ISSN 0233-7657. Biopolymers and cell. 2008. vol. 24. N 1. Translated from Ukrainian

Optimization of enzyme bioselective elements as components of potentiometric multibiosensor

O. O. Soldatkin, O. A. Nazarenko, O. S. Pavluchenko[1], O. L. Kukla[1], V. M. Arkhipova, S. V. Dzyadevych, O. P. Soldatkin, A. V. El'skaya

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Zabolotny Str., Kyiv, 03680, Ukraine

[1]V.E. Lashkaryov Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine
41, prospect Nauky, Kiev 03028,Ukraine

alex_sold@yahoo.com

The investigation presents the development of highly sensitive and selective multibiosensor based on different immobilized enzymes as bioselective elements and the matrix of ion-selective field effect transistors as transducers. To develop bioselective elements of multibiosensor, such enzymes as acetylcholinesterase, butyrylcholin esterase, urease, glucose oxidase, and three-enzyme system (invertase, mutarotase, glucose oxidase) were used. The bioselective elements obtained were shown to demonstrate high sensitivity to corresponding substrates in direct enzymatic analysis, which lasted 10 min. Dynamic range of substrate determination (0.1 mM – 1.5–10 mM) was shown to depend on an enzymatic system and differ specifically in the upper detection limit. The dependence of multibiosensor response on pH, ionic strength, and buffer capacity was investigated; optimal conditions for simultaneous operation of all bioselective elements of the multibiosensor were selected; the data on cross-influence of substrates for all the enzymes used were obtained. The developed multi-analyzer was shown to demonstrate sufficient signal reproducibility.

Keywords: multibiosensor, immobilized enzymes, ion-selective field-effect transistors, glucose oxidase, direct substrate analysis, inhibitor analysis

Introduction. Every year ecological monitoring of the environment becomes more and more important around the world [1] due to the significant development of chemical industry, intensive use of chemical preparations in agriculture, and the increase in the application of different chemical products in other fields of human activity. The mentioned compounds, often toxic, add to the pollution of large territories, mixing up with air, ground, water, entering food products of human and animals. The latter results in weakening human health and occurrence of different diseases [2].

It is commonly known that heavy metals and pesticides occupy a special place among toxic environment pollutants. Heavy metals and their complexes are specific for high resistance to the

degradation in environment, solubility in the atmospheric precipitations, capability to ground sorption and accumulation by plants. They can be accumulated in different organisms, be poisonous to humans and differ in wide spectrum and variety of harmful influences [3].

Along with heavy metals, pesticides are another high risk factor for human health [4, 5]. Toxic organophosphorous pesticides, resistant to decomposition, are widely used in agriculture of many countries worldwide; they, as well as their toxic residues [6], are specific for the high degree of penetration and are capable of entering human food products [7].

In regards to the mentioned above, permanent effective control of the toxins presence in the environment and food products is of great need for the nature protection and improvement of life quality [8]. Modern standard methods of reliable determination of toxic compounds, namely gas and liquid chromatography, spectrophotometry, various chemical and physical methods, require high-skilled personnel and complicated and expensive equipment [9, 10]. Another disadvantage of standard methods of analysis is the need for complex pretreatment of samples, which is rather time-consuming.

Alternative solution for the mentioned problems is the application of biosensors, novel bioanalytical devices. Currently, a series of monobiosensors for determination of different toxic compounds has been designed, some of which are developed for direct enzymatic [11] and some for inhibitory analysis [12]. However, they can be used for determination of only one toxic compound or for one class of toxic compounds. Nowadays the concept of multibiosensor for environment monitoring has been proposed and the principal possibility of its elaboration has been demonstrated [6]. There are already several laboratory prototypes of multibiosensor devices with different types of transducers [13, 14]. Yet, to the authors' opinion, the most promising way is the development of multibiosensor based on several immobilized enzymes and the matrix of ion-selective field-effect transistors (ISFET) for inhibitory analysis of toxic compounds, which has not been used up to now.

Therefore, the development of enzyme multibiosensor was initiated for inhibitory determination of different toxic compounds. To develop bioselective elements of a multibiosensor, the most applicable enzymes were selected, namely, acetyl- and butyrylcholinesterases, urease, glucose oxidase, and three-enzyme system with invertase, mutarotase and glucose oxidase. The application of above-mentioned enzymes allows selective determination of toxic compounds like organophosphorous and carbamat pesticides, and heavy metal ions. At the first stage of multibiosensor development, presented in the current paper, the immobilization method optimal for all the above-mentioned enzymes was selected, the conditions of enzyme concurrent operation were optimized, substrates cross-influence was tested, a possibility of the multibiosensor usage for direct analysis of corresponding substrates was verified.

Materials and Methods. The following frozen-dried preparations of enzymes: soybean urease (activity index of 31 U/mg) (Fluka, Switzerland); acetylcholinesterase (AChE) (activity index of 292 units/mg) of electric eel (Sigma-Aldrich Chemie, USA); butyrylcholinesterase (BuChE) (activity index 13 U/mg) of horse blood serum (Sigma-Aldrich Chemie), glucose oxidase (GOD) of Penicilium vitale (activity index 130 U/mg) (Diagnosticum, Ukraine); baker's yeast invertase (activity index of 355 U/mg) (Sigma-Aldrich Chemie), pig kidney mutarotase (activity index of 100 U/mg) (Biozyme Laboratories Ltd, UK). Bovine serum albumin (BSA) (fraction V) and 50% aqueous solution of glutaraldehyde (GA) were purchased from Sigma-Aldrich Chemie. The following substrates were used: urea, butyrylcholine chloride BuChCl, acetylcholine chloride AChCl, glucose, and succhrose. Phosphate solution (KH2PO4-NaOH) was chosen as a working buffer. Other inorganic compounds used were of domestic production and of analytical grade.

*Preparation of bioselective membranes.*To produce working bioselective elements based on AChE, BuChE, urease, and GOD, the solutions of the following composition were prepared: 5% enzyme + 5% BSA, and for three-enzyme system – 3% invertase + 2% mutarotase + 4.5% GOD + 1.5% BSA

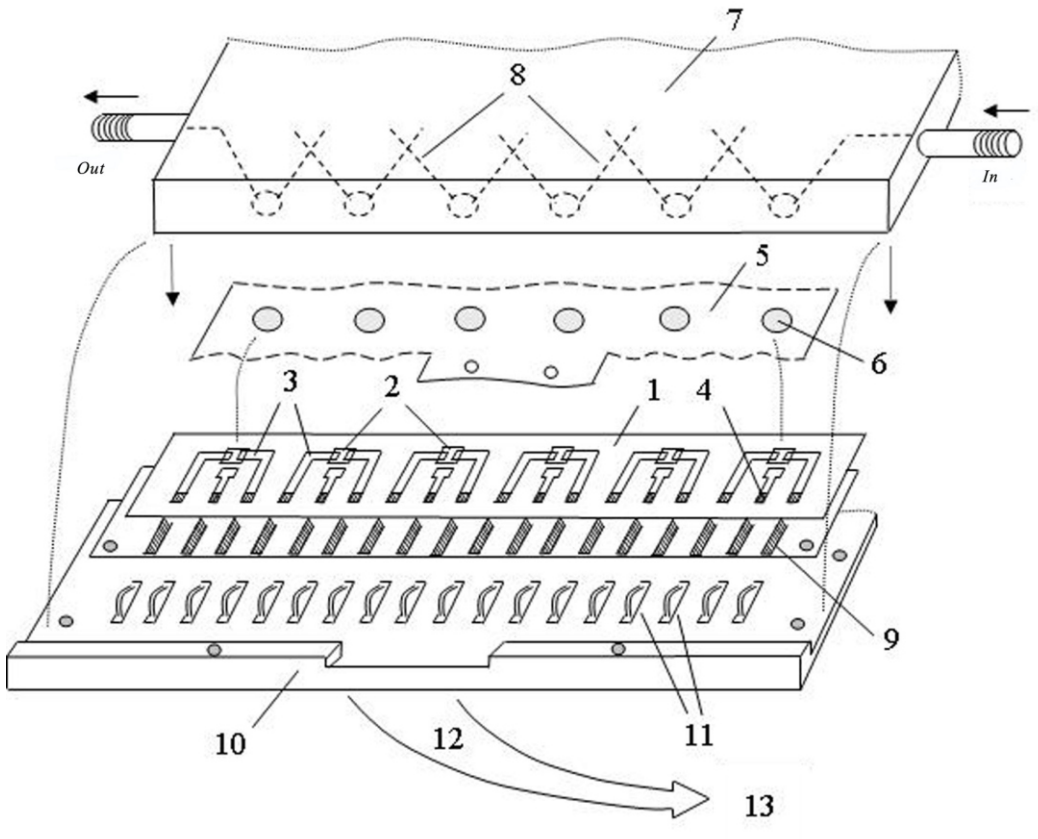

Fig.1. Scheme of components of polychannel multibiosensor: 1 – integral silicon line with 6-element array of ion-selective field-effect transistors; 2 – regions of transistor gates with pH-sensitive silicon nitride surface layer; 3 – diffusive low-resistance strip by which contacts of transistors source and drain are adjusted at the plate edge; 4 – additional contact strip to integrated referent electrode; 5 – rubber seal; 6 – holes in rubber strip for liquid contact with active parts of gates; 7 – clamp cover with liquid flow system; 8 – inlet-outlet system of integrated zigzag flow channels; 9 – intermediate circuit board of contacts to electric outputs of ISFET-elements; 10 – fluoroplastic case; 11 – integrated system of spring metal contacts to corresponding electric outputs of sensor line; 12 – strip for electrical connection; 13 – polychannel unit of transducers.

(hereinafter three-enzyme solution). Reference membrane mixture was made in the same way but instead of enzymes BSA was added to final concentration of 10%. Prior to deposition on the transducer surface, the solutions for reference and working membranes were mixed with 1% aqueous solution of GA (1:1). The obtained solutions were deposited immediately on the transducer working parts using Eppendorf sampler (total volume 0.1–2.5 мl) till complete covering of work surfaces of transistors. The volume of each deposited mixture was ~0.1 мl. All membranes contained the same amount of protein. Next, the membranes were dried for 12 h at room temperature, and prior to use, the membranes were washed off unbound GA.

*ISFET-based multisensor device.*The device with 12-channel integral sensor array on the basis of ion-selective (pH-sensitive) filed-effect transistors (Fig.1) consists of two independent 6-channel sensor lines and includes four basic blocks: 1. flow system block, presented as two independent flow channels with fluid contact for each separate sensor line, which contains inlet and outlet for solution supply; 2. signal measuring block, which consists of two sensor lines, six ISFETs each; 3. 12-channel electronic block for measuring output signal from each ISFET; 4. interface input-output block for PC serial port for processing the signals obtained.

The device operation is based on the formation of multisignal response of the electrochemical sensors

array on the base of ISFETs with pH-sensitive layer of silicon nitride. The
multisensor functions on the bases of measuring changes of surface potential at interface of electrolyte-transducer gate for each sensor element of the array. Simultaneously, subsequent processing of the data obtained from the measuring array is performed using special mathematical methods resulting in formation of the unique chemical pattern of the investigated liquid.

The 12-channel multisensor matrix case (2x6 cm) was made of hard isolating material with low absorbability (fluoroplast), which eliminates the bending and contains integrated zigzag channels system of liquid flow. The hydraulic system sustaining chemical effect of acids and alkali is specific for high thermal capacity and low heat conductivity. The mechanical part provides uniform pressing of flow system to the silicon plate via compaction, which provides continuous solution flow in the presence of sensitive membranes. The outlet of flow system is connected to external referent electrode.

The electronic part of the device consists of two modules. 12-channel transducers module works according to the scheme of maintaining dc voltage of each ISFET channel, while signal response of each sensor is formed in real time as corresponding potential on the transistor gate. Microprogramme control module, manufactured on the basis of 8-discharge microcontroller of C51 series, consists of 12-channel analogue commutator, 12-discharge sequential analogue-digital converter, and RS-232 standard port of data serial input-output.

Measurement procedure. Measurement was basically carried out in 2 mM phosphate buffer, pH 7.2, at room temperature using flow system. To determine optimal pH of multibiosensor operation, the universal buffer was used which consisted of a mixture of different buffer solutions (phosphorous, acetic, boronic acids of 2.5 mM concentration), pH 3.5 - 8.5 [15]. Substrates concentration in the cell was varied by addition of portions of the substrates stock solutions of standard concentration into the working buffer. Mesurements were performed at least three times. Nonspecific changes in output signal caused by temperature and medium pH oscillations, as well as by electric interference, were avoided by using differential mode of measurement.

Results and discussion

Operation of bioselective elements of multibiosensor for direct substrates analysis is based on single-enzyme reactions and cascade of enzymatic reactions:

Urease
$$\text{Urea} + 2H_2O + H^+ \xrightarrow{} 2NH_4^+ + HCO_3^- \quad (1)$$
BuChE
$$\text{Butyrylcholine} + H_2O \xrightarrow{} \text{choline} + CH_3(CH_2)_2COO^- + H^+ \quad (2)$$
AChE
$$\text{Acetylcholine} + H_2O \xrightarrow{} \text{Choline} + CH_3COO^- + H^+ \quad (3)$$
GOD
$$\text{-D-glucose} + O_2 + H_2O \xrightarrow{} \text{D-gluconolactone} + H_2O_2 \quad (4.1)$$
$$\text{D-gluconic acid} + H_2O \leftrightarrows \text{acid residue} + H^+ \quad (4.2)$$
Invertase
$$\text{Succhrose} + H_2O \xrightarrow{} \text{-D-fructose} + \text{- D-glucose} \quad (5.1)$$
Mutarotase
$$\text{-D-glucose} \xrightarrow{} \text{-D-glucose} \quad (5.2)$$
GOD
$$\text{- D-glucose} + O_2 \xrightarrow{} \text{D-gluconolactone} + H_2O_2 \quad (5.3)$$
$$\text{D-gluconic acid} + H_2O \leftrightarrows \text{acid residue} + H^+ \quad (5.4)$$

Reactions (1-3) and cascade of reactions (4, 5) are followed by the changes in proton concentration (and corresponding local pH change in membrane). It permits to use the matrix of ion-selective field-effect transistors as a transducer.

The typical multibiosensor responses to injection of mixture of corresponding substrates (10 mM ACh, 5 mM BuCh, 5 mM urea, 5 mM succhrose, and 2 mM glucose) into analyzed medium are presented in Fig. 2. As seen, the responses differ both in sign and in absolute values. Their different orientation is determined by the basic enzymatic reactions. Thus, reactions (2-5) result in proton accumulation (positive response) while reaction (1) – in proton consuming (negative response). The reason of biosensor responses difference in their absolute values can be different activity of the enzymes as

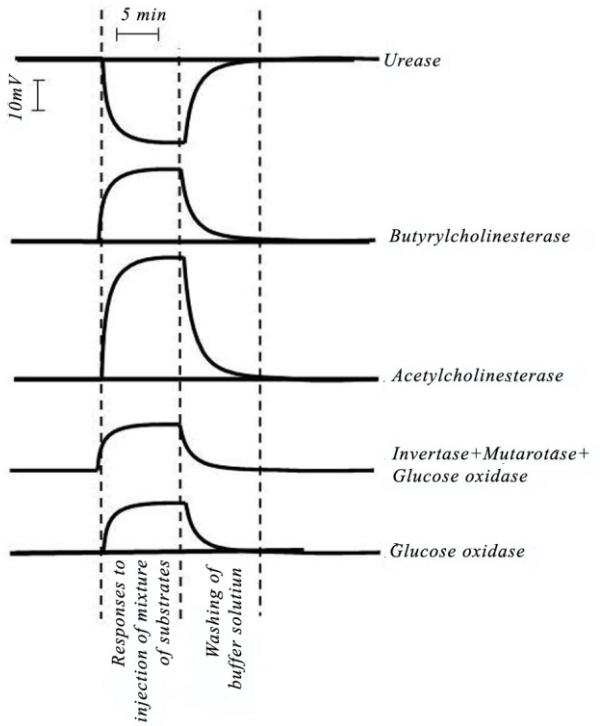

Fig. 2. Typical multibiosensor responses to injection of mixture of corresponding substrates in analyzed medium

well as dependence of some reactions (4, 5) on concentration of oxygen as a co-substrate in these reactions.

Calibration curves characterizing the enzymes immobilized on the transducer surface are shown in Fig. 3. Acetycholinesterase based bioselective element is seen to have the highest sensitivity, butyrylcholinesterase- and urease- based ones - to be less sensitive; glucose oxidase based element and three-enzyme system for succhrose analysis were the least sensitive. Nevertheless, sensitivity of all these membranes was sufficient for further experiments. The dynamic ranges of measurement were also determined from these calibration curves being 0.1 – 1.5-10 mM. The detection limits were almost similar for all selective enzyme elements. In further experiments on inhibitory analysis the substrates will be used at the concentrations corresponding to maximum sensor responses (under conditions of maximum enzyme saturation by the substrates). For example, for glucose oxidase based element 2 mM glucose was used, for urease based one – 5 mM urea.

It is known that operation of ion-selective field-effect transistors is based on measurement of changes in pH of analyzed solution. The local pH change can depend on the enzymatic reactions as such, as well as on characteristics of the solution in which these reactions take place. Therefore, an influence of solution parameters on the multibiosensor response was studied first of all.

One of the important buffer characteristics which can modulate the value of ISFET response is ion strength [16]. To study this effect, the responses of multibiosensor bioelements to injection of substrates (10 mM ACh, 5 mM BuCh, 5 mM urea, 5 mM succhrose, and 2 mM glucose) were measured depending on KCl concentration which varied from 1 mM to 40 mM (Fig. 4). The plotted curves show that at increase in KCl concentration, and correspondingly – in ion strength, the responses to the substrates injection dropped exponentially in almost similar way that enables concurrent work of the selected enzymes with the same dependence on the ion strength of tested sample. It is seen an intense decrease in response value at the beginning, and at KCl concentration of 10 mM the signals dropped by 5 – 40% depending on the enzyme while at further increase in KCl concentration they remained stable. Some explanations can be suggested for this phenomenon. On the one hand, increasing ion strength of tested solution can change the membrane density owing to the screening of membrane charges and corresponding changes in membrane permeability and activity of immobilized enzymes. On the other hand, rise in ion strength can result in some change in the rate of proton association-dissociation on the ion-selective membrane of field-effect transistor, which also can cause modulation of multibiosensor responses. Therefore, control over ion strength of analyzed samples is of importance in measurement by the ISFET matrix.

Change in concentration of working buffer causes the variation in both solution ion strength (which is important by itself) and buffer capacity of the sample analyzed which is also substantial for potentiometric measurement [17]. Dependence of multibiosensor responses on concentration of the

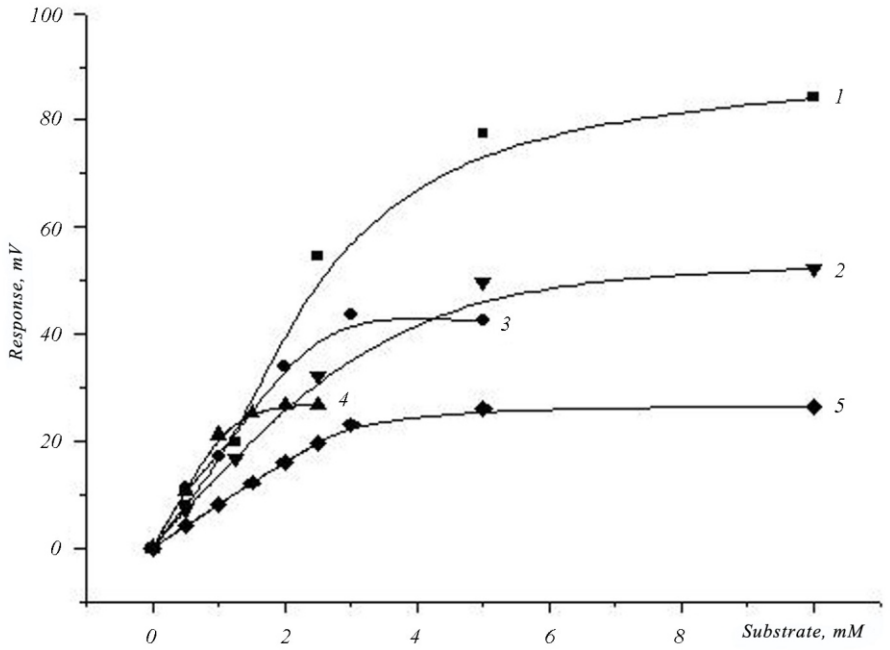

Fig. 3. Dependence of response of multibiosensor with enzymes immobilized on sensitive surfaces of transducers line (AChE (1), urease (2), BuChE (3), GOD (4) and three-enzyme system (5)) on concentration of corresponding substrates.

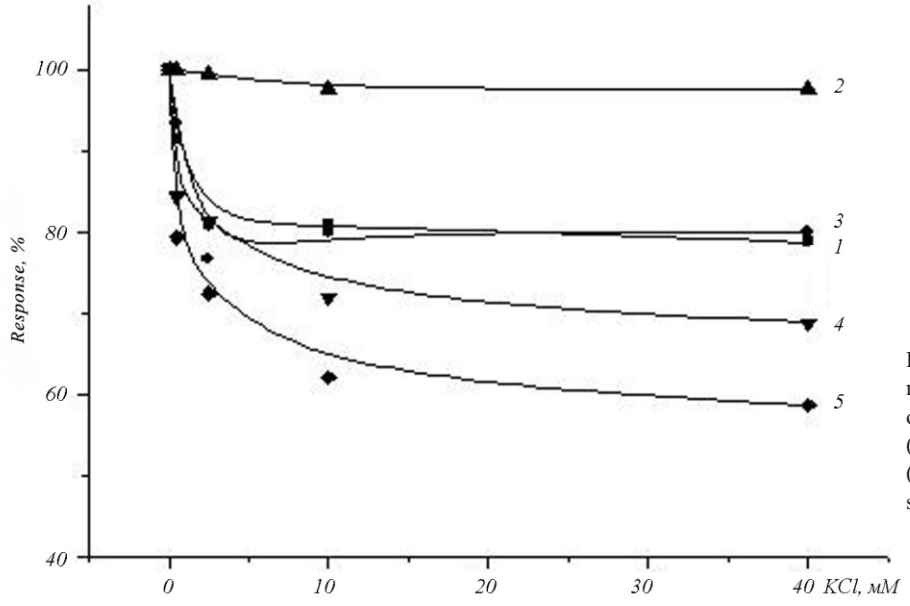

Fig. 4. Dependence of response of multibiosensor with enzymes immobilized on sensitive surfaces of transducers line (AChE (1), urease (2), BuChE (3), GOD (4) and three-enzyme system (5)) on ion strength of phosphate buffer.

phosphate buffer (different buffer capacity) is shown in Fig. 5. As seen, the strongest dependence was observed for the three-enzyme system, the weakest – for the glucose oxidase, but the tendency of changes in sensitivity of the bioselective elements in general was the same. The maximum sensitivity of sensor elements toward the presence of substrates was revealed in 1 mM phosphate buffer, minimum – in 10 mM one.

It is well known that each enzyme has particular optimal pH. Immobilization of enzymes can shift their optimum pH in either alkaline or acid region. In our case, there are several enzymes with different optimum pH immobilized on the transducer surfaces. Therefore, at the next stage optimum pH was found for each of these immobilized enzymes, and subsequent analysis of the obtained data permitted to determine close to optimal pH value at concurrent

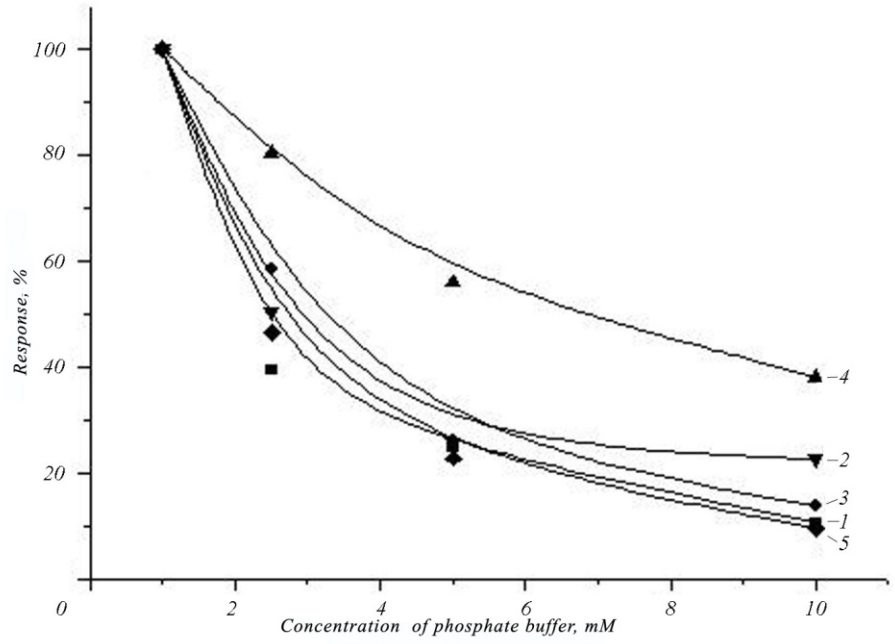

Fig. 5. Dependence of response of multibiosensor with enzymes immobilized on sensitive surfaces of transducers line: (AChE (1), urease (2), BuChE (3), GOD (4) and three-enzyme system (5)) on concentration of phosphate buffer solution.

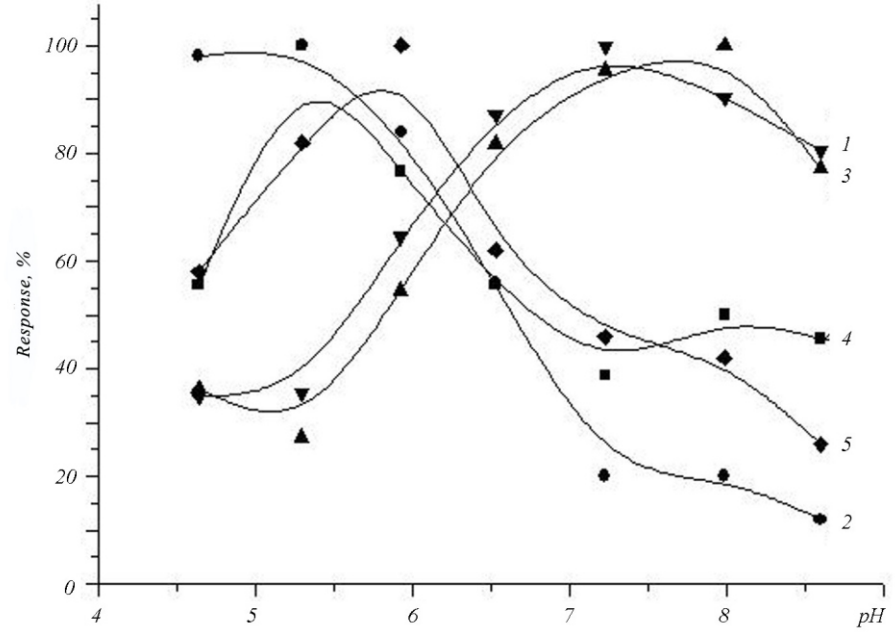

Fig. 6. Dependence of response of multibiosensor with enzymes immobilized on sensitive surfaces of transducers line: (AChE (1), urease (2), BuChE (3), GOD (4) and three-enzyme system (5)) on pH of 2 mM universal buffer solution.

operation of all the enzymes in the multibiosensor structure. As known, a single-component buffer changes its capacity if pH varies [18]. That is why, the dependence of multibiosensor response on pH of universal multicomponent buffer with the same buffer capacity in a wide range of pH values was investigated to avoid additional effects of buffer capacity on the multisensor responses [18]. pH-dependences of sensor signals toward substrates injection were bell-shaped with different positions of maximum (Fig. 6). For example, buffer pH = 5-6 was optimal for the sensor elements based on urease, GOD and three-enzyme system, while for those based on cholinesterases optimum pH = 7-8.

As the functioning of each bioselective element differs in optimum pH, in case of concurrent operation of all elements two considerations are to be taken into account to select the multibiosensor

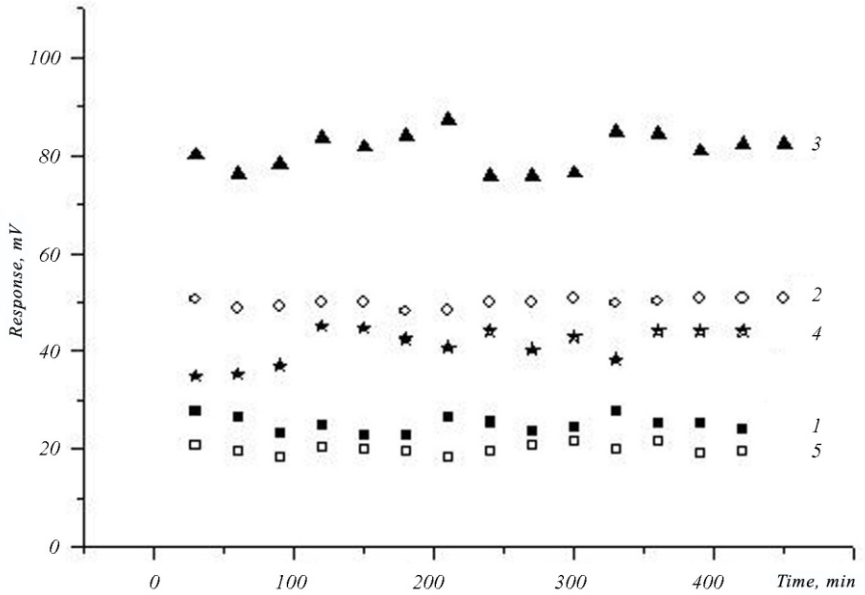

Fig. 7. Reproducibility of responses of multibiosensor with enzymes immobilized on sensitive surfaces of transducers line (GOD (1), urease (2), AChE (3), BuChE (4) and three-enzyme system (5)) during one working day. Measurement in 2 mM phosphate buffer, pH 6.

optimal pH. First, optimal (acceptable) pH value at simultaneous work of all enzymes, second, relative activity of each enzyme. The higher is response of a sensor element, the more likely pH shifts toward non-optimal values. Since the cholinesterase based elements demonstrated higher responses compared with the elements based on urease, GOD and three-enzyme system (invertase, mutarotase and GOD), there is a necessity to shift pH value of working buffer into weak-acid region. Thus, in the further investigation of multibiosensor characteristics pH = 6 was taken as an optimal value for 2 mM phosphate working buffer.

Another important biosensor characteristic is operational stability and response reproducibility. To study this aspect the experiment was performed as follows: the responses to the same mixture of substrates (10 mM ACh, 5mM BuCh, 5 mM urea, 5 mM succhrose, and 2 mM glucose) were measured in a course of one working day with 45 min intervals, the ISFET matrix with immobilized enzymes being during intervals kept in working buffer solution at room temperature. The selected analyte concentrations corresponded to the saturation regions on the calibration curves (see Fig. 3). As seen from Fig. 7, the multibiosensor responses under these quasi-continuous conditions were highly reproducible.

Since the matrix with bioselective elements works in the same medium under similar conditions, the substrate cross-effect on each separate bioselective element should be controlled. The data on responses of the matrix consisting of five bioselective elements to each substrate in particular and to their mixture are presented in Table 1. As seen, only the urease- and GOD-based elements were highly selective to their substrates, urea and glucose. The bioselective elements based on AChE and BuChE had weak cross-sensitivity to substrates acetylcholine and butyrylcholine and no sensitivity at all to the substrates of other enzymes. The bioselective element based on three-enzyme system (invertase, mutarotase and GOD) for succhrose determination was not sensetive to all cross-substrates except for glucose. The response to the latter was significant which is reasonable as GOD is a part of this bioselective element. The responses of all biosensor elements of the multibiosensor toward injection of the substrates mixture were the same as to injection of each substrate in particular.

All the results presented in Table 1 are important for the evaluation of direct substrates determination. They will be also essential for further practice of the inhibitory analysis of toxins with a multibiosensor though in this case substrate

Effect of particular substrates and their mixture on responses of bioselective elements of the multibiosensor

Type of bioselective element	Substrates, %					
	10 mM ACh	5 mM BuCh	5 mM urea	2 mM glucose	5 mM succhrose	Substrates mixture
AChE	100	15	0	0	0	100
BuChE	20	100	0	0	0	100
Urease	4	3	100	0	0	100
GOD	4	3	0	100	0	100
Three-enzyme system	4	3	0	75	100	100
BSA	4	3	0	0	0	8

cross-effect is not as critical as at direct determination. For the inhibitory analysis it is important to work with saturating concentrations of substrates that ensures stability and reproducibility of the responses of multisensor elements, and consequently dependence on the inhibitor concentration will be strongly pronounced.

Conclusion. The multibiosensor based on the matrix of ion-selective field-effect transistors with immobilized corresponding enzymes was first developed for the determination of acetylcholine, butyrylcholine, glucose, succhrose, and urea. The optimal conditions of its operation were studied, as well as the main analytical characteristics for the direct substrate measurement. The multibiosensor developed is promising for the inhibitory enzyme analysis of toxins in aqueous samples after appropriate adaptation. The parameters determined as optimal in epy direct substrate analysis can be also taken into consideration for epy inhibitory analysis of toxic agents

The investigation was carried out at financial support of National Academy of Sciences of Ukraine in the frame of Scientific and Technical Programme “Sensor systems for medical-ecological and industrial-technological problems”.

О. О. Солдаткін, О. А. Назаренко, О. С. Павлюченко, О. Л. Кукла, В. М. Архипова, С. В. Дзядевич, О. П. Солдаткін, Г. В. Єльська

Оптимізація роботи ферментних біоселективних елементів як складових потенціометричного мультибіосенсора

Резюме

Розроблено високочутливий та селективний мультибіосенсор на основі низки іммобілізованих ферментів як біоселективних елементів та матриці іоноселективних польових транзисторів – перетворювачів біохімічного сигналу в електричний. Для створення біоселективних елементів мультибіосенсора використано ферменти ацетилхолінестеразу, бутирилхолінестеразу, уреазу, глюкозооксидазу та триферментну систему (інвертаза, мутаротаза, глюкозооксидаза). Отримані біоселективні елементи в прямому ферментному аналізі демонструють високу чутливість до відповідних субстратів. Час проведення аналізу складає 10 хв. Динамічний діапазон визначення субстратів значною мірою залежить від застосованих ферментних систем і знаходиться в межах від 0,1 мМ до 1,5–10 мМ. Досліджено також залежності відгуків мультибіосенсора від pH, іонної сили та буферної ємності розчину. Підібрано оптимальні умови для одночасної роботи всіх біоселективних елементів мультибіосенсора, наведено дані з перехресного впливу субстратів усіх використаних ферментів. Розробленому мультианалізатору притаманна задовільна відтворюваність сигналів.

Ключові слова: мультибіосенсор, іммобілізовані ферменти, іоноселективні польові транзистори, глюкозооксидаза, прямий аналіз субстратів, інгібіторний аналіз.

А. А. Солдаткин, Е. А. Назаренко, А. С. Павлюченко, А. Л. Кукла, В. Н. Архипова, С. В. Дзядевич, А. П. Солдаткин, А. В. Ельская

Оптимизация работы ферментных биоселективных элементов в составе потенциометрического мультибиосенсора

Резюме

Разработаны высокочувствительный и селективный мультибиосенсор на основе ряда иммобилизованных ферментов как биоселективных элементов и матрицы ионоселективных полевых транзисторов в качестве преобразователей биохимического сигнала в электрический. Для создания биоселективных элементов мультибиосенсора использовали ферменты ацетилхолинэстеразу, бутирилхолинэстеразу, уреазу, глюкозооксидазу и трехферментную систему (инвертаза, мутаротаза, глюкозооксидаза). Полученные биоселективные элементы в прямом ферментном анализе демонстрировали высокую чувствительность к выявленным субстратам. Время проведения анализа составляло 10 мин. Динамический диапазон определения субстратов зависел от ферментной системы, особенно

отличался верхней границей определения и находился в пределах от 0,1 мМ до 1,5–10 мМ. Проверена зависимость отклика мультибиосенсора от pH, ионной силы и буферной емкости раствора, подобраны оптимальные условия для одновременной роботы всех биоселективных элементов мультибиосенсора, представлены данные по перекрестному влиянию субстратов всех использованных ферментов. Разработанный мультианализатор также характеризуется хорошей воспроизводимостью сигнала

Ключевые слова: мультибиосенсор, иммобилизованные ферменты, ион селективные полевые транзисторы, глюкозооксидаза, прямой анализ субстратов, ингибиторный анализ.

REFERENCES

1. *Yatsenko V*. Determining the characteristics of water pollutants by neural sensors and pattern recognition methods // J. Chromatogr. A,–1996.–**722**.–P. 233–243.
2. *Castillo J., Gaspar S., Leth S., Niculescu M., Mortari A., Bontiden I., Soukharev V., Dorneanu S. A., Ryabov A. D., Csoregi E*. Biosensors for life quality design, development and applications // Sensors and Actuators.–2004.–**102**.–P. 179–194.
3. *Горобец П. Ю., Ильченко И. Н., Ляпунов С. М., Шугаева Е. Н*.Распространенность экологически зависимых нарушений нервно-психического развития у детей в возрасте 4–7 лет при хроническом воздействии тяжелых металлов в малых дозах // Профилактика заболеваний и укрепление здоровья.–2005.–№ 1.–P. 14—20.
4. *Schuman S. H., Simpson W. M*. A clinical historical overview of pesticide health issues // Occup. Med.–1997.–**12**.–P. 203–207.
5. *Johnson B. L*. A review of health-based comparative risk assessments in the United States // Rev. Environ. Health–2000.–**15**.–P. 273–287.
6. *Arkhipova V. N., Dzyadevych S. V., Soldatkin A. P., El'skaya A. V., Jaffrezic-Renault N., Jaffresic H., Martlet C*. Multibiosensor based on enzyme inhibition analysis for determination of different toxic substances // Talanta–2001.–**55**.–P. 919–927.
7. *Verscheuren K*. Handbook of environmental data on organic chemicals.–New York: Van Norstrand Reinhold, 1983.–673 p.
8. *Dzantiev B. B., Yazynina E. V., Zherdev A. V., Plekhanova Yu. V., Reshetilov A. N., Chang S. C., McNeil C. J*. Determination of the herbicide chlorsulfuron by amperometric sensor based on separation-free bienzyme immunoassay // Sensors and Actuators–2004.–**98**.–P. 254–261.
9. *Sherma J., Zweig G*. Pesticides // Anal. Chem.–1983.–**55**.–P. 57.
10. *Tran-Minh C., Pandey P. C., Kumaran S*. Studies on acetylcholine sensor and its analytical application based on the inhibition of cholinesterase // Biosensors & Bioelectronics.–1990.–**5**.–P. 461–471.
11. *Солдаткін О. О., Сосовська О. Ф., Бенілова І. В, Гончар М. В., Корпан Я.І.* Ензимний кондуктометричний сенсор для визначення формальдегіду у модельних розчинах // Біополімери і клітина.–2005.–**21**, № 5.–С. 425–432.
12. *Zhylyak G. A., Dzyadevich S. V., Korpan Y. I., Soldatkin A. P., El'skaya A. V*. Application of urease conductometric biosensor for heavy-metal ion determination // Sensors and Actuators.–1995.–**24–25**.–P. 145–148.
13. *Kukla A.L., Kanjuk N.I., Starodub N. F., Shirshov Yu. M*. Multienzyme electrochemical sensor array for determination of heavy metal ions // Sensors and Actuators.–1999.–**57**.–P. 213–218.
14. *Moreno L., Merlos A., Abramova N., Jimтenez C., Bratov A*. Multi-sensor array used as an «electronic tongue» for mineral water analysis // Sensors and Actuators B.–2006.–**116**.–P. 130–134.
15. *Лурье Ю. Ю*. Справочник по аналитической химии.–М.: Химия, 1967.–Т. 3.–230 с.
16. *Soldatkin A. P., El'skaya A. V., Shul'ga A. A., Netchiporouk L. I., Nyamsi Hendji A. M*., *Jaffrezic-Renault N., Martelet C*. Glucose-sensitive field-effect transistor with additional Nafion membrane // Anal. Chim. Acta–1993.–**283**.–P. 695–701.
17. *Dzyadevich S. V., Arhipova V. A., Soldatkin A. P., El'skaya A. V., Shul'ga A. A*. Glucose conductometric biosensor with Potassium hexacyanoferrate(III) as an oxidizing agent // Anal. Chim. Acta–1998.–**374**.–P. 11–18.
18. *Архипова В. Н., Дзядевич С. В., Солдаткин А. П., Ельская А. В*. Ферментные биосенсоры для определения пенициллина на основе кондуктометрических планарных электродов и pH-чувствительных полевых транзисторов // Укр. биохим. журн.–1996.–**68**.–С. 26–31.

UDC 577.15.543.555
Received 21.05.07