CONDUCTOMETRIC ENZYME BIOSENSORS AND CALIXARENE-BASED CHEMOSENSOR FOR DETERMINATION OF ARGININE IN AQUEOUS SOLUTIONS

Saiapina O.Y.[1*], Soldatkin O.O.[1], Soldatkina O.V.[2], Marchenko S.V.[1], Yarynka D.V.[1], Cherenok S.O.[3], Kalchenko V.I.[3], Dzyadevych S.V.[1,2]

[1]Department of Biomolecular Electronics, Institute of Molecular Biology and Genetics of the NAS of Ukraine. 150, Academician Zabolotnyi Str., Kyiv, 03680, Ukraine. E-mail: osayapina4@gmail.com; [2]Institute of High Technologies, Taras Shevchenko National University of Kyiv. 4H Academician Hlushkov Ave., Kyiv, 03022, Ukraine; [3]Phospohoranes Chemistry Department, Institute of Organic Chemistry of the NAS of Ukraine. 5, Murmanska Str., Kyiv, 02660, Ukraine.

◄► **A**rginine (2-amino-5-guanidinovaleric acid, Arg) is a semi-essential amino acid, which is being a building block of proteins also offers immense clinical and quality control significance. In food industry, possibility to monitor the concentration of Arg in the raw agricultural commodities and finished products allows to control their authenticity, efficiency of production and storage. Adulterated food, in turn, shows the altered levels of Arg within their amino acid profiles (Fang et al., 2012). The fruit juice adulteration occurs with diluting a more expensive product with a less expensive one (Tezcan et al., 2017). Data from food biochemistry show that some industrially important fruits, berries and legumes have definite concentrations of arginine. By knowing this, the level of Arg in the analyzed food can be used to distinguish between the authentic or original products from their adulterated versions. It has particular relevance for the juices from the tropical or exotic fruits that are typically of a higher price than other fruit juices and at the same time have higher consumption demand on the market. From the standpoint of government, food dealers and end consumers, it is necessary to have reliable, affordable and easy-to-use inspection methods enabling testing the juices and other foodstuffs for their authenticity in a timely manner.

Despite the satisfied criteria of analytical performance, the commonly used laboratory methods of analysis of the complex samples such as fruit juices, is frequently associated with a time consuming sample pre-treatment and needs to be performed by skilled personnel using costly and bulky equipment and reagents. The hybrid methods that harness the properties of bio- and nanomaterials coupled with the electrochemical methods of detection have a potential to overcome the challenges faced by the field. In particular, development of the arginine biosensors with the modified biomembranes and exploring the impact of the natural zeolite clinoptilolite and arginine-sensitive calixarene methylenebisphosphonic acid for the quantification of Arg will be considered here.

PRINCIPLE OF ARGININE DETERMINATION USING ENZYME BIOSENSORS BASED ON THE CONDUCTOMETRIC TRANSDUCERS:

$$\text{L-arginine} + H_2O \xrightarrow{\text{Arginase}} \text{L-ornithine} + \text{urea}$$

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^-$$

ΔG (conductometric electrodes)

CHARACTERISTICS OF CLINOPTILOLITE

Unit cell formula of the clinoptilolite sample (CLT):

$(Na_{0.10}K_{0.57})(Ca_{0.47}Mg_{0.15})(Al_{1.97}Fe_{0.12})(Si_{9.96}Ti_{0.02})O_{24},7H_2O$

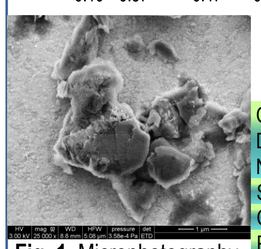
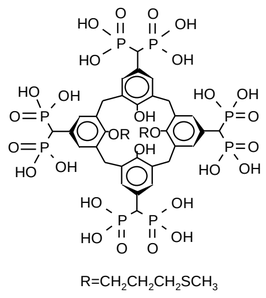


Fig. 1. Microphotography of the CLT particles obtained using SEM (instrument FEI Quanta™ 50)

Properties of the natural Romanian clinoptilolite ZN-C1BF-R (Mediterranean Society of Zeolites)

Classification according to Dana 8th edition (Dana's New Mineralogy)	77.1.4.2: Tectosilicates Zeolites, Zeolite group – True zeolites
Specific surface area	101 $m^2\ g^{-1}$
Cation exchange capacity	2.6 meq g^{-1}
Porosity	Microporous material, total pore volume 0.036 $cm^3\ g^{-1}$
Average size of particles	0.4 μm (90% between 0.2 μm and 1.0 μm)
Morphology	Monoclinic crystal form with platelets of 10–20 nm thick

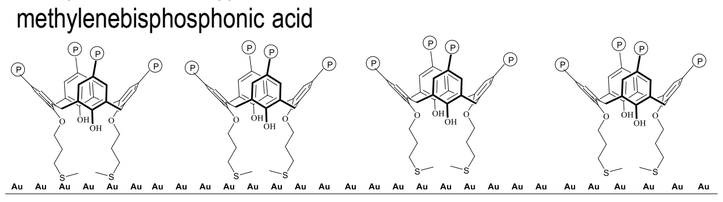
CALIXARENE METHYLENEBISPHOSPHONIC ACID SENSITIVE TO ARGININE



R=$CH_2CH_2CH_2SCH_3$

The arginine-sensitive conductometric chemosensor was based on 25,27-di(3-methylsulfdopropoxy) calixarene tetrakis-methylenebisphosphonic acid (Fig. 2), which contained the methylenebisphosphonic acid moieties capable of complexation with arginine at the upper rim and methylsulfdopropyl moieties capable of adhesion to the transducer gold surface at the lower rim (Fig. 3).

Fig. 2. 25,27-di(3-methylsulfidopropoxy)calixarene tetrakis-methylenebisphosphonic acid



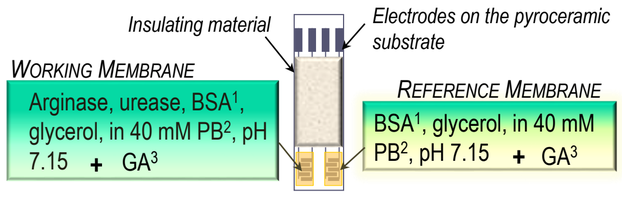
Ⓟ = $CH(P(O)(OH)_2)_2$

Fig. 3. Schematic representation of the surface of gold electrodes coated with a monomolecular layer of calixarene

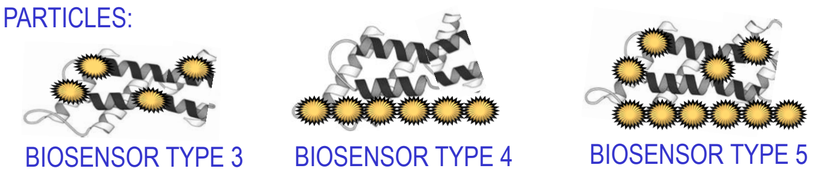
SENSOR ELEMENTS IMMOBILIZED ON CONDUCTOMETRIC MICROELECTRODES

Each conductometric transducer consisted of two identical pairs of gold interdigitated thin-film electrodes (150 nm thick) deposited onto a non-conducting pyroceramic substrate (5×30 mm). The sensitive area of each pair of electrodes was ~ 2.9 mm^2. One pair of electrodes, covered with a relatively inert layer, constituted the **reference sensor**. Another pair of electrodes, covered with an arginine-selective material (arginase and urease; calixarene), represented the **working sensor**. The sensor responses in a differential measuring mode were obtained using a portable differential conductometer (Institute of Electrodynamics of National Academy of Sciences of Ukraine) and in a single-channel mode – using the electrochemical impedance spectroscopy (VoltaLab®, Radiometer Analytical, France).

ENZYME-BASED BIOSENSORS (TYPES 1,2):



WORKING MEMBRANES OF THE ARG BIOSENSORS INCORPORATING CLT PARTICLES:



BIOSENSOR TYPE 3

Deposition of arginase, urease and CLT in one layer

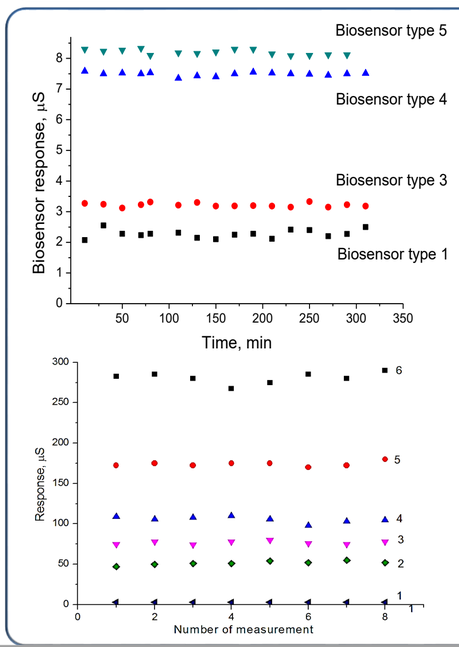
BIOSENSOR TYPE 4

Deposition of arginase, urease and CLT in two layers where CLT is an adlayer

BIOSENSOR TYPE 5

Deposition of enzymes concurrently in one layer with CLT and on the CLT adlayer

[1]BSA – bovine serum albumin [2]PB – phosphate buffer [3]GA – glutaraldehyde ● – CLT particles



◄ **Fig. 8.** Reproducibility of signals of CLT-based biosensors. Measurements in 5 mM phosphate buffer solution, pH 6.15

◄ **Fig. 9.** Reproducibility of sensors signals. Calixarene concentrations in the membrane: 0 mg/mL (1), 1 mg/mL (2), 5 mg/mL(3), 25 mg/mL (4), 50 mg/mL (5) and 100 mg/mL (6). Measurements in 5 mM phosphate buffer, pH 7.4

INFLUENCE OF THE WORKING BUFFER PARAMETERS ON THE SENSITIVITY OF ARGININE DETECTION WITH THE DEVELOPED (BIO)SENSORS

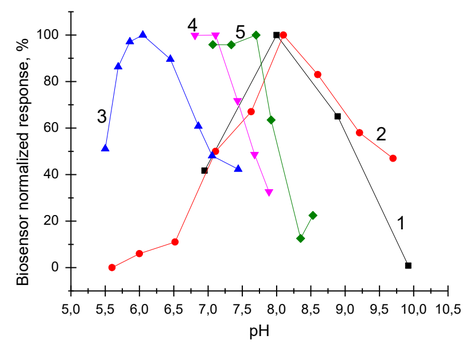
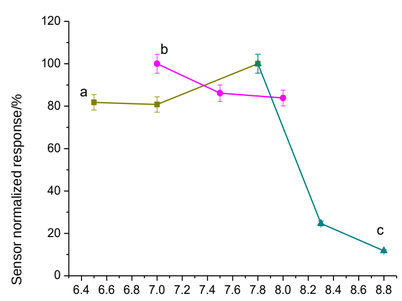


Fig. 4. Dependence of responses of arginase-urease biosensor on pH. The biosensor responses to arginine in:
– multicomponent buffer solution: curve 1 – for the biosensor comprising both enzymes in a single layer, and curve 2 – for the biosensor comprising each enzyme in the individual layer,
– phosphate buffer solution (curve 3)
– HEPES-NaOH buffer (curve 4)
– Tris-HCl buffer (curve 5)



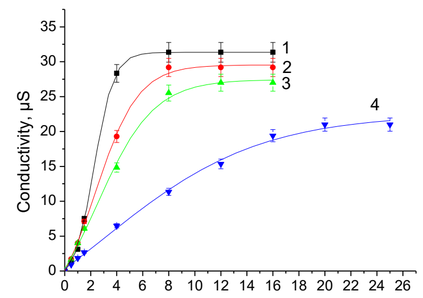


Fig. 5. Sensitivity of CLT-based conductometric sensor to ammonium at different pH. The sensor responses to 1 mM ammonium in phosphate buffer solution (a), HEPES-NaOH buffer (b) and Tris-NaOH buffer (c).

Fig. 6. Dependence of responses of arginase-urease biosensor on buffer capacity of phosphate buffer solution. Biosensor responses to arginine in 5 mM PB (1), 7.5 mM PB (2), 10 mM PB (3) and 20 mM PB (4)

TABLE 1. ANALYTICAL CHARACTERISTICS OF THE ENZYME-BASED CONDUCTOMETRIC BIOSENSORS FOR ARGININE DETERMINATION OBTAINED IN THE OPTIMIZED BUFFER SOLUTION

Method of immobilization	Linear range (mM)	Dynamic range (mM)	LOD[a] (mM)	Response time (s)	CV[b] (%)	Advantages over other elaborated here biosensors
Cross-linking with GA, two enzymes in a single layer (biosensor type 1)	0.01–4	0–8	0.0005	120±5	3.9	Low limit of detection
Cross-linking with GA, two-layer membrane (biosensor type 2)	0.0025–0.5	0-5	0.0025	20±5	2.43	Short response time
Cross-linking with GA, arginase, urease and CLT in one layer (biosensor type 3)	0.01–6	0–15	0.01	58±0.6	1.91	Wide linear and dynamic ranges
Cross-linking with GA, arginase, urease and CLT in two layers where CLT is an adlayer (biosensor type 4)	0.5–6	0–16	0.5	46±0.3	0.76	Wide dynamic range, low coefficient of signal variation
Cross-linking with GA, enzymes concurrently in one layer with CLT and on the CLT adlayer (biosensor type 5)	0.01–0.1	0–2	0.01	47±0.6	1.04	Low coefficient of signal variation

[a]LOD – limit of detection; [b]CV – coefficient of signal variation; [c]n/a- data not available

OPTIMIZATION OF THE SENSITIVITY OF THE CALIXARENE-BASED SENSOR TO ARGININE

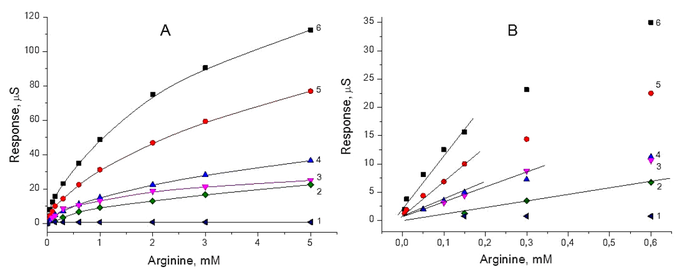


Fig. 10. Calibration curves of calixarene-based sensor prepared with different concentration of calixarene in the membrane: 0 mg/mL (1), 1 mg/mL (2), 5 mg/mL (3), 25 mg/mL (4), 50 mg/mL (5) and 100 mg/mL (6). The measurements were carried out in 5 mM phosphate buffer solution, pH 7.4

TABLE 2. COMPARISON OF OPERATIONAL CHARACTERISTICS OF SENSORS WITH DIFFERENT CONCENTRATION OF CALIXARENE IN MEMBRANE

Analytical characteristics of the chemosensor	Concentration of calixarene in sensitive element, mg/mL				
	100	50	25	5	1
Sensitivity, μS/mM	37.5	23.45	11.25	9.37	6.5
Limit of detection, mM	0.005	0.005	0.03	0.04	0.1
Linear range, mM	5-150	5-150	50-150	100-300	150-600
Response time, s	150	50	60	80	150
Baseline noise, μS	0.375	0.25	0.25	0.5	0.5
Baseline drift, μS/min	0.0625	0.125	0.075	0.25	0.75
RSD, %	2.5	1.7	3.9	2.7	4.8

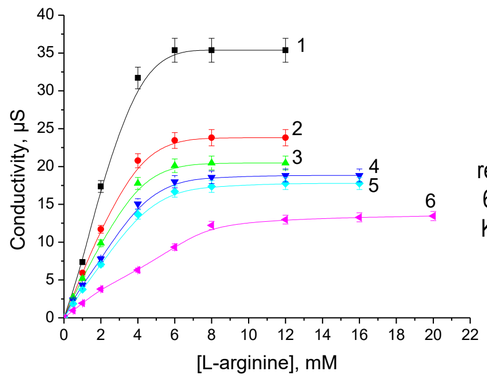


Fig. 7. Dependence of biosensor responses on ionic strength of phosphate buffer solution. Biosensor responses to arginine in 5 mM PBS, pH 6.0 (1), and in the presence of 2.5 mM KCl (2), 5 mM KCl (3), 7.5 mM KCl (4), 10 mM KCl (5), 20 mM KCl (6)

CONCLUSIONS. As seen from the obtained data, the arginase-urease biosensors, prepared under different approaches, differed significantly in the main analytical characteristics. The CLT-based biosensors for Arg demonstrated improved operational stability, wider dynamic range and slightly shorter response time in comparison with unmodified biosensors. That we explain by a combination of high adsorption properties of natural zeolite clinoptilolite toward proteins. Together, each group of biosensors can offer useful potentialities to the analysis. By varying the procedure of immobilization as described above, it seems to us possible to overcome the majority of the detection challenges when analyzing the real samples using the conductometric biosensors. A possibility of creation of chemosensor with calixarene-based sensitive element for arginine detection was also evaluated. The optimal concentration of calixarene for preparation of chemosensitive element was determined to be 100 mg/mL. The analytical characteristics of the corresponding chemosensor were as follows: sensitivity – 37.5 μS/mM, limit of detection 5 μM, linear range 0.005–150 μM, response time 150 s.

Acknowledgements:

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