AMPEROMETRIC BIOSENSORS FOR GLUCOSE DETERMINATION

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Abstract: *The paper presents an experimental laboratory research target on using enzyme biosensor with glucose oxidase and amperometric detection and high concentrations of glucose and also target on eliminating single-use chips by using a measuring cell with unlimited use.*

Key words: *biosensors, glucose oxidase, amperometric detection.*

Introduction

In order to determine rapidly and in situ the concentration, the weight or thickness of the layer of chemical or biological species in addition to classical analytical methods are increasingly used biosensors, which are biological-electronical selective integrated systems, consisting in a biologically active receiver, a transducer and an electronic amplification, processing and displaying data. Biologically active receiver gives specific analytical information enabling the recognition and quantitative or semi-quantitative determination of a particular biological or chemical species in a complex mixture that consists matter under review, the active biological component of the receiver it can be formed from an enzyme, an antibody, DNA, or even whole cells, and the transducer converts the value of concentration of reaction products in an electrical quantity proportional compatible with the system of processing and displaying data.

For enzyme field representative are biosensors using as active biological component type oxidase enzyme that catalyzes the transformation of species specific reaction in the reaction product followed in this regard are given below some specific applications of such biosensors:

$$\beta - D - Glu\cos e + O_2 \xrightarrow{Glu\cos oxidase} \Delta - Gluconolactone + H_2O_2 \quad (1)$$

$$Colesterol + O_2 \xrightarrow{Colesteroloxidase} Colestenone + H_2O_2 \quad (2)$$

$$Glutamate + O_2 \xrightarrow{D-Glutamateoxidase} Oxiglutarate + NH_3 + H_2O_2 \quad (3)$$

$$Lactate + O_2 \xrightarrow{Lactatoxidase} Pyruvate + H_2O_2 \quad (4)$$

$$Pyruvate + HPO_4 \xrightarrow{Pyruvatoxidase} \text{acetil phosphate} + CO_2 + H_2O_2 \quad (5)$$

In all reactions presented results besides the specific reaction products and hydrogen peroxide which is determined quantitatively by amperometric method or conductivity measurement, the amount of hydrogen peroxide generated in reaction and consumed in electrolysis is proportional to the concentration stoechiometric species analyzed, so a measure of its, transformation of current or conductivity values in concentration units it's automatically done by making a calibration curve stored in the microprocessor device. The most representative is the glucose biosensor enzyme widely used for rapid determination of blood sugar in diabetics and athletes for this purpose it's enough one drop of blood that brings a single-use plastic media that are two electrodes and a dry deposition of a gel containing a certain amount of glucose oxidase that has catalytic role in transforming the sugar drop in a few seconds blood into gluconolactone and hydrogen peroxide. Applying a continuous and constant voltage from electronic side to those two electrodes allows the amount of electrolysis current installed and Faraday's law to determine the amount of hydrogen peroxide resulting from the reaction in turn, under stoechiometric of chemical (1) – (5) and electrochemical processes of decomposition of hydrogen peroxide and adverse results above, is a measure of the concentration of specific reactants whose oxidation is catalyzed by oxidas. [1-3]

Materials and method. [4-8]

In the experimental work the authors have proposed to extende the principle of amperometric measurement of blood glucose, as described above at concentrations higher than that of blood glucose, at appropriate concentrations of industrial fermentative processes. The basic idea is to put on a glucose biosensor for laboratory and field, working on the amperometric principle and which does not use single-use classical kits but works with accurate liquid dose of glucoseoxidase that are injected or mixed with a certain volume solution containing glucose for analysis. After mixing the reaction components, namely one for about 10 seconds, the liquid solution is analyzed with amperometric detector it carried out with the hundreds of electronic determinations of a few seconds over. The measured values are statistically processed in the electronics, the Gaussian distribution curve of the tip are eliminated and those belonging to the central area of the curve is performed statistical average value which is converted from a calibration curve the concentration of glucose values. Used for experimental determinations thermostatic electrochemical cell with a volume of 100 ml and two working electrodes of electrolytic copper. Current measurements were made with an electronic miliampermeter, accuracy class 0.1 using an electrolysis cell and a thermostatic adjustable current source belonging to the instrumental analysis laboratory of the Faculty of Food Engineering Suceava. The principle scheme of arrangement experiment is shown in Figure 1.

In the experimental research were used two glucose concentrations:

- 1% , using 0,4% glucose oxidase concentration,
- 10 % using 1,0% glucose oxidase concentration,

The first concentration of glucose covers the application of biosensors in medicine and in sports for the determination of blood glucose, the second concentration covers the fermentative processes from food engineering.

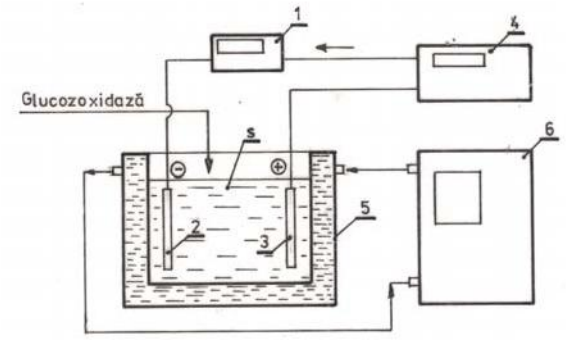

Figure1. The principle scheme of the experimental stand used for amperometric determination of values in different regimes of temperature and concentrations of glucose oxidase. 1-electronic miliampermeter, 2-cathode, 3- anode, 4- adjustable continiung current source, 5- thermostatic electrolysis cell, 6-thermostat

The main purpose of experimental research was the determination of minimum required for glucose oxidase used for catalytic reaction of glucose under conditions of low temperature and a high
enough resolution to ensure high precision of the method.
All experimental determinations were performed with a glucose oxidase provided by Enzimes & Derivates Company. Work temperatures ranged from 35°C to 60°C measurements from 5 to 5°C. Results were played by curves like those in Figure 2. Given the large number of experimental determinations maded, the electrolysis current graphic evolution of the glucose oxidase volume used to a certain temperature and concentration of glucose was done as a family of curves represented in Figure 3 and Figure 4. These representations provide a better comparability of different conditions.

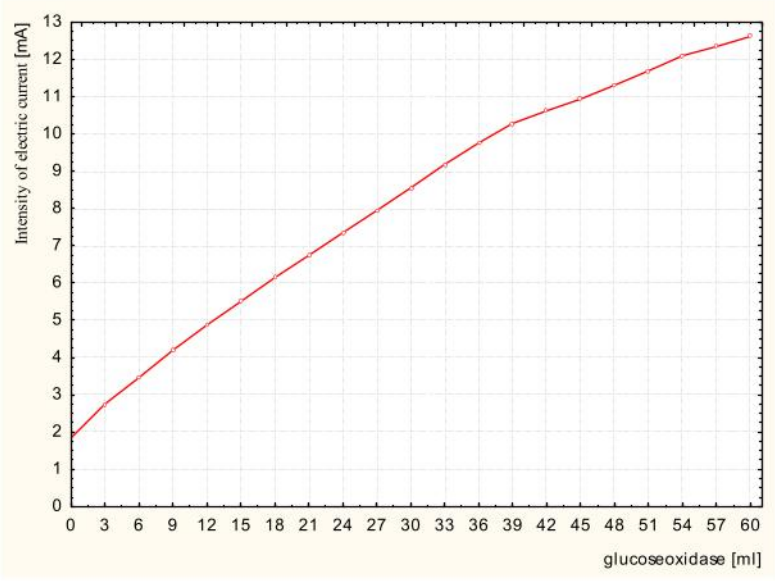

Figure 2. Variation of intensity of electric current to the solution of glucose, concentration 1%, the addition of different volumes of glucose oxidase, concentration 0.4%, the working temperature of 35°C

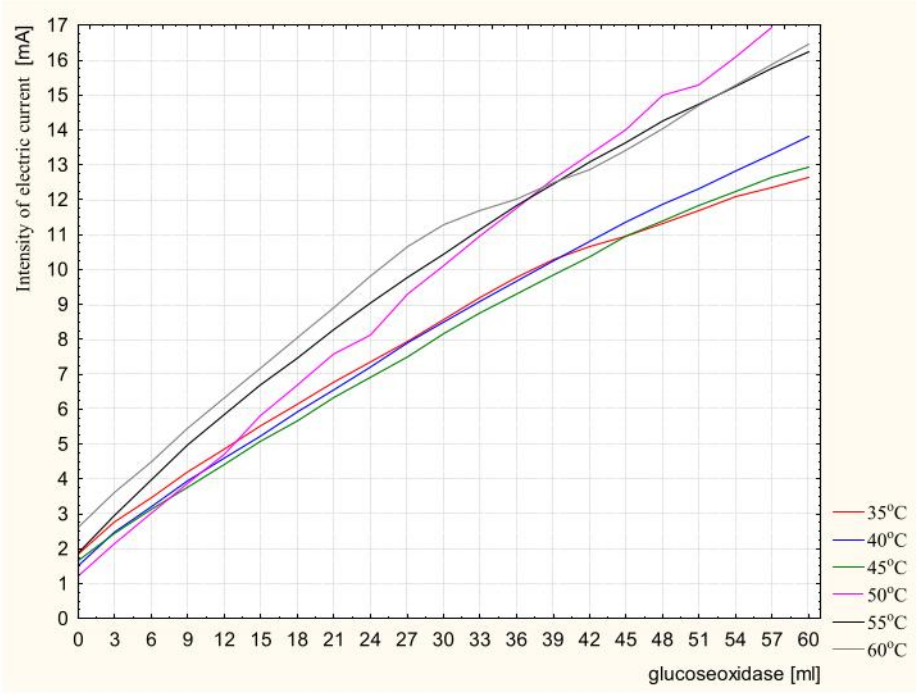

Figure 3. Family of curves representing the variation of intensity of electric current to the solution of glucose concentration 1%, the addition of different volumes of glucose oxidase, concentration 0.4%, at different working temperatures

Electric current intensity variation of glucose solution (1%) at the addition of glucose oxidase (0.4%) to work temperaturatures: 35°C, 45°C, 45°C, 50°C, 55°C,60°C.

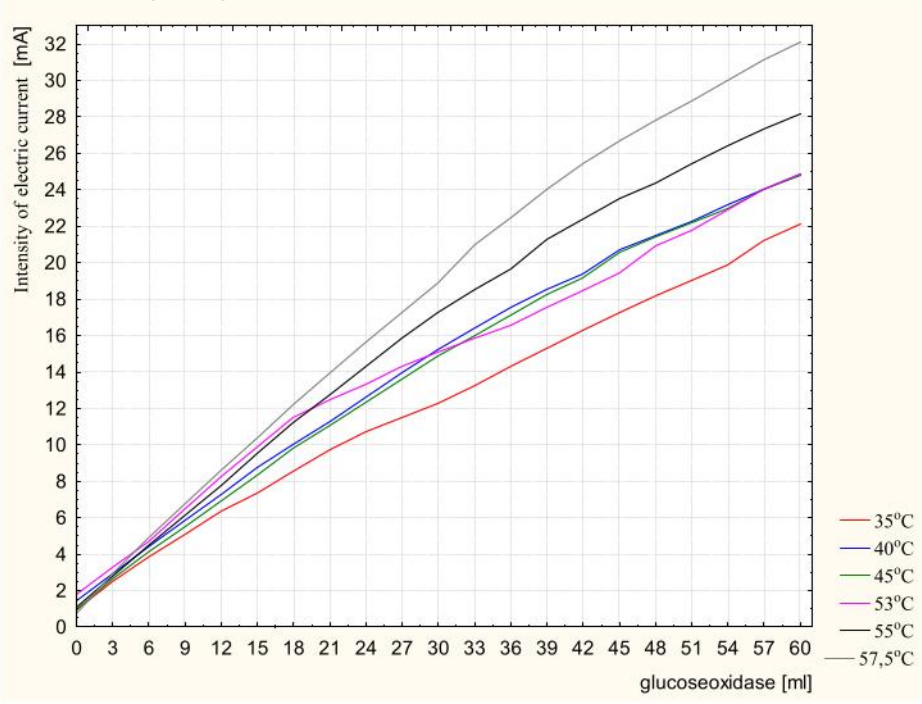

Figure 4. Family of curves representing the variation of intensity of electric current to the solution of glucose, 10% concentration, the addition of different volumes of glucose oxidase concentration 1%, working at different temperatures

An important aspect of research was the determination whether glucose oxidase has a sufficiently high enzyme activity at lower temperatures, in terms of the manufacturer enzyme shows its maximum activity at temperatures about 50°C temperature that would complicate the current analysis with biosensors in particular the analysis in situ, in conditions close to the working temperature of ambient.

To make the study of temperature was achieved by a rearrangement of current values depending on the temperature at different values of glucose oxidase at the two concentrations of glucose,1% and 10%. Experimental data are redistributed in Figure 5 and Figure 6.

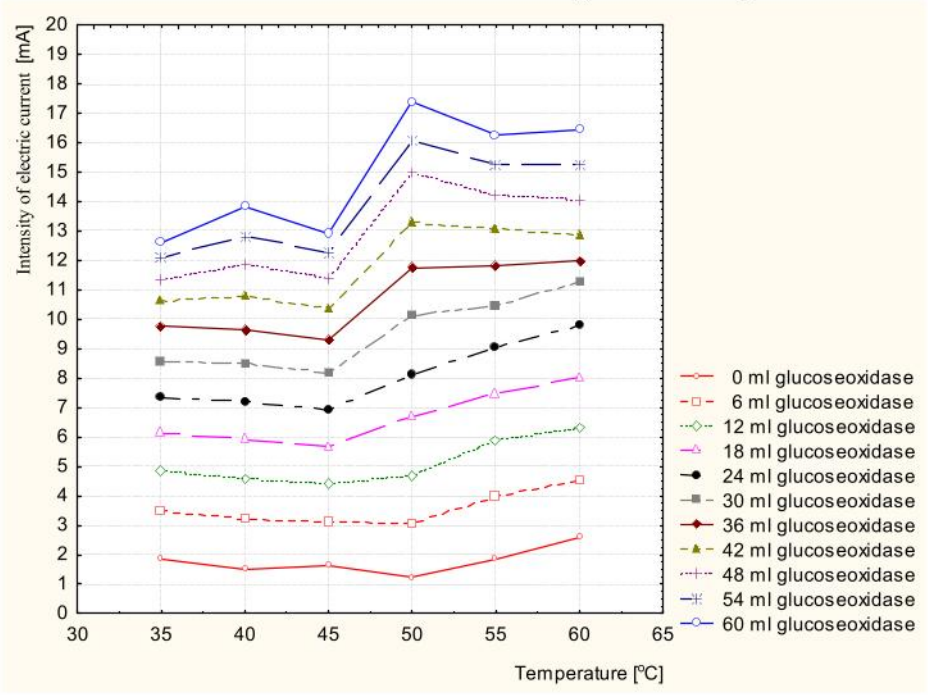

Figure 5. Variation of electric current intensity of glucose solution (1%) depending on the working temperature at different concentrations of glucose oxidase

For comparative study of graphics in Figure 5 and Figure 6 is an increase in electrolysis current intensity with increasing temperature. This increase, however, is relatively small but which do not justify high-temperature work. In exchange for a good resolution requires a volume of 60 ml glucose oxidase, concentration about 1% to a volume of 100 ml 10% glucose concentration. This means a consumption of 0.6 ml 1% glucose oxidase concentration for one determination in conditions in which biosensor is using 1 ml 10% glucose solution , this is expressed by mass means 0.006 g glucose per determination.

Conclusions

Experimental research has shown that it is possible the achievement of a biosensor, enzyme catalyzed with glucoseoxidase and having amperometric detection, for determining the concentration of glucose at concentrations specific to fermentative processes in food chemistry.

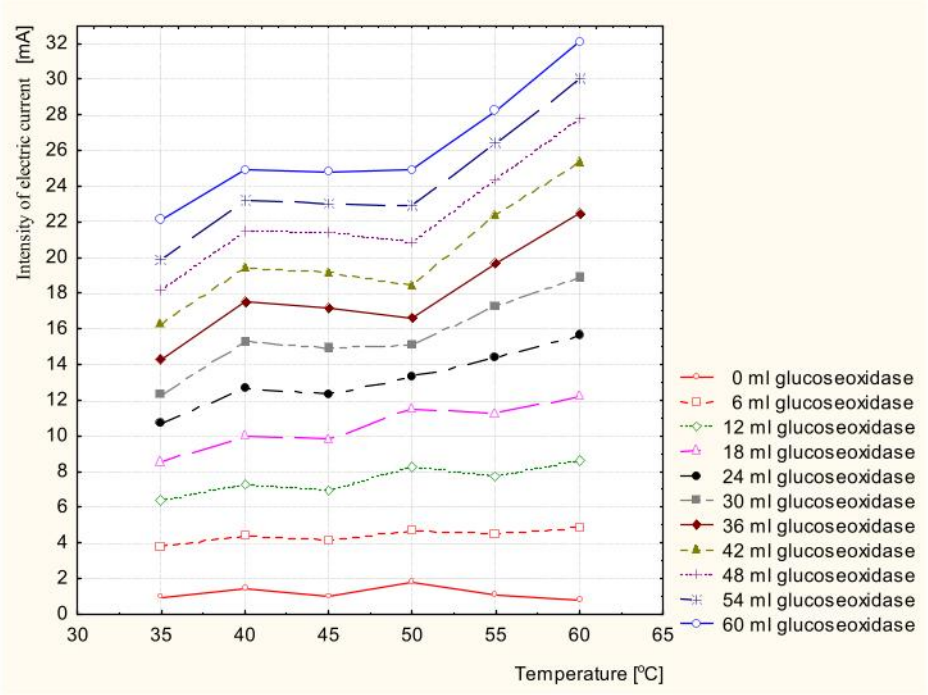

Figure 6. Variation of electric current intensity of glucose solution (10%) depending of working temperature at different concentrations of glucose oxidase

Consumption of glucose oxidase at high concentrations of glucose is much higher than the specific concentrations in the blood, measured values for comparison readed the same current intensity. Glucose oxidase used has a sufficient activity for working in conditions of sensitivity and good precision and near ambient temperature, while amperometric method gives good results working under liquid-liquid which enables the use of this principle for the construction of a biosensor without putty single-use liquid samples using glucose and glucose oxidase all liquid.

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