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www.elsevier.com/locate/talanta

PII: S0039-9140(13)00380-9
DOI: <http://dx.doi.org/10.1016/j.talanta.2013.04.065>
Reference: TAL13858

To appear in: *Talanta*

Received date: 17 January 2013
Revised date: 17 April 2013
Accepted date: 24 April 2013

Cite this article as: O.O. Soldatkin, V.M. Peshkova, O.Y. Saiapina, I.S. Kucherenko, O.Y. Dudchenko, V.G. Melnyk, O.D. Vasylenko, L.M. Semenycheva, A.P. Soldatkin, S.V. Dzyadevych, Development of conductometric biosensor array for simultaneous determination of maltose, lactose, sucrose and glucose, *Talanta*, <http://dx.doi.org/10.1016/j.talanta.2013.04.065>

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Development of conductometric biosensor array for simultaneous determination of maltose, lactose, sucrose and glucose

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The aim of this work was to develop an array of biosensors for simultaneous determination of four carbohydrates in solution. Several enzyme systems selective to lactose, maltose, sucrose and glucose were immobilised on the surface of four conductometric transducers and served as bio-recognition elements of the biosensor array. Direct enzyme analysis carried out by the developed biosensors was highly sensitive to the corresponding substrates. The analysis lasted 2 min. The dynamic range of substrate determination extended from 0.001 mM to 1.0 - 3.0 mM, and strongly depended on the enzyme system used. An effect of the solution pH, ionic strength and buffer capacity on the biosensors responses was investigated; the conditions of simultaneous operation of all biosensors were optimised. The data on cross-impact of the substrates of all biosensors were obtained; the biosensor selectivity towards possible interfering carbohydrates was tested. The developed biosensor array showed good signal reproducibility and storage stability. The biosensor array is suited for simultaneous, quick, simple, and selective determination of maltose, lactose, sucrose and glucose.

Keywords: conductometric transducer; biosensor array; sucrose; glucose; maltose; lactose.

Introduction

Reliable control of mono- and disaccharides is of great importance in food processing, agriculture, pharmacy, etc. In agriculture, the sucrose content in sugar beet at all stages, from

beet cultivation and preservation to its technological processing, is a key characteristic since in the end it determines an efficiency of sugar production [1]. The content of lactose, one of the major milk components, is an important indicator of quality of dairy products [2]. Maltose is also of significance in food industry since its content in molasses affects the final quality of production, in particular beer and kvass. Information on the presence and concentration of mono- and disaccharides in drinks and food is important for the assessment of their quality [3, 4]. Control of carbohydrates is essential for monitoring the processes of fermentation, etc.

Currently, there are a number of different methods of saccharides determination; however, they have considerable disadvantages. Some standard methods require skilled personnel, expensive and cumbersome equipment, are time-consuming and complex (chromatography, spectrophotometry), others are simple and fast, but less accurate and selective (qualitative chemical methods, refractometry, polarimetry). Therefore it is an urgent need to develop simple, low-cost, and precise analytical express methods for determination of carbohydrates. Implementation of such systems will enhance the organization of control of mono- and disaccharides in agriculture, food production and pharmaceutical industry, the regulation of biotechnological processes, etc [5, 6]. For today, biosensing technologies is a promising way to meet the challenges of traditional methods of saccharide analysis, since compared with the latter the biosensor analysis has such advantages as fast procedure, low cost, high sensitivity and selectivity [7, 8].

Nowadays, there are laboratory prototypes of biosensors for the maltose determination [9-18] with different enzymes immobilised on the electrode surface: amyloglucosidase and glucose oxidase (GOD) [9-10]; amyloglucosidase, mutarotase (MUT), GOD and peroxidase [11]; α -glucosidase (α -GLA) and GOD [12-14]; α -GLA and pyranose oxidase [15]; α -GLA, MUT and GOD [16]; α -GLA and glucose dehydrogenase [14, 17]; α -GLA and glucokinase [18]. The developed electrochemical maltose biosensors have different working characteristics depending on the ratio of certain enzymes, mediators, stabilizing agents in the bioselective membrane and the type of enzyme immobilisation onto the electrode surface. For example, the amperometric sensor system based on carbon electrodes is developed for simultaneous maltose and glucose determination in the solution [10]. Maltose was determined using two enzymes, amyloglucosidase and GOD, while glucose - only GOD. 1,1'-ferricyanide methanol was used as a mediator. Optimum pH of the sensor system was 4.8. The linear range of measurements was up to 40 mM glucose and 20 mM maltose. The enzyme electrodes did not lose their activity during four months of dry storage at 4 °C.

In [16] the amperometric biosensor was developed, in which maltose measurement was based on determination of the activity of α -amylase hydrolyzing starch to maltose. When

creating a bioselective membrane, GOD, MUT, α -glucosidase, and bovine serum albumin were immobilized on the electrode surface using glutaraldehyde. The developed biosensor was characterized by linear dependence of responses on the maltose concentration in the range of 0.1 - 3 mM.

In [12] the authors report the development of amperometric enzyme biosensor for determination of maltose in the culture fluid while studying the process of fermentation, which is accompanied by changes in the maltose concentration. An impact of α -glucosidase and amyloglucosidase on the efficiency of maltose conversion into glucose was examined. The authors stated that amyloglucosidase was more effective; therefore its mixture with GOD was used for maltose determination. The range of measurable maltose concentrations was found to be 0.2 - 4 mM.

Authors [19] developed the biosensor for the determination of α -amylase in human saliva which is based on the quantity of maltose generated by hydrolysis of maltopentose in the presence of salivary α -amylase. The biosensor is fabricated by co-immobilization of the α -GLA, GOD, and MUT on screen-printed electrodes modified with Prussian Blue. A linear relationship is found for the range from 5 to 250 units per mL, with an LOD of 5 units per mL. The biosensor is stable for at least one month and over this time retains 80% of its original activity.

At present, a number of biosensors have been developed for the sucrose determination [20-29]. Various biological materials and methods of enzyme immobilization were used. For example, immobilisation of invertase, MUT and GOD by electrochemical polymerization of phenylenediamine on the electrode surface is reported in [27]. In [28, 29] phosphate ions instead of mutarotase were used to convert glucose from α - into β -form. In [23] the authors used yeast cells co-immobilised with GOD as a source of invertase. A new method of enzymes immobilization on the electrode surface developed in [25] consists in the binding of enzymes (GOD, invertase, peroxidase) with chelating sepharose through various metal ions and lectin concanavalin A. This method allows the elution and re-immobilization of various enzymes on the biosensor surface. Such reversible immobilization enables determination of various substrates in a multicomponent solution.

The enzyme immobilization in polyvinyl acetate matrix [26] resulted in the formation of double enzyme layer on the electrode surface which was shown to extend the linear range of sucrose and glucose determination as compared to a single-layer membrane.

There is information in literature on the development of different versions of biosensors for lactose determination [30-41]. Most of them are of amperometric type with different enzymes immobilized on the electrode surface: β -galactosidase (β -GAL) and galactooxidase [30], β -GAL

and GOD [31, 32, 41], peroxidase, GOD and β -GAL [33], β -GAL, MUT and glucose dehydrogenase [34], β -GAL and galactose dehydrogenase [35], cellobiose dehydrogenase [36].

Authors [37] have investigated the deposition of β -GAL and GOD using alternating current electrophoretic deposition (AC-EPD) to manufacture a lactose sensor. Using the optimal parameters the sensor has a large linear range up to 14 mM lactose, high sensitivity, fast response time (8 s) and reasonable stability without employing any stabilizers or outer polymer membrane.

Some authors also report the development of biosensors for lactose determination using different mediators [33, 38]. For example, in [38] the graphite electrode with adsorbed ferrocene mediator served as a working electrode, in [33] aminosalicic acid was used as a mediator. The ISFET-based biosensor for lactose determination described in [40] included thermophilic glucokinase and β -galactosidase, which do not lose their activity at the temperatures of +50 °C.

Along with numerous biosensors for different single carbohydrates, the multibiosensor systems for simultaneous measurement of several carbohydrates are also known. In [42] the amperometric multibiosensor was designed for maltose, lactose, sucrose and glucose.

The potentiometric biosensor array for sucrose, maltose and glucose determination in solution [43] is based on thermostable enzymes. In [35] the amperometric multibiosensor was successfully tested for determination of glucose, galactose and lactose in milk.

To date, most of the developed biosensors and multibiosensors for carbohydrates determination are amperometric. So far, conductometric biosensors have not been applied despite a number of their important advantages over other electrochemical transducers. They have no technologically complex reference electrode; alternating voltage of small amplitude is used for operation which results in the absence of Faraday processes at the electrodes; they are light-insensitive; miniaturization is possible; inexpensive standard thin-film technology can be applied for their production [44]. However, conductometric biosensors are highly affected by sample matrix, what is a significant disadvantage. Thus, the presence of charged substances can greatly affect biosensor measurements. For this reason, differential mode of measurements should be applied, that enables to reduce non-specific signals. In general, conductometric biosensors can be considered economically reasonable even in case of their single request, in particular in the field.

In the paper, the conductometric biosensor system is proposed for simultaneous determination of sucrose, maltose, lactose and glucose. Its application can significantly simplify and improve the monitoring of these carbohydrates in food and pharmaceutical industry, biotechnology, agriculture, etc.

Materials and Methods

Materials

The following enzymes and reagents were used: mutarotase (EC 5.1.3.3) from pig's kidney with activity of 100 U/mg from Biozyme Laboratories Ltd. (UK); glucose oxidase (GOD) from *Penicillium vitale* (EC 1.1.3.4) with activity of 130 U/mg from Diagnosticum (L'viv, Ukraine); invertase (EC 3.2.1.26) from baker's yeast with activity of 355 U/mg from "Fluka" (Switzerland); β -galactosidase from *E. coli* (EC 3.2.1.23) with activity of 149 U/mg from „Sigma-Aldrich Chemie" (Germany); α -glucosidase from *Bacillus stearothermophilus* (EC 3.2.1.20) with activity of 109 U/mg, „Sigma-Aldrich Chemie" (Germany); α -glucosidase from baker's yeast (EC 3.2.1.20) with activity of 5.7 U/mg, „Sigma-Aldrich Chemie" (Germany); bovine serum albumin (BSA) (V fraction), „Sigma-Aldrich Chemie" (Germany); 50% aqueous solution of glutaraldehyde (GA), „Sigma-Aldrich Chemie" (Germany); glucose, maltose, lactose and sucrose were from „Sigma-Aldrich Chemie" (Germany).

Other non-organic compounds were of analytical grade.

Design of conductometric transducers

The fabrication of conductometric transducers has been performed at Lashkarev Institute of Semiconductor Physics of National Academy of Sciences of Ukraine (NASU). The conductometric transducers were consisted of two identical pairs of gold electrodes onto ceramic substrate, that have dimensions of 5×30 mm². The deposition is thermo vacuum sputtering of chromium (5 nm) and gold (150 nm) on glassceramic substrate. Photomask was positive, photolithography was contact optical positive. After exposition under UV plate was placed in 0.5% KOH and washed-off the photoresist from exposed area. After this, wet etching of gold in nitrohydrochloric acid was done, and etching of chromium in special solution was conducted. Placement of two electrode pairs on one transducer enabled differential mode of measurements (see sections below). The sensitive surface of each pair of electrodes was about 1×1.5 mm²; the distance between neighbor digits, as well as the width of each digit was 20 μ m (Fig. 1).

Experimental setup for conductometric measurements

A portable conductometric analyzer developed in Institute of Electrodynamics (NASU) was used to determine changes in conductivity in the near-electrode buffer layer of two pairs of electrodes of each conductometric transducers (Fig. 2): 4 conductometric transducers (2) were fixed in the plastic holder (1) and connected to the module of secondary transducers (7) and measuring and control module (8), that processed signal and transmitted it to a personal

computer via RS-232C interface [45]. Module of secondary transducers includes 4 channels on the basis of an ac bridge and provides measurements in differential mode. Each of these bridge circuits includes both electrode pairs (working and reference) of conductometric transducer. The applied sinusoidal potential with frequency of 36 kHz and amplitude of 12 mV allowed avoiding such effects as faradaic processes, double-layer charging and polarization of the microelectrodes. Bridge circuits can be balanced on reactive and active components of impedance of conductometric transducers. This reduces the influence of non-informative parameters of transducers on the measurement results and allows obtaining stable metrological characteristics of the instrument. The analyzer is able to process the signals of all 4 channels simultaneously.

Enzyme immobilisation onto the electrode surface

Immobilisation of the enzymes and BSA on the electrodes surface was performed using glutaraldehyde, a bifunctional reagent that formed covalent bonds with amino groups of proteins. To obtain the bioselective membranes for determination of sucrose, lactose and maltose three enzymes – GOD, mutarotase and suitable glycosidase (invertase, β -galactosidase or α -glucosidase respectively) with BSA were mixed with 1 % solution of glutaraldehyde in 1:1 ratio and then this mixture was deposited onto the first pair of electrodes. GOD membrane with BSA was applied for glucose determination. The BSA (10 – 20 %) instead of enzymes was immobilized on the second pair of electrodes which was used as reference electrodes. Both membranes (reference and bioselective) had a volume of 0.1 μ l and had the same protein content. Then biosensors were dried during 30 minutes in the air at room temperature and after that washed from unbound reagents in the working buffer. After washing, biosensors were ready for use.

The composition of each solution for preparing bioselective membrane consisted of appropriate concentration of enzymes in 20 mM phosphate buffer, pH 7.5, with 20 % glycerol and 5 % BSA. Glycerol was added to the solutions to stabilize the immobilized enzymes and to prevent early drying of solution on the transducer surface. BSA as a membrane component stabilized the enzymes, promoted formation of intermolecular bindings and prevented intramolecular interaction of glutaraldehyde with the enzyme that can cause undesirable structural changes in enzyme and complete loss of its activity [23]. The composition of bioselective membrane of sucrose biosensor was: 5 % invertase, 4 % mutarotase, 5 % GOD, 20 % glycerol and 5 % BSA in 20 mM phosphate buffer, pH 7.5; lactose biosensor – 6 % β -galactosidase, 8 % mutarotase, 6 % GOD, 20 % glycerol and 5 % BSA in the same buffer; glucose biosensor – 5 % GOD, 5 % BSA, 20 % glycerol in the same buffer; maltose biosensor –

5 % α -glucose oxidase, 5.5 % mutarotase, 5 % GOD, 4 % BSA, 20 % glycerol in the same buffer.

Measurement procedure

Measurements were carried out in the potassium-phosphate buffer solution and universal buffer solution of different molarity and pH, with constant stirring at room temperature. The sensors were first placed into the 4 ml measuring cell filled with buffer solution to obtain a steady-state primary signal, i.e. the sensor base line. A certain aliquot of the substrate stock solution was then injected. Non-specific changes in the output signal associated with fluctuations of temperature, medium pH, and applied voltage were compensated by using differential mode, i.e. measurement of the difference between the signals from two pairs of electrodes (with enzyme and referent membranes), placed on the same transducer. The experiments were performed at least in three series.

Results and discussion

The cascades of enzymatic reactions for sucrose, lactose, maltose and glucose detection by conductometric biosensors are presented in Fig. 3.

Three enzymes are used for measurement of sucrose, lactose and maltose while only one, glucose oxidase – for glucose. The enzymes invertase, β -galactosidase and α -glucosidase decompose corresponding substrates (sucrose, lactose and maltose), to α -D-glucose, which is transformed into β -D-glucose by mutarotase. β -D-glucose is decomposed by GOD to hydrogen peroxide and D-glucolactone. In its turn, D-glucolactone is spontaneously hydrolyzed to gluconic acid, which dissociates to the acid residue and a proton. These reactions result in changes in solution conductivity that can be registered by a conductometric transducer.

The conductometric method is based on measurement of the changes in conductivity of the solution. These changes depend on the enzyme reaction and on characteristics of the solution where this reaction takes place. Therefore, the influence of the solution parameters (pH, buffer capacity, and ionic strength) on the biosensor response was studied firstly.

Any enzyme is characterized by working range of pH and optimal pH value. For example, free invertase is most effective at pH 4.5 [48, 49], free β -galactosidase - at pH 7.4, α -glucosidase – at pH 6.8-7.0, mutarotase – at pH 7.4, GOD – at pH 5.5. However, pH-optimum of some enzymes changes after immobilisation, shifting to either alkaline or acid region [48, 50, 51]. Sometimes, pH working range becomes essentially wider after immobilisation [48]. In our case, enzyme membranes of lactose, maltose, and sucrose biosensors consist of a mixture of

three immobilised enzymes, each with different pH optimum. Therefore, the next step of our study was selection of an optimal pH of working buffer for simultaneous work of all four biosensors.

It is known that one-component buffer (i.e. phosphate buffer) has different buffer capacity at different pH values, what can influence on the work of conductometric biosensor. The multicomponent universal buffer has the similar buffer capacity in a wide range of pH. Thus it was used to study biosensors work at different pH values. The universal buffer consisted of a mixture of phosphoric, acetic, and boric acids [47]. Measurements of sucrose, maltose and glucose were carried out in 10 mM universal buffer solution. In lactose biosensor we did not use universal buffer solution because lactose is isomerised to lactulose in the presence of borate salts [38]. Since the activity of lactose biosensor in phosphate buffer is higher than in acetate one [42] we carried out the measurements in 10 mM phosphate buffers with pH from 6.0 to 8.0. The highest biosensors responses were obtained at pH 6.5 for lactose and pH 6.0 for maltose, glucose and sucrose biosensors (Fig. 4). Thus, pH 6.0 was chosen as optimal for simultaneous work of all the biosensors in one system.

A change in concentration of working buffer solution automatically results in a change of ionic strength and buffer capacity, what can affect the conductometric measurements [52]. That is why influence of buffer concentration on biosensor work was investigated.

As seen in Fig. 5, the biosensor responses decrease significantly at higher concentrations of phosphate buffer solution. Decrease of responses is a result of increase in background conductivity and capacity of buffer solution. For all tested conductometric biosensors, the tendency of changes in sensitivity was the same and had an exponential character. The most significant decrease in biosensor responses was revealed in the concentration range from 2.5 mM to 15 mM, while further increase in the buffer concentration resulted in minor change of the responses. In further experiments 10 mM phosphate buffer solution, pH 6.0, was mostly used as a working buffer since it had sufficient buffer capacity and at the same time high and stable signals to specific saccharides were observed.

Ionic strength is one of the basic buffer characteristics which can affect results of measurements made by conductometric biosensor. To study this effect, signals to the 0.5 mM of substrate were measured in the buffer solution which contained different concentration of KCl (0-50 mM) (Fig. 6). As seen, an increase in ionic strength results in exponential decrease of the biosensor responses: at the beginning, a remarkable reduction was revealed and at 20 - 50 mM KCl the signal value was less than 50 % in comparison with the buffer without KCl.

This phenomenon can be explained in several ways. One of key reasons of this effect is an increase in the solution background conductivity [44]. On the other hand, higher ionic

strength of the tested solution can provoke changes in density of bioselective membranes due to the screening of membrane charges and consequently – changes in membrane permeability and immobilised enzymes activity. Thus, the addition of a sample with high ionic strength will result in smaller responses to the substrates compared with responses to model solutions of the substrates. For this reason, we should maximally dilute samples with high ionic strength, and the ionic strength of the samples analyzed by conductometric biosensors should be strictly controlled.

Operational stability and signal reproducibility are essential characteristics of biosensors. There are numerous factors that influence on enzyme stability – temperature, pH, ionic strength, composition of buffer solution, cofactors, etc. Rather high stability is a trait of enzymes obtained from microorganisms adapted to extreme conditions as well as from some genetically modified microorganisms [50, 53]. However, immobilised enzymes are usually more stable than soluble ones.

Next, reproducibility of the biosensor array was tested. Responses to the same concentration of four substrates were measured for several hours every 10 min, the biosensors all time were kept in the continuously stirred buffer. Substrates' concentrations were chosen from the linear segment of calibration curves for all the tested biosensors. All four biosensors were characterised by high signal reproducibility, the relative standard deviation of the responses did not exceed 5 %.

Reproducibility of biosensor construction was 20-30 % and was measured as R.S.D. of responses of different biosensors. Such big deviation of responses is caused by the shortages of the method of immobilization that was used for biosensor construction. Cross-linking with glutaraldehyde strongly depends on the environmental conditions, such as temperature, so it is not very reproducible. Furthermore, active regions of conductometric transducers were quite large, so it was difficult to put identical bioselective elements manually. However, we calibrate each biosensor after enzyme immobilization.

Then, storage stability of the biosensor array was studied. Right after fabrication of lactose, maltose, glucose and sucrose biosensors, their responses to the corresponding substrates (0.5 mM) were measured and these values taken as 100 %. Then the biosensors were stored in dry conditions at +4 °C. Next measurements were carried out with 3–8-day intervals. The obtained results showed that lactose, glucose and sucrose biosensors demonstrated only 10 % decrease of response values during first month of storage. At further storage, activity of these sensors remained on rather high level, for example, the activity of lactose biosensor decreased during 3-month storage only by 20 %. Stability of the maltose biosensor was the worst – after a month of storage its response decreased to 50 % of its initial value. Thus, it is necessary to

investigate better conditions for maltose biosensor storage in case of producing such biosensors (for example, transducers can be stored at $-20\text{ }^{\circ}\text{C}$, or in the working buffer at $+4\text{ }^{\circ}\text{C}$); however, these experiments were beyond our work.

Selectivity is an important analytical characteristic of biosensor. It depends on a choice of both biological recognition element and transducer [54]. Selectivity of enzyme biosensors directly depends on specificity of enzymes that form their bio-recognition elements. Biosensors which respond to one substance only are unique. However, a wide range of enzyme activity can be an advantage when the total content of specific substances should be measured in a solution [55]. In our experiments we used bioselective three-enzyme membrane comprising glucose oxidase because GOD is known by its high selectivity toward β -D-glucose [55, 56]. Other enzymes, i.e. invertase, α -galactosidase and β -glucosidase, have lower selectivity towards corresponding disaccharides [56, 57]. For example, it is known that among natural substrates not only sucrose can be decomposed by invertase but raffinose and inulin as well. However, decomposition of raffinose results in the formation of fructose and melibiose which cannot significantly influence the biosensor response because they do not change solution conductivity and they are not the substrates for the subsequent enzymes, mutarotase and glucose oxidase. Thus, all carbohydrates, decomposition of which by the mentioned glycosyl-hydrolase does not result in glucose formation or in formation of charged substances, have no essential effect on responses of the biosensors developed.

An influence of interfering components (some carbohydrates and ascorbic acid) on response value was studied to test biosensor selectivity. For the purpose, glucose, maltose, sucrose, lactose, fructose, arabinose, ramnose, mannose, and ascorbic acid in concentration of 0.5 mM were added to a working cell (Fig. 7). Responses of each biosensor to specific substrate (0.5 mM maltose, sucrose, lactose, and glucose) were taken as 100 %.

Biosensors for maltose, lactose and sucrose determination are highly sensitive not only to corresponding disaccharides but also to glucose because enzyme membranes of all these biosensors contain glucose oxidase. For this reason, a biosensor array should be used for analyzing real samples in order to distinguish between glucose and disaccharides. Except for this fact, all the biosensors were quite selective to the corresponding disaccharides. If a higher accuracy of analysis is required, simultaneous determination of several carbohydrates by the biosensor array should be carried out.

In general, all conductometric biosensors appeared to be sufficiently selective, thus, they can be further used for analysis of real samples.

The biosensors for sucrose, maltose, lactose, and glucose determination were successfully tested for simultaneous determination of several carbohydrates. The signals to injection of

corresponding saccharides were obtained from four biosensors dipped into one measuring cell and connected to the portable device (Fig. 8). As seen, the lactose, maltose and sucrose biosensors are sensitive only to the corresponding disaccharide and glucose, while glucose biosensor – only to glucose.

An application of the developed conductometric biosensors as parts of a portable biosensor array for simultaneous saccharides determination provides high rate measurement as well as high accuracy of comparative analysis of working characteristics of the biosensors (sensitivity, response time, stability, kinetic parameters of the enzymatic reaction, etc.) which is necessary for correct optimization and upgrade of the biosensor system.

Calibration curves for sucrose, lactose, maltose and glucose determination are presented in Fig. 9.

The linear relationship between the response and substrate concentration was observed up to 1.0 – 3.0 mM depending on immobilisation method, enzymes concentration in bioselective membrane, etc. Detection limit of the all substrates was 0.001 mM.

The signal noise was 0.03 – 0.06 μ S in 10 mM phosphate buffer, pH 6.0, and somewhat increased while the buffer concentration dropped. Measurement time was 1-2 min and depended on the membrane thickness – the thicker membrane, the higher response time, which is due to diffusion processes in the membrane.

The biosensors developed for determination of the most important natural saccharides can be a basis for industrial design and production of measuring devices for selective analysis of maltose, sucrose, lactose, and glucose. In comparison with traditional methods of saccharides determination, an analysis with conductometric biosensor methods is quite simple, quick, easy-to-use, and accurate; biosensor array permits to respond to some research and industrial challenges which cannot be solved with convenient approach. Application of conductometric biosensors for analysis of main natural saccharides can essentially simplify and improve the monitoring of these carbohydrates in food production, pharmaceutical and biotechnology industry, agriculture, etc.

Conclusions

In this work we developed a portable array of conductometric biosensors for simultaneous determination of four saccharides (glucose, sucrose, lactose, and maltose). Analytical characteristics and working conditions of the developed biosensors were thoroughly studied. Time of determination of carbohydrates in solution was 1 – 2 min, a linear range of biosensor work extended from the limit of detection to 1.0 – 3.0 mM depending on

immobilisation method, enzyme concentration in a membrane, etc. The detection limit of each substrate was 0.001 mM and depended on the biosensor activity and measurement conditions.

The developed conductometric enzyme biosensors are characterized by high selectivity, operational stability, and signal reproducibility. Standard relative deviation of the responses did not exceed 5 %. Responses of all the sensors, except maltose one, during one-month dry storage at +4 °C reduced by no more than 10 % compared with the initial values. Thus, the biosensor array is perspective for further elaboration and analysis of carbohydrates in real samples. Another possible extension of this work is an addition of biosensor for fructose determination, because this important disaccharide is often present in food and beverages.

Acknowledgements

This work was supported by National Academy of Sciences of Ukraine (complex scientific-technical program “Sensor devices for medical-ecological and industrial purposes: metrology and trial performance”).

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Figure captions:

Fig. 1. Overall view of gold-based conductometric transducers and image of gold interdigitated electrodes obtained by scanning electron microscopy.

Fig. 2. Scheme of portable conductometric analyzer: 1 – fixing block, 2 – biosensors, 3 – buffer solution (4 ml), 4 – working cell, 5 – magnetic stirrer, 6 – holder, 7 – module of secondary transducers, 8 – measuring and control module, 9 – power supply.

Fig. 3. Basic enzymatic reactions for sucrose, lactose, maltose and glucose detection by conductometric biosensors.

Fig. 4. Dependence of responses of sucrose, lactose, glucose and maltose biosensors on buffer pH. Sucrose, maltose and glucose were measured in 10 mM universal buffer solution, lactose – in 10 mM phosphate buffer. Concentrations of the substrates in the cell – 0.5 mM.

Fig. 5. Dependence of responses of lactose (1), sucrose (2), maltose (3) and glucose (4) biosensors on concentration of phosphate buffer solution, pH 6.0. Concentration of substrates in the cell – 1 mM.

Fig. 6. Dependence of responses of lactose (1), sucrose (2), maltose (3) and glucose (4) biosensors on KCl concentration in 10 mM phosphate buffer solution, pH 6.0. Concentration of corresponding substrates in the cell – 0.5 mM.

Fig. 7. Selectivity of the biosensors for determination of glucose, sucrose, maltose and lactose. Measurements were carried out in 10 mM phosphate buffer, pH 6.0. Concentration of carbohydrates in the cell was 0.5 mM.

Fig. 8. Responses of maltose, sucrose, lactose, and glucose biosensors to 0.5 mM of lactose, maltose, sucrose and glucose. Measurements were carried out with portable conductometric biosensor device in 10 mM phosphate buffer solution, pH 6.0.

Fig. 9. Calibration curves of lactose, glucose, sucrose and maltose biosensors. Measurements were carried out with portable conductometric biosensor device in 10 mM phosphate buffer solution, pH 6.0.

Highlights

- We developed an array of 4 conductometric biosensors based on hydrolytic enzymes.
- Conditions for simultaneous operation of all biosensors were selected.
- Biosensor array showed good signal reproducibility and storage stability.
- The possibility of simultaneous selective determination of carbohydrates was shown.

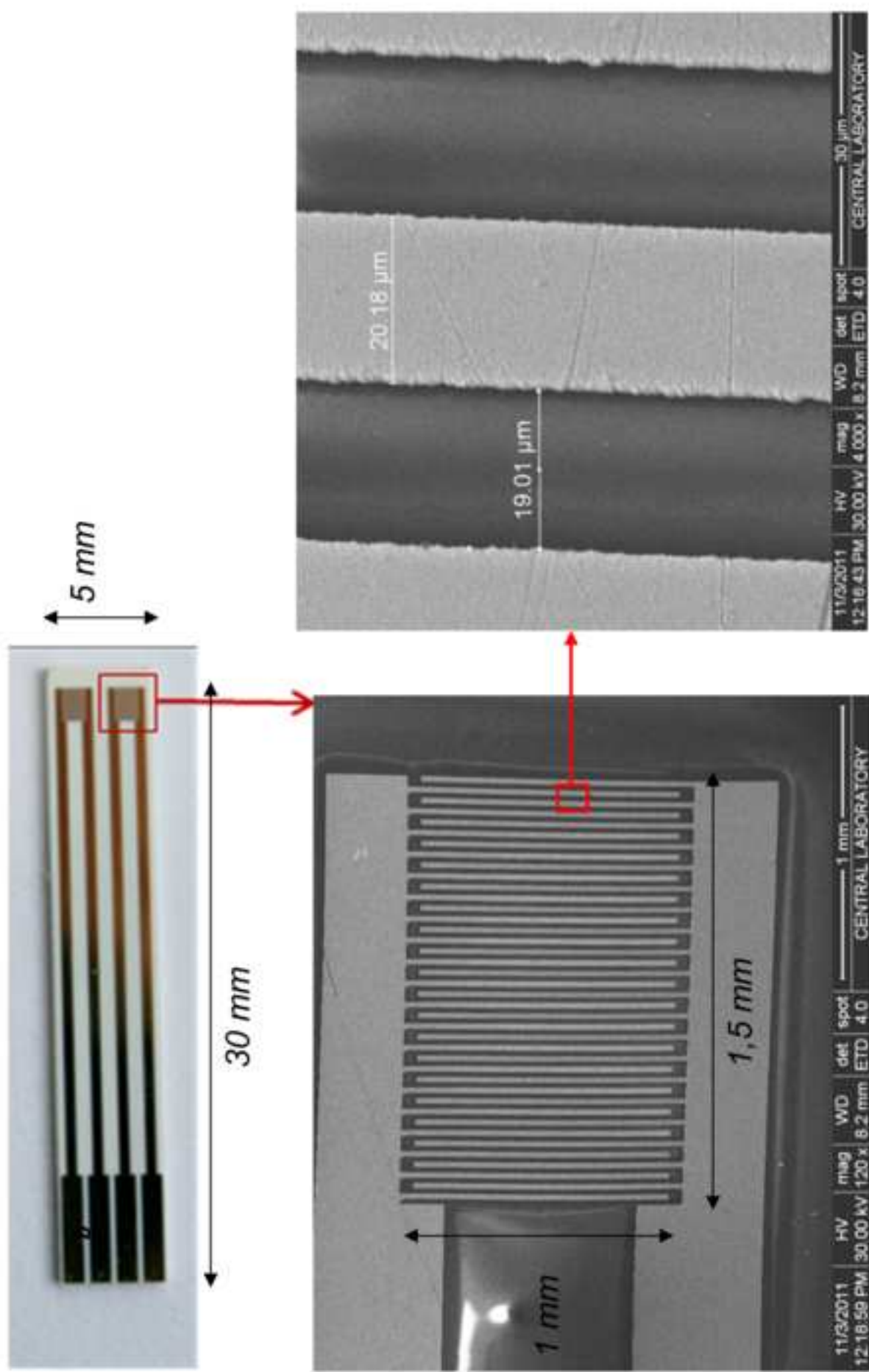


Figure 1

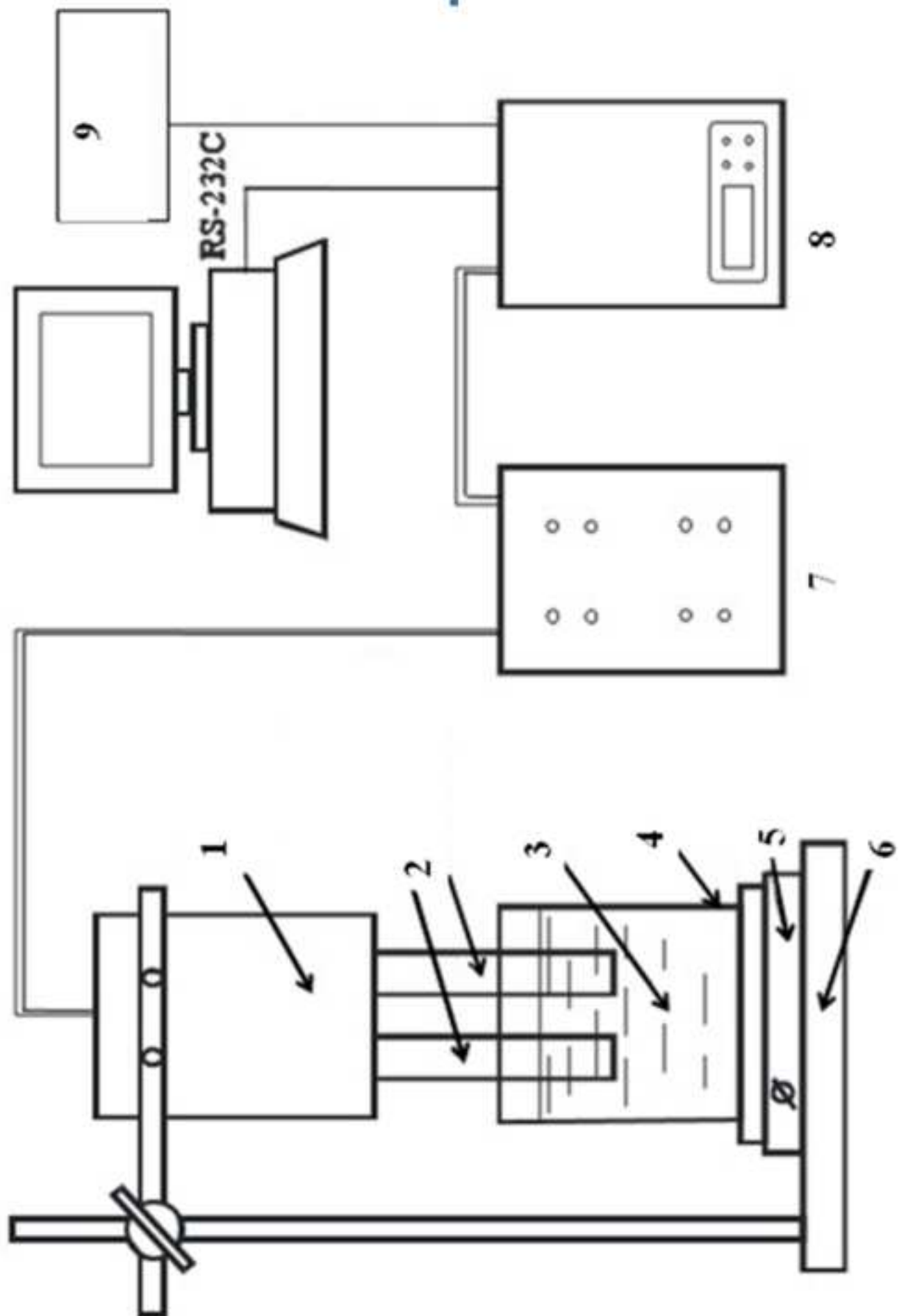


Figure 2

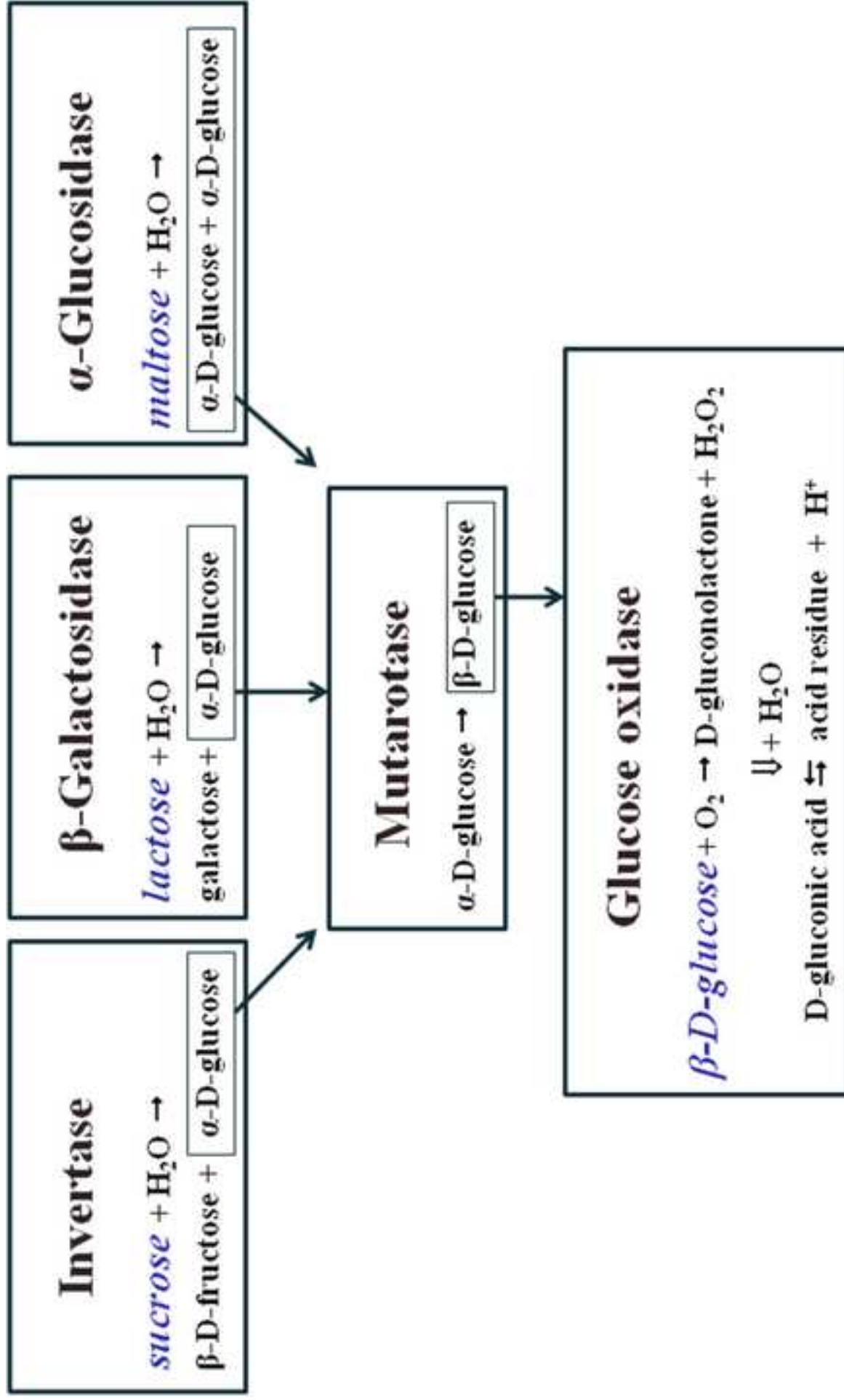


Figure 3

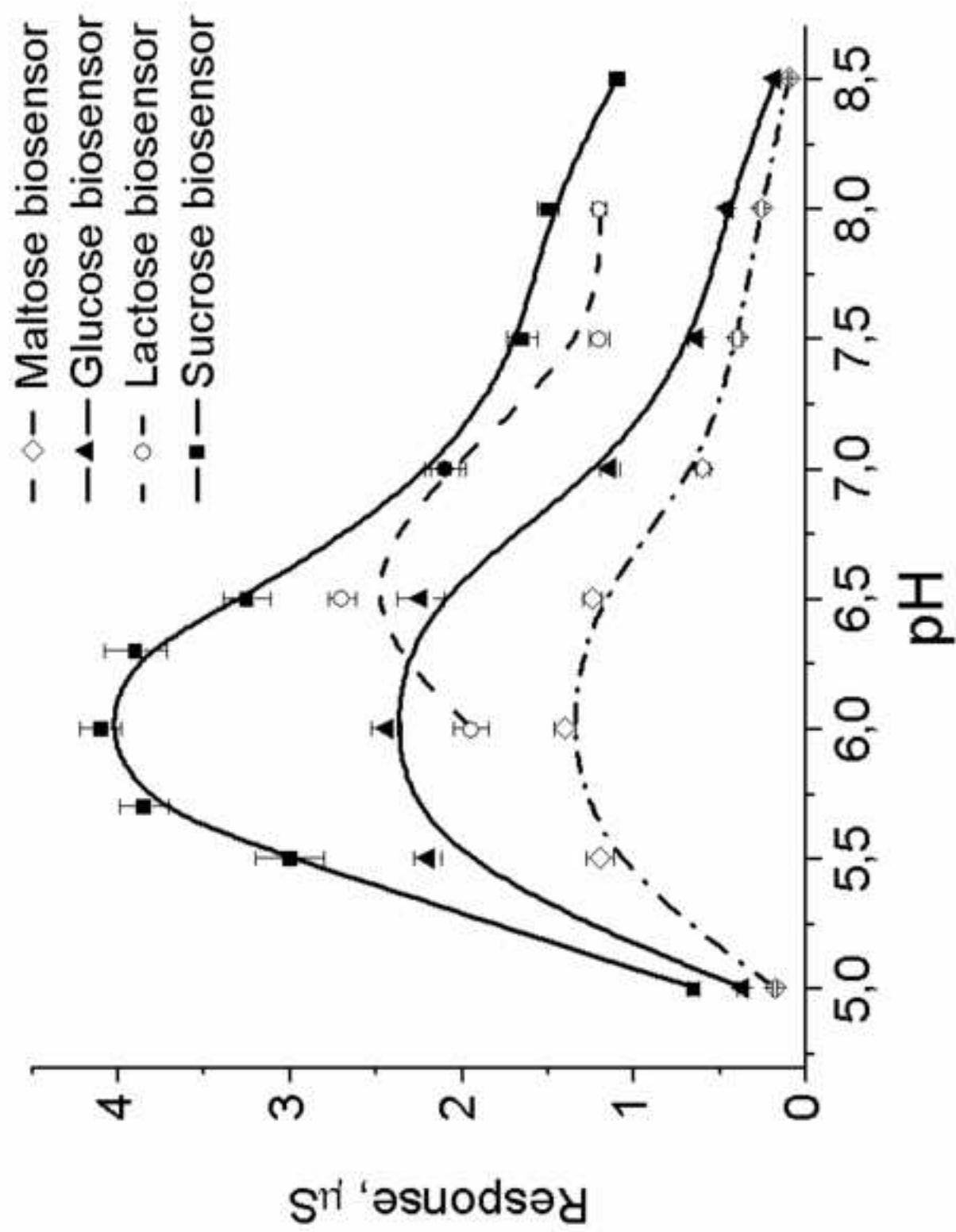


Figure 4

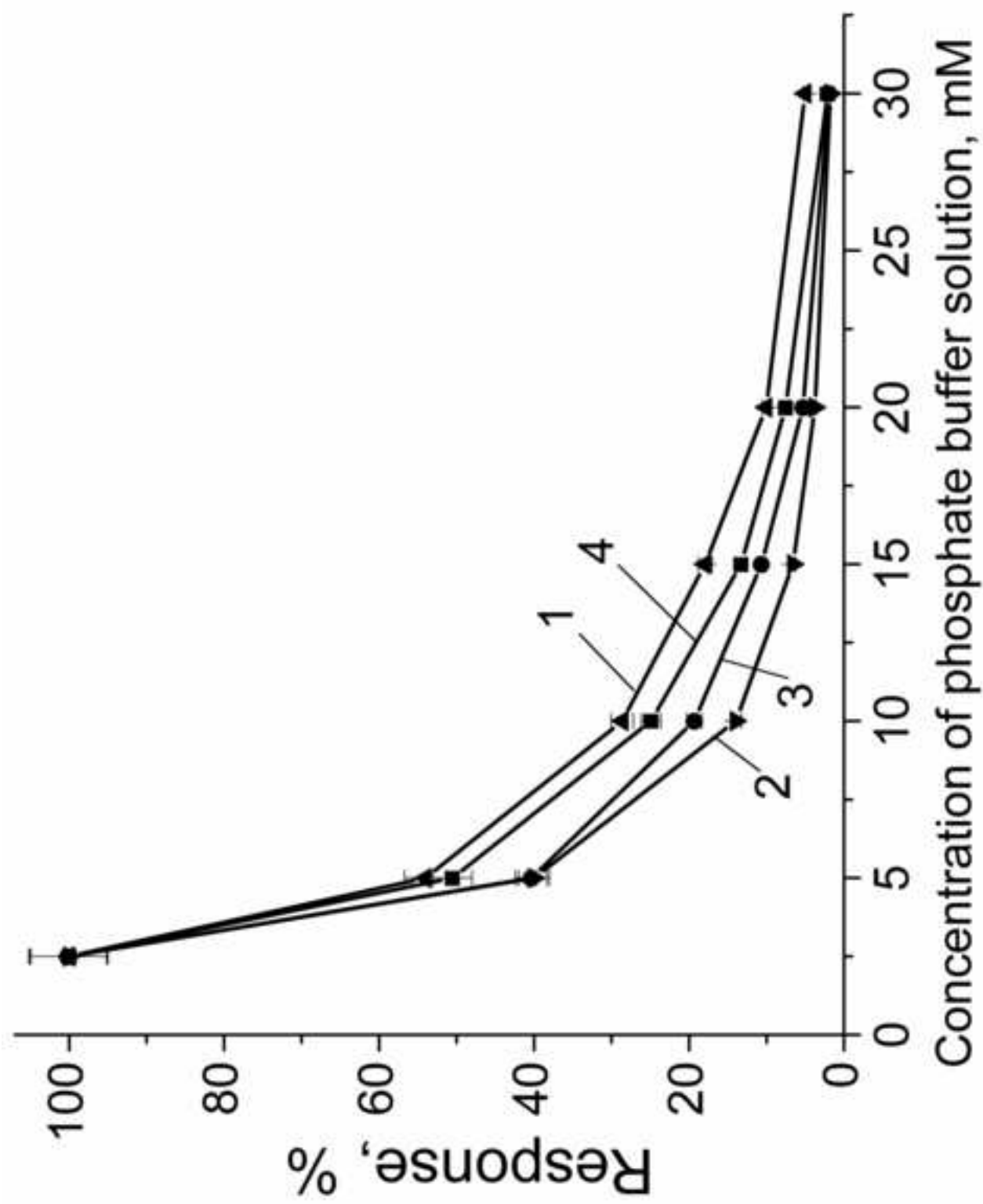


Figure 5

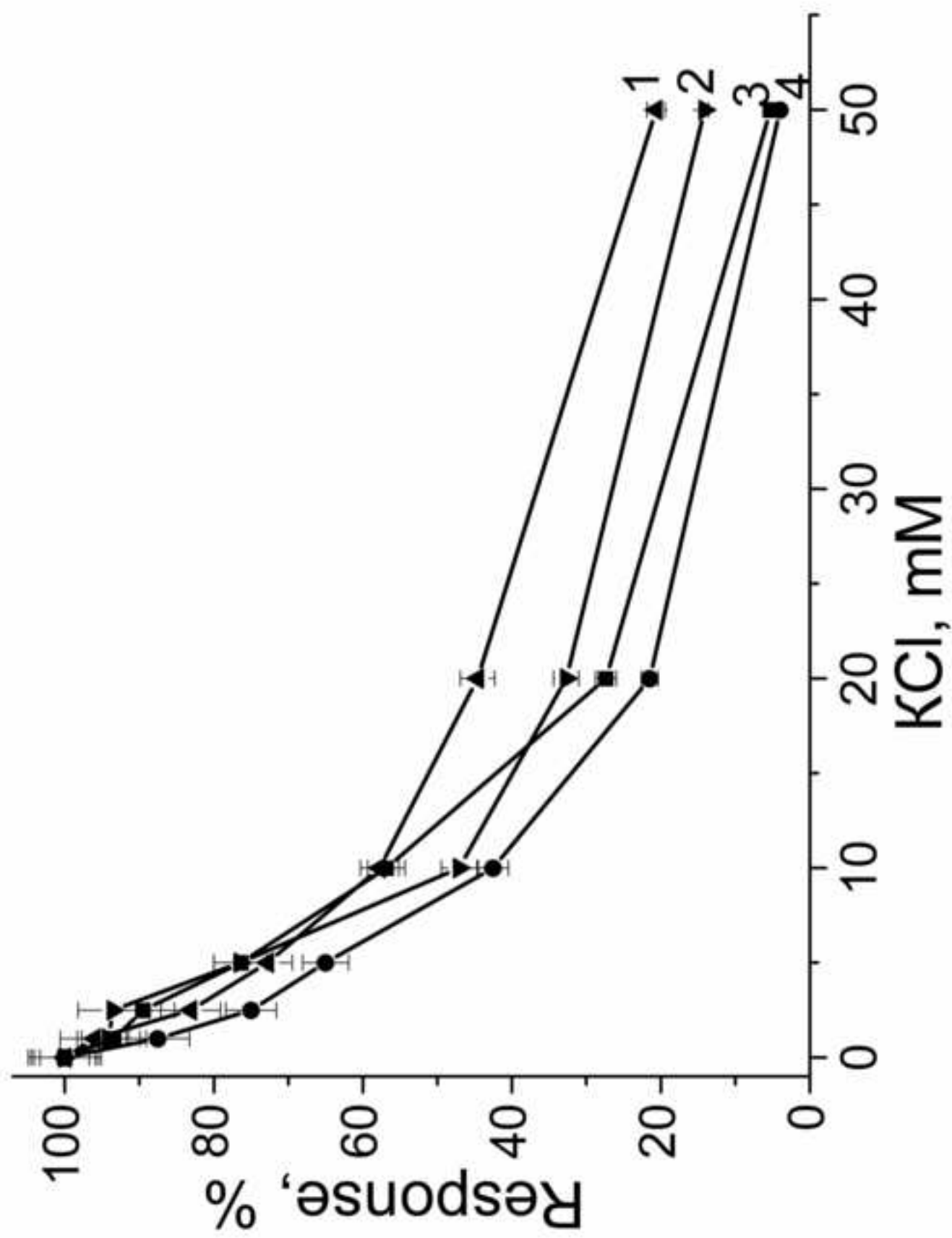


Figure 6

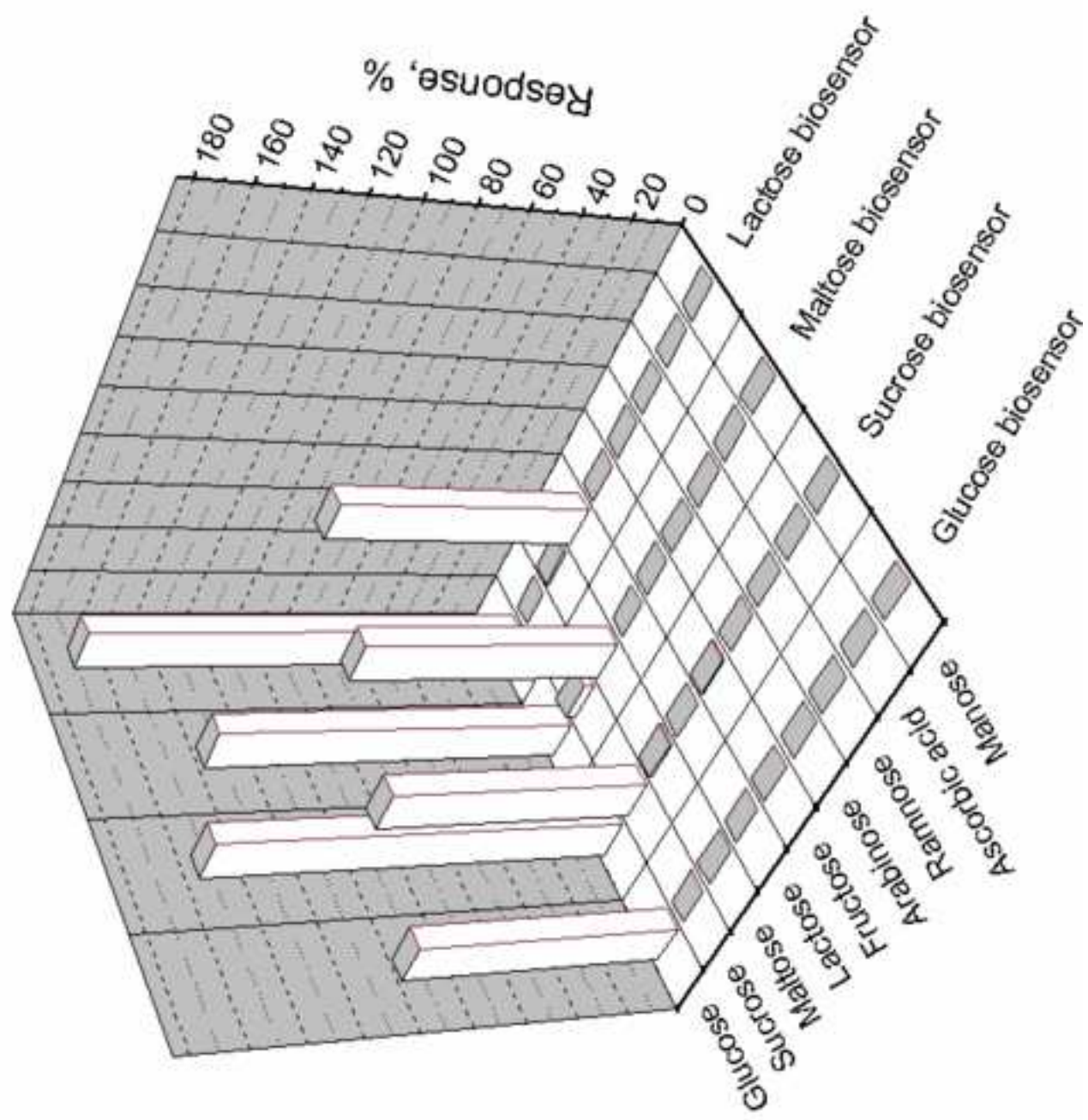


Figure 7

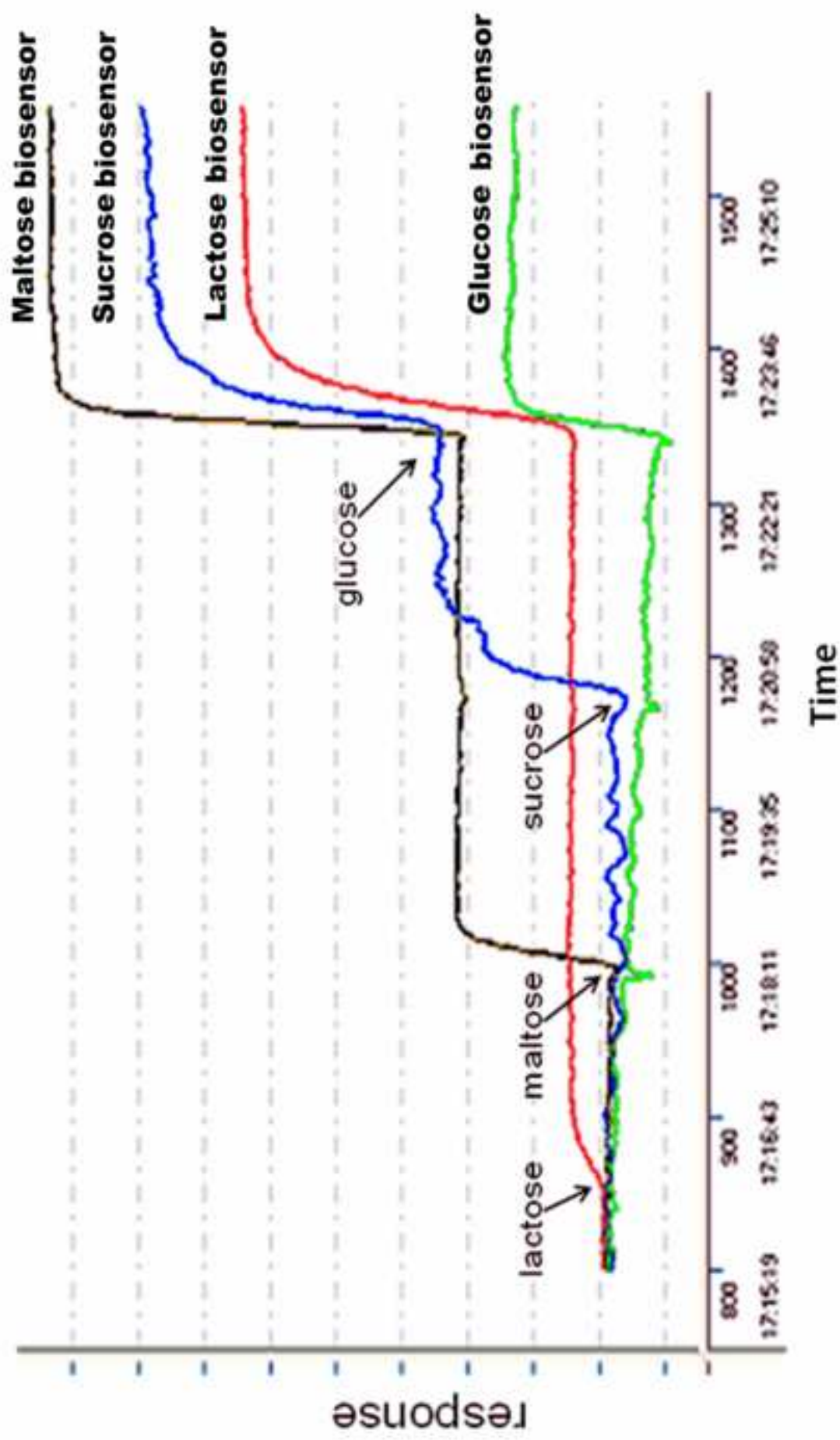


Figure 8

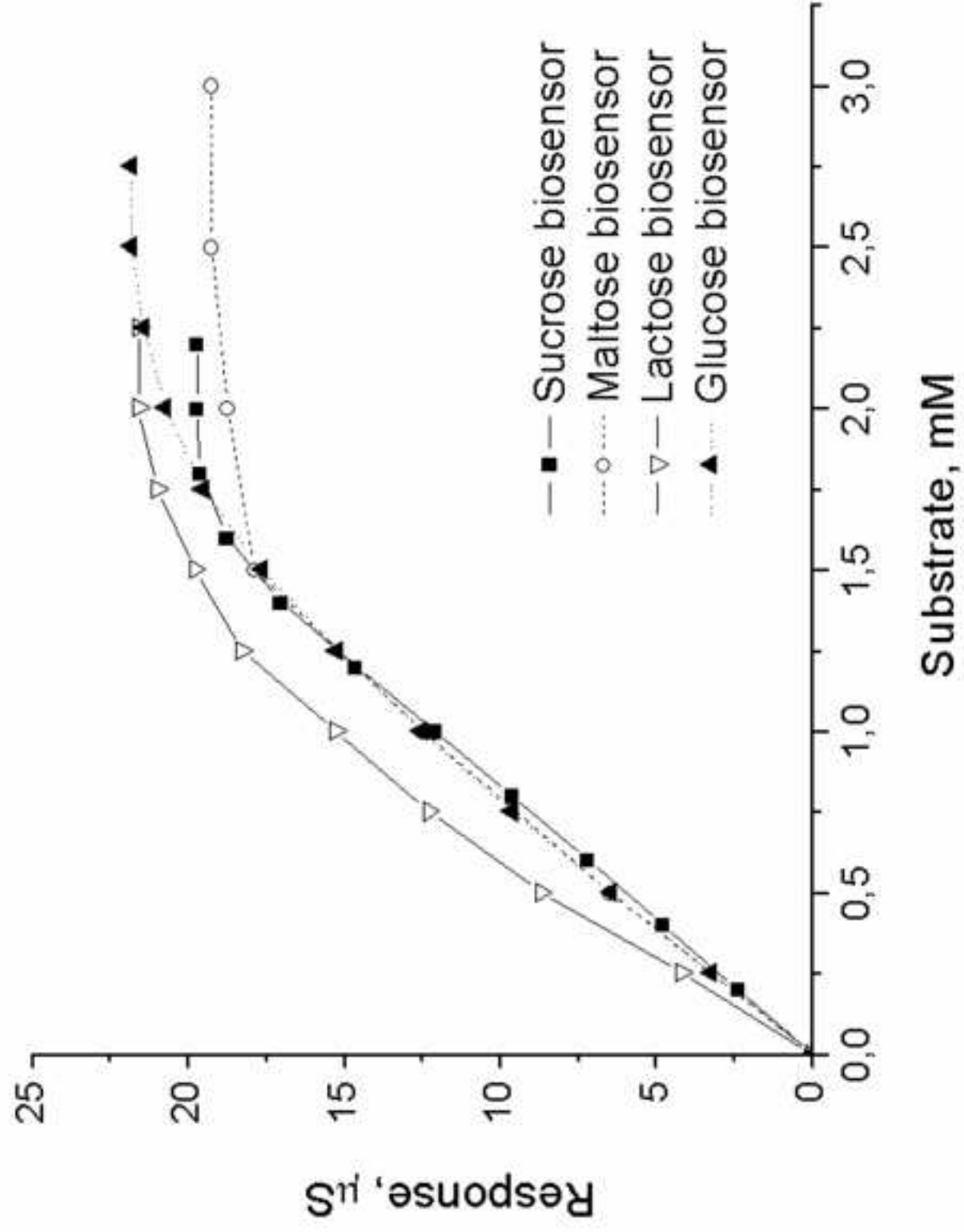


Figure 9