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β-Galactosidase monitoring by a biosensor based on Clark electrode: Its optimization, characterization and application

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ABSTRACT

β-Galactosidase is an hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. Substrates of different β-galactosidases include ganglioside GM1, lactosylceramides, lactose, and various glycoproteins. A novel aspect of the activity determination of β-galactosidase was presented. A glucose oxidase biosensor based on Clark electrode was utilized in order to monitor β-galactosidase. Immobilization of glucose oxidase was made by gelatin and glutaraldehyde as cross-linker. Several parameters such as glucose oxidase activity, gelatin amount, and glutaraldehyde percentage for cross-linking were optimized. The most important parameter, lactose concentration in working buffer was studied in detail. Optimum temperature, thermal stability, optimum pH, buffer system and its concentration effect on the biosensor system, repeatability, reproducibility, and storage and operational stabilities of the biosensor were identified. A linear detection range for β-galactosidase was observed between 9.4×10^{-5} and 3.2×10^{-2} U/ml. Finally, β-galactosidase activity in artificial intestinal juice was investigated by the biosensor and the results obtained were compared with a reference spectrophotometric method.

1. Introduction

β-Galactosidase is an enzyme that initiates the breakdown of the sugar lactose. β-Galactosidase is coded by a gene (lac z) in the lac operon of *Escherichia coli*. It is a metalloenzyme and hydrolyzes terminal, non-reducing β-D-galactose residues in β-D-galactosides. The β-galactosidase tetramer is comprised of four polypeptide chains (Fowler and Zabin, 1978). As can be known, β-galactosidase has two catalytic activities. First, it hydrolyzes the disaccharide lactose to galactose plus glucose. Second, it converts lactose to another disaccharide, allolactose, which is the natural inducer for the lac operon (Koshland, 1953; Stokes and Wilson, 1972). β-Galactosidase deficiency is a rare inherited biochemical disorder characterized by the accumulation of mucopolysaccharides (glycosaminoglycans) in various body tissues due to insufficient amounts of the enzyme needed to break it down. Enzymatic hydrolysis of lactose by β-galactosidase is one of the most popular technologies to produce lactose-reduced milk and related dairy products for consumption by lactose-intolerant persons whose metabolism exhibits a decline in the level of β-galactosidase needed for proper metabolization of lactose (Kretchmer, 1971; Heyman, 2006).

Recent efforts in the development of activity detection systems for β-galactosidase are primarily focused on spectrophotometric methods. This is not a surprise because the enzyme, β-galactosidase, is a member of hydrolyses. The reaction rate of β-galactosidase can be monitored with the help of a few alternative ways. Most of these alternative ways are based on the spectrophotometric methods. Many literatures report that the activity determination of β-galactosidase has been achieved by use of several chromogenic or fluorogenic substances such as *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG), *para*-nitrophenyl-β-D-galactopyranoside (PNPG), (Li et al., 1975; Burestedt et al., 2000; Seeber and Boothroyd, 1996; Manafi, 2000; Pelisek et al., 2000; McGuire et al., 2002; Masson et al., 1995; Perez et al., 2001; Madoz et al., 1997; Scott et al., 1997; Zinselmeyer et al., 2003; Gary and Kindell, 2005). Moreover, such alternative techniques that have been explored include scanning electrochemical microscopy (Zhao et al., 2004), a voltammetric sensor prepared by the immobilization of metallophthalocyanine complexes onto a glassy carbon electrode (Wutor et al., 2007). Although these technologies have demonstrated successful β-galactosidase activity determination, many require expensive laboratory instrumentation, operator expertise and may not be easily incorporated into routine analysis. Because of the reasons mentioned above, the biosensor developed for the activity determination of β-galactosidase was very important. By the biosensor, it became possible to analyze β-galactosidase activity

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in the levels of μU. Moreover, if the level expresses as protein amounts, the detection range of the biosensor was in the level of nanograms.

In this work, a different viewpoint for the activity determination of β-galactosidase is presented. A glucose oxidase biosensor based on Clark dissolved oxygen probe was utilized to determine the activity of β-galactosidase. The rate of consumption of oxygen was proportional to the level of glucose produced from the hydrolysis of lactose by β-galactosidase, and was directly related to the activity of β-galactosidase. Measurements were carried out by standard curves that were obtained by the determination of decrease in the consumed oxygen level related to activity amount of injected β-galactosidase.

2. Experimental

2.1. Apparatus

YSI 57 A model oxygen meter and YSI 5700 series dissolved oxygen (DO) probes (YSI Co. Inc., Yellow Springs, OH, USA) were used. A water bath was used for preparation of bioactive material (Stuart scientific linear shaker bath, SBS 35, UK). All the measurements were carried out of constant temperature using a thermostat (Haake JF, Germany). Magnetic stirrer (IKA-Combimag, RCO) and pH meter with electrode (WTW, pH 538, Germany) for preparing buffer solutions were used. The temperature was maintained constant in the reaction cell by circulating water at appropriate temperature around the cell compartment during the experiment.

2.2. Procedure

2.2.1. Dissolved oxygen probe

To construct the biosensor a dissolved oxygen probe was covered with high-sensitive teflon membrane by using on O-ring and then the teflon membrane which is selective for oxygen was pretreated with 0.5% sodium dodecylsulphate in phosphate buffer (50 mM, pH 7.5) to reduce the tension on the membrane surface.

2.2.2. Biosensor preparation

36 U glucose oxidase and 5 mg gelatine were dissolved in 200 μl, pH 7, 50 mM phosphate buffer. The enzyme and gelatine mixture was dispersed over the dissolved oxygen probe membrane surface and allowed to dry at 4 °C for 15–30 min. For cross-linking with glutaraldehyde, the probe carrying bioactive layer was immersed in to 5% (v/v) glutaraldehyde solution (in phosphate buffer, 10 ml, 50 mM and pH 7.5) and was allowed to wait for 5 min. At the end of this time, the biosensor was washed with distilled water and it was ready to use. In order to prevent to dry the bioactive layer of the biosensor, it was stored in a flask that contained some distilled water at 4 °C. The biosensor was not in contact with distilled water. This condition provided a moisture medium for the biosensor.

2.2.3. Measurement procedure

The biosensor based on glucose oxidase was put in to the thermostatic reaction cell containing working buffer (pH 4.8, 50 mM citrate buffer containing 100 mM lactose) and the magnetic stirrer was fixed at a constant speed. A few minutes later, dissolved oxygen concentration was equilibrated because of the diffusion of dissolved oxygen between working buffer and dissolved oxygen probe. At this time, β-galactosidase was injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease and a few minutes later (approximately 5 min later) it reached constant dissolved oxygen concentration due to the enzymatic reaction equilibration above. At this moment, dissolved oxygen concentration was recorded. Measurements were carried out by the change of dissolved oxygen concentration related to β-galactosidase activity added to the reaction cell.

3. Results and discussion

3.1. Optimization studies of the biosensor

3.1.1. The effect of glucose oxidase activity on β-galactosidase determination

In the optimization studies of the biosensor, first of all, the activity of glucose oxidase immobilized on the dissolved oxygen probe was investigated. For this purpose, five different glucose oxidase activities were used to construct the biosensor. Glucose oxidase amounts, linear ranges and R^2 values of the different biosensors are given in Table 1.

As can be seen from the table, more increase in glucose oxidase activity did not mean the best biosensor results. In glucose oxidase activities higher than 36 U, the linear ranges for β-galactosidase activity determination were narrowed. This was probably caused by the negative diffusion effects when the biosensor used glucose oxidase activities higher than 36 U. The best biosensor signals for β-galactosidase activity were obtained with the biosensor that containing 36 U glucose oxidase. Besides, the slope of a position versus β-galactosidase activity can reveal pertinent information about the performance of the biosensors. For example, a small slope means a small biosensor signal. Thus the shape of the line on the graph can be descriptive of the biosensor performance. As can be seen in Table 1, the slopes of the biosensors prepared with different glucose oxidase activities were very close to each other. Although the biosensor prepared with 36 U glucose oxidase presented the smallest slope, the widest linear range for β-galactosidase activity was obtained by this biosensor. As a result, in further measurements 36 U of glucose oxidase was used.

3.1.2. The effect of gelatine amount on β-galactosidase determination

In order to reveal optimum immobilization conditions for the biosensor, the effect of quantity of gelatine in the bioactive layer

Table 1
The effects of glucose oxidase activity, gelatine and glutaraldehyde percentage, and lactose concentration on the biosensor response

	R^2	Slopes (y)	Linear ranges (U/ml)
Glucose oxidase activity[a] (U)			
9	0.982	$80.173x + 0.2203$	5.6×10^{-4}–2.4×10^{-2}
18	0.9747	$79.941x + 0.2665$	9.4×10^{-5}–2.4×10^{-2}
36	0.9988	$63.596x + 0.1494$	9.4×10^{-5}–3.6×10^{-2}
54	0.9992	$75.782x + 0.1427$	9.4×10^{-5}–3×10^{-2}
72	0.9897	$95.377x + 0.2439$	9.4×10^{-5}–2.4×10^{-2}
Gelatine amount[b] (mg)			
2.5	0.997	$63.445x + 0.1448$	9.4×10^{-5}–3.6×10^{-2}
5	0.9989	$63.371x + 0.1552$	7.5×10^{-4}–3.6×10^{-2}
7.5	0.9988	$62.032x + 0.0907$	7.5×10^{-4}–3.8×10^{-2}
Lactose concentration (mM)			
10	0.9987	$11.918x + 0.0938$	9.4×10^{-3}–1.88×10^{-1}
25	0.9916	$16.19x + 0.2106$	9.4×10^{-3}–1.41×10^{-1}
50	0.9814	$25.229x + 0.2043$	1.88×10^{-4}–1.88×10^{-2}
100	0.9949	$90.387x + 0.1858$	9.4×10^{-5}–4.7×10^{-2}
150	0.9776	$118.85x + 0.1582$	9.4×10^{-5}–3.2×10^{-2}
200	0.9941	$86.828x + 0.1681$	9.4×10^{-5}–3.2×10^{-2}

[a] Gelatin and glutaraldehyde percentage were kept constant as 2.5 mg and 5%, respectively. Working buffer was 0.05 M and pH 4.8 citrate solution and contained 0.1 M lactose substrate of β-galactosidase, $T = 35$ °C.

[b] Glucose oxidase activity and glutaraldehyde percentage were kept constant as 36 U and 5%, respectively. Working conditions were same as mentioned above.

was determined. In these studies 1.25, 2, 2.5, 5 and 7.5 mg gelatine amounts were tested on biosensors. Detail information is shown in Table 1. Optimization studies for gelatine amount showed that there was no change in the biosensor activity related to gelatine amount. However, to obtain gelation of the bioactive layer in a good form, at least, 2.5 mg of gelatine was needed. The amounts higher than 2.5 mg of gelatine (5 and 7.5 mg) the biosensor signals did not alter seriously. Consequently, 2.5 mg of gelatine was chosen as optimum gelatine amount.

3.1.3. The effect of glutaraldehyde percentage on the biosensor response

In the investigation of the effect of differing glutaraldehyde amounts on glucose oxidase biosensor for the activity determination of β-galactosidase, experiments were carried out by keeping the amount of glucose oxidase activity (36 U) and gelatin (2.5 mg) constant while changing the amount of glutaraldehyde. In these studies, glutaraldehyde solutions in the concentration range of 1.25–10% were employed as a cross-linking agent for glucose oxidase immobilization in gelatine. The highest biosensor response was observed when 2.5% glutaraldehyde solution was used. The response of the biosensor increased as the glutaraldehyde percentage was increased until optimum value of 5%. At glutaraldehyde concentration greater than 5%, the biosensor response decreased, which may be attributed to excessive cross-linking between the immobilization materials, glucose oxidase and gelatine, where the active sites of the enzyme were blocked and thus a decrease in the activity of the immobilized enzyme. Moreover, the decrease in the biosensor signal may be caused by the negative diffusion barrier effects.

3.1.4. The effect of concentration of lactose as β-galactosidase substrate

This was an experiment to examine how the concentration of the substrate lactose affected the rate of reaction of the enzyme β-galactosidase and, of course, the relationship between this rate and its effect on the glucose oxidase biosensor. The investigations showed that the optimization of the lactose concentration in the buffer solution was the most important parameter that had to be identified. Because the activity determination of β-galactosidase was strictly dependent on the lactose concentration of the working buffer solution. This effect is given in Fig. 1.

It is clear from the figure, increasing lactose concentration improved the linear range of the β-galactosidase activity (U) determination. Initially, the more substrate caused enzyme activity to increase, up to a certain point. Of course, the biosensor signal obtained for β-galactosidase activity unit increased with glucose product of β-galactosidase. Parallel to this result, the detection limit of the β-galactosidase activity was decreased by increase in the lactose concentration. Increasing lactose concentration any further had no effect on activity. So again, initially increasing lactose concentration caused biosensor signals to increase but up to a maximum. In this experiment the maximum lactose concentration value was 100 mM. A summarizing of the effect of lactose concentration on β-galactosidase activity determination is seen in Table 1. Adding any extra lactose had no effect on the detection limit of the enzyme. However, in the lactose concentrations higher than 100 mM, linear detection range for β-galactosidase activity were affected negatively. Moreover, if we examine how the actual slope value of any straight line on the graph (Table 1), the results support the information given above. An increase in the lactose concentration resulted in an increase in the slope of the line. It was meant to increase biosensor signals. Consequently, the optimum lactose concentration in the working buffer was accepted as 100 mM.

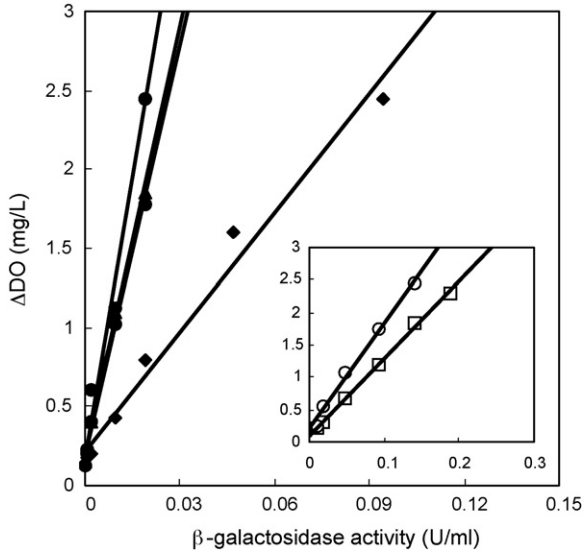

Fig. 1. Lactose concentration dependence of the biosensor response (lactose concentrations tested: -□-, 0.01 M; -○-, 0.025 M; -♦-, 0.05 M; -■-, 0.1 M; -●-, 0.15 M; -▲-, 0.2 M. Biosensor components: glucose oxidase activity, gelatin and percentage of glutaraldehyde were kept constant as 36 U, 2.5 mg and 5%, respectively. Working buffer was 0.05 M, pH 4.8 citrate solution, T = 35 °C).

3.1.5. Optimum temperature

Biosensors have a temperature range in which a maximal rate of reaction is achieved. This maximum is known as the temperature optimum of the biosensor. In this biosensor system for β-galactosidase activity determination, the temperature optimization was also very important because there were two different enzymes in the measurement process, one from sample, β-galactosidase, and the other from the biosensor, glucose oxidase. In the temperature range 15–55 °C, the biosensor signals increased with increasing temperature. The highest biosensor response was obtained when worked with 55 °C. At higher temperatures than

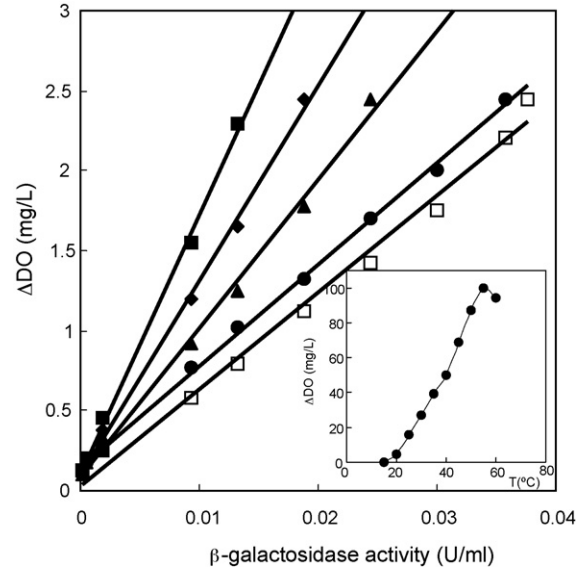

Fig. 2. β-Galactosidase activity calibration graphs obtained under different temperatures and optimum temperature for the biosensor (the graph in the inner area) (temperatures (°C): -□-, 35; -●-, 40; -▲-, 45; -♦-, 50; -■-, 55. Biosensor components: glucose oxidase activity, gelatin and percentage of glutaraldehyde were kept constant as 36 U, 2.5 mg and 5%, respectively. Working buffer was 0.05 M, pH 4.8 citrate solution, T = 35 °C).

55 °C, the biosensor response was started to decrease. These results are given in the inner area of Fig. 2 as an optimum temperature graph. Moreover, the calibration graphs for β-galactosidase activity were drawn at different temperatures. Fig. 2 shows these graphs.

Although the highest response was obtained at 55 °C, to avoid, as much as possible, the denaturation process of glucose oxidase in the bioactive layer of the biosensor, a series of further experiments were carried on. These experiments are explained in the following sections.

3.1.6. Thermal stabilities

In these experiments, first of all, the thermal stability of β-galactosidase was determined. For this purpose, the enzyme β-galactosidase was incubated at different temperatures 35, 40, and 45 for 5 h. There was no activity change in the enzymes incubated at temperatures of 35 and 40 °C. However, the enzyme incubated at the temperature of 45 °C was lost 6% of its initial activity. Besides, thermal stability of the biosensor was also tested by the help of incubation of the biosensor at various temperatures and then making measurements. The biosensor showed no thermal deactivation in the temperatures of 40 and 45 °C at the end of the incubation period of 5 h. At the temperatures of 50, 55 and 60 °C, 18%, 34% and 53% activity decreases were observed, respectively. After these studies, operational stability experiments were carried out to help to choose the correct working temperature. These studies are discussed in Section 3.2.4. After all measurements made for determining the working temperature, 40 °C was chosen as working temperature.

3.1.7. Optimum pH

pH value of working buffer was also one of the most important parameters that could dramatically affect the biosensor response. Optimum pH for free glucose oxidase is 5.1 with a broad range 4–7 (Bright and Appleby, 1969). Besides, an optimum pH for free β-galactosidase is 4.5 (Tanaka et al., 1975). So, an optimum pH scan was done in the range of pH 4–6. Fig. 3 shows optimum pH graph.

These results showed that the best biosensor signal was obtained at pH 4.8. In this experiment, optimum pH value of β-galactosidase probably played a determinative role. Its optimum pH value reported was 4.5 while glucose oxidase could work in the range of pH 4–7. The results were agreed with the optimum pHs of both enzymes. As can be seen clearly from the figure, for a pH of 4.6, the biosensor signal was about 3% lower than that found at pH 4.8 and its value decreased for pH values higher than 4.8. The biosensor response was dramatically decreased at pH values higher than 5.2. This was probably caused from leaving optimum pHs of both enzymes, glucose oxidase and β-galactosidase.

3.1.8. Appropriate buffer system

Buffer system can result in the change of the capacity of the buffer or ion strength and can change the biosensor response. Especially, in our measurement system, the effect of different buffer systems could definitely be investigated because the system used two different enzymes. For this purpose, three different buffer systems which were adjustable to pH 4.8, sodium–phosphate, Citric acid/NaOH, and acetic acid/NaOH were used to investigate the effect of the buffer system on the biosensor. The results showed that the buffer systems did not alter the biosensor response significantly. Consequently, sodium–phosphate buffer system was accepted as the working buffer.

3.1.9. The effect of the buffer concentration

The increase of buffer concentration often contributes to the reproducibility of biosensor response together with the decay of the response value and the lifetime of the immobilized enzyme or tissue. So we also investigated the optimum concentration of the working buffer solution. An increase in buffer concentration resulted in a signal decrease, probably justified by an ionic strength increase in reaction cell, resulting in an inhibiting effect on glucose oxidase and β-galactosidase activity. The highest biosensor signal was obtained at a concentration of 0.05 M. Consequently, the appropriate buffer concentration was 0.05 M.

3.2. Characterization studies of the biosensor

3.2.1. Calibration graph for β-galactosidase activity

A calibration curve for β-galactosidase activity is presented in Fig. 4. The biosensor showed a linear response for an interval of β-galactosidase activity between 9.4×10^{-5} and 3.2×10^{-2} U/ml.

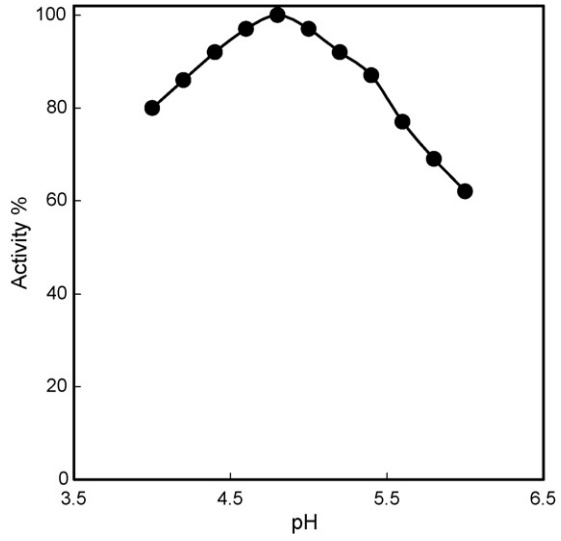

Fig. 3. Optimum pH for the biosensor (biosensor components: glucose oxidase activity, gelatin amount and percentage of glutaraldehyde were kept constant as 36 U, 2.5 mg and 5%, respectively. Working buffer was 0.05 M with different pHs citrate solution and contained 0.1 M lactose substrate of β-galactosidase. A 9.4×10^{-3} U/ml standard solution of β-galactosidase was used, $T = 40$ °C).

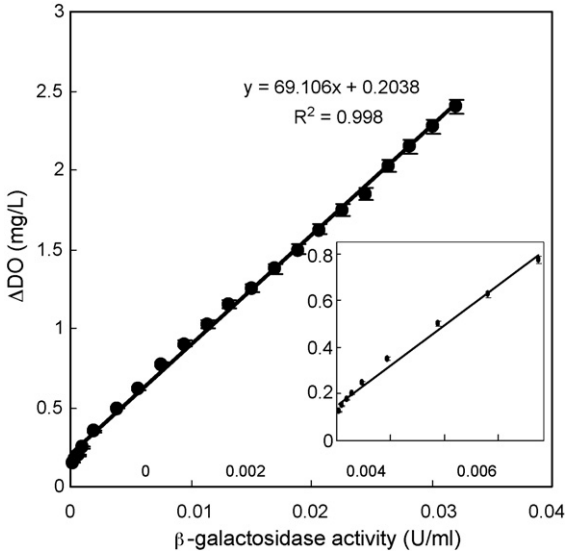

Fig. 4. β-Galactosidase activity calibration graph (biosensor components: glucose oxidase activity, gelatin amount and percentage of glutaraldehyde were kept constant as 36 U, 2.5 mg and 5%, respectively. Working buffer was 0.05 M pH 4.8 citrate solution and contained 0.1 M lactose substrate of β-galactosidase, $T = 40$ °C).

Table 2
β-Galactosidase activity determination in artificial intestinal juice by the biosensor and by a spectrophotometric reference method

Added	Found by the biosensor	Found by the reference method	Recovery (%)	Relative difference (%)
β-Galactosidase (U/ml)				
0.0018	0.0018	0.0019	100	5

The minimum detectable activity amount of β-galactosidase was estimated to be 9.4×10^{-5} U/ml. In the studies, a commercial *A. oryzae* β-galactosidase was used. Its activity amount was in the level of 9400 U/g. So, the detection limit of the biosensor for β-galactosidase could be given as g/ml in the reaction cell. The lowest detection limit of the biosensor was 10 ng/ml of β-galactosidase. This detection limit was perfect for the biosensor.

3.2.2. Repeatability

Repeatability is another factor, which must be determined, especially for a biosensor. The signal changes of the biosensor were investigated when it was alternately exposed to a 9.4×10^{-3} U/ml β-galactosidase standard solution for 10 times. R^2 was 0.998 and repeatability of the measurements was very good considering that the correlation coefficient on measurements was 2.2%, and average value and standard deviation were calculated as 9.7×10^{-3} and $\pm 2.1 \times 10^{-4}$ U/ml, respectively. The results showed that the biosensor exhibited a fairly desirable analytical feature of repeatability.

3.2.3. Reproducibility of the biosensor

The reproducibility is an important parameter for the evaluation of the performance of the biosensor. The reproducibility of the same electrode in the measurements, expressed as the relative standard deviation (R.S.D.), was less than 3.8%, obtained by recording the signals through calibration curves for β-galactosidase. The reproducibility of five biosensors with the same composition was examined under the same working conditions. The linear calibration ranges of 5 biosensors were same as 9.4×10^{-5} to 3.57×10^{-2}. And R^2 values of these graphs were 0.9991, 0.9982, 0.9992, 0.998, and 0.9995. Moreover, for a selected β-galactosidase standard, 0.0188 U/ml, average value, standard deviation, and variation coefficient were also calculated. And the values calculated were 0.0189, $\pm 3 \times 10^{-3}$ U/ml, and 3.7%, respectively.

3.2.4. Operational stability of the biosensor

The operational stability of the biosensor was investigated by consecutive measurements of its response to β-galactosidase activity standard, 9.4×10^{-3} U/ml. These investigations were carried out by working in two different temperatures, at 40 and 45 °C. 95% initial activity had been retained after 23 measurements when the measurements were done at 40 °C. On the other hand, when the biosensor was run at temperature of 45 °C, it retained 94% of its initial activity at the end of the 10th measurement. The results showed that the operational stability of the biosensor was perfect when it was operated at 40 °C.

3.2.5. Storage stability

The purpose of storage stability testing was to provide evidence on how the performance of the biosensor based on glucose oxidase for β-galactosidase activity varies with time under the influence of environmental factors such as temperature, humidity.

Two different ways were used to investigate correctly the storage stability of the biosensor. In the first way, the biosensors were stored at +4 °C for altering periods such as 10, 20, 30, 60, and 90 days. The measurements were done at the beginning and end of the storage period. The difference between the signals obtained at first and last was used to understand the storage stability of the biosensor. The biosensor response was constant for the first 30 days. When the biosensor was stored for 60 days, the response of the biosensor decreased to about 87%. At the 90th days of storage period, the biosensor retained about 78% of its initial activity. The results indicated the excellent long-term stability of the biosensor. In the second way, like the first way the biosensors were stored at +4 °C however, the measurements were done more frequently than the first one. After 22 days storage period the biosensor lost no activity. At the end of the 24th, 34th, 45th, and 51st days, the remaining activity of the biosensor were 96, 89, 81, and 76, respectively.

3.3. Sample analysis

Artificial intestinal juice was prepared to use for sample analysis studies. It was containing 10 mM sodium–phosphate buffer (pH 7.2), 150 mM sodium chloride, 1 mg/ml bovine serum albumin, and 1% (v/v) methanol. A determined unit of β-galactosidase-spiked artificial intestinal juice was determined by the help of the biosensor. A reference method was used to compare the results (Bahl and Agrawal, 1969). The results are shown in Table 2.

Experimental results indicated good recovery was observed. Besides, good agreement between the results of the biosensor presented and the reference method was achieved. These results indicate that the proposed biosensor can be applied successfully for activity determination of β-galactosidase.

4. Conclusion

In this work, we demonstrate an enzyme biosensor to investigate the activity of β-galactosidase. This biosensor is the first approach to the utilization of a biosensor system for β-galactosidase activity determination. This kind of biosensor was a novel aspect of both β-galactosidase activity determination and a biosensor design. As mentioned previously, β-galactosidase activity can frequently be monitored by spectrophotometric methods utilizing chromogenic or fluorogenic substrates. Consequently, alternative methods to monitor the enzyme should be developed in order to overcome some barriers results from the spectrophotometric methods. For this purpose, the biosensor presented here was optimized and characterized in terms of its important parameters. All these suggest one conclusion: the biosensor based on glucose oxidase can successively be used to monitor the enzyme, β-galactosidase. Moreover the biosensor offers some serious advantages with respect to spectrophotometric methods such as a perfect linear range (9.4×10^{-5} to 3.2×10^{-2} U/ml) and a very low detection limit (9.4×10^{-5} U/ml or differently 10 ng/ml), the absence of a chromogenic or flourogenic substrates, a good long lifetime, a very high operational stability, a very simple measurement procedure and low cost of an analysis. In addition, the simplicity of immobilization of the components of the biosensor and the use of a very cheap enzyme, glucose oxidase, demonstrate a considerable serious economic advantage.

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