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Development of a New Amperometric Biosensor for Lactose Determination

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Abstract

A new amperometric biosensor for lactose determinaion in raw milk was developed through the simultaneous immobilization of β-galactosidase and galactose oxidase on a derivatised polyethersulphone membrane. β-galactosidase catalyses the hydrolysis of lactose into galactose and glucose and galactose oxidase catalyses the oxidation of galactose into galactonic acid and H_2O_2. The membranes with the two immobilized enzymes were then used in an amperometric sensor, by oxidation of the H_2O_2 formed, at a Pt electrode of an Universal Sensors electrode base system. The sensitivity and the reproducibility of the biosensor thus formed were found to be 6.81 and 0.72 $nA.M^{-1}$, respectively. Biosensors were found to be stable for 20 days.

Keywords: biosensor, lactose, amperometric, stabilization, β-galactosidase, galactose oxidase.

Introduction

The need to quantify lactose, a disaccharide formed by glucose and galactose, came up with the knowledge of its importance in the human diet. Besides, its quantification in food is vital for people who are intolerant to this carbohydrate.

Various methods are available for the determination of lactose. These include polarimetry, gravimetry, spectrophotometry, chromatography (HPLC and GLC) [1-4]. The majority of these methods is tedious and time consuming, and may require expensive equipments in addition to considerable technical skills.

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Therefore, it is of great importance to get a rapid and reliable method of quantifying lactose in food. Biosensors are promising tools in that respect since they combine the specificity of the biological reconnaissance of relevant analytes with the versatility of the transducers. Moreover, a good biosensor will require fewer operations than conventional methods.

Lactose biosensors based on immobilized enzymes with electrochemical detection have been used before [5-12]. Most of them use the enzymes β-galactosidase (GAL), which catalyses the dissociation of lactose into galactose and glucose (Fig. 1), and glucose oxidase (GOD), which catalyses the oxidation of glucose into gluconolactone and hydrogen peroxide (which can be detected electrochemically). The detection of lactose based on glucose, a carbohydrate also present in milk and its derivates, can cause some problems of interference. Others use the enzyme galactose oxidase (GAD), which catalyses the oxidation of galactose (which is also present in milk in small concentrations) into galactonic acid and hydrogen peroxide (Fig. 2), instead of GOD. Most of the immobilization procedures reported in the literature need several steps for the enzyme immobilization [6] or immobilize the enzymes directly on the electrodes, what makes difficult their reutilization [8-13].

In the present work, a biosensor with the β-galactosidase and galactose oxidase immobilized directly on a derivatized polyethersulphone membrane is developed, based on a method described by Climent et al., 2001 [14]. The biosensor sensitivity and reproducibility were analysed. Lifetime of the biosensor was also studied.

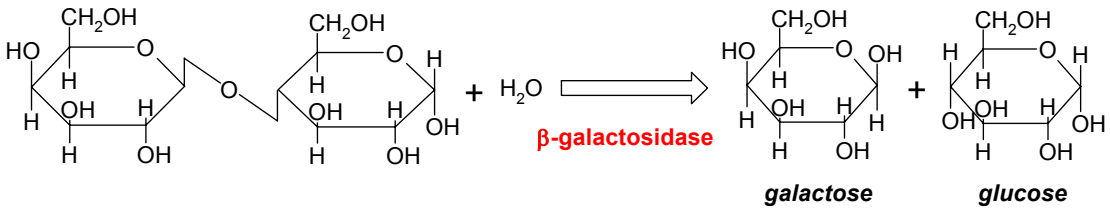

Figure 1. Hydrolysis of lactose catalysed by the enzyme β-galactosidase.

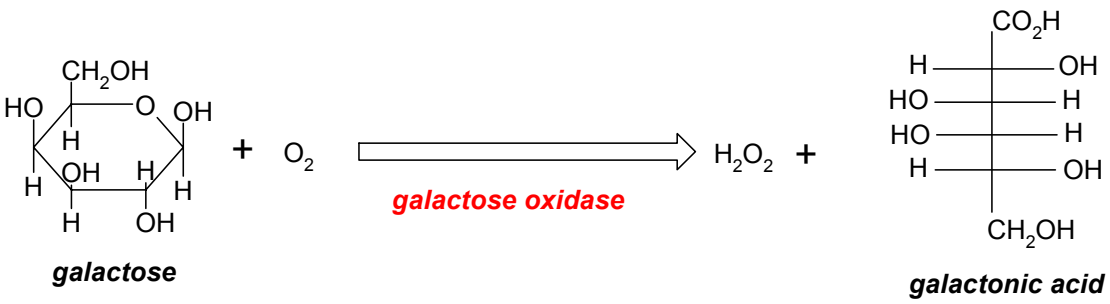

Figure 2. Oxidation of galactose catalysed by the enzyme galactose oxidase.

Experimental

Materials

β-galactosidase (E.C. 3.2.1.23) and galactose oxidase (E.C. 1.1.3.9) were purchased from Fluka (127 U/mg) and Sigma (5920 U/g), respectively. Peroxidase (E.C. 1.11.1.7) was obtained from Fluka (147 U/mg). β-Lactose, orto-nitrofenil-β-D-galactopiranósido (ONPG), orto-nitrofenol (ONP), and Guaiacol were purchased from Sigma.

Polyethersulphone membrane (Ultrabind™, US450, 0.45 μm) for the enzyme immobilization was obtained from Gelman Sciences, PALL. Discs with 18 mm of diameter were used.

The biosensor was composed of platinum electrode and an Ag/AgCl anode electrode from Universal Sensors, Model 4006. The amperometric measurements were carried out using an amperometric biosensor detector - ABD - from Universal Sensors, Model 3001. The measurements were recorded on a recorder from Pharmacia Biotech, Mode REC 102.

Methods

β-galactosidase and galactose oxidase immobilization

A solution containing 6.7 μg/μL of each enzyme dissolved together in phosphate buffer (0.1 M , pH 7.0) was prepared. Thirty μL of this solution were deposited on the polyethersulphone membrane surface and the enzymes were allowed to

react during 1 hour at 22 ºC, in order to assure an efficient immobilization. The membranes were then ready to use.

Preparation of the biosensor

The membrane side containing the immobilized enzymes was applied on the internal membrane of the electrode jacket in contact with the platinum electrode and secured with an O-ring. The electrode jacket was filled with physiological phosphate buffer. The reference electrode was an helix of Ag/AgCl which was housed in the same stem as the Pt electrode. The biosensor, thus prepared, was then dipped in 5 mL of phosphate buffer saline (PBS).

A voltage of +700 mV (*vs.* Ag/AgCl) was applied from an amperometric biosensor detector. The background current was allowed to reach a steady value, and 100 μL of the substrate (lactose) were added to the solution, under stirring (100-300 rpm). The transient current-time was registered until the system was stable again. The variation of the current was calculated, and this variation is proportional to the substrate concentration.

Sensitivity and reproducibility studies

In the study of the sensitivity of response, the same procedure described before was done repeating the addition of 100 μL of the substrate (0.292 mol dm^{-3}; 100 mg lactose/mL) to the solution after stabilization of the current response. This procedure was repeated three times to analyze the reproducibility of the method.

Enzymatic stabilization studies

Stabilization was studied under the following conditions: membranes with enzymatic system immobilized were stored dry at room temperature and at 4 ºC, in glycerol 30 % (v/v) at room temperature, and in PBS at room temperature, following the procedure described before in the "*Amperometric Detection*"(addition of 100 μL of lactose solution 0.292 mol dm^{-3}).

Results and discussion

Sensitivity and reproducibility studies

The relationship between the current intensity and lactose concentration is shown on Fig. 3. It can be seen that the biosensor response is proportional to the substrate concentration. The sensitivity and the reproducibility of the biosensor can be obtained through the study of the Figure. The linear range for the biosensor response to lactose was verified to be between 0.0043 and 0.031 mol dm^{-3}. This linear range is similar to the one reported in the literature [6,7,11-13]. Sensitivity and reproducibility, were, respectively, 6.81 and 0.72 nA.mol dm^{-3}. This value of sensitivity is superior to the one found in the literature [8,11].

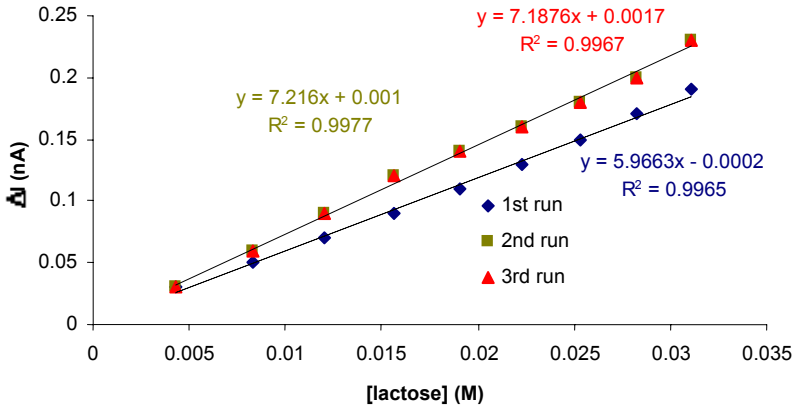

Figure 3. Current response of the biosensor to the substrate concentration (22 ºC).

Enzymatic stabilization studies

To analyse the effect of the immobilization on the enzyme stability, several membranes with the enzymatic system were stored under different conditions during 20 days. At different time intervals the membranes were used to determine the remaining activity of the biosensor, for lactose detection. The activity observed was compared with the activity presented immediately after the membrane was prepared (activity at time zero). The results obtained are shown in Fig. 4. From Fig. 4 it can be seen that the best condition to store the membranes is to leave them dry, at 4 ºC. Under this condition, it is also noted an increase in the system activity during the first day. With this immobilization procedure the

stability of the enzymes used in this biosensor is increased, which is an advantage for the characteristics of this biosensor for lactose detection.

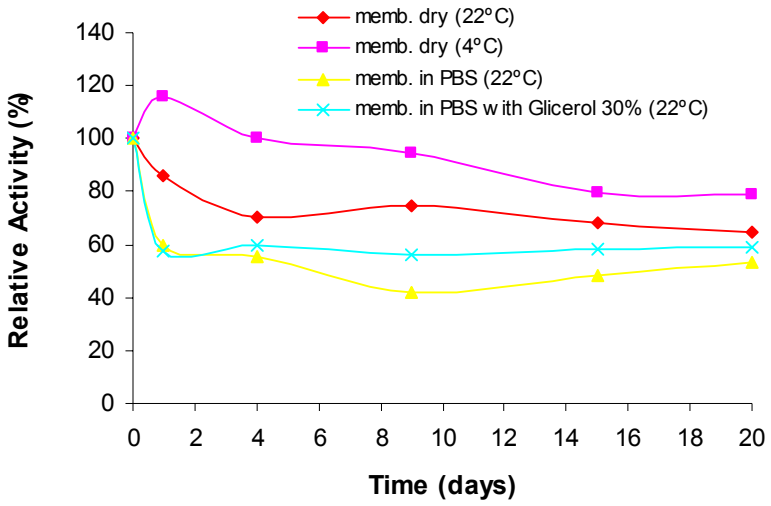

Figure 4. Relative activity of the biosensor as a function of time (days) for membranes stored dry at 4 ºC, dry at room temperature, in PBS and in PBS with glycerol 30% at room temperature (22 ºC).

Conclusions

The enzymatic system studied was effective in detecting lactose.

The method of enzyme immobilization was very rapid and easy. The biosensor sensitivity and reproducibility were 6.81 and 0.72 nA.mol dm^{-3}, respectively. This value of the sensitivity is superior to that reported by other authors [2] e [3]. The membranes showed an enzymatic stability for a period of 20 days, when stored at 4 ºC.

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