RESEARCH ON ACHIEVEMENT AN CONDUCTOMETRIC ENZYME BIOSENSOR

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Abstract: *Starting from the idea of the usefulness of enzyme biosensors in food fermentative processes, where glucose concentration is high, the authors have conducted studies and experimental research on the design and implementation of an enzyme biosensor based on conductometric measurement principle, a principle that eliminates several disadvantages of amperometric measurement used to classical enzyme biosensors using glucose oxidase as a catalyst enzyme. Research were performed at different temperatures and different concentrations of glucose oxidase to the concentration of 1% and 10% glucose concluded that biosensors enzyme conductometric detection with clear advantages over enzyme biosensors with amperometric detection .*

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

Introduction

Enzyme biosensor with glucose oxidase is important for rapid determination of sugar concentration in fermentative processes where glucose concentrations are over an order of magnitude higher than in blood. But having in mind that the classical amperometric method used to produce biosensors are some disadvantages such as:

- feature non-linear current density-concentration at high concentrations reactants
- specific electrode polarization process of electrolysis reduces accuracy
- amperometric method have relatively low sensitivity
- the nature of the liquid matrix has influence sensitivity accuracy

Amperometric method is not recommended in high concentrations prompted us to seek an alternative to replace it with another instrumental method which do not present these disadvantages. The fact that the enzyme catalyzed reactions resulting hydrogen peroxide, whose concentration is stoechiometric proportional to the concentration of glucose shows all recommended as an electrochemical method namely measuring the electrolytic conductivity alternatively current reaction of hydrogen peroxide from the oxidation of glucose. By applying this method disadvantages mentioned above are eliminated.

Materials and method [1-4]

Conductometric method is an electrochemical method of quantitative analysis based on the dependence between electrical conductivity of a solution and an ion concentration of that solution. Conductivity of the electrolyte column length (l) and section (s) that is located between two electrodes, figure 1, is defined as the inverse of electrical resistance:

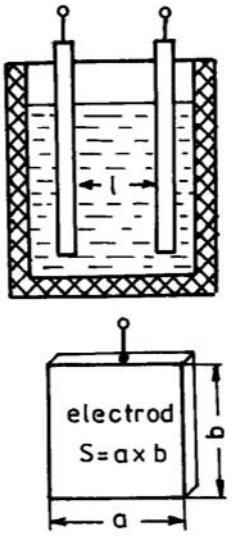

Figure 1. Electrochemical for conductivity measurement

For electrolytes is defined electrolyte conductivity (χ) the inverse resistivity (1 / ρ) of electrolyte and expressed in [Ohm $^{-1}$ x cm^{-1}] or Siemens [S]

$$\chi = \frac{1}{\rho}[S] \tag{2}$$

$$R = \frac{\ell}{\chi \cdot s} \tag{3}$$

Conductivity is a specific size of electrolyte concentration and depends on its temperature. Another expression of the conductivity is as equivalent conductivity (λ) and it is defined to be the conductivity of a cm3 of solution containing one gram equivalent of substance.

$$\lambda = 100\frac{\chi}{c} \tag{4}$$

where: C - the equivalent concentration in g / 1000 cm$^{3.}$ Replacing the value of conductivity (χ) in relation (3) the value of conductivity (χ) in relation (4) is obtained:

$$R = \frac{1000 \cdot l}{\lambda \cdot c \cdot s} \tag{5}$$

or :

$$\lambda = \frac{1000 \cdot l}{c \cdot R \cdot s} \tag{6}$$

Conductivity cells are constructed so that the ratio l / s to have a constant value:

$$\theta = \frac{l}{s} \tag{7}$$

In this case, the relation (6) becomes:

$$\lambda = \frac{1000 \cdot \theta}{R \cdot C} \tag{8}$$

determining value:

$$\theta = \frac{\lambda \cdot R \cdot c}{1000} \tag{9}$$

is by measurement of resistance R of an electrolytical solution with conductivity χ familiar with KCl. For strong electrolytes, Kolrausch established empirical relationship between equivalent conductivity λ and concentration c:

$$\lambda = \lambda_0 - K_d \cdot \sqrt{C} \tag{10}$$

For weak electrolytes, Ostwald has established a relationship equivalent to:

$$K_d = \frac{\lambda^2 \cdot C}{\lambda_0(\lambda_0 - \lambda)} \tag{11}$$

where :

Kd - dissociation constant of the electrolyte

- λ_o conductivity limit (infinite) amount proportional to the relative mobility at infinite dilution (μ^0) of ions in solution.

For a binary electrolyte results :

$$\lambda_o = \lambda^0_+ + \lambda^0_- \tag{12}$$

where: $\lambda^0 = F\,\mu^0$ and is equivalent ionic conductivity

Relationship (12) expresses the Kohlrausch's law of independent migration and can be written as:

$$\lambda_O = \sum_{i=1}^{n} \lambda_i^0 \tag{13}$$

or :

$$\lambda = \sum_{i=1}^{n} \lambda_i \tag{14}$$

If the electrolyte with (n) ion species, substituting relation (14) in relation (5) is obtained :

$$R = \frac{1000 \cdot \theta}{n} \Big/ \sum_{i=1}^{n} \lambda_i \cdot C_i \qquad (15)$$

This relationship establishes a dependence between the electrical resistance of electrolyte and concentration that the total conductivity of the solution and ionic species contribution (i) to achieve the conductivity. Relationship (15) represents the relationship underlying the calibration apparatus for determining glucose concentration on conductivity determination of reaction products, that of hydrogen peroxide, the base. Conductivity can perform both DC and AC as well.

Experimental [5-10]

From relation (14) results that the measurement of electrolyte conductivity that is proportional to the concentrations of all ionic species in electrolyte and measured the electrical resistance of the electrolyte, depending on the intake of certain ionic species in solution measured, is given by relation (15). On these findings were based on experimental research using conductivity detection for the concentration of hydrogen peroxide resulted in different reactions catalyzed by oxidase

Further experimental determinations are presented for consideration of the possibility of using conductometric method for measuring glucose concentration in optimal conditions, is presented a proposed biosensor to achieve rapid analysis and conclusions resulting series. Experimental tests were performed with a glucose oxidase from Enzimes & Derivates company and experimental measurements with an electronic microchip conductometer using a thermostat beaker. Taking into account that the determination of DC conductivity electrodes polarization occurs and leads to errors of measurements, a conductometric cell was used to measure conductivity in alternative current. For comparative study of the amperometric method with conductometric method was used a thermostatic electrolysis cell volume of 100 ml and an adjustable current source, belonging to the Instrumental Analysis Laboratory of the Faculty of Food Engineering Suceava. Scheme of principle stand of comparative measurements is presented in Figure 2.

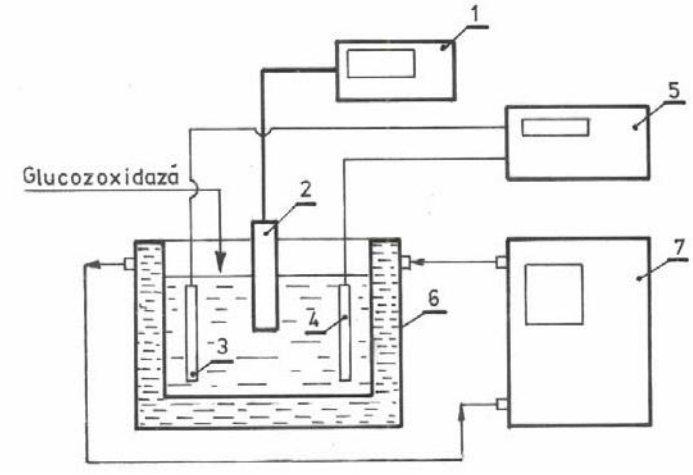

Figure 2. The principle scheme of the experimental stand used for amperometric and conductometric comparative studies to determine the concentration of glucose by the enzyme catalyzed oxidation method of the glucose oxidase in different regimes of temperature and concentrations of glucose oxidase. 1-conductometer, 2-probe conductivity, 3-cathode, 4-anode, 5-current source, 6-cell electrolysis thermostatic, 7-electronic thermostat

Two glucose concentrations were used: 1% and 10%, first concentration covering biosensors with applications in medicine and in sports for the determination of blood glucose, the second application covering fermentative processes in food engineering. To determine the maximum enzyme activity but also for determining the optimal scope of work, considering the use of enzyme determinations were performed experiments that have targeted conductivity measurement depending on the concentration of glucose oxidase using each time a volume of 100 ml of glucose, concentration of 1% and 10% at temperatures between 35^0C and 60 ^{0}C measurements in 5 out of 5 ^{0}C. The result of these determinations was represented graphically in figures like the ones in Figure 3 where the electrolytic conductivity was represented by the amount of glucose added. Considering the large number of experimental determinations representations have been cumulative as family of curves for working temperatures between 35^0C and 60 ^{0}C.

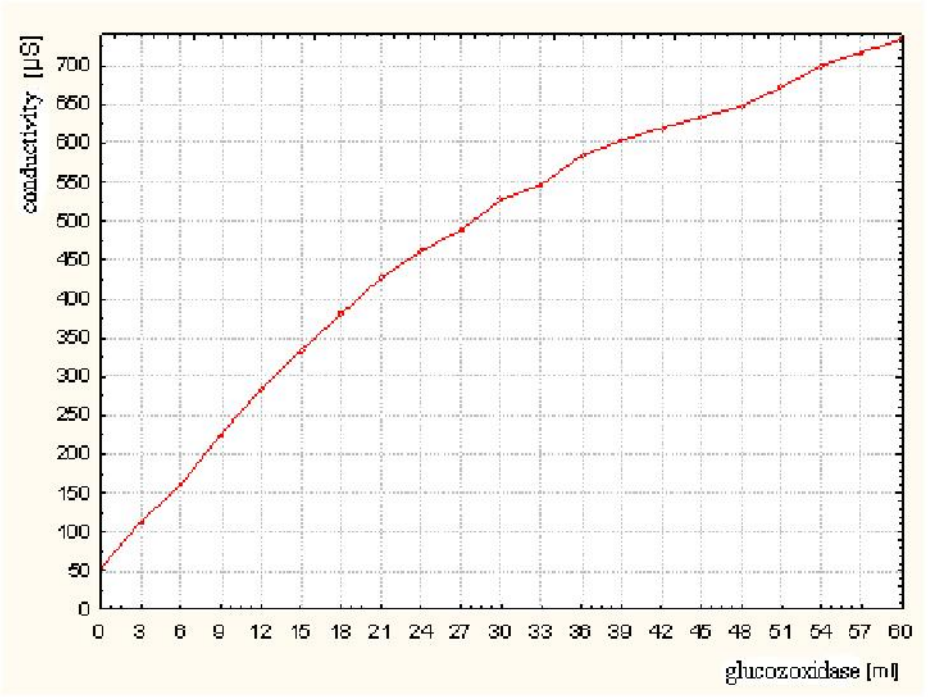

Figure 3. Variation of electrolyte solution conductivity of glucose (10%) according to the different volumes of glucose oxidase concentration (1%) at 50**°C** work temperature

Graphic developments are given in Figure 4 for the concentration of 1% glucose and in Figure 5 for the concentration of 10% glucose. The purpose of this research experiments being to determine primarily the minimum amount of glucose oxidase required for catalytic reaction of glucose oxidation in terms of a resolution high enough to ensure high precision of the method. Secondly had investigated whether glucose oxidase has a sufficiently high enzyme activity at lower temperature of 50-60 ^{0}C values which are indicated as the optimum working for this enzyme.

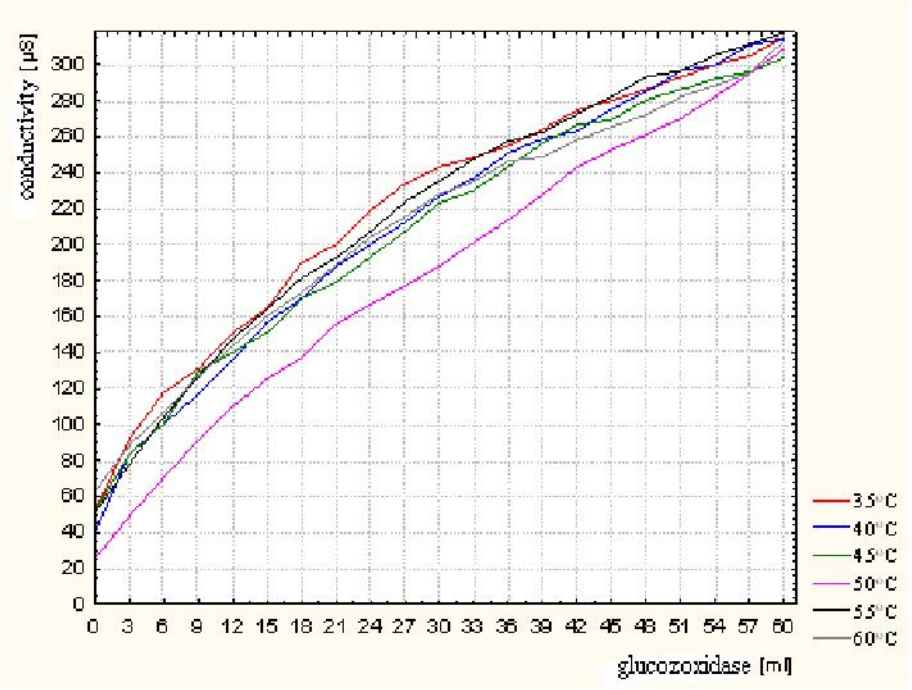

Figure 4. Variation of the electrolyte solution conductivity of glucose (1%) according to the different volumes of glucose oxidase concentration 0.4% at different working temperatures

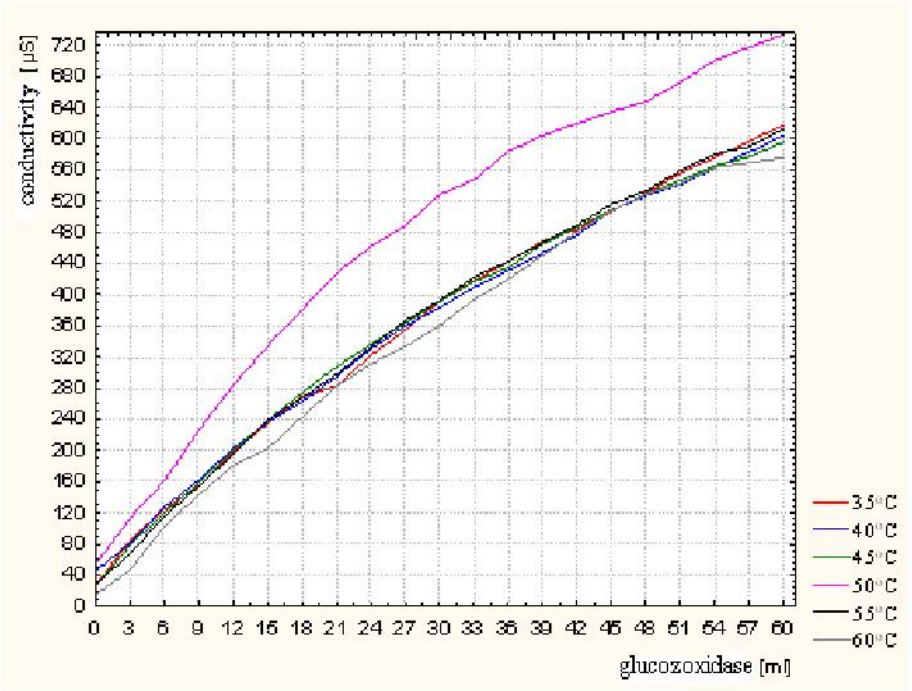

Figure 5. Variation of the electrolyte solution conductivity of glucose (10%) according to the different volumes of 0.4% glucose oxidase concentration and different working temperatures.

For completion of the conclusions it is necessary to be determined whether glucose oxidase has sufficient enzyme activity outside of the optimum recommended by the manufacturer. In this sense was studied conductivity evolution versus temperature at various concentrations for the two glucose concentrations . For the experimental data

were rearranged to match the graphics that have the abscissa temperature distribution resulting in graphics Figure 6.

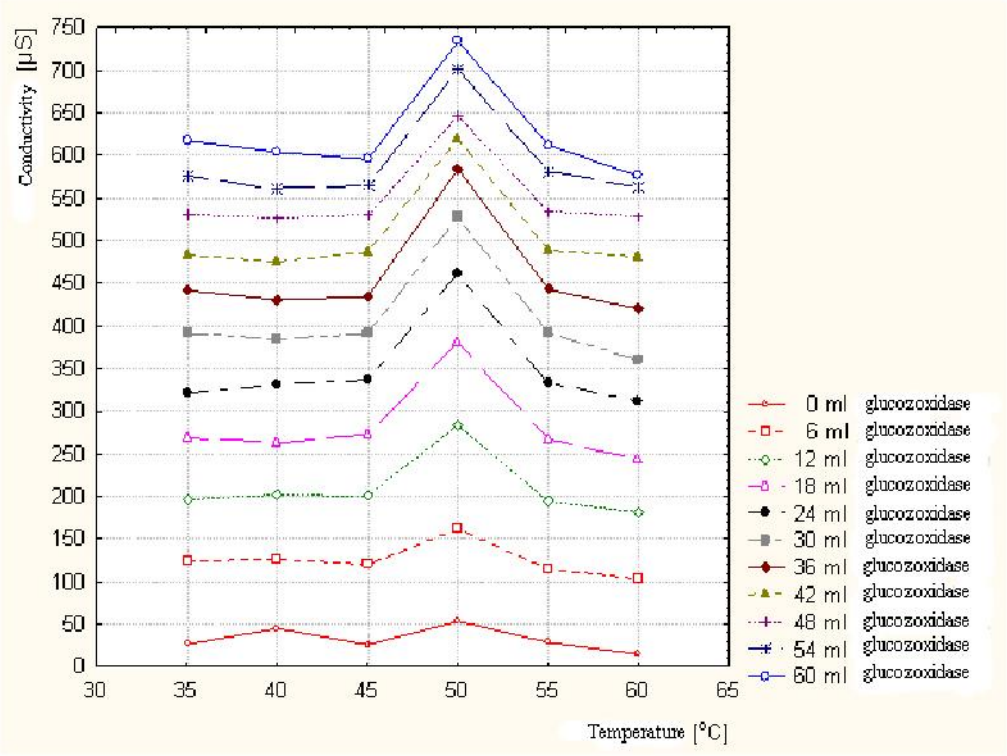

Figure 6. Variation of the electrolyte solution conductivity of glucose (10%) in the temperature of working at different concentrations of glucose oxidase concentration 1%.

The study compared the results of Figure 6 shows an insignificant influence of operating temperature on the conductivity of hydrogen peroxide the only significant variations in temperature are at 50 ^{0}C and the optimum working of the manufacturer indicated glucose [9] to use it in different processes. Taking into account how consumption and less glucose in the interpretation of results as Figure 6 optimum temperature conditions are at 35 ^{0}C and added 30 ml of 1% glucose oxidase concentration in a volume of 100 ml, 10 % glucose concentration. It is obvious that the conditions put up a biosensor based on this principle having such measuring cell volume of 1 ml is needed volume of 0.3 ml of 1% for glucose oxidase concentration measurement, which means 0.003 g glucose oxidase mass insignificant in terms of the cost of this enzymes.

Conclusions

Experimental results shown that the use of electrolytic conductivity values of hydrogen peroxide resulted as a product of reaction of catalytic oxidation reaction of glucose with the enzyme glucose oxidase is an expression of the concentration of glucose and by extension the method can be used in other products that used oxidation reaction by oxidase type enzymes. Projected for the design and the method of conductometric biosensors can draw the following conclusions:

- conductometric method have read resolutions better than amperometric method

- method using the AC conductivity is eliminated the influence of mass transport on the accuracy and selectivity of the method of measurement
- measurement of conductivity can be achieved automatically compensate for temperature changes
-value of conductivity at temperature 35^0C and the volume of 6 ml of glucose oxidase ensures a good reading resolutions and a sufficiently high precision method
- cost of an electronic conducttomertic system do not exceed that amperometric one.

Low price of conductometric and amperometric systems makes possible the design and production of biosensors including in a unitary system both detectors.

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