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A FIBER OPTIC BIOSENSOR FOR SULFITE ANALYSIS IN FOOD

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Summary—The sulfite fiber optic biosensor developed herein is based on the enzymatic oxidation reaction of sulfite catalyzed by sulfite oxidase (SOD). The consumption of O_2 is measured with an O_2 fiber optic sensor which monitors the fluorescence quenching of the indicator, perylene, by molecular oxygen. Perylene is immobilized into a polymer matrix and attached to the end of a fiber bundle forming an O_2 sensor. The enzyme, sulfite oxidase is immobilized on a pre-activated membrane and mounted onto the O_2 sensor. Several analytical characteristics of this sulfite biosensor were investigated including dynamic range, reversibility, reproducibility, stability and selectivity. The sulfite contents of various food samples, e.g. dried fruits, potato flakes, lemon juice were determined and the results obtained were in good agreement with the standard AOAC method.

Sulfite is commonly used in the food industry as a preservative. It is added to food products to prevent oxidation and bacterial growth. Since sulfite is also considered as a hazard to human health, the Food and Drug Administration has set a 10 ppm limit of sulfite in certain foods. Therefore, the development of a sensitive and convenient method for sulfite analysis is very important for food assurance and quality control.

The AOAC methods used for sulfite analysis in food usually need extensive sample pretreatment and reagent preparation.[1,2] Several new enzymatic methods have been reported in the literature recently.[3–7] Smith and co-workers constructed a sulfite-sensing electrode by physically trapping sulfite oxidase, which catalyzes the oxidation of sulfite to sulfate,

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{SOD} SO_4^{2-} + H_2O_2 \quad (1)$$

at the tip of an oxygen electrode[3]. The decrease in oxygen during the enzymatic reaction was measured amperometrically. Using the same enzyme, Masoom *et al.* developed a flow injection analysis system for sulfite determination based on the amperometric measurement of the enzymatically generated hydrogen peroxide.[4] Another method developed by Keil *et al.* was based on the use of a coupled enzyme assay (sulfite oxidase and NADH-dependent peroxidase) and the subsequent fluorescence measurement of the decrease in NADH.[5] The product (NAD) was amplified by enzymatic cycling. This technique improved sensitivity with a reported detection limit down to femtomole level. However, additional NADH-peroxidase, NADH and the enzymes necessary for the cycling had to be used leading to an increase in the cost. Coury and co workers described another electrochemical method for the anaerobic determination of sulfite, where they evaluated electron mediators for transporting electrons between enzyme and electrode.[6]

Most optical oxygen sensors have been based on fluorescence quenching. Polycyclic aromatic hydrocarbons and ruthenium complex compounds have been used as fluorophores,[7–10] and different methods were developed for embodying the fluorophore into a polymeric matrix.

The approach for constructing a fiber optic sulfite biosensor described here is based on the fluorescence quenching measurement of oxygen consumed during the enzymatic reaction (1). Perylene, a polycyclic aromatic hydrocarbon fluorophore was used as an indicator and was

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embedded in a siloprene matrix (a silicone rubber). Hydrophobic silicone polymer is considered as an excellent support because of its good solubility for oxygen and mechanical stability. Also silicone matrix is useful for indicator entrapment where the indicator can be physically retained. The analytical characteristics of the sensor as well as its application to some food analysis were studied.

EXPERIMENTAL

Reagents and apparatus

Sulfite Oxidase (SOD, E.C. 1.8.3.1) from chicken liver as a suspension in ammonium sulfate solution (1780 units/ml) and ascorbate oxidase were obtained from Sigma Chemicals Co. (St. Louis, MO). Perylene was bought from Aldrich Chemical Company, Inc. (Milwaukee, WI). Siloprene K 1000 and siloprene Crosslinking agent K-11 were purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Immunodyne membrane was from Pall Biosupport (Glen Cove, NY). The rest of chemicals were of reagent grade and used without further purification. Deionized water was used through the whole experiment.

Fluorescence measurements were made with a Perkin-Elmer model 650-10s fluorescence spectrophotometer modified to facilitate a fiber optic arrangement as described earlier.[11] The bifurcated fiber optic bundle used was from Oriel model 77533. A research pH meter (Radiometer, model PHM 84) was used for all the pH measurements. A Beckman model DB-G grating spectrophotometer was also employed to measure the sulfite contents in food samples by the AOAC method for evaluating the proposed method.

Preparation of the indicator polymer membrane

Perylene (0.5 mg) was dissolved in 1 ml of siloprene crosslinking reagent K-11 in a small vial. In another vial, 60 mg of siloprene K 1000 was weighed and mixed with 20 μl of the perylene-K-11 mixture with a glass stirring rod. A few seconds later, 4 μl of the mixture was spread on a piece of polyethylene membrane and allowed to polymerize overnight. A thin transparent polymer membrane was formed and was peeled from the supporting polyethylene membrane.

Construction of a sulfite sensor

Eight to 10 microlitres of sulfite oxidase was pipetted onto an immunodyne membrane. After drying in air for 20 min, the enzyme membrane was covered with a dialysis membrane, then both membranes were mounted on the end of a Teflon tube with an o-ring to form a cap which fitted the fiber end exactly. To construct the sulfite sensor, the perylene siloprene membrane was first attached to the common end to a bifurcated fiber bundle, then the fiber was slowly inserted into the Teflon cap placing the indicator layer between the fiber end and the enzyme membrane.

Measurement procedure

The sulfite fiber optic sensor was first immersed in air-saturated 0.2*M* phosphate buffer solution (pH = 7.5). A relatively low fluorescence signal was observed due to the quenching by oxygen in the buffer and a baseline was established. Upon the injection of sulfite solution, the signal was increased, where the change was related to sulfite concentration. Fluorescence measurement was done at the excitation wavelength of 413 nm and emission of 473 nm. Sulfite solutions are not stable, and a fresh solution had to be prepared daily.

Sulfite determination in food samples

Ten different food samples were bought from a local supermarket including apple chunks, apricots, extra large prunes, California figs, figlet, golden raisins (from Sun-Diamond Growers of California, Pleasanton, CA); mashed potato flakes (from Idahoan Foods, Lewiville, Idaho); Red rose wine (from Gall Vineyards, Modest, CA); red wine vinegar from Nabisco Brand, Inc., East Hanover, NJ) and lemon juice (from Borden, Inc.-T, Columbus, OH). In a typical determination for a solid sample, 10 g of sample was blended with 290 ml of deionized water for five minutes. Then the sample solution was centrifuged and the supernatant was analyzed by the proposed sulfite sensor and the AOAC standard method, para-rosaniline colorimetric method. Liquid samples were analyzed without pretreatment.

RESULTS AND DISCUSSION

Perylene siloprene membrane

The amount of perylene used for making an indicator membrane is very critical to the sensor's performance. Since fluorescence quenching by molecular oxygen reduces the fluorescence intensity, a large initial fluorescence intensity is desirable. Too much perylene,

Table 1. Interference study for sulfite fiber optic biosensor ($C_{sulfite} = 5 \times 10^{-4}$ M)

Interferant	Concentration (M)	Relative response (%)
NaCl	0.05	100
KCl	0.05	100
NaF	0.05	100
NaI	0.05	100
$NaNO_2$	0.05	60
Succinic acid	0.05	100
Glucose	0.05	75
L-Maleic acid	0.05	92
Sucrose	0.05	86
Fructose	0.05	72
Ascorbic acid	0.05	120
Ethanol	0.05	64
Benzoic acid	0.01	84
Salicylic acid	0.01	84
Thiourea	0.01	60

however, can result in reduced fluorescence intensity because of concentration quenching and/or inner cell effect. The best responses were achieved when the concentration of perylene in K-11 was 0.05%.

The ratio of K-11 to K 1000 has also pronounced effect on both the final concentration of perylene in siloprene matrix and the physical property of the siloprene polymer. High ratios resulted in the polymer crunching which was broken down easily. However, too low ratios led to a decrease in perylene concentration and the polymer became sticky and difficult to dry. The best ratio was determined to be 20 μl of K-11 containing 0.05% of perylene with 60 mg of K 1000.

In addition, the thickness of the indicator polymer membrane affected the sensor's performance. It depended on the amount of final mixture to be spread on the polyethylene support membrane and the spread area. Optimum response was obtained when 4 μl of the mixture was spread on a polyethylene membrane circular disc (5 mm in diameter).

pH effect

The pH effect of the sulfite biosensor was investigated over the range of 6–8.5. The response increased from pH 6 to pH 7.5, then decreased. The maximum response pH of the sensor is very close to that of soluble enzyme.[12]

Sensitivity and response dynamic range

The dynamic range of this sulfite fiber optic biosensor depended on the sulfite oxidase activity. At high enzyme activity, the sensitivity of the sensor increased, however, the response was saturated at the sulfite concentration of 100 ppm as shown in the calibration curve (Fig. 1). Since the sensor was based on the fluorescence quenching by molecular oxygen, the response was limited by the maximum quenching efficiency for the proposed system which was determined to be 20–25%. When the activity

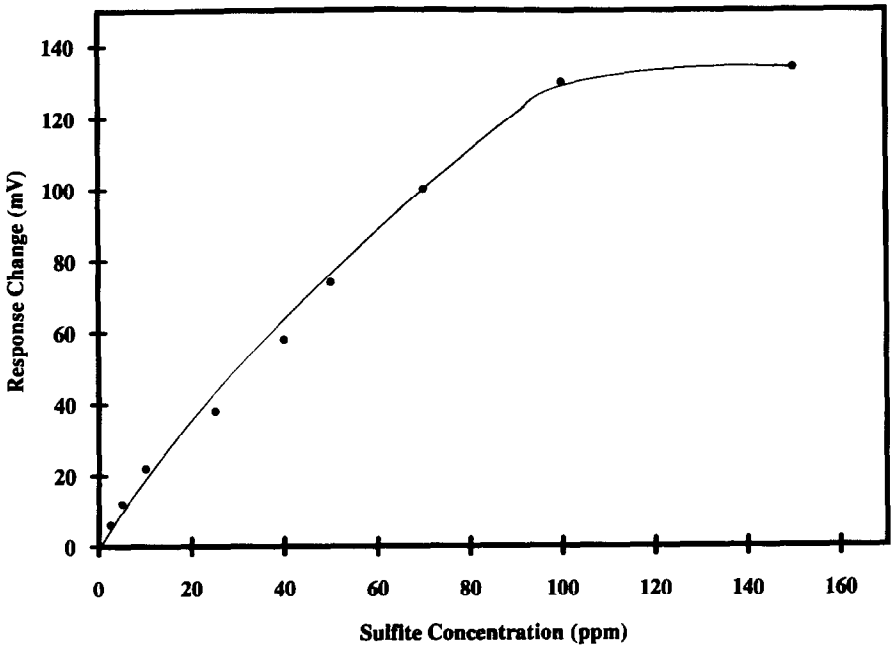

Fig. 1. Calibration curve of sulfite fiber optic biosensor; 18 units of sulfite oxidase was used.

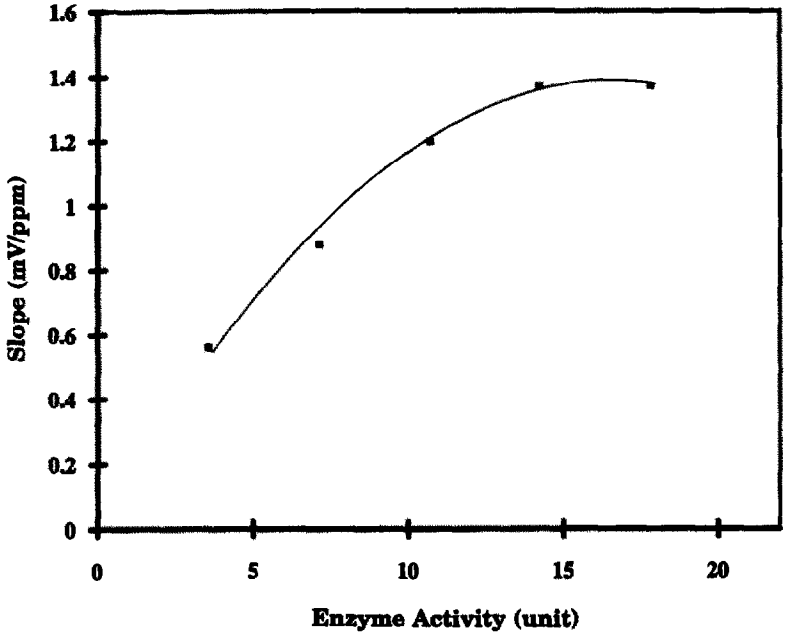

Fig. 2. Slope of calibration curve at sulfite concentration of 50 ppm *vs* activity of immobilized enzyme.

decreased, the sensitivity decreased, but the dynamic range was extended up to 250 ppm of sulfite. Figure 2 shows the sensitivity (slope of calibration curve at the sulfite concentration level of 50 ppm) change with enzyme activity.

Reproducibility and stability

The reproducibility of the proposed sensor was studied by measuring a 50 ppm sulfite solution 10 times. The coefficient of variation was 5.0%. The stability of this sensor is affected

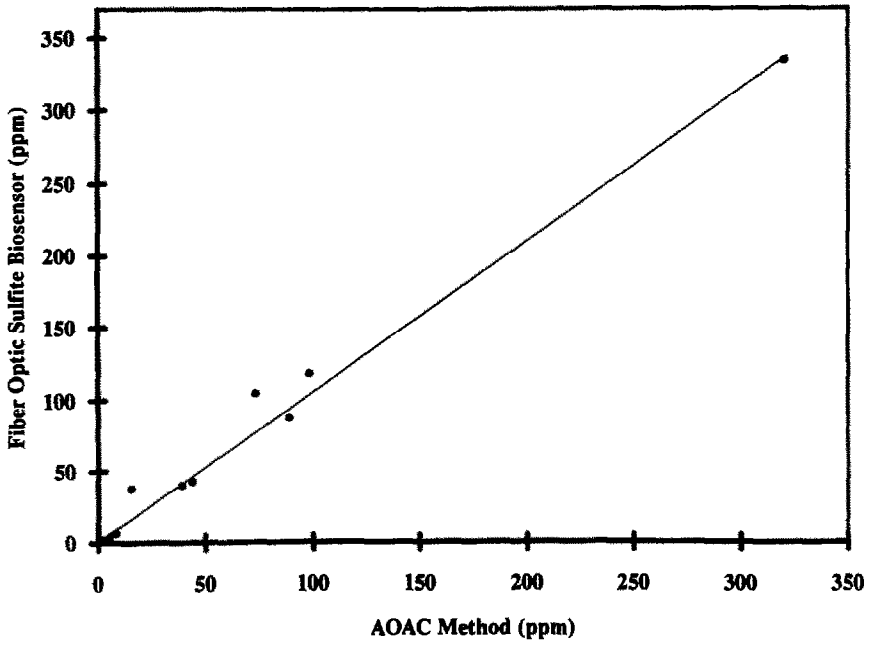

Fig. 3. Correlation of the sulfite fiber optic biosensor with AOAC para-rosaniline method for the sulfite determination in food samples. (1) Figlet. (2) Prune. (3) California figs. (4) Rose wine vinegar. (5) Raisin. (6) Potato flakes. (7) Apricots. (8) Apple chunks. (9) Rose wine. (10) Lemon juice.

by the stability of the enzyme membrane and the indicator in the polymeric membrane. The indicator membrane was very stable and could be used for several months. However, the sulfite oxidase membrane was not very stable. It only retained 50% of its initial activity after 3 days. Since the preparation of the enzyme membrane was simple and quick, the technique is still useful for practical applications. Other immobilization methods such as molecular cross-linking using glutaraldehyde may increase the stability, however, a much longer time is needed for making an enzyme membrane.

Interference study

Similar to other enzymatic methods,[3-5] a positive response to the presence of ascorbic acid was noticed. This is due to the oxidation of ascorbic acid which consumes oxygen in solution. To eliminate the interference from ascorbic acid, samples were pre-treated with ascorbate oxidase.

Food analysis

Sulfite contents in 10 different food samples were analyzed by both the sulfite sensor and standard AOAC methods. The correlation of these two methods is shown in Fig. 3. The numbers reported for apple chunks, apricots, raisin, figlets, California fig, prunes and potato flakes are the sulfite concentration of their aqueous solutions (10 g of solid sample in 290 ml of water).

The correlation between the two methods can be described by a linear regression equation with a slope of 1.040, an intercept of 0.045 ppm, and a coefficient (r^2) of 0.985. This good agreement suggests that the proposed method can be used successfully for food analysis.

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