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ORIGINAL PAPER

Titania sol–gel-derived tyrosinase-based amperometric biosensor for determination of phenolic compounds in water samples. Examination of interference effects

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Abstract For detection of phenolic compounds in environmental water samples we propose an amperometric biosensor based on tyrosinase immobilized in titania sol–gel. The analytical characteristics toward catechol, *p*-cresol, phenol, *p*-chlorophenol, and *p*-methylcatechol were determined. The linear range for catechol determination was 2.2×10^{-7}–1.3×10^{-5} mol L^{-1} with a limit of detection of 9×10^{-8} mol L^{-1} and sensitivity 2.0×10^{3} mA mol^{-1} L. The influence of sample matrix components on the electrode response was studied according to Plackett–Burman experimental design. The potential interferents Mg^{2+}, Ca^{2+}, HCO_3^-, SO_4^{2-}, and Cl^-, which are usually encountered in waters, were taken into account in the examination. Cu^{2+} was also taken into account, because $CuSO_4$ is sometimes added to a water sample, as a preservative, before determination of phenolic compounds. It was found that among the ions tested only Mg^{2+} and Ca^{2+} did not directly affect the electrode response. The developed biosensor was used for determination of catechol in spring and surface water samples using the standard addition method.

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Introduction

Phenolic compounds are important contaminants in ground and surface water. Due to their toxicity and persistence in the environment phenols are regarded as priority pollutants. Many efforts have been made to develop simple and effective methods of determination of phenols.

Determination of phenol and its derivatives is commonly accomplished by chromatographic and spectrometric methods (Ref. [1] and references cited therein). These methods are expensive, usually time-consuming, include complicated sample pretreatment, and are inadequate for in-situ monitoring. Amperometric biosensors based on phenol oxidases, tyrosinase, or laccase have been regarded as promising methods for determination of phenols because of their effectiveness and simplicity [2]. The biosensors most sensitive to phenols are those based on tyrosinase, a copper-containing polyphenol oxidase (Ref. [3] and references cited therein). Tyrosinase 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. Numerous biosensors based on tyrosinase have been elaborated for detection of phenols [4–14]. A phenol biosensor based on tyrosinase encapsulated in silica sol–gel composite film was reported by Wang et al. [4]. Rajesh et al. proposed immobilization of tyrosinase on to an electrochemically prepared novel copolymer, poly(*N*-3-aminopropyl pyrrole-co-pyrrole), film [5]. Rijaravanich et al. developed a microcylinder biosensor based on layer-by-layer immobilization

of tyrosinase on latex particles [6]. Application of polyacrylamide microgels for entrapment of tyrosinase was reported by Perez et al. [7]. Development of a high analytical performance tyrosinase biosensor based on a composite graphite–Teflon electrode modified with gold nanoparticles was described by Caarralero et al. [8]. A reagentless tyrosinase biosensor based on 1,6- hexanedithiol and nano-Au self-assembled monolayers has been reported [9]. Yildiz et al. proposed a biosensor based on electrochemically controlled integration of tyrosinase in a redox polymer [10]. Sonogel-Carbon materials have been used as a basis for development of enzyme biosensors for monitoring phenols and polyphenols [11]. Laponite clay–chitosan nanocomposite matrix has been employed for entrapment of tyrosinase in a phenol biosensor [12]. Temble et al. proposed an electrochemical biosensor for catechol using agarose-guar gum-entrapped tyrosinase [13]. Multiwalled carbon nanotubes – Nafion nanobiocomposites – have been reported as a tyrosinase immobilization matrix [14].

Immobilization of bioreceptors on a matrix plays an important role for biosensor application and stability. Sol–gel glasses are attractive methods for immobilization of biomolecules, e.g. enzymes, because matrices can be prepared under ambient conditions and can retain the catalytic activity of enzymes [15–17]. Compared with other immobilization matrices, sol–gel films have many advantages such as entrapment of large amounts of enzymes, thermal and chemical stability, simple preparation without covalent modification, and flexibility of control of pore size and geometry [18]. Most reported biosensors with a sol–gel matrix have been based on silica [17]. In recent years, a number of new sol–gel-derived materials have been designed as matrices in the construction of biosensors [17]. Some biomolecules entrapped in titania sol–gel matrix have been reported, for example horseradish peroxidase [19], haemoglobin [20], and glucose oxidase [21]. Few biosensors for determination of phenolic compounds based on tyrosinase immobilized in a sol–gel matrix have been proposed. Kim et al. described an amperometric biosensor based on tyrosinase entrapped in a sol–gel silicate/Nafion film [22]. A vapour deposition method was employed for immobilization of tyrosinase on a titania sol–gel matrix [23].

The objective of this study was to develop an amperometric tyrosinase-based biosensor for determination of phenolic compounds in environmental water samples. The enzyme was encapsulated in a titania matrix prepared by a simple sol–gel method. Experimental conditions affecting the response of the biosensor were examined, including enzyme loading, temperature of measurement, operating potential of electrode, and pH of background electrolyte. The performance of the biosensor was checked for detection of phenolic compounds such as catechol, *p*-cresol, phenol, *p*-chlorophenol, and *p*-methylcatechol. The analytical characteristics of the biosensor toward these analytes were determined and the influence of natural water matrix components on the biosensor response was examined. The experiments were carried out according to the Plackett–Burman experimental design. Potential interferents usually encountered in waters – Mg^{2+}, Ca^{2+}, HCO_3^-, SO_4^{2-}, and Cl^- – were taken into account in the examination. Cu^{2+} was also taken into account because $CuSO_4$ is sometimes added to a water sample, as a preservative, before determination of phenolic compounds. The developed biosensor was used for determination of catechol in spring and surface water samples.

Experimental

Chemicals

Tyrosinase (E.C. 1.14.18.1, 5370 U/mg) from mushrooms was purchased from Sigma. Electrokarbon Topolcany (Slovak Republic) carbon electrodes were used for construction of biosensors. Paraffin used for impregnation of carbon electrodes was produced by Merck. Acetone and 2-propanol were purchased from Z.B.P. Chemed (Poland). Nitric acid, hydrochloric acid, sodium chloride, and ammonia were from Lach-Ner (Poland). Disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, magnesium nitrate hexahydrate, sodium sulfate, sodium nitrate, and phenolic the compounds *p*-methylcatechol, phenol, *p*-chlorophenol, and *p*-cresol were from Merck. The precursor titanium isopropoxide was from Fluka Chemie (Switzerland). Sodium hydrogen carbonate, copper(II) nitrate trihydrate, calcium nitrate tetrahydrate, L-(+)-ascorbic acid, and EDTA were from POCh (Poland). Ethanol was purchased from Eurochem BDG (Poland) and catechol from BDH Chemicals (UK). All chemicals were analytical grade and were used as received. Solutions were prepared in ultra-pure water.

Apparatus and measurements

Amperometric and cyclic voltammetric experiments were performed in a thermostatic cabinet Pol-Eko-Aparatura (Poland) using an EMU/O multimeter (Poland) connected to a computer. The conventional three-electrode system used comprised a carbon working electrode coated with an enzyme layer, an Ag/AgCl (3 mol L^{-1} KCl) reference electrode, and a platinum wire counter-electrode. A homemade electrochemical cell and magnetic stirring bar were used. The supporting electrolyte was 0.1 mol L^{-1} phosphate buffer solution. For voltammetric measurements the solution was purged free from oxygen by bubbling with laboratory-grade nitrogen (99.99%). Determination of phenols was carried out amperometrically, in batch mode, by

measuring the intensity of current which corresponded to electrochemical reduction of enzymatically generated quinones. Under the optimized conditions the steady-state current was reached in approximately 3 min for all tested compounds except for the supporting electrolyte, phosphate buffer, for which a steady-state baseline current was observed after 2 min. It was not necessary to activate the sensor before measurements; additional voltammetric cycles were not performed. Between measurements the sensor was rinsed with ultra-pure water. In the examination of interference effects EDTA was added to each synthetic water matrix in order to avoid precipitation of magnesium, calcium, and copper(II) phosphates. To adjust the ionic strength of the solution appropriate amounts of sodium nitrate were added.

Preparation of biosensor

A carbon rod of 6 mm diameter was impregnated in paraffin for 30 min at 80 °C and placed in a Teflon holder. A stainless steel wire, a current lead, was fastened to the end of the electrode. The working surface of the electrode was polished with α-alumina powder and rinsed thoroughly with ultra-pure water. Next, the electrode was successively sonicated in ultra-pure water, ethanol, nitric acid (1:1), aqueous ammonia solution, a saturated solution of L-(+)-ascorbic acid, and acetone. Electrodes were rinsed with ultra-pure water after each sonication and finally dried at room temperature.

A homogeneous titania sol was prepared by mixing 250 μL titanium isopropoxide, 2.5 mL 2-propanol acidified with 10 μL HCl (concentrated diluted 1:9 with 2-propanol), and 20 μL concentrated CH_3COOH. The solution of the precursor (titanium isopropoxide) was then instilled into 3 mL cold water, with constant vigorous stirring. The sols prepared by this procedure were stable for many months if stored at 4 °C, and were used for preparation of biosensors.

The appropriate amount of tyrosinase was dissolved in phosphate buffer of pH 7 and shaken with an equal volume of titania sol. This mixture (20 μL) was deposited on the surface of a pretreated electrode in portions of 10 μL. After each portion of sol had been added, the surface of the electrode was dried in air for 10 min. Finally the electrode was allowed to dry over saturated disodium phosphate solution for 20 h at 4 °C. The biosensors were stored at 4 °C in phosphate buffer, their active surfaces touching the surface of the buffer solution. Before measurements, electrodes were immersed in phosphate buffer at room temperature for 15 min.

Results and discussion

Optimization of experimental conditions

The response of the enzyme electrode may be affected by enzyme loading, pH of the buffer solution used as carrier solution, applied potential of working electrode, and temperature of analysis.

The optimum enzyme loading were chosen in accordance with our previous results. Briefly, electrodes containing 25, 50, 75, and 100 μg of tyrosinase were prepared. After 20 h, and 2, 3, and 6 days their performance was tested in solution containing 1.0×10^{-5} mol L^{-1} catechol. High current intensity and the best repeatability of signals were achieved for sensors with 50 μg tyrosinase entrapped in 20 μL titania gel.

To choose the best medium for determination, phosphate buffers of different pH were used as carrier solutions. The highest current was obtained in solution containing 1.0×10^{-5} mol L^{-1} catechol at pH 6. This corresponds to the optimum pH for tyrosinase immobilized in agarose–guar gum [13], entrapped in alumina sol–gel matrix [24], and in composite biopolymeric film [25]. Consequently pH 6 was selected in this study.

The effect of applied potential on the amperometric signal of the sensor was tested in the range between −200 and 200 mV vs. Ag/AgCl (Fig. 1). As expected, the

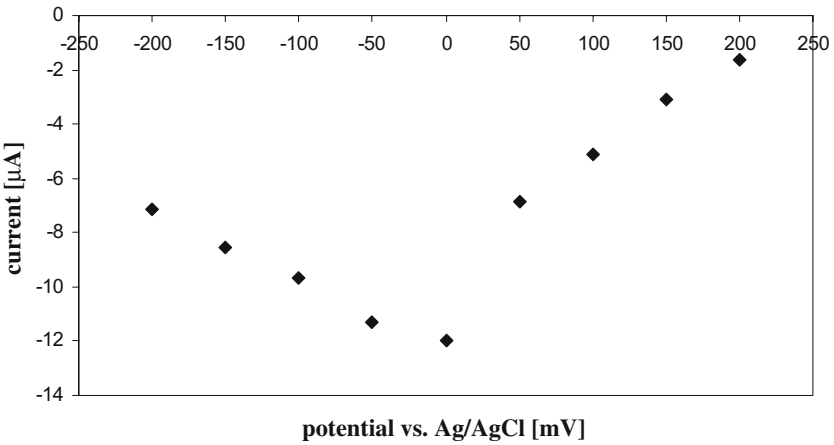

Fig. 1 Effect of the potential of working electrode on the sensor response to 1×10^{-7} mol L^{-1} catechol in 0.1 mol L^{-1} phosphate buffer solution (pH 6)

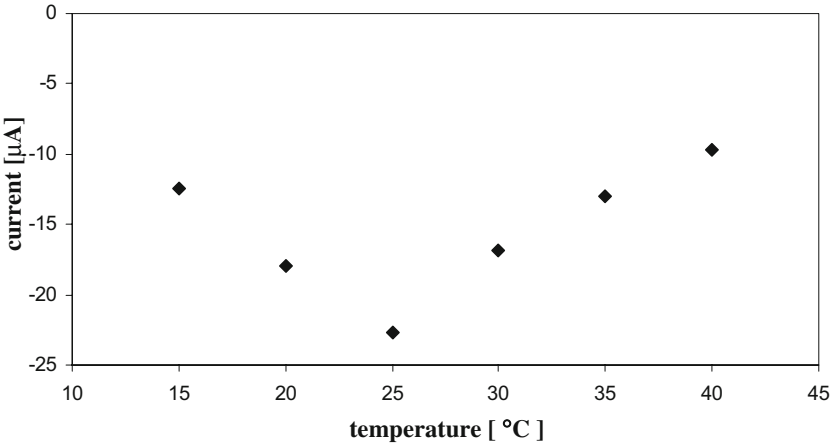

Fig. 2 Effect of temperature on the response of the biosensor for 1×10^{-7} mol L^{-1} catechol in 0.1 mol L^{-1} phosphate buffer solution (pH 6); working potential 0.0 mV

reduction current increased rapidly as the potential decreased from 200 to 0 mV, which is due to the increased driving force for the fast reduction of *o*-quinones at low potential [4]. A decrease in the response was observed when applied potential was more negative than 0.0 mV. The decrease in the cathodic current at potentials below 0.0 mV can be attributed to polymerization of *o*-quinones accompanying the enzymatic reaction at such negative potentials [26].

The influence of measurement temperature on the activity of tyrosinase immobilized in titania gel is shown in Fig. 2. The optimal temperature was found to be 25 °C. It seems that the optimal temperature for activity of the enzyme varies depending on the source. For tyrosinases originating from plants the optimal activity temperature may be lower than that for the mammalian enzymes.

Analytical characteristics of biosensor

Five phenolic compounds – catechol, *p*-cresol, phenol, *p*-chlorophenol, and *p*-methylcatechol – were determined using the biosensor. Table 1 presents the response characteristics of the enzyme electrode including linear range, detection limit, and sensitivity. The linear concentration range was obtained as log I (μA) = a + $b$log C (μmol L^{-1})

Table 1 Response characteristics of the biosensor to the tested phenolic compounds

Analyte	Sensitivity (mA mol^{-1} L)	Limit of detection (mol L^{-1})	Linear range (mol L^{-1})
Catechol	2.0×10^{3}	9×10^{-8}	2.2×10^{-7}–1.3×10^{-5}
Phenol	1.4×10^{3}	1.3×10^{-7}	4.4×10^{-7}–1.1×10^{-5}
p-Cresol	1.3×10^{3}	1.4×10^{-7}	2.2×10^{-7}–7.7×10^{-6}
p-Chlorophenol	1.1×10^{3}	1.7×10^{-7}	2.2×10^{-7}–1.3×10^{-5}
p-Methylcatechol	5.7×10^{2}	3.2×10^{-7}	4.4×10^{-7}–1.2×10^{-5}

in accordance with Z. Liu et al. [24]. Limit of detection (LOD) was calculated according to the formula $3s_b/b$, where s_b is the standard deviation of blank measurements (n = 10) and b is the slope of the calibration curve [11]. The widest linear range, 2.2×10^{-7}–1.3×10^{-5} mol L^{-1}, was obtained for catechol and *p*-chlorophenol. The highest sensitivity was observed for catechol – 2.0×10^{3} mA mol^{-1} L (7.1×10^{3} mA mol^{-1} L cm^{-2}). The sensitivity increases in the order catechol > phenol > *p*-cresol > *p*-chlorophenol > *p*-methylcatechol. This sequence for catechol, phenol, and *p*-cresol is the same as that reported for a tyrosinase biosensor based on 1,6-hexanedithiol and nano-Au self-assembled monolayers [9], for tyrosinase immobilized in multiwalled carbon nanotubes – Nafion nanobiocomposite matrix [14], and tyrosinase entrapped in mediator-free alumina sol–gel matrix [24]. The different sensitivity for different phenolic compounds may depend on the hydrophobic characteristics of the immobilization matrix and molecular steric hindrance [27].

The repeatability of the developed enzyme sensor was good. It was evaluated for five sensors in 6.7×10^{-7} mol L^{-1} catechol solution; relative standard deviations (RSD) were 0.3%, 0.5%, 1.4%, 1.5%, and 2.4%. For a series of five electrodes prepared at different time, RSD 3.2% was obtained for 6.7×10^{-7} mol L^{-1} catechol solution (reproducibility). These satisfactory results may be attributed to the uniform structure of the gel matrix formed.

The operating stability of the biosensor was tested by successive measurement of its response to 2.2×10^{-7} mol L^{-1} catechol solution. It was found that the electrode retained of 100% its original response after ten measurements, and 90% after twenty measurements. That decrease of enzyme activity could be explained by the decrease of the accessibility of active centres of the enzyme. The quinone products of the enzymatic reaction inactivate the enzyme, due to the interaction between the enzyme's active sites and phenoxy radicals, or passivate the electrode

surface by formation of non-conducting polyether films with consequent alteration in the stability of operation of the biosensor [12]. The storage stability of the biosensor was also studied. The enzyme electrode was stored in buffer solution at pH 6, at 4 °C. The results showed that the activity of the biosensor decreased gradually. The biosensor retained 75% of its initial current response after 7 days, and only 55% after 2 weeks. The decrease of response in the long term can be associated with enzyme leaching from the titania gel matrix and enzyme inactivation.

Ten successive measurements were carried out with the biosensor. To prepare a new electrode the exhausted gel layer was mechanically removed (scratched) and, before deposition of new sol layer, the working surface of the electrode was polished and sonicated as described above (Section: "Preparation of biosensor").

Taking catechol as an example, the performance of the proposed biosensor is compared in Table 2 with biosensors based on tyrosinase immobilized in other matrices. It is apparent that the sensitivity of the proposed biosensor for catechol is substantially higher than that of other biosensors (matrices), indicating that tyrosinase entrapped in titania gel has greater catalytic activity. The LOD for catechol determination is also better (lower) than that of other biosensors and in same cases the linear range is wider [5, 7, 13, 14, 22]. However, the stability of response of the proposed biosensor is worse than that reported for other biosensors.

Examination of the effects of interference

The effect of sample matrix components on the response of the biosensor was studied according to Plackett–Burman

Table 2 Comparison of analytical characteristics toward catechol for some reported tyrosinase biosensors

Electrode	Sensitivity	Linear range (mol L^{-1})	LOD (mol L^{-1})	Stability	Ref.
Tyrosinase encapsulated in silica sol–gel composite film	59.6 mA mol^{-1} L	1×10^{-7}–1×10^{-4}	4×10^{-8}	Maintained 73% of initial activity after intermittent use for 3 weeks	[4]
Tyrosinase immobilized in silicate/Nafion composite film	200 mA mol^{-1} L	1×10^{-6}–1×10^{-4}	3.5×10^{-7}	Retained 74% of initial activity after 14 days of storage	[22]
Tyrosinase covalent immobilized on to copolymer poly(*N*-3-aminopropyl pyrrole-co-pyrrole) film	3.46 mA mol^{-1} L	1.6×10^{-6}–1.2×10^{-4}	1.2×10^{-6}	Retained 80% of the enzyme activity for 4 months of storage	[5]
Tyrosinase layer-by-layer immobilized on latex particles	150 mA mol^{-1} L cm^{-2}	2×10^{-6}–2.0×10^{-5}	n.r.	Not reported	[6]
Tyrosinase entrapped in polyacrylamide microgels	469.3 mA mol^{-1} L cm^{-2}	5.0×10^{-7}–2.4×10^{-5}	3.0×10^{-7}	Not reported	[7]
Tyrosinase immobilized onto graphite–Teflon composite electrode modified with gold nanoparticles	746 mA mol^{-1} L	1.0×10^{-8}–8.0×10^{-6}	3×10^{-9}	39 days without apparent loss of enzyme activity	[8]
Tyrosinase immobilized using 1,6-hexanedithiol and nano-Au self-assembled monolayers	3.94 mA mol^{-1} L cm^{-2}	4.0×10^{-7}–7×10^{-5}	6×10^{-8}	Response current decreased to 70% of initial response after one month	[9]
Tyrosinase immobilized within Os-complex-functionalized electrodeposition polymer	6.10 mA mol^{-1} L	Not reported	1×10^{-8}	Not reported	[10]
Tyrosinase immobilized on a Sonogel-Carbon matrix	82.5 mA mol^{-1} L	Not reported	6.4×10^{-8}	Not reported	[11]
Polyphenol oxidase entrapped in laponite clay-chitosan nanocomposite matrix	674 mA mol^{-1} L cm^{-2}	5.3×10^{-9}–4.0×10^{-5}	5.3×10^{-9}	Retains 88% of the original activity after 60 days	[12]
Tyrosinase entrapped in agarose-guar gum	Not reported	6×10^{-5}–8×10^{-4}	6×10^{-6}	Marginal loss of enzyme activity was observed after 2 months of storage	[13]
Tyrosinase immobilized in multiwalled carbon nanotubes–Nafion nanobiocomposite matrix	346 mA mol^{-1} L	1×10^{-6}–2.3×10^{-5}	2.2×10^{-7}	Not reported	[14]
Proposed biosensor Tyrosinase immobilized in titania sol–gel matrix	2010 mA mol^{-1} L (7100 mA mol^{-1} L cm^{-2})	2.2×10^{-7}–1.3×10^{-5}	9×10^{-8}	Retains only 75% of initial current response after 7 days of storage	

Table 3 Levels of catechol concentration (mg L^{-1}) assumed in the Plackett–Burman plan

Factor	Concentration levels [mg L^{-1}]	
	−1 (lower)	+1 (upper)
Mg^{2+}	8	40
Ca^{2+}	30	155
HCO_3^-	100	800
SO_4^{2-}	50	200
Cl^-	100	300
Cu^{2+}	0	260

design. The Plackett–Burman factorial is a two-level orthogonal design [28]. The design minimizes the number of experiments required to allow exploratory study of a large number of factors to see whether they have a significant effect on the response. The main factors' effects are determined independently of each other.

In our studies five potential interferents which are usually met in waters were selected – Mg^{2+}, Ca^{2+}, HCO_3^-, SO_4^{2-}, and Cl^-. Cu^{2+} was also taken into account because $CuSO_4$ is sometimes added to a water sample, as a preservative, before determination of phenolic compounds [29]. In the examination each potential interferent occurred at two concentrations, high (+) and low (−). The actual values of six tested factors (concentrations of interferents) are shown in Table 3. The higher concentration levels of Mg^{2+}, Ca^{2+}, HCO_3^-, SO_4^{2-}, and Cl^- were correlated with concentrations of these ions in natural waters of medium purity [29].

The experimental design (concentrations of interferents) is presented in Table 4, together with response values, each response being the mean catechol concentration obtained from two parallel determinations carried out on the corresponding solution. The main effect of variable "*i*" (interferent), $E(X_i)$, was calculated as the difference between the average of responses measured at the high settings (+) and the average measured at the low settings (−) of the variable "*i*". Student's *t*-test was employed to determine the significance of the effects of the interferents. Student's *t* values, $t(X_i)$ (i = 1,...,8), presented in Table 4 (bottom), were obtained by dividing the effects by their standard error, *SE* (the same for all effects): $t(X_i) = E(X_i)/SE$. The error *SE* was calculated from the formula: $SE = 2S_b/\sqrt{N}$, where S_b=0.1039, the response standard deviation, was estimated from differences between the pairs of results of repeated determinations, as explained above, and N=8 − number of trials. For eight degrees of freedom the critical *t*-values are $t_{(0.01;8)}$=3.36 and $t_{(0.05;8)}$=2.31 at significance levels α = 0.01 and 0.05, respectively. From Table 4 it is apparent that for most of the effects absolute *t*-values were greater that 2.31, and they were regarded as significant. Only the main effects of Mg^{2+} and Ca^{2+} seemed to be negligible.

Table 4 Plackett–Burman design for six factors (concentration of interferents) and eight trials. Actual concentration of catechol: 0.75 μmol L^{-1}

Solution	Factors, *X* (concentrations of interferents)						Response (μmol L^{-1})
	Mg^{2+}	Ca^{2+}	HCO_3^-	SO_4^{2-}	Cl^-	Cu^{2+}	
1	+1	+1	+1	−1	+1	−1	0.16
2	+1	−1	+1	+1	−1	+1	0.28
3	−1	−1	+1	+1	+1	−1	0.30
4	−1	+1	−1	+1	+1	+1	0.75
5	+1	−1	−1	−1	+1	+1	0.70
6	−1	+1	+1	−1	−1	+1	0.00
7	+1	+1	−1	+1	−1	−1	0.37
8	−1	−1	−1	−1	−1	−1	0.08
E(*X*)	0.095	−0.02	−0.29	0.19	0.296	0.206	
|*t*(*X*)|	1.29	0.27	3.95	2.59	4.30	2.80	

E(*X*) – main effect of factor *X*; *t*(*X*) – Student's *t*-value for factor *X*; critical *t*-values for f = 8 degrees of freedom at significance levels α= 0.01 and α=0.05: $t_{(0.01;8)}$=3.36 and $t_{(0.05;8)}$=2.31

Analysis of real water samples

Because of significant matrix effects found in the Plackett–Burman experiment we decided to check the usefulness of the proposed biosensor in determination of catechol added

Table 5 Determination of spiked catechol in the matrix of natural waters

Water sample	Catechol concentration (mol L^{-1})		Recovery (%)
	added	found	
Spring water	4.4×10^{-7}	5.0×10^{-7}	113.6
	8.7×10^{-7}	1.01×10^{-6}	116.1
	1.74×10^{-6}	1.89×10^{-6}	108.6
	3.91×10^{-6}	4.13×10^{-6}	105.6
	8.21×10^{-6}	9.51×10^{-6}	115.8
River water 1 (Rudawa)	4.4×10^{-7}	5.4×10^{-7}	122.7
	8.7×10^{-7}	1.08×10^{-6}	124.1
	1.74×10^{-6}	2.04×10^{-6}	117.2
	3.91×10^{-6}	4.17×10^{-6}	106.6
	8.21×10^{-6}	9.36×10^{-6}	114.0
River water 2 (Vistula)	4.4×10^{-7}	5.4×10^{-7}	122.7
	8.7×10^{-7}	1.05×10^{-6}	120.7
	1.74×10^{-6}	2.04×10^{-6}	117.2
	3.91×10^{-6}	4.08×10^{-6}	104.3
	8.21×10^{-6}	9.38×10^{-6}	114.3
	4.4×10^{-7}	5.3×10^{-7}	120.5
	8.7×10^{-7}	1.00×10^{-6}	114.9
	1.74×10^{-6}	1.90×10^{-6}	109.2
	3.91×10^{-6}	4.25×10^{-6}	108.7
	8.21×10^{-6}	9.87×10^{-6}	120.2

to a matrix of natural river or spring water, using the standard addition method. No special sample pretreatment was performed – water samples were filtered and diluted fivefold with buffer, as proposed by Solna et al. [30]. It was assumed that concentrations of phenolic compounds in the water samples tested was below the detection limit. The water samples were therefore spiked with different amounts of catechol. The results of analysis obtained for different types of water are presented in Table 5. It is seen that, in general, the concentrations found were higher than those added. For spring water (simple matrix) the excess of catechol found (recovery 105.6–116.1%) was slightly lower than for river waters (recovery 106.6–124.1%). That phenomenon could be connected both with an effect of the water matrix which could not be compensated by the standard addition method and with a complex water matrix which contained a tyrosinase substrate.

Conclusion

A biosensor based on tyrosinase is proposed for determination of phenols in environmental water samples. The developed immobilization procedure based on a titania sol–gel matrix resulted in biosensor with high sensitivity towards phenolic compounds. It was demonstrated that the reproducibility of the biosensor was good and that the corresponding LOD, 10^{-8}–10^{-7} mol L^{-1}, compared favourably with those of other tyrosinase biosensors recently described in the literature. In order to study matrix interference effects the Plackett–Burman factorial design was employed. It is apparent from Table 4 that the interferents studied substantially affect the determination of catechol. A thorough inspection of the data in Table 4 leads to a conclusion that interactions between interferents play an important role. The Plackett–Burman design, though economical, offers determination of only main effects of the interferents, the second-order and higher-order interactions being confined. Nevertheless, the results obtained (Table 4) resulted in our deciding to use the standard addition method to determine catechol in natural water samples. The method results, in principle, in correct analytical results for multiplicative interference effects. Application of the standard addition method enabled determination of catechol in surface and spring waters at very low concentrations (10^{-7}–10^{-6} mol L^{-1}). Although recoveries of catechol were higher than 100% (up to 124%) the results can be regarded as satisfactory as they fulfil a need for monitoring of contaminants of natural waters. More experiments will be performed, focusing on improvement of the storage stability of the biosensor, for example by organic modification of the sol–gel matrix [17]. In our opinion, preparation of natural water samples for determination of phenolic compounds should also be optimized. The efficiency of the biosensor proposed will be compared with that of other methods for determination of phenols in industrial waters.

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