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APPLICATION OF GLUTAMATE-SENSITIVE BIOSENSOR FOR ANALYSIS OF FOODSTUFF

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The aim of the work were the optimization of an amperometric glutamate-sensitive biosensor and its utilization for the determination of the glutamate concentrations in food samples. Amperometric method of measurements was used. The biosensor was based on immobilized glutamate oxidase and platinum disc electrode. The biosensor was connected to the working cell with auxiliary (platinum wire) and reference (Ag/AgCl) electrodes. The biosensor exhibited high sensitivity to glutamate, duration of one analysis was about 5 min. An influence of the ionic strength, pH, and buffer capacity on the biosensor operation was investigated. The sensitivity of biosensor to various possible interfering substances, including amino acids, was studied; high selectivity to glutamate was shown. The reproducibility of analysis of food samples and an impact of sample dilution was evaluated. Glutamate concentrations in different sauces and seasonings were measured by the developed biosensor; the results correlated well with those obtained by the spectrophotometric method ($R^2 = 0{,}988$).

Thus, the amperometric biosensor for glutamate determination was successfully optimized and used for measurement of glutamate concentrations in sauces and seasonings.

Key words: amperometric biosensor, glutamate oxidase, poly(phenylenediamine), glutamate, food samples.

Glutamate (in the form of glutamic acid sodium salt) is a well-known nutritional supplement often used as a flavor enhancer in soups, sauces, chips, seasonings and other types of foods. It can become habit-forming, which makes people consume more and more products with high glutamate level leading to obesity and cardiovascular disease. In some people, excess of glutamate in the body can cause dizziness, headache, numbness, chest pain [1, 2]. Moreover, excessive intake of glutamate can result in disturbance of the endogenous glutamate metabolism, which is associated with the emergence of such diseases as Alzheimer's and Parkinson's, and amyotrophic lateral sclerosis [3, 4].

One more cause of necessity to control the glutamate content is the prevention and detection of falsified products. Considering the glutamate property to give a pleasant taste to almost any product, dishonest manufacturers might add glutamate to inferior foodstuff. Therefore, to avoid the negative consequences of consuming poor-quality products, it is necessary to develop new express methods for glutamate monitoring.

The methods mainly used for glutamate determination are following: high-performance liquid chromatography, capillary electrophoresis, potentiometric and conductometric titration, gas chromatography [5–7]. Combination of enzymatic methods with spectrophotometry can be used too [8]. Additionally, the method of chemiluminiscent determination is reported involving luminol and potassium ferricyanide and using a luminophotometer [9].

However, the above methods involve difficult and time-consuming procedures and complicated sample pretreatment, they are unsuitable for quick analysis of a large number of samples and require complex expensive equipment and skilled personnel [10].

The use of biosensors can be an alternative to these methods. Amperometric biosensors are the most promising and frequently used for glutamate determination. However, high

working potential of amperometric biosensors can cause the oxidation of electrochemically active compounds present in the sample on the transducer surface. To avoid the influence of this effect, different methods are used: modification of the electrode surface, application of various mediators, bienzyme electrodes, combination of several membranes, etc. Several types of glutamate biosensors were reported, including those based on L-glutamate oxidase, L-glutamate dehydrogenase, L-glutamate synthase and L-glutamate decarboxylase [11–15]. L-glutamate oxidase, as compared with the rest of mentioned enzymes, provides a relatively high specificity to the substrate and does not require additional expensive coenzyme NAD^+, thus it is used most frequently for the glutamate determination [15–17].

The glutamate concentration is most often evaluated via detecting hydrogen peroxide or ammonia using electrochemical transducers, occasionally — via detecting oxygen consumption by a fiber optic sensor, which registers the changes in luminescence of a special layer sensitive to the oxygen concentration [18].

An optical biosensor in the test-strip format was created for glutamate analysis in food samples [19]. The peculiarity of this biosensor consists in simultaneous immobilization of several sensitive agents (one indicator dye — 3,3′,5,5′-tetramethylbenzidine, and two enzymes — L-glutamate oxidase and horseradish peroxidase) using a composite membrane system with non-covalent bonding of sensitive components. Several sensors combined with flow-injection systems were created for glutamate determination. For example, a biosensor based on a gold electrode with immobilized glutamate oxidase and a polyionic complex for preventing electrochemical interferents was described [20]; in another work a solid-state electrode based on non-plasticized chitosan was proposed for glutamate determination in food samples (seasoning and soups) [21]. However, the mentioned works do not present reproducibility of biosensor preparation, and have insufficient examination of the biosensors selectivity towards possible interfering substances. Furthermore, the possibility of application of the biosensors for multiple measurements of food samples was not shown in the most cases. We have earlier developed a biosensor for the monitoring glutamate uptake and release from the isolated brain nerve terminals and showed good correlation of the biosensor results with those obtained using radiolabelled glutamate assay, spectrofluorimetric glutamate dehydrogenase assay and amino acid analyzer [22, 23]. In this work we wanted to optimize and apply the biosensor for a food analysis.

The purposes of this work were optimization of the amperometric glutamate-sensitive biosensor based on glutamate oxidase, comprehensive analysis of its selectivity, reproducibility of preparation and other characteristics and application for measurements of the glutamate concentration in food products — sauces and seasonings.

Materials and Methods

Materials. In this work recombinant glutamate oxidase (GluOx, EC 1.4.3.11) from *Streptomyces* sp. with activity of 7 U/mg (Yamasa Corporation, Tokyo, Japan) was used for biosensor creation. For spectrophotometric measurements, horseradish peroxidase (EC 1.11.1.7) with activity of 150 U/mg from Sigma-Aldrich Chemie (USA) was used. Bovine serum albumin (BSA, fraction V), glycerol, HEPES, *m*-phenylenediamine, 4-aminoantipyrine, 3-(N-Ethyl-3-methylanilino)-2-hydroxypropanesulfonic acid sodium salt (TOOS), dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA) disodium salt, NaN_3 and 25% aqueous glutaraldehyde solution were obtained from Sigma-Aldrich Chemie (USA). Monosodium L-glutamate (as a substrate of glutamate oxidase) was from Sigma-Aldrich Chemie (USA). Other inorganic compounds were domestically produced and had analytical grade of purity.

Sauces and seasonings were bought in supermarkets in Kyiv (Ukraine).

Preparation of biosensor. In the work, platinum disk electrode was utilized as amperometric transducer. Its scheme and preparation procedure was presented in [23]. The transducers were used repeatedly; their sensitive surfaces were cleaned with ethyl alcohol and cotton wool before every procedure of enzyme immobilization.

First, the transducers were modified with phenylenediamine membrane to improve the selectivity of the biosensor. The membrane was deposited according to the procedure described in [24].

Biorecognition elements of the biosensors were obtained by covalent immobilization of the GluOx in BSA membrane onto the sensitive surface of amperometric transducers. The

initial solution contained 8% of GluOx (hereafter — mass fraction), 4% of BSA, and 10% glycerol in 100 mM phosphate buffer, pH 6.5. Glycerol was added to stabilize the enzyme during its immobilization, to prevent early drying of the solution and to improve the membrane adhesion to the transducer surface. This solution was mixed with 0.8% aqueous solution of glutaraldehyde (crosslinking agent) in a ratio of 1:1 and immediately afterwards deposited (drop-casted) onto the transducer, which were next air dried for 30 min at room temperature. Final deposited volume of the enzyme/glutaraldehyde mixture was approximately 0.1 µl. After immobilization, the biosensors were washed in a working buffer solution from unbound components of the biomembrane and excess of glutaraldehyde.

Measuring procedure. The three-electrode circuit of amperometric analysis was used in this work. Working amperometric electrodes, auxiliary platinum electrode and Ag/AgCl reference electrode were connected to PalmSens potentiostat (Palm Instruments BV, the Netherlands). General view of the biosensor setup is presented in Fig. 1. The measurements were carried out at room temperature in a 3.5 ml open measuring cell at continuous stirring and constant working potential of +0.6 V vs Ag/AgCl reference electrode, which corresponds to the anodic oxidation of hydrogen peroxide. 25 mM HEPES buffer, pH 7.4, served as a working buffer. Concentration of substrates in the working cell was obtained by adding aliquots of the substrate stock solutions. All experiments were performed at least in three replicates. The data shown in the tables and figures are the average of three experiments ± standard deviation.

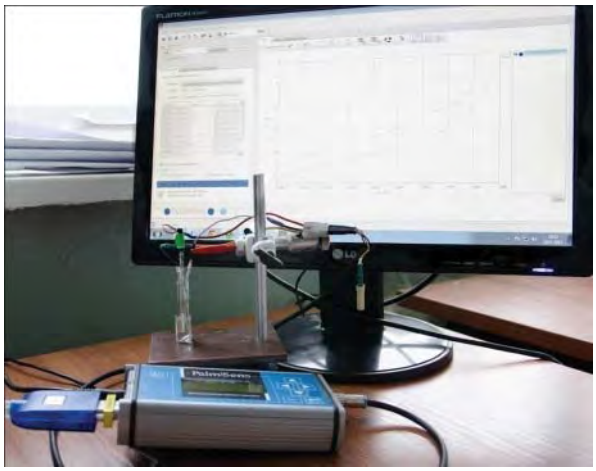

Fig. 1. **General view of the biosensor setup (potentiostat, working cell with three electrodes, and computer)**

Methods of biosensor analysis of glutamate in real samples. Before measurements, the sauces were 10-fold diluted with distilled water. The aliquots of this solution were next added to the measuring cell containing working buffer. The seasonings (dry powder) were dissolved in hot distilled water; the mass fraction of seasoning in solution was 1%. After cooling, the seasoning solutions were filtered through a filter paper for removal of dried vegetables and other insoluble components.

Two methods were utilized for measurement — comparison with the calibration curve and method of standard additions. In the first method, the glutamate concentration was determined by comparing the biosensor response after the sample addition to the measuring cell with the previously obtained calibration graph. In the standard additions method, first a biosensor response to the sample was measured, afterwards the glutamate model solution was added to the working cell three times in turn and responses were measured (without washing between the measurements). Thus, four sequential responses were obtained (in the form of a stairs). Based on the data received, a line chart was plotted — the glutamate concentrations in solution were put on the X-axis, the biosensor responses (in nA) — on the Y-axis; the unknown glutamate concentration (the first response) was taken as a zero X value. Linear extrapolation of this curve crosses the X-axis at a point corresponding to the analyte concentration in the tested sample.

Determination of glutamate in real samples by spectrophotometric method. Spectrophotometric measurements were carried out in 1 ml disposable plastic cuvettes. The composition of solution in the cuvette was as follows: 25 mM HEPES buffer, pH 7.4, 0.3 mM TOOS, 0.1 mM 4-aminoantipyrine, horseradish peroxidase (1.8 U), and an aliquot of glutamate sample (sauce, seasoning or model solution). The reaction started after the addition of GluOx (0.112 U) to the cuvette. In the course of reaction, GluOx oxidized glutamate to α-ketoglutarate and produce hydrogen peroxide. Horseradish peroxidase in the presence of hydrogen peroxide formed a colored (violet) product through oxidation of TOOS and 4-aminoantipyrine. The intensity of the solution color was proportional to the hydrogen peroxide concentration, and hence to the glutamate concentration. The reaction was carried out for 8 min (the time of incubation was determined previously to attain the maximum level of the solution color).

The intensity of light absorption by solution was measured by the Thermo Electron Corporation Bio Mate 5 spectrophotometer. The light wavelength was 555 nm. It was chosen because the highest peak of light absorption by the reaction product was observed at this wavelength. Before the GluOx addition, the blank light absorbance was determined for each solution. This value was subtracted from the value of post-reaction absorbance (in most cases, the basic absorbance was insignificant and could be ignored, except for the most colored sauces).

To obtain a calibration curve, the aliquots of a model glutamate solution were sequentially added to the cuvette with 8-min intervals and the absorbance values were fixed. Totally, six additions were made up to the final glutamate concentration of 120 μM. The calibration curve of dependence of absorbance on the glutamate concentration was then plotted; actually, it was a straight line . The glutamate concentration in sauces and seasonings was determined comparing the samples absorbance with this calibration curve.

The glutamate concentration in the sample was also determined by a proportion (simplified method of standard additions). After measurement of the post-reaction absorbance of the food sample, a model glutamate solution was added to the same cuvette up to the final glutamate concentration of 20 μM; after 8-min interval, the sample absorbance was measured again. The initial (unknown) glutamate concentration in the sample was determined by the ratio between the values of absorbance before and after addition of 20 μM glutamate.

Results and Discussion

Principle of biosensor operation. Determination of glutamate by the amperometric biosensor is based on the enzymatic reaction (1) in the biorecognition membrane, which results in the oxidation of glutamate and the formation of hydrogen peroxide. A positive potential (+0.6 V vs Ag/AgCl) was applied to the transducer, and for this reason hydrogen peroxide was decomposed in reaction (2), resulting in the formation of electrons, which were directly registered by the amperometric transducer:

$$\text{Glutamate} + O_2 \xrightarrow{\text{GluOx}} \alpha\text{-ketoglutarate} + NH_3 + H_2O_2 \qquad (1)$$

$$H_2O_2 \xrightarrow{+600\ \text{mV}} 2H^+ + O_2 + 2e^- \qquad (2)$$

The biosensor response occured immediately after the glutamate addition and the maximum response was observed in 1.5 min.

Influence of the working buffer parameters on the biosensor operation. It is known that the work of any biosensor depends on both its own characteristics and the properties of working buffer solution, in which the measurements are carried out, namely, ionic strength, pH and buffer capacity. Food products, in particular sauces and seasonings, are characterized by a significant ionic strength due to the presence of nutritional additives such as sodium chloride, various acids, preservatives, etc. The ionic strength of solution also depends on the buffer concentration. Therefore, the biosensor operation was studied depending on the value of ionic strength. As a source of ions, different aliquots of 3.3 M NaCl solution were added to the working cell. Next, the biosensor responses to 100 μM glutamate were measured. No significant changes in the biosensor response to glutamate were observed at various NaCl concentrations in the working cell, which is typical for amperometric method of detection.

Optimum pH of enzymes can be changed during immobilization. Therefore, the effect of buffer pH on the work of developed amperometric biosensor was studied. A universal buffer (containing Tris-HCl, KH_2PO_4, citric acid and sodium tetraborate in concentrations of 10 mM) was used. This buffer has the same capacity in a wide pH range. The pH values in experiments ranged from 5 to 10. The highest biosensor responses were observed in the pH range of 7–8.5.

The effect of buffer concentration (buffer capacity) was also investigated. The experiment showed that an increase of buffer concentration from 5 to 100 mM did not affect significantly the sensitivity of biosensor to glutamate, which allows utilization of the developed biosensor to determine glutamate in samples characterized by various buffer capacities.

Response reproducibility and biosensor storage. Response reproducibility is one of the main working characteristics of a biosensor as it is necessary to get accurate results over all the period of measurements. Therefore, the reproducibility of biosensor responses to glutamate was investigated over a prolonged

period of continuous work. One measurement of glutamate took 3–5 min, the intervals between measurements were about 10 min. During intervals, the biosensors were washed from the substrate with the working buffer changing it several times. No noticeable drop in the responses was observed over 10 measurements. The relative standard deviation of responses was 3%.

To determine the optimum conditions of storage, the developed biosensor was tested when storing for a long time. The total period of storage was 65 days. The biosensors were stored under the following conditions: at +25 °C and +4 °C in a dry state and in 25 mM HEPES buffer; in a dry state at −18 °C. Additional substances (1 mM DTT, 1 mM EDTA and 0.1% NaN_3) were added to the buffer during storage to improve stability of GluOx.

When stored at + 25 °C, the biosensors lost their sensitivity during several days. At storage temperatures of +4 °C and −18 °C, the biosensors were stable for a much longer time. After 65-day storage, the highest responses to glutamate demonstrated the biosensors stored in the buffer at +4 °C, the responses decreased by only 15%. However, over the storage period the biosensor was unstable, its sensitivity to glutamate changed to a greater or lesser extent due to the interaction of bioselective membrane with the components of buffer solution.

Significantly worse results were observed when the biosensor was stored in a dry state at +4 °C; by the end of the storage period the responses equaled 35% of their initial values. However, this method of storage can be used if necessary since the response decreases most significantly during the first 20 days of storage, afterwards the biosensor was quite stable. The most predicted was storage at −18 °C since the biosensor sensitivity to glutamate decreased at approximately the same speed, and a decrease in responses at the end of storage was 30%. These results prove the possibility of using biosensor after prolonged storage, but for accurate results recalibration of the biosensor is required before use.

Selectivity of biosensor. The developed GluOx-based biosensor is intended for glutamate determination in food products. Nevertheless, there are many additional substances (e.g. ascorbic acid, cysteine, benzoic acid, etc) in these food products, which can be either oxidized on the electrode upon application of the potential (electroactive substances) or can be substrates for GluOx; in both cases, presence of such interfering substances can cause non-specific biosensor response and lead to errors in measurements. To avoid impact of electroactive substances on the biosensor response, permselective membrane based on polymerized phenylenediamine (PPD) was deposited on the electrodes before immobilization of GluOx. Effectiveness of the PPD membrane was tested earlier [25, 26].

To test the biosensor selectivity, we tried to obtain biosensor responses to possible interfering substances. It turned out that there was no biosensor response to glucose, citric acid, benzoic acid, sodium azide, α-ketoglutarate, urea, EDTA, NaCl, KCl and $CaCl_2$ in concentration of 1 mM, and the presence of these substances in a working cell did not affect the biosensor response to glutamate.

The biosensor sensitivity to amino acids was also examined (Fig. 2).

The biosensor did not respond to most amino acids. Low sensitivity to asparagine, aspartic acid, glutamine and histidine was observed, but sensitivity to glutamate was 50–100-fold higher; therefore, the presence of amino acids in the sample even in high concentrations could not lead to measurement errors. These results coincide with the data obtained by another team of researchers for a different GluOx-based biosensor [27]. The biosensor sensitivity to some amino acids can be explained by the fact that GlOx is not perfectly selective to glutamate; additionally, trace amounts of other enzymes, such as L-amino acid oxidase, remained after GluOx purification.

According to these results, GluOx-based biosensor is sufficiently selective to glutamate and is suitable for the measurements of real food samples.

Analytical characteristics of developed biosensor. The limit of detection of biosensor for glutamate is defined as the glutamate concentration, the response to which equals three-fold the baseline noise; when using 25 mM HEPES buffer, pH 7.4, it was 0.5–2 μM. The linear working range was from 2 to 700 μM, the sensitivity to glutamate 200–230 nA/mM. The biosensor response time was 5–20 s.

A typical calibration curve of the biosensor for glutamate determination is shown in Fig. 3. The linear part of this calibration curve is described by the equation $I = 210*C + 0.3$ ($R^2 = 0.999$), where I is the steady-

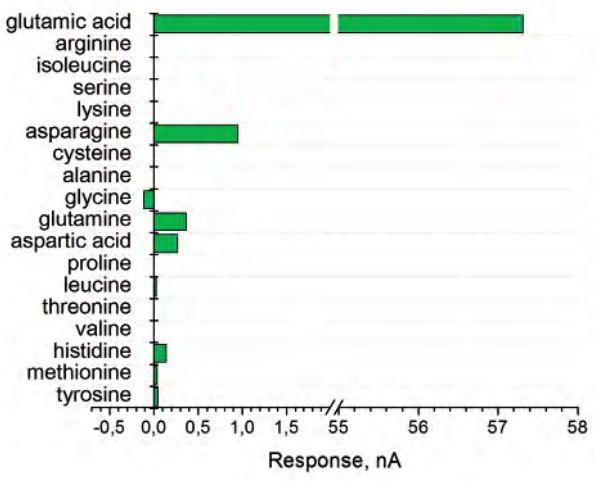

Fig. 2. **Biosensor responses to various amino acids:**
concentration of each amino acid was 1 mM. Measurements were carried out in 25 mM HEPES buffer, pH 7.4, at a constant potential of +0.6 V vs Ag/AgCl reference electrode

state current (nA), C is the concentration of glutamate (mM).

Reproducibility of biosensor preparation. Reproducibility of preparation of biosensors is very important for their large-scale manufacturing. To study this characteristic 11 different biosensors were prepared and the responses of these biosensors to 200 μM glutamate were compared. The relative standard deviation of responses of various biosensors to glutamate was 6%, which is acceptable. The calibration curves of the created biosensors for glutamate determination were studied. They had the same shape and the same linear range. Given that the biosensors were prepared manually, the received reproducibility of characteristics of different biosensors is very good. The difference in the response values did not pose a problem since before use each biosensor was calibrated.

Determination of glutamate concentrations in food samples. To confirm the possibility of practical use of the biosensor, glutamate concentration in real samples was analyzed. The sauces and seasonings were purchased in the supermarket. Before the measurements the sauces were 10-fold diluted with distilled water, and 1% solutions of dry seasonings were prepared. Two methods of glutamate determination (using calibration curve and standard additions) were used, which are described in section 2.5. In total, 6 sauces and 4 seasonings were analyzed. The spectrophotometric method of glutamate determination was used as control. The results are shown in Table 1. The results are expressed not in moles but in mass fraction since it is not possible to determine molar concentration in dry seasonings. Noteworthy, the method of standard additions showed a better correlation with the spectrophotometric one than using the calibration curve. This is because the method of standard additions takes into account an impact of components of the real sample on the biosensor sensitivity to glutamate. However, use of this method often requires preliminary measurement of the glutamate concentration in the tested sample by the calibration curve. It is necessary in order to determine needed dilution of the tested sample, at which the response to the sample and three responses to the model glutamate solution (according to the method of standard addition) were within the linear range of biosensor analysis. A correlation graph was plotted (Fig. 4). As seen, the results of biosensor measurement well correlated with the control spectrophotometric method (R^2 = 0,988); results are well distributed along the theoretical line.

Reproducibility of analysis of glutamate in a food sample. To study the biosensor accuracy, the reproducibility of glutamate determination in a single sample was evaluated. The aliquots of 10-fold dissolved sauce were added 12 times to the biosensor working cell (with washing of the biosensor between the additions), and the obtained responses were analyzed (Fig. 5).

In the working cell, the sample was 50-fold diluted, thus, the initial sample was diluted in total by 500 times. The relative standard deviation of the biosensor responses was 2.7%, no decrease of responses during

Table 1. **Results of determination of glutamate concentrations in sauces and seasonings**

Measured samples	Biosensor, mass fraction (%)		Spectrophotometry, mass fraction (%)	
	Calibration curve	Standard additions	Calibration curve	Proportion
Sauce #1	0.49	0.52	0.54	0.68
Sauce #2	0.36	0.45	0.39	0.46
Sauce #3	1.08	1.23	1.41	1.57
Sauce #4	1.74	2.11	2.32	2.45
Sauce #5	1.17	1.27	1.14	1.13
Sauce #6	1.92	2.45	2.39	2.22
Seasoning #1	5.39	5.64	5.31	6.65
Seasoning #2	9.68	9.73	9.16	10.18
Seasoning #3	5.62	5.56	5.17	6.33
Seasoning #4	4.61	4.57	4.16	4.74

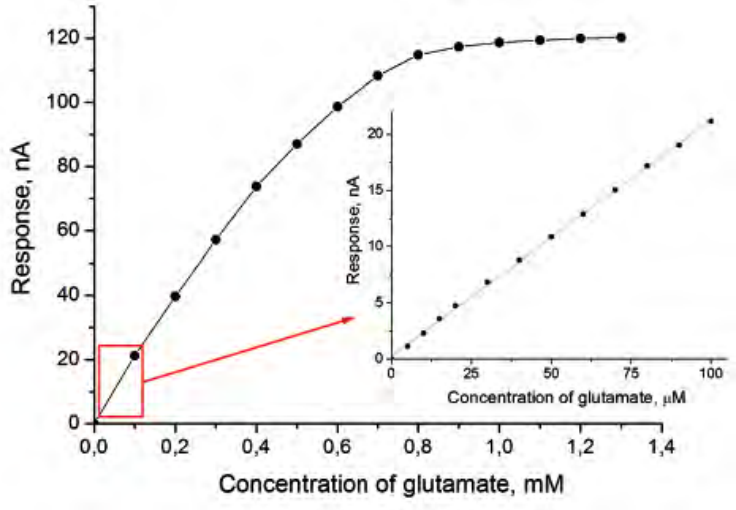

Fig. 3. **Calibration curve of biosensor for glutamate determination:**
measurements were carried out in 25 mM HEPES buffer, pH 7.4, at a constant potential of +0.6 V vs Ag/AgCl reference electrode

12 measurements was observed. This indicates high accuracy and stability of the biosensor during real samples analysis. However, after a larger number of measurements, the biosensor recalibration is required, since the components of samples likely interact with the bioselective membrane and could change the biosensor sensitivity to glutamate.

Determination of glutamate at different dilutions of food samples. For correct determination of the analyte, its concentration in the working cell should be within the linear range of biosensor analysis. When determining glutamate in food samples, significant dilution of the food samples is commonly required due to high concentration of glutamate in it. Use of different dilutions might affect the results of measurement of glutamate concentration; therefore, an impact of the dilution rate was investigated. In the experiment, different volumes of non-diluted sauce were added to the cell and glutamate concentration in the samples was

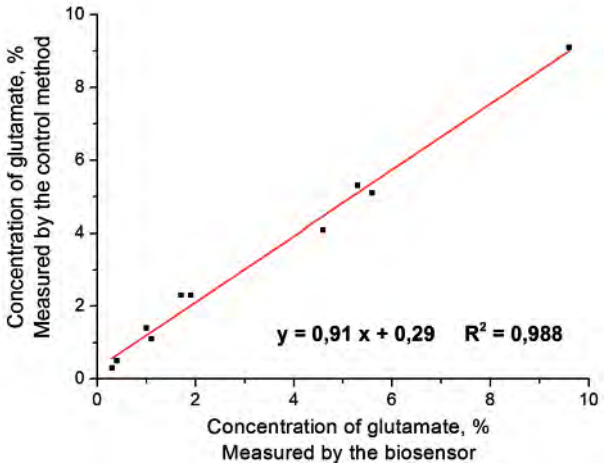

Fig. 4. **Correlation between biosensor and spectrophotometric determination of glutamate concentrations in food samples**

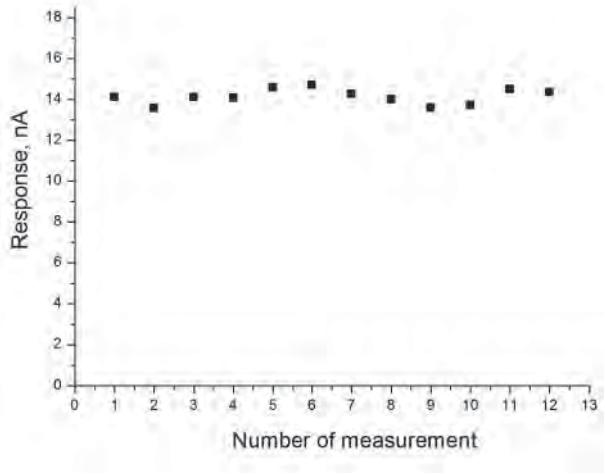

Fig. 5. **Reproducibility of glutamate determination in a single sample of sauce: measurements were carried out in 25 mM HEPES buffer, pH 7.4, at a constant potential of +0.6 V vs Ag/AgCl reference electrode**

determined. Four different rates of sample dilution were further used. Since initially the sauce was not diluted, small volumes of the sample were added to get within the linear range of biosensor analysis.

The results of experiments are presented in Table 2. As seen, the glutamate concentrations in the sauce determined at different dilutions are practically the same. This demonstrates the possibility of using different dilutions of samples. The main condition of accurate results is hitting of the glutamate concentration (in the working cell) in the linear range of biosensor analysis.

Thus, the amperometric biosensor for glutamate determination was optimized and used for measurement of glutamate concentrations in sauces and seasonings.

An influence of the composition of working buffer on the biosensor analytical characteristics was investigated. The highest responses were in the pH range of 7–8.5. Buffer capacity and ionic strength did not affect the biosensor response. The reproducibility of biosensor responses to glutamate over one day was studied; the relative standard deviation of response was 3%. Different conditions of the biosensor storage were evaluated. The storage at –18 °C was preferable, since in this case the biosensor sensitivity to glutamate decreased at approximately the same speed, and the drop in responses by the end of storage period

Table 2. **Results of the determination of glutamate concentration in sauce obtained with different dilutions of the sauce in working cell**

Sample dilution, times	Sample volume, added to the working cell, μL	Response of biosensor, nA	Concentration of glutamate in sauce, mM
2917	1.2	8.6	166.1 ± 10.9
1522	2.3	15.2	167.4 ± 10.7
1000	3.5	23.6	168.7 ± 6.9
700	5	29.1	165.1 ± 2.4

(2 month) was 30%. The results indicate the possibility of using biosensor after prolonged storage, but recalibration is necessary for repeated application.

The reproducibility of biosensor preparation was checked. The calibration curves of all tested biosensors were of the same form and had the same linear range of detection. The relative standard deviation of responses to glutamate for various biosensors was 6%, which is acceptable. The biosensor sensitivity to various interfering substances, including amino acids, was studied and it shown that the biosensor is highly selective to glutamate.

Concentrations of glutamate in 6 sauces and 4 seasonings were measured using the developed biosensor and spectrophotometric method, good correlation (R^2 = 0,988) of the results was demonstrated. Duration of analysis of one sample was about 5 min.

Additionally, it was shown that the relative standard deviation of the biosensor responses to the glutamate in food sample was 2.7%; the level of sample dilution did not influence the results of measurement of glutamate concentration.

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ВИКОРИСТАННЯ ГЛУТАМАТ-ЧУТЛИВОГО БІОСЕНСОРА | ДЛЯ АНАЛІЗУ ХАРЧОВИХ ПРОДУКТІВ

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Метою роботи були оптимізація амперометричного глутаматчутливого біосенсора і його використання для визначення концентрацій глутамату в зразках їжі. Застосовували амперометричний метод вимірювань. Біосенсор базувався на іммобілізованій глутаматоксидазі та платиновому дисковому електроді. Біосенсор був підключений до робочої комірки з допоміжним електродом (платиновим дротом) і електродом порівняння (Ag/AgCl). Біосенсор мав високу чутливість до глутамату, тривалість одного аналізу становила близько 5 хв. Досліджено вплив іонної сили, pH і буферної ємності на роботу біосенсора. Вивчено чутливість біосенсора до різних можливих інтерферуючих речовин, включаючи амінокислоти; показана висока селективність до глутамату. Було оцінено відтворюваність аналізу зразків харчових продуктів та вплив розведення зразків. Концентрації глутамату в різних соусах і приправах вимірювали за допомогою розробленого біосенсора; результати добре корелювали з результатами, отриманими спектрофотометричним методом ($R^2 = 0,988$).

Таким чином, амперометричний біосенсор для визначення глутамату було успішно оптимізовано і використано для вимірювання концентрації глутамату в соусах і приправах.

Ключові слова: амперометричний біосенсор, глутаматоксидаза, полі(фенілендіамин), глутамат, харчові зразки.

ИСПОЛЬЗОВАНИЕ ГЛУТАМАТ-ЧУВСТВИТЕЛЬНОГО БИОСЕНСОРА ДЛЯ АНАЛИЗА ПРОДУКТОВ ПИТАНИЯ

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Целью работы были оптимизация амперометрического глутаматчувствительного биосенсора и его использование для определения концентраций глутамата в образцах пищи. Применяли амперометрический метод измерений. Биосенсор был основан на иммобилизованной глутаматоксидазе и платиновом дисковом электроде. Биосенсор был подключен к рабочей ячейке со вспомогательным электродом (платиновой проволокой) и электродом сравнения (Ag/AgCl). Биосенсор имел высокую чувствительность к глутамату, продолжительность одного анализа составляла около 5 мин. Исследовано влияние ионной силы, pH и буферной емкости на работу биосенсора. Изучена чувствительность биосенсора к различным возможным интерферирующим веществам, включая аминокислоты; показана высокая селективность к глутамату. Была оценена воспроизводимость анализа образцов пищевых продуктов и влияние разведения образцов. Концентрации глутамата в разных соусах и приправах измеряли с помощью разработанного биосенсора; результаты хорошо коррелировали с результатами, полученными спектрофотометрическим методом ($R^2 = 0,988$). Таким образом, амперометрический биосенсор для определения глутамата был успешно оптимизирован и использован для измерения концентрации глутамата в соусах и приправах.

Ключевые слова: амперометрический биосенсор, глутаматоксидаза, поли(фенилендиамин), глутамат, пищевые образцы.