

Biosensors for determination of lactose by immobilized beta-galactosidase: A Review

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***Biosensors for determination of lactose by immobilized beta-galactosidase. A Review:** The increasing demand for on-line measurement of milk composition directs science and industry to search for practical solutions, and biosensors may be a possibility. According to this fact, it is important to have a method that is fast, simple, and specific for lactose. The specific objective of this work was to compare literature sources about biosensors, commonly used for determination of lactose, to show pros and cons of these methods, for practical using.*

Key words: Biosensor, β -Galactosidase; Immobilization; Lactose hydrolysis;

INTRODUCTION

Although the canaries used in coal mines could qualify as the first biosensors, the area of biosensors research started in the year 1962 with the development of enzyme electrodes by L.C. Clark. Since then, scientists from the fields of physics, chemistry, biochemistry, molecular biology and material science have contributed to this multidisciplinary field developing more reliable and robust biosensing devices for applications in the fields of medical/clinical analysis [20, 34], veterinary [39], agriculture [37], food quality [32], environmental analysis [27], and bioterrorism prevention [5]. Biosensors are chemical sensors in which the recognition system utilizes a biochemical mechanism [33]. The coupling between the biorecognition molecule and the transducer is often a critical step in biosensor development and can be performed by membrane entrapment, physical adsorption, matrix entrapment, or covalent binding among others. It is important to maintain the biorecognition capacity during such procedures while at the same time guarantee the robustness and reproducibility of the sensor.

The past few years have seen a dramatic change in biosensor technology [22]. This change can be attributed to the convergence of three different strands of research:

- a. Researchers have focused their work on producing sensors or related technologies that meet the needs of industrial and governmental analysis;
- b. The rapid advancement in the field of molecular biology has helped create a new generation of biosensors. The biocomponents can be optimized, on the molecular level, to be more rugged, efficient, thermally stable, cheaper or more rapid to produce;
- c. Continued development on how to immobilise biocomponents, while maintaining optimal activity.

These three strands are explored in terms of their effects on the underlying technology used in biosensors.

In this review, the work is concentrated on the some types of biosensors which can be used in some biotechnological process especially in the dairy industry for determination of lactose by immobilized beta-galactosidase. It also explores the possibility of taking advantage of molecular biology to create generically biorecognition elements that can readily transduce their biomolecular interactions in electrochemical signals.

DISCUSSION

Enzymes are commonly used in biosensors because of their high specificity. Biosensor applications require a highly active immobilized enzyme system that allows the maintenance of an efficient connection between the sensing molecule and the transduction component of the biosensor. Because of their moderate stability, many alternative strategies to immobilize and stabilize enzymes have been explored to improve the feasibility and applicability of a wide range of biosensor applications. Such strategies include covalent immobilization, physical adsorption, cross-linking, encapsulation, or entrapment.

Application of beta-galactosidase for determination of lactose in dairy food

β Galactosidases have been obtained from microorganisms such as fungi, bacteria and yeasts; plants, animals cells, and from recombinant sources. The enzyme has two main applications; the removal of lactose from milk products for lactose intolerant people and the production of galactosylated products. In order to increase their stability, reusability, and use in continuous reactors, these enzymes have been immobilized on both organic and inorganic support via adsorption, covalent attachment, chemical aggregation, microencapsulation, and entrapment. Free and immobilized preparations of β galactosidases have been exploited in various applications such as industrial, biotechnological, medical, analytical, and in different other applications. β galactosidase is widely used in food industry to improve sweetness, solubility, flavor, and digestibility of dairy products. Immobilized β -galactosidases are employed for the continuous hydrolysis of lactose from whey and milk in a number of reactors such as hollow fiber reactors, tapered column reactors, packed bed reactors, fluidized bed reactors etc [10].

Recent demands that high quality food products meet customer needs have opened up new improved techniques and sensor technologies that are coupled with production processes for quality control and consumer assurance. Sensor-based systems, have been more often used in food industries [21, 22]. With the sensors fitted within the production process, a nearly instant reading can be achieved. Milk is one of the important bulk consumer products from a dairy industry that need constant assurance of quality. Milk contains lactose and other carbohydrates in small quantities [12].

Both traditional and modern methods of cheese manufacture produce a large amount of cheese whey (about 83% of the entire volume of milk used). Due to the high concentration of organic substances, mainly lactose and proteins (70% and 20% of total solids, respectively), cheese whey causes values of biochemical oxygen demand in wastewater treatment plants to be high, varying from 30,000 to 60,000 mg/L [25]. The biotechnological utilization of this valuable feedstock is largely limited by lactose due to its poor solubility, insufficient sweetness and the problem of lactose intolerance. Hydrolysis of lactose to glucose and galactose by β -galactosidase (commonly known as lactase) would overcome some of these limitations and permit greater usage of whey [31], including enzyme production.

Routine analysis in the food industry and the on-line control of bioprocesses require determination of several compounds in a fast and trustworthy way. Often these measurements are carried out by gas chromatography, high performance liquid chromatography, thin layer chromatography, enzymatic reactions or colorimetric determinations, but these analyses are time-consuming and require off-line procedures. During the past years, the development of flow injection analysis and biosensors [8, 14, 15, 23, 28, 36] have contributed to reducing these difficulties.

Lactose biosensors based immobilized beta-galactosidase

Many alternative strategies to immobilize and stabilize enzymes have been investigated in recent years for applications in biosensors. Betancor et al. report a strategy for chemically associating silica nanospheres containing entrapped enzyme to a silicon support. β -galactosidase from *E. coli* was used as a model enzyme due to its versatility as a biosensor for lactose. The immobilization strategy resulted in a three-dimensional network of silica attached directly at the silicon surface, providing a significant increase in surface area and a corresponding 3.5-fold increase in enzyme loading compared to enzyme attached directly at the surface. The maximum activity recovered for a silicon square sample of 0.5 x 0.5 cm was 0.045 IU using the direct attachment of the enzyme through glutaraldehyde and 0.16 IU when using silica nanospheres. The immobilized β -galactosidase prepared by silica deposition was stable and retained more than 80% of its initial activity after 10 days at 24 degrees C. The ability to generate three-dimensional

structures with enhanced loading capacity for biosensing molecules offers the potential to substantially amplify biosensor sensitivity [3].

A microdialysis-coupled flow injection amperometric Sensor (μ FIAS) was used to determine glucose, galactose, and lactose in milk (Fig. 1). The sensor is based on enzymecatalyzed reaction in combination with the three well-established analytical techniques, namely; microdialysis sampling, flow injection analysis (FIA), and amperometric detection. With the multianalyte sensor it was possible to detect glucose and galactose by sequential injection of their corresponding oxidase enzymes: glucose oxidase and galactose oxidase, while lactose was determined by injection of a mixture of beta-galactosidase and glucose oxidase enzymes. The sensor showed a linear response between 0.05 and 10 mM for glucose, between 0.1 and 20 mM for galactose and between 0.2 and 20 mM for lactose, respectively. The relative standard deviation values of the sensor measurements for glucose, galactose, and lactose were 3– 4% ($n = 3$). The sensor measurements for lactose content in milk were compared with a standard method with an infrared spectrophotometer [26].

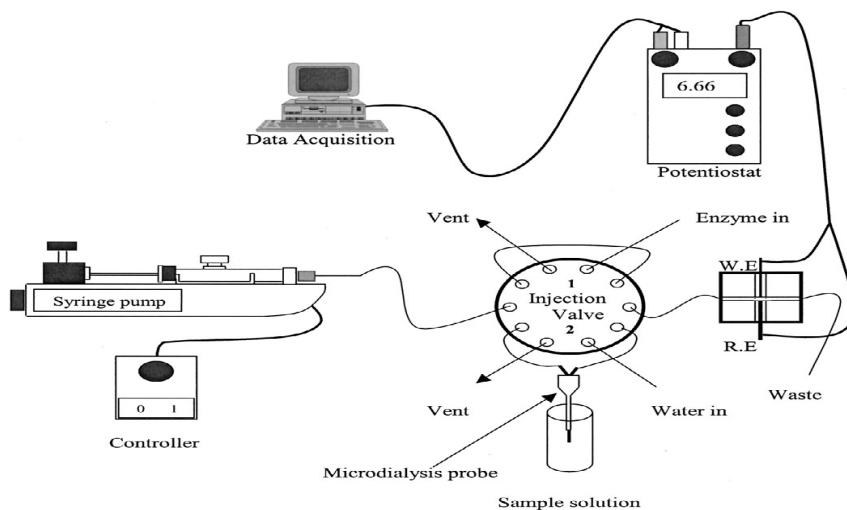


Figure 1. Schematic representation of the microanalysis-coupled flow injection amperometric sensor. W.E is working electrode and R.E is reference electrode in the electrochemical cell. One represents valve position 1 and two represents valve position 2, respectively

It employs two immobilized enzymes, β -galactosidase and glucose oxidase and the quantitative analysis of lactose is based on determination of oxygen consumption in the enzymatic reaction. The influence of temperature on the biosensor signal was experimentally studied. It was observed that a nonlinear relationship exists between the electric response of the biosensor – provided by CAFCA (Computer Assisted Flow Control & Analysis - ANASYSCON, Hannover) - and lactose concentration. In this work, attempts were made to correlate these variables using a simple nonlinear model and multilayered neural networks, with the latter providing the best modeling of the experimental data [7].

An amperometric lactose biosensor was developed by immobilizing lactase (EC 3.2.1.23) and galactose oxidase (GaO) (EC 1.1.3.9) in Langmuir–Blodgett (LB) films of poly(3-hexyl thiophene) (P3HT)/stearic acid (SA) for estimation of lactose in milk and its products to prevent “lactose intolerance”. The enzyme immobilized LB film was used as working electrode and platinum as reference electrode. The enzyme electrodes show a linearity 1–6 g/dL of lactose and have a shelf life more than 120 days. The reusability of electrode was found ten times with 3% loss in current response. The enzyme electrode was characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and kinetic parameters such as pH, temperature and stability. The working electrode may be used for the estimation of lactose/galactose in food and biological fluids [29].

A new amperometric biosensor for lactose determination 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 [17].

According to some resources of literature, hybrid biosensors for the determination of lactose were developed. *Saccharomyces cerevisiae* was entrapped in agar. Lactases (Lactozym and β -galactosidase of *Enterobacter agglomerans*) were adsorbed onto diethylaminoethyl cellulose (DE-52). Catalytic components were confined between the surface of a gas-permeable membrane of a CO_2 electrode and a dialysis membrane. Lactases hydrolysed lactose and *Saccharomyces cerevisiae* fermented glucose and galactose producing CO_2 . This gas was measured by a CO_2 electrode. These biosensors showed Nernstian characteristics. The semilogarithmic concentration range was between 0.5% and 10%. The lifetime of these biosensors was two weeks and two months, respectively. The results were applied to lactose analysis in milk samples. Validation of the assays was made on milk of several dairy brands by comparison with a spectrophotometric method [2].

Also, in literature the scientific team describes the preparation of a new lactose biosensor based on electrode coating with β -galactosidase and glucose oxidase immobilized gelatin. For this purpose, β -galactosidase and glucose oxidase enzymes were immobilized onto gelatin by crosslinking with glutaraldehyde. Lactose biosensors were subjected to continuous repeated use in order to observe reusability and shelf life; where standard lactose and milk samples were used as substrate solutions. Continuous reuse experiments showed that most of the lactose biosensors activities were retained even after the 10th use in a period of 30 days [16].

Mariotti et al. studied the hydrolysis of whey lactose by an immobilized enzyme reactor. β -Galactosidase from *Aspergillus oryzae* was immobilized on silica and activity and stability were evaluated. The best immobilization results were attained by using glutaraldehyde as support's activator and enzyme stabilizer. The optimized enzyme proportion for immobilization was 15-20 mg g⁻¹ of support. Treatments of whey were performed (microfiltration, thermal treatment and ultrafiltration), seeking the elimination of sludge, and the effects on operating the fixed bed reactor were evaluated. Ultrafiltration was the best treatment towards a proper substrate solution for feeding the reactor [18].

An amperometric biosensor sensitive to glucose and lactose has been developed by immobilizing glucose oxidase (GOD), β -galactosidase, mutarotase and ferrocene in β -cyclodextrin polymer. The ferrocene is included in the cavities of the β -cyclodextrin polymer through a host-guest chemical reaction whereas glucose oxidase, β -galactosidase and mutarotase are cross-linked with the β -cyclodextrin polymer. Cyclic voltammetry and

amperometric measurement have been employed for the first time to show the efficacy of electron transfer between immobilized glucose oxidase and a glassy carbon electrode via ferrocene included in the cavities of β -cyclodextrin polymer. Performance and characteristics of the biosensor were evaluated with respect to response time, detection limit, selectivity, and dependence on applied potential, temperature and pH as well as operating and storage stability. The stability of the enzyme membrane was greatly enhanced by cross-linking of the enzymes with β -cyclodextrin polymer because of the water absorbability of the β -cyclodextrin polymer [14].

The specific objective of work [6] was to develop an electrochemical biosensor to determine lactose concentration in fresh raw milk. The sensor is based on serial reactions of three enzymes--beta-galactosidase, glucose oxidase, and horseradish peroxidase--immobilized on a glassy carbon electrode. The sequential enzymatic reactions increase the selectivity and sensitivity of the sensor. The sensor requires dilution of the raw milk and the addition of 5-aminosalicylic acid. Lactose concentrations in raw milk measured by the sensor were in good agreement with those measured by a reference laboratory using infrared technology. The results were obtained in milk samples that varied in fat and protein composition. From the results, we conclude that an electrochemical biosensor for determination of lactose concentration in fresh raw milk can be developed, and that the biosensor presented in this study maintained the qualities required for further development into an online sensor in the milking parlor.

There are some contributions from the literature on lactose biosensors with their technical characteristics.

Table 1

Some contributions from the literature on lactose biosensors

Analytes	Biological component	Detector	Linear range	Reference
lactose	glucose oxidase, β -galactosidase	Pt – Ag/AgCl oxygen electrode	0,02 – 3 mM	24
lactose, glucose	β -galactosidase, mutarotase, glucose oxidase	Oxygen electrode	58,5 – 181,3 mM	38
lactose	β -galactosidase, glucose oxidase	Screen-printed electrode	0 – 4,7 mM	11
lactose	β -galactosidase, galactose oxidase, + <i>NAD as a cofactor</i>	Field effect transistor	0 – 1,5 mM	13
lactose	β -galactosidase, <i>Saccharomyces cerevisiae</i>	CO ₂ electrode	no dates	2
glucose, sucrose, lactose	<i>Gluconobacter oxydans</i> , <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i>	Oxygen electrode	0 – 4,1 mM	30
lactose	A - β -galactosidase, galactose oxidase B - β -galactosidase, glucose oxidase C - β -galactosidase, galactose oxidase, glucose oxidase	H ₂ O ₂ electrode	0,9 – 5,0 mM 2,05 – 10,0 mM 0,9 – 5,0 mMg/L	1

lactose	β -galactosidase, glucose oxidase, peroxidase	Graphite electrode	0,044 – 0,339 mM	35
glucose, galactose, lactose	glucose oxidase, galactose oxidase, β -galactosidase	Platinum electrode	0,05 – 10 mM 0,1 – 20 mM 0,2 – 20 mM	26
lactose	β -galactosidase, galactose oxidase	Langmuir- Blodgett film was working electrode	29,2 – 175,4 mM	17
lactose	glucose oxidase, β -galactosidase	Glassy carbon electrode	0,1 – 3,5 mM	9
lactose	glucose oxidase, β -galactosidase	Gold interdigitated microelectrode	0,06 – 0,8 mM	19
lactose	glucose oxidase, β -galactosidase, peroxidase	Gold electrode	0,0015 – 0,12 mM	4

From Table 1, it was obvious that the lowest detection of lactose concentration (0,0015 – 0,12 mM) has been reported at gold electrode/glucose oxidase, β -galactosidase, peroxidase [4]. Similarly the lowest detection of lactose concentration (0,06 – 0,8 mM; 0,044 – 0,339 mM) have also been reported at gold interdigitated microelectrode/glucose oxidase, β -galactosidase [19] and at graphite electrode/ β -galactosidase, glucose oxidase, peroxidase [35].

CONCLUSIONS

In summary it is possible to draw the conclusion - according to the facts of the permanently increasing amount of the processed milk and there from the increasing amount of waste of whey in the dairy industry, the problem of the determination of concentration of lactose is actual nowadays. It is necessary to create new types of biosensors on a basis of immobilized β - galactosidase to define too low concentration of lactose.

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