RESEARCH AND ACHIEVEMENTS FOR NEW ELECTROCHEMICAL BIOSENSORS

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Abstract. *The paper presents results and research of a team involved in instrumental analysis from Faculty of Food Engineering of Suceava University in biosensors field, for food, health and environment issues. Starting from previous achievements were developed electrochemical performance biosensors, extensive use, for analysis both in situ as well as in the laboratory, mainly in pursuing the universal use of such equipment in all reactions using as catalyst type oxidase enzymes. Another aim was to increase sensitivity of measurement and accuracy of such equipment, also by proposed solutions were removed single-use kits, commonly used in the realization of biosensors by using watertight vials containing oxidase which is enough for hundreds of tests. Combined biosensor described in this paper has the great advantage of using in the amperometric and conductometric methods only their advantages since the two electrochemical methods are complementary, also the dosing system of oxidazes is simple and accurate ensuring a good reproducibility of experimental data. By the avant-garde research and achievements of the team itis opened the way for development of new types of biosensors.*

Keywords: *amperometric and conductometric biosensor, universal biosensor, dual biosensor*

Introduction.

Biosensors are biological-selective electronic integrated systems, consisting of a biological active receiver, a transducer and an electronic amplification, processing and display data. Biological active receptor provides specific analytical information enabling recognition of certain biological or chemical species in a complex mixture that consists matter under review, followed by quantitative or semi-quantitative determination of it.

Following the interference of biological active receptor and analytes resulted changes of raport between reactants and products of the reaction that causes in turn, depending on active biological used systems, proportional evolution of physico-chemical measurements values such as: electric charge, absorption / photon emission, temperature, refractive index, layer thickness, values that are converted by transducers type amperometric, pH meters, photometers, thermal, refractive, piezo oscillator, surface plasmon resonance, in proportional electrical signals to the concentration of the species sought in the matter under review.

Basic applications for biosensors are in medicine, environmental quality control issue, pharmaceutical industry and cosmetics, food industry and in biotechnology. Experimental measurement with biosensors are simple and require no specialized advanced knowledge but only the user browsing of short successive stages in time.

New Methods for achieving electrochemical amperometric biosensors

Amperometric biosensors are used for the detection of reaction products or metabolites that oxidizes or shall slightly reduces. Amperometric method consists in measuring the electrical current of a miniature electrolysis cell electrolysis (this is a biosensors system), fed at a constant voltage.

According to Faraday's law, a mass m of a chemical species discharged from an electrode of an electrochemical cell at a time t is proportional to current I of electrolysis and with electrochemical constant k:

$$m = k \cdot I \cdot t \qquad (1)$$

Currently, one of the most popular applications in biosensors amperometric detection is a portable glucose biosensors used to determine in situ blood sugar for diabetics and athletes. Glucose sensor has as an active biological material a glucosoxidase enzyme used as biocatalysts in oxidation reaction of glucose with oxygen in the air with obtaining gluconolactone and hydrogen peroxide (H_2O_2 -hydrogen peroxide) as products of reaction. Biologically active receptor for rapid analysis of blood is in the form of single-use plastic strips that has at one end two miniature plate electrodes between which is deposited glucoseoxidase in a form of dry gel that containes a conductive polymer and at the other end the strip has electrical pine connection with the electronic system. When submitting a drop of blood on the area covered by the glucoseoxidase the folowing reaction occurs:

$$\beta - D - Glu\cos e + O_2 \xrightarrow{Glucozoxidase} \Delta - Gluconolactone + H_2O_2 \qquad (2)$$

Hydrogen peroxide is highly reactive and breaks down at the electrodes of electrochemical cell, current intensity of electrolysis is proportional to the amount decomposed and proportional to the amount of glucose in analysed blood .

Such biosensors systems can be used for determining concentration of phenols, polyphenols, flavonoids and for the development of substances with antioxidant activity [3], [4]

Below are listed some typical examples of biosensors that use different enzymes from oxidase type, as a catalyst, the result being also hydrogen peroxide obtaining:

$$Colesterol + O_2 \xrightarrow{Colesteroloxidase} Colestenone + H_2O_2 \qquad (3)$$

$$Glutamate + O_2 \xrightarrow{D-Glutamatoxidase} Oxiglutarate + NH_3 + H_2O_2 \qquad (4)$$

$$Lactate + O_2 \xrightarrow{Lactatoxidase} Piruvat + H_2O_2 \qquad (5)$$

$$Piruvat + HPO_4 \xrightarrow{Piruvatoxidase} Acetilfosfate + CO_2 + H_2O_2 \qquad (6)$$

The concerns of analytical instrumental group from the Faculty of Food Engineering of Suceava University focused on improving the amperometric

biosensors. Research focused on the following directions:

1. achievement a conductometric biosensor as a superior alternative to amperometric biosensors
2. design and implementation a conductivity-amperometric biosensor combined with concurrent analysis by both methods, biosensor that can be used in reactions 1-6 using oxidase enzyme as catalyst
3. extending limits of concentrations of a new type of biosensors from maximum 500mg/dl, as they are encountered in blood, to limit of 20% glucose, the latter concentration being specific to analytical food, particular applications in fermentative processes
4. elimination of chemical kit for single use from typical biosensors

Research have focused initially only on glucose biosensors [1], [2], [4], [6] and were mainly intended to find an alternative to low reproducibility of typical portable biosensors used to determine blood glucose

This low reproducibility is due to electrolysis phenomenology, electrolysis current, so the indication of glucometer, depending on the addition of hydrogen peroxide concentration and other parameters relating primarily to ionic transport phenomena that lead to advanced electric polarization at electrodes that alter current yield.

Very promising results obtained in experimental research [1], [2] have confirmed that using conductometric method with frequency of 5 kHz eliminates the phenomenon of polarization and also allows determination both of glucose low concentration, as they are present in blood, as well as the concentration at high levels up to 20% , specific to analitical food. Using conductometric method presents also a disadvantage, namely that the value of conductivity is an adder for all electrolytical species of blood.

In this issue is shown that using amperometric method, in which electrolysis current is also adder, enables reducing the error by limiting electrolysis voltage of hydrogen peroxide at the Nernst voltage.

To combine the advantages of both methods and to eliminate the disadvantages of each of them the team has developed a combined biosensor type conductometric - amperometric [3], [4] for the clinical laboratory and analytical food

In addition to those mentioned this biosensor has a great advantage of not working with expensive kits of single-use but with glucoseoxidase and pure liquid colesteroloxidase which is precisely dosed[5], [6] using the equipment designed by collective

Starting from the results of theoretical and applied research has been developed a portable dual biosensor [7], [8] that allows concurrent determination of both glucose and cholesterol from a single drop of blood, but it is not the subject of this paper.

Contributions to conception, design and implementation of combined electrochemical biosensor with superior performance

Research team has designed and developed a prototype of enzyme biosensor that uses oxidase type enzymes as a catalyst in liquid form, biosensors being capable of rapid determinations and in situ of chemical or biological concentration of species without using of single-use biological kits [4], [4]. The advantages of using this measurement system are:

- obtaining an universal and reliable biosensor a long-term usable in all types of reactions catalyzed by enzymes type oxidase
- eliminating single-use kits and thereby significantly lowering of cost price of the analysis
- combined use of both amperometric

and conductometric method in the same determinations, it ensure a high a precision of measurements

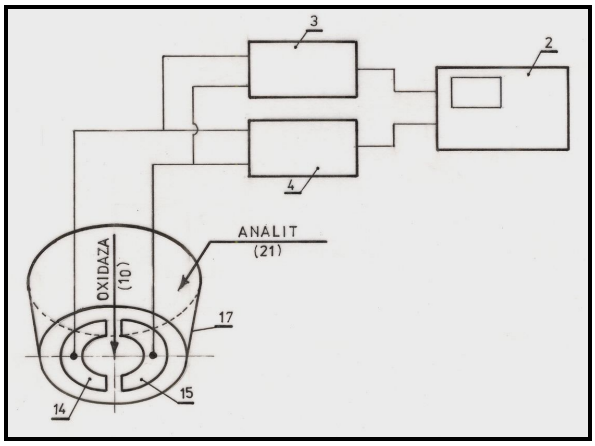

Fig.1.Amperometric and conductometric scheme of measurement principle with enzyme biosensor. 2- acquisition electronics unit, data processing and display, 3-amperometric electronic unit, 4- conductometric electronic unit, 10- silicone hose, 14,15-platinum electrodes, 17-cup, 21- pipette or manually electronic unit.

In Figures 1.3 are represented elements of principle, construction and function of combined biosensors [4].

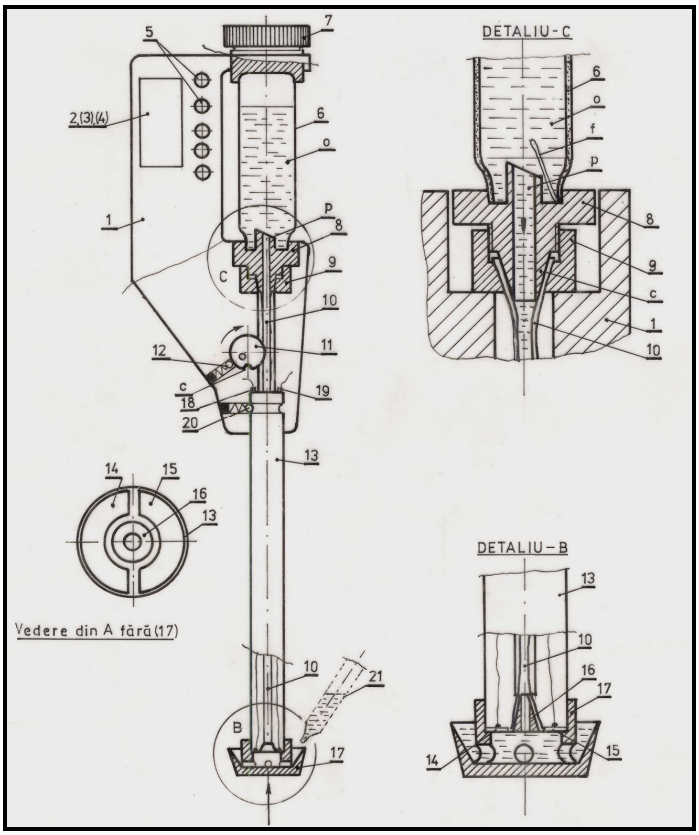

Fig.2.Sectional view of enzyme biosensors. 1-body 2- electronics unit of acquisition, data processing and display, 3-electronic amperometric unit, 4-conductometric electronic unit, 5-keyboard, 6- plastic capsule, o- oxidase solution, 7-bolt, 8-marker, p-drill, f-strip, c-con, 9-nut, 10-silicone hose, 11- supported eccentric cylinder l-conical seat, 12- ratchet, 13-bar 14.15-platinum electrodes, 16-con, 17-cup, 18.19- electric contacts, e-slot 20-ratchet ball, 21- pipette or manually hopper.

The procedure to work with biosensors is the folowing: it is maximum screwed up 7 cav screw then returned device to 180^0 over normal working position, place the cap with oxidase in cavity of the screw 7 and after it is screwed by hand until it is sensitive mechanical strength which means that strip of capsule 6 was pierced by the knife p of the marker 8 and capsule neck 6 sealing made with the marker 8, then returned again biosensors in working position and screw up removable cup 17 on rod 13, it is running several full rotations to the right of the cylinder 11 eccentric supported until on the perforated cone 16 it is a drop of oxidase

After these operations the cup 17 is screwed and it is executed a full rotation of eccentric cylinder to dosing, by peristaltic system, of required volume for oxidase in cup 17, with the stop of cylinder 11 eccentric supported on full shuttered position of silicone hose, position indicated by arrow signs on the click swing button but also by the ball's ratchet 12.

It follows the dosage in cup 17 of the prescribed volume for examined species by a dosing pipette 21 or manually hoppers after the device is keeped upright and digital display is intended. Triggering of catalyzed reaction lead to the emergence of first quantities of hydrogen peroxide, a threshold of its concentration causes initiation amperometric and conductometric measurements, these are alternatively carrying by an automatic electronic switching.

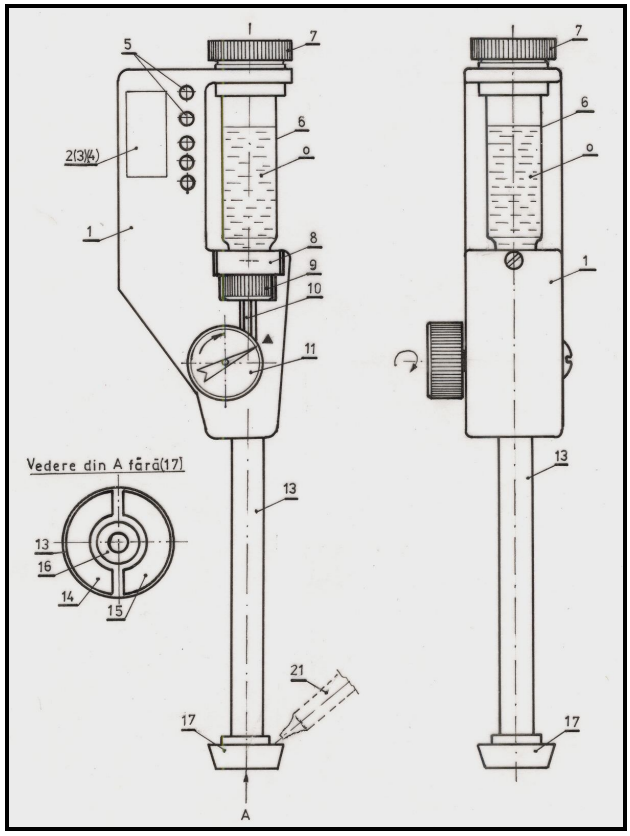

Fig.3.Exterior front and side of enzyme biosensors, 1-body, 2- electronic unit acquisition, data processing and display, 3-amperometric electronic unit, 4- conductometric electronic unit, 5-keyboard, 6- plastic capsules, o- oxidase solution, 7-bolt, 8-marker, p-drill, f-strip, c-con, 9-nut, 10-silicone hose, 11-cylinder eccentric supported, l-conical seat, 13-rod 14.15 platinum electrodes, 16-con, 17-cup, 21- pipette or manually hoppers.

After 10 seconds is displayed concentration value resulting from the average of dozens of amperometric and conductometric measurements statistically processed by biosensors microprocessor.
The volume of oxidase capsule reaches hundreds of determinations, between two determinations with the same oxidase is not required than washing under running water of cup 17. On oxidase changing the whole route is washed using for this purpose a capsule with double distilled water which is fixed, as already was described, on the body 1 of biosensors and run more full rotation of cylinder 11 supported eccentric supported for pumping water through the circuit. In some situations it is necessary to replace the silicone hose 10, these cases occur in specific applications where the volume of dosing oxidase is different from the previous application. This requires replacement of silicone hose with another with an greater inside diameter, that smaller, the operations arising in a few seconds. The replacement of silicone hose 10 is required and its wastage in compression zone-stretching as a result of numerous pumping of eccentric supported cylinder 11. At hose replacement it unwind the nut on the opposite side of the actuating button of cylinder 11, after which it is extracted from the body 1 of biosensors, it follows the return with 180^0 of the device from the working position, is unscrew the screw 7, extract of capsule 6 and marker 8 from its slot, full unscrew of nut 9, extraction of silicone hose on the marker 8, followed by extraction of the con 16 from rod 13, retrieval of hose 10 from cone 16, at the mounting of new siliconic hose operations are repeated in reverse of of the dismantling of 10 silicone hose.

Conclusions

Using both amperometric and conductometric principle it made possible the achievement of combined performance biosensor that combines the advantages of both electrochemical methods of measurement.
Devising a simple and accurate dosing system for catalytic enzymes type oxidase and its implementation on the biosensor allowed elimination of single-use kits that determine the decisive price of analysis. At the same time other design and construction solutions embedded in the architecture of biosensors allow its universal use in all catalyzed reactions by oxidase
Theoretical and applied research carried out allowed the design of new biosensors one being a dual portable biosensor that allows co-determination both of glucose and cholesterol in blood using for this purpose a single drop of blood. This type of biosensor is not the subject of this work, research and prototype implementation are still pending.

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