UDC 577.15, 577.114, 573.6

APPLICATION OF AMPEROMETRIC BIOSENSOR FOR DETERMINATION OF GLUCOSE CONTENT IN BEVERAGES

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Introduction. Glucose is one of the main animal and plant carbohydrates. Its quantitative determination is essential in biochemistry, clinical chemistry, food industry, etc. [1].

Not only glucose, but also content of others carbohydrates at different stages of the production process is an important task in various industries: food industry (sugar, dairy, brewing, winemaking), agriculture, pharmaceutical production and even in ecology [4-5]. In agriculture, the sucrose content of sugar beet is one of the indicators that determines the efficiency of the technological process at all stages - from growing and storing beet to complete processing and sugar production. In dairy production, the lactose content, one of the main components of milk and dairy products, is an indicator of quality. Maltose is also of great importance in the food industry, as its content in molasses determines the quality of the final product (beer, kvass, etc.). Fructose, maltose, and other monosaccharides and disaccharides are often used as sucrose substitutes in the production of a wide range of products. Information on the presence and concentration of these carbohydrates in beverages and food is an important indicator of the quality of the latter [5].

Today, there are many different methods for determining carbohydrates, but each of them has certain disadvantages, some of which require qualified personnel and complex and expensive equipment, while others are simple and fast, but less accurate and selective. For quantitative glucose analysis: gas and high-performance liquid chromatography, spectrophotometry, refractometry, enzymatic analysis, NMR and mass spectrometry, capillary electrophoresis [2] are the most commonly used methods. However, the analytical procedures using most of these methods is time-consuming, labour-intensive, costly and often requires a significant amount of chemicals, some of which are hazardous to the environment [1]. In order to avoid these analytical complications, simple but reliable electrochemical biosensors are currently being developed which can meet the growing need for quantitative determination necessitates; a faster, more convenient and cheaper detecting methods. In this study we introduce an amperometric biosensor which is based on enzyme recognition element what provides high selectivity and sensitivity and does not require complex equipment and sample preparation. In addition, the response of amperometric biosensors does not depend on the buffer capacity and ionic strength of the solution in which the measurement is performed, which is undoubtedly a great advantage when analysing real samples [3].

In this paper the advantages of electrochemical analysis are demonstrated using the glucose amperometric biosensor. To ensure selectivity, a membrane was created with immobilized Glucose oxidase (GOx), which formed a biosensor when placed on a Pt electrode. The reliable determination of glucose is only an example of how widely an amperometric glucose biosensor can be applicable, since by replacing the

glucose-sensitive membrane with one, sensitive to other carbohydrates, we will easily measure other carbohydrates.

For such a purpose, it is only necessary to immobilize an enzyme in the membrane, such as PQQ-dependent glucose dehydrogenase, which catalyzes other monosaccharide and/or disaccharides.

The aim of the study. The aim of the work is to design the glucose amperometric biosensor and confirm it reliability of action by determining the concentration of glucose in some soft drinks that are often used by consumers and usually have a high content of this carbohydrate.

Methods of research. The Eksan-GM glucose analyzer was used for testing of the glucose biosensor. The extension of the functional responsibilities of this device may include the development of methods for determining both too low and too high glucose concentrations in various biological and food samples.

The principle of operation of the analyzer is based on the electrochemical amperometric determination on a working electrode of the products depending on the enzyme used in a reaction.

The amperometric biosensor was adjusted to operate in a three-electrode electrochemical cell of the analyzer.

During the measurement, the potentiostat will maintain the required voltage across the working electrodes and record an electrical signal proportional to the glucose concentration in the samples.

The following were used as testing samples: carbonated drink «Coca Cola», non-alcogolic beer «Carlsberg», non-alcoholic cider «Somersby», non-alcogolic Champagne, carbonated drink «Schweppes», sport drink «Vita minerale», energy drink «Monster» sugar-free, energy drink «Monster», kvass (black). To determine the glucose content, some beverages were diluted 10 times to provide a more accurate measurement. This is because we assume a high glucose concentration in these samples, but the minimum detection limit for glucose was between 2 and 30 mmol-l.

The determination of glucose in soft drinks and a control experiment were carried out using Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4) and/or Catalase from bovine liver. Aiming to design the biosensor the membrane containing immobilized GOx was mechanically adjusted to working (Pt) electrode.

Main results.

It is known that at a potential of 0.6 V at Pt electrode, glucose is oxidized and products are formed to hydrogen peroxide and D-glucone-δ-lactone. Thus, to confirm the selectivity of the glucose determination by the biosensor the enzyme catalase was added to the test sample. Since content of H_2O_2 must be reduced by catalase, the current of response of the biosensor must decrease over time close to zero.

The study was carried out on a carbonated Coca Cola drink, which was diluted by a factor of 20. GOx (1 mg in 1 ml) and catalase (5 μl) were added to the drink. Within half an hour, the glucose concentration was tested with the biosensor (Fig. 1).

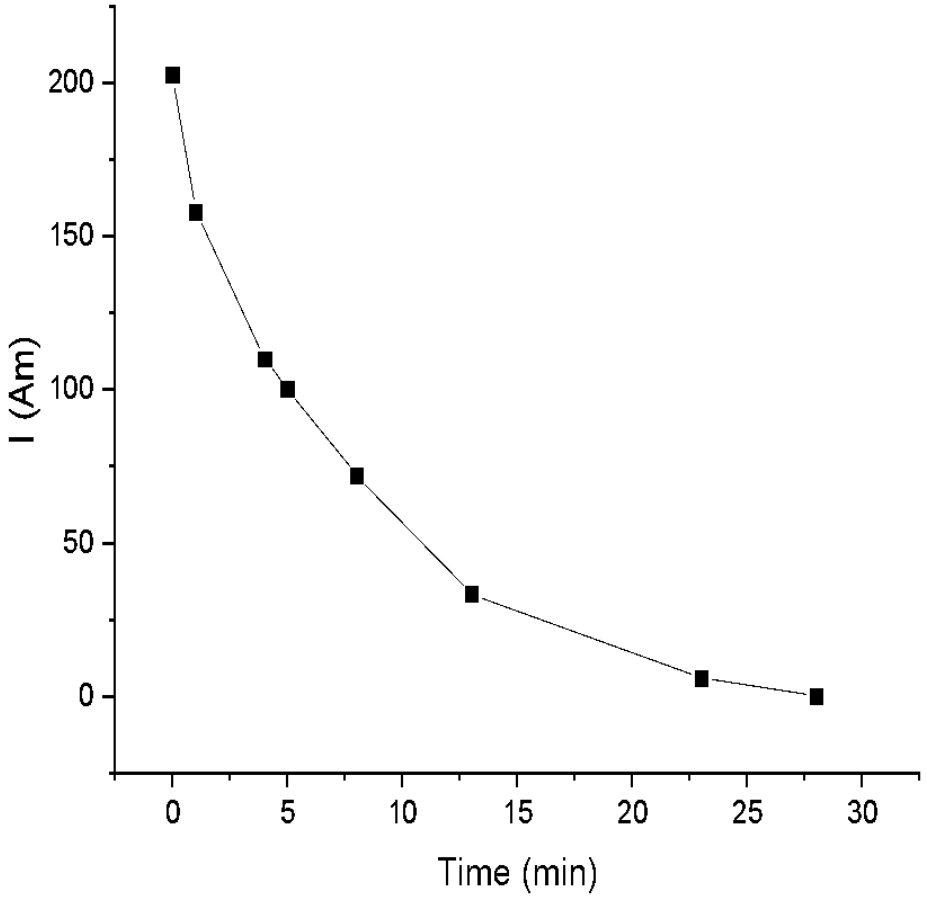

Figure 1. Changes in glucose concentration in the presence of GOx **and Catalase.**

The data shown in Figure 1 indicates the presence of glucose in the sample, and a zero value after 28 minutes confirms that there is no any influence/ response to interfering compounds.

After being convinced that the biosensor selectively measures glucose, a number of beverages were tested using the biosensor. When beverages were infused into the electrochemical cell of the analyzer, the biosensor measured the concentration of glucose. The results are shown in table 1.

Table 1

Glucose content in soft drinks obtained using glucose biosensor

№	Test liquid	Glucose concentration (mM)	Background signal (%)*
1	Standard glucose solution (10 mM)	10.00±0.50	0.093
2	Carbonated drink «Coca Cola»	180.00±4.45	0.24
3	Non-nalcogolic beer «Carlsberg»	13.48±1.01	0.25
4	Non-alcoholic cider «Somersby»	19.51±0.85	0.78
5	Non-alcogolic Champagne	14.13±0.95	0.067
6	Carbonated drink «Schweppes»	10.73±0.85	0.029
7	Sport drink «Vita minerale»	7.53±0.92	0.13
8	Energy drink «Monster» sugar-free	2.40±0.80	0.07
9	Energy drink «Monster»	147.96±23.66	0.70
10	Kvass (black)	21.49±0.79	0.39

* Response to glucose in beverages obtained with a control sensor without GOx. The response is calculated by taking the biosensor response as 100 percent.

Conclusions.

The amperometric biosensor selectively measures glucose, so it is suitable for evaluating the glucose content in beverages. As the glucose biosensor has already been validated for use in blood and serum, this experiment demonstrates its expanded potential for use in the beverage industry as well.

Since the design of the biosensor allows easy adjustion of the new membrane with other immobilized enzymes, this type of biosensor can be easily adapted for the analysis of various carbohydrates other than glucose.

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