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DEVELOPMENT OF ORGANIC PHASE BIOSENSORS FOR ANALYSING FOOD SAMPLES

PhD thesis

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The use of enzymes in organic solvents goes back to 1913 (Laane et al., 1987). An active area of current research in biotechnology is biocatalysis in solvents with much lower polarity than that of water. Many enzymes in their natural form bind to nonpolar cells or membranes; water or other highly polarized environments are often unfavorable for the biocatalyst, leading to decreased activity, specifity and stability. Research of the last decades proved that enzymatic activity was higher in hydrophobic solvents than in hydrophilic ones (Kazandjian, Klibanov, 1985, Klibanov, 1986).

Our research at the Analytical Department of the Central Food Research Institute has focused on developing enzyme based biosensors for the last 15 years. In the course of this research, we developed methods for measuring glucose in fruit juices, maltose and galactose in fermentation broth, lactose in milk products, alcohol from beer samples and the rate of D- and L- amino acids in foods. We developed an enzyme based thin-layer cell using one or two enzymes, and measured the hydrogen peroxide produced during the enzymatic reaction by amperometric detector. On the basis of this experience, we aimed to widen the use of biosensors for organic phase applications.

The objective of our research was to develop organic phase enzyme based biosensors applied in an FIA system. We developed methods for determining glucose, hydrogen peroxide and cholesterol. We investigated the conditions of enzyme activity, and optimized the chemical and biochemical parameters of the biosensor. The methods were applied to the analysis of different food samples, and simple sample pre-treatment was developed.

During the development of glucose measuring system

- It was found, by studying different organic solvents, that the highest signals could be obtained with acetonitrile and 2-propanol; so we used these solvents for further measurements.
- We studied the effect of conducting salts as ferrocene monocarboxil acid (FMCA) and tetra-butyl-ammonium-4-sulphonate (TBATS) on glucose measurement by adding to the organic solvents 6% v/v acetate buffer (pH 5). The concentration of the organic salts was optimised on the base of the amperometric signal.
- The effect of percentage volume and pH value of buffer in the carrier solution was studied.
- The optimal flow rate was found at 0.8 mL min^{-1}.
- After optimising measurement parameters, the linear measuring range, the statistical parameters of the calibration curves, and the repeatability of glucose standard were compared.
- We determined glucose content in food samples such as mayonnaises, salad dressings, mustards, sauces. Glucose concentration of the samples was measured and compared with results obtained by the reference UV-photometric method performed in water phase. The correlation between the results measured by the two methods was very good with correlation coefficient (r) as high as 0.976.

During the development of hydrogen peroxide measuring system

- A stopped-flow type injection analyser system was developed in order to ensure that samples spend the time

required to complete the enzymatic reaction in the enzyme cell. Investigating the reaction rate of the enzyme, after two minutes the reaction was completed and the signal became stable when acetonitrile with conducting salts (5% buffer) was used.

- The results obtained by immobilizing the catalase enzyme with and without polyethylene glycol (PEG) 6000 were compared by preparing different enzyme cells.
- The effect of conducting salts as FMCA and TBATS in the organic solvents was studied. The concentration of the organic salts was optimised on the base of the amperometric signal and on the activity of the enzyme.
- The effect of percentage volume and pH value of buffer was studied in the carrier solution. A significant difference was found between immobilizing enzyme with PEG 6000 and without it.
- The signals were the highest when the enzyme was immobilized without any PEG. However, when using the enzyme cell containing PEG, the relative standard deviation became much smaller than in the other case. For measuring hydrogen peroxide content the enzyme immobilized together with PEG showed reliable and repeatable results, and in addition this enzyme cell could be used for the longest time. For measuring hydrogen peroxide content in oily samples (e.g. cosmetics), the enzyme cell using PEG is highly preferable.
- Using enzyme cells without PEG, we developed an indirect method to determine the water content in various oily food samples by maintaining a fixed substrate concentration, following small changes in water (activator) content. In the indirect measurement, the amperometric signal obtained for the fixed substrate concentration closely followed the water

content of the standard. The optimal measuring range was between 0,05-1 % water content.

- Water content of different sorts of commercial butter, fat and margarine products was measured and compared with results obtained by the gravimetric reference method (AOAC Method 920.116). The correlation between the two methods was very good, with a correlation coefficient (r) of 0.993. The results agreed also with the declared maximal value stated on the products.

During the development of free and total cholesterol measuring system

- We determined the kinetics of cholesterol oxidation as a function of elapsed time as the sample remained in the enzyme cell with only COD immobilized. The increase of the signals showed that after four minutes the reaction rate was sufficiently high to measure the hydrogen peroxide produced.
- The optimal temperature of the enzymatic reaction was found at 28 °C.
- As COD activity is known to be higher in apolaric solvents, Toluene was added to the acetonitrile carrier solution. This had a significant effect on the amperometric signs: the peaks increased significantly when the toluene concentration was changed between 10-40% v/v.
- Effect of adding salts such as FMCA and TBATS to the organic solvents was studied. The concentration of the organic salts was optimised on the basis of the amperometric signal and the activity of the enzyme.
- Using acetonitrile with FMCA and 40% toluene, we observed the stability of the solution when adding different

quantities of acetate buffer in a narrow range. In our measurements 2.4 % buffer was used.

- Since CE and COD were immobilized together in the enzyme cell, the conversion of cholesterol-oleate to cholesterol was studied. During the measurements the conversion rate increased significantly when the toluene concentration was changed between 10-50 %.
- After the biochemical and electrochemical parameters were optimised for cholesterol measurement, the linear concentration range of free and total cholesterol determination and the statistical parameters of the calibration curves were determined. The linear measuring range was between 0.05-0.5 mM for both standards. The correlation coefficients for the regression curve for free and total cholesterol were 0.967 and 0.972, respectively.
- Total cholesterol content in two different egg yolks was measured. The recovery of 0.1 mM cholesteryl oleate standard added to the samples was 86-93%, which showed that the sample preparation was adequate for a quick determination. The total cholesterol content was 1135 and 1310 mg $100g^{-1}$, respectively, the results being in agreement with the data from food composition tables.
- Total cholesterol content of commercially available margarine, lard and butter samples was determined. The results agreed with the data from food composition tables.
- A linear relationship between the total cholesterol content of pasta and the amount of egg content (0, 4, 6, 8) was found. Our results showed that this measurement could be used for quality control of pasta.

New scientific results of the thesis

1. A flow-through measuring apparatus for the determination of glucose content was developed as a model system in organic media; properties of the biosensor were compared in organic and in aqueous solutions. On the basis of these results, I concluded that biosensors using enzymes with covalent immobilization can be used in an organic phase FIA system with the eluent containing the optimised quantity of buffer.
2. A simple stopped-flow system was developed in order to ensure that samples spend the time required to complete the enzymatic reaction in the enzyme cell; different immobilization methods were compared. For measuring hydrogen peroxide content, the enzyme immobilized together with PEG showed reliable and repeatable results, and in addition this enzyme cell could be used for the longest time.
3. An indirect way to determine moisture content in different food samples was also developed, and the results obtained by our method were compared to those of reference.
4. For determining free cholesterol, a cholesterol oxidase containing enzyme cell was developed. The effect of apolar solvent on the activity of the enzyme was studied.
5. For determining total cholesterol, a bienzyme cell containing cholesterol oxidase and cholesterol esterase was developed. The rate of conversion of cholesterol oleate was investigated.
6. The biosensor methods developed were used successfully to measure the chemical composition of different food samples, only partly soluble in water. Easy and rapid methods were developed for food sample pre-treatment.

Most important publications in the last five years related with the thesis

Váradi, M., **Adányi, N**., Szabó, E.E., Trummer N. (1999): Determination of the ratio of D-and L-amino acid oxidase enzyme reactor coupled to amperometric detection, *Biosensors & Bioelectronics* **14** 335-340.

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Adányi, N., Szamos J., Szabó E.E., Váradi M. (1999): Interfacial enzyme partitioning as a tool for constructing biosensors. *Acta Alimentaria* **28** 329-338.

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Adányi, N., Váradi, M. (2003): Development of organic phase amperometric biosensor for measuring cholesterol in food samples. *Int J Food Research* (submitted)

Adányi, N., Szabó, E.E., Tóth-Markus, M., Váradi, M. Sammartino, M.P., Tomassetti, M., Campanella, L. (2003): Investigation of organic-phase amperometric biosensor for measuring glucose in FIA system. *Anal Chim Acta* (submitted)

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Most important publications in the last five years related with other topics

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