Development of Enzyme Amperometric Biosensor Based on Poly(meta-phenylenediamine) Film for Determination of Lactose

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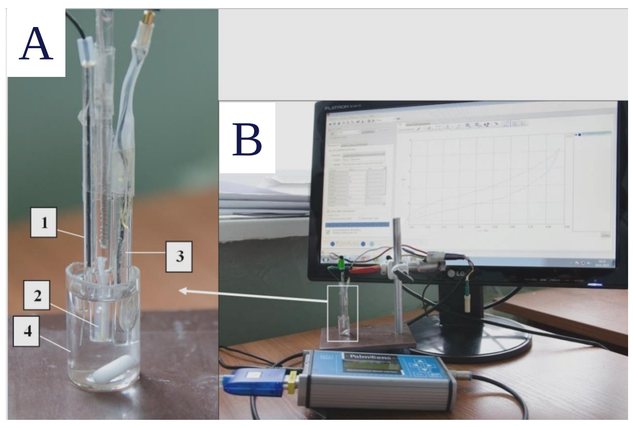
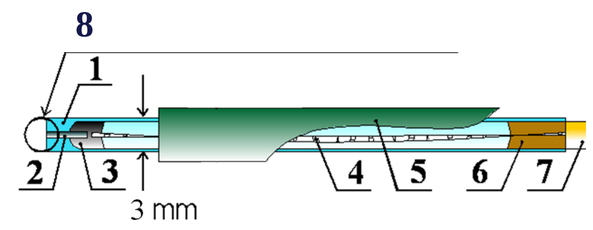
The lactose content of normal cow milk is generally between 4.4 and 5.2%. Express and accurate determination of lactose is very important in process control of cheese whey fermentation and also in membrane separation of lactose from cheese whey. In addition, lactose content is a basic indication for evaluating milk quality and detecting abnormal milk. It has been reported that milk from cows suffering from mastitis shows lower lactose levels. Besides, there is a specific disease called lactose intolerance, which caused by total or partial deficiency of enzyme β-galactosidase in the digestive system. Patients with lactose intolerance are not able to digest lactose from milk, so they should exclude lactose from their diet. Thus, the quantitative control of lactose level is needed in production of lactose-free milk.

As a rule, conventional methods for lactose measurement are laborious, time-consuming, expensive and require highly qualified technicians. Among innovative technologies proposed, biosensors are promising alternative to conventional analytical methods. The aim of the current work was to develop rapid, sensitive and selective lactose biosensor based on improved semipermeable polyphenylene diamine film for determination of lactose in milk and dairy products.



(A) Amperometric transducer:
1 – counter electrode,
2 – reference electrode (Ag/AgCl in saturated KCl),
3 – working electrode,
4 – cell with 2 ml of buffer solution
(B) PalmSens potentiostat connected with amperometric transducer and PC.



Structure of working electrode based on platinum wire:
1 – Glass capsule (d=3,5mm);
2 – Platinum wire (d=0,4mm, l=3 mm);
3 – Wood's metal;
4 – Silver wire;
5 – Protective cover;
6 – Epoxy resin;
7 – Contact pad.

Principle of lactose biosensor functioning

β-Galactosidase

α-D-Lactose + H_2O → β-D-Galactose + α-D-Glucose (1)

Mutarotase

α-D-Glucose → β-D-Glucose (2)

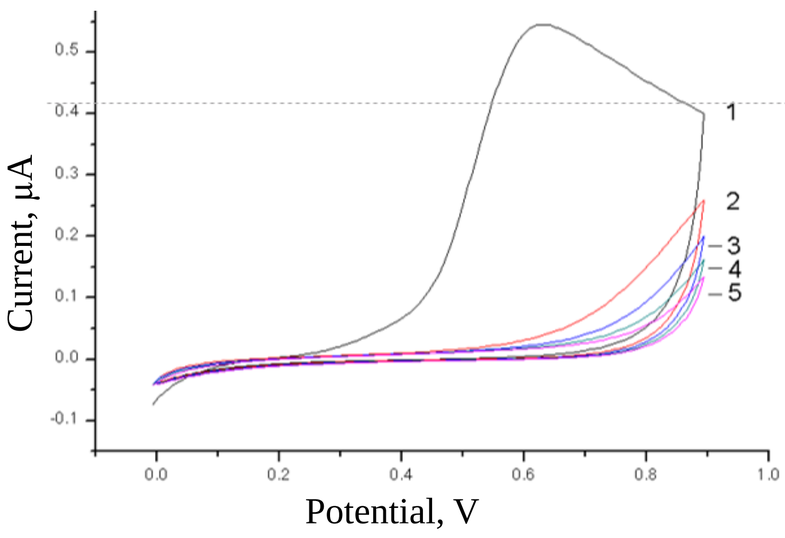
Glucose Oxidase

β-D-Glucose + O_2 → Gluconic acid + H_2O_2 (3)

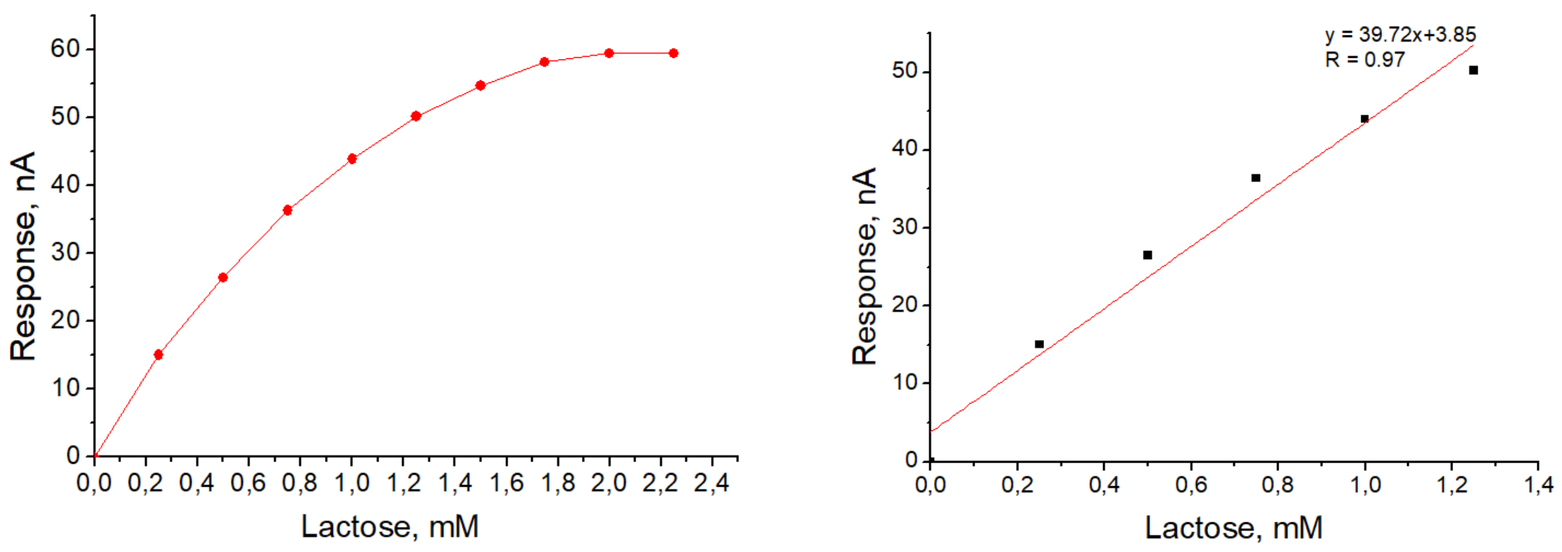
(+0.6 V)

$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$ (4)

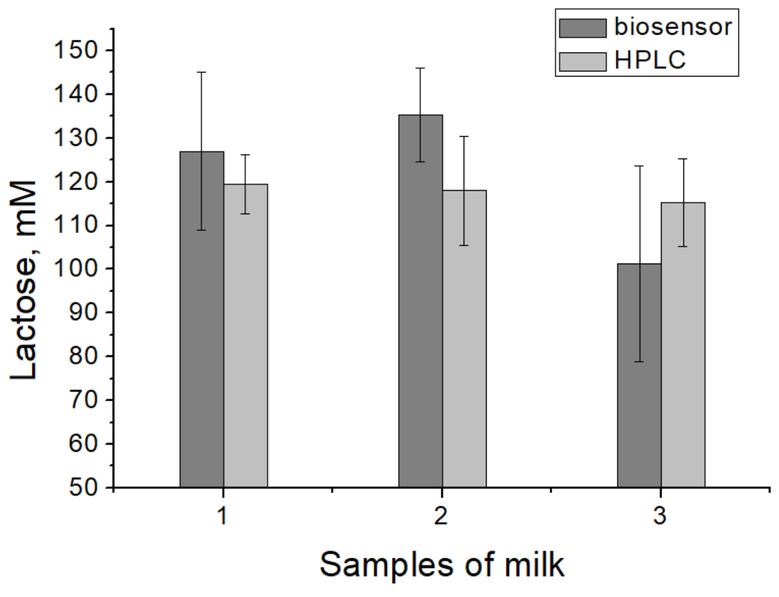
New amperometric enzyme biosensor based on platinum disk electrodes modified by nanosized poly (meta-phenylenediamine) (PmPD) film for determination of lactose was developed in this work. The m-PD was used to form a semipermeable film on the electrode surface to prevent influence of interfering substances. Due to the presence of certain pores in such film, it has a property of the semipermeable membrane. This membrane is able to pass compounds with low molecular weight (e.g. hydrogen peroxide) and to retain larger molecules (e.g. ascorbic acid). The bioselective membrane of biosensor was obtained by immobilization of three enzymes (β-Galactosidase, Mutarotase and Glucose Oxidase) using glutaraldehyde on the surface of amperometric transducer (platinum electrode modified by PmPD film). To prepare PmPD film m-phenylenediamine was dissolved in 40 mM phosphate buffer, pH 7.4. Cyclic voltammetry was carried out in the same measuring cell without stirring. Start potential was 0 V, final potential +0.9 V, scan rate (rate of potential change) 20 mV/s, and step of potential change 5 mV. The bare working electrodes were immersed in 5 mM m-phenylenediamine solution, afterwards 10–12 cyclic voltammograms were obtained. Next, the enzymes were immobilized onto the PmPD membrane.



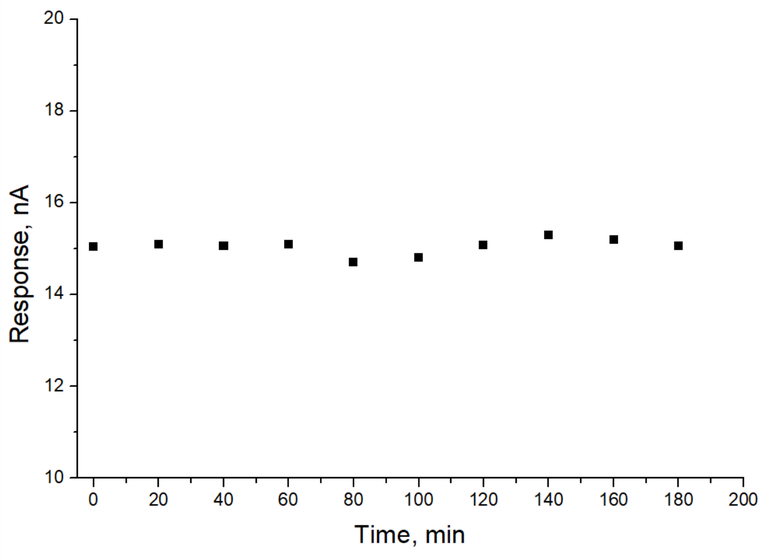
Cyclic voltammogram obtained during the process of m-PD electropolymerization. Measurements were carring out in 10 mM phosphate buffer solution, pH 7.4.



Calibration curves of lactose biosensor. Measurements were carring out in 10 mM phosphate buffer solution, pH 6.5.



Results of lactose determination in milk obtained by biosensor and HPLC



Reproducibility of biosensor response to 0.25 mM lactose.

Selectivity of lactose biosensor with PmPD coating

Analyzed substance, Concentration, mM	Relative response, %
Lactose 0.25 mM	100
Glucose 0.25 mM	242.35
Lysine-HCl 0.125 mM	0
L-Arginine 0.125 mM	0
Histidine-HCl 0.125 mM	0
Ascorbic acid 0.125 mM	1.12
Citric Acid 0.25 mM	0
Sucrose 0.25 mM	0.71
Fructose 0.25 mM	0
Arabinose 0.25 mM	0
Galactose 0.25 mM	3.53
Sorbitol 0.25 mM	0

Conclusion

New amperometric enzyme biosensor for determination of lactose was developed. The linear range of lactose determination was from 0.005 mM to 1.25 mM. The time of lactose determination was around 30 seconds. The biosensor showed high sensitivity, operational stability, selectivity and signal reproducibility. The developed biosensor was applied for lactose determination in milk samples. It was shown high correlation between results obtained with lactose biosensor and HPLC. Thus, the developed enzyme biosensor based on nanosized PmPD film is suited for rapid, inexpensive, sensitive, selective and simple determination of lactose in milk samples.