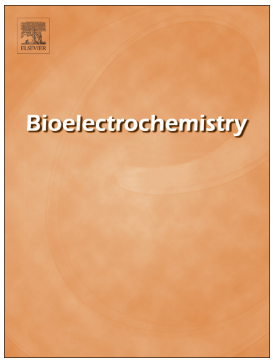

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Conductometric biosensor for arginine determination in pharmaceutics

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Abstract

A new conductometric biosensor based on coimmobilized urease and arginase has been developed for arginine determination in pharmaceutics. First, the main parameters of the selected method of immobilization (concentrations of arginase, urease, and glutaraldehyde, time of incubation) were optimized. An influence of the solution parameters (buffer ionic strength, capacity, pH, Mn^{2+} concentration) on the biosensor operation was studied, working conditions were optimized. After biosensor optimization, the main analytical characteristics were as follows. The limit of detection - 2.5 μM, the linear range - 2.5 - 500 μM, the sensitivity to arginine 13.4 ± 2.4 μS/mM, the response time - 20 s. The signals repeatability and operational stability in continuous exploitation were studied over one working day and during one week. Additionally, the selectivity of the developed biosensor towards arginine was essayed relative to other aminoacids.

The developed biosensor has been used to measure arginine concentrations in some drugs. The results obtained were in high correlation with the characteristics declared by producers.

Keywords: arginine, arginase, urease, biosensor, conductometry

Introduction

L-Arginine is a semiessential amino acid regulating a lot of metabolic processes. It is characterized by a wide range of biological properties [1]. It is a precursor of L-Ornithine, L-Citrulline, L-Glutathione, γ-Amino Butyric Acid, spermine; L-Arginine is one of the most polarized positively charged amino acids [2].

In humans, L-Arginine plays an important role in energy and plastic metabolism, is involved in the synthesis of nitric oxide, certain hormones, polyamines, creatine, and serves as a precursor for the formation of other amino acids, including proline, glutamate and glutamine. L-Arginine is known to be capable of independent synthesis in the body; however, the synthesis significantly reduces with the age-related changes and in certain diseases [3].

L-Arginine is vitally important for the organism because it is a precursor to nitric oxide (NO). The NO molecule is present in all types of tissues of the human body and plays an essential role in the functioning of the cardiovascular, immune and nervous systems. NO inhibits the platelet aggregation, proliferation of vascular smooth muscle cells and production of reactive oxygen species. Under physiological conditions NO is involved in the vascular system adaptation to enhanced physical activity, and in diseases NO is responsible for increased peripheral vasodilation. In turn, the NO deficiency negatively affects the general state of the immune system and causes the development of serious diseases, in particular, hypertension, coronary heart disease and atherosclerosis [4].Therefore, continuous replenishment of the body with L-arginine is required for normal functioning of the organism.

Currently, pharmacological market offers numerous L-arginine-based preparations. A wide range of effects, which arginine shows on the functioning of different systems, makes its use promising in treatment of various disorders, particularly, in cardiology practice, diseases of the central nervous system and liver, and even respiratory disorders [5]. Unfortunately, in the last years along with high quality drugs the counterfeit medicines often happen, which contain active ingredients at wrong ratio or no active substances at all [6].Therefore, control of arginine concentration in pharmaceuticals is an issue of essential importance. The drug production is not the only area, where the monitoring of arginine concentration is required. The L-arginine level in biological fluids should be determined when diagnosing and controlling the course of diseases of the endocrine system [7], hepatocellular carcinoma [8, 9], melanoma [10], and colorectal cancer [11]. In laboratory diagnostics, the L-arginine level in urine is evaluated for the diagnosis of homozygous biochemical cystinuria [12].

To date, a number of methods are known for determination of L-arginine concentration in solutions, in particular, fluorometry [13], spectrophotometry [14-16], ion-exchange chromatography [17, 18], polarography [19], capillary [20] and zone electrophoresis [21]. Flow-

injection analysis [22], enzymatic analysis [23-25], high performance liquid chromatography (HPLC) [26] are also used. However, most of the known physico-chemical and chemical methods have disadvantages, such as low selectivity and sensitivity, high-cost and complex equipment. Additionally, the measurement procedure is time-consuming and requires highly qualified personnel.

To simplify and reduce the costs of analysis, it is necessary to develop new methods, which would allow fast determination of arginine concentration in pharmaceuticals and biological fluids. Use of novel analytical devices, biosensors, can be a promising example of such methods.

Currently, there are numerous reports on the development of biosensors for arginine determination worldwide [27]. Some of them, mostly amperometric and potentiometric, are based on electrochemical transducers of various types. In amperometric biosensors, one-enzyme systems based on oxidase of L- and D-amino acids are often used.

Unfortunately, these biosensors are not selective enough to determine arginine, especially in complex multicomponent samples (blood, juices, etc.). There is information about the amperometric biosensor based on arginase, urease and electrodeposited polyaniline (PA) developed for arginine quantification [28]. When creating the ammonium-sensitive chemosensor based on PA platform, Nafion was used to compensate the negative charge occurring due to the anode polymerization of PA. In case of potentiometric ISFET biosensors, the two-enzyme (arginase/urease) system is often utilized [29-31]. A significant drawback of this biosensor system is a certain loss of sensitivity because of the use of two enzymes; besides, it is impossible to analyze complex samples containing urea. The biosensor with ion-selective electrode is known for determination of L-arginine in fruit juices using only one enzyme - arginine deiminase [32]. However, it has some disadvantages: the requirement of technologically complex reference electrode, light sensitivity, difficulties in further miniaturization, impossibility of using inexpensive thin-film technique for its manufacture. Notably, conductometric biosensors are rarely used for arginine determination. Both one- (arginine deiminase) [33] and bi- (arginase and urease) enzyme biosensors [34], are characterized by high sensitivity to arginine. Conductometric transducers have a number of advantages compared to other electrochemical sensors. In particular, they are light insensitive, do not need any reference electrodes, can be miniaturized, low-cost thin-film manufacturing technology is applicable.

However, actually none of numerous known biosensors attains the stage of commercialization; therefore the development of novel biosensors for arginine determination still remains a challenge.

Materials and methods

Materials

Enzymes urease from soya beans, activity 66 U/mg ("Fluka", Germany), and arginase from bovine liver, activity 105 U/mg (Sigma-Aldrich Chemie, USA) were used. Bovine serum albumin (BSA, fraction V), 50% aqueous solution of glutaraldehyde (GA), glycerol, and urea were from “Sigma-Aldrich Chemie” (Germany).

The working buffers, 5 mM TRIS $(HOCH_2)_3CNH_2$, pH=8.1, and 5 mM phosphate, pH=7.4, were of domestic production. Other inorganic compounds were of domestic production and had analytical purity grade.

Conductometric transducers

The conductometric transducers were manufactured in accordance with our recommendations at the V.Ye. Lashkarev Institute of Semiconductor Physics, NASU (Kyiv, Ukraine). They consist of a sital (fused Al_2O_3) substrate 5 × 30 mm in dimension with a couple of gold interdigitated electrodes deposited on its surface. A titanium 0.1 μm thick sublayer was used for better adhesion to the substrate. More information can be found in the previous paper [35].

Experimental setup for conductometric measurements

The experimental setup was used to measure the changes in conductivity of the near-electrode layer of conductometric transducer. To increase the sensor sensitivity and minimize noise due to nonspecific effects, the differential mode of measurements was used. The detailed information has been presented earlier [36]

Enzymes immobilization in a glutaraldehyde drop

For immobilization in GA drop two-layer working and reference membranes were prepared. To form the first layer of the working membrane, the solution of 10% of urease and 10% of BSA in 20 mM phosphate buffer, pH=7.4, with 20% of glycerol was used [37]. For the second layer the same solution was preapared but 10% of arginase was taken instead of urease. The mixture for reference two-layer membranes was prepared in the same way, but without any enzyme and 20% of BSA instead. The solutions for working and reference membranes were

mixed with aqueous solutions of GA (2% - for the urease-based layer and 0.1-0.5% - for the arginase-based one) in the ratio of 1:1. Immediately thereafter, the mixtures (0.15 µl each) were deposited on the working surfaces of conductometric interdigitated electrodes, which then were air dried for 45 min at room temperature. Next, the biosensors were immersed in the working buffer for washing from unbound GA.

Measuring procedure

The biosensor functioning is based on the enzymatic *reactions*, which occur in the urease- and arginase-containing membrane deposited on the surface of conductometric transducer:

$$\text{L-arginine} + H_2O \xrightarrow{\text{Arginase}} \text{urea} + \text{L-ornithine} \quad (1)$$

$$\text{Urea} + H_2O + H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^- \quad (2)$$

Arginase cleaves L-arginine to urea and L-ornithine by the enzymatic reaction (1). Then urease cleaves urea to ammonia and carbon dioxide - reaction (2). Thus, the enzymatic reactions lead to the changes in solution conductivity, the value of which is proportional to the arginine concentration. These changes are recorded by a conductometrcic transducer.

Measurements were carried out in 5 mM phosphate buffer, pH=7.4, and 5 mM TRIS, pH=8.1, at room temperature in the 2 ml open cell at constant stirring. The substrate concentrations were specified by addition of aliquots of the substrate stock solution to the working cell. All experiments were carried out in three or four replicates. Non-specific changes in the output signal caused by fluctuations of environmental pH and temperature and electrical noise, were avoided due to use of differential mode of measurements.

Results

Optimization of basic parameters of immobilization.

The first step should be optimization of the basic parameters of the method of immobilization, i.e. the concentrations of both enzymes and GA, incubation time and sequence of immobilization procedure.

To determine the optimal urease concentration, the biosensor responses to urea of different concentrations were measured depending on the urease concentrations in the bioselective membrane, which were 0.25%, 0.5%, 1%, 3%, 5%. As seen from Fig. 1,A, an increase in the enzyme concentration from 0.25% to 0.5% resulted in many-fold rising of the biosensor responses, whereas further increase up to 5% causes gradual growth of the signal. The largest value was at urease concentration of 5%. To determine the optimal concentration of arginase in the bioselective element, the biosensor responses to arginine of different concentrations were measured changing the arginase concentrations: 1.5%, 2.5%, 3%, 5%, 6.6%, and 7.5%. As seen (Fig. 1,B), the largest responses of bienzyme biosensors were observed at the arginase concentration of 5%. This value was used as optimal in further experiments.

At the next stage, an effect of glutaraldehyde concentration as well as an influence of immobilization time on the biosensor operation were studied. The biosensor responses to arginine of different concentrations were measured depending on the glutaraldehyde concentration during immobilization. According to the results (Fig. 1,C) a conclusion can be made that GA concentrations 0.1% - 0.5%, are the most efficient; thus, they were used in further works on creating bienzyme (urease + arginase) biosensors. Additionally, the biosensor sensitivity to arginine (1 mM) was evaluated depending on duration of immobilization. It was revealed that 30 min is optimum for the formation of enzyme membranes on the surface of conductometric transducer using glutaraldehyde (Fig. 1, D).

Fig. 1.

Effect of solution characteristics on biosensor function

The biosensor work can depend on both its own characteristics and parameters of the solution, in which measurements are carried out. The next stage was to study the influence of parameters of the working buffer on the operation of the proposed biosensor for arginine determination, including the measurement of biosensor responses depending on the buffer capacity, ionic strength, pH and Mn^{2+} concentration.

All conductometric biosensors are characterized by a strong dependence of the value of their response on the ionic strength of the solution, in which the measurements are performed. Therefore, an impact of the ionic strength (KCl concentration) was evaluated. The biosensor responses to 1 mM arginine were measured at a gradual increase of the KCl concentration from 1 mM to 120 mM (Fig. 2,A). As seen, the biosensor responses significantly decreased along with

an increase in the solution ionic strength, which means a necessity of control of the ionic strength in further experiments with real samples to reduce the measurement error.

This phenomenon can be explained in several ways. One of the key reasons is an increase in the background conductivity of solution. In previous studies it was shown that sensitivity of conductometric transducers to the conductivity changes, when working with the samples with high ionic strength is essentially. This is because detection limits are ultimately dependent on the ratio dG/G, where G is the medium conductivity and dG is its change in the enzymatic process [38, 39].

On the other hand, a higher ionic strength of the solution tested can provoke changes in density of bioselective membranes due to the screening of membrane surface charges and the consequent changes in membrane permeability and immobilised enzymes activity. Therefore the ionic strength of the samples analyzed by conductometric biosensor is to be strictly controlled.

The value of responses of conductometric biosensors also depends on the buffer capacity. Therefore, the biosensor responses to 1 mM arginine were measured at various concentrations of the buffer solution: 1 mM, 2.5 mM, 5 mM, 10 mM, 25 mM, 50 mM (Fig. 2,B). As seen, the biosensor responses are considerably larger at low concentrations of buffer solution. However, in further experiments 5 mM buffer was used because at low concentrations the buffer loses its activity.

It is known that immobilization can change optimal pH of the enzyme. It is especially important to consider for the proposed bienzyme-based biosensor. To find optimal working conditions of biosensor, the dependence of its responses to 1 mM arginine on pH of working solution was investigated. In the experiments a universal buffer was used, which has the same buffer capacity in a wide range of pH values (Fig. 2,C). As seen, the responses were of largest values at pH=8.1. In further work, 5 mM TRIS, pH=8.1, was used.

To intensify the arginase functioning, the working buffer should contain Mn^{2+} ions. To determine the optimum Mn^{2+} concentration, the biosensor was tested using the working buffer with Mn^{2+} of concentrations 0.01 - 1 mM. The biosensor responses to arginine, as could be expected, increased along with an increase in the Mn^{2+} concentration up to 0.1 mM (Fig. 2, D). At the Mn^{2+} concentration of 0.1 mM to 1 mM, an opposite effect, i.e. the enzymes inhibition, was observed. Therefore, in future work 0.1 mM Mn^{2+} was added to the working buffer.

Fig. 2.

Determination of signal repeatability and operational stability of the biosensor

Signal repeatability and operational stability are the basic working characteristics of any biosensor. To test the signal repeatability of the proposed biosensor to arginine, the responses to 1 mM arginine were measured over some time at intervals of 10-15 min. During intervals the biosensor was kept in the buffer at continuous stirring (Fig. 3,A). As seen, the biosensor was characterized by high signals repeatability. The measurement error did not exceed 5%.

To test the biosensor for operational stability, the experiment was carried out as follows. The biosensor responses to 1 mM arginine were measured in 4-signal series every 10-15 min over 4-day period (Fig. 3,B). Between series the biosensor was stored dry at 2 – 6 °C. In the course of experiment only a slight decrease in the average response to arginine was observed. Therefore in general the developed biosensor for determination of arginine can be characterized by high operational stability during 4 days.

Fig. 3.

Study of the biosensor selectivity

An influence of interfering components (other amino acids) on response value was studied to test biosensor selectivity. For the purpose, amino acids of concentration of 0.5 mM were injected into a working cell (Fig. 4). Measurements were carried out in 5 mM TRIS, pH=8.1. Selectivity is expressed as the ratio of the signal output with the arginine alone to that with the interfering substance alone, at the same concentration as that of the arginine (0,5 mM).

The ratio of response of the developed conductometric biosensor to some amino acids / arginine amounted to 0.18. Considering that the developed biosensor is supposed to be used mainly for arginine determination in pharmaceuticals, which usually contain the active ingredient arginine in much higher concentrations than admixtures and additives, it could be assumed that the proposed biosensor is highly selective.

Fig. 4.

Investigation of basic analytical characteristics of the developed biosensor for arginine determination.

Under optimal working conditions when using 5 mM TRIS buffer, pH = 8.1, with 0.1 mM Mn^{2+}, the limit of arginine detection was 2.5 μM (it was measured as the arginine concentration, the response to which is three times larger than the baseline noise). The linear range was 2.5 -

500 μM, sensitivity to arginine was 13.4 ± 2.4 μS/mM. The linear portion of calibration curve is described by the ΔG = 13*C (for arginine determination; R^2 = 0.98), where ΔG is the change in solution conductivity (μS), C is the arginine concentration (mM). Noteworthy, the arginine determination strongly depended on the bioselective element composition and the parameters of working buffer solution. Thus, changing these parameters it is possible to obtain biosensors with a variety of analytical characteristics considering the tasks to be fulfilled.

Further, the analytical characteristics of the biosensor were compared with those of known enzyme biosensors. A series of works on arginine determination using biosensors based on various electrochemical transducers and enzyme systems were analyzed. The results of the comparison are given in Table 1. As seen, the biosensor proposed in this paper had advantages over most analogues by higher selectivity and sensitivity to arginine, as well as a wider working linear range.

Table 1

Measurement of arginine concentration in samples of drugs using developed biosensor

Six drugs were chosen to test the biosensor for analysis of arginine concentration: Veyron, Tivomax, Glutargin, Tivortin, Citrarginine, Aminoplasmal. The method of standard additions was applied as follows. First, an aliquot of the pharmaceutical preparation was introduced to the working cell with biosensor. Then three aliquots of standard solution with known arginine concentration were added. By the received responses a straight-line graph was plotted. OriginPro program was used for plotting the graph and analyzing the results; the concentration of arginine in the test solution (sample pharmaceuticals) was calculated. Next, the values of a,b were inserted to the equation of straight line y=ax+b obtained by the measured biosensor responses, and calculated x at y = 0. Then, it was calculated the arginine concentration in aliquots, i.e. in the analyzed drug considering the dilution. The analysis of every medication was carried out in seven replications.

To test the developed arginine biosensor, the data obtained were compared with concentration of arginine declared by producers (Fig. 5). We have estimated a correlation between the data on arginine concentration in some drugs measured by the biosensor and declared by producers. The correlation coefficient was 0.9888.

Fig. 5.

Conclusions.

To develop a conductometric biosensor for determination of arginine concentration a series of researches were carried out. First, the optimal conditions of immobilization of urease and arginase on gold interdigitated electrodes were determined, such as the concentrations of GA and enzymes in biomembranes and duration of immobilization. The influence of parameters of working solution (buffer capacity and ionic strength, pH) on the function of arginine biosensor was evaluated. The operational stability and signal repeatability of the developed biosensor were studied over 4 working days. It was also shown that the proposed biosensor is highly selective towards arginine as compared with other amino acids.

The developed biosensors were used to analyze the arginine concentration in six pharmaceutical preparations. The results were in high correlation with the concentrations specified by the drug manufacturers.

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References

[1] M.M Cox, A.L Lehninger, D.L Nelson. Lehninger principles of biochemistry, third ed., New York: Worth Publishers; 2000.

[2] J. C. Marini, Arginine and ornithine are the main precursors for citrulline synthesis in mice, J. Nutr. 142(3) (2012) 572–580.

[3] A. Vretlind, A. Sujyan, Clinical nutrition. Stockholm-Moscow, 1990 (in Russian).

[4] R. H. Boger, S. M. Bode-Boger, The clinical pharmacology of L-Arginine, Ann. Rev. Pharmacol. Toxicol. 41 (2001) 79–99.

[5] N. K. Murashko, Actual Interview, Medical Newspaper "Health of Ukraine - XXI Century" Neurology (2014) p.27 (in Ukrainian).

[6] V. Pashkov, Substandard and counterfeit medicines: the issue of bringing to criminal and administrative liability, Pharmacy 810 (39) (2011). (in Ukrainian).

[7] S. W. Brusilow, A. L. Horwich, C. R Scriver, The metabolic and molecular bases of inherited disease, eighth ed., New York: McGraw-Hill, 2001, pp.1909–1963.

[8] T. L. Lam, G. K. Wong, H. C. Chong, P. N. Cheng, S. C. Choi, T. L. Chow, S. Y. Kwok, R. T. Poon, D. N. Wheatley, W. H. Lo, Y. C. Leung, Recombinant human arginase inhibits proliferation of human hepatocellular carcinoma by inducing cell cycle arrest, Cancer Lett. 277 (1) (2009), 91–100.

[9] E. S. Glazer, E. M. Stone, C. Zhu, K. L. Massey, A. N. Hamir, S. A. Curley, Bioengineered human arginase I with enhanced activity and stability controls hepatocellular and pancreatic carcinoma, Transl. Oncol. 4(3) (2011) 138–146.

[10] T. L. Lam, G. K. Wong, H. Y. Chow, H. C. Chong, T. L. Chow, S. Y. Kwok, P. N. Cheng, D. N. Wheatley, W. H. Lo, Y. C. Leung, Recombinant human arginase inhibits the in vitro and in vivo proliferation of human melanoma by inducing cell cycle arrest and apoptosis, Pigment Cell Melanoma Res. 24(2) (2011) 366–376.

[11] S. Y. Leu, S. R. Wang, Clinical significance of arginase in colorectal cancer, Cancer. (70) (1992) 733–736.

[12] S. M. Morales, Cystinuria: diagnosis and therapeutic approach, An Sist. Sanit. Navar. 34(3) (2011) 453–461.

[13] M. I. Parniak, G. Lange, T. Viswanatha, Quantitative determination of monosubstituted guanidines: a comparative study of different procedures, J. Biochem. Biophys. Meth. 7(4) (1983) 267–276.

[14] R. B. Yamasaki, D. A. Shimer, R. E. Feeney, Colorimetric determination of arginine residues in proteins by p-nitrophenylglyoxal, Anal. Biochem. 111(2) (1981) 220–266.

[15] S. Sakaguchi, Colorimetric determination of arginine, J. Biochem. (5) (1950) 25–32.

[16] F. Casadebaig, J. P. Dupin, P. Mesnard, The Sakaguchi reaction: Analytical developments and application to drug control, Ann. Pharm. Fr. 37(7–8) (1979) 313–324.

[17] S. E., Spayd, R. L. Wample, R. G. Evans., R. G. Stevens, B. J. Seymour., C. W. Nagel, Nitrogen fertilization of white Riesling grapes in Washington. Must and wine composition, Am. J. Enol. Vitic. (45) (1994) 34–42.

[18] Z. Huang, C. S. Ough, Effect of vineyard locations, varieties and rootstocks on the juice amino acid composition of several cultivars, Am. J. Enol. Vitic. (40) (1989) 135–139.

[19] Y. Rong, H. Junru, C. Dayi, Determination of L-arginine in drug by single sweep oscillopolarography, J. Sichuan Containing Educ. Coll. Med. Sci. (12) (1999) 1219–1236.

[20] L. Zhang, Y. Liu, G. Chen, Simultaneous determination of allantoin, choline and L-arginine in Rhizoma Dioscoreae by capillary electrophoresis, J. Chromatogr. A. 1043(2) (2004) 317–321.

[21] E. V. Narezhnaya, O. I. Askalepova, A. A. Nikashina, I. I. Krukier, T. N. Pogorelova, Determination of L-arginine in amniotic fluid by capillary disk electrophoresis, Zh. Anal. Chim. 65(12) (2010) 1309–1312. (In Russian).

[22] A. Alonso, M. J. Almendral, M. D. Baez, M. J. Porras, C. Alonso, Enzyme immobilization on an epoxy matrix. Determination of L-arginine by flow-injection techniques, Anal. Chim. Acta. 308(1–3) (1995) 164–169.

[23] J. G. Mayoral, F. J. Alarcón, T. F. Martínez, P. Barranco, F. Noriega, An Improved End-point Fluorimetric Procedure for the determination of low amounts of trypsin activity in biological samples using rhodamine-110-based substrates, Appl. Biochem. Biotechnol. (160) (2010) 1–8.

[24] O. R. Mira, Quantitative determination of L-arginine by enzymatic End-Point analysis, J. Agric. Food Chem. 49(2) (2001) 549–552.

[25] G. Gaede, M. Grieshaber, A rapid and specific enzymatic method for the estimation of L-arginine, Anal. Biochem. 66(2) (1975) 393–399.

[26] K. M. Chu, P. W. Huang, Li H. Pao, Determination of arginine, asymmetrical dimethylarginine, and symmetrical dimethylarginine in human plasma by high performance liquid chromatography, J. Med. Sci. 23(4) (2003) 201–206.

[27] G. Z. Gaida, N. Y. Stasyuk, M. V. Gonchar, Methods of analysis of arginine, Biotechnologia acta, 7(1) (2014) 31-39. (In Russian).

[28] N. Stasyuk, O. Smutok, G. Gayda, B. Vus, M. Gonchar, Ye. Koval'chuk, Bi-enzyme L-arginine-selective amperometric biosensor based on ammonium-sensing polyaniline-modified electrode, Biosens Bioelectron. 37(1) (2012) 46–52.

[29] S. Karacaoglu, S. Timur, A. Telefoncu. Arginine selective biosensor based on argi naseurease immobilized in gelatin, Artif Cells Blood Subst Immob Biotechnol. (31) (2003) 357–363.

[30] S. Komaba, Y. Fujino, T. Matsuda, K. Osaka, I. Satoh, Biological determination of Ag (I) ion and arginine by using the composite film of electroinactive polypyrrole and polyion complex, Sens Actuator. (52) (1998) 78–83.

[31] L. Lvova, A. Legin, Y. Vlasov, G.S. Cha, H. Nam, Multicomponent analysis of Korean green tea by means of disposable all-solid-state potentiometric electronic tongue micro system, Sens Actuator. (95) (2003) 391–395.

[32] N. Verma, A.K. Singh, P.J. Kaur, Biosensor based on ion selective electrode for detection of L-arginine in fruit juices, Anal Chem. (70) (2015) 1111-1115.

[33] O.O. Soldakin, D.M. Oliynyk, I.S. Kucherenko, SV Marchenko, MA Kuibida, VO Prilipko, O.P. Soldakin, S.V. Dzyadevich, Conductometric biosensor based on arginine deiminase for quantitative analysis of arginine in liquids of biological origin, Patent of Ukraine Bul. №7 (10.04.2017). (in Ukrainian).

[34] O. Y. Saiapina, S. V. Dzyadevych, N. JaffrezicRenault, O. P. Soldatkin. Development and optimization of a novel conductometric bi-enzyme biosensor for L-arginine determination, Talanta (92) (2012) 58–64.

[35] S.V. Dzyadevich, O. P. Soldatkin, Scientific and technological principles of creation of miniature electrochemical biosensors. Naukova Dumka, Kyiv, 2006, pp. 71-72

[36] O.O. Soldatkin, I.S. Kucherenko, V.M. Pyeshkova, A.L. Kukla, N. Jaffrezic-Renault, A.V. El'skaya, S.V. Dzyadevych, A.P. Soldatkin, Novel conductometric biosensor based on three-enzyme system for selective determination of heavy metal ions, Bioelectrochemistry. (83) (2012) 25-30.

[37] O.O. Soldakin, O.A Nazarenko, O.S. Pavlyuchenko, O.L Kukla, V.M. Arkhipova, S.V Dzyadevych, O.P. Soldakin, G.V Elska, Optimization of the work of enzyme bioselective elements in the potentiometric multibiosensor, Biopolymers and Cell. 24(1) (2008) 42-50.

[38] A.M. Nyamsi, N. Jaffrezic–Renault, C. Martelet, A.A. Shul'ga, S.V. Dzydevich, A.P. Soldatkin, A.V. El'skaya, Enzyme biosensor based on a micromachined interdigitated conductometric transducer: application to the detection of urea, glucose, acetyl- and butyrylcholine chlordes. Sensors and Actuators B: Chemical 21(2) (1994) 123-129.

[39] L.D. Watson, P. Maynard, D.C. Cullen, R.S. Sethi, J. Brettle, CR. Lowe, A microelectronic conductimetric biosensor, Biosensor and Bioelectronics, 3 (1987) 101-115.

[40] N. Stasyuk , O. Smutok , G. Gayda , Y. Koval'chuk, M. Gonchar, A new bi-enzyme potentiometric sensor for arginine analysis based on recombinant human arginase 1 and commercial urease. J. Mater. Sci. Eng. A. 1 (2011) 819–827.

[41] N. Stasyuk , G. B. Gayda, M. Gonchar L-arginine-selective microbial amperometric sensor based on recombinant yeast cells over-producing human liver arginase 1. Sensors and Actuators B: Chemical 204 (2014) 515-521.

[42] M. Sheliakina, V. Arkhypova, O. Soldatkin, O. Saiapina, B. Akata, S. Dzyadevych Urease-based ISFET biosensor for arginine determination Talanta 121 (2014) 18–23

[43] V. Kacaniklic, K. Johansson, G. Marko-Varga, L. Gorton, G. Jonsson-Pettersson, E. Csoregi. Amperometric biosensors for detection of L- and D-amino acids based on coimmobilized peroxidase and l- and d-amino acid oxidases in carbon paste electrodes, Electroanalysis 6 (1994) 381-390

[44] R. Domı´nguez, B. Serra, A. J. Reviejo, J. M. Pingarro´n Chiral Analysis of amino acids using electrochemical composite bienzyme biosensors, Analytical Biochemistry 298 (2001) 275–282

Figure captions:

Fig. 1. Dependence of biosensor responses to urea and arginine on concentration of urease (A), arginase(B), GA(C) in biomembrane and time of immobilization (D). Measurements were carried out in 5 mM phosphate buffer, pH=7.4

Fig. 2. Dependence of biosensor responses on ionic strength (A), buffer capacity (B), pH (C) and Mn^{2+} concentration (D) of working buffer. Measurements were carried out in 5 mM phosphate buffer, pH=7.4 (for A and B) in 2,5 mM universal buffer (for C) in 5 mM TRIS, pH=8.1 (for D)

Fig. 3. Repeatability (A) and operational stability (B) of biosensor signals. Measurements were performed in 5 mM TRIS, pH=8.1, arginine concentration 1 mM.

Fig. 4. Selectivity of the biosensor. Measurements were performed in 5 mM TRIS buffer, pH=8.1, concentration of injected substrates in cell – 0.5 mM.

*Fig. 5. Correlation between the data on arginine concentration in drugs obtained by biosensor procedure and declared by producers. Equation of the line: Y=7.5723+1.0082*X. Correlation coefficient: R=0.9888. Measurements were performed in 5 mM TRIS buffer, pH=8.1.*

Table 1. Analytical characteristics of enzyme biosensors for arginine determination

Table 1

Analytical characteristics of enzyme biosensors for arginine determination

Method	Biomaterial	Sensitivity	Response time/s	Linear range/ mM	Substrate specificity/ %	Reference
Potentiometric	arginase/urease	34.7 mV/ decade	300	0.5–50	Can-100, Arg-100, Val-18, Cys-17, Orn-16, Lys-10	[40]
	ArginaseNPs/ urease	26.2 mv/ decade		0.12-40		
Amperometric	arginase/urease	110±1.3 $A \times M^{-1} \times m^{-2}$	10	0.07-0.6	Can -100, Arg- 95, Pro- 4	[28]
Amperometric	Permeabilized cells/urease	14±1.2 $A \times M^{-1} \times m^{-2}$	60	till 0.6	Arg -100, Can -65, Lys-27, Ser-7, Pro-5	[41]
Conductometric	arginase/urease	4.2 $\mu S \times mM^{-1}$	120	0.01–4	Arg-100, Can-81, His-49, Lys-102, Gly-1, Pro-1,Gln-1, Met-1, Thr-1, Ile-1, Val-1, Pro-1, Ala-1, Orn-1, Ser-1, Cys-1	[34]
Potentiometric	Inhibition of urease	No data	300	0.1-2	Arg-100, Gly-76, Val-60, Lys-19, Cys-2, His-2, Pro-2	[42]
Potentiometric	Arginine deiminase	No data	30	10–6-100	No data	[32]
Conductometric	arginine deiminase	64±3.1 $\mu S \times mM^{-1}$	50	0.03-0.8	No data	[33]
Amperometric	oxidase of L- and D-amino acids	No data	100-200	till 5	Gly-20, Ala-20, Val-35, Leu -112, Ile-45, Ser-35, Thr-27,Asp-14, Asn-22, Glu-29, Gln-28, Lys-34, His-38, Arg-28, Phe-100, Tyr-92, Try-65, Met-133, Pro-32	[43]
Amperometric	oxidase of L- and D-amino acids/ peroxidase	140±2 $\mu A \times M^{-1}$	50-100	0.1-1	Try-98, Arg-29, Phe-49, Met-55, Leu-100, Ser-31,Val-80	[44]
Conductometric	arginase/urease	13.4±2.4 $\mu S \times mM^{-1}$	20	0.0025-0.5	Arg- 100, Met-3, Gln-4, Ala-2, Cys-17, His-14, Gli-4, Lys-12, Tyr-5, Leu-3, Val-2, Asn-6, Ser-3, Ile-3, Asp-10, Glu-9, Thr-3, Prol-3, Phe-0,Trp-0	This work

Highlights

- We developed a conductometric arginine biosensor based on arginase and urease
- Biosensor showed good signal reproducibility, sensitivity and selectivity
- Biosensor was used to analyze the arginine concentration in pharmaceutical samples
- Results obtained were in good correlation with concentrations declared by producers

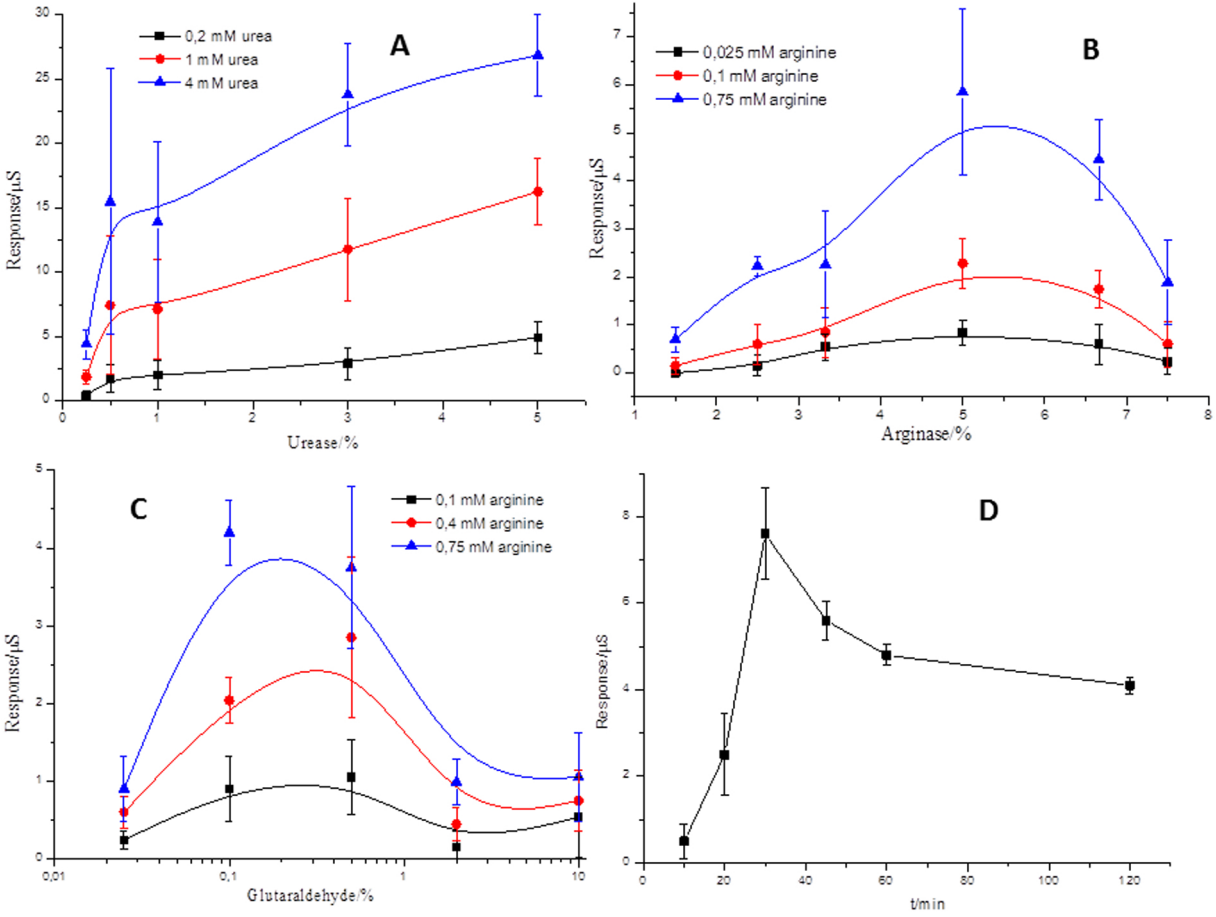

A
0,2 mM urea
1 mM urea
4 mM urea
Response/μS
Urease/%
B
0,025 mM arginine
0,1 mM arginine
0,75 mM arginine
Response/μS
Arginase/%
C
0,1 mM arginine
0,4 mM arginine
0,75 mM arginine
Response/μS
Glutaraldehyde/%
D
Response/μS
t/min

Figure 1

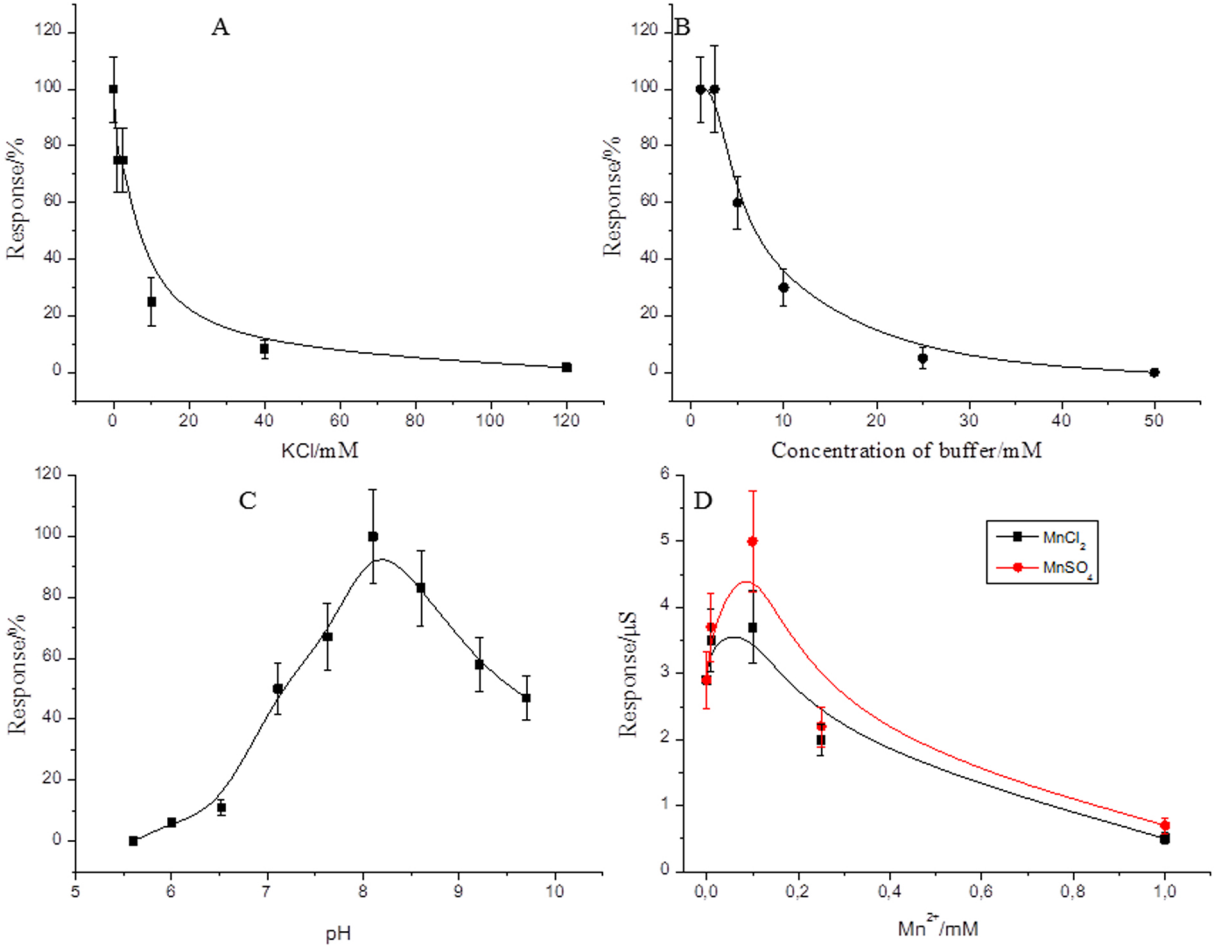
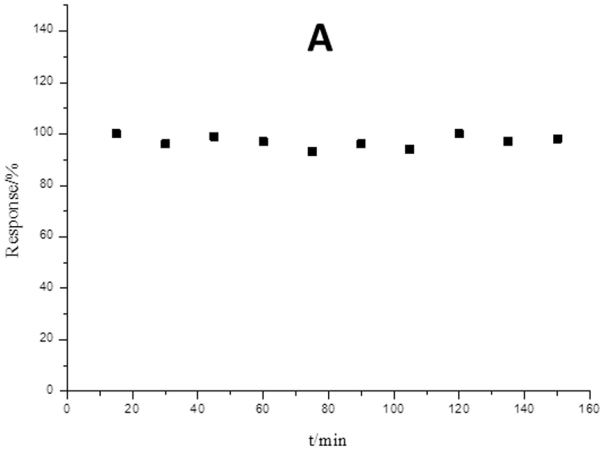

A
Response/%
KCl/mM
B
Response/%
Concentration of buffer/mM
C
Response/%
pH
D
MnCl2
MnSO4
Response/μS
Mn2+/mM

Figure 2


A
Response/%
t/min
0
20
40
60
80
100
120
140
160

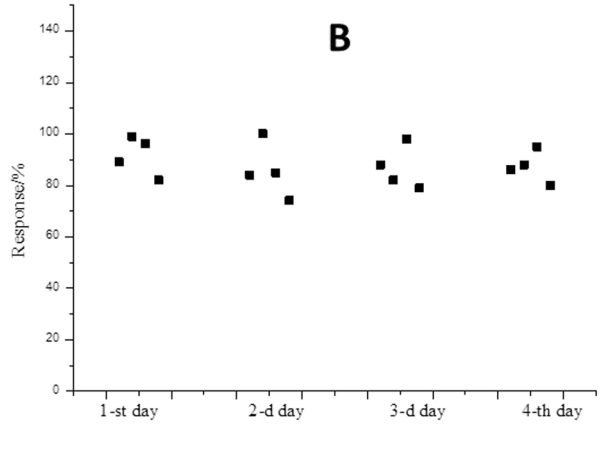

B
Response/%
1-st day
2-d day
3-d day
4-th day

Figure 3

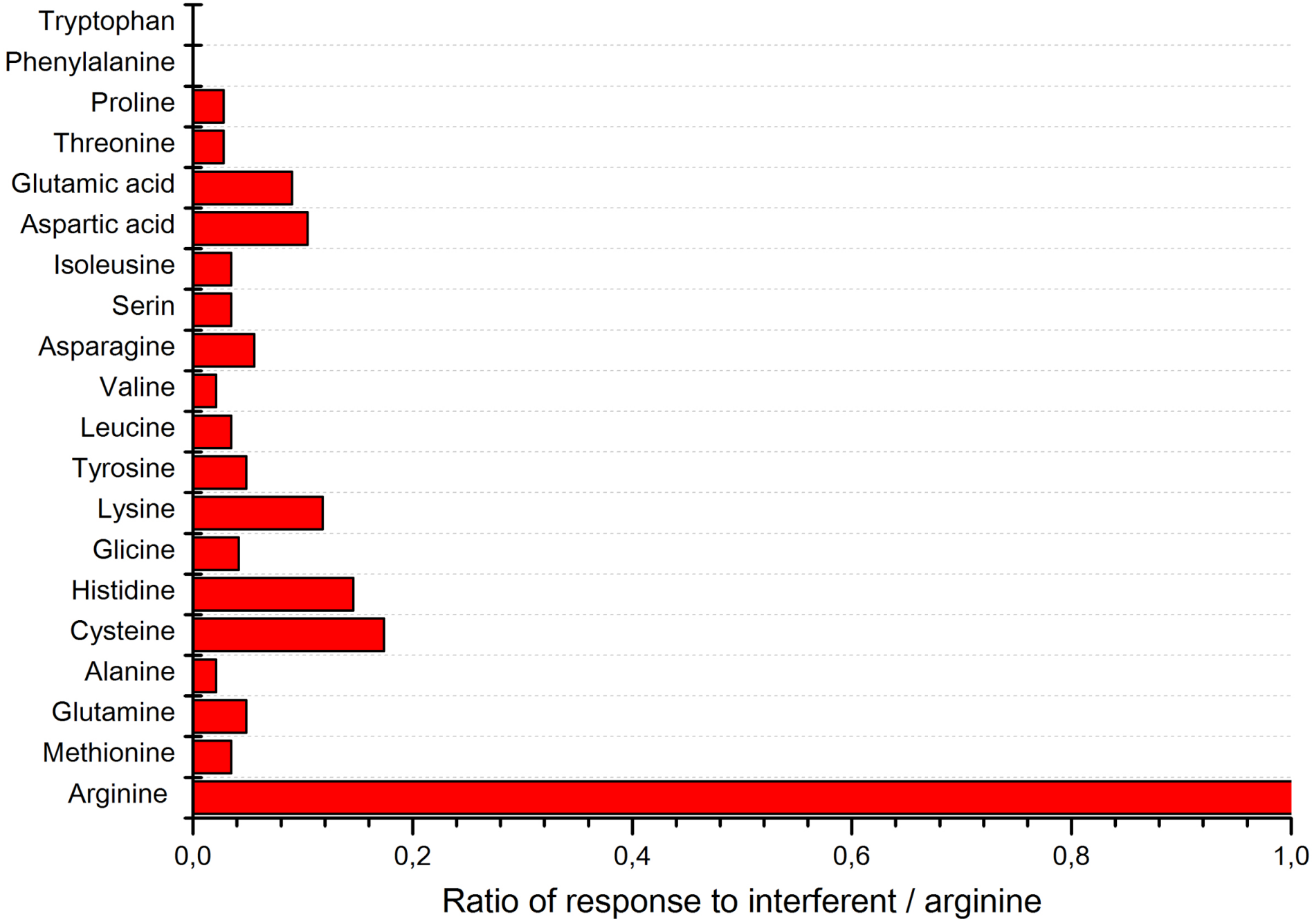

Tryptophan
Phenylalanine
Proline
Threonine
Glutamic acid
Aspartic acid
Isoleusine
Serin
Asparagine
Valine
Leucine
Tyrosine
Lysine
Glicine
Histidine
Cysteine
Alanine
Glutamine
Methionine
Arginine
0,0
0,2
0,4
0,6
0,8
1,0
Ratio of response to interferent / arginine

Figure 4

Drugs	Concentration declared by producers/mM	Concentration obtained by biosensor/mM
«Veyron»	1050	1026 ± 153
«Tivomaks»	200	238 ± 31
«Glutargin»	70	66 ± 19
«Betargin»	270	262 ± 46
«Tivortin»	654	665± 49
«Aminoplasmal»	66	84 ± 26

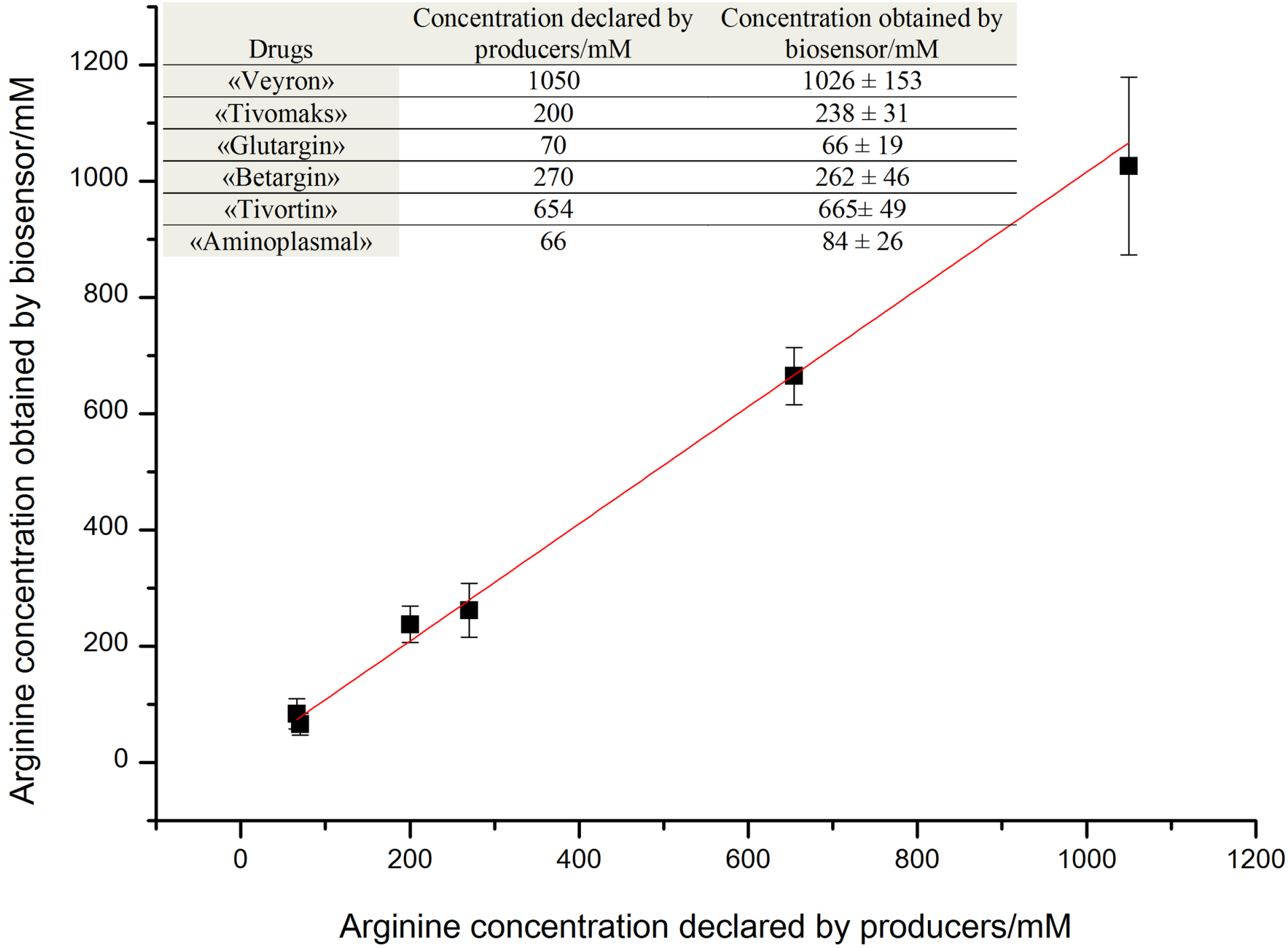


Figure 5