Conductometric enzyme biosensor for determination of heavy metal ions in water solution

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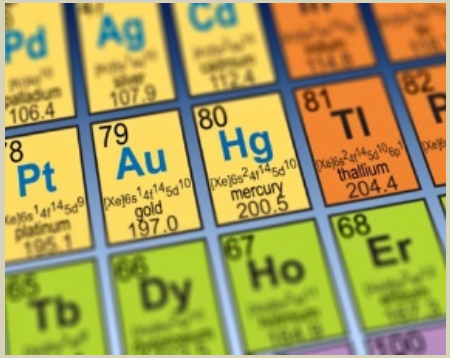
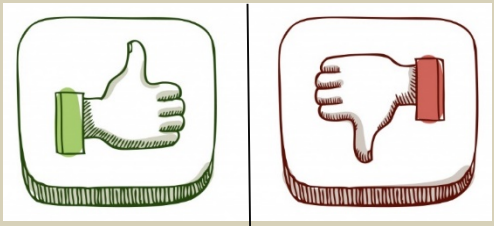
Conventional methods of HMIs detection:

- gas and liquid chromatography,
- spectrophotometry,
- atomic absorption spectroscopy,
- chemical and physical techniques





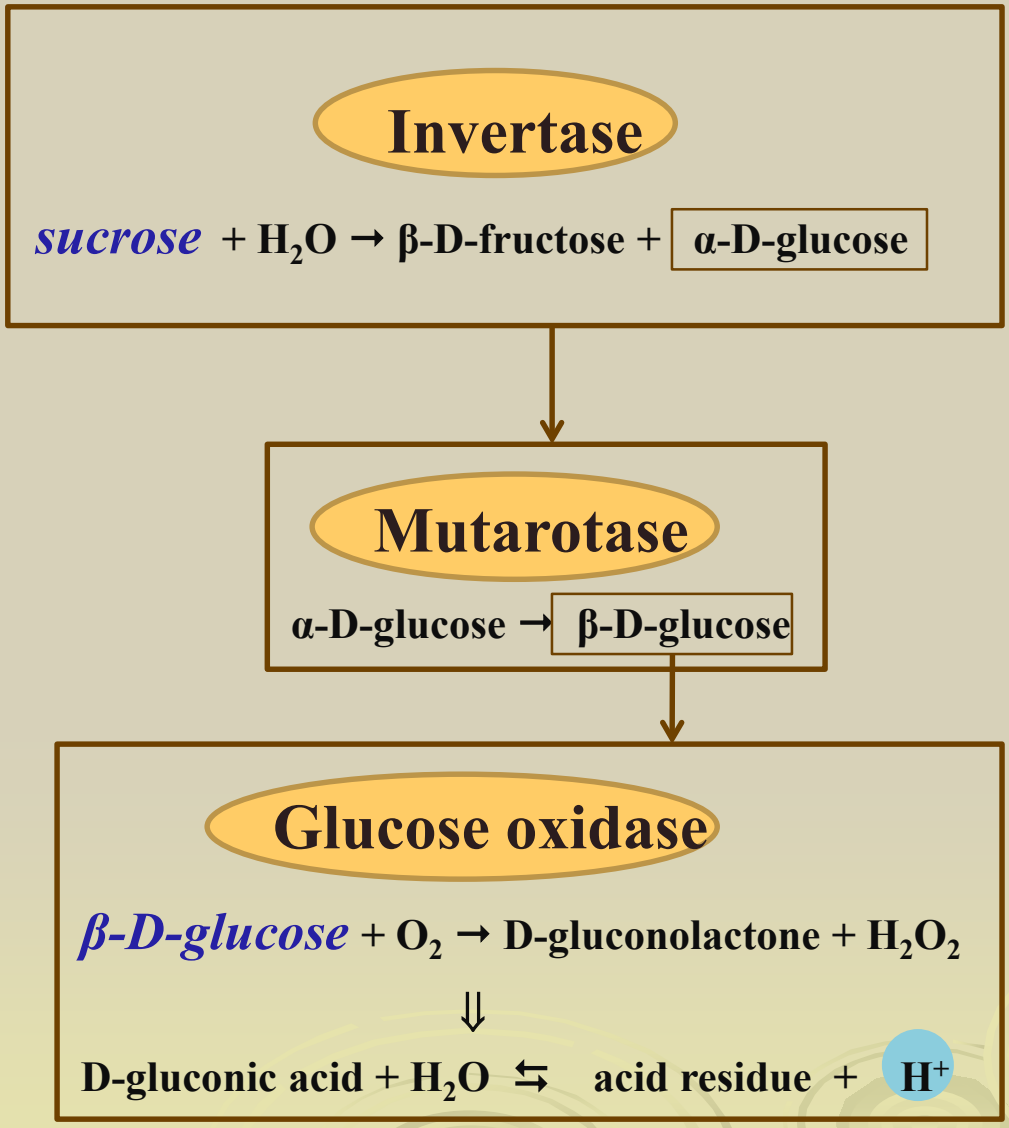
- Accurate
- High resolution power of separation
- Can separate very complex solution

-Expensive
-Complex
-Time consuming
-Require high-skilled staff
-Require a lot of sample pretreatments

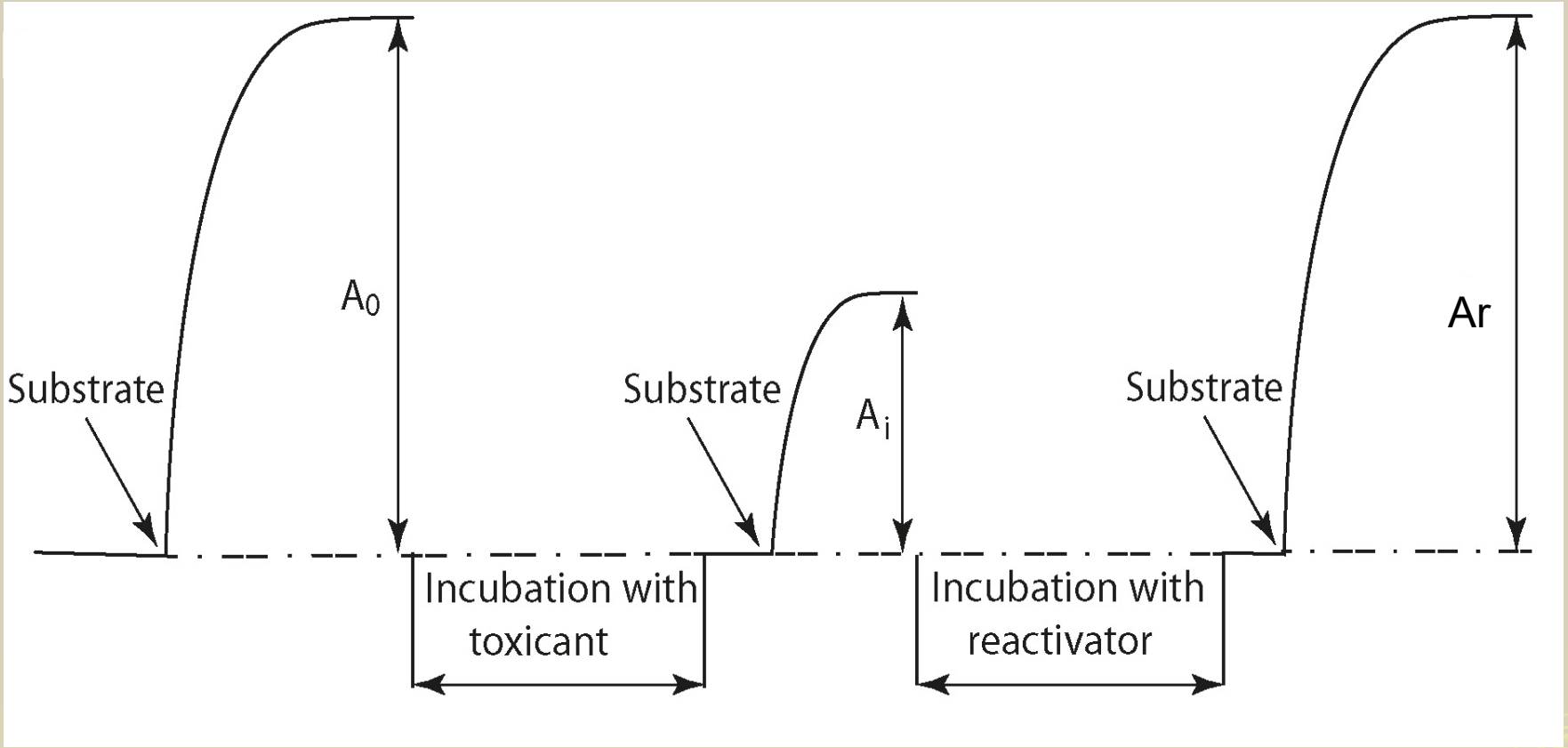
The advantages of biosensor analysis comparing to conventional methods:

- Simple;
- Accurate;
- Specific;
- Sensitive;
- Convenient;
- Fast;
- Cheap;
- Don’t require a lot of pretreatments.

Enzyme reactions



Inhibitory analysis



Biosensor signals:

A_0 — before inhibition

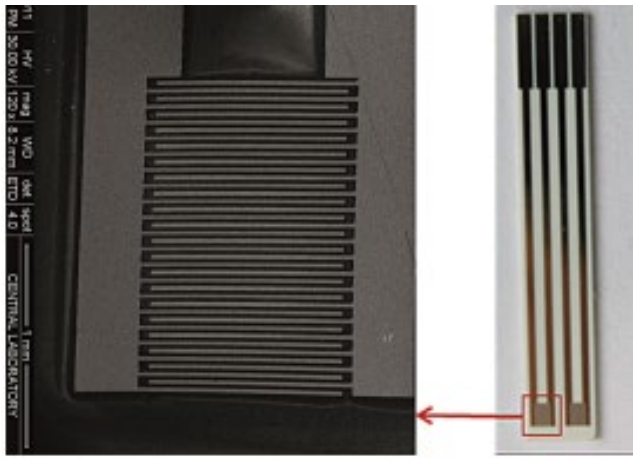
A_i — after inhibition

Ar — after reactivation

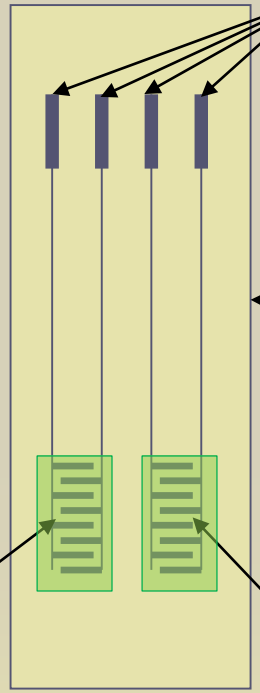
Z — residual activity

$$Z = A_i * 100 / A_0$$

Transducer



Immobilization of enzymes on the surface of electrodes



Electrodes (gold or platinum)

Ceramic or glass substrate

Enzyme (work) membrane:

Invertase 10%, Mutarotase 10%, Glucose oxidase 10% , 10% BSA, 20%Glycerine, in 40mM PBS, pH 6,5.

2.5% Glutare aldehyde +

Reference membrane:

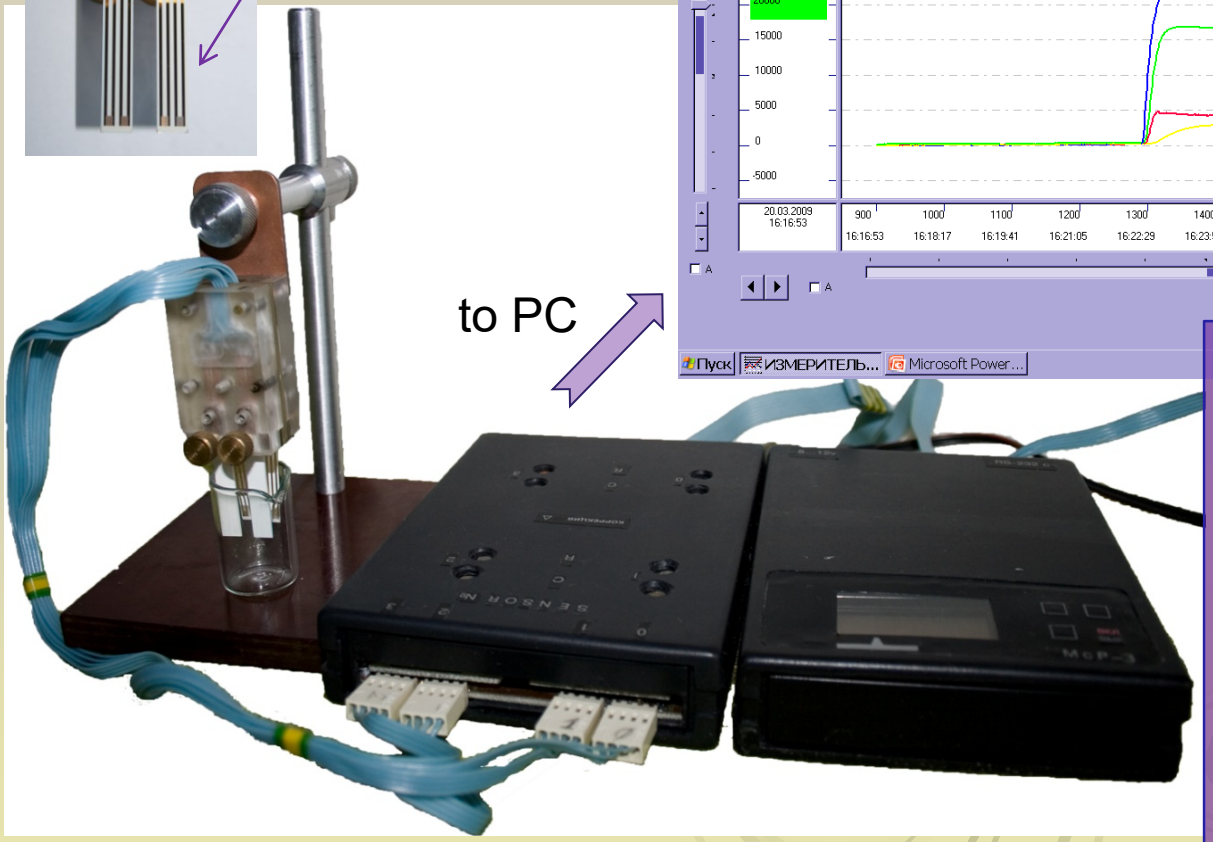
40% BSA, 20%Glycerine, in 40mM PBS. +

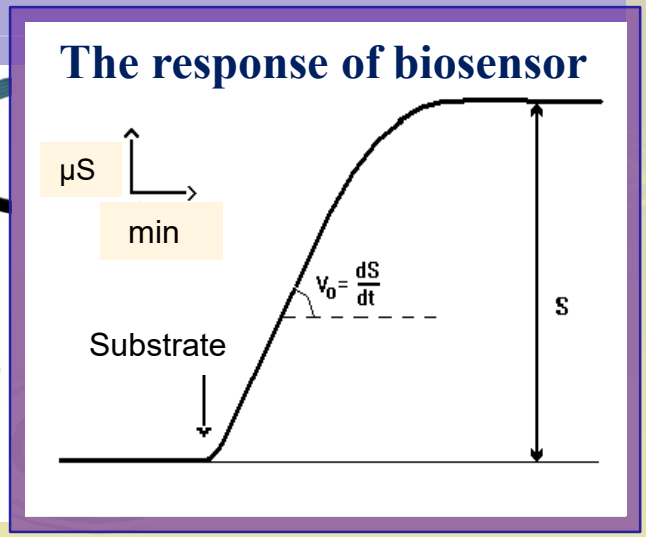
2.5% Glutare aldehyde

Conductometric multibiosensor system

conductometric trancducers

to PC





Part 1

Study of biosensor characteristics in direct analysis:

Effect of pH,
Buffer capacity,
Ionic strength on responses of biosensor, reproducibility,
Operation stability,
Storage stability,
Linear range,
Limit of detection, etc.

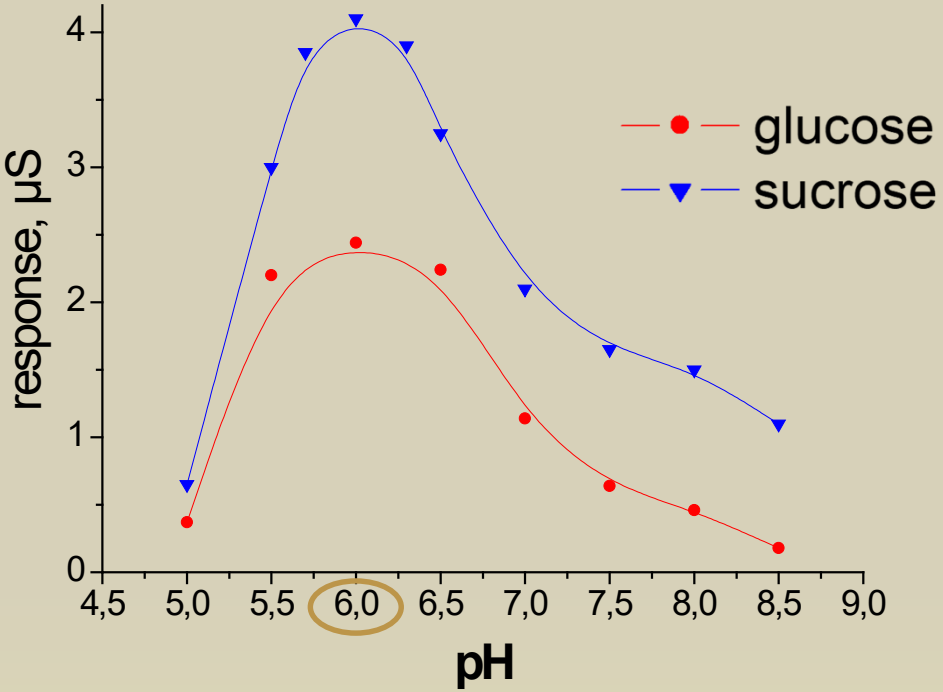
Part 2

Study of biosensor characteristics in inhibitory analysis:

Optimal substrate concentration in inhibitory analysis,
Optimal time of biosensor inhibition,
Selectivity to different HMIs,
Sensitivity to different HMIs,
Study of reactivation of biosensor, etc.

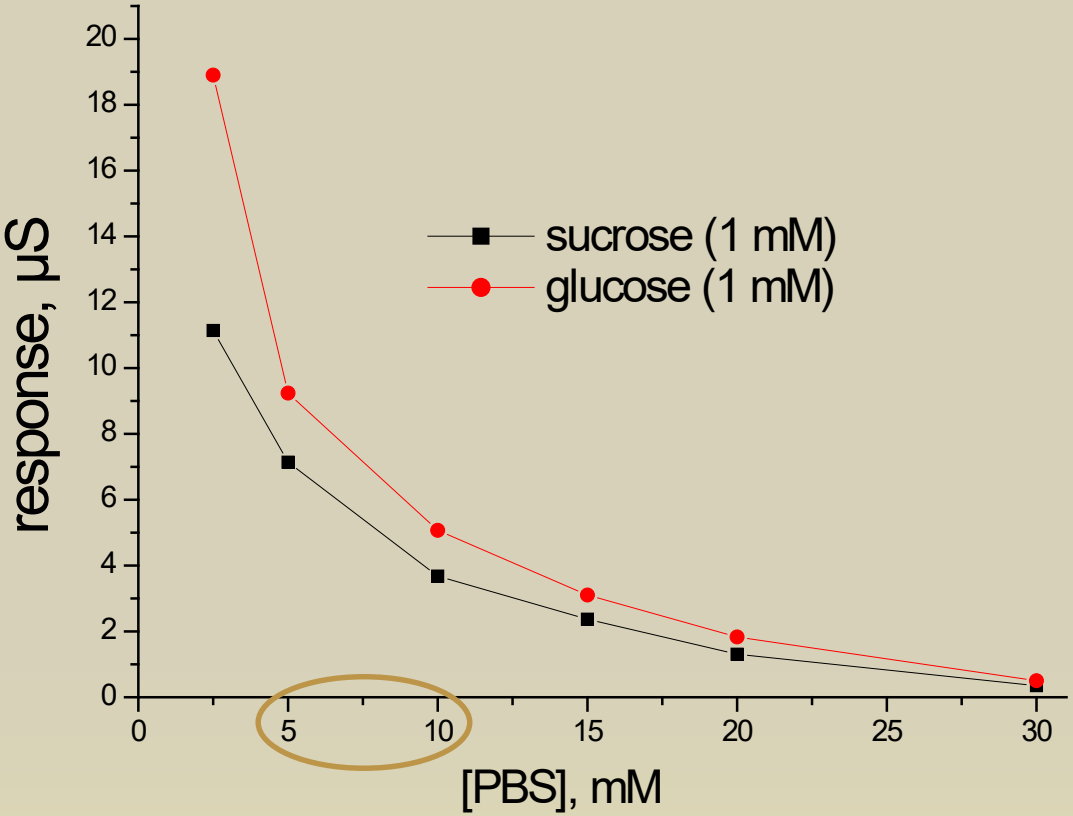
Part 1

Effect of pH on responses of biosensors



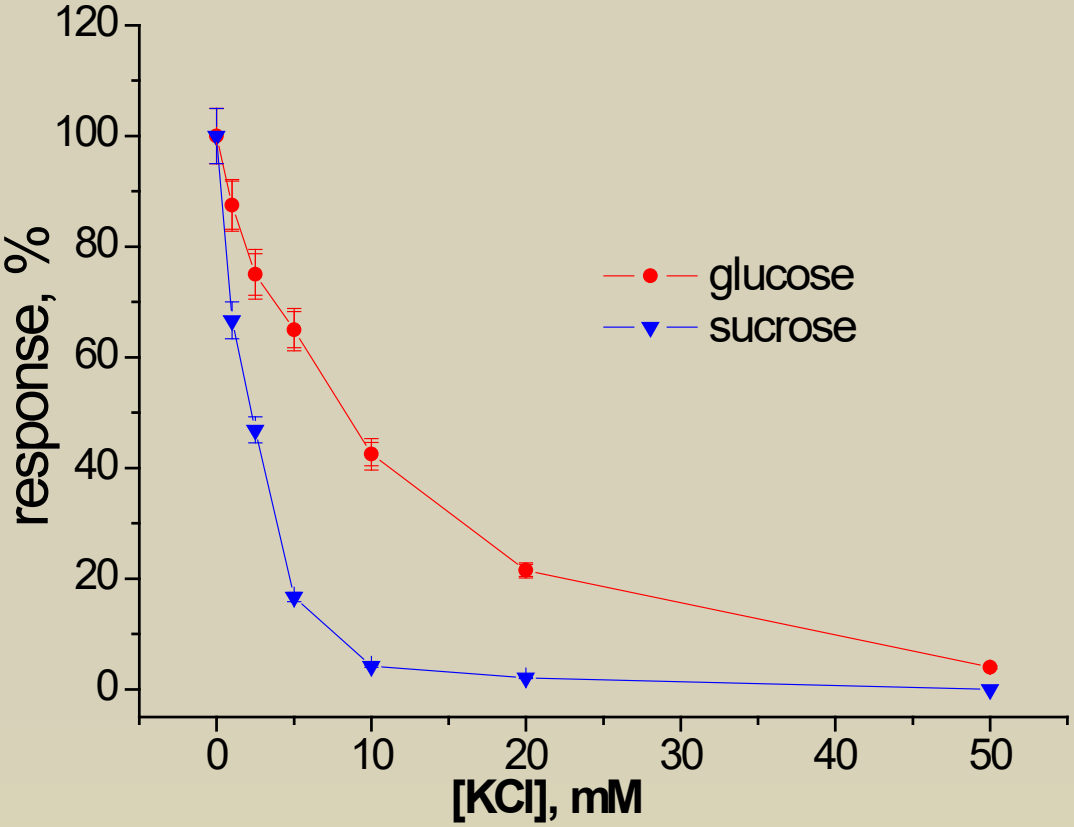
Concentration of substrate was 0.5 mM.
Measurement in polymix buffer solution

Effect of buffer capacity on responses of biosensor



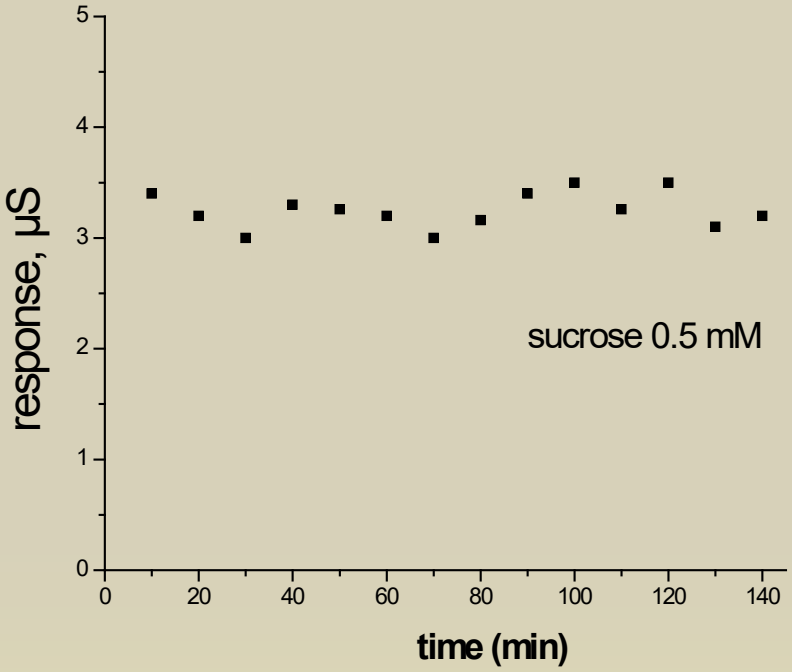
Dependences of biosensors responses on buffer concentration.
Measurement were carried out in phosphate buffer, pH 6,0.

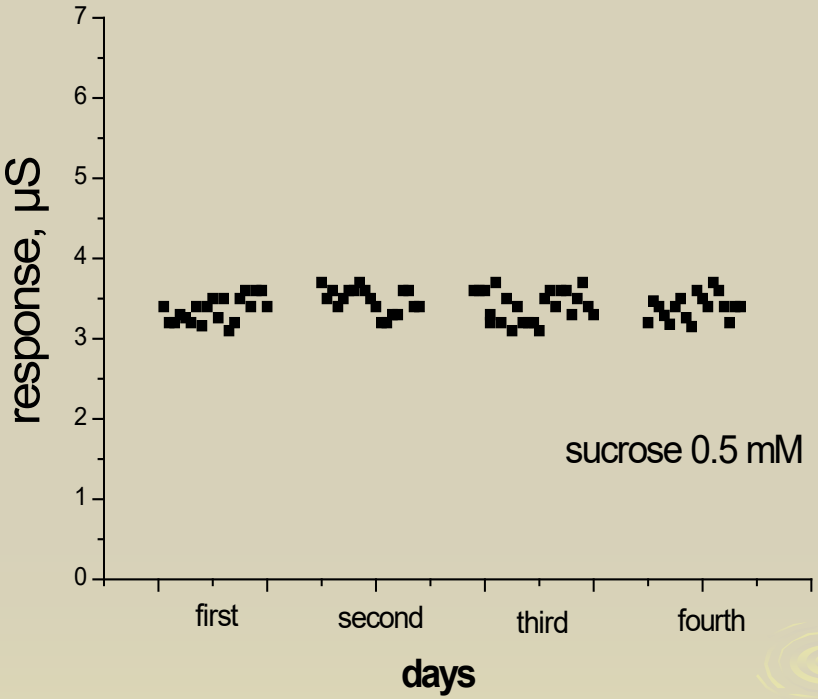
Effect of ionic strength on responses of biosensors



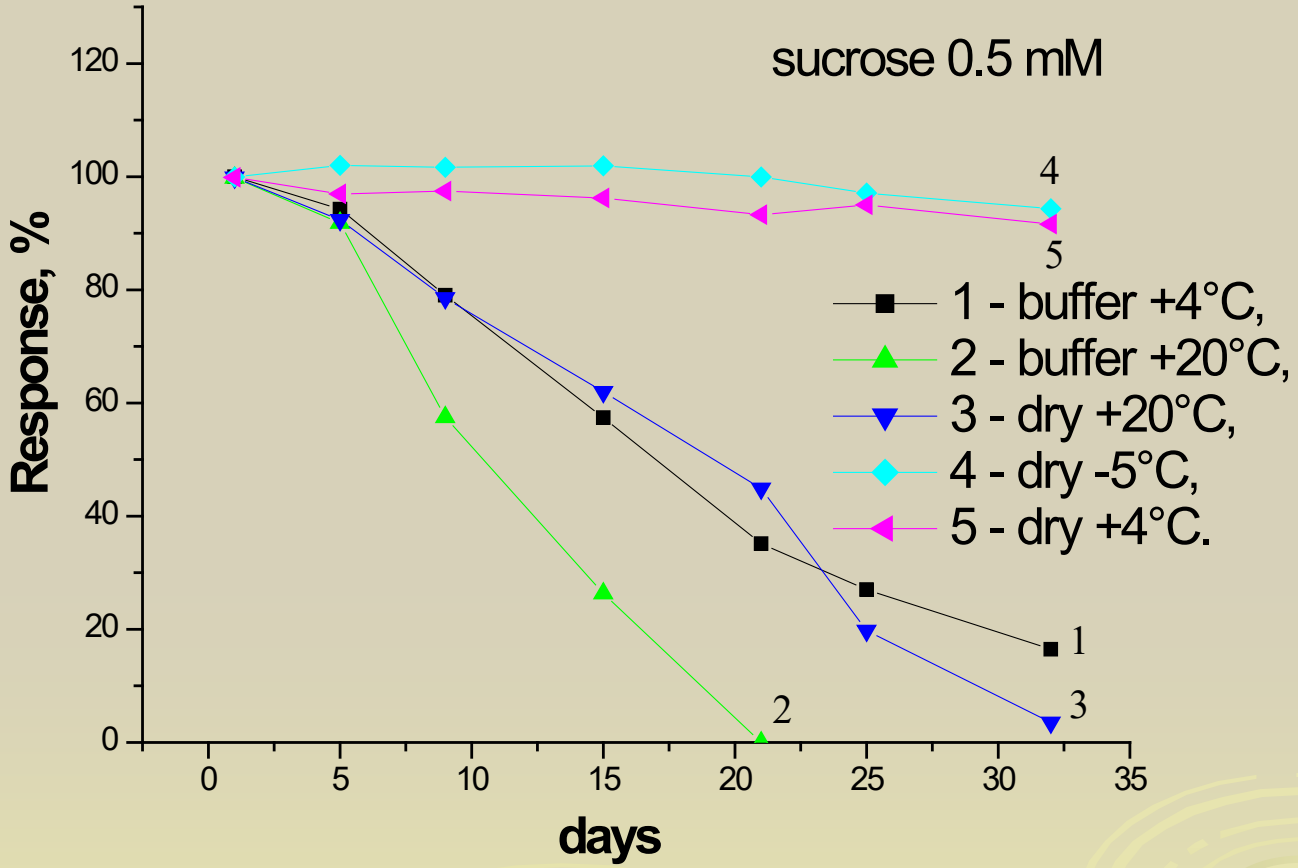
Effect of KCl on response of biosensor.
Measurement were carried out in 10 mM phosphate buffer, pH 6,0.
Concentration of substrate was 0.5 mM.

Reproducibility and operation stability

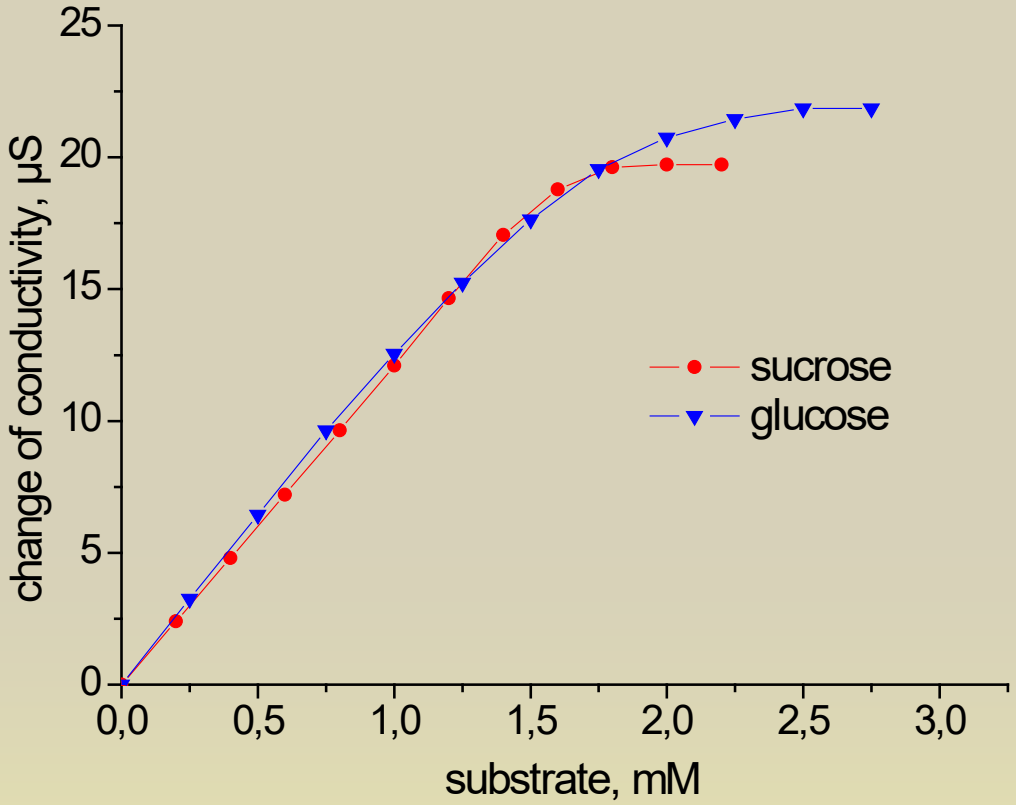




The storage stability of biosensors



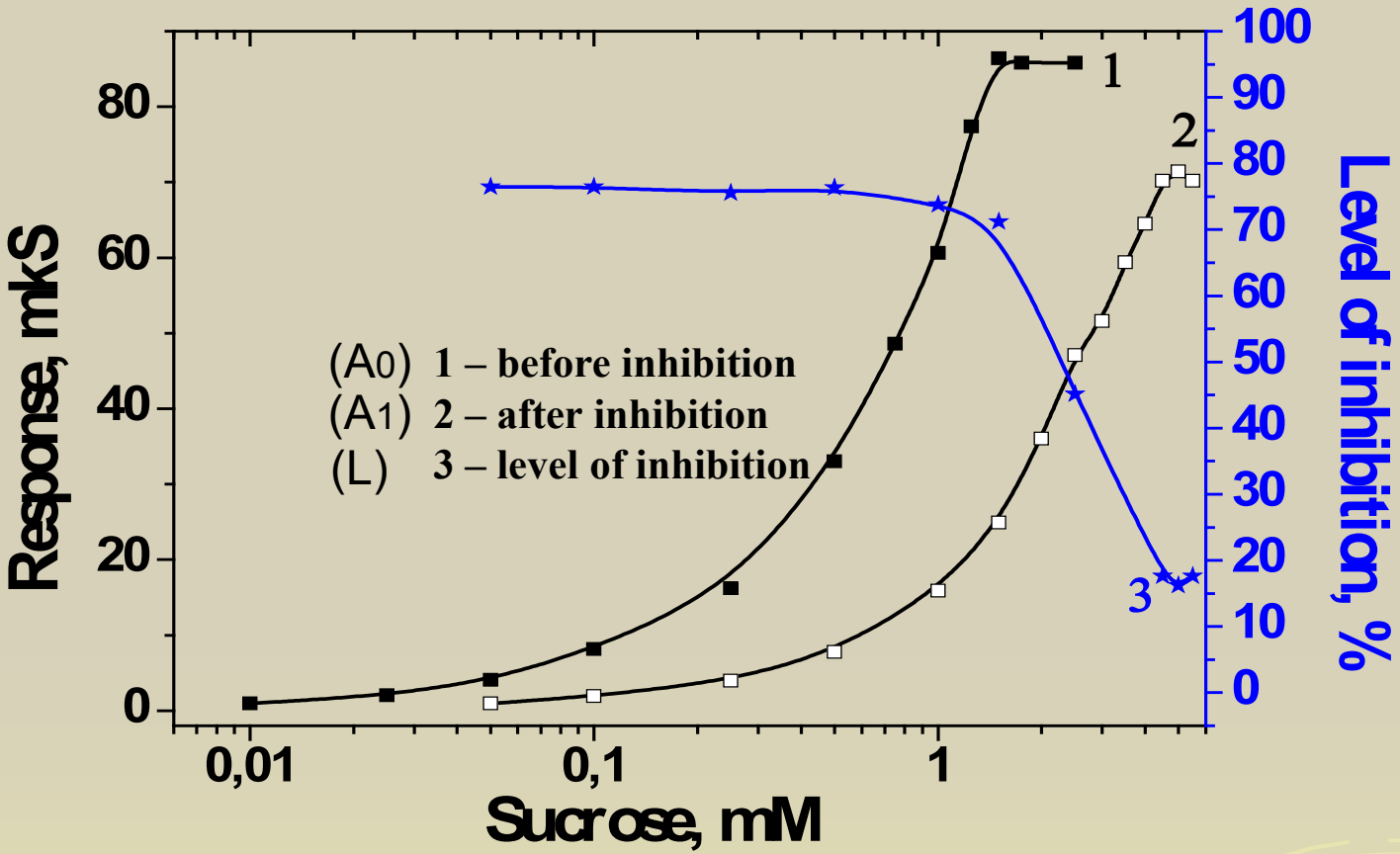
Calibration graphs of dependences of biosensors responses on concentration of substrates



Measurements were carried out in 10 mM PBS, pH 6,0.

Part 2

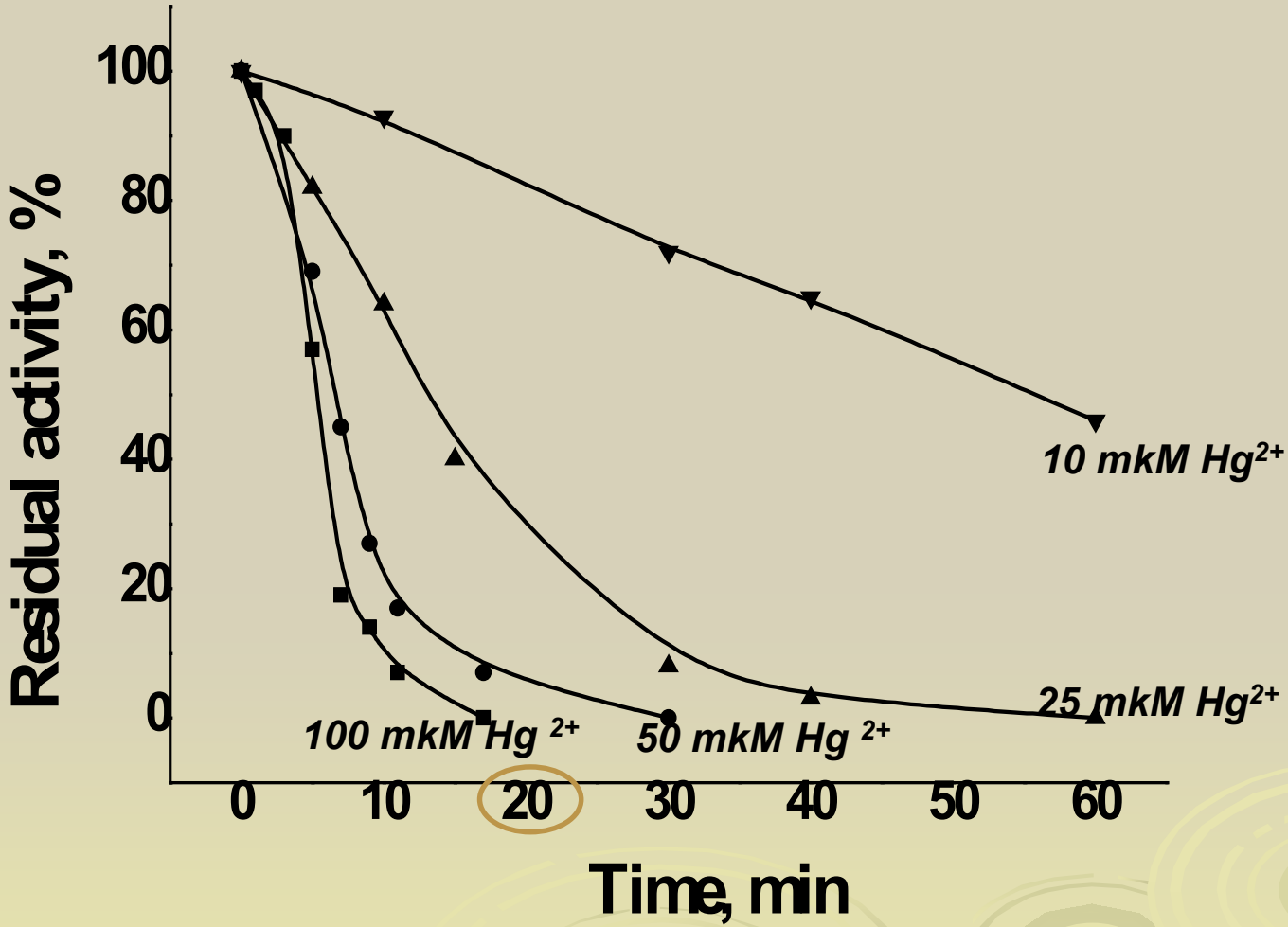
Finding of optimal sucrose concentration for inhibitory analysis



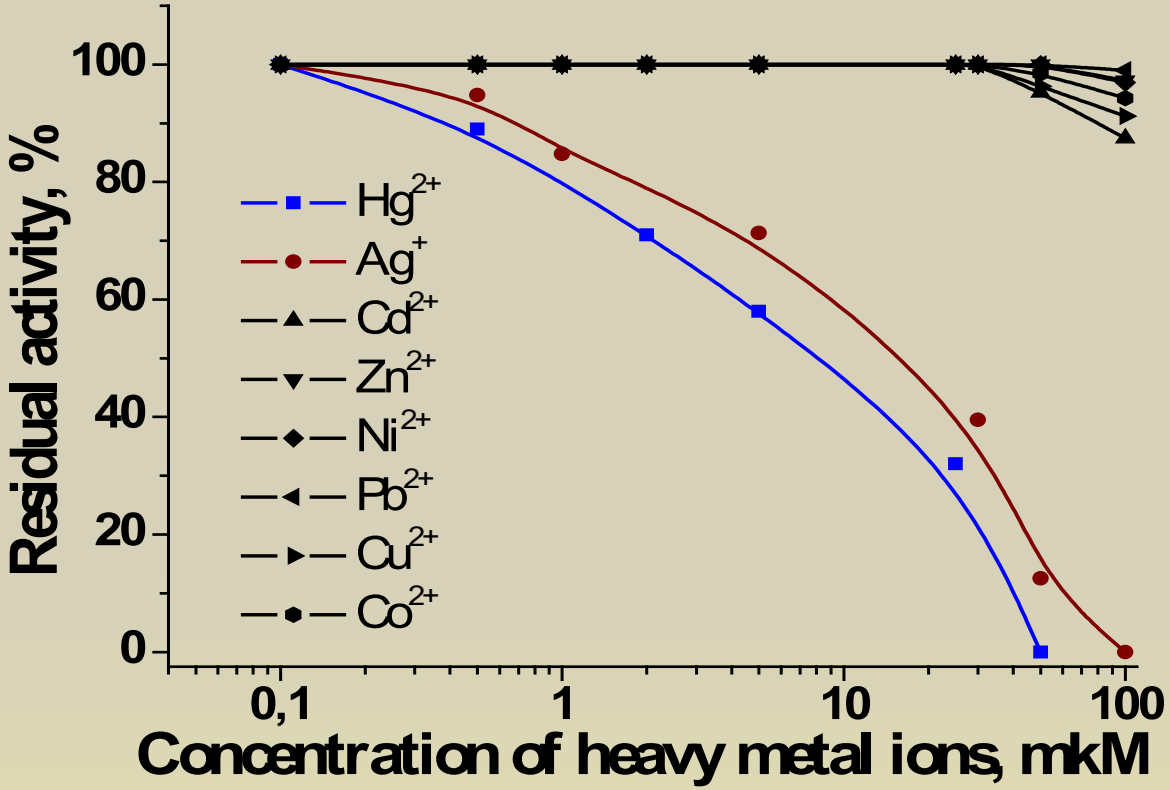
$L=(A_0-A_1)\cdot 100\%/A_0$

Optimal concentration of substrate – 1,25 mM
Inhibition - 30 min with 50 μM Hg2+ solution

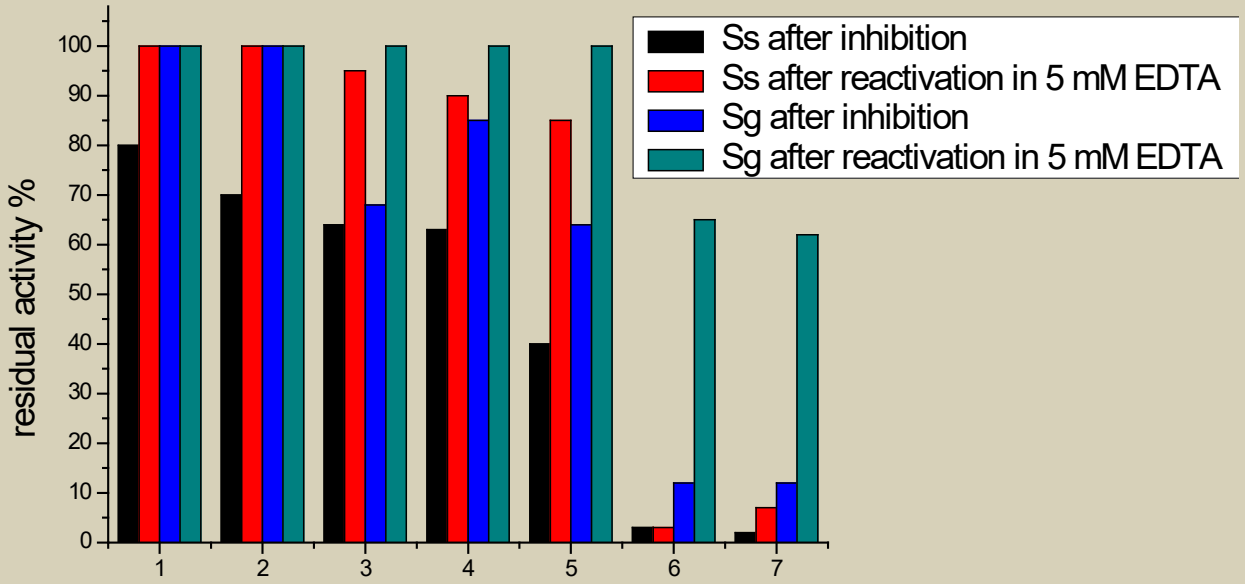
Finding of optimal time of biosensor incubation in solution with inhibitor

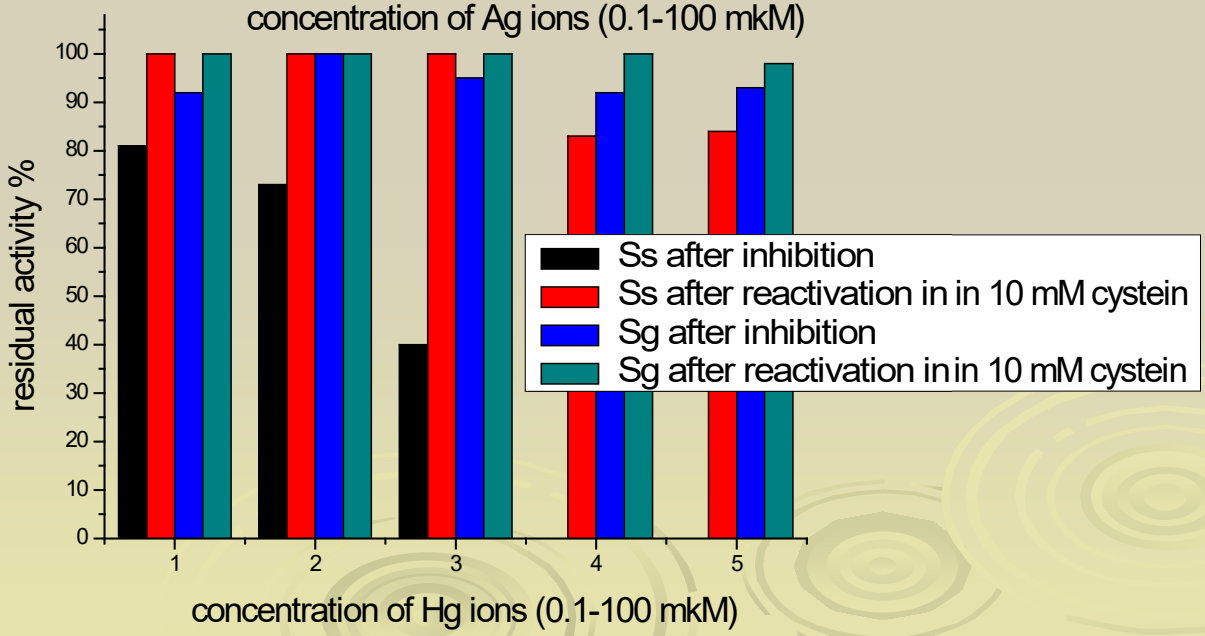


Study of influence of HMI on biosensor response



Study of reactivation of biosensors





Another enzyme reactions in inhibitory analysis

Urease

$$\text{Urea} + 2H_2O + H^+ \longrightarrow 2NH_4^+ + HCO^{3-} \quad (1)$$

BChE

$$\text{Butyrylcholine} + H_2O \longrightarrow \text{Choline} + CH_3(CH_2)_2COO^- + H^+ \quad (2)$$

AChE

$$\text{Acetylcholine} + H_2O \longrightarrow \text{Choline} + CH_3COO^- + H^+ \quad (3)$$

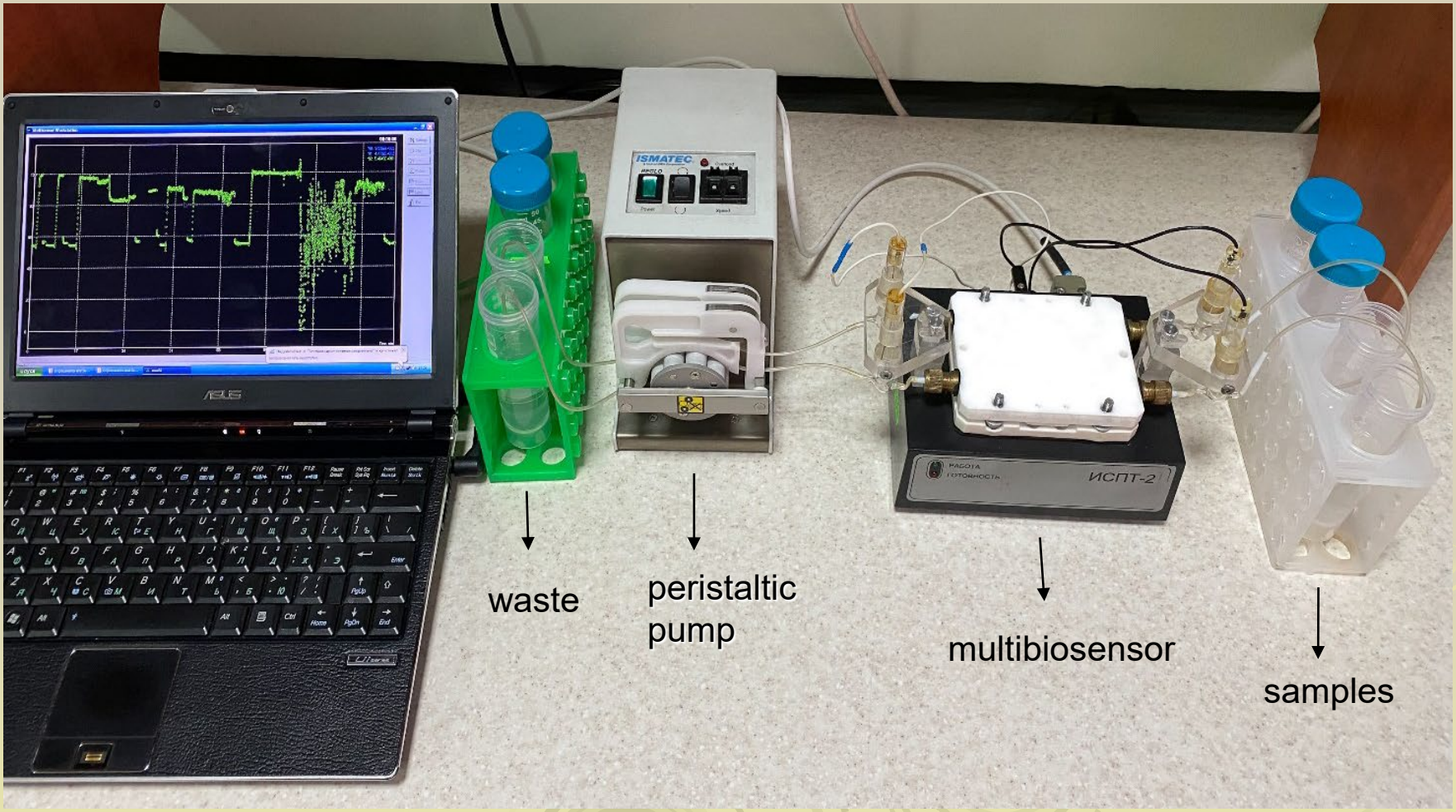
GOD

$$\beta\text{-D-glucose} + O_2 + H_2O \longrightarrow \text{D-gluconolacton} + H_2O_2 \longrightarrow$$
$$\text{D-gluconic acid} + H_2O \leftrightarrows \text{Acid residue} + H^+ \quad (4)$$

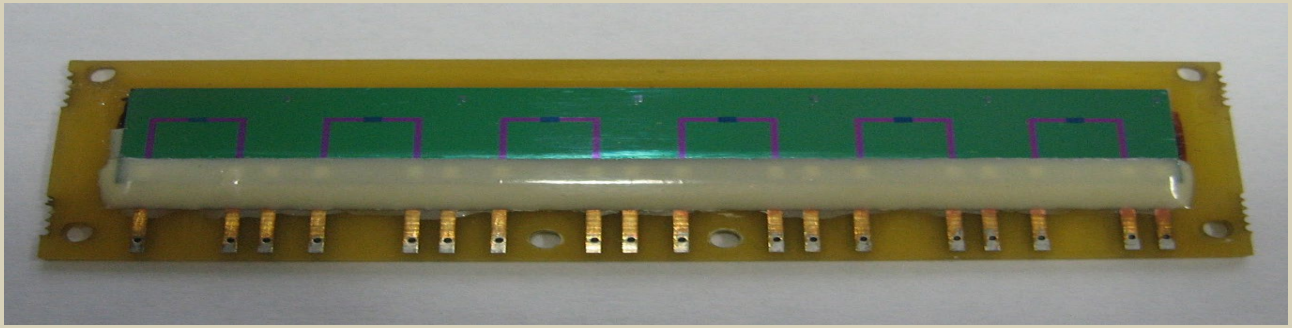
Invertase Mutarotase GOD

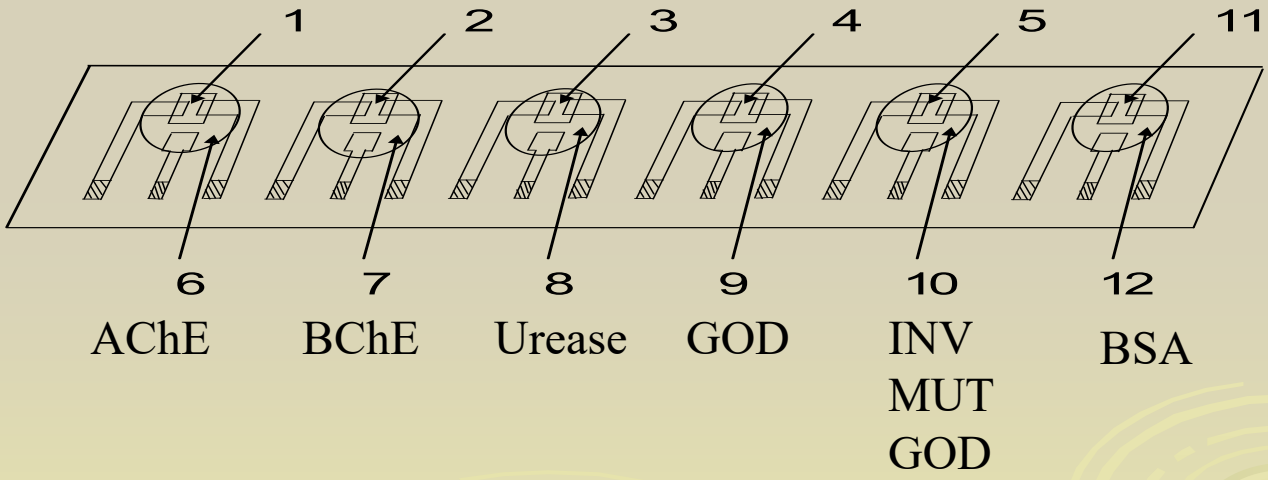
$$\text{Sucrose} + H2O \longrightarrow \beta\text{-D-fructose} + \alpha\text{-D-glucose} \longrightarrow \beta\text{-D-glucose} + O2 \longrightarrow$$
$$H2O2 + \text{D-gluconolacton} \longrightarrow \text{D-gluconic acid} + H2O \leftrightarrows \text{Acid residue} + H+ \quad (5)$$

Multi-biosensor ISFET device and flow injection analysis (FIA) system

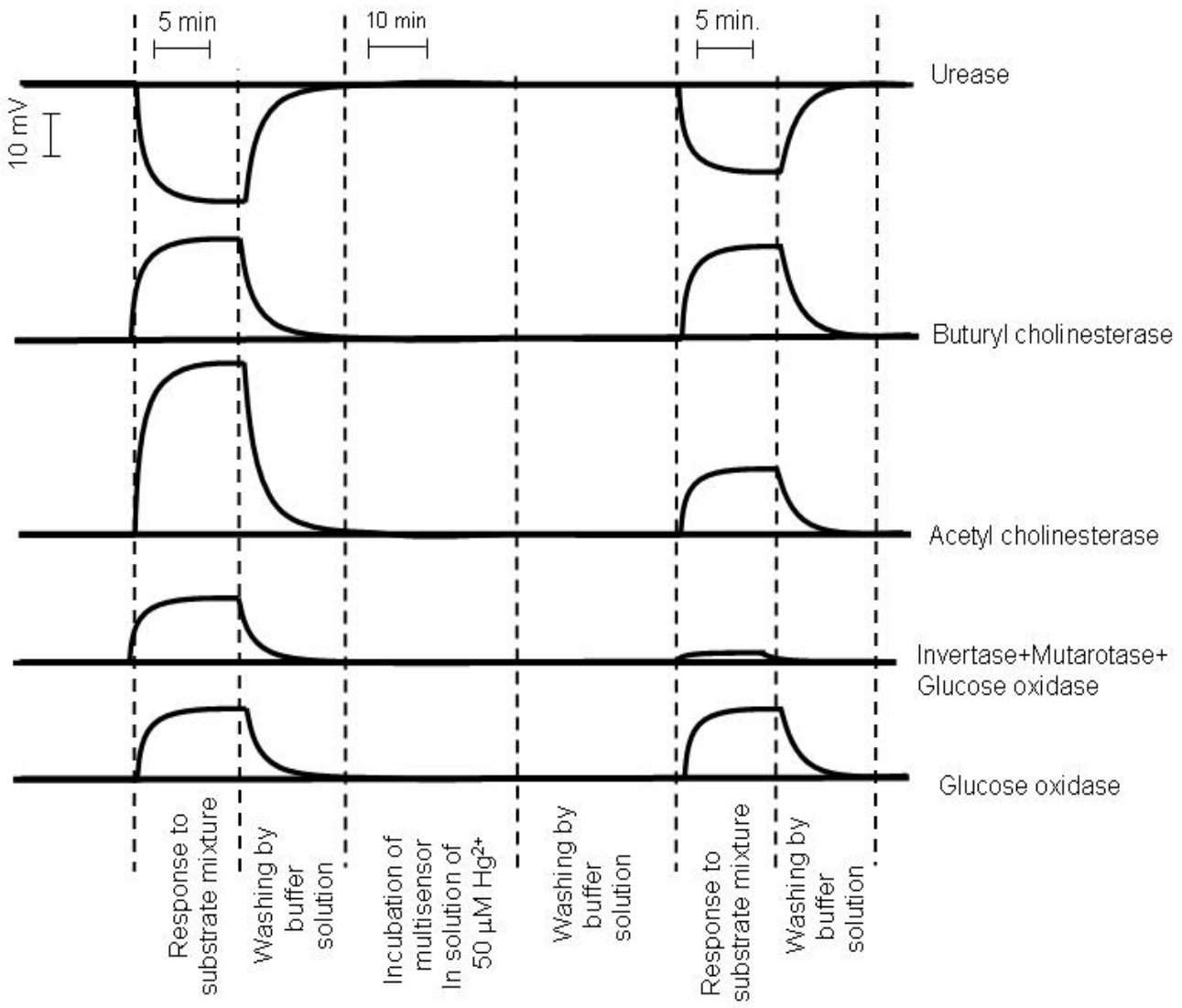


Sensor strip with pH-sensitive ISFETs





ISFET multibiosensor responses to injection of mixture of substrates before and after enzymes inhibition by the mercury ions



Inhibitor	Level of inhibition of bioselective element, %				
	Urease	BChE	AChE	GOD	INV MUT GOD
1 mkM Ag^{+}	0	0	5	15	1
10 mkM Ag^{+}	0	3	25	60	65
0,2 mkM Hg^{2+}	0	0	0	0	5
1 mkM Hg^{2+}	4	0	0	10	22
10 mkM Hg^{2+}	25	3	10	50	70
10 mkM Cu^{2+}	10	0	0	0	0
50 mkM Cu^{2+}	30	0	0	0	5
10 mkM Cd^{2+}	12	0	0	0	5
50 mkM Cd^{2+}	65	0	15	10	30

Conclusion

- New enzyme conductometric and ISFET biosensors based on inhibitory analysis for determination of heavy metal ions were developed.
- The main working characteristics of the biosensors in direct analysis were determined. The developed biosensors are characterized by high sensitivity, high storage stability, reproducible responses, and show good operation stability.
- The measurement conditions were optimized. The effect of buffer capacity, ionic strength and pH of buffer solution on biosensor responses were studied.
- The main working characteristics of the biosensors in inhibitory analysis were studied. The optimal time for incubation of biosensor in solution of toxic compounds was 20 minutes. The optimal concentration of sucrose for inhibitory analysis was 1.25 mM.
- The biosensor reactivation with EDTA and cysteine solutions was shown and can be used also in selective determination of different HMIs.
- The developed biosensors were tested in the work with water contaminated by HMIs and waste-water samples from different origin. The obtained results showed good correlation with results obtained by standard method of HMIs determination (atomic absorption spectroscopy). In future, the developed biosensors can be used for express evaluation of the presence of different HMIs in water samples and monitoring of water toxicity.

Thank you for attention!