

Jolanta KOCHANA^{1*}, Juliusz ADAMSKI¹ and Andrzej PARCZEWSKI¹

A CRITICAL VIEW ON THE PHENOL INDEX AS A MEASURE OF PHENOL COMPOUNDS CONTENT IN WATERS. APPLICATION OF A BIOSENSOR

KRYTYCZNA OCENA PRZYDATNOŚCI INDEKSU FENOLOWEGO JAKO MIARY ZAWARTOŚCI ZWIĄZKÓW FENOLOWYCH W WODZIE. ZASTOSOWANIE BIOSENSORA

Abstract: Phenol index is considered as an important indicator of water purity and quality. Usually phenol index is determined by a spectrophotometric method the calibration being based on phenol standards. Unfortunately, the absorptivities of different phenols compounds differ from each other. This leads to significant uncertainty concerning content of phenols in water. It is shown that the same shortage of the phenol index appears also if it is determined using an amperometric biosensor based on tyrosinase. The sensitivity of the biosensor response to four phenol compounds: phenol, catechol, 3-cresol and 4-chlorophenol was examined, as well as possible interactions between phenols, according to 2⁴ factorial experiment. It was proved that individual phenols affect phenol index independently from each other, *ie* no significant interaction between phenols was detected. However, sensitivity of the biosensor to different phenols is not the same. Relationship between phenol index and concentrations of phenols in water is discussed.

Keywords: phenol index, tyrosinase, biosensor, factorial designs

Phenolic compounds belong to organic pollutants, which are widely distributed in the environment. They may be present in waste waters and natural environmental waters. Phenols are introduced to the environment in variety of ways like wastes from paper manufacturing, agriculture, petrochemical industry, coal processing or as municipal wastes [1]. The phenolic micropollutants generally include chloro-, bromo-, nitro- and alkylphenols [2]. Due to their toxicity and persistence in the environment, numerous phenolic compounds are considered priority pollutants and they appear in a list of dangerous substances of the US *Environmental Protection Agency* (EPA) [3]. According to the EU directive maximum concentration of total phenols in drinking water is 0.5 µg/dm³, while individual concentrations should be under 0.1 µg/dm³ [4]. Because phenolic compounds can easily penetrate into skin and trough cellular membrane, some of them show toxic effects in animals and plants. In drinking water, even at low concentration, phenolic compounds give

¹ Department of Analytical Chemistry, Jagiellonian University, ul. R. Ingardena 3, 30-060 Kraków, Poland, phone +48 12 663 20 14, fax +48 12 663 22 32

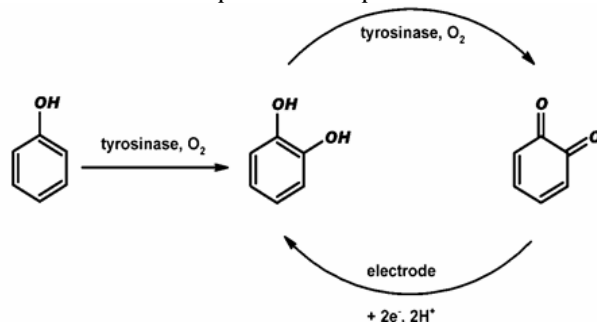
*Corresponding author: kochana@chemia.uj.edu.pl

off strong taste and odor. Moreover, during chlorination process, various chlorophenols and chlorinated *p*-benzoquinones are produced, some of them thought to be mutagenic [1]. Therefore, an easily applied, sensitive and selective method is required for monitoring of phenolic compounds concentration in environmental waters.

In the past decade a variety of analytical methods were proposed for determination of phenol and its derivatives in natural environmental waters and waste waters. The most widely used are gas chromatography [5-7], high performance liquid chromatography [8-10] and electrochemical methods [11-13]. These methods allow to detect individual phenolic compounds, however they often include complicated sample pretreatment, involve complicated and time consuming procedures, and require expensive equipment. Moreover, they are inadequate for in-situ monitoring. Therefore, analytical methods for determination of the total concentration of phenols are preferred.

Phenol index is supposed to be an indicator of water pollution with phenols. According to the international standard ISO 6439 (Water quality. Determination of phenol index. 4-aminoantipyrine spectrometric method after distillation) it is determined spectrophotometrically ($\lambda = 460$ nm) using 4-aminoantipyrine, 4-AAP, as a color making agent, after distillation [14]. 4-AAP was chosen as the photometric reagent for phenols due to the highest rate of colored compound formation and the rather high values of its molar absorption coefficient [15]. However, not all phenol derivatives form color products with 4-aminoantipyrine, *eg* 4-alkylo- and 4-nitrophenols. Moreover, absorptivities of color products are not the same for different phenol derivatives. It is important because in determination of phenol index the calibration is based on phenol standards only. Then the total concentration of phenols cannot be deduced from the phenol index.

Many efforts have been made to develop the simple and effective methods for determination of phenols [3, 16]. Electrochemical biosensors, in particular those based on polyphenol oxidases, are regarded as promising tools for determination of phenols because of their effectiveness and simplicity [17-21]. The biosensors most sensitive to phenols are those based on tyrosinase, a copper-containing polyphenol oxidase. It catalyses the oxidation of monophenols by molecular oxygen to form *o*-biphenols, which are subsequently oxidized to *o*-quinones. Quinones can be electrochemically reduced to enable convenient low-potential detection of phenolic compounds:



An amperometric biosensor based on tyrosinase immobilized in titania sol-gel has been proposed [22]. The developed biosensor shows high sensitivity towards phenolic compounds (catechol, phenol, *p*-cresol, *p*-chlorophenol and *p*-methylcatechol), low limit of detection (0.012 mg/dm^3 for phenol) and satisfying linear range ($0.04 \div 1.03 \text{ mg/dm}^3$).

In the present paper the influence of composition of phenols mixture on phenol index determined with the use of the biosensor was examined. The following phenols were taken into account in the examination which differ in substituents and their positions in benzene ring: phenol, catechol, 3-cresol and 4-chlorophenol. Studies were carried out according to 2⁴ factorial experimental designs [23-25]. The relationship between total concentration of phenols and phenol index was examined and discussed.

Experimental

Chemicals

Tyrosinase (E.C. 1.14.18.1, 5370 U/mg) from mushrooms was from Sigma-Aldrich; graphite electrodes were from ZEW Raciborz (Poland); the precursor: titanium isopropoxide was from Fluka Chimie (Switzerland); paraffin (used for impregnation of electrodes), disodium hydrogen phosphate dehydrate, potassium dihydrogen phosphate, acetone, phenol, 4-chlorophenol and 3-cresol were obtained from Merck; ethanol and L-(+)-ascorbic acid were purchased from POCh (PL), nitric acid and ammonia were from Lach-Ner (PL), catechol was obtained from BDH Chemicals (UK). All chemicals were analytical grade and were used as received. Solutions were prepared in ultra-pure water.

Apparatus and measurements

Voltammetric and amperometric measurements were performed using an EMU/O multimeter (Poland) in thermostatic cabinet Pol-Eko-Aparatura (Poland). Experimental conditions such as: enzyme loading, pH of supporting electrolyte, potential of working electrode and temperature of analysis were optimised and reported in our previous paper [22]. A conventional three-electrodes system was employed consisting of the enzyme electrode as a working electrode, a platinum wire as a counter electrode and Ag/AgCl (3 M) reference electrode. Phosphate buffer solution (pH = 6; 0.1 M) was used as supporting electrolyte. Cleanness of electrode surface was checked in deaerated 0.1 M phosphate buffer solution (pH 7.0) by recording voltammetric curve; for these measurements the buffer solution was purged from oxygen by bubbling with laboratory-grade nitrogen (99.99%). Amperometric experiments were performed under constant stirring with magnetic bar and under free access of air. The enzyme electrode worked at a potential 0.0 V vs. Ag/AgCl (based on earlier research [22, 26]). It was not necessary to activate the sensor before measurements, additional voltammetric cycles were not performed. All experiments were performed at 25°C.

Construction of the biosensor

A graphite rod was impregnated in paraffin and placed into a Teflon holder with stainless steel wire as a current lead. The working surface of electrode was polished with emery paper, then with α -alumina powder and finally rinsed with ultra-pure water. Next, the electrode was successively sonicated in the following media: ultra-pure water, ethanol, nitric acid (1:1), ammonia water, saturated solution of ascorbic acid and acetone. Electrodes were rinsed with ultra-pure water after each sonification and finally dried at room temperature.

In order to prepare titania sol, titanium isopropoxide (250 mm³) was added to propan-2-ol (2.5 cm³) and stirred with addition of concentrated acids: HCl (10 mm³) and CH₃COOH (20 mm³). The prepared solution of precursor was then slowly instilled into cold

water (3 cm³), under constant intensive stirring. The solution of tyrosinase in phosphate buffer (pH = 7; 0.1 M) was prepared, and shaken with titania sol (1:1 v/v). The volume of 20 mm³ of so prepared mixture was deposited on the surface of a pretreated electrode in portions of 10 mm³. After each portion of sol had been added, the surface of electrode was dried in air for ca 10 min. Finally the electrode was allowed to dry over saturated disodium phosphate solution for 20 h at 4°C. Loading of enzyme was 50 µg of tyrosinase per electrode.

The biosensors were stored at 4°C in phosphate buffer, their active surface touching the surface of solution. Before measurements electrodes were immersed in buffer at room temperature for 15 min.

Phenol index determination

The biosensor calibration was performed using standard solutions of phenol. Then amperometric measurements were carried out in solutions containing phenolic compounds which concentrations corresponded to 2⁴ factorials (see below: Experimental plans applied). Phenol index was determined from the calibration graph as a formal concentration of phenol corresponding to the biosensor signal measured in a solution tested.

Methodology

Experimental design applied

Experiments, aimed at determination of influence of the tested phenols on the phenol index, were carried out according to 2⁴ factorial experimental designs. While elaborating experimental results, 2⁴⁻¹ fractional factorials (with contrasts “+” and “-“) and Plackett-Burman plans were extracted from 2⁴ factorial. The plans are presented in Table 1. Concentrations of phenols tested were assumed as controlled factors: phenol (X₁), catechol (X₂), 4-chlorophenol (X₃) and 3-cresol (X₄). For all phenolic compounds the lower concentration level (-1) equaled to 0 µmol/dm³ and the upper level (+1) was assumed to be 1 µmol/dm³. The latter concentration level (+1) corresponded to 0.094, 0.110, 0.129 and 0.108 mg/dm³ for phenol, catechol, 4-chlorophenol and cresol, respectively.

Effects of factors

The main and interaction effects of factors X₁, X₂, X₃ and X₄ (concentrations of phenols tested) were considered as coefficients B_i (i ≠ 0) in the following statistical models (Y denotes phenol index):

from 2⁴ factorial:

$$\hat{Y} = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{14}X_1X_4 + B_{23}X_2X_3 + B_{24}X_2X_4 + B_{34}X_3X_4 + B_{123}X_1X_2X_3 + B_{124}X_1X_2X_4 + B_{134}X_1X_3X_4 + B_{234}X_2X_3X_4 + B_{1234}X_1X_2X_3X_4$$

from 2⁴⁻¹ factorials:

$$\hat{Y} = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{14}X_1X_4$$

from Plackett-Burman plan:

$$\hat{Y} = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + (B_{12}X_1X_2 + B_{23}X_2X_3 + B_{24}X_2X_4).$$

Table 1

2^4 (points 1-16), 2^{4-1} fractional factorials with contrast “ \ominus ” (points 1-8) and contrast “ \oplus ” (points 9-16), and the appropriate fragment of the Plackett-Burman plan (points 5-12, as presented within bold frame); experimental conditions see Experimental design applied

No	X_1	X_2	X_3	X_4	$X_1 X_2$	$X_1 X_3$	$X_1 X_4$	$X_2 X_3$	$X_2 X_4$	$X_3 X_4$
1	-1	-1	-1	1	1	1	-1	1	-1	-1
2	1	-1	-1	-1	-1	-1	-1	1	1	1
3	-1	-1	1	-1	1	-1	1	-1	1	-1
4	-1	-1	1	1	-1	1	1	-1	-1	1
5	1	1	1	-1	1	1	-1	1	-1	-1
6	-1	1	1	1	-1	-1	-1	1	1	1
7	-1	1	-1	-1	-1	1	1	-1	-1	1
8	1	1	-1	1	1	-1	1	-1	1	-1
9	-1	-1	1	1	1	-1	-1	-1	-1	1
10	1	-1	-1	1	-1	-1	1	1	-1	-1
11	1	-1	1	-1	-1	1	-1	-1	1	-1
12	-1	-1	-1	-1	1	1	1	1	1	1
13	1	1	-1	-1	1	-1	-1	-1	-1	1
14	-1	1	-1	1	-1	1	-1	-1	1	-1
15	1	1	1	1	1	1	1	1	1	1
16	-1	1	1	-1	-1	-1	1	1	-1	-1

No	$X_1 X_2$ X_3	$X_1 X_2$ X_4	$X_1 X_3$ X_4	$X_2 X_3$ X_4	$X_1 X_2$ $X_3 X_4$	total conc. of phenols [$\mu\text{mol}/\text{dm}^3$]	phenol index found [$\mu\text{mol}/\text{dm}^3$]	total conc. of phenols [mg/dm^3]	phenol index found [mg/dm^3]
1	-1	1	1	1	-1	1.00	0.58	0.108	0.054
2	1	1	1	-1	-1	1.00	1.10	0.094	0.104
3	1	-1	1	1	-1	1.00	0.64	0.129	0.060
4	-1	-1	1	-1	-1	3.00	1.75	0.331	0.165
5	1	-1	-1	-1	-1	3.00	2.28	0.333	0.215
6	-1	-1	-1	1	-1	3.00	1.86	0.347	0.175
7	1	1	-1	1	-1	1.00	1.13	0.110	0.106
8	-1	1	-1	-1	-1	3.00	2.23	0.312	0.210
9	1	1	-1	-1	1	2.00	0.78	0.237	0.073
10	1	-1	-1	1	1	2.00	1.39	0.202	0.131
11	-1	1	-1	1	1	2.00	1.26	0.223	0.119
12	-1	-1	-1	-1	1	0.00	0.00	0.000	0.000
13	-1	-1	1	1	1	2.00	2.03	0.204	0.191
14	1	-1	1	-1	1	2.00	1.37	0.218	0.129
15	1	1	1	1	1	4.00	2.62	0.441	0.247
16	-1	1	1	-1	1	2.00	1.28	0.239	0.120

In case of Plackett-Burman design, because only 4 factors (concentrations: X_1 , X_2 , X_3 and X_4) were considered in the present investigation, there are still 3 degrees of freedom which enables estimation of the following second order interaction effects: B_{12} , B_{23} and B_{24} (see equation above). The latter coefficients are not confounded with the main effects of factors (B_1 , B_2 , B_3 and B_4).

A coefficient B_i in the above models was determined by scalar multiplication of the column vector of the appropriate design matrix, C_i , and the column vector of responses measured at the points of the corresponding experimental plan, y (phenol index found in Table 1): $B_i = C_i^T \cdot y$.

Significance of the effects

The significance of the effects B was tested according to the Students' t-test:

$$t = \frac{|B|}{s_B} \quad \text{where} \quad s_B = \frac{s_Y}{\sqrt{N}}$$

In the above equations s_Y denotes standard error of response Y determination, N is the number of experimental points in the corresponding design: $N = 16$ in case of 2^4 factorial, $N = 8$ in case of 2^{4-1} fractional factorials and the Plackett-Burman plan. $s_Y = 0.087$ was determined on the basis of the results of twofold measurements made at 16 experimental points of 2^4 factorial (homogeneity of variance was assumed).

Results and conclusions

In Table 2 the main and interaction effects of factors are presented, the significant effects ($\alpha = 0.01$) being distinguished in bold. It is seen that only main effects, B_1 , B_2 , B_3 and B_4 , are significant at $\alpha = 0.01$ and the corresponding effects obtained according to different experimental designs appear similar.

Table 2

Comparison of factors' effects obtained according to different experimental designs

Effect	Design			
	2^4	$2^{4-1} \ominus$	$2^{4-1} \oplus$	Plackett-Burman
B₀	1.394	1.446	1.341	1.366
B₁	0.439	0.394	0.484	0.424
B₂	0.456	0.429	0.484	0.509
B₃	0.165	0.186	0.144	0.179
B₄	0.179	0.159	0.199	0.199
B₁₂	0.001	-0.014	0.016	-0.044
B₁₃	-0.020	-0.011	-0.029	-
B₁₄	-0.014	-0.009	-0.019	-
B₂₃	-0.005	-	-	0.016
B₂₄	-0.009	-	-	-0.029
B₃₄	0.015	-	-	-
B₁₂₃	0.020	-	-	-
B₁₂₄	-0.021	-	-	-
B₁₃₄	0.028	-	-	-
B₂₃₄	0.045	-	-	-
B₁₂₃₄	-0.053	-	-	-

Indices: 1, 2, 3 and 4 at B's correspond to phenol, catechol, 4-chlorophenol and 3-cresol, respectively.

From Table 2 it is seen that sensitivity of the biosensor to phenol (B_1) and catechol (B_2) are similar and it is about two times higher than sensitivity of the sensor to 4-chlorophenol (B_3) and 3-cresol (B_4). It is why one can predict that the standard way of phenol index determination will lead to significant systematic difference between total concentration of phenols and phenol index as the index is obtained from the calibration graph based on phenol only. The difference just mentioned depends on phenols content in a water sample

tested. This can easily be seen by inspecting data in Table 1 where found phenol index values and total concentrations of phenols are presented.

It is worthwhile to add that catalytic activity of polyphenol oxidizes varies depending on the source of enzyme [13]. Generally, the highest sensitivity of mushroom tyrosinase is usually observed for catechol [9, 13]. Substituted phenols showed lower response than phenol, low sensitivity toward chlorophenols may be explained by the deactivating influence of Cl substituent on benzene ring. Above statements are in good agreement with calculated values of B's coefficients presented in Table 2.

The above observations concerning discrepancy between phenol index and total concentration of phenols in water are intrinsic characteristic of the method of phenol index determination in waters. This results from different sensitivity of the biosensor to different phenols. This is true not only when biosensor is applied but also if photometric method with 4-aminoantipyrine is used in determination of phenol index. It is because the absorptivities of the phenols differ, eg 0.98, 0.64 and 0.41 $\text{dm}^3/(\text{mg cm})$ for phenol, 3-cresol and 4-chlorophenol, at $\lambda = 460 \text{ nm}$, respectively [27].

An interesting and important result of the present investigation is that different phenols influence the biosensor response independently from each other; their influence has additive character. Then it was also proved that Plackett-Burman plan is sufficient in such investigations which minimize the number of necessary experiments. The final conclusion is that neither method of phenol index determination, spectrophotometric nor electroanalytical, gives certain information on the total amount of phenols in waters, however, the proposed biosensor enables much easier and direct determination of the index.

References

- [1] Adamski J, Nowak P, Kochana J. Simple sensor for determination of phenol and its derivatives in water based on enzyme tyrosinase. *Electrochim Acta*. 2010;55:2363-2367. DOI: 10.1016/j.electacta.2009.11.099.
- [2] Kim K-R, Kim H. Gas chromatographic profiling and screening for phenols as isobutoxycarbonyl derivatives in aqueous samples. *J Chromatogr A*. 2000;866:87-96.
- [3] Sousa AR, Trancoso M. Validation of an environmental friendly segmented flow method for the determination of phenol index in water as alternative to the conventional one. *Talanta*. 2010;79(3):796-803. DOI: 10.1016/j.talanta.2009.05.009.
- [4] EU/4 Drinking Water Directive 80/778/EEC.
- [5] Padilla-Sanchez JA, Plaza-Bolanos P, Romero-Gonzalez R, Barco-Bonilla N, Martinez-Vidal JL, Garrido-Frenich A. Simultaneous analysis of chlorophenols, alkylphenols, nitrophenols and cresols in wastewater effluents, using solid phase extraction and further determination by gas chromatography-tandem mass spectrometry. *Talanta*. 2011;85:2397-2404. DOI: 10.1016/j.Talanta.2011.07.081.
- [6] Sanchez-Avila J, Fernandez-Sanjuan M, Vicente J, Lacorte S. Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography-tandem mass spectrometry. *J Chromatogr A*. 2011;1218:6799-6811. DOI: 10.1016/j.chroma.2011.07.056.
- [7] Kovacs A, Mortl M, Kende A. Development and optimization of a method for the analysis of phenols and chlorophenols from aqueous samples by gas chromatography-mass spectrometry, after solid-phase extraction and trimethylsilylation. *Microchem J*. 2011;99:125-131. DOI: 10.1016/j.microc.2011.04.007.
- [8] Alcudia-Leon MC, Lucena R, Cardenas W, Valcarcel M. Determination of phenols in waters by stir membrane liquid - liquid - liquid microextraction coupled to liquid chromatography with ultraviolet detection. *J Chromatogr A*. 2011;1218:2176-2181. DOI: 10.1016/j.chroma.2011.02.033.
- [9] Tanigawa T, Watabe Y, Kubo T, Hosoya K. Determination of bisphenol a with effective pretreatment medium using automated column-switching HPLC with fluorescence detection. *J Sep Sci*. 2011;34:2840-2846.

- [10] Segovia-Martinez L, Moliner-Martinez Y, Campins-Falco P. Direct capillary liquid chromatography with electrochemical detection method for determination of phenols in water samples. *J Chromatogr A*. 2010;1217:7926-7930. DOI: 10.1016/j.chroma.2010.10.078.
- [11] Jing T, Xia H, Niu J, Zhou Y, Dai Q, Hao Q, Zhou Y, Mei S. Determination of trace 2,4-dinitrophenol in surface water samples based on hydrophilic molecularly imprinted polymers/nickel fiber electrode. *Biosens Bioelectron*. 2011;26:4450-4456. DOI: 10.1016/j.bios.2011.05.00.
- [12] Vidal L, Chisvert A, Canals A, Psillakis E, Lapkin A, Acosta F, Edler KJ, Holdaway JA, Marken F. Chemically surface-modified carbon nanoparticle carrier for phenolic pollutants: extraction and electrochemical determination of benzophenone-3 and triclosan. *Anal Chim Acta*. 2008;616:28-35. DOI: 10.1016/j.aca.2008.04.011.
- [13] Wu Y. Nano-TiO₂/dihexadecylphosphate based electrochemical sensor for sensitive determination of pentachlorophenol. *Sens Actuators B*. 2009;137:180-184. DOI: 10.1016/j.snb.2008.11.005.
- [14] International Organization of Standardization: Water quality. Determination of phenol index. 4-Aminoantipyrine spectrometric methods after distillation. 1990:6439.
- [15] Moskvin AL, Mozhukhin A, Mukhina EA, Moskvin LN. Flow-injection photometric determination of the phenol index of natural waters in the presence of humic acids. *J Anal Chem*. 2005;60(1):79-84.
- [16] Maya F, Estela JM, Cerda V. Flow analysis techniques as an effective tools for the improved environmental analysis of organic compounds expressed as total indices. *Talanta*. 2010;81(1-2):1-8. DOI: 10.1016/j.talanta.2010.01.028.
- [17] Arecchi A, Scampicchio M, Drusch S, Mannino S. Nanofibrous membrane based tyrosinase-biosensor for the detection of phenolic compounds. *Anal Chim Acta*. 2010;659(1-2):133-136. DOI: 10.1016/j.aca.2009.11.039.
- [18] Zhang Y, Ji C. Electro-induced covalent cross-linking of chitosan and formation of chitosan hydrogel films: Its application as an enzyme immobilization matrix for use in a phenol sensor. *Anal Chem*. 2010;82(12):5275-5281. DOI: 10.1021/ac100714s.
- [19] Chen J, Jin Y. Sensitive phenol determination based on co-modifying tyrosinase and palygorskite on glassy carbon electrode. *Microchim Acta*. 2010;169(3-4):249-254. DOI: 10.1007/s00604-010-0320-6.
- [20] Lu L, Zhang L, Zhang X, Huan S. A novel tyrosinase biosensor based on hydroxyapatite-chitosan nanocomposite for the detection of phenolic compounds. *Anal Chim Acta*. 2010;665(2):146-151. DOI:10.1016/j.aca.2010.03.033.
- [21] Zhang J, Lei J, Liu Y, Zhao J. Highly sensitive amperometric biosensors for phenols based on polyaniline-ionic liquid-carbon nanofiber composite. *Biosens Bioelectron*. 2009;24(7):1858-1863. DOI: 10.1016/j.bios.2008.09.012.
- [22] Kochana J, Gala A, Parczewski A, Adamski J. Titania sol-gel-derived tyrosinase-based amperometric biosensor for determination of phenolic compounds in water samples. Examination of interference effects. *Anal Bioanal Chem*. 2008;391(4):1275-1281. DOI: 10.1007/s00216-007-1798-6.
- [23] Bayne ChK, Rubin IB. Practical experimental designs and optimization methods for chemists. Weinheim: VCH Publishers; 1986.
- [24] Parczewski A. Chemometric aspects of environmental analytics. Proc Summer School organized by CEEAM. Gdańsk, Poland; 2005.
- [25] Otto M. Chemometrics. Statistics and computer application in analytical chemistry. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA; 2007.
- [26] Kochana J, Nowak P, Jarosz-Wilkolazka A, Biedroń M. Tyrosinase/laccase bienzyme biosensor for amperometric determination of phenolic compounds. *Microchem J*. 2008;89(2):171-184. DOI: 10.1016/j.microc.2008.02.004.
- [27] Ettinger MB, Ruchhoft CC, Lishka R. Sensitive 4-aminoantipyrine method for phenolic compounds. *Anal Chem*. 1951;23(12):1783-1788.

KRYTYCZNA OCENA PRZYDATNOŚCI INDEKSU FENOLOWEGO JAKO MIARY ZAWARTOŚCI ZWIĄZKÓW FENOLOWYCH W WODZIE. ZASTOSOWANIE BIOSENSORA

Zakład Chemii Analitycznej, Uniwersytet Jagielloński, Kraków

Abstrakt: Indeks fenolowy jest ważnym wskaźnikiem czystości i jakości wody. Oznacza się go zwykle metodami spektrofotometrycznymi z 4-aminoantypiryną, stosując fenol jako wzorzec. Ponieważ współczynniki absorpcji różnych związków fenolowych różnią się, wyznaczona wartość indeksu fenolowego obarczona jest znaczną niepewnością. Podobny efekt występuje, gdy indeks fenolowy oznaczany jest przy użyciu biosensora amperometrycznego opartego na tyrozinazie. W pracy wyznaczono czułości biosensora w stosunku do czterech związków fenolowych: fenolu, katecholu, 3-krezolu oraz 4-chlorofenolu oraz zbadano możliwe interakcje pomiędzy fenolami. Doświadczenia prowadzono według planów czynnikowych 2^4 . Wykazano, że poszczególne fenole wpływają na indeks fenolowy niezależnie od siebie, tzn. nie stwierdzono istotnych interakcji pomiędzy fenolami. Jednak czułość biosensora jest różna w stosunku do różnych fenoli. Przedyskutowano zależność pomiędzy indeksem fenolowym a stężeniami fenoli w wodzie.

Słowa kluczowe: indeks fenolowy, tyrozinaza, biosensor, plan czynnikowy