*Anal. Bioanal. Electrochem., Vol. 4, No. 2, 2012, 153 - 162*

**Analytical & Bioanalytical Electrochemistry**



*Full Paper*

# A Hybrid Enzyme Biosensor Based on Sulfite Oxidase and Glucose Oxidase for the Analyzing of Sulfite and Glucose

**Mustafa Kemal Sezgintürk[1,*], Sencer Alaçam[2], Çağrı Altuğ[2], Pınar Akbayirli[2] and Erhan Dinçkaya[2]**

*[1]Namık Kemal University, Faculty of Arts and Science, Chemistry Department, 59100, Tekirdağ/Turkey*
*[2]Ege University, Faculty of Science, Biochemistry Department,35100Bornova- İzmir/Turkey*

*Corresponding Author, Tel: +90-282-2643513; Fax: +90-282-2643513
E-Mail: msezginturk@hotmail.com

*Received: 7 March 2012 / Accepted after minor revision: 21 April 2012 /*
*Published online: 30 April 2012*

**Abstract**- In this study, we developed a hybrid biosensor based on sulfite oxidase and glucose oxidase for determination of sulfite and glucose. The principle of the measurements was based on the determination of the decrease in the differentiation of oxygen level which had been caused by the catalytic reactions of the enzymes in the bioactive layer of the biosensor. The biosensor showed a linear response for an interval of sulfite concentration between $1\times10^{-4}$ and $1.75\times10^{-3}$ M and for glucose concentrations between $2.5\times10^{-5}$ and $1.25\times10^{-3}$ M. After some optimum and characterization studies, the proposed biosensor was applied to the determination of sulfite and glucose in certain real samples, blood and pickle water.

**Keywords-** Sulfite, Glucose, Biosensor, Food Additives, Diabetes, Sulfite Allergy

## 1. INTRODUCTION

Sulfites or sulfur dioxide is a fruit preservative widely used in dried fruits as well as wine. It is also produced by the human body at the level of about 1000 mg per day. Consumption of food preserved with sulfites is generally not a problem except for a few people who are

deficient in the natural enzyme to break it down. For these people, the additional sulfites from food can be a problem. Sulfites cause little to no problems in most people without allergies and asthma, even when large amounts are consumed [1,2]. Sulfites are known to increase asthma symptoms in approximately 5% of asthmatics, particularly in adults with severe disease. Numerous well-controlled studies show that some asthmatics can have severe asthma symptoms with eating sulfite-containing foods/beverages or inhaling sulfite fumes or vapors. Less is known about hives/swelling and anaphylaxis as a result of sulfites, although various cases have been described in which the consumption of sulfite-containing foods/beverages leads to severe allergic reactions. Some of these people even had positive skin tests to sulfites, suggesting the presence of allergic antibodies to the preservative.

Glucose is by far the most common carbohydrate and classified as a monosaccharide, an aldose, a hexose, and is a reducing sugar. It is also known as dextrose, because it is dextrorotatory (meaning that as an optical isomer is rotates plane polarized light to the right and also an origin for the D designation. Glucose is also called blood sugar as it circulates in the blood at a concentration of 65-110 mg/mL of blood [3-6]. Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans. Glucose is critical in the production of proteins and in lipid metabolism. In plants and most animals, it is also a precursor for vitamin C (ascorbic acid) production. Glucose is used as a precursor for the synthesis of several important substances. Starch, cellulose, and glycogen ("animal starch") are common glucose polymers (polysaccharides). Lactose, the predominant sugar in milk, is a glucose-galactose disaccharide. In sucrose, another important disaccharide, glucose is joined to fructose. These synthesis processes also rely on the phosphorylation of glucose through the first step of glycolysis [7,8].

In this study, a hybrid enzyme biosensor was developed for the alternately analyzing of sulfite and glucose. For this purpose, sulfite oxidase and glucose oxidase were immobilized on a Clark type oxygen electrode together. In the study some optimization and characterization studies were carried out for buth enzymes sulfite oxidase and glucose oxidase. Finally, the hybrid biosensor based sulfite oxidase and glucose oxidase was validated by the reference methods for sulfite [9] and glucose determinations.

## 2. EXPERIMENTAL

### 2.1. Materials

Bidistilled water was used to prepare the solutions. All reagents were analytical grade. Pickle water samples were obtained from a Turkish commercial source. Blood samples were provided from a national health centre.

### 2.2. 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 comportment during the experiment.

**2.3. Procedure**

*2.3.1. 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 .

*2.3.2. Biosensor preparation*

5 mg glucose oxidase, 0.5 U sulfite oxidase and 5 mg gelatine were dissolved in 200 μL, pH 7, 50 mM phosphate buffer. The enzymes and gelatin 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 2.5 % (v/v) glutaraldehyde solution (in phosphate buffer, 10 ml, 50 mM and pH 7.5) and was allowed to wait 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.

*2.3.3. Measurement procedure*

The biosensor based on sulfite oxidase and glucose oxidase was put in to the thermostatic reaction cell containing working buffer (pH 7.5, 50 mM phosphate buffer) 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, sulfite or glucose was injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease and a few minutes later (approximately 3 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 sulfite or glucose activity added to the reaction cell. Enzymatic reactions occur in the biosensor active layer are shown below.

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{Sulfite oxidase}} H_2O_2 + SO_4^{2-}$$

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{D- Glucanolactone} + H_2O_2$$

## 3. RESULTS and DISCUSSION

### 3.1. Optimum pH values for the determination of sulfite and glucose

In this biosensor design one of the most important parameter is pH value of the working buffer. Because the pH value of the working buffer must be suitable for both enzymes on the bioactive layer of the biosensor. So that, an optimum pH scan was done in the range of pH 6-8. Fig. 1 shows optimum pH graph below.

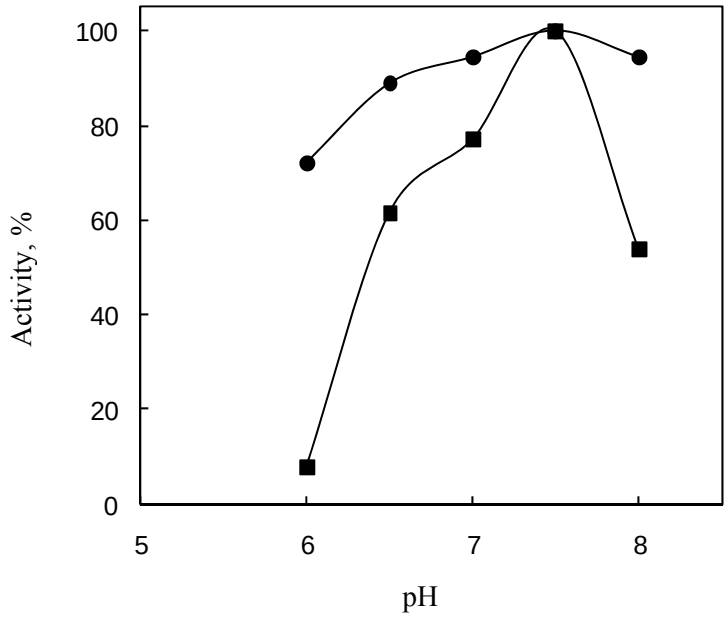


**Fig. 1.** The effect of pH value on the biosensor response [ -•-•-: glucose oxidase, -■-■-: sulfite oxidase. The amount of sulfite oxidase, glucose oxidase, glutaraldehyde and gelatin were kept constant at 0.5 U, 5 mg, 2.5% and 5 mg, respectively. Standard sulfite (750 μM) and glucose (250 μM) solutions were used in these experiments. Working conditions: Phosphate buffer, 50 mM, pH 7.5, T:38 $^{o}$C]

These results showed that the best biosensor signals were obtained at pH 7.5 for glucose oxidase and at 7.5 for sulfite oxidase. In this experiment, optimum pH value of sulfite oxidase

probably played a determinative role. The activity of the biosensor toward sulfite was changed dramatically by change of pH value of working buffer. However the performance of glucose determination of the biosensor was almost same in the range of 6.5-8. Moreover it was our lucky that the best results were obtained at pH 7.5 of both substances.

### 3.2. Temperature dependence of the hybrid biosensor

It is clear that 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 sulfite and glucose determination, the temperature optimization was also very important like optimum pH because there were two different enzymes in the bioactive layer of the biosensor, sulfite oxidase and glucose oxidase. The results were summarized in Fig. 2 below.

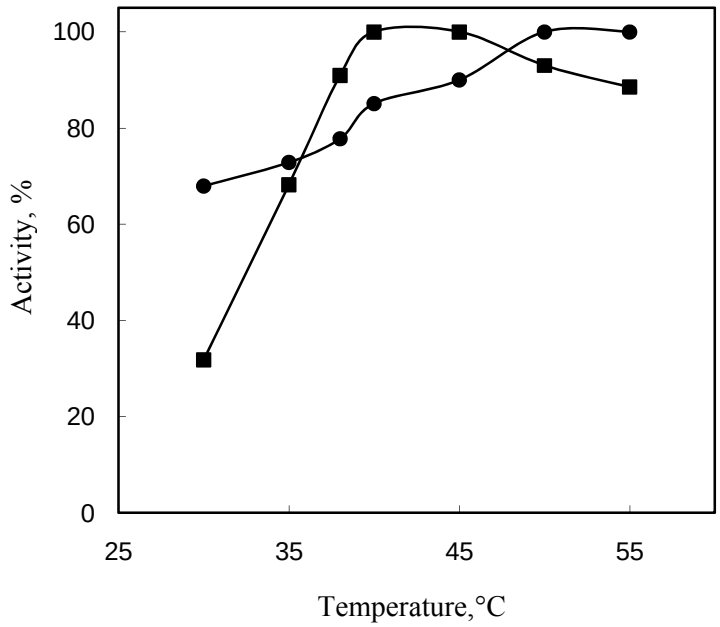


**Fig. 2.** The effect of temperature on the biosensor response [ -•-•-: glucose oxidase, -■-■-: sulfite oxidase. The amount of sulfite oxidase, glucose oxidase, glutaraldehyde and gelatin were kept constant at 0.5 U, 5 mg, 2.5% and 5 mg, respectively. Standard sulfite (750 μM) and glucose (250 μM) solutions were used in these experiments. Working conditions: Phosphate buffer, 50 mM, pH 7.5]

In the temperature range 30-55 $^{o}$C, the biosensor signals for glucose increased with increasing temperature. Until 55 $^{o}$C, the biosensor response increase with the temperature

increase. However, the biosensor performance in the determination of sulfite didn't look like glucose performance. In the temperature range 30-40 $^{o}$C, the biosensor performance for sulfite increased with increasing temperature linearly. On the other hand, at the temperatures higher than 40 $^{o}$C the biosensor signals for sulfite were become stable. Moreover, further increase in temperature decreased the biosensor response for sulfite. Probably this was caused from the thermal denaturation or insufficient thermal stability of sulfite oxidase on the bioactive layer of the biosensor. Finally, our experiments showed that at the temperatures higher than 40 $^{o}$C, the gelatine layer of the biosensor was become physically insufficient. Although the highest response was obtained at 55 °C for glucose determination, to avoid, as much as possible, the denaturation process of sulfite oxidase and time dependent thermal denaturation of glucose oxidase in the bioactive layer of the biosensor and also biosensor destruction caused from gelatine layer insufficiency, 38 $^{o}$C was chosen as working temperature of the hybrid biosensor.

### 3.3. Calibration graphs for sulfite and glucose

Two calibration curves for sulfite and glucose are presented in Fig.3 and 4, respectively. The biosensor showed a linear response for an interval of sulfite concentration between $1\times10^{-4}$ and 1, $75\times10^{-3}$ M .

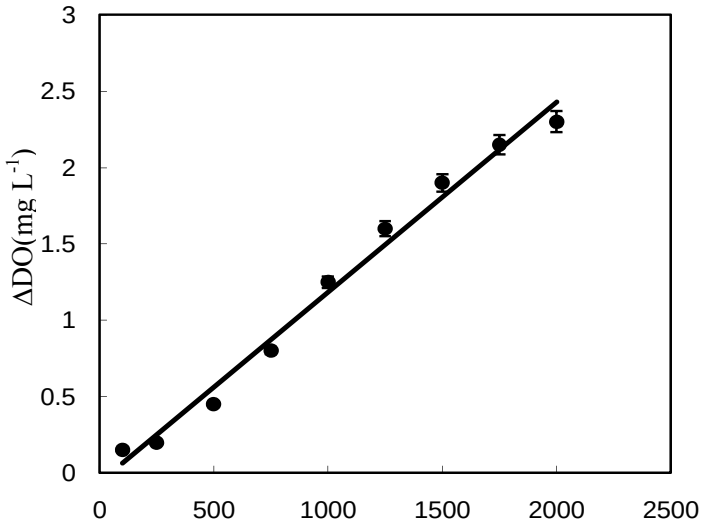


**Fig. 3.** Calibration graph for sulfite [The amount of sulfite oxidase, glucose oxidase, glutaraldehyde and gelatin were kept constant at 0.5 U, 5 mg, 2.5% and 5 mg, respectively. Working conditions: Phosphate buffer, 50 mM, pH 7.5, T:38 $^{o}$C]

The minimum detectable concentration amount of sulfite was estimated to be $1\times10^{-4}$ M. This detection limit was perfect for the biosensor. The combination of sulfite oxidase and

glucose oxidase with an oxygen electrode led to a biosensor with a linear response for glucose concentrations between $2.5\times10^{-5}$ and $1.25\times10^{-3}$ M. This calibration graph is shown below.

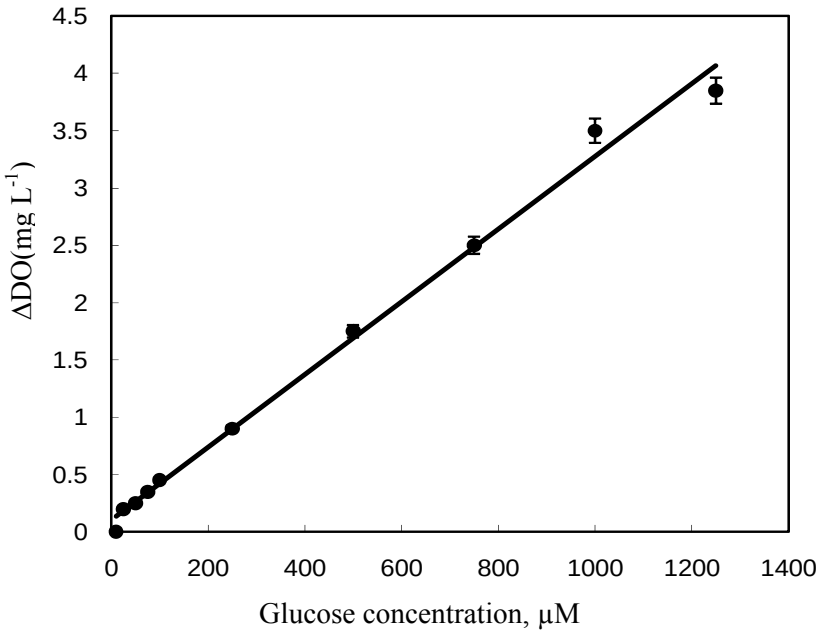


**Fig. 4.** Calibration graph for glucose [The amount of sulfite oxidase, glucose oxidase, glutaraldehyde and gelatin were kept constant at 0.5 U, 5 mg, 2.5 % and 5 mg, respectively. Working conditions: Phosphate buffer, 50mM, pH 7.5, T:38 $^{o}$C]

In table below, a summary of biosensor performances for both substances is given.

**Table 1.** Results obtained from the biosensor based on sulfite oxidase and glucose oxidase

| Compound analyzed | Activity % | Linear range (μM) | $R^2$ | y |
|---|---|---|---|---|
| Sulfite | 100 | 100-1750 | 0.9876 | 0.0012x–0.0593 |
| Glucose | 100 | 25-1250 | 0.9935 | 0.0032x+0.1031 |

### 3.4. Repeatability experiments

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 $5\times10^{-4}$ M sulfite standard solution for 10 times. Like sulfite, we measure glucose for 10 times.

However in the repeatability studies of glucose we used $5\times10^{-5}$ M standard solution. The results are shown in Table 2 below.

**Table 2.** Repeatability studies for sulfite and glucose

| Compound analyzed | Standard concntration (μM) | Xi | C.V. (%) | S.D |
|---|---|---|---|---|
| Sulfite | 500 | 509 | 5.4 | ±2.2 |
| Glucose | 50 | 57 | 3.3 | ±0.95 |

The results showed that the hybrid biosensor based on sulfite oxidase and glucose oxidase exhibited a fairly desirable analytical feature of repeatability.

### 3.5. Real Sample Analyses

To demonstrate the applicability of the hybrid biosensor for sulfite and glucose analyses, three different blood samples for glucose analysis and three different commercial pickle water samples were analyzed. The results obtained by the biosensor were supported by reference methods. For sulfite determination a spectrophotometrically reference method was used [9]. In this method, sulfite oxidase, when reduced by sulfite, the heme prosthetic group of the enzyme is oxidized in the steady state achieved by the presence of ferricyanide. This process should be monitored by spectrophotometrically. Moreover the glucose contents of blood samples were validated by a biochemical laboratory of a hospital. The performance of the biosensor is shown in Table 3.

In these studies, the feasibility of sulfite oxidase and glucose oxidase on a Clark type oxygen electrode as hybrid enzyme based biosensor was evaluated.

**Table 3.** Sulfite and glucose contents of some real blood samples and commercial pickle water samples and comparisons with reference methods

| Found sulfite (ppm) | | | | |
|---|---|---|---|---|
| Sample | Reference [9] | Biosensor[a] | % Recovery | S.D. |
| Pickle water 1 | 4456 | 4418 | -0.85 | ±1.12 |
| Pickle water 2 | 6578 | 6533 | -0.68 | ±0.95 |
| Pickle water 3 | 3467 | 3396 | -2.04 | ±1.2 |
| Found glucose (mg/dL) | | | | |
| Sample | Reference[b] | Biosensor[a] | % Recovery | S.D. |
| Blood 1 | 77 | 79 | 2.59 | ±0.8 |
| Blood 2 | 81 | 82 | 1.23 | ±1.2 |
| Blood 3 | 96 | 100 | 4.16 | ±1.5 |

[a]Mean of three determinations

[b] These values were obtained from a local hospital

## 4. CONCLUSIONS

The proposed sulfite oxidase and glucose oxidase hybrid biosensor based on Clark oxygen electrode allowed the rapid determination of sulfite and glucose. This biosensor showed good characteristics such as its low cost, fast preparation due to its simplicity, response time, and its low detection limit. Finally its applicability to blood and pickle water samples for sulfite and glucose determinations was agreed well with the references reference methods.

## REFERENCES

[1] A. F.Gunnison, and D. W. Jacobsen, CRC Cri. Rev. Toxicol. 17 (1987) 185.

[2] R. K. Bush, S. L. Taylor, and W. Buse, J. Aller. Clin. Immun. 78 (1986) 191.

[3] Calculation of the Energy Content of Foods–Energy Conversion Factors, Food energy -methods of analysis and conversion factors, FAO Food and Nutrition Paper 77, Rome: Food and Agriculture Organization, 2003, ISBN 92-5-105014-7.

[4] S. H. Fairclough, and K. Houston; Biol. Phys. 66 (2004) 1772 .

[5] M. T. Gailliot, R. F. Baumeister, C. N. DeWall, E. A. Plant, L. E. Brewer, B. J. Schmeichel, D. M. Tice, and J. K. Maner, J. Person, Soc. Psych. 92 (2007) 325.

[6] M. T. Gailliot, R. F. Baumeister, and J. Person, Soc. Psych. Rev. 11 (2007) 303.

[7] E. J. Masicampo, and R. F. Baumeister, Psych. Sci. 19 (2008) 255.

[8] R. P. Ferraris, Biochem. J. 360 (2001) 265.
[9] H. J. Cohen, and I. Fridovich, J. Biol. Chem. 246 (1971) 359.