potato) and choline oxidase. Choline oxidase was also co-immobilised with pseudo-cholinesterase (Fig. 10) to assay choline esters due to the course of reactions:

$$\text{butyrylcholine} \xrightarrow{\text{pseudocholinesterase}} \text{choline} + \text{butiric acid} \tag{8}$$

$$\text{choline} + 2\,O_2 \xrightarrow{\text{choline oxidase}} \text{betaine} + 2\,H_2O_2 \tag{9}$$

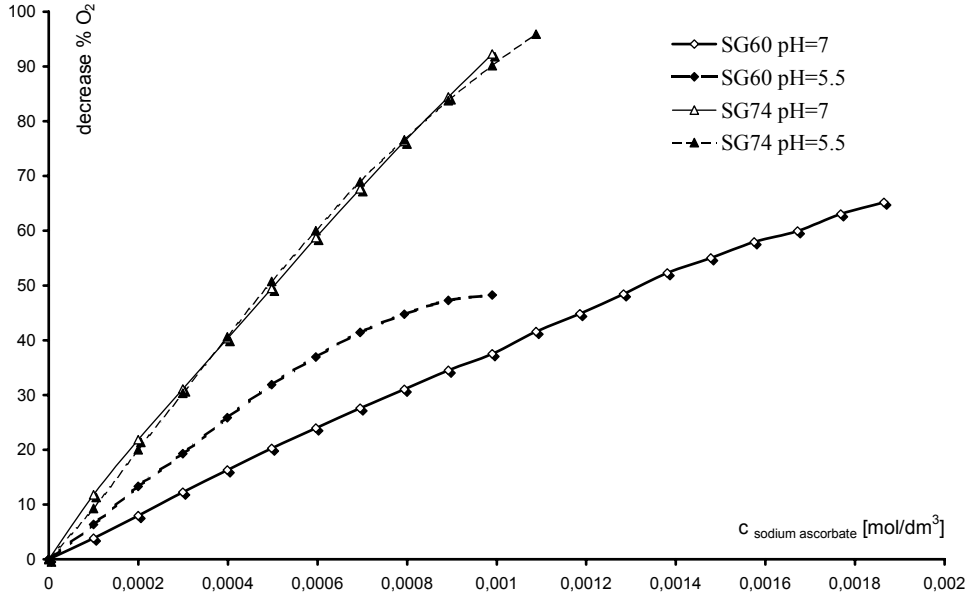

Fig. 8. Calibration curves of electrode with ascorbate oxidase for vitamin C

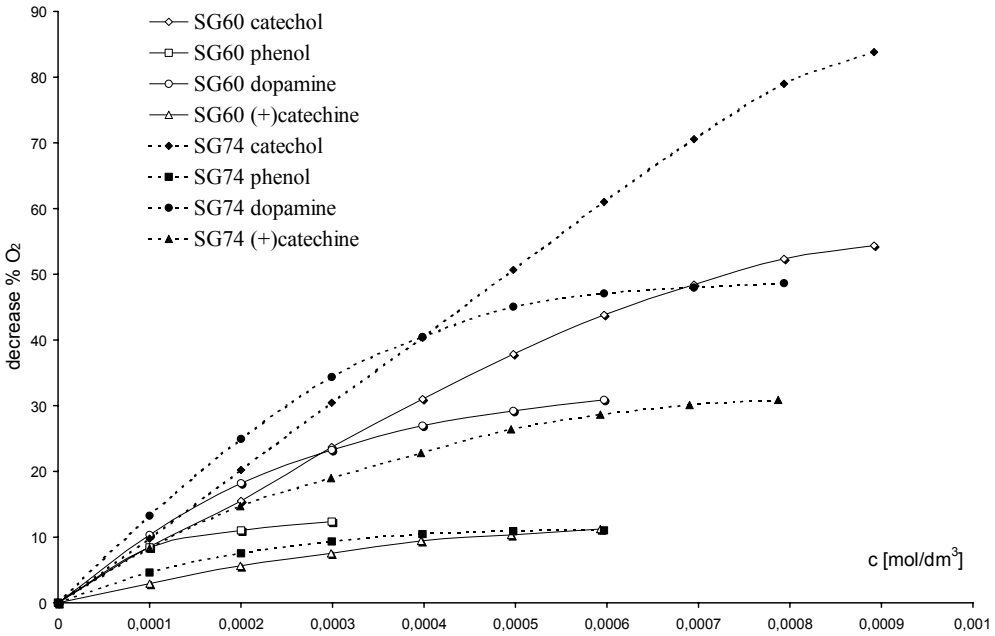

Fig. 9. Calibration curves of electrode with tyrosinase for different substrates at pH 7 (4U of enzyme immobilised on electrode)

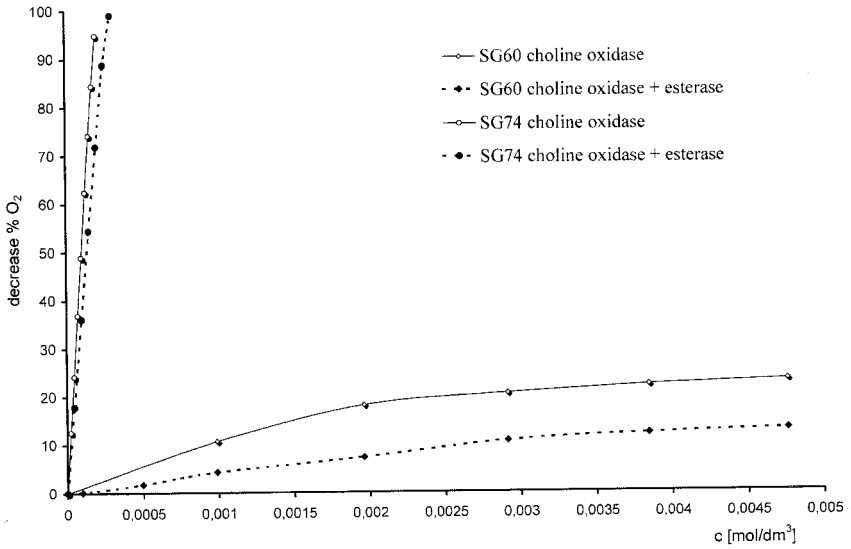

Fig. 10. Calibration curves of electrodes with choline oxidase without and with added pseudocholinesterase at pH 7

Such an electrode is very promising for indirect monitoring of pesticides, which inhibit catalytic properties of esterase [26]. For all enzymes except alcohol oxidase, the compositions of casting solutions tested were SG60 and SG74. Some examples of the calibration curves are shown in Figs. 8–10, and analytical parameters of calibration curves are collected in Table 4.

For all enzymes, the measured electrode signal was greater for sol to buffer ratio 1:4. The electrode with galactose oxidase was tested also for lactose because this enzyme can oxidise not only free galactose, but also galactosides but with lower rate. For some enzymes, the precipitation of the protein could be observed during formation of gel with sol to buffer ratio 1:1 causing a very low electrode signal or even none. It is especially characteristic of choline oxidase and esterase. The ratio of sensitivities for sol SG74 and SG60 is 51.5 and 96.8 for choline oxidase and co-immobilised choline oxidase and esterase, respectively (Fig. 10). The reason is that in sol SG60 the concentration of ethanol is much higher as compared with SG74. It could be also seen that for the same enzyme the response for the substrate with greater molecule is much lower for the denser gel (compare the results with tyrosinase for catechol and (+)catechine, Fig. 9). For sol composition SG60, the ratio of sensitivities for (+)catechine and catechol is 0.34 and for SG74 0.74. It is caused by greater diffusional resistivity of denser gel.

For all oxidases tested, the calibration curves are not linear and their linear ranges are sometimes very narrow. The reason of such non-linearity is that the response of the electrode is controlled by the kinetics of enzyme reaction not by the diffusion of the substrate and oxygen [21]. For some enzymes, there is also another reason of non-

linearity. Polyphenolases, especially tyrosinase, and ascorbate oxidase belong to the group of the enzymes that undergo suicide inactivation by the substrates [27, 28]. The higher is the concentration of the substrate and the longer is the time of enzyme contact with it the lower is the activity of the enzyme. This effect is especially significant for tyrosinase and ortodiphenols. It causes that during prolonged time of measurements and for higher concentrations of substrate, the measured signal of the enzyme electrode with immobilised tyrosinase begun to increase instead decrease after addition of substrate [29, 30]. This was observed for all polyphenolases tested and for ascorbate oxidase at pH 5.5.

Table 4. Analytical parameters of some electrodes with immobilised oxidases

Composition	Enzyme	Activity /U*	Substrate	pH	Linear range /mol/dm^3	Sensitivity /% of O_2/mol·dm^{-3}	*r*
SG60	Galactose oxidase	2.5	galactose	7	0–0.0012	5615	0.997
			lactose	6	0–0.005	1144	0.953
SG74			galactose	7	0–0.004	16745	0.994
			lactose	6	0–0.006	791	0.998
SG74	alcohol oxidase	0.2	ethanol	7	0–0.0016	11880	0.985
			ethanol	7	0–0.0004	42810	0.976
SG60	ascorbate oxidase	4	sodium ascorbate	7	0–0.0014	38363	0.999
				5,5	0–0.0006	63568	0.999
SG74				7	0–0.0007	99356	0.998
				5,5	0–0.0007	100051	0.999
SG60	choline oxidase	2	choline	7	0–0.002	9530	0.994
SG74				7	0–0.000175	490562	0.999
SG60	choline oxidase + pseudo-cholinesterase	2 + 6	butyryl choline chloride	7	0–0.003	3704	0.998
SG74				7	0–0.00025	358494	0.999
SG60	tyrosinase	1.6	catechol	7	0–0.0003	69275	0.996
		4	catechol	7	0–0.0004	78457	0.999
			dopamine	7	0–0.0002	93830	0.997
			(+)catechine	7	0–0.0002	26373	0.996
SG74		4	catechol	7	0–0.0007	101755	0.999
			dopamine	7	0–0.0002	126361	0.999
			(+)catechine	7	0–0.0003	75739	0.997
SG74	phenolase	4	catechol	7	0–0.0004	39218	0.989
SG74	laccase	4	catechol	7	0–0.002	15674	0.979

*Activity of enzyme immobilised on electrode.

To diminish the effect of suicide inactivation the activity of immobilised tyrosinase must be very high. For sol composition SG60 the effect of inactivation by catechol was observed at concentration 0.5 mM when 1.6 U of tyrosinase is immobilised and at

0.9 mM for 4U. Also the sensitivity of the electrode is in first case lower and linear range is narrower.

The presented results for oxidases are only preliminary and are continued especially for tyrosinase.

4. Conclusions

- The technique of immobilisation by sol-gel transition on the tip of oxygen electrode can be applied to different enzymes to obtain enzyme electrodes.
- The ratio of water to sol during gel formation must be high to avoid the denaturation of the enzyme by ethanol during immobilisation, to diminish the diffusional resistance of the gel layer and thus, to obtain electrodes with high sensitivity.
- The addition of APTES does not improve significantly the properties of the electrodes.
- The glucose oxidase entrapped in silica hydrogel is rather unstable and some leakage of the enzyme during storage is observed. The stability could be improved either by crosslinking the protein with for example glutaraldehyde or covering the gel with outer membrane.
- The calibration curves are not linear. The non-linearity is caused by different reasons depending on the enzyme type and immobilised amount of the enzyme.

The investigations of enzyme electrodes with enzymes immobilised by sol-gel technique will be continued especially for tyrosinase. The aim of the further work is to optimise the composition of the casting solution for this particular enzyme to improve stability and avoid inactivation by the substrate, to check the operation parameters of the resulted electrode, influence of pH and temperature and selectivity spectrum.

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References

[1] BRAUN S., RAPPOPORT S., ZUSMAN R., AVNIR D., OTTOLENGHI M., Mater. Lett., 10 (1990), 1.
[2] DAVE B.C., DUNN B., VALENTINE J.S., ZINK J.I., Anal. Chem., 66 (1994), 1120 A.
[3] WANG J., Anal. Chim. Acta, 399 (1999), 21.
[4] LIN J., BROWN C.W., Trends Anal. Chem., 16 (1997) 200.
[5] LEE W.-Y., KIM S.-R., KIM T.-H., LEE K.S., SHIN M.-C., PARK J.-K., Anal. Chim. Acta, 404 (2000), 195.

[6] NARANG U., PRASAD P.N., BRIGHT F.V., RAMANATHAN K., KUMAR N.D., MALHOTRA B.D., KAMALA-
SANAN M.N., CHANDRA S., Anal. Chem., 66 (1994), 3139.
[7] WU X., CHOI M.M.F., XIAO D., Analyst, 125 (2000), 157.
[8] KUMAR A., MALHOTRA R., MALHOTRA B.D., GROVER S.K., Anal. Cim. Acta, 414 (2000), 43.
[9] LIU Z., LIU B., KONG J., DENG J., Anal. Chim. Acta, 392 (1999), 135.
[10] LEV O., GLEZER V., J. Am. Chem. Soc., 115 (1993), 2533.
[11] PANDLEY P.C., UPADHYAY S., PATHAK H.C., Electroanalysis, 11 (1999), 59.
[12] PANDLEY P.C., UPADHYAY S., PATHAK H.C., Sens. Actuators B, 60 (1999), 83.
[13] PARK T.-M., IWUOHA E.I., SMYTH M.R., FREANEY R., MCSHANE A.J., Talanta, 44 (1997), 973.
[14] WANG J., PAMIDI P.V.A., PARK D.S., Anal. Chem., 68 (1996), 2705.
[15] TATSU Y., YAMASHITA K., YAMAGUCHI M., YAMAMURA M., YAMAMOTO H., YOSHIKAWA S., Chem. Lett., (1992), 1615.
[16] Lee Y.H., Tsao G.T., [in:] *Advances in Biochemical Engineering*, T.K. Ghose (Ed.), A. Fiechter & N. Blakenbrough, Springer, Berlin, 1979 Vol. 13, 34–86.
[17] Przybyt M., Sugier H., Anal. Chem. (Warsaw), 34 (1989), 63.
[18] Filipiak M., Fludra K., Gościmińska E., Biosens. Bioelectr., 11 (1996), 355.
[19] THÈVENOT D.R., TOTH K., DURST R.A., WILSON G.S., Biosens. Bioelectr., 16 (2001) 121.
[20] LEV O., Analusis, 20 (1992), 543.
[21] SCHELLER F., SCHUBERT F., *Biosensors*, Elsevier, Amsterdam, 1992, p. 55.
[22] LI J., CHIA L.S., GOH N.K., TAN S.N., Anal. Chim. Acta, 362 (1998) 203.
[23] YAO T., TAKASHIMA K., Biosens. Bioelectr., 13 (1998), 67.
[24] WILSON R., TURNER A.P.F., Biosens. Bioelectr., 7 (1992), 165.
[25] NARINESINGH D., STOUTE V.A., DAVIS G., NGO T.T., Analyt. Biochem., 194 (1991) 16.
[26] BERNABEI M., CHIAVARINI S., CREMISINI C., PALLESCHI G., Biosens. Bioelectr., 8 (1993), 265.
[27] CÉNOVAS F.G., TUDELA J., MADRID C.M., VARÊN R., CARMONA F.G., LOZANO J.A., Biochim. Biophys. Acta, 912 (1987), 417.
[28] TOKUYAMA K., DAWSON C.R., Biochim. Biophys. Acta, 56 (1962), 427.
[29] ZHANG J., LI B., XU G., CHENG G., DONG S., Analyst, 124 (1999), 699.
[30] RIVAS G.A., SOLIS V.M., Electroanalysis, 6 (1994), 1136.

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