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Seasonal Variability of Water Parameters and Construction of Laccase Biosensors Using Ureasil Polymers for Analysis of Water Pollution

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Physical and chemical indicators of underground waters of the Skole region were studied. The samples were taken from natural water sources of Skole district of Lviv region and analyzed within organoleptic and physico-chemical indicators of the quality of water. The organoleptic and physico-chemical indicators of the quality of natural water sources of the studied area meet the requirements of the State sanitary norms and rules. Organic-inorganic ureasil polymers with different precursors were used as holding matrixes in construction of laccase biosensors for analysis of water pollution. Cyclic voltammetry and chronoamperometric measurements were carried out to estimate the main parameters of the constructed biosensors. It is found that the sensitivity of the constructed biosensors correlates well with the network properties (free volume and crosslinking) of the investigated polymers studied using positron annihilation lifetime spectroscopy and swelling measurements. The same correlation was also earlier reported for ureasil composites of different prehistory and photopolymers and it seems to be universal that allows controlled biosensing according to the required needs of analysis.

Keywords: underground water, organoleptic and physico-chemical indicators, polymers, positron annihilation, free volume, crosslinking, laccase, biosensors, water pollution.

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Introduction

Ensuring the protection of natural water resources is the main prerequisite for the economic and social development of West Part of Ukraine, in particular, Skole district of Lviv region. Good quality of drinking water is a guarantee of ecological safety and good health of residents and guests of the district [1, 2]. Because of technogenic pressure on the environment it is important to control the quality of drinking water or level of water

pollution. Especially dangerous are xenobiotics – products of the chemical and pharmaceutical industry, which negatively impact on the physiological state of living organisms and have carcinogenic properties even at very low concentrations. Conservation and restoration of water resources is a huge problem for modern society. Some xenobiotics, apart from wastewater treatment plants, are also in the surface and underground waters, because they were only partially removed in the process of the existing technological schemes for cleaning of wastewater. One of

such innovations is creation of highly sensitive biosensors for analysis of the level of water pollution. The one of the most dangerous pollutants of water are xenoestrogens. For example, xenoestrogen Bisphenol A is a monomer that is used for the manufacture of polycarbonate plastic and epoxy resins, which are raw materials for the production of packaging materials for food and drinks. Xenoestrogens, classified as carcinogens, are toxic to healthy compounds that cause disruption of the endocrine system of human and animals.

The commercial laccase was used in the role of catalytic bioselective element of amperometric enzyme biosensor sensitive to different aromatic phenols and amines [3-6]. Laccase (EC 1.10.3.2 p-diphenol: benzenediol oxygen oxidoreductase from *Trametes versicolor*) is a copper containing enzyme which is able to catalyze the oxidation of several phenolic compounds and aromatic amines [7-9]. Laccase is one of the first enzymes, which was proved to have the ability to direct transfer of electrons on the surface of amperometric transducer (see [10-13], for review). A direct electron transfer is possible only with molecules of monolayer of enzyme that is directly in contact with the surface of the electrode in case of the proper orientation of the active center of enzyme in sufficient distance to the electrode for electron transfer. This ability of laccase was used for creation of mediatorless biosensor, and, for the first time, using the urea-silicate or ureasil-based composites as holding matrixes for immobilization of laccase [14]. The constructed laccase biosensor based on the ureasil-chalcogenide glass composite was characterized by a very high sensitivity, but a weak point of the biosensor was very strong unexpected electrochemical noise at chronoamperometric measurement [14].

New perspectives of the ureasil-based polymers for construction of amperometric enzyme biosensors were further found [15-17]. In particular, a correlation between the network properties of the biosensor sensing layers (e.g., free-volume V_h at glass transition temperature T_g and coefficients for the thermal expansion of free-volume voids α_{F1} , α_{F2} as well as their difference ($\alpha_{F2} - \alpha_{F1}$), and swellability or crosslinked density) based on the pure ureasil and ureasil-chalcogenide glass composites of different history (fresh and aged samples) and biosensor characteristics (e.g., a maximal current at substrate saturation I_{max} , apparent Michaelis-Menten constant K_M^{app} to ABTS chosen as a substrate, the slope of the calibration curve B , and the sensitivity of bioelectrodes obtained by means of cycle voltammetry and chronoamperometric analysis) was established. On the other hand, vegetable oil-based photopolymers were also used as a holding matrix in biosensors [18-21]. Amperometric laccase biosensors for analysis of phenol derivatives were constructed using graphite rods (type RW001) as working electrodes and the photocross-linked polymers as a matrix [18]. Such matrix consisted of epoxidized linseed oil (ELO), bisphenol A diglycidyl ether (RD) as reactive diluent and 50 % mixture of triarylsulfonium hexafluorophosphate in propylene carbonate as photoinitiator (PI). The synthesis was made by the reaction of ELO and 10 mol.% or 30 mol.% of RD, using 3 mol.% of PI (ELO/10RD and ELO/30RD, respectively). Again, a correlation between the network properties of the

biosensor sensing layers based on the photocross-linked polymers and biosensor characteristics was observed [18].

Recently, novel sol-gel organic-inorganic hybrids, obtained by co-condensation of two ureasilicate stoichiometric precursors (Precursor 1, consisting siloxane clusters and long polymer chains, and Precursor 2, consisting siloxane clusters and short polymer chains) were prepared and characterized on nanoscale [22], showing possibilities to obtain nanostructured materials with predictable properties for their potential practical application, in particular for analytical biotechnology in construction of ureasil-based amperometric enzyme biosensors.

Thus, the aim of the present work is two-fold: 1) to study the physical and chemical indicators of underground waters taken, as an example, from the Skole district of Lviv region; and 2) to develop the laccase-biosensors for analysis of water pollution using new organic-inorganic ureasil polymers with different precursors (Precursor 1 and Precursor 2) as holding matrixes.

I. Experimental

Samples of natural water sources were taken in the following settlements of Skole district, namely: Source № 1 (Skole town), Source № 2 (Kozyova town), and Source № 3 (V. Sinyovydyne village). Samples of these natural water sources were taken seasonally (winter, spring, summer, and autumn). It is worth noting that these natural water sources are located near the Kyiv-Chop international highway and are often used by motorists and pedestrians to meet their water needs.

These samples were analyzed for organoleptic and physicochemical indicators in accordance with the requirements of the relevant standards, namely: State sanitary norms and rules – “Hygienic requirements for drinking water intended for human consumption” (DSanPin 2.2 .4-171-10) and DSTU 7525: 2014 Drinking water. Requirements and methods of quality control” [1, 2].

For the construction of amperometric biosensors using organic-inorganic ureasil polymers with different precursors, 5 μ l of *Trametes zonate* laccase solution with an activity of 12 U·mg⁻¹ was applied to the surface of graphite rod electrodes (diameter 3.05 mm, working surface area 7.35 mm²) and dried for 10 min at room temperature. The formed enzyme layer was covered with 5 μ l of polymer solution and dried for 15 min at room temperature. The constructed electrodes were washed with 50 mM acetate buffer (AB), pH 4.5 and kept at 4 °C until use.

Cyclic voltammetry and chronoamperometric measurements of laccase-based biosensors with various polymers were performed using an ABTS solution as a calibrator. Ureasil polymers with different precursors (Precursor 1 (ICPTES+Jeffamine ED-600) and Precursor 2 (ICPTES+APTES)) marked as Ureasil-1 (100 mol% Precursor 1 : 0 mol% Precursor 2), Ureasil-2 (30.8 mol% Precursor 1 : 69.2 mol% Precursor 2), and Ureasil-3 (0 mol% Precursor 1 : 100 mol% Precursor 2) were used as prepared in [22]. The measurements were carried out at room temperature in a glass electrochemical cell with a

working volume of 50 ml, filled with 10 ml of 50 mM acetate buffer, pH 4.5. The bioelectrodes were placed in a stirred solution and, after establishing the base signal against Ag/AgCl, increasing concentrations of the analyte were introduced into the cell.

II. Results and Discussion

2.1. Physical and chemical indicators of underground waters.

The research results on physical and chemical indicators of underground waters are presented in Table 1 (numerator-winter, denominator-spring) and Table 2 (numerator-summer, denominator-autumn), respectively.

Diagrams 1-6 present the dynamics of changes in

physico-chemical and organoleptic quality indicators of natural water sources (such as hydrogen index, total hardness, chloride, sulfate, calcium and magnesium content) depending on the season.

The value of the hydrogen pH indicator (diagram 1) does not exceed the norm and practically does not depend on the seasons. However, the highest hydrogen level can be observed in the water source selected in Skole. As can be seen from diagram 2, the total hardness of the studied natural springs does not exceed the norm and practically does not depend on the season. The maximum hardness is observed in the water body of the city of Skole. Diagrams 3 and 4 present the content of chlorides and sulfates in the selected water samples. As can be seen from the obtained diagrams, the highest content of chlorides and sulfates is observed in the natural source of the city of Skole, the

Table 1.

Sanitary and chemical indicators of the quality of drinking water in the winter and spring period.

№	Name of metrics	Units of measurement	Standard	Source №1	Source №2	Source №3
1	smell at 20 °C	points	up to 3	1/1	1/1	1/1
2	coloration	degrees	up to 35	6/7	8/10	9/10
3	feculence	units of turbidity	up to 2.6	0.8/0.9	0.9/1.0	1.1/1.3
4	taste and flavor	points	up to 3	1/1	1/1	1/1
5	pH	units of pH	6.5-8.5	8.1/8.2	7.4/7.6	7.3/7.4
6	total hardness	mmol/dm ³	up to 10	8.5/8.6	7.2/7.5	7.5/7.6
7	chloride content	mg/dm ³	up to 350	46/42	34/36	36/37
8	sulfate content	mg/dm ³	up to 500	64/62	52/54	58/60
9	calcium content	mg/dm ³	not normal	112/110	90/93	89/87
10	magnesium content	mg/dm ³	not normal	67/69	34/35	57/58

Table 2.

Sanitary and chemical indicators of the quality of drinking water in the summer and autumn period

№	Name of metrics	Units of measurement	Standard	Source №1	Source №2	Source №3
1	smell at 20°C	points	up to 3	1/1	1/1	1/1
2	coloration	degrees	up to 35	8/9	11/12	10/12
3	feculence	units of turbidity	up to 2.6	0.8/0.9	0.8/1.0	1.0/1.2
4	taste and flavor	points	up to 3	1/1	1/1	1/1
5	pH	units of pH	6.5-8.5	8.0/8.1	7.4/7.5	7.4/7.4
6	total hardness	mmol/dm ³	up to 10	8.4/8.5	7.2/7.4	7.3/7.4
7	chloride content	mg/dm ³	up to 350	48/49	32/33	38/37
8	sulfate content	mg/dm ³	up to 500	63/32	52/51	58/59
9	calcium content	mg/dm ³	not normal	113/114	91/92	91/92
10	magnesium content	mg/dm ³	not normal	69/71	36/37	58/59

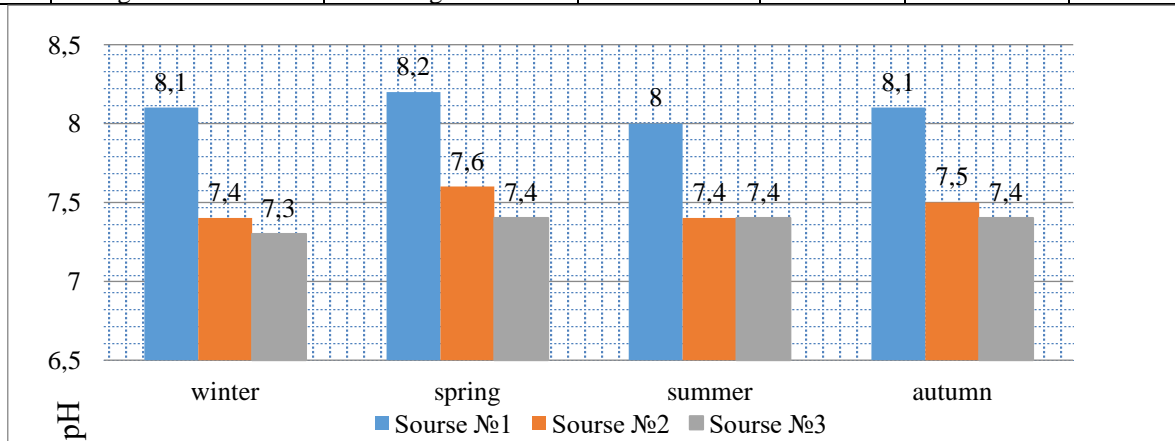


Diagram 1. Season dependence of the pH value of the studied water sources.

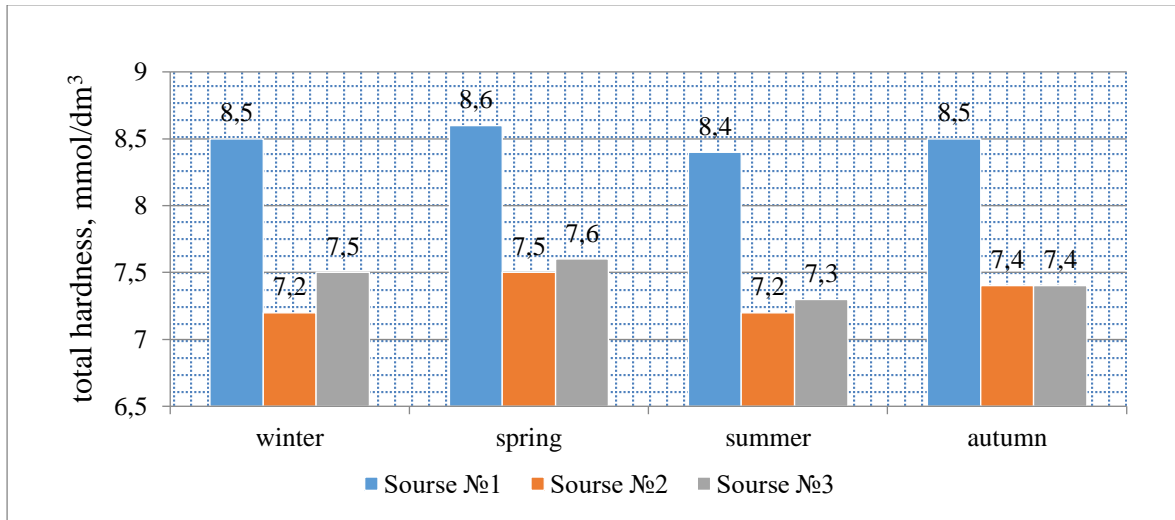


Diagram 2. Season dependence of the value of the total hardness of the studied water sources.

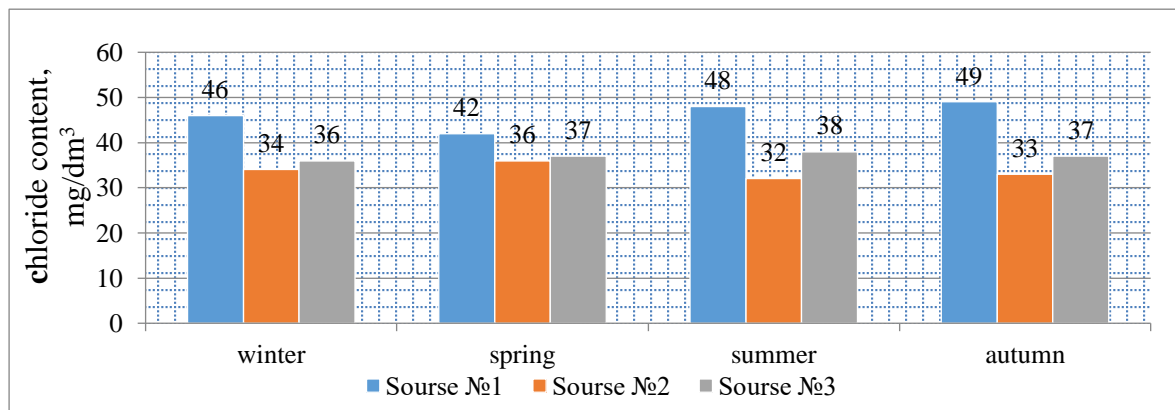


Diagram 3. Season dependence of the chloride content of the investigated water sources.

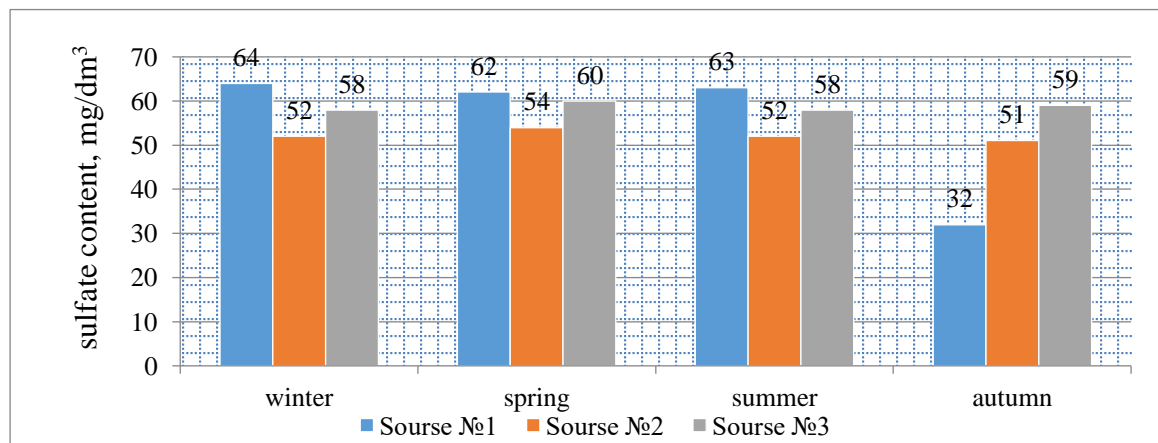


Diagram 4. Season dependence of the sulfate content of the studied water sources.

lowest – in the settlement of Kozyova. Diagrams 5 and 6 show the content of calcium and magnesium ions in the studied water samples. The highest calcium content can be observed in water samples from Skole. Water sampled in Skole and V. Sinyovyadne has the highest content of magnesium ions.

On the basis of the conducted research, it can be concluded that the organoleptic and physico-chemical indicators of the quality of natural water sources of the studied area meet the requirements of the State sanitary norms and rules “Hygienic requirements for drinking

water intended for human consumption” (DSanPin2.2.4-171-10) and DSTU 7525: 2014 Drinking water. Requirements and methods of quality control” [1, 2].

2.2. Ureasil polymers in construction of laccase biosensors.

A working potential was estimated for ureasil samples as -100 mV vs. Ag/AgCl (reference electrode) toward ABTS (Fig. 1). The dependence of the operational parameters of three laccase biosensors with polymers was evaluated according to four main parameters: I_{\max} is the

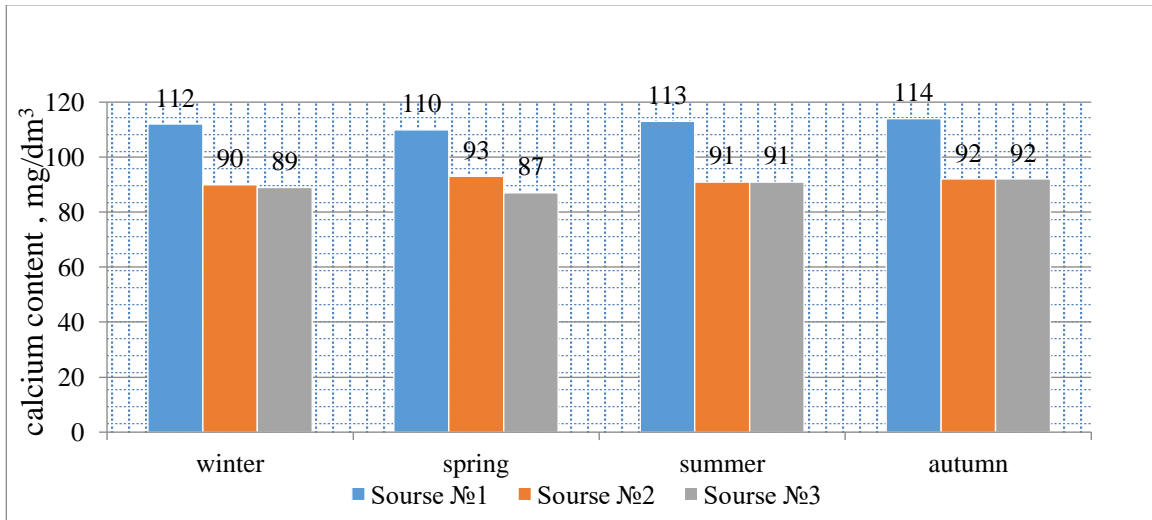


Diagram 5. Season dependence of the amount of calcium content in the studied water sources.

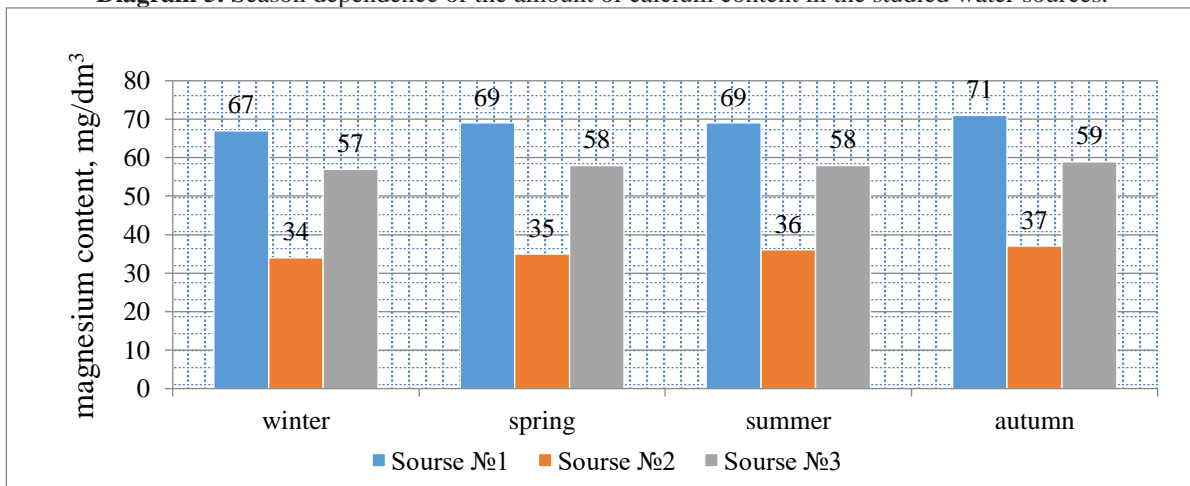


Diagram 6. Dependence of the amount of magnesium content in the studied water sources.

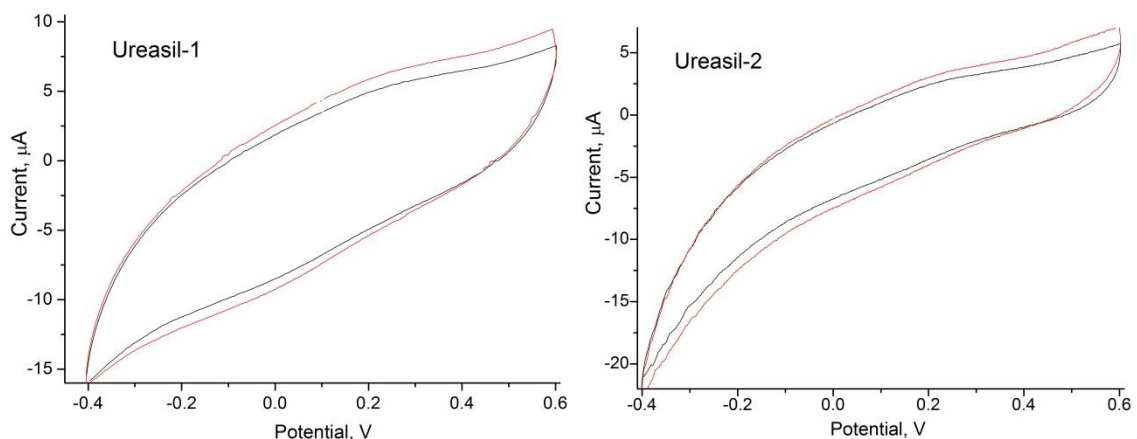


Fig. 1. Cyclic voltammetric response of the bioelectrodes constructed based on laccase immobilized by ureasil samples (Ureasil-1 and Ureasil-2) after addition of an increasing concentration of ABTS.

value of the response of the biosensor when saturated with the substrate; K_M^{app} is the value of the apparent Michaelis-Menten constant; limits of linearity and sensitivity (Table 3).

Figure 2 shows the chronoamperogram and calibration response curves for the laccase/ureasil tested bioelectrodes for adding analyte of ABTS. It was not possible to obtain a good signal using laccase/Ureasil-3 polymer. But it can be seen that sensors with

laccase/Ureasil-1 and laccase/Ureasil-2 polymers demonstrated a high sensitivity and a wide range of linearity (Table 3).

Amperometric characteristics of bioelectrodes using laccase and ureasil composites of different prehistory (fresh and aged samples) [16] and photocross-linked polymers based on epoxidized linseed oil (ELO) [18] were also gathered in Table 3 for comparison.

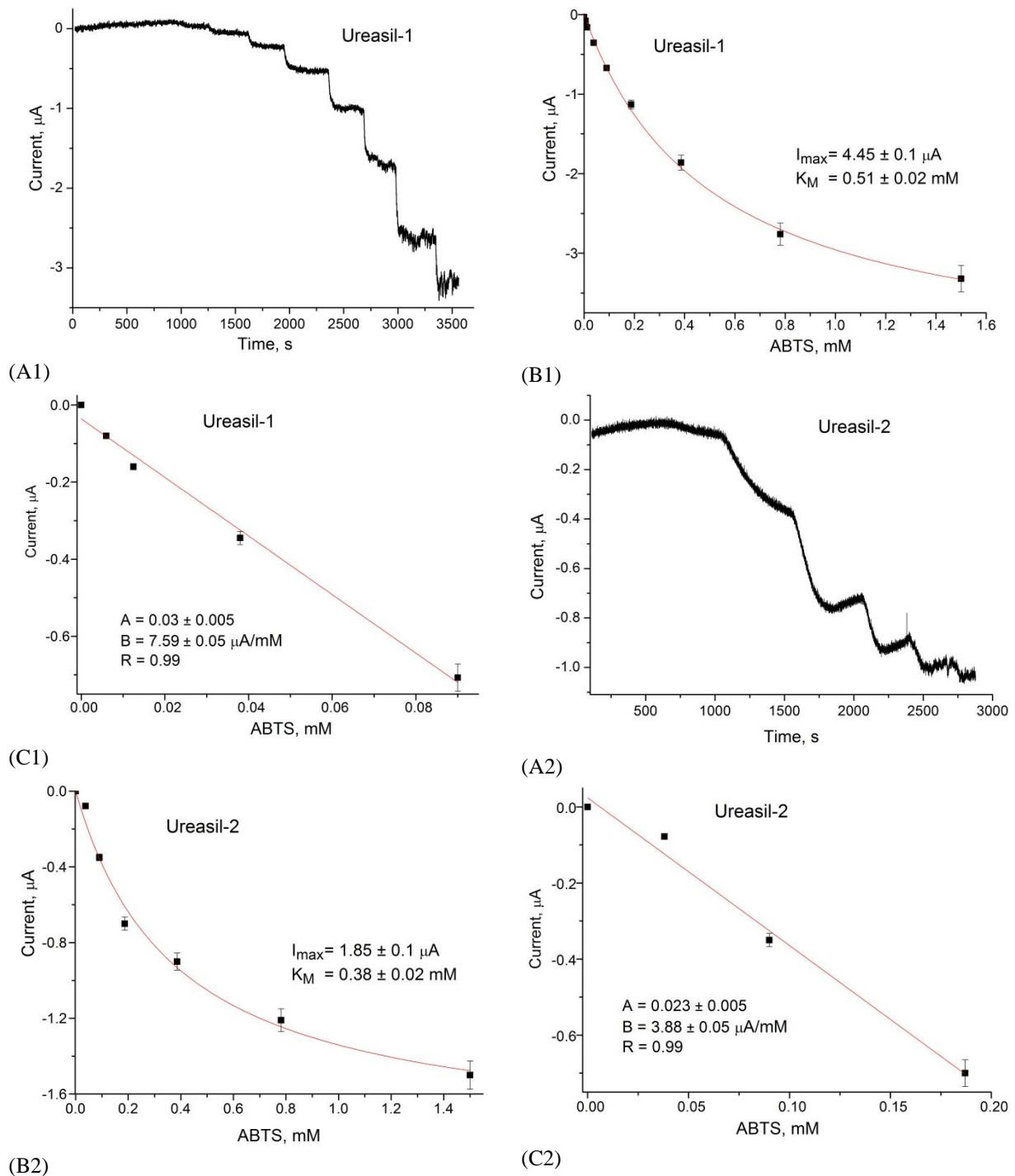


Fig. 2. Amperometric characteristics of bioelectrodes: laccase/Ureasil-1 (A1, B1, C1) and laccase/Ureasil-2 (A2, B2, C2), where chronoamperograms (A1, A2), dependencies of the amperometric signal on the concentration of ABTS (B1, B2), and calibration curve for determining ABTS (C1, C2). Conditions: working potential -100 mV vs. Ag/AgCl/3 M KCl in 50 mM acetate buffer, pH 4.5. Notes: *B* is the slope of the calibration curve; *R* is the linear regression correlation coefficient.

The network properties (free volume and crosslinking) of the investigated ureasil polymers with different precursors, earlier studied using positron annihilation lifetime spectroscopy technique and swelling measurements [22], were taken into account for comparative analysis (Table 4).

A correlation of network properties of polymer matrixes with parameters of electrochemical biosensors was found in a good agreement with the previously

reported data for ureasil composites of different prehistory (fresh and aged samples) [16, 17] and photocross-linked polymers based on epoxidized linseed oil (ELO) [18]. It is concluded that the above mentioned correlation has fundamental origin that allows controlled biosensing according to the required needs of analysis.

Table 3.

Analytical properties of the fabricated laccase-based biosensors toward ABTS

Polymers	I_{\max} (μA)	K_M^{app} (mM)	B ($\mu\text{A}\cdot\text{mM}^{-1}$)	Sensitivity ($\text{A}\cdot\text{M}^{-1}\cdot\text{m}^{-2}$)	Linearity, up to (mM)
Ureasil-1 (this work)	4.45 ± 0.1	0.51 ± 0.02	7.59 ± 0.05	1080	0.10
Ureasil-2 (this work)	1.85 ± 0.1	0.38 ± 0.02	3.88 ± 0.05	552	0.18
Ureasil-3 (this work)	n/a	n/a	n/a	n/a	n/a
K0-fresh [16]	7.62 ± 1.7	0.64 ± 0.17	10.0	794	0.15
K0-aged [16]	10.96 ± 3.4	0.35 ± 0.14	22.2	1762	0.15
K4-fresh [16]	43.77 ± 2.7	0.045 ± 0.005	501.7	39,817	0.04
K4-aged [16]	86.8 ± 0.9	0.030 ± 0.008	761.2	60,413	0.06
ELO/10RD [18]	4.9 ± 0.19	0.36 ± 0.03	12.3	1,673	0.15
ELO/30RD [18]	1.25 ± 0.17	0.11 ± 0.04	9.07	1,234	0.10

Table 4.

Hole volume V_h at glass transition temperature T_g , swellability S in EtOH, and slopes α_{F1} , α_{F2} of the $V_h(T)$ dependences in the regions below and above T_g , respectively, as well as their differences for the ureasil-based [16, 17, 22] and photocross-linked polymers [18]. Values for heating and cooling cycles are in the top and bottom part of the boxes, respectively

Polymers	$V_h(T_g)$ (nm^3)	S (%)	α_{F1} (10^{-4}K^{-1})	α_{F2} (10^{-4}K^{-1})	$\alpha_{F2} - \alpha_{F1}$ (10^{-4}K^{-1})
Ureasil-1 [22]	0.050 ± 0.009	37.8	53 ± 11	213 ± 40	160
	0.050 ± 0.005		44 ± 5	181 ± 20	137
Ureasil-2 [22]	0.049 ± 0.005	22.3	39 ± 5	163 ± 19	124
	0.048 ± 0.001		34 ± 9	131 ± 27	97
Ureasil-3 [22]	n/a	-	n/a	n/a	n/a
	n/a		n/a	n/a	n/a
K0-fresh [16, 17]	0.123 ± 0.002	23.0	25 ± 3	286 ± 21	261
K0-aged [16, 17]	0.123 ± 0.003	11.0	53 ± 22	273 ± 99	220
K4-fresh [16, 17]	0.104 ± 0.001	24.0	48 ± 10	344 ± 63	296
K4-aged [16, 17]	0.123 ± 0.002	19.0	56 ± 22	237 ± 84	181
	0.134 ± 0.001		46 ± 17	206 ± 56	160
ELO/10RD [18]	0.057 ± 0.002	24.09	3.53 ± 0.30	13.02 ± 0.60	9.49 ± 0.67
	0.068 ± 0.002		3.31 ± 0.32	11.16 ± 0.55	7.85 ± 0.64
ELO/30RD [18]	0.051 ± 0.002	24.81	3.47 ± 0.33	12.42 ± 0.64	8.95 ± 0.72
	0.049 ± 0.002		3.87 ± 0.83	8.96 ± 0.48	5.09 ± 0.96

Conclusions

The investigating the physical and chemical indicators of underground waters taken from the Skole district of Lviv region it can be concluded that the organoleptic and physico-chemical indicators of the quality of natural water sources of the studied area meet the requirements of the State sanitary norms and rules – “Hygienic requirements for drinking water intended for human consumption” (DSanPin2.2.4-171-10) and DSTU 7525: 2014 Drinking water. Requirements and methods of quality control”.

Using comprehensive analysis of the results obtained on the nanostructure and detection properties of ureasil polymers with different precursors (Precursor 1, consisting siloxane clusters and long polymer chains, and Precursor 2, consisting siloxane clusters and short

polymer chains) and prehistory (fresh and aged samples) as well as photocross-linked polymers of different composition, a correlation of network properties of polymer matrixes with parameters of electrochemical biosensors is verified and it seems to be universal. The development of the predictable laccase biosensor can be used for assay of phenolic compounds in the wastewater and drinking water according to the required needs of analysis (i.e., for the high-content samples (technological wastewater) the biosensor should be less sensitive to avoid multistep dilution affecting the assay accuracy, while for the assay of low-content samples (drinking water) it is critically important to use a highly sensitive biosensor with a wide linearity).

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Сезонна мінливість параметрів води та конструювання лакказних біосенсорів з використанням уреасильних полімерів для аналізу забруднення води

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Досліджено фізико-хімічні показники підземних вод Сколівського району. Проби відібрано з природних джерел води Сколівського району Львівської області та досліджено на органолептичні та фізико-хімічні показники якості води. Органолептичні та фізико-хімічні показники якості природних джерел води досліджуваної території відповідають вимогам державних санітарних норм і правил. Органічно-неорганічні уреасильні полімери з різними прекурсорами використовувалися як утримуючі матриці при конструюванні лакказних біосенсорів для аналізу забруднення води. Для оцінки основних параметрів сконструйованих біосенсорів проведено циклічні вольтамперометричні та хроноамперометричні вимірювання. Виявлено, що чутливість сконструйованих біосенсорів добре корелює з сітковими властивостями (вільний об'єм і зшивання) досліджуваних полімерів, вивчених за допомогою спектроскопії часу життя анігіляції позитронів і вимірювань набухання. Така ж кореляція була також раніше повідомлена для уреасильних композитів різної передісторії та фотополімерів, і вона, мабуть, є універсальною, що дозволяє контролювати біодетекцію відповідно до необхідних потреб аналізу.

Ключові слова: підземні води, органолептичні та фізико-хімічні показники, полімери, позитронна анігіляція, вільний об'єм, зшивання, лаккази, біосенсори, забруднення води.