MOLECULAR AND CELL BIOTECHNOLOGIES

Application of enzyme multibiosensor for toxicity analysis of real water samples of different origin.

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Aim. The analysis of toxicity of different water samples with the multibiosensor developed earlier. **Methods**. *The potentiometric multibiosensor with several immobilized enzymes as bioselective elements and the matrix of pH-sensitive field effect transistors as transducers of the biochemical signal into the electric one was applied for the analysis.* **Results**. *The bioselective elements of the multibiosensor were developed using acetylcholinesterase, butyryl- cholinesterase, urease, glucose oxidase, and three-enzyme system (invertase, mutarotase, glucose oxidase). The measurement of toxic compounds in water samples of different origin was performed using the constructed sensor. The results obtained were compared with those obtained by the conventional methods of toxic agent's analysis (atomic absorption spectrometry, thin-film chroma-tography, and atomic absorbic analyser of mercury).* **Conclusion**. *A strong conformity between the results obtained with the multibiosensor and traditional methods has been shown.*

Keywords: multibiosensor, pH-sensitive field-effect transistors, enzymes, inhibitory analysis, pesticide, ions of heavy metal, toxins.

Introduction. A remarkable interest paid to biosensors during two last decades is determined by their advantages comparing with traditional physical-chemical and biochemical methods of analysis: relatively low cost and simple application along with high sensitivity, specificity and possibility to analyze colored samples [1, 2]. Biosensors can be used in the fields of medical diagnostics, environmental protection and agriculture [3]. Today an
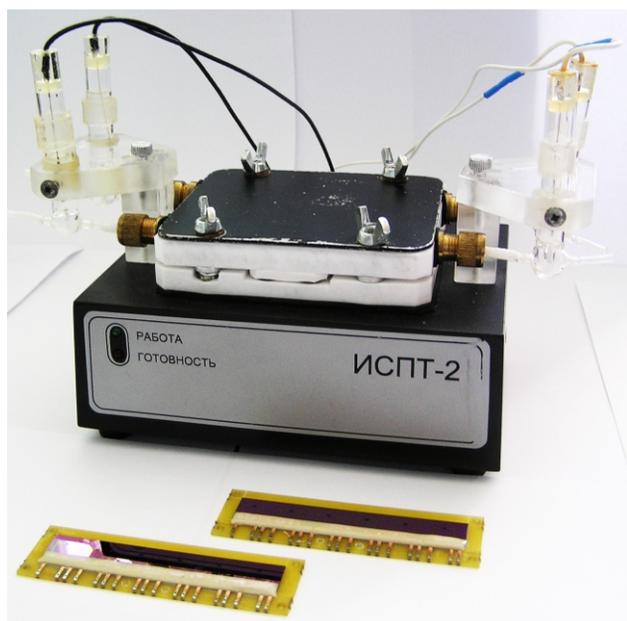
Fig. 1. General view of multisensor device

advanced challenge is the development of biosensor systems for toxins determination.

At present, there are a number of commercial enzyme biosensor devices for analysis of glucose, ethanol, urea, etc. [4], however, none is known to be applied for the determination of heavy metal ions and pesticides. The authors have developed a laboratory prototype of multibiosensor for express identification of toxins in aqueous solutions [5, 6]. The characteristics obtained are promising in respect to usage of this model as a basis for designing a commercial version of measuring device for both integral and, to some extent, selective determination of toxic substances.

Comparing to known standard methods, the multibiosensor system suggested is essentially advantageous [7, 8] because of:

a possibility of measurements of toxins in water samples without their pretreatment;

short duration of analysis, the procedure does not exceed 40 minutes;

small quantity of reagent consumption and low price of working elements at industrial production;

a simple measuring procedure and, thus, no particular requirements toward personnel qualification.

The goal of the investigation presented was to compare the results of determination of toxin

concentration in real water samples from different sources, obtained with the developed multibiosensor and traditional analytical methods.

Materials and Methods. The following frozen-dried preparations of enzymes were used: soybean urease (activity index of 31 U/mg) (Fluka, Switzerland); acetylcholinesterase (AChE) (activity index of 292 units/mg) from Electric Eel (Sigma-Aldrich Chemie, USA); butyrylcholinesterase (BuChE) (activity index 13 U/mg) from horse blood serum (Sigma-Aldrich Chemie), glucose oxidase (GOD) from *Penicilium vitale* (activity index 130 U/mg) (Diagnosticum, Lviv, 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 (KH_2PO_4-NaOH) was chosen as a working buffer. Other inorganic compounds used were of domestic production and of analytical grade.

Multisensor device. General view of the multisensor device with 12-channel integral sensor array on the basis of ion-selective (pH-sensitive) filed-effect transistors (ISFET) is shown in Fig.1.

The device functions due to the formation of multisignal response of the array of electrochemical sensors based on ISFETs with pH-sensitive layer of silicon nitride. The change in surface potential at electrolyte-transducer gate interface is measured by each sensor element of the array. The data obtained are processed using methods of discriminate analysis, which results in generation of the unique chemical pattern of the liquid sample tested.

Measurement procedure. The measurement was carried out at room temperature in 2 mM phosphate buffer, pH 6.5, using flow system. The substrates concentration in a cell was varied by addition of portions of the stock solutions of standard concentration into the working buffer. The bioselective elements were incubated for 20 min in solutions of water samples of different origin. The results of
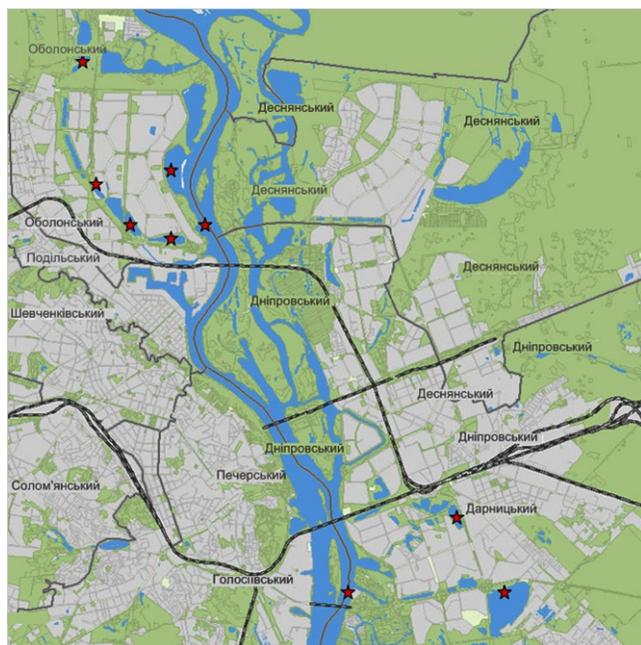
Fig. 2. Fragment of Kiev map (the places where the samples were taken are indicated with asterisks)

samples toxicity obtained by the multibiosensor were compared with those got by traditional analytical methods, i.e. atomic absorption spectrometry, thin-layer chromatography and absorption mercury analyzer. Each measurement was performed at least three times. Nonspecific changes in the output signal caused by temperature and medium pH oscillations, as well as by electric noise, were eliminated due to differential mode of measurement.

Results and discussion. To examine operational capability of the multibiosensor developed, natural water samples were tested as to the presence of toxins. The water samples were taken from several Kiev water pools, especially in the most popular places of people recreation, i.e. municipal beaches of Obolon and Darnitsa districts (see a fragment of Kiev map – Fig. 2). They are: the lakes Vyrlytsa, Sonyachne, Ministerske, Opechen, Lower Opechen, as well as the Dnipro River in the vicinity of Moscow and Pivdenny (South) bridges and Obolon bay.

A potential of the multibiosensor for analysis of polycomponent complex samples was evaluated, for which along with above mentioned samples, the

aqueuos assays from the landfill of solid domestic waste in Pidgirtsi village (Kyiv region) were analyzed.

Analyzing the selected samples with the multibiosensor we have revealed that none of the bioselective elements are inhibited by the water samples from the Kiev pools (Table 1).

To prove an adequacy of multibiosensor operating, some toxins of known concentrations were added to the samples tested. As seen from Table 1, a distinct signal of corresponding bioselective elements was revealed due to the inhibition by certain toxins. It testifies to the absence of pesticides and heavy metal ions in the water pools in Kiev region.

A more complicated situation arises at inhibitory analysis of the sample taken from Kiev landfill No. 5 of solid domestic waste. After the multibiosensor incubation in the assays tested, the sensitive elements completely lost their ability to respond to the substrate addition. The reason for this could be either 100% inhibition of all enzymes used or/and the blocking of pores of bioselective membranes with colloidal particles present in the sample, which decreases their permeability.

To avoid mechanical barrier, the assay was filtered and centrifuged for removal of large-scale particles. The best result was obtained by means of filtrating through «Sartorius» filter with 20 µm pores (Table 2). Thus, assay pretreatment is necessary to remove large-scale particles decreasing permeability of the bioselective membranes. After their eliminating the residual inhibitory activity of such sample may be considered definitely as a result of the toxins presence. According to the results of inhibition of the bioselective membranes based on urease, GOD and three-enzyme system, these toxins are heavy metal ions [6].

Control analysis of toxins content in water samples by traditional methods. All samples tested with the multibiosensor were controlled in L.I. Medved' Institute of ecohygiene and toxicology (Kiev, Ukraine) using traditional analytical methods (Table 3).

Pesticides were measured in the samples, taken from Kiev No. 5 landfill of domestic waste and from Kiev water pools, by thin-layer chromatography in

Table 1
Bioselective elements inhibition with water samples originating from different areas (100% - complete inhibition)

Screened area	Level of inhibition (%) of bioselective elements				
	Urease	BuChE	AChE	GOD	Three-enzyme system
Vyrlytsa lake (Poznyaky)	0	0	0	0	0
Vyrlytsa lake (Poznyaky) (**400 nM Hg^{2+} added**)	0	0	0	5	10
Dnieper river (Osokorky)	0	0	0	0	0
Dnieper river (Osokorky) (**10 µM trichlorfon added**)	0	50	5	0	0
Sonyachne lake (Osokorky)	0	0	0	0	0
Sonyachne lake (Osokorky) (**5 µM Cu^{2+} added**)	7	0	0	0	0
Ministerske lake (Obolon)	0	0	0	0	0
Opechen lake (Obolon)	0	0	0	0	0
Lower Opechen lake (Obolon)	0	0	0	0	0
Verbne lake (Obolon)	0	0	0	0	0
Dnipro River, Obolon bay (Obolon)	0	0	0	0	0
Dnipro River , vicinity of Moscow bridge (Obolon)	0	0	0	0	0
Kiev landfill No. 5 of domestic waste	100	100	100	100	100

accordance with the manual operation [9]; no pesticides were revealed

Mercury was determined on the atomic absorption mercury analyzer "Julia-2". To measure other heavy metal ions, the samples were placed one by one into a crucible and burnt in a muffle furnace. The residues were dissolved in nitric acid, and heavy metals were determined in the obtained solution by atomic absorption spectroscopy on the apparatus Z-8000 (Hitachi, Japan).

Analysis of the data presented in Table 3 shows their good correlation with the results obtained with the multibiosensor. As could be expected an excess of concentration of toxins over maximum allowable content was revealed in the samples from Kiev water pools to which corresponding toxin aliquots were deliberately added, i.e. for mercury (line 2), copper (line 6) and trichlorfon (line 4). No toxins in

hazardous concentration were revealed by traditional methods (as well as with the multibiosensor) in the rest of the samples. As to the samples from Kiev landfill No. 5 of domestic waste, by traditional methods an excess of the maximum allowable content was registered for copper, zinc and chromium, while mercury and pesticides were not revealed at all (line 13) which is in good agreement with the data obtained with the biosensor.

Thus, the results of analysis of all water samples obtained by traditional methods confirm the data of multibiosensor measurement.

Conclusion. A number of experiments concerning determination of toxicity of assays taken from Kiev water pools and from Kiev landfill No. 5 of domestic waste were performed. The content of toxic substances in the assays was analyzed by traditional methods and with the developed multibiosensor; the

Table 2
Inhibition of bioselective elements of multobiosensor with samples taken from Kiev landfill No. 5 of solid domestic waste after different procedures of sample pretreatment (100% - complete inhibition)

Procedure of sample pretreatment	Level of inhibition (%) of bioselective elements				
	Urease	BuChE	AChE	GOD	Three-enzyme system
Without pretreatment	100	100	100	100	100
Centrifugation	75	45	50	70	75
Filtration through filter paper	60	20	30	55	60
Filtration through standard filter "Sartorius", d=45 µm	45	5	8	30	40
Filtration through standard filter "Sartorius", d=20 µm	40	3	8	20	30

Table 3
Toxins concentration in water samples measured by traditional methods

Screened area	Mercury, mg/l	Copper, mg/l	Cadmium, mg/l	Cobalt, mg/l	Zinc, mg/l	Chromium, mg/l	Pesticides, µM
Vyrlytsa lake (Poznyaky)	undetected	undetected	0.007	undetected	0.044	undetected	undetected
Vyrlytsa lake (Poznyaky) (400 nM Hg+2added)	0.079	undetected	0.007	undetected	0.044	undetected	undetected
Dnipro River (Osokorky)	undetected	undetected	0.001	undetected	0.035	0.014	undetected
Dnipro River (Osokorky) (10 µM trichlorfon added)	undetected	undetected	0.001	undetected	0.035	0.014	10
Sonyachne lake (Osokorky)	undetected	undetected	0.007	undetected	0.011	0.016	undetected
Sonyachne lake (Osokorky) (5 µM Cu2+ added)	undetected	0.321	0.007	undetected	0.011	0.016	undetected
Ministerske lake (Obolon)	undetected	0.005	0.006	undetected	0.021	0.043	undetected
Opechen lake (Obolon)	undetected	undetected	0.003	undetected	undetected	0.016	undetected
Lower Opechen lake (Obolon)	undetected	undetected	0.010	undetected	0.019	0.008	undetected
Verbne lake (Obolon)	undetected	undetected	0.004	undetected	0.041	undetected	undetected
Dnieper river, Obolon bay (Obolon)	undetected	undetected	0.008	undetected	0.040	0.058	undetected
Dnieper river, vicinity of Moscow bridge (Obolon)	undetected	undetected	0.021	undetected	undetected	0.011	undetected
Kiev landfill No. 5 of domestic waste	undetected	0.317	undetected	0.034	1.471	0.988	undetected

data obtained were compared; a good correlation of the results was shown. Therefore, the developed multibiosensor system was experimentally proved to be potentially applicable as an express analyzer of toxicity of water assays. It is valid for fast determination of sample toxicity under field conditions, identification of toxins or toxin groups, and proposition of using the optimal traditional method for further, precise determination of the toxins revealed.

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Використання ферментного мультибіосенсора при аналізі токсичності реальних водних зразків різного походження

Резюме

Мета. З використанням розробленого мультибіосенсора зробити аналіз токсичності реальних водних зразків. **Методи.** Застосовано потенціометричний мультибіосенсор з низкою іммобілізованих ферментів та матриці іоноселективних польових транзисторів як перетворювачів біохімічного сигналу в електричний. **Результати.** Біоселективні елементи у складі мультибіосенсора створено на основі ацетилхолінестерази, бутирилхолінестерази, уреази, глюкозооксидази та триферментної системи (інвертаза, мутаротаза, глюкозооксидаза). За допомогою розробленого аналізатора виконано експерименти з визначення токсичних речовин у водних зразках різного походження. Отримані дані порівняно з результатами, одержаними стандартними традиційними методами аналізу токсичних речовин (атомна абсорбційна спектроскопія, тонкошарова хроматографія та атомно-абсорбційний аналізатор ртуті). **Висновки.** Показано кореляцію результатів, одержаних мультибіосенсорним і традиційними методами.

Ключові слова: Мультибіосенсор, іоноселективні польові транзистори, ферменти, інгібіторний аналіз, пестициди, іони важких металів, токсичні речовини.

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Использование ферментного мультибиосенсора при анализе токсичности реальных водных образцов разного происхождения

Резюме

Цель. С использованием разработанного мультибиосенсора провести анализ токсичности реальных водных образцов. **Методы**. Применен потенциометрический мультибиосенсор с рядом иммобилизованных ферментов и матрицы ионоселективных полевых транзисторов как преобразователей биохимического сигнала в электрический. **Результаты**. Биоселективные элементы в составе мультибиосенсора созданы на основе ацетилхолинэстеразы, бутирилхолинэстеразы, уреазы, глюкозооксидазы и трехферментной системы (инвертаза, мутаротаза, глюкозооксидаза). С помощью разработанного анализатора выполнен ряд экспериментов по определению токсичных веществ в водных образцах разного происхождения. Собственные данные сравнивали с результатами, полученными стандартными традиционными методами анализа токсичных веществ (атомная абсорбционная спектроскопия, тонкослойная хроматография и атомно-абсорбционный анализатор ртути). **Выводы**. Показана корреляция результатов, полученных мультибиосенсорным и традиционными методами.

Ключевые слова: мультибиосенсор, ионоселективные полевые транзисторы, ферменты, ингибиторный анализ, пестициды, ионы тяжелых металлов, токсические вещества.

REFERENCES

1. *Coulet P. R.* What is a biosensor // Biosensor principles and application / Eds L. J. Blum, P. R. Coulet.–New York: Marcel Dekker, 1991.–P. 1–6.
2. *Thevenot D. R., Toth K., Durst R. A., Wilson G. S.* Electrochemical biosensors: recommended definitions and classification (Technical report) // Pure Appl. Chem.–1999.–**71**.–P. 2333–2348.
3. *Hall E. A. H.* Recent progress in biosensor development // Int. J. Biochem.–1988.–**20**, № 4.–P. 357–362.
4. *Scheller F. W., Pfeiffer D.* Commercial Devices based on amperometric biosensors // Handbook of biosensors and electronic noses: medicine, food, and environment / Ed. E. Kress-Rogers.–New York: CRC press, 1997.–P. 245–256.
5. *Soldatkin O. O., Pavluchenko O. S., Kukla O. L., Arkhipova V. M., Dzyadevych S. V., Soldatkin O. P., El'skaya A. V.* Optimization of enzymatic bioselective elements as components of potentiometric multibiosensor // Biopolymers and Cell.–2008.–**24**, N 1.–P. 42–50.
6. *Soldatkin O. O., Pavluchenko O. S., Kukla O. L., Arkhipova V. M., Dzyadevych S. V., Soldatkin O. P., El'skaya A. V.* Optimization of multibiosensor operation for inhibitory analysis of toxins // Biopolymers and Cell.–2008.–**24**, N 6.–P. 494–502.
7. *Sherma J., Zweig G.* Pesticides // Anal. Chem.–1983.–**55**.–P. 57.
8. *Tran-Minh C., Pandey P. C., Kumaran S.* Studies on acetylcholine sensor and its analytical application based on the inhibition of cholinesterase // Biosensors and Bioelectronics.–1990.–**5**.–P. 461–471.
9. *Metodicheskie* ukazaniya po opredeleniyu pestizidov v vode, produktach, kormach i tabachnych izdeliyach metodom chromotografii v tonkom sloe № 2142-80 ot 28.01.1980 g.