FOUR-CHANNEL BIOSENSOR-ANALYZER OF SACCHARIDES

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Abstract. The problems of realization of highly sensitive and precise conductometric biosensor systems are considered. Composition, structural schemes, software functions of multisensor analyzer of saccharides are described, general view is presented. Preliminary experimental research testifies that the system suggested allows separate determination of concentrations of saccharose, glucose, lactose and maltose with commercially necessary sensitivity. It can be a basis for development of modern analytical equipment for efficient concurrent measurement of concentrations of several saccharides in food industry.

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ЧОТИРЬОХКАНАЛЬНИЙ БІОСЕНСОРНИЙ АНАЛІЗАТОР САХАРИДІВ

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Анотація. Розглянуто проблеми реалізації високочутливих і точних кондуктометричних біосенсорних систем. Наведено склад, структурні схеми, функції програмного забезпечення та зовнішній вигляд мультисенсорного аналізатора сахаридів. Попередні експериментальні дослідження свідчать, що розроблена система дозволяє визначати роздільно концентрації цукрози, глюкози, лактози та мальтози з необхідною для промисловості чутливістю і може бути основою для створення сучасного аналітичного обладнання для одночасного операцівного визначення концентрації декількох сахаридів в харчовій промисловості.

Ключові слова: Біосенсор, аналізатор, вимірювальна система, сахариди
Introduction

Permanent control of saccharides concentration is vital in various branches of food industry and farming production. In biotechnology, saccharides monitoring is necessary for fundamental comprehension of processes of cultivation and fermentation, their optimization and regulation.

Determination of saccharose concentration is essential at all stages of sugar production — from its monitoring in white-beet roots during growing and storage and throughout the whole technological cycle of complete processing to the final product [1]. Lactose is intensively used in production of baby foods (along with saccharose), human milk substitutes, medicinal substances, antibiotics and food additives [2]. At present, maltose assumes ever greater importance in food production. Syrup containing maltose as a basic component is featured by high thermo-stability, low hygro-scopics also being less allergenic and viscous, it has sweet taste and do not crystallize at storage [3]. Maltose is used, in particular, in baby foods production as a saccharose constituent since its allergenic effect is essentially lower than that of the saccharose.

In sugar production technology, boiling water should be strictly controlled regarding the presence of invert (glucose and fructose) which causes pipeline corrosion and boiler failure and, as a result, defect and accidental additional cost.

Currently, saccharides concentration is regularly measured by analytical methods. Most traditional methods (liquid- and gas chromatography, chemical and optical methods) need expensive and complicated equipment and highly skilled personnel for its operation and maintenance, samples should be pretreated in a rather complex way [4, 5]. For instance, the refractometry is used for saccharose analysis in beet pulp; this method is experienced labour- and time-consuming because of the requirement of samples pretreatment with harmful reagents (e.g., lead acetate) for over than 40 min.

Nowadays, the application of conductometric biosensors is a promising approach in development of apparatus for determination of saccharide concentration. Conductometric methods are sufficiently simple, easy-to-use and precise in terms of application for both research and commercial purposes. Conductometric transducers are advantageous as compared with electrochemical transducers of other types by following characteristics:

— absence of technologically complex and large-sized reference electrode;
— application of low-amplitude alternative current (which allows to avoid Faraday effect on electrodes);
— light insensitivity (in contrast to ion-selective field-effect transistors);
— a potential of miniaturization and high-rate integration assuming usage of inexpensive thin-film technology;
— low costs at mass production [6].

The four-channel biosensor analyzer of saccharides, described in this article, is suggested for determination of glucose, saccharose, lactose and maltose in aqueous solutions.

Design of conductometric biosensor analyzer and principles of operation

Measurement of tested analytes concentration by conductometric biosensors (CBS) is carried out in a buffer solution with ion conductivity. CBS consists of a selective biochemical transducer in the form
of a thin membrane deposited on planar electrodes placed on a thin plate. CBS is immersed in the buffer solution. The tested substrate being added in buffer solution, penetrates the selective membrane and the chemical reaction takes place resulting in change of ion concentrations. Consequent change in specific electric conductivity is proportional to the tested substrate concentration.

The system of planar electrodes transforms the changes of solution conductivity into a CBS output informational parameter — changes of active conductivity. However, along with this useful component, there are non-informative components in the CBS output signal: considerable background temperature-dependent (2%/°C) active conductivity of buffer solution and reactive conductivity connected with the processes at electrode/electrolyte interface.

To suppress non-informative components and ensure required measurement stability, a differential sensor is used which consists of two conductometric transducers (CT) included in a compensation-bridge circuit of a secondary transducer (ST). Considering difference in electric properties of CT and membranes to be negligible, the circuit can be taken as balanced. Both CT are geometrically identical, each CT consists of a pair of thin-film interdigital electrodes on an insulating support. Addition of the tested solution into buffer solution causes changes in specific conductivity of the active membrane while that of passive membrane remains the same. It causes the bridge circuit disbalance and the constituent of ST output voltage occurs which is proportional to the tested solution concentration.

On the other hand, the dependencies of CT equivalent circuit parameters on electrode circuit geometry, electrodes material, frequency of bridge applied voltage, concentration of buffer solution have been shown. The tangent of the phase angle can range from several tenths to one. At increase of frequency till 20 — 30 kHz, the tangent value drops (that is characteristic for the series equivalent circuit) while at higher frequencies, either stabilization, slight decrease or increase of this parameter is revealed. The frequency characteristics of the phase angle tangents for 6 pairs (firm lines and dashed lines, respectively) of planar conductometric transducers with 20 × 20 μm inter-digital topology of gold electrodes are presented at Fig. 1. The data obtained testify the complication of the equivalent circuit and failure to improve characteristics via increasing frequency.

An influence of non-informative parameters of measuring transducers is especially challenging at development of multi-channel (multi-sensor) systems since these effects are to be taken into account and corrections have to be done for each channel separately. In the course of the research, causes and characteristics of the reported dependencies were studied; novel methods and means of transformation of differential CBS impedance parameters were suggested to ensure required stability of the transformation coefficient of measuring circuit as regards to the effect of non-informative parameters of the circuit elements [9, 10].

Basically, new measuring methods suggested distinguishing of the compensation (equilibration) of the voltage drop on the capacitive (non-informative) component of CT impedance which allows the normalization of test voltage on the active (informative) component at working frequencies of 20 — 30 kHz for CT of any kind. As a result are attained:
the considerable increase in measuring channel sensitivity;
− decrease of its variation range;
− stability of transformation coefficient are attained.

Further sensitivity increase and stabilization can be obtained by complete equilibration of the bridge circuit and use of its output signal module as an informative parameter. Main transformation methods and functional schemes of some ST with compensation-bridge circuits have been reported in [9, 10].

Below the hardware and software packages are considered as a basis for realization of four-channel biosensor analyzer of saccharides composition in food production and allied industries.

At Fig. 2 the analyzer composition and interaction of its modules and units are shown. The sensor block consists of a stand with fixed block of holders (BH) containing four conductometric biosensors (CBS) with membranes, each of which is selective to either glucose, saccharose, lactose or maltose. CBS are immersed into a vessel filled with buffer solution; the sample solution is added in order to be measured. A magnetic stirrer (MS) ensures solution homogeneity throughout the measurement procedure. An electronic measuring block consists of two modules:

− the module of secondary transducers (MST);
− the basic measurement-control module (BMCM) [11].

A structural diagram of the measuring channel is presented at Fig. 3. MST module consists of four secondary transducers ST1 — ST4; each has a compensation-bridge circuit [9]. Output impedances (Z) of each CT pair are connected into the loops of certain ST. The sensors are supplied with sinusoidal potential, frequency of 20 — 30 kHz and amplitude of 10 mV, generated by the generator G. The output potential of each ST is proportional to CT impedance difference of the particular CBS. Output potentials of ST are connected in turn into BMCM module input by means of an electronic switch K1. There are two synchronous detectors CD1 and CD2 intended for separation of ST output signal into two components, synchronous and meander, relative to G potential. These components in turn, via electronic switch K2, enter the input of the integrating analogue-digital converter (ADC), and then are connected, as the digital code, into the microcontroller (MC) for subsequent processing. MC is regulated with the software at lower level which guarantees the data exchange with ADC and PC, and control over G and switches K1- K2 operation. BMCM is provided with keyboard (KB) and indication block (IB) for autonomous (without PC) work of the analyzer.

The operational algorithm consists of two stages: preliminary balancing of bridge circuit and determination of results of biochemical reaction.

At the first stage, CBS is placed into buffer solution without tested substrate. By means of regulation elements, the bridge circuits of ST1 — ST4 are in turn balanced with respect to two components (in-phase and meander) of the output potential. The results of output signals measurement from ST1 — ST4 are processed and displayed at the indicator (I) and are used as a balanced parameter.

At the second stage, the solution under test, containing saccharides of certain concentration, is added into the buffer solution. The biochemical reactions in selective membranes result in generation
of the unbalance potentials on ST outputs. Their in-phase components proportional to the concentration of particular saccharide are transformed into digital codes which enter PC via interface port RS-232C; they are displayed on digital indicator in an autonomous mode.

The personal computer with software of higher level (SHL) performs automatic control of the measuring complex, and complete processing, accumulation and plotting of the measurement results. The software consists of two modules. The basic one (BSHL) is a unified module which could be used for multi-channel measuring systems with rather wide range of regular functions. Another module takes into account the peculiarities of actual measuring complex intended for specific task.

SHL is featured in following functions:
- compensation of the voltage drop on a capacitive component of CT impedance and coarse balance of bridge circuit by in-phase components of a disbalance signal; accurate bridge balancing by meander components of a disbalance signal (for measurements with the equilibrium method);
- realization of multi-channel mode of measurement;
- calibration of measuring channels for correction of transfer functions and determination of saccharides concentration;
- regulation of the equivalent noise transmission band by the data averaging.

For realization of specific functions of a multi-channel biosensor analyzer, the BSHL is supplemented with:
- the software ensuring interface with a user of the analyzer;
- generation and transmission of the commands to equilibrium;
- BMCM and MST control;
- obtaining and specific processing of information.

Besides, the tracer programs are available for experimental investigation of apparatus and software of the conductometric complex.

Basic software is a set of various functional programs started by the operator via a system of multi-level menus. BSHL is a Windows applications with the menu and pictograms; it gives to users the wide scope of facilities for processing electric informative characteristics (U, I, R, etc.) obtained by transducers of various kinds as well as for computation of the parameters to be determined (solution concentration, specific conductivity, temperature, humidity, mass, pressure, etc.) with the fourth power polynomial or specific formula.

BSHL functions are similar to those of other measuring systems:
- tuning for required number of channels (1 to 32);
- data exchange with the measuring block via interface RS-232C;
- summarizing measured and computed values of the parameters and other information (date, time, experiment number, notes) in the Excel tables with regard to the time of tabulation;
- saving information in a file in the text format convenient for other applets (Excel, Word, etc.);
- realization of different modes of measurement (single, continuous, cyclic).

BSHL allows performing the routine procedures of processing measurement information:
- accounting of the initial parameters;
- comparison with threshold values;
- calibration by external factors;
- determination of sum (difference) of the values obtained on various channels;
- plotting the results of measurement and calculation.

It provides the output of an arbitrary combination of measured or computed values to one or two axes, scaling of indicated values (by the user or automatic), viewing the data on horizontal and vertical axes with the possibility of selecting and scaling of a part of the graph.

The view of the main window of the developed BSHL on the PC display is presented at Fig. 4.

Fig. 4. View of main window of basic program
**Results**

The system was tested regarding determination of the saccharides such as saccharose, lactose, maltose, glucose. The basic enzymatic reactions are presented at Fig. 5.

Three enzymes are required for measurement of saccharose, lactose and maltose, whereas only glucose oxidase is necessary for glucose determination. The enzymes invertase, β-galactosidase and α-glucosidase decompose their substrates: saccharose, lactose and maltose to α-D-glucose. The latter is decomposed upon mutarotase action to β-D-glucose, then by glucose oxidase — to hydrogen peroxidase and D-gluconolactone which is spontaneously hydrolyzed to gluconic acid with subsequent dissociation into acid residue and proton generation. Therefore, the solution conductivity changes which can be registered by conductometric transducer.

Calibration curves obtained by the developed conductometric saccharides analyzer (Fig. 6) could be used for efficient concurrent measurement of concentrations of four saccharides, i.e. saccharose, lactose, maltose, and glucose.

The general view of the analyzer is presented at Fig. 7.

**Conclusions**

As the result of the experimental results discussion we could state the following:

1. The conductometric biosensor system using novel methods and measuring means is an example of convenient advanced analytical equipment for concurrent efficient determination of concentration of several saccharides in raw materials and semi-processed goods, in final production and in technological media.

2. The preliminary experimental research presented testifies that the system ensures separate determination of concentration of saccharose, lactose, maltose and glucose with commercially available sensitivity.

3. The soft hardware applied provides high technical and economic parameters of the developed analyzer and could be used for solution of various tasks.

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![Fig. 5. Basic enzymatic reaction of conductometric analyzer of saccharides](image)

![Fig. 6. Calibration curves of responses dependence for glucose, saccharose, maltose and lactose sensors on concentrations of glucose, saccharose, maltose and lactose, respectively](image)

![Fig. 7. General view of conductometric analyzer of saccharides](image)
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