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An inhibitory enzyme electrode for hydrogen sulfide detection

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ABSTRACT

An enzymatic biosensing system has been developed to study the capability of ascorbate oxidase (AOx), EC (1.10.3.3), in hydrogen sulfide (H_2S) detection, based on the inhibition of AOx activity. The immobilization parameters including glutaraldehyde (GA) concentration and pH were optimized using experimental design. The optimized values of GA concentration and pH were found to be 12.5% (w/w) and 7, respectively, where the enzymatic reaction reached the steady-state level within 55 s. A linear relationship was observed between the decrease in the oxygen concentration and H_2S concentration, where H_2S concentration is in the range of 1–15 mg/L. Moreover, to investigate the selectivity of the biosensor, a certain H_2S concentration (9 mg/L) was used against different ions. The results indicated that Fe^{3+} and SO_4^{-2} ions had no significant (11% error) effect on the H_2S detection. The operational stability of the biosensing system was determined in terms of response to H_2S concentration, at optimal working conditions. The enzyme electrode could retain 73% of its original sensitivity after this period, which has made it possible for the system to measure H_2S with concentrations as low as 0.5 mg/L.

1. Introduction

Chemical composition of gases is an important concept in fields such as healthcare and environment [1]. H_2S gas emission in environmental and industrial settings such as swamps [2], paper manufacturing industries; waste water treatment, natural gas, coking coal, and food processing units [3] can be extremely dangerous to human health, depending on its concentration. The detection of H_2S can be done based on its physicochemical and biological properties. H_2S is water soluble, colorless, and flammable, which inhibits enzyme activities and is in equilibrium with bisulfide (HS^-) and sulfide (S^{-2}) in aqueous solutions [4]. Several methods have been developed to monitor the hydrogen sulfide concentration including (but not limited to) chromatographic [5], spectrophotometric [6] polarographic [7], amperometric [8], and potentiometric [9].

Biosensors have also been developed for specific determination of chemical compounds with simplicity, specificity, and accuracy. In recent years, enzymatic biosensors have become more practical than other detection methods, as a result of their selectivity and sensitivity [10–12]. Biosensors that are based on enzyme inhibition are used for detection and concentration measurement of the inhibitory compounds [13]. H_2S sensors with an electrochemical transducer and cytocrome c oxidase as the recognition element have been studied by many researchers [14–16]. Yang et al. [17] and Liu et al. [18] developed an amperometric biosensor for measuring sulfide concentration based on horseradish peroxidase inhibition. They have reported sulfide detection in the range of 0.5–12.7 μm, with a detection limit of 0.3 μm. They concluded that the detection limit was improved by this method but the poor selectivity remained as a problem yet to be solved, which has restricted its applicability. Shahidi Pour Savizi et al. [19] fabricated an ampprometric biosensor based on the inhibition of *Coprinus cinereus* peroxidase against sulfide. Their sensor had a linear response in the

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range of 1.09–16.3 μm (detection limit of 0.3 μm) and a response time of 43 s. Kariminia et al. [20] constructed an optical biosensor based on fungal peroxidase inhibition for sulfide detection. To the best of our knowledge, there is no published report on the development of an amperometric biosensor according to the inhibitory effect of H_2S on the activity of ascorbate oxidase.

Ascorbate oxidase (AOx), EC (1.10.3.3) catalyzes the four-electron reduction of oxygen to water, using ascorbic acid as substrate [21]. This enzyme can be inhibited by small inorganic anions such as azide and fluoride, or organic molecules acting as competitive inhibitors toward ascorbate [22]. Ascorbate oxidase catalyzes the oxidation of ascorbic acid via molecular oxygen reduction. This reaction occurs on the sensing element of Clark dissolved oxygen electrode. Therefore, local oxygen depletion is resulted [12]. For the purpose of ascorbic acid detection, this enzyme is covalent binded on collagen [23], gelatin [12] nylon net [24] or adsorbed on a carbon felt [25].

The purpose of this study was to consider enzyme as the sensing element of a H_2S biosensor, according to its inhibitory effect on the activity of AOx. The enzyme was immobilized on the nylon membrane by Mascini's method [26] and the immobilization process was optimized [27]. The oxygen depletion was then measured in the absence and presence of the H_2S gas, using a dissolved oxygen electrode. The calibration curve, linear range, and detection limit were reported as well.

2. Materials and methods

2.1. Materials

Ascorbate oxidase (EC 1.10.3.3; 215 U/mg solid AOx) and GA were purchased from Sigma, USA. L-ascorbic acid, $Na_2HPO_4{\cdot}2H_2O$, $NaHPO_4{\cdot}2H_2O$, and DMS were bought from Merck (Darmstadt, Germany) and used as received without any further purification. Ascorbic acid solutions were prepared in 0.1 mol L^{-1} phosphate buffer pH 7.

2.2. Apparatus

The Clark type dissolved oxygen electrode (model 8401) was purchased from AZ Instruments, Taiwan. The nylon membrane from A. Bozzone was kindly supplied by Prof. M. Mascini (University of Florence, Italy) finally the tested biosensor was made at University of Tehran.

2.3. Preparation of bioactive layer

In this study, 200 μl of GA solution 12.5% (wt./wt.) was saturated in 0.1 M of borate buffer, pH 8.5. The prepared solution was mixed with 200 μl of the AOx (50 U). The enzyme solution was immobilized onto nylon membrane according to the Mascini's method [26]. The nylon membrane was stored overnight at 4 μl, was washed with 0.1 M phosphate buffer (pH 7), and then it was attached to the Clark oxygen electrode as a transducer.

2.4. Biosensor instruction

All the experiments were done in a bioreactor with a detection chamber, an oxygen electrode as transducer, two inputs for H_2S and air injection, and a computer to record dissolved oxygen concentration (Fig. 2). The oxygen sensor was inserted into the detection chamber containing 200 mL of phosphate buffer (pH 7.0, 100 mM); while the immobilized enzyme was fixed on the top of the dissolved oxygen probe by an O-ring and the solution was stirred with a magnetic bar.

2.5. Measurement of enzyme activity

The activity of the immobilized enzyme was determined by measuring the reduction current of oxygen. Since, in the enzymatic reaction, the steady-state current depends on the oxygen consumption; the oxygen depletion was recorded after the injection of 1 mM ascorbic acid (as the substrate), the air-saturated mixture of free enzyme (AOx), and 0.1 M phosphate buffer (as enzymatic solution (50 U)).

3. Theory

3.1. Optimization of the immobilization process

Optimization of AOx immobilization process on the nylon membrane was carried out at room temperature. The GA concentration (X_1) and pH (X_2) were considered as effective factors for this process and the influence of these parameters on the steady-state time of reaction was studied. CCD and RSM were used in order to investigate the relationship between the former variables and their optimum levels. For this purpose, 10 experimental runs were required as per three-level two-factor fractional factorial CCD (Table 1).

Table 1
The analytical parameters for optimization of enzyme immobilization.

NO	GA concentration (wt/wt)%	pH of enzyme solution	Steady-state time (s)
1	7.5	5.5	82
		7	63
		8.5	152
2	10	5.5	77
		7	60
		8.5	124
3	12.5	5.5	68
		7	55
		8.5	136

Table 2
Analytical parameters of H_2S injection.

NO	H_2S concentration ppm	Acid ascorbic mM	Inhibition %
1	9	1	93
		3	78
		5	68
2	5	1	80
		3	74
		5	70
3	1	1	44
		3	12
		5	<

Data from CCD was subjected to a second-order multiple regression analysis to explain the behavior of the system using the least squares regression methodology to obtain the estimators of the mathematical model [26]. The result can be expressed as follows:

$$Y = \beta_0 + \sum \beta_i \times X_i + \sum \beta_{ii} \times X_i^2 + \sum \beta_{ij} \times X_{ij} \quad (1)$$

where Y, X_i, β_0, β_i, β_{ii}, and β_{ij} are respectively the response, the independent variable, a constant, the slope or linear effect of the input factor, the quadratic effect of the input factor, and the linear by linear interaction effect between the input factors.

Statistical software was used to analyze the obtained data and to determine the optimum values of the variables.

3.2. Enzyme inhibition measurement

Oxygen depletion of the enzymatic reaction was measured in absence (A_0) and presence (A_i) of hydrogen sulfide (inhibitor). Gas with a certain concentration ($\leq$10 ppm) was injected into the bioreactor using a mass flow controller. The inhibition percentage (I %) was calculated using the following equation:

$$I\% = \frac{A_o - A_i}{A_o} \quad (2)$$

A different concentration of substrate (X'_1) and inhibitor (X'_2) were used to optimize the enzyme capability for H_2S detection by statistical software. For this purpose, 10 experimental runs were required as per three-level two-factor fractional factorial CCD (Table 2). All measurements were carried out in a batch system containing 300 ml of oxygen-saturated phosphate buffer (pH 7) solution.