Enzyme Biosensor for Determination of Glucose in Honey Bee

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***Abstract* — an enzyme biosensor for determination of glucose in honey was developed by using enzyme glucose oxidase. The measuring range of the biosensor and its response are determined. A measurement with real samples was made.**

***Index Terms* — biosensor, glucose, honey**

I. Introduction

Honey is a product that can be easily counterfeited, with sugar being the most difficult to identify. Thus, instead of honey being produced from plant nectar, bees are artificially fed with table sugar to increase the volume. This reduces the usefulness of honey and changes its properties and qualities. When bees are fed with ordinary sugar, they first break it down to invert and only then absorb it and turn it into honey. To perform this whole process of breaking down ordinary sugar into invert sugar, bees need extra time and energy. This, respectively, exhausts them and reduces the effect of their work. That is why it is important to have a way to quickly and reliably measure the sugar content of honey.

Honey contains simple sugars - glucose, fructose and sucrose. These substances make up 95% of all dry trace elements. Glucose (grape sugar) and fructose (fruit sugar) are included in the group of monosaccharides. Their content in honey varies from 65-80%. Sucrose refers to a group of disaccharides. The content in honey is from 1 to 6%. Sucrose under the influence of invertase enzymes is gradually broken down into glucose and fructose, so that its ratio in the mature product is negligible. The content of sucrose in honey above the stated 6% indicates the addition of sugar. Such honey can be dangerous, for example, for people with diabetes.

Different methods are used to measure sugars in honey - optical, spectrophotometric, biosensor [1, 2]. According to ORDINANCE 48 of 11.11.2003 on the procedure and methods for sampling and the methods used for analysis of honey, high-performance liquid chromatography is used to determine the sugar content in honey [3]. This method requires pre-treatment of the sample as well as the use of special reagents to perform the analysis.

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The aim of the present work is to develop an enzyme biosensor for measuring glucose in honey. The enzyme glucose oxidase was used to make the biosensor. The measurements are performed in the established mode.

II. Apparatus and Materials

A Clark type electrochemical electrode is connected to the Hanna Instruments 9146-04 oxygen meter to perform the measurements. The electrode measures the dissolved oxygen content in the measuring cell, which is visualized on the oxygen meter display. A Hanna Instruments pH digital meter HI 251 is used to control the pH of the medium. A PL 100 digital pipette is used to place a substrate in the measuring cell. Cole Parmer RZ 11700-42 analytical balance and BOECO MSH 300 electromagnetic stirrer are also used. Chemically pure substances are used - glucose oxidase, glucose, honey.

III. Construction of Enzyme Biosensor and Principle of Operation

The base transducer used to construct the biosensor is a Clark-type oxygen electrode that operates with an external polarization voltage. An additional active membrane and a dialysis membrane are placed on the semi-permeable membrane of this electrode. The forehead of the electrode is flat. two mg of the enzyme glucose oxidase are weighed with the analytical balance. The weighed enzyme is carefully placed on the front of the oxygen electrode. The enzyme portion is coated with a dialysis membrane. The dialysis membrane is 25 micrometers thick and serves to capture the enzyme on the oxygen teflon membrane. It also serves to separate the biological material, ie. the enzyme glucose oxidase, from the research medium. In this way it also prevents foreign bodies to enter into the active membrane. In order not to displace the elements of the biosensor transducer, compression O-rings are used.

The biosensor thus constructed is immersed vertically in the measuring cell. A special stand allows it to be fixed at the desired level. The measuring cell itself is placed on a magnetic stirrer MSC M-300, which maintains a constant speed of stirring the solution.

The enzyme biosensor is used to measure glucose concentration. The measurements are performed in a fixed mode using the method of successive additions. The principle of operation is based on the occurs of a biochemical reaction between the analyte and the enzyme, which is associated with a change in the oxygen content in the reaction volume.

When the enzyme biosensor is immersed in the measuring cell in its active membrane the following enzyme-catalyzed

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reaction takes place, which is accompanied by oxygen consumption:

$$glucose + O_2 + H_2O \xrightarrow{GOD} H_2O_2 + gluconic\ acid \quad (1)$$

When a certain amount of substrate - glucose is placed in the measuring cell, it diffuses through the dialysis membrane and enters in the active membrane. Under the action of the enzyme glucose oxidase, glucose is broken down into hydrogen peroxide and gluconic acid, and the reaction proceeds with the consumption of oxygen. The concentration of glucose in the measuring cell is judged by the amount of oxygen depleted.

IV. Experimental Research

The oxygen meter shown in Figure 1 is used to process and output the information obtained from the oxygen electrode. The device is digital - manufactured by Hanna Instruments, model HI 9146 - 04, with ± 1.5% accuracy and serves for continuous monitoring of oxygen content and temperature in the research environment.

Fig. 1. Digital oximeter HANNA INSTRUMENTS

The analyzer has the following technical characteristics when measuring oxygen:

- Measurement range of dissolved oxygen concentration measurement: 0,0 ÷ 45,00 mg/l O_2
- Resolution: 0,01 mg/l O_2
- Accuracy: ±1,5% on the scale mg/l O_2
- Automatic temperature compensation: 0 ÷ 50^0C

The experimental measurements are performed in an established mode. The method of successive additives is used, which consists in the following:

1. The establishment of the output signal of the biosensor system is pending.
2. After the output signal reaches a set value, an injection with a certain volume of the tested substrate is placed in the measuring cell.
3. A new establishment of the output signal of the biosensor is awaited - due to the course of biochemical reactions in the active membrane of the biosensor system, its output signal changes. Record the value of the output signal that corresponds to the concentration of the measured substrate in the measuring cell.

Each subsequent injection of substrate is placed after the output signal of the biosensor system reaches a set value corresponding to the oxygen concentration in the reaction volume. The value of the measured substrate is determined by the read oxygen concentration.

The measuring cell has a volume of 20 ml. It is placed on a magnetic stirrer, which ensures continuous stirring of the research medium at a constant speed of 500 rev/min. The buffer solution, which is placed in the measuring cell and in which the analyte is placed, has a pH value 6.25. The process of initial operation of the biosensor lasts 30-40 minutes. This time ensures maximum saturation of the active membrane with oxygen, which is reported by the established reading of the oxygen meter.

The concentration of oxygen in the buffer solution at the initial detection of the oxygen meter is 4.55 mgO_2/ l. Once the output signal is established injections of the pre-prepared starting substrate - glucose - are started to be placed in the measuring cell. The injection volume is 40 microliters. After placing the first additive with the digital pipette, reaction (1) occur, in which the oxygen content in the reaction volume decreases. After reaching a new equilibrium state, a new additive is placed from the initial substrate with the same volume. The process continues until the oxygen in the measuring cell is depleted. The output signal of the biosensor is shown in fig. 2. whereas fig. 3 shows its conversion function. According to the ordinate of the graph of fig. 3 the calculated values for oxygen are plotted, which are obtained by subtracting the measured current values from the saturated oxygen concentration without the presence of glucose.

Based on the obtained results and the obtained function from figure 3, the linear measuring range of the enzyme biosensor is determined - from 0.4 µM to 2.1 µM. Saturation is obtained after 2.2 µM glucose concentration.

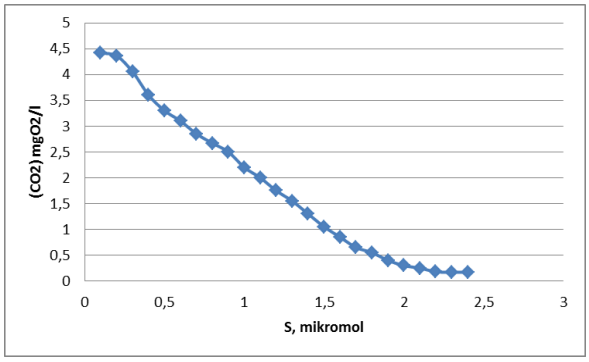

Fig. 2. Enzyme biosensor output signal

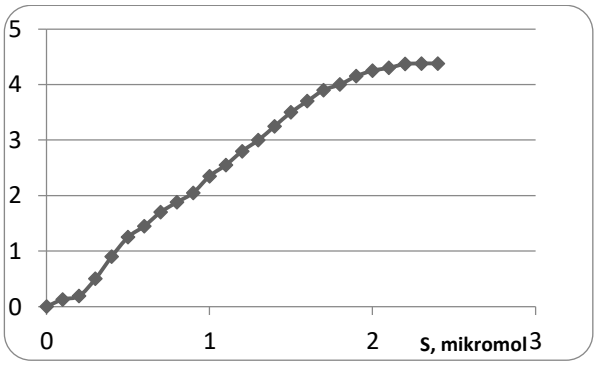

Fig. 3. Enzyme biosensor conversion function biosensor

A. Measurement of glucose in real samples

The developed glucose biosensor was tested with real samples of honey. Two samples are home made honey, tentatively called “sample 1” and “sample 2”. The third sample was purchased from the trade network, where packaged small doses of honey are offered. The third sample is tentatively called “sample 3”. As the honey has a very thick consistency, it had to be diluted with distilled water in order to be able to perform the measurement itself. To do this, dilute 1 ml of the honey sample with 5 ml of distilled water. After complete dissolution of the honey, the glucose content of each sample is measured. The measurement is performed under the following conditions: measuring cell with a volume of 20 ml, injection volume 40 µl. The results obtained are shown in Table 1.

TABLE I
MESUREMENT OF GLUCOSE IN HONEY BEE

	Sample 1	Sample 2	Sample 3
C_{O2}^{*}	4,76	3,80	3,78
C_{O2}, mgO2/l	3,46	2,79	1,62
$\Delta(C_{O2})$ mgO2/l	1,30	1,01	2,16
Glucose, µM	0,50	0,4	0,9

The measurement time for all three samples is almost the same - about 5 minutes. Table 1 shows that the sample purchased from the commercial network shows the highest glucose content. The concentration of glucose in the samples of home-made honey is almost the same - 0.5 µM and 0.4 µM.

V. CONCLUSION

The presented results show that the constructed enzyme biosensor for glucose analysis has good sensitivity and a wide measuring range. This makes it possible to measure very low glucose concentrations, as well as to perform measurements on small samples. A sensitivity threshold of 0.4 µM glucose concentration was reached. The results obtained are close to the results obtained by an accredited laboratory.

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