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Biosensors for the evaluation of lipase activity

N.F. Starodub

A.V. Palladin Institute of Biochemistry of the Ukrainian National Academy of Sciences, 9 Leontovicha Str., 01601 Kiev, Ukraine

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Abstract

In the review a comprehensive analysis is given of instrumental analytical approaches to control the activity of lipolytic enzymes and their substrates. A special attention is attached to the methods based on the biosensor technology. A number of electrochemical, optical and mechanical biosensors that were developed in several laboratories are analyzed in detail. In addition, perspectives are evaluated for the development of these biosensors and their practical application. It was concluded that biosensors may provide all practical demands which are in enzymology field at the express determination of lipase activity and triacylglycerol levels in biological objects.
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1. Introduction

Lipases (triacylglycerol lipase, EC 3.1.1.3.) are serine hydrolases, which are able to catalyze the hydrolysis of fatty acyl esters. The natural substrates of lipase are triacylglycerols. However, a wide range of structurally diverse esters, alcohols and carboxylic acids may also serve as substrates of this enzyme [1]. Today a lot of different biochemical methods exist for the determination of lipase activity and triacylglycerol level. Among them it is necessary to distinguish colorimetric methods with *p*-nitrophenyl-butyrate as a substrate, and HPLC chromatography on C-18 reversed-phase column and β-naphtol as substrate [2,3]. Titration methods for the determination of the lipase activity using its tributyrin substrate [4] or olive oil [5] are wide spread too. A number of methods vary in the process of substrate solubilization, or in the activity marker employed, or in the detection system [6,7]. A new colorimetric microassay which is based on the activation and inhibition of *Candida rugosa* and *Bacillus*-related lipases by saturated fatty acids has recently been described [8]. Highly sensitive active-site titration of lipase in microscale culture media using fluorescent organophosphorus esters such as diethyl 4-methylumbelliferyl phosphate, ethyl hexyl 4-methylumbelliferyl phosphate and ethyl 4-methylumbelliferyl heptylphosphonate was proposed [9]. The most detailed overview of the methods to determine the activity of lipases and the level of their substrates is given in a recently published review [10]. It was analyzed titrimetric, colorimetric, fluorimetric, turbidometric, chromatographic, radiometric, enzymatic, physical and immunological procedures. We support the opinion of the authors cited in refs. [6,8,10], that the majority of the existing methods to control the lipase activity are not yet suitable for non-purified samples and for large scale analyses due to their cost and time requirements. Further automation, miniaturization, simplicity, increased sensitivity and resettability of obtained results remain to be essential improved. This could be achieved through development of new assays based on chromogenic and fluorogenic substrates with enhanced characteristics. Nevertheless, the most complete fulfillment of all practice demands may be done with the application of instrumental approaches based on the principles of biosensors.

2. Biosensors-based approaches

International Union of Pure and Applied Chemistry defines biosensor as autonomic integrated device intended to obtain quantitative and semi-quantitative information using biological recognition element (biochemical receptor), which is in a space contact with a transducer [11]. At the same time it was recommended to distinguish biosensor from a bioanalytical system, i.e., when additional facilities are needed to add a sample. Biosensor differs from biotest, which may be used for single analysis only. If recognizing and transforming elements are integrated in a unique compact system it is a biosensor in a form of a biochip as analog of a computer chip, which denotes silicon structure made for miniaturization of electronic circuit. As

E-mail address: nikstarodub@yahoo.com.

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a rule, biochip is an individual sensor, and a combination of several such structures is a multisystem. The latter may be presented as a set of biochips with individual transducers and combined by computer programs, or as several analyzing units based on a single transducer and using a joint computer program. Biosensors may be classified according to a biological recognition material, a way of transformation of a physical–chemical signal into electrical one, a type of transducer, a registered reaction or an analyzed substance.

Biosensors may be grouped, based on the types of transducers, in the following categories: electrochemical (using macro-, planar electrodes or ion sensitive field effect transistors—ISFETs), mechanical (on the basis of quartz microbalance, surface active wave), optical (with fiber optics and with signal registration by means of "evanescent" wave, non-emitting energy transfer, or electronic devices that use effects of ellipsometry, surface plasmon resonance—SPR, holography and others), calorimetrical (with thermistors, microcalorimeters and thermopairs) and magnetic. Electrochemical biosensors, in their turn, are divided into conductometric, potentiometric and amperometric types in accordance with the way they record generated signal. Amperometric biosensors may have special mediator for transferring electrons from a redox enzyme center to the transducer.

To determine the lipase activity and the quantity of its substrates, different biosensors have been proposed; their description is given below.

2.1. Electrochemical biosensors

This type of biosensors is the most well known for the analysis of different types of substrates. The first reports describing biosensors for the determination of triglyceride levels date back to 1977–1979 [12,13]. It was a flow-through pH electrode biosensor for the determination of activity of neutral lipase. A bit later electrode biosensor was constructed, in which lipase, glycerol dehydrogenase and peroxidase were used simultaneously [14]. Potentiometric triglyceride enzymatic biosensor has been prepared with the use of pH glass electrode [15]. The linear range was 1.91 mM with a slope of 32.7 mV/pC in the pH 8.5 Tris–HCl buffer. The response time was approximately 10 min and biosensor was relatively stable for 25 days.

The next step in the developed of this type of biosensor was connected with ISFETs. Therein lipase from *Candida cylindracea* was photoimmobilized on the gate surface with the help of 4,4′-diazidostilbene-2,2′-disulfonic acid (sodium salt) and polyvinylpyrrolidone [16]. The response time of such a biosensor was 2 min. The linear response was in the range of 100–400, 3–50 and 0.6–3 mM for triacetin, tributyrin and triolein, respectively. In case of triolein the detection limit was 9 μg/ml at the signal-to-noise ratio of 3:1. Such a biosensor was used for the determination of triglycerides in solution [17,18]. The newly created ISFETs-based biosensor was able to determine triglycerides in the range of concentration from 0.1 up to 2 mM. The sensitivity of biosensor was equal to 0.1 mM and time required for a measurement was 2 min. Sodium phosphate buffer (1 mM, pH 7.3) was used to provide the optimal environment for the registration of triglyceride catalytic reaction. A similar buffer was employed to register a variety of analytes by this type of biosensor. It is essential to highlight that lipase from porcine pancreas, type 11 (Sigma, USA) was immobilized on the ISFET gate with the help of glutaraldehyde, which was used for a preliminary activation of nitride silicon according to the procedure proposed in ref. [19].

During further development of biosensors, a new system of microreactor containing silica gel with surface immobilized lipase and ISFET, as a pH-recording element, was included [20]. The enzyme was immobilized through its entrapment within alginate gel beads and subsequent glutaraldehyde mediated linking to the surface of the keratin coated glass beads followed by adsorption onto nitrocellulose sheets. The last methods were found to be effective. The inefficacy of the first method in this work may probably be prescribed to non-optimized conditions of the entrapment process, since recently [21] it was demonstrated that *C. rugosa* lipase may be well immobilized on Ca-alginate gel beads, especially when they were preliminary coated with chitosan and silica. A steady-state response of a biosensor system for the triacetin concentration range from 0 to 2.56 mM was about 110 min. At the concentration of less than 4 mM, the sensitivity of this system had the following indices: for tributyrin—0.478 pH/mM, for triolein—0.128 pH/mM and for triacetin—0.022 pH/mM. At the same time the linear range of response was within 30.0, 4.0 and 1.1 mM for triacetin, tributyrin and triolein, respectively.

In general, an ISFET-based biosensor works on the principal of potentiometry. On the basis of this principle, a novel type of biosensor was developed that used porous structure obtained by thermal oxidization of p-type (1 0 0) crystalline silicon with resistance ranging from 5 to 10 Ω cm [22]. This biosensor detected changes in pH during the hydrolysis of triglycerides, e.g., fatty acid esters, by lipase to glycerol and fatty acids. A shift in capacitance–voltage (*C–V*) characteristics was measured by an electrolyte-oxidized porous silicon–crystalline silicon structure. The lipase was immobilized on the porous structure by physical sorption. The sensor was calibrated by tributyrin dissolved in 0.1 M phosphate buffer (pH 7.0) at different concentrations. The device sensitivity was characterized as 30 mV to a change in one pH unit. The pH (from 2 to 9) response function was linear in the range of tributyrin concentrations of 6.0–21.6 mM. The time of analysis was about 15 min. The biosensor shown reproducible results (about 2% error) during 6 months if it was washed with phosphate buffer (0.1 M, pH 7) and if it was stored at 0–5 °C. The examination of biosensor work in real conditions, e.g., during blood sample analysis, revealed a shift in its *C–V* characteristics, which was in the order of 10 mV. Despite the described characteristics and optimism of the authors about practical perspective of this biosensor, the problem of sensitivity at low concentrations of triglycerides remains. It is essential to point out that the drawback of all potentiometric biosensors lies in their dependence on conditions, in which sample is presented, in particular, pH, buffer capacity, ionic strength, etc. [23,24]. Mirsky et al. [25] developed an electrode capacitive biosensor for monitoring the activities of lipases, phospholipases hydrolyzing water-insoluble substrates into water-soluble

products. This biosensor was based on a sandwich structure: Au/S$(CH_2)_{17}CH_3$/substrate/electrolyte. Hydrolysis of the substrate leads to the formation of water-soluble products and desorption of these compounds from the electrode. These processes cause an increase in the electrode capacitance. The detection limit of the determination of phospholipase A_2 is about 50 pg/ml (or about 5×10^{-5} U/ml). It has a higher sensitivity than a monolayer with Langmuir technique [26] (10^{-3} U/ml) and electrostatistical (2×10^{-4} U/ml) methods, and about 500 times lower than the sensitivity of bioluminescence and radioactive methods. It was demonstrated [26] that the developed capacitive biosensor in comparison with monolayer one has a much higher volume/surface ratio. Recently a novel potentiometric biosensor based on silicon was proposed for the estimation of tributyrin and urea [27]. It is an electrolyte–insulator–semiconductor capacitor (EISCAP) that shown a shift in the measured *C–V* with changes in pH of the electrolyte. The pH sensitivity is determined by calculating the parallel shift in *C–V* curves in the depletion region along the voltage axis at a point constant capacitance (usually at the midpoint of the *C–V* curve). The voltage value at that point is termed as U_{bias}. A plot for this value versus pH showed a maximum slope of 55 mV per unit change in pH. Sensitivity of this biosensor was on the level of millimolar concentrations of the tributyrin or urea. In spite of the fact that the developed biosensor has the satisfactory sensitivity the fulfillment of analysis with its help is not entirely convenient since the work with electrolyte system is complicated. Moreover, it might to be too difficult to combine a recording system with a measuring cell.

Potentiometric electrode biosensor based on the unusual reversible sol–gel transition phenomenon and lipase was used for the determination of olive oil [28]. It was mentioned high activity and good stability of this lipase electrode.

Biosensors that generate signals based on an amperometrical principle are capable of avoiding interference of a sample environment with the measured signals. One of such a biosensor [29] consisted of a platinum anode and a silver cathode and exploited glycerol dehydrogenase immobilized on a collagen membrane. Triglycerides were first hydrolyzed by lipase in solution and the resulting glycerol was determined by the measurement of the steady-state oxidization current generated at a platinum electrode by NADH produced in the enzyme-catalyzed reaction. Olive oil containing 78% triolein was used as source of triglycerides. For both glycerol and triglycerides the calibration plots were linear in the same range from 0 to 12 μM, with detection limits of 0.2 and 0.7 μM, respectively. The time of analysis was 10 min. The immobilized glycerol dehydrogenase retained high operational activity for a period longer than 30 days. Membrane with immobilized enzyme was reusable. It appears that this biosensor's characteristics could be improved if both enzymes were co-immobilized on the membrane. Another amperometric biosensor was developed on the basis of three-electrode cell configuration consisting of a working carbon electrode, a saturated Ag/AgCl reference electrode, and a Pt auxiliary electrode [30]. Working electrode was modified with adsorbed Meldola Blue, Nile Blue or Toluidine Blue O and glycerol dehydrogenase entrapped into gelatin gel immobilized in polylysine gel or trapped in two types of organic salts: poly(dimethylaminoethyl)methylmethacrilate amylobromide with suspension of polyvinylacetate or poly[*N*,*N*′-sulfono-(*p*-phenylene-terephthalamide)]. The sensitivity of such a biosensor varies from 2 to 9 nA/mM glycerol with steady state achieved from 20 s and 8 min depending on the method of immobilization. Tryglycerides were determined after pre-incubation during 5 min in the mixture of lipase.

Since polyaniline exhibits a strong dependence of electronic behaviors on the redox and protonation state it was utilized to develop a generic biosensor concept at the creation of next types of analytical systems: glucose/glucose oxidase, urea/urease, neutral lipid/lipase and Hb/pepsin [31].

It was described the amperometric analytical system included triglyceride sensor with an enzymatic hydrolysis unit and sensor unit with a thin polyurethane membrane contained glycerol kinase and glycerol-3-phosphate oxidase sandwiched between two dialysis membranes and mounted onto a platinum electrode in a flow-through cell combined with pumps, autosampler and potentiostat [32]. Complete hydrolysis of triglyceride samples is attained within 15 min with the aid of a previously freeze-dried mix containing triacylglycerol acylhydrolase, carboxylic-ester hydrolase, detergent, ATP and buffer. Linear sensor signal was in frame of 5–1000 μmol/l and 25 samples/h can be analyzed at 2% of standard deviation.

Amperometric biosensor on the basis of non-specific lipase isolated from *C. rugosa* and intact *Gluconobacter oxydans* cells containing membrane-bound glycerol gehydrogenase was developed and investigated at the analysis of pre-hydrolyzed samples and kinetic regime fulfillment [33]. Using *G. oxydans* cells it was obtained the next characteristics of biosensor: detection limit—20 μM, linear range—up to 2 mM and response time—84 s (90% of steady state). Triglyceride assay of pre-hydrolyzed samples was based on 20 min hydrolysis. For triolein samples the calibration curve linear was up to 12 mM. At the kinetic regime of analysis glycerol was determined during 10 min with linear range up to 30 mM and detection limit of 50 μM. In this case free glycerol presented in the sample did not effect on the results of analysis. Half biosensor activity was lost through 7 days when trehalosee was used as a stabilizer.

Biosensor with flow-injection analytical (FIA) system and amperometric detection of oxygen consumption during enzymatic reaction at the lipoxygenase catalyzed oxygenation was proposed for the control of essential fatty acids in such real samples as vegetable oils and margarines [34]. Biosensor showed different sensitivities for linoleic and α-linoleic acids, the most common essential fatty acids. In the presence of detergent the triglycerides of the hydrophobic food samples were converted into water-soluble glycerol and free fatty acids by 15 min incubation with a ready to use lipase/esterase mixture, thus avoiding the use of organic solvents for analysis.

Recently it was developed new variant of FIA system for the determination of glycerol and triacylglycerol based on enzymatic reactions with electrochemical detection [35]. The hydrogen peroxide produced in this system was monitored by electrochemically with platinum electrode. The best and most effective analytical configuration was the tandem of lipase column and silica-fused capillary with glycerokinase (GK) and

glycerol-3-phosphate oxidase (GPO). Lipase helps the breakdown of triacylglycerol to yield free fatty acids and glycerol, while glycerokinase catalyzes the adenosine-5-triphosphate-dependent phosphorylation of glycerol to yield α-glycerol phosphate, which can subsequently be oxidized by 3-glycerol phosphate oxidase to produce hydrogen peroxide. The optimum conditions of the system are 2 mM ATP in 0.1 M carbonate buffer (pH 11.0), flow rate of 0.18 ml/min, temperature of 35 °C, 20 μl of sample injection and applied voltage of 0.650 V. The proposed biosensing system using lipase, GK and GPO exhibited a flow-injection analysis peak response of 2.5 min and a detection limit of 5×10^{-5} M glycerol with acceptable reproducibility (<4.30%). It also had linear working ranges from 10^{-4} to 10^{-2} M for glycerol and from 10^{-3} to 10^{-2} M for triacylglycerol. The capillary enzyme reactor was stable up to 2 months in continuous operation, and it was possible to analyze up to 15 samples/h. The resent biosensing system holds promise for on-line detection of triacylglycerol in serum and glycerol content in fermented products.

Despite a significant improvement in the design in comparison to early samples, the latter biosensor was still hampered by the cumbersome nature of the detection method requiring different enzymatic reactions. Nevertheless, amperometric biosensors have the best practical perspectives out of all electrochemical biosensors after issues of multiple steps, long time of analysis, costs and sensitivity are resolved.

2.2. Optical biosensing analysis

The reagentless pH-based biosensing system has been recently developed using a fluorescently labelled dextran co-entrapped with a lipase from *C. rugosa* in sol–gel derived nanocomposite films (500 nm) [36]. Fluorescein (FC) or carboxy-seminaphtharhodafluor (SNARF-1) was used as fluorescent labels. The films also contained various additives, such as methyltrimethoxysilane (MTES), dimethyldimethoxysilane (DMDMS), polyethylene glycol (PEG) or polyvinyl alcohol (PVA), to optimize the entrapped enzyme. SNARF-1 was more photostable than FC and for the stability of the lipase activity the presence of PVA was more preferable than PEG. For MTES–PVA-based film the response curve was linear in concentrations of glyceryl tributyrate (GTB) and provided a sensitivity corresponding to a change in the emission intensity ratio of 0.01 per 50 mM change in GTB.

Original optical biosensor based on the SPR was proposed using biodegradable polymer films: a poly(ester amide), dextran hydrogel and poly(trimethylene) succinate for α-chymotrypsin, dextranase and lipase from *Pseudomonas fluorescens*, respectively [37,38]. The most sensitive analysis was found for α-chymotrypsin. The analyzed concentration range was from 4×10^{-11} to 4×10^{-7} mol/l of enzyme. Degradation of the films was complete in less than 20 min for the enzyme concentrations greater than 9×10^{-9} mol/dm^3. Enzyme concentrations as low as 4×10^{-11} mol/dm^3 were detected in less than 30 min. The transducer has great potential for the detection of enzyme concentrations as well as for use in immunosensing where the enzyme degrading the polymer would be the enzyme label. Unfortunately, the rate of degradation and sensitivity of analysis for lipase was not given.

The SPR technique was found to be very effective for the investigation of interactions between biological substances. It was used to explore the interaction of lipoproteins with heparan sulfate proteoglycans and with lipoprotein lipase [39]. With the help of this technique a contribution of the carboxyl-terminal domain of lipoprotein lipase into interaction with heparin and lipoproteins was demonstrated [40].

2.3. Mechanical biosensor

Discovery of a linear relationship between the frequency shift of the SAW sensor system and the conductivity and dielectric constant of solution made possible development of a new type of biosensors, which could also be used for detection of lipase activity [41]. It was shown that such biosensor has a linear relationship between the change in frequency and the enzyme activity up to 500 U/l, with the detection limit reaching 0.3 U/l. The sensitivity of this biosensor was found to be much higher in comparison with the other methods, in particular, flow-through pH-state, turbidimetric and colorimetric methods.

2.4. Thermal biosensor

These biosensors are based on the measurement of heat generated by the enzymatic catalysis [42]. The level of enthalpy in this case may achieve up to −83 kJ/mol and it allows conducting sensitive measurement of the concentration of reagents. This approach has also been used for the determination of lipase activity [43]. Unfortunately, practical application of thermometric biosensors requires new efforts directed toward increasing sensitivity and simplicity of recording devices.

3. Conclusion

The analysis of the above-presented data illustrates a widespread effort devoted to the problem of measurement of the lipase activity and lipase substrates. The biosensor technology has a great promise in this respect. Undoubtedly, all types of biosensors can find their practical application depending on the measurement conditions and task in hand. Some comparative characteristics of different biosensor types are given in Table 1. The main attention there is paid to the sensitivity, linear range of analysis and response time.

The electrochemical biosensors appear to have most favorable characteristics, for example, amperometric biosensors do not depend on the source of sample. The thermometric and optical biosensors are also very attractive, but require improvement in sensitivity and simplicity of analysis. Despite very high sensitivity of the SAW-based biosensors they remain sufficiently complicated. The potentiometric biosensors based on ISFETs and electrolyte–insulator–semiconductor structure (EIS) deserve special attention. The EIS-based biosensors have previously been used for simultaneous multiparametrical analysis of some pesticides and heavy metal ions [24,44]. The ISFETs may also be combined to form a multiparametrical biosensor.

Table 1
Some characteristics of biosensors for the estimation of lipase activity and triacylglycerol levels

Biosensor based on	Analyzed substance	Sensitivity	Linear range (mM)	Response time (min)	Working period (month)	References
Electrode, potentiometric	Triglyceride		1.91	10	25	[15]
ISFET, potentiometric	Triacetin	–	100–400	2		[16,17]
	Tributyrin	–	3–50			
	Triolein	9 μg/ml	0.6–3			
Idem	Triglycerides	0.1 mM		2		[18]
Idem	Tributyrin	0.478 pH/mM	30.0	Steady-state response—110		[20]
	Triolein	0.128 pH/mM	4.0			
	Triacetin	0.022 pH/mM	1.1			
Idem	Tributyrin		6.0–21.6	15	6	[21]
Electrode, capacitive	Phospholipase A_2	50 pg/ml				[24]
Electrode, potentiometric	Tributyrin	1 mM				[26]
Electrode, amperometric	Glycerol	0.2 μM	0–0.012	10		[28]
	Triglycerides	0.7 μM				
Idem	Glycerol	2–9 nA/mM		0.3–8	1	[29]
Idem	Triglycerides	5–1000 μmol/l		15		[31]
Idem						
Pre-hydrolyzed sample	Glycerol	20 μM	Up to 2	1.4		[32]
Kinetic regime		50 μM	Up to 30	10		
Idem	Glycerol	5×10^{-5} M	0.1–10	2.5	2	[34]
	Triacylglycerol		1–10			
SPR, optical	Chymotrypsin		4×10^{-8} to 4×10^{-4}	20		[36,37]
	Lipase					
SAW, mechanical	Lipase	0.3 U/l	Up to 500 U/l			[40]
Calorimeter, thermal	Lipase	Up to −83 kJ/mol				[41,42]

Both these biosensors can allow simultaneous monitoring of the lipase activity from several sources or evaluation of influence of a number of inhibitors on particular enzymes.

The success in the development of biosensors lies in the efficacy of combining biological sensitive elements with the transducer surface. This problem has recently attracted significant interest, and may benefit from a big experience obtained from the lipase immobilization for biotechnological processes [21,45–47]. Recent announcement of creation of monoclonal antibodies against lipase from *C. rugosa* [48] opens new perspectives to develop immune biosensors for very selective and rapid identification and quantitative determination of this enzyme in biological objects.

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References

[1] R. Sharma, et al., Biotehnol. Adv. 119 (2001) 627.
[2] M. Yordanov, et al., J. Med. Microbiol. 54 (2005) 1.
[3] M.T. Gatto, et al., Bioorg. Med. Chem. 10 (2002) 269.
[4] W. Torsten, et al., Enzyme Microb. Technol. 17 (1995) 218.
[5] M. Tenkanen, et al., Appl. Microbiol. Biotechnol. 60 (2002) 120.
[6] D. Wahler, J.L. Reymond, Curr. Opin. Chem. Biol. 5 (2001) 152.
[7] F. Beisson, et al., Eur. J. Lipid Sci. Technol. 28 (2000) 133.
[8] C. Ruiz, et al., Biochem. Biophys. Acta 1672 (2004) 184.
[9] R. Fujii, et al., Biochem. Biophys. Acta 1631 (2003) 197.
[10] H.C. Deeth, V. Touch, Austr. J. Dairy Technol. 55 (2000) 153.
[11] International Union of Pure and Applied Chemistry, Talanta 43 (1996) 503.
[12] T.A. Kelly, G.D. Christian, Analyst 109 (1984) 453.
[13] I. Satoh, et al., J. Solid-Phase Biochem. 2 (1977) 1.
[14] I. Satoh, et al., Anal. Chim. Acta 106 (1979) 369.
[15] Y.-S. Wang, et al., Hengyang Med. Coll. 26 (1999) 144.
[16] M. Nakako, et al., Anal. Chim. Acta 185 (1986) 179.
[17] S.P. Ivashkevich, et al., Ukr. Biochem. J. 72 (2001) 88.
[18] S.P. Ivashkevich, et al., Proceedings of VI Scientific Conference "Optical and Electronic Sensors", vol. 1, Gliwice, Poland, 2000, p. 165.
[19] D.L. Harame, et al., IEE Trans. Electron Devices ED-34 (1987) 1700.
[20] D.G. Pijanowska, et al., Sens. Actuators B 78 (2001) 263.
[21] K. Won, et al., Process Biochem. 40 (2005) 2149.
[22] R.K. Reddy, et al., Biosens. Bioelectron. 16 (2001) 313.
[23] N.F. Starodub, et al., Electrochem. Soc. Proc. 97-19 (1997) 799.
[24] N.F. Starodub, et al., Anal. Chim. Acta 385 (1999) 461.
[25] V.M. Mirsky, et al., Thin Solid Films 284 (1996) 939.
[26] G. Zografi, et al., Chem. Phys. Lipids 7 (1971) 185.
[27] I. Basu, et al., Sens. Actuators B 107 (2005) 418.
[28] H.X. Rong, et al., Chin. Chem. Lett. 12 (2001) 453.
[29] T. Fonong, Anal. Lett. 20 (1987) 783.
[30] V. Laurinavicius, et al., Anal. Chim. Acta 330 (1996) 159.
[31] A.Q. Contractor, et al., Electrochim. Acta 39 (1994) 1321.
[32] R. Feldbruegge, et al., Sens. Actuators B 19 (1994) 365.
[33] J. Tkac, et al., Anal. Lett. 33 (2000) 2441.
[34] M. Schoemaker, et al., Biosens. Bioelectron. 12 (1997) 1089.
[35] L.-C. Wu, C.-M. Cheng, Anal. Biochem. 346 (2005) 234.
[36] M.D. Gulcev, et al., Anal. Chim. Acta 457 (2002) 47.

[37] C. Sumner, et al., Anal. Chem. 72 (2000) 5225.
[38] C. Summer, et al., Biosens. Bioelectron. 16 (2001) 709.
[39] A. Lookene, et al., Biochemistry 36 (1997) 5267.
[40] A. Lookene, et al., Biochem. Biophys. Res. Commun. 271 (2000) 15.
[41] K. Ge, et al., Frasenius J. Anal. Chem. 354 (1996) 118.
[42] O.N. Bevza, et al., Ukr. Biochem. J. 74 (2002) 10.
[43] I. Satoh, et al., Anal. Chim. Acta 131 (1981) 255.
[44] N.F. Starodub, et al., Anal. Chim. Acta 385 (1999) 461.
[45] Z. Knezevic, et al., Process Biochem. 39 (2004) 1377.
[46] H. Rahimi, et al., J. Mol. Catal. B: Enzym. 28 (2004) 71.
[47] N.W. Fadnavis, K. Koteshwar, Biotechnol. Prog. 15 (1999) 98.
[48] E. Calleri, et al., J. Pharm. Biomed. Anal. 32 (2003) 715.