

Direct determination of sulfite in food samples by a biosensor based on plant tissue homogenate

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Abstract

In the work described here, a biosensor was developed for the determination of sulfite in food. *Malva vulgaris* tissue homogenate containing sulfite oxidase enzyme was used as the biological material. *M. vulgaris* tissue homogenate was crosslinked with glutaraldehyde and fixed on a pretreated Teflon membrane. Sulfite was enzymatically converted to sulfate in the presence of the dissolved oxygen, which was monitored amperometrically. Sulfite determination was carried out by standard curves, which were obtained by the measurement of consumed oxygen level related to sulfite concentration. Several operational parameters had been investigated: the amounts of plant tissue homogenate and gelatin, percentage of glutaraldehyde, optimum pH and temperature. Also, some characterization studies were done. There was linearity in the range between 0.2 and 1.8 mM at 35 °C and pH 7.5. The results of real sample analysis obtained with the biosensor agreed well with the enzymatic reference method using spectrophotometric detection.

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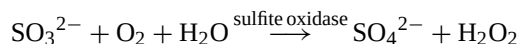
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1. Introduction

Determination of sulfite is important particularly from biological and industrial point of view [1]. Sulfite is widely used as additive in food and beverages to prevent oxidation and bacterial growth and to control enzymatic reactions during production and storage. Nowadays due to the reported harmful effects towards hypersensitive people, in many countries, the sulfite content in food and beverages have been strictly limited [2]. The useful properties of sulphiting agents are generally due to the nucleophilicity of the sulfite ion that may react by addition to carbonyl groups, carbon–carbon double bonds, quinines, heterocyclic nitrogen compounds or by cleaving disulfide bounds [3]. Sulfite is also known to present some cytotoxic, mutagenic and antinutritional effects [4]. In particular, it interacts with some vitamins, i.e. pyridoxal, nicotin amide, thiamine, folic acid, reducing the nutritional quality of treated food [5].

The quantitative determination of sulfite in different types of samples has been reported by employing a range of analytical techniques, which include iodimetry [6], spectrophotometry [1,7–10], anodic stripping voltammetry [11], reciprocal oscillographic chronopotentiometry [12], ion chromatography [13], enthalpimetry [14], chemiluminescence [15,16], gravimetry [17], ion selective electrode [18,19], gas chromatography [20] and biosensor [25,26]. The samples analyzed in these experiments ranged from water, wastewater and food materials to inorganic compounds.

In this paper, we present an amperometric biosensor based on plant tissue homogenate to determine sulfite in food sample. The plant used in the preparation of the biosensor was *Malva vulgaris* that contained sulfite oxidase enzyme (E.C.1.8.3.1) abundantly. Sulfite oxidase catalyses the final reaction in oxidative degradation of sulphur-containing amino acids cysteine and methionine.



The enzyme is physiologically important; its absence may even lead to death [21]. The principle of the measurements

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was based on the determination of the decrease of oxygen concentration, which had been caused by the activity of sulfite oxidase in the bioactive material.

2. Materials and methods

2.1. Chemicals

Chemicals were obtained from either E. Merck (Germany) or Sigma-Aldrich Chemical Co. (USA) as the analytical grades. The *M. vulgaris* used was harvested from a local garden. After harvesting, they were kept at -20°C . The foodstuffs tested were purchased from Turkish manufacturers. (Pickle water from İpekeli Co., Biscuits from Ülker Co., Beer from Tuborg Co., Soup from Lezzo Co. and Vinegar from Fersan Co.)

2.2. Apparatus

YSI 54 A model oxygen meter and YSI 5700 series dissolved oxygen (DO) probes (YSI Co Inc., Yellow Springs, Ohio, 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 a temperature of 35°C around the cell compartment during the experiment.

2.3. Procedure

2.3.1. Dissolved oxygen probe

To construct the biosensor, a dissolved oxygen probe was covered with highly sensitive Teflon membrane by using an 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. Preparation of the bioactive layer material

M. vulgaris leaves were firstly homogenized with 1 ml phosphate buffer (pH 7.5 and 50 mM) by a manual glass homogenizer. Five milligrams of gelatin (Type 3, 225 Bloom) was weighed and added to a test tube. Then, 250 μL *M. vulgaris* tissue homogenate was pipetted into the test tube. This mixture was incubated at 38°C for 5–10 min to dissolve gelatin.

2.3.3. Biosensor preparation

Two hundred microlitres gelatin of *M. vulgaris* tissue homogenate mixture was dispersed over the dissolved oxygen probe membrane and allowed to dry at 4°C for 15–30 min.

For crosslinking with glutaraldehyde, the probe carrying bioactive layer was immersed into 2.5% (v/v) glutaraldehyde solution (in phosphate buffer, 10 ml, 50 mM, and pH 7.5) and was allowed to wait 5 min in this solution. At the end of this time, the biosensor was washed with distilled water and it was ready to use.

In order to prevent drying out of 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.

2.3.4. Measurement procedure

The biosensor based on *M. vulgaris* tissue homogenate was put into the thermostatic reaction cell containing 49 ml of 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 standards or samples (1 ml totally) were injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease, and a few minutes 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 standard curves which were obtained by the determination of consumed oxygen level (ΔDO : Δ dissolved oxygen)) by the enzymatic reaction of sulfite oxidase in bioactive layer of the biosensor.

2.3.5. Measurement time

Measurement time was the passing time from the injection of the sample or standard into the reaction cell to reach equilibration of enzymatic reaction of sulfite oxidase. This period was varied from 6 to 8 min for low sulfite concentrations and for higher sulfite concentrations in the linear range of the biosensor the measurement time was varied from 8 to 10 min.

3. Results and discussion

3.1. Optimization studies of the biosensor

In the optimization studies of the biosensor, the effects of the quantity of *M. vulgaris* tissue (50 mg, 100 mg and 200 mg) and the quantity of gelatin (2.5 mg, 5 mg and 10 mg) on the biosensor, and the effect of the crosslinking agent glutaraldehyde (1.25, 2.5 and 5%) on the biosensor were investigated. Measurements were accomplished by using each of the standard curves obtained under these conditions. The results of the optimization studies are displayed in Figs. 1–3, respectively.

In the optimization studies of the amount of *M. vulgaris* tissue, using more than 200 mg tissue was not possible

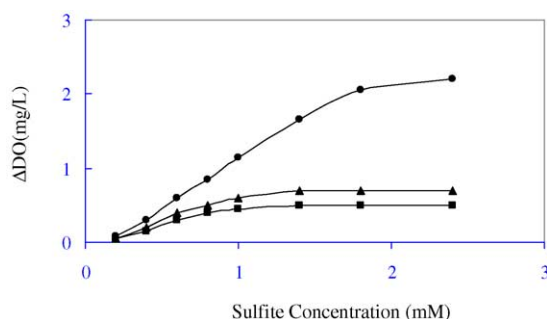


Fig. 1. Optimization of the quantity of *Malva vulgaris* tissue. [Phosphate buffer; pH 7.5, 50 mM; T 35 °C. The amount of *Malva vulgaris* tissue; ●: 200 mg, ▲: 100 mg and ■: 50 mg. The amount of glutaraldehyde and gelatin were kept constant at 2.5% and 5 mg, respectively.]

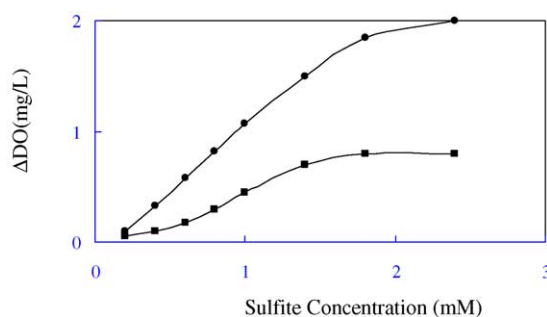


Fig. 2. Optimization of the gelatin amount. [Phosphate buffer; pH 7.5, 50 mM; T 35 °C. The amount of gelatin; ●: 5 mg gelatin and ■: 10 mg gelatin. The percentage of glutaraldehyde and *Malva vulgaris* amount were kept constant at 2.5% and 200 mg, respectively.]

because that amount of the plant to homogenate was very concentrated. So, it was not formed a suitable bioactive layer on the electrode. Moreover, at the end of the investigation of the effects of gelatin on the biosensor response, we saw that the biosensor prepared by using 2.5 mg gelatin was destroyed because of the lack of physical stability. Consequently, it was not able to make measurement with this biosensor.

These studies showed that optimum *M. vulgaris* tissue quantity, optimum gelatin quantity and optimum glutaraldehyde percentage were 200 mg, 5 mg and 2.5%, respectively. In all experiments, *M. vulgaris* tissue and gelatin quantity,

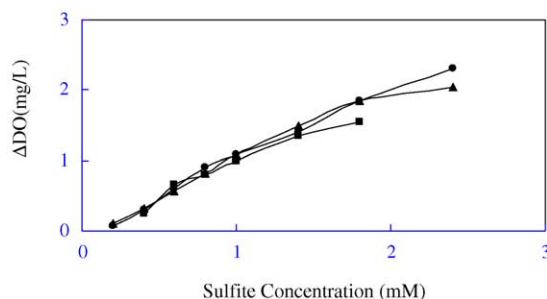


Fig. 3. Optimization of the glutaraldehyde percentage. [Phosphate buffer; pH 7.5, 50 mM; T 35 °C. Glutaraldehyde percentages; ●: 2.5%, ▲: 5% and ■: 1.25%. *Malva vulgaris* and gelatin amounts were kept constant at 200 mg and 5 mg, respectively.]

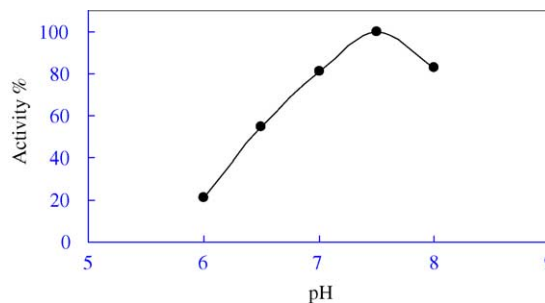


Fig. 4. The effect of pH on the biosensor response. [Phosphate buffers, 50 mM and pH 6.0, 6.5, 7.0, 7.5, 8.0. Sulfite was injected to a final concentration of 1 mM. The activity at pH 7.5 was set to 100% in each buffer, T 35 °C.]

and glutaraldehyde percentage were kept constant at 200 mg, 5 mg and 2.5%, respectively.

3.1.1. Effect of pH

All buffers had a concentration of 50 mM. Optimal biosensor response was obtained at pH 7.5 (Fig. 4). The response at pH 7.5 was set as 100% for each buffer. The oxygen consumption rate was 83% at pH 8 and 81% at pH 7 compared to pH 7.5. In contrast to that, the absolute signal height decreased dramatically to 21% at pH 6. Consequently, optimum pH was accepted as 7.5.

3.1.2. Dependence on temperature

Fig. 5 shows the influence of temperature on the response of the biosensor. The best biosensor responses resulted between 30 and 50 °C. To prolong the life of the biosensor, the measurements were done at a temperature of 35 °C. At this temperature also, the biosensor showed its highest signal. As a result, the working temperature was 35 °C.

3.2. Characterization studies of the biosensor

3.2.1. Linear range

The combination of *M. vulgaris* with an oxygen electrode led to a biosensor with a linear response for sulfite concentrations between 0.2 and 1.8 mM with a linear correlation coefficient of 0.9967. And the limit of detection (LOD) was

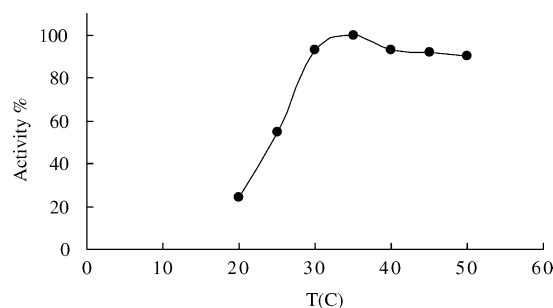


Fig. 5. The effect of temperature on the biosensor response. [Phosphate buffer, 50 mM, pH 7.5. Sulfite was injected to a final concentration of 1 mM. The temperature of 35 °C was set to 100%.]

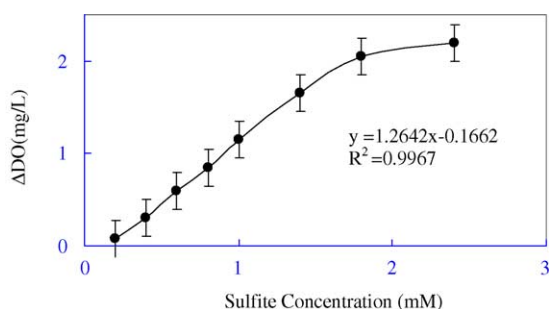


Fig. 6. Calibration curve for sulfite. [Measurements were done in 50 mM phosphate buffer at 35 °C and pH 7.5.]

0.2 mM. The linear calibration curve of the biosensor is given in Fig. 6.

3.2.2. Repeatability

Repetitive injections with 1 mM sulfite standard were made. Seven injections were made with sulfite without loss of activity ($n = 7$). The average value ($\bar{x}$), the standard deviation (S.D.) and variation coefficient (C.V.) were calculated as 1.00 mM, ± 0.04 mM and 3.86%, respectively.

3.2.3. Storage stability

The performance of the biosensor was maintained after 10 days storage. The stability of the biosensor was acceptable with no decrease of the response after 4 days of storage at 4 °C. At the end of the day of 7th day, the biosensor maintained 62.5% of its initial activity. However, on the 10th day, the bioactive material was damaged physically. Preparation of the biosensor did not require any expensive chemicals, enzymes and apparatus. Because of this, 10-days storage stability was not so bad. However, it can be concluded that since storage stability is an important feature, this may be improved in the future development of the biosensor.

3.2.4. Sulfite determination in some food samples

The proposed biosensor based on the *M. vulgaris* tissue homogenate may be used directly determination of sulfite in food samples without any pretreatment, separation and derivatization steps. The accuracy of the sample analysis was validated by using enzymatic reference method [22]. Table 1

Table 1

Sulfite contents of some commercial products by enzymatic reference method [22] and the present biosensor

Sample	Found sulfite (ppm)		
	Reference ^a	Biosensor ^a	Relative error (%)
Pickle water	7812	7900	101.1
Biscuit a	102	108	105.5
Biscuit b	138	133	96.2
Beer	250	242	96.7
Soup	155	148	95.2
Vinegar	1345	1394	103.5

^a Mean of three determinations.

shows the results of the sample obtained with different two methods.

4. Conclusion

The results presented in this work demonstrate that plant-tissue homogenate biosensor, based on *M. vulgaris*, is feasible for the detection of sulfite. The biosensor is readily prepared from inexpensive and commercially available materials through a general method. At the end of this work, the biosensor showed very good performance for detection of sulfite. A wide linear response range between 0.2 and 1.8 mM of sulfite was verified. However, the detection limit of the biosensor was capable of monitoring of sulfite in food samples studied. Another parameter that made the present method advantageous was measurement time. Total measurement time was about 10 min for the present method. However, some methods' measurement times usually range from 20 min to few hours [1,5,23,24]. Besides these methods, two biosensor-utilizing pure sulfite oxidase were also reported [25,26]. The detection limits of both of the biosensors were better than that of the biosensor presented. However, using the pure enzyme in these biosensors was a disadvantage in terms of the cost of the biosensor. Moreover, the immobilization procedure used was much easier than the procedure used by other authors. Consequently, the simplicity in the bioactive material immobilization demonstrates a considerable economic advantage because of low cost, which is desirable in routine analysis. In this respect, the system described is a simple and inexpensive method for monitoring of sulfite in food.

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