

BIORECOGNITION ABILITY OF POLYSACCHARIDES AS PIEZO QUARTZ BIOSENSORS

* E.N. Kalmykova^{1,2} and C. A. Mgina³

1 - Lipetsk State Technical University, Russia,

2 - School of Physics, Chemistry Department, College of Natural Science and Mathematics,

University of Dodoma, P.O. Box 259, Tanzania

3 - Chemistry Department, University of Dar es Salaam

P.O. Box 35065, Tanzania

*Corresponding author's e-mail: veter1407@rambler.ru

ABSTRACT

Piezoquartz biosensors (PQB) which are analytical devices for recognition of biochemical interactions have recently attracted increasing interest from different researchers such as analysts, immune-chemists, medical doctors, environmentalists, etc. This is due to the advantages of PQB for having high detection sensitivity (at ng and mg level depending on the mass of sorbates or micro-organisms) and the ability to monitor biochemical reactions in turbid or highly coloured liquids (including in biological fluids) in real time. The use of additional markers and preliminary sample preparation are not required in this biorecognition process. This paper reports on the principle of the PQB and the design units to perform analysis in static and dynamic conditions using different types of biomolecules (immunoglobulins, DNA, lipopolysaccharides, glycoconjugates, hapten-protein conjugates and polysaccharides). Research results performed at the Department of Chemistry, Lipetsk State Technical University (Russia) and School of Physics, College of Natural Sciences, UDOM (Tanzania) are presented. The report aims to review various examples of use of carbohydrate molecules and glycoconjugates in the design of the PQB. These examples include the following: use of sulphated polysaccharides to increase the strength of the sensor's bio-layer; use of glycolipids such as O-antigens of bacteria (Yersinia enterocolitica) with different chemical structures in the development of immunosensors for the determination of specific immunoglobulins at 3-100 mg/ml levels in serum; and the use of polysaccharide hydrogels of the first chemical structure (hyaluronic acid, zosteran and neutral and acidic polysaccharide fractions isolated from water hyacinth Eichhornia crassipes) in studying their effectiveness in Pb²⁺ ions sorption.

Key Words: Quartz crystal microbalance, piezoquartz biosensors (PQB), bioreceptor molecules, polysaccharide hydro gel, liquid media, "dip and dry", flow injection analysis (FIA), adsorption, heavy metals.

INTRODUCTION

One of the actively developing directions of modern bioanalytical chemistry is in researches dealing with production of sensors of different nature including piezoquartz biosensors (O'Sullivan 1999, Berg 2003, Shen 2005, Ermolaeva 2008 & Hunter 2009). Biosensors provide analytical devices for rapid and sensitive analysis in gas and liquid environments. A distinctive

feature of this type of analytical devices is the ability to determine the contents of target components in multiple matrices of the samples to be analyzed, with little or without preparation of probes for analysis. Biosensors contain two major functional blocks: a bio-selective receptor and a detector directly in contact with the receptor. Since biochemical interactions lead to a change in voltage, current, light properties

(such as refractive index), acoustics, etc., different physical converters (optical, electrochemical, acoustic and piezoquartz transducer) can be used for their registration.

The high selectivity of analysis is due to using biomolecules such as different types of antibody (Ab) and antigens (Ag) (Cooper 2007), lectins (Lebed 2007), nucleic acids (Minunni 2001), aptamers (Yao 2010) as well as synthetic polymers (Molecularly Imprinted Polymers, MIP) (Alexander C. 2006) as receptors.

The receptor interacts with a defined substance, the detector, which is a physical

transducer, and transforms the generated signal of biochemical interactions into an analytical signal.

The detector, a quartz crystal resonator which is known as micro- or nanobalance (Fig. 1) has a high detection sensitivity at a nano- or microgram level, is composed of a thin plate of quartz crystal of AT-cut which is characterized by thermostability in the range 10 – 50°C, and metallic electrodes (Au, Ag, Ni, Al, Cr or others) which are deposited by thermal spraying on both sides of the quartz disc.

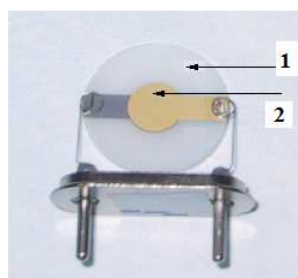


Figure 1: Quartz crystal resonator (1) metallic electrodes , (2) quartz disc.

An analytical signal of these sensors is inversely proportional to the added mass of the electrode's coating and can be calculated according to Sauerbrey equation (Sauerbrey 1959):

$$\Delta f = - 2.3 \cdot 10^6 \cdot f_0^2 \cdot \Delta m / A,$$

where: Δf is the change of frequency of oscillation of the crystal in Hz, f_0 is the frequency of oscillation of the crystal in MHz, Δm is the change in mass of tin receptor film on the electrode surface in g, and A is the area of the surface of the sensor's electrode in cm^2 .

The advantages of Quartz Crystal Biosensor are not only their high sensitivity but also their ability to determine high and low molecular weight biologically active

substances, their economy due to multiple use after regeneration of the bio-receptor layer, their simplicity of construction and possibility of registration of biochemical interactions without any labeling (enzymatic, radioactive, fluorescence, luminescent, etc.). It is not surprising therefore that the designing and use of piezoquartz biosensors has attracted active interest of various researchers in the world.

Since 1998 there have been active researches in the application of piezoquartz biosensors, especially immunosensors, for the determination of biologically active substances and micro organisms in liquid media in the Department of Chemistry of Lipetsk State Technical University, Russia. Thus, techniques capable of analysis have

been used in the determination of low molecular weight haptens such as sulphonamides (toxic phenolic compounds) and cotinine (a nicotine metabolite). Direct quantification was used to determine the high molecular weight substances and micro organisms. In each case, it was necessary to use different types of receptor molecules.

Sensitivity, reproducibility and duration of the exploitation of the sensor depend on the quality of the receptor layer. This quality requires the following: high activity of immobilized biomolecules; the firm attachment of electrodes on the surface of the crystal; physical and chemical stability of the receptor in contact with the sample to be analyzed and the regenerating solution; minimal mass of the receptor layer that allows the detection and registration of an analytical signal without overloading the sensor; long term of operation of biosensor.

We have investigated the conditions needed for obtaining bioreceptor coatings based on DNA and Ab, hapten-protein conjugates on metal surface by covalent immobilization on the electrode's coating using various cross linkers such as glutaraldehyde (GA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), N,N'-dicyclohexylcarbodiimide (DCC) (Liedberg 1997, Su 2000, Kalmykova 2006, 2007a-c, Shashkanova OY. 2007).

The nature of the metal should be taken into account when choosing a modifier of the resonator electrode. Compounds containing sulphur (Protein A, thioctic acid) are preferred for gold electrodes since sulphur atoms form coordination bonds with gold atoms.

The recent emergence of "nanotechnology"

contributed in intensifying research on the development of biosensors using carbohydrate and glycoconjugate molecules. The possibility of using a sulphated polysaccharide (S-PS) as an activator of gold electrode of quartz crystal resonator has been reported by us (Kalmykova 2009). This technique improves the resistance of the bio layer by 5-7 times compared with protein A substrate. This can be explained by the presence of more quantity of coordination bonds for a linear polysaccharide structure compared with protein molecules where sulphur atoms are localized inside globules.

The use of lipopolysaccharides (LPS) as receptor molecules of piezoelectric crystal biosensor for the determination of peptides was previously reported by Chang (1997). We have proposed the method of immobilization of LPS with orientation assignment of ligands (Kalmykova 2007a, b). LPS are found on the outer membranes of cell walls of gram-negative bacteria. Structurally, LPS are biopolymers consisting of three structural units: a lipid which is responsible for the manifestation of toxicity, an oligosaccharide bark common for all the bacteria, and an O-specific polysaccharide (O-PS) that defines the diversity of serovars of bacteria (Westphal 1983). Use of diphylic LPS macromolecules allows excluding the application of cross-phase reagents. It was shown that the hydrophobic activator of the electrode orients immobilization of the carbohydrate part of LPS molecules in the direction of hydrophilic eluent. This allows the use of biosensor for the determination of concentrations of specific antibodies for diagnosis of yersiniosis, an infectious disease caused by a bacteria, *Yersinia enterocolitica* (Kalmykova 2007c) (Fig. 2 a)

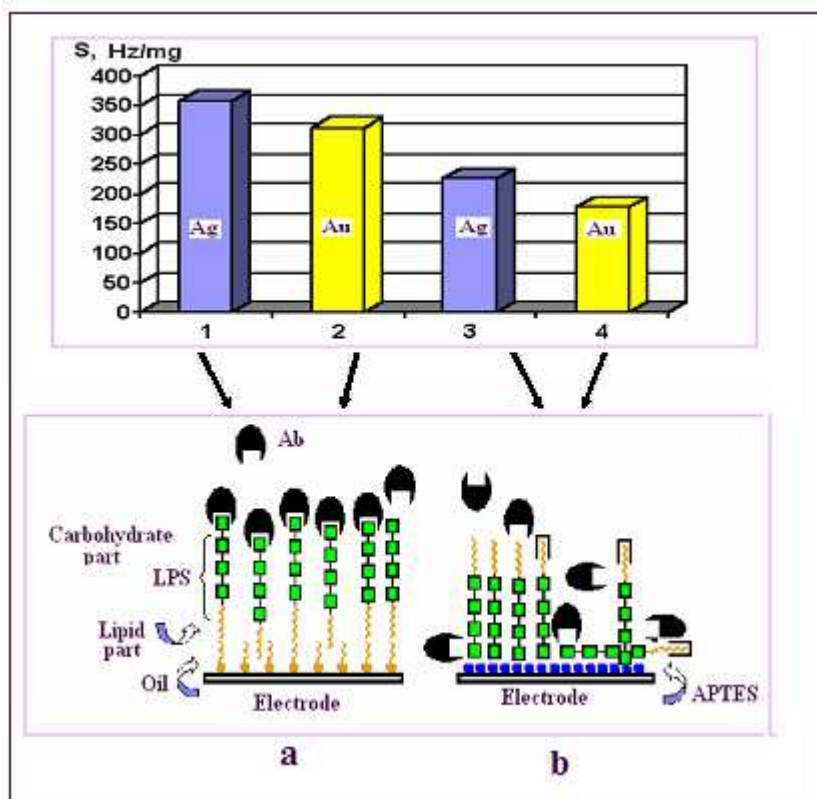


Figure 2: Assignment orientation of LPS molecules.
 (a) – hydrophobic activator; (b) – hydrophilic modifier of electrode of quartz crystal resonator

The Hydrophilic surface causing the opposite orientation of LPS molecules becomes the bioreceptor layer of the sensor which can be used to evaluate anti-toxic medications (Fig. 2b). The sensor with immobilized LPS has also been proposed for quantification of bacteria *Francisella tularensis* by using succinimide (Pohonka 2007).

The analyses of liquids using PQB have both static (Thompson 1986, Bunde 1998) and flow-injection modes (Barnes 1992, Saber 2002). The use of the sensor as a detector in flowing injection analysis improves the speed of the analysis, as well as it provides

the ability to monitor high-risk biochemical reactions in real time.

When the sensor is used in “dip & dry” method (Shons 1972) the mass increment of the receptor layer is measured before and after the exposure of the sensor in the analyzed solution and then drying it in air to constant weight. An advantage of the method is not only having low detection limits of analytes, but also in its ability to be used in both laboratory and field conditions (Su 2002).

Conditions of analysis. The sensitivity of detection using biosensor depends largely on the conditions of analysis of the fluids

(pH, temperature and flow rate of the solution for flowing injection analysis). It has been found out that the pH of the buffer solution has an influence not only on the analytic signal but also on the linear range

of analyte concentrations (Kalmykova 2007c). For example, pH 7.2 possesses a higher value of analytic signal and a wider linear range of defined concentrations (Fig. 3).

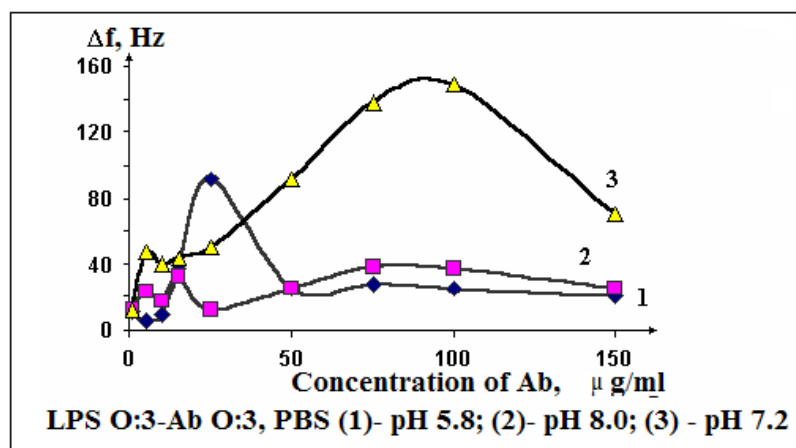


Figure 3: Influence of pH of buffer solution effectivity of binding immobilized LPS as receptor molecules with antibodies.

For immobilized LPS most fully immunochemical reactions occur when the pH is 7.2 with a maximum value of an analytic signal. For neutral media the process of linking of LPS with antibodies is noted over a wide linear range of concentrations (5-100 μg/ml). Changes of pH to weak-acid region (pH 5.8) and particularly to alkaline region (pH 8) lead to the reduction of effectiveness of linking immune-reactants and dissociation of the immune complex that is accompanied by a

decrease of the analytical signal and reduction of the linear range of concentrations (10-25 μg/ml at pH 5.8).

Ionic strength of the solution affects the stability of an immune complex. Changing it can result in the deletion of the sorption analyte and regeneration of the biolayer. Studies of various solutions (Figure 4) show distilled water as the most effective and soft acting recycling reagent.

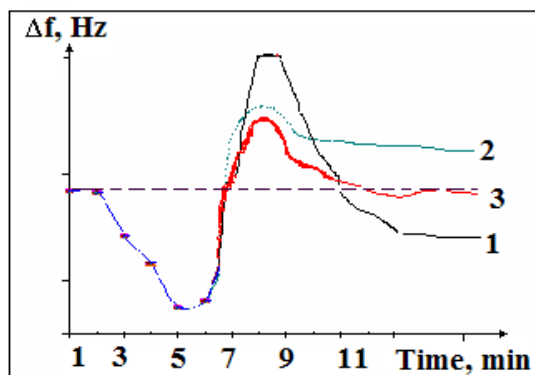


Figure 4: Influence of ionic strength of buffer solution. Regenerating solution:
 1 – 0.03 mM KCNS - low effectivity of regeneration,
 2 - 0.1 M HCOOH, NaOH - aggressive action causing destruction of not only the immune complex, but also the substrate of biolayer;
 3 - H₂O optimal action: effective dissociation of immune complex without destruction of substrate if bioreceptor layer (more then 20 measurement cycles).

Since the limited stage for immunochemical reaction on the surface of the electrode sensor is phase diffusion, the influence of duration of the exposure of the sensor in the

analyzed solution under static conditions and rate of solution for the analytical signal in flow-injection determination of biologically active compounds was investigated (Fig. 5).

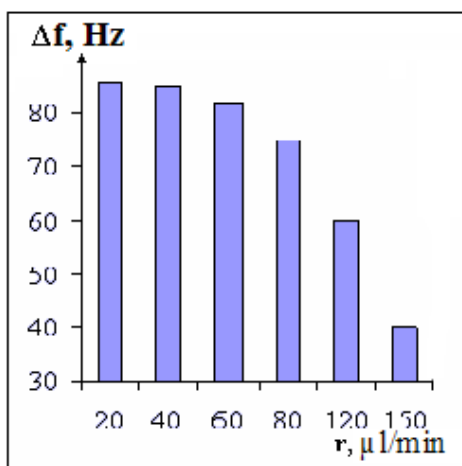


Figure 5: Influence of the flow rate of solution (r) for analytical signal of flow-injection quantifications.

Reducing the solution flow (30 $\mu\text{l}/\text{min}$) contributes to the enhancement of the analytic signal but increases the duration of

one cycle of measurement. The optimum rate established was 60-90 $\mu\text{l}/\text{min}$.

The maximal reduction of signal (20-22 Hz) is noted only after 10-15 min exposure of the sensor for “dip & dry” conditions.

Change of temperature in the range 18-26°C does not provide significant influence on the value of the signal, so it’s possible to carry out an analysis at room temperature without need of temperature stabilization.

Establishment of selectivity of biosensor is particularly important for carrying out of

clinical research. Selectivity was assessed as a coefficient of cross-interaction of haptens and their structural analogs with antibodies by the formula:

$$CR, \% = \Delta f (B) 100 \text{ per cent} / \Delta f (A),$$

Where: $\Delta f (B)$ and (A) frequency Δf improvements when binding antibodies with a structural analogue of haptens and immunogens, respectively, Table 1.

Table 1: Cross-reactivity (CR, %) of LPS with Ab to bacteria *Y. enterocolitica*, determined by Precipitation Reaction (+ or -) and Piezoquartz Biosensors

LPS (serovar)	CR, %					
	Ab O:3	Ab O:5	Ab O:6,30	Ab O:7,8	Ab O:8	Ab O:19,8
O:3	98-100	4-6	6-8	-	-	-
O:5	3-6	96-99	4-9	-	-	-
O:6,30	4	7-9	96-98	-	-	-
O:7,8	-	-	39	99-100	84	61
O:8	-	-	23	47-55	86-99	24
O:19,8	-	-	24	91-100	65-72	100

The high specificity of interaction of LPS with homologous serum allows using immunosensors for clinical diagnosis of the infectious diseases yersiniosis (Kalmykova 2007, Ermolaeva 2008, Kalmykova 2011). Results obtained with PQB are correlated with serological tests (Precipitation Reaction) but are characterized by higher sensitivity and expression.

Studies have enabled the development of sensors for the determination of low-molecular haptens such as cotinine (a metabolite of nicotine, drugs such as sulphonamides and toxic compounds such as nonylphenol (Melikhova 2006, Kalmykova 2002, Ermolaeva. 2006) Table. 2.

Table. 2: Quantification of Low-molecular Haptens in Enviromental Samples, Foodstuffs, Drugs, Biological fluids

Sample	Introduced (ng/ml)	Determined (ng/ml)	Calculated (ng/ml)	S _r
Sulfamethoxazole (Standard additions method)				
River Voronezh water	10	14±2	4±2	0.2
Soil	10	22±3	12±3	0.2
Human milk	10	15±1	5±1	0.06
Natural cow milk	10	11.5±0.4	1.5±0.4	0.04
Pasteurized cow milk	10	11±0.2	0.6±0.2	0.02
Chicken meat	10	14±1	1.6±0.6	0.1
Chicken eggs	10	11.6±0.4	0.8±0.4	0.1
Biseptol (Poland)	20	16.±0.8	-	0.04
Co-trimoxazole (Russia)	20	18±2	-	0.1
Cotinine (Calibrated graph method)				
Urine - probe 1	-	2300±300	-	0.04
Urine - probe 2	-	4500±500	-	0.05
Nonylphenol (Calibrated graph method)				
River Voronezh water	15.2±1	-	-	0.02

The PQBs were tested in the analysis of real substances (foodstuffs, medicines, body fluids). The accuracy of determinations was evaluated by the method “introduced-found” and by comparing the results obtained with those from other methods of analysis such as polarization-fluorescent immunoassay (PFIA) and HPLC.

The determination of cotinine was carried out with the aim of identifying active smokers. The results obtained were consistent with data obtained through PFIA.

Sulfonamides and phenolic compounds were found in water samples from river Voronezh, matching with sewage from industries, but the content of these substances did not exceed the approved standard.

The concentration of sulphamethoxazole in pharmaceutical products was determined and was found to correspond with those in documents thus confirming the satisfactory quality of pharmaceutical products. Sulphamethoxazole was also detected in one sample of women milk, as well as in all

samples of cow's milk (from 0.6 to 1.5 ng/ml), chicken meat (1.6 ng/g), and eggs (0.8 - 1.2 ng/g). These amounts are far below the standard indicators for European Union countries (Melikhova 2006).

High-molecular analytes *Yersinia enterocolitica* bacteria that are also referred to as "bacteria out of the refrigerator", are causative agents of gastro enteric diseases such as widespread yersiniosis that can be passed through contaminated water and food stored in the refrigerator. These analytes were determined by direct form of analysis with specified biosensors. When you define a linear function in microorganisms, the range of concentrations of the graduating function is sufficiently narrow due to the large size determined by the particles. The liner range for bacteria is 0.1 – 4.9 cells/ml (Kalmykova 2007c).

The presence Ab-DNA in the blood at varying specificity can serve as a marker to identify various somatic and infectious diseases. Ab-DNAs are formed in the organism in the development of autoimmune diseases such as Lupus Erythematosus,

Rheumatoid Polyarthritis, etc. Since the identification of specific Ab allows for early detection of pathological processes, we have proposed an immunosensor based on DNA immobilized molecules to determine the Ab-DNA. As a standard, a solution of Ab serum of a patient with symptoms of Systemic Lupus Erythematosus was used. The activity was detected by ELISA -320 IU. The linear range of definitions was 25 - 0.1 µg/ml (0.03-8 IU) while the LOD was 0.01 µg/ml (0.003 IU) (Kalmykova 2002).

The necessity of quantification of Ab to bacteria arises due to the polymorphism of clinical forms of yersiniosis (arthritic, enteric, miocarditic, etc.) which makes it difficult to identify the disease. Therefore, in order to confirm the diagnosis microbiological and immuno-chemical methods of analysis are now used. These methods are characterized by long-run tests (several hours for reaction of passive hemagglutination), and the possibility of non-refillable bio reagents (ELISA). We, therefore, propose sensors based on immobilized LPS *Y. enterocolitica* serovar O:3 for quantification of antibodies. The sensor is characterized by high expression, sensitivity and efficiency for the determination of antibodies in the blood sera at concentrations range from 3-150 µg/ml (Kalmykova 2007c).

The method of piezo quartz microbalance detection is promising not only for identifying the various biologically active substances in aquatic environments but for studying of sorption processes of toxins, ions of heavy metals, etc. by biosorbents of different nature (proteins, polysaccharides). The advantages of biosorbents are: safety for

people and other living organisms, relative low cost, high stability in extreme conditions, ability to biodegrade quickly enough, possibility of wider practical applications. Sorption activity and selectivity of some biosorbents are the same as synthetic ionites and in many cases better.

MATERIALS AND METHODS

Zosteran was kindly proposed by Dr R.P. Ovodova, Institute of Physiology, Komi Science Centre, the Urals Branch of the Russian Academy of Sciences.

Hyaluronic acid was isolated from animal tissue (umbilical cord) using water-acidic extraction.

Neutral and acidic polysaccharide fractions were isolated from Water Hyacinth (*Eichhornia crassipes*), collected from Lake Victoria, Tanzania.

All polysaccharides were tested for effectiveness of sorption of Pb⁺² ions in water solutions using PQB by “dip and dry” detection. Resonators of AT – cut running at 9 MHz resonance frequency with silver electrodes of 5 mm diameters from CAS “ETNA”, Russia were used as detectors for measuring sorption activity of thin films formed at the surface of the electrode of the resonator. Each polysaccharide investigated was immobilized (as a thin film) on the electrodes by cross-linking with water-formaldehyde solution. The process of polysaccharide-film formation was controlled by quartz crystal microbalance method. The sorption activity of polysaccharide fractions was tested using diluted solutions of Pb(NO₃)₂. The results are given in Table 3.

RESULTS AND DISCUSSION

Table 3: Sorption Activity of Polysaccharides for Pb⁺² Characterized with using PQB

C, %	Linear interval, mg/ml	C _{min} , mg/ml	a _∞ , Hz
Based on Hyaluronic Acid			
1.00	0.32-8.33	0.27	5019.1
2.00	0.32-10.00	0.25	6942.2
Based on Zosteran			
1	0.31 – 8.83	0.062	60050
1.5	0.31 – 10.00	0.060	70700
2	0.31 – 10.00	0.059	70800
Based on Polysaccharide fractions from Water Hyacinth (<i>Eichhornia crassipes</i>)			
Neutral PS 1.5	0.0005 – 0.019	0.0004	-
Acidic PS 1.5	0.0005 – 0.025	0.0004	-

a_∞ - limiting sorption of biolayer of the sensor, Hz

The influence of concentration of polysaccharide solution was investigated and the optimal concentration for obtaining a bioreceptor layer was determined to be 1.5 %. Hyaluronic acid and zosteran were found to exhibited high sorption activity for Pb⁺² ions. The linear ranges were found to be of 0.31 - 8.00 and 0.32 - 10.00 mg/ml for hyaluronic acid and zosteran, respectively.

Compared to purified polysaccharides (zosteran and hialuronic acid) sorption activity of polysaccharide fractions isolated from Water Hyacinth (*Eichhornia crassipes*) exhibited lower activity. This may be due to the chemical nature and the presence of admixtures in the fractions obtained. It was also found out that acidic polysaccharide exhibited higher activity than neutral polysaccharides The linear range was found to be 0.5 - 18.5 µg/ml and 0.5 - 25 µg/ml for neutral and acidic polysaccharides respectively. The high sorption activity of acidic polysaccharide fractions can probably be attributed to the space attractable carboxyl groups of uronic acids present in glycans.

CONCLUSION

Given their effectiveness sensors, piezoquartz biosensors should be more widely used in many areas such as:

- Medicine in assessing the dynamics of immunological interactions, the growth of microbial populations, changes in the viscose-elastic properties of bioliquids to identify infectious, autoimmune, of cancer at early stages
- Food and perfume industry for identifying genetically-modified or counterfeit products);
- Pharmaceutical chemistry
- Agriculture (veterinary, horticulture, plant breeding)
- Biotechnology;
- Monitoring of environmental toxic agents, and of bacteriological pollutants of water, soil and sewage.

Of particular importance is the promising use of PQBs for the detection of drugs and doping which can be carried out on samples of urine, saliva and other biological fluids on the content of alkaloids of hormonal preparations and other medicinal substances taken by athletes.

REFEENCES

- Alexander C, Andersson HA, Fndersson LI, Ansell RJ, Kirsch N, Nicholls IA, O'Mahony J, Whitcombe MJ. 2003 Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. *J. Mol. Recognit.* **19**: 106-180.
- Babacan S, Pivarnik P, Letcher S, Rand AG 2000 Evaluation of antibody immobilization methods for piezoelectric biosensor application. *Biosens. Bioelectron.* **15**: 615-621.
- Barnes C, D'Silva C, Jones JP, Levis TJ 1992 *Sens. Actuators.* **B7**: 347-350.
- Bunde RL, Jarvi EJ, Rosentreter JJ 1998 Piezoelectric quartz crystal biosensor. *Talanta* **46**: 1223-1236.
- Chang HC, Yang C, Yeh TM 1997 Detection of lipopolysaccharide binding peptides by the use of a lipopolysaccharide-coated piezoelectric crystal biosensor *Anal. Chim. Acta.* **340**: 49-54.
- Cooper MA and Singleton VT 2007 A survey of the 2001 to 2005 quartz crystal microbalance biosensor literature: applications of acoustic physics to the analysis of biomolecular interactions *J. Mol. Recognit.* **20**: 154-184.
- Ermolaeva T N, Dergunova ES, Kalmykova EN, and Eremin SA 2006 Flow-Injection Determination of Nonylphenol in Liquid Media Using a Piezoelectric Immunosensor. *J. Anal. Chem. Russia* **61**(6): 1-6.
- Ermolaeva TN, Kalmykova EN, Shashkanova OYu 2008 Piezoquartz biosensors for the analysis of environmental objects, foodstuff and for clinical diagnostic. *Russ. J. Gen. Chem.* **78**(12): 2430-2444.
- Kalmykova EN, Eremin SA, Ermolaeva TN 2002 Flow-Injection Immunosensors With Quartz Crystal Microbalance For Analysis Of Aqueous Solutions And Biological Liquids. International Conference Biocatalysis –: Fundamentals & Applications, June 2002, Moscow. ABSTRACTS. 107-108.
- Kalmykova EN, Eremin SA, Ermolaeva TN. 2002 Elaboration of Quartz Crystal Microbalance Immunosensors for Flow-Injection Assay of High- and Low – Molecular Compounds. Vest. Moscow University (*Russia*). *Seria 2. Khimia.* **43**(6): 399-403.
- Kalmykova EN, Ermolaeva TN. 2006 Quartz Crystal Immunosensors for Clinical Diagnostics. International Congress On Analytical Sciences ICAS-2006 June 25-30, 2006, Moscow, Russia Abstracts. p.133.
- Kalmykova EN, Garbuzova AV, Shashkanova OYu, Zubova NYu, Ermolaeva TN. 2007a Piezoquartz Immunosensors on the base of immobilized Antibodies for determination of bacteria *Yersinia enterocolitica* in water media *Izv. Vuzov. Khim. Chimtechnol.* **50**(9): 10-15.
- Kalmykova EN, Dergunova ES, Ermolaeva TN, Gorshkova RP, Komandrova NA. 2007b Piezoquartz immunosensors for assessing the interactions between *Yersinia enterocolitica* lipopolysaccharides and antibodies to them. *Appl. Biochem. Microbiol.* **44**(4):372-377.
- Kalmykova EN, Dergunova ES, Zubova NYu, Gorshkova RP, Komandrova NA, Ermolaeva TN. 2007c Determination of Antibodies to *Yersinia enterocolitica* Bacteria Using a Piezoelectric Quartz Crystal Immunosensor. *J. Anal. Chem. Russ.* **62**(10): 970–976.
- Kalmykova EN, Pushilina MYu, Rogozhnikov NA, Poletaeva Yu V, Tereshenkova AA, Novikova OD. 2009 Quartz Crystal Microbalance Biosensors on the Base of Carbohydrates International Conference Biocatalysis-2009: Fundamental & Applications 19-

- 24 June. Arkhangelsk. Russia. pp115-116.
- Kalmykova EN, Vuai SA, Tomshich SV, Komandrova NA, Zubova NYu. 2011 Quartz Crystal Immunosensors For Diagnostic Of Yersiniose Infection. IX European symposium of The Protein Society, May, 22 – 26. 2011. Stockholm. Sweden. Abstracts. P. 75.
- Liedberg B, Gopel W. Antibody immobilization using heterobifunctional crosslinkers 1997 *Biosens. Bioelectron.* **12**(8): 691-695.
- Lebed K, Kulik AJ, Forro L and Lekka M 2007 Atomic Force Microscopy and Quartz Crystal Microbalance Study of the Lectin-Carbohydrate Interaction Kinetics. *Acta Physica Polonica A* **111**(2): 273-286.
- Melikhova EV, Kalmykova EN, Eremin SA, Ermolaeva TN. 2006 Using Piezoelectric Flow Immunosensor for Determining Sulfamethoxazole in Enviromental Samples. *J. Anal. Chem. Russ.* **61**(76): 744-750.
- Minunni M, Tombelli S, Pratesi S, Mascini M, Piatti P, Bogani P, Buiatti M. 2001 A piezoelectric affinity biosensor for genetically modified organisms (GMOs) detection *Anal. Lett.* **34**: 825-840.
- O'Sullivan CK, Guilbault GG. 1999 Commercial quartz crystal microbalances – theory and applications. *Biosens. Bioelectron.* **14**(8-9): 663-670.
- Pohonka M, Skladal P. 2007 Piezoelectric Immunosensor for the Direct and Rapid Detection of *Francisella tularensis*. *Folia Microbiol.* **52**(4): 325-330.
- Sauerbrey G. 1959 Verwendung von Schwingquarzen zur Wagung dunner Schichten und zur Microwagang. *Z. Phys.*, **155**: 206-212.
- Saber R, Mutlu S, Piskin E. 2002 Glow-discharge treated piezoelectric quartz crystals as immunosensors for HSA detection *Biosens. Bioelectron.* **17**: 727-734.
- Shashkanova OY, Dergunova ES, Kalmykova EN, Ermolaeva TN. Flow-Injection Piezoquartz Immunosensors for Determination of Bacteriophages. 2007 *Sorpt. Chromatogr. Proc. Russ.* **7**(4): 548-555.
- Shen Z, Stryker GA, Mernaugh RL, Yu L, Yan HP, Zeng XQ 2005 Single-chain Fragment Variable Antibody piezoimmunosensors. *Anal. Chem.* **77**(3): 797-805.
- Shons A, Dorman F, Najarian J. 1972 An immunospecific microbalance *J. Biomed. Mater. Res.* **6**: 565-570.
- Su XD, Chew FT, Li SF Piezoelectric quartz crystal based label-free analysis for allergy disease 2000 *Biosens. Bioelectron.* **15**(11-12): 629-639.
- Su XD. 2002 Covalent DNA immobilization on polymer-shielded silver-coated quartz crystal microbalance using photobiotin-based UV irradiation. *Biochem. Biophys. Res. Commun.* **290**(3): 962-966.
- Su X, Dai C, Zhan J, O'Shea SJ 2002 Quartz tuning fork biosensor *Biosens. Bioelectron.* **17**: 111-117.
- Thompson M, Arthur CL, Dhaliwal GK 1986 Liquid-phase piezoelectric and acoustic transmission studies of interfacial immunochemistry *Anal. Chem.* **58**: 1206-1209.
- Westphal O, Jarn K, Himmelspach K 1983 Chemistry and Immunochemistry of Bacterial lipopolysaccharides as cell wall antigens and endotoxin. *In: Prog. Allergy (Eds Ishizaka K, Kallos P, Waksman BH, de Wesk AL) Karger: Basel* **33**: 9-39.
- Yao C, Zhu T, Qi Y, Zhao Y, Xia H, Fu W. 2010 Development of a Quartz Crystal Microbalance Biosensor with Aptamers as Bio-recognition Element. *Sensors.* **10**(6): 5859-5871.