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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.

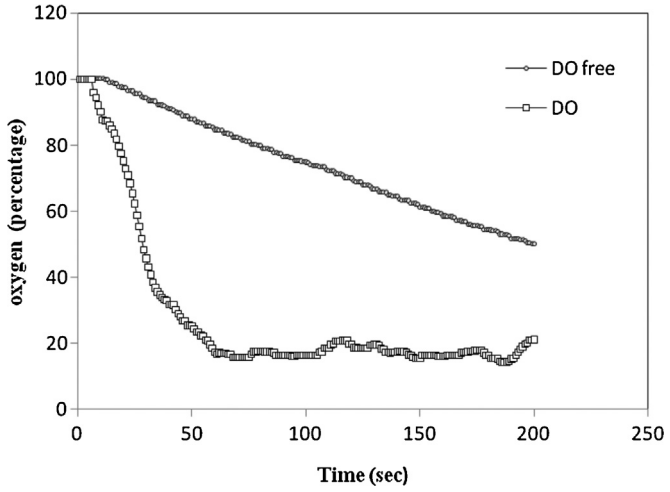

Fig. 1. Comparison between free (○) and immobilized (□) AOx. The steady-state time for immobilized enzyme was 60 s. 1 mM of acid ascorbic was used as a substrate.

4. Results and discussion

The H_2S detection capability of AO_X was studied in a batch system. The activity of AO_X was calculated using oxygen depletion before and after injection of H_2S to the biosensor.

4.1. Enzyme activity measurement

It has been assumed that AOx has been homogeneously distributed on the nylon. It was observed from Fig. 1 that the immobilized enzyme has better activity in comparison with the free one. Since the immobilized enzyme was fixed near the oxygen probe, the rate of oxygen depletion illustrated an increase, which decreased the steady-state level to 60 s. Moreover, the steady state response for the free enzyme sample was not reached till 100 s.

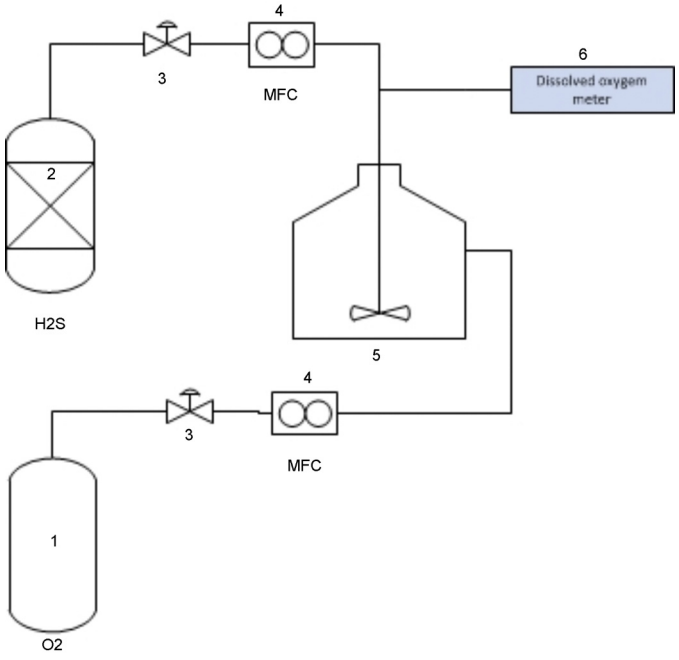

Fig. 2. The schematic diagram of the H_2S biosensing system.

Table 3
Parameters of the model for the bioreaction steady-state time.

p-value Prob > *F*	*F* Value	Mean square	Term
0	345.96	5704.2	X_2
0.007	25.27	416.7	X_1
0.160	2.97	49	X_1X_2
0	307.88	5189.4	X_2^2
0.763	0.10	1.7	X_1^2

4.2. The optimization of immobilization process

The results of the statistical design based on steady-state time are presented in Table 1. The quadratic polynomial model was established on the experimental results of CCD to identify the relationship between responses and variables. Parameters obtained through modeling are listed in Table 3.

According to Table 3, the first- and second-order terms of X_1 and the first-order term of X_2 were found to be significant ($P < 0.1$). The proposed model based on the regression coefficients for the steady-state time can be stated as:

$$R_1 = 60 + 30.83X_2 - 8.33X_1 + 46.5X_2^2 \quad (3)$$

where R_1, X_1 and X_2 were the steady-state time of the bio-reaction (s), the concentration of GA (w/w %), and pH, respectively. A positive coefficient in the equation represents a synergistic effect, while the negative sign indicates an antagonistic effect.

At constant GA concentration, reaction rate was defined based on the steady-state duration. The rate of reaction increased while the pH of the enzyme solution decreased. As can be seen from Table 1 and Fig. 3, the optimized condition for the immobilization process was recorded to be a high GA concentration (12.5%) and a low enzyme solution pH (6.7).

Fig. 4 indicates the effect of two variables (GA concentration and pH) on the steady-state time of the bio-reaction. Moreover, Fig. 5 compares the calculated and experimental values of steady-state time. Eq. (3) correlates 99% of the data with 11% error.

4.3. Effect of H_2S on bio-reaction

After enzyme immobilization, the effects of H_2S injection on bio-reaction were evaluated. Fig. 6 shows the variation of steady-state time in terms of dissolved oxygen reduction in a batch system versus time, both in the absence (a) and the presence of 10 ppm (b) H_2S. It can be seen that AOx has been inhibited in the presence of H_2S. In addition, the inhibitory action was also studied at lower

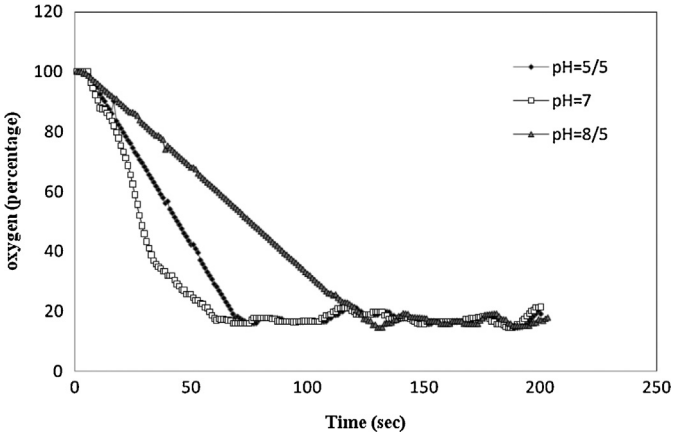

Fig. 3. Influence of pH on immobilization process at GA 12.5%.

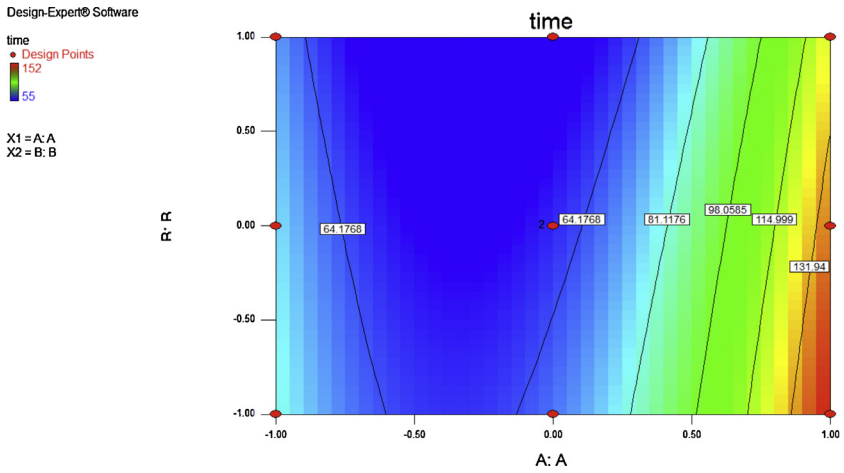

Fig. 4. Effect of GA concentration and pH on the steady-state time (at optimum conditions).

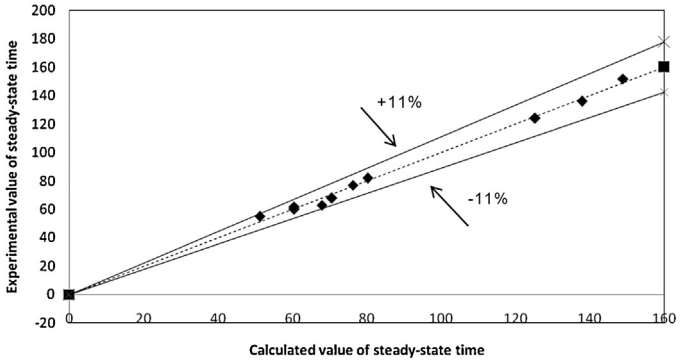

Fig. 5. Comparison between calculated and experimental values of steady-state time.

H_2S concentrations in order to optimize the inhibition percentage, influence of ascorbic acid concentration, and H_2S amount.

4.4. Influence of substrate and H_2S concentrations on steady-state time

As can be seen from Table 2 and Fig. 7, at constant inhibitor concentration (9 ppm H_2S), a decrease in the substrate concentration has resulted in an increase in the inhibition percentage. At low H_2S concentration (1 ppm) and high substrate concentration (5 mM), the inhibition percentage was not measurable (<12%). The results

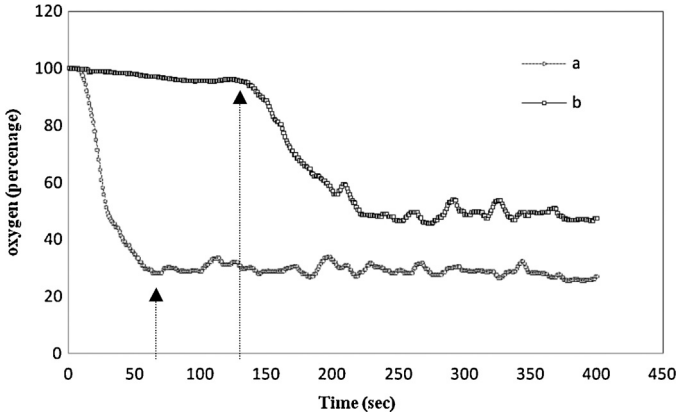

Fig. 6. Electrode response for 1 mM of ascorbic acid in the presence (□) and in the absence (○) of 10 ppm hydrogen sulfide. The value of inhibition percentage was 97.

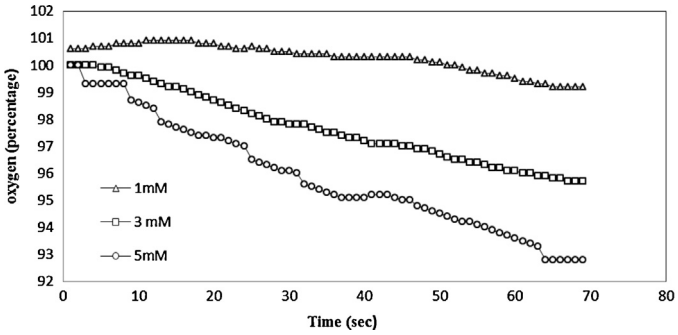

Fig. 7. Influence of acid ascorbic concentration on the inhibition rate at 9 ppm H_2S.

of optimization process pointed out that the best sensitivity was obtained at 1 mM and 9 ppm concentration of ascorbic acid and H_2S, respectively.

According to Table 4, the linear and square effects of X_1 and the linear effect of X_2 were found to be significant ($P < 0.1$). The proposed model based on the regression coefficients for inhibition percentage can be expressed as:

$$R'_1 = 72.55 + 31.83X'_2 - 11.67X'_1 - 21.43X'^2_2 \quad (4)$$

where R'_1 was the inhibition percentage of the bioreaction, X_1 and X_2 were the concentration of acid ascorbic (mM) and H_2S (ppm), respectively. Fig. 8 illustrates the effect of the two variables (acid ascorbic and H_2S concentration) on the inhibition percentage. Fig. 9 shows the comparison between the calculated and experimental values of inhibition effect. Eq. (4) correlated 99% of the data with 11% error.

Reports of using ascorbate oxidase as an enzyme electrode have been summarized in Table 5. Ascorbate oxidase has been used in many different biosensing systems, however sufficient data have not yet been provided for H_2S monitoring. Having said that

Table 4
Parameters of the model for the bioreaction inhibition percentage.

p-value Prob > *F*	*F* value	Mean square	Term
0.0001	156.6	6080.17	X'_2
0.0025	21.04	816.67	X'_1
0.5413	0.41	16	$X'_1X'_2$
0.0007	32.68	1268.51	X'^2_2
0.3137	1.18	45.73	X'^2_1
–	–	271.70	Residual

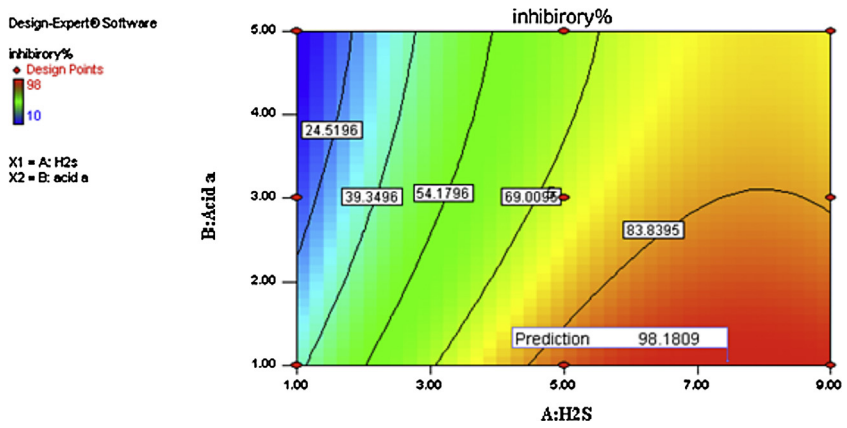

Fig. 8. Effect of acid ascorbic and H_2S concentration on inhibition percentage.

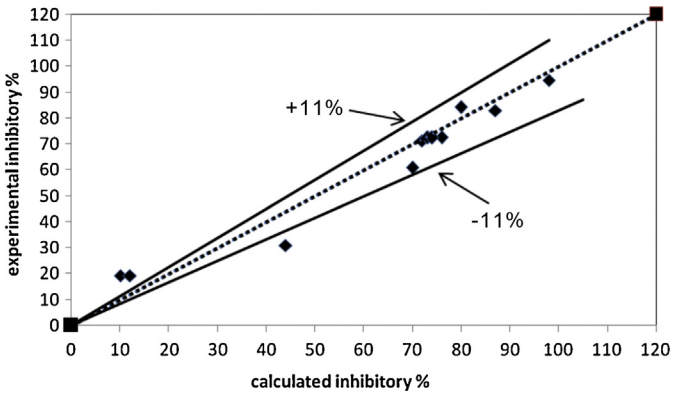

Fig. 9. Comparison between calculated and experimental values of inhibition percentage.

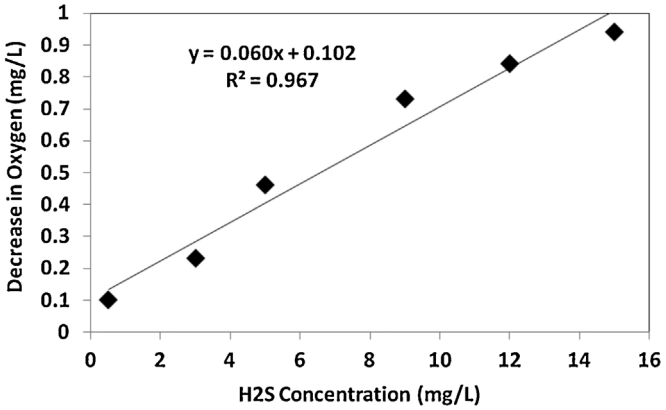

Fig. 10. Calibration curve for H_2S determination with AOx inhibition biosensor.

Albery et al. used free cytochrome c oxidase for H_2S monitoring; in this study, the steady-state time was shown to be better than cytochrome c oxidase electrode [14–16]. Also, Shahidi Pour Savizi et al. [19] has used *Coprinus cinereus* peroxidase (CIP), immobilized on screen printed electrode, for sulfide detection. In this case, the response time has reached 43 s, which is quicker than HRP sensor developed by some researchers (65 s) [19]. In addition, Mirzaei et al. [29] used a microbial bio-sensing device for H_2S sensing, which has the response time of 80 s, similar to other researchers [28].

4.5. Calibration curve, detection limit and life time

The calibration curve was derived using the effective factors at their optimal levels. A linear relationship was observed between the oxygen concentration (the concentration difference between the initial and the steady state) and the hydrogen sulfide concentration in the range of 1–15 mg/L (Fig. 10). In this way, the response time reaches 55 s which is shorter than 65 s reported by Liu et al. [18]. It is worth noting that the response time reported by Shahidi Pour Savizi et al. was 43 s [19] and the detection limit was 0.5 mg/L. The aim of this work was to show the AOx capability as a sensing element for the production of a reliable new enzyme electrode for H_2S detection.

In order to determine the operational stability, the biosensing system was used to assess its response to H_2S concentration at optimal working conditions. The results demonstrated that the enzymatic biosensing system still maintains its initial sensitivity at the end of the operational period. The storage stability is an important consideration for long-term applications of biosensors. The storage stability of inhibitory enzyme electrode was determined using a periodically assessment of its sensitivity to H_2S concentration, at two weeks intervals for a two months period. The enzyme electrode could retain 73% of its original sensitivity after this period.

4.6. Selectivity

Selectivity is an important parameter in determination of components used in enzyme inhibition biosensors. Several anions and cations were selected from environmental matrix components to investigate their possible interference on the determination of

Table 5
Comparison of different enzyme electrodes.

References	Steady-state time(s)	Immobilized system	Biosystem	Monitoring substrate
Tomita et al. [24]	60	Nylon membrane	Ascorbate oxidase	*Acid ascorbic*
Akyilmaz et al. [12]	45	Teflon membrane	Ascorbate oxidase	*Acid ascorbic*
Albery et al. [14–16]	600–1200	Gold disk	Cytochrome c oxidase	H_2S
Shahidi Pour Savizi et al. [19]	43	On screen printed electrode	CIP	S^-
Liu et al. [18]	65	–	HRP	S^-
This work	65	Nylon membrane	Ascorbate oxidase	H_2S

sulfide. In order to investigate the influence of various anions and cations on the biosensor performance, a specific hydrogen sulfide concentration (9 mg/L) was used against different ions. Fe^{3+} Cations and SO_4^{-2} anions had the less (11% error) interference on H_2S detection. This interference results were in agreement with the previous results reported by Kariminia et al. [20].

Moreover, CH_4, N_2, and CO_2 interference were also considered during H_2S measurement. The results indicated the fact that in a constant response time, gas interference on the response time of H_2S enzymatic biosensor can be neglected.

5. Conclusion

Ascorbate oxidase was found to be inhibited by H_2S in a batch system using a Clark electrode as the transducer. It has proven to be a simple method for monitoring bio-reaction before and after H_2S injection. In this study, nylon membrane has been used as a carrier due to its high strength. The best steady-state time (55 s) was obtained in 12.5% GA concentration and a pH equal to 7. After H_2S injection, the inhibition percentage could be measured at low concentrations of H_2S ($\leq$10 ppm). 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 with a detection limit of 0.5 mg/L. At low ascorbic acid concentrations and high H_2S concentrations, the inhibition percentage reached the optimized value. The results show the best capability of ascorbate oxidase due to the H_2S inhibition effect.

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References

[1] Hulko M, Hospach I, Krasteva N, Nelles G. Cytochrome C biosensor, a model for gas sensing. Sensors 2011;11:5968–80.
[2] Lomans BP, Pol A, Op den Camp HJ. Microbial cycling of volatile organic sulfur compounds in anoxic environments. Water Sci Technol 2002;45:55–60.
[3] Ramirez M, Gomez JM, Aroca G, Cantero D. Removal of hydrogen sulfide by immobilized *Thiobacillus thioparus* in a biotrickling filter packed with polyurethane foam. Bioresour Technol 2009;100:4989–95.
[4] Brouwer H. Acid volatile sulfides (AVS) in sediment. An environmental chemistry experiment. J Chem Educ 1995;72:182–3.
[5] Howard AG, Yeh CY. Sulfide measurement by flow injection analysis with flame photometric detection. Anal Chem 1998;7:4868–72.
[6] Guenther EA, Johnson KS, Coale KH. Direct ultraviolet spectrophotometric determination of total sulfide and iodide in natural waters. Anal Chem 2001;73:3481–7.
[7] Canterford DR. Simultaneous determination of cyanide and sulfide with rapid direct current polarography. Anal Chem 1975;47:88–92.
[8] Lawrence NS, Jiang L, Jones TGJ, Compton RG. A thin-layer amperometric sensor for hydrogen sulfide. Anal Chem 2003;75:2499–503.
[9] Hassan SSM, Marzouk SAM, Sayour HEM. Methylene blue potentiometric sensor for selective determination of sulfide ions. Anal Chim Acta 2002;466:47–55.
[10] Chen S, Chen Z-j, Ren W, Ai H-w. Reaction-based genetically encoded fluorescent hydrogen sulfide sensors. J Am Chem Soc 2012;134:9589–92.
[11] Sasakura K, Hanaoka K, Shibuy N, Mikami Y, Kimura Y, Komatsu T. Development of a highly selective fluorescence probe for hydrogen sulfide. J Am Chem Soc 2011;133:18003–5.
[12] Akyilmaz E, Dinckaya E. New enzyme electrode based on ascorbate oxidase immobilized in gelatin for specific determination of L-ascorbic acid. Talanta 1999;50:87–93.
[13] Luque de Castro MD, Herrera MC. Enzyme inhibition-based biosensors and biosensing systems: questionable analytical devices. Biosens Bioelectron 2003;18:279–94.
[14] Albery WJ, Cass AEG, Shu ZX. Inhibited enzyme electrodes. Part 1: Theoretical Model. Biosens Bioelectron 1990;5:367–78.
[15] Albery WJ, Cass AEG, Shu ZX. Inhibited enzyme electrodes. Part 2: The kinetics of the cytochrome oxidase system. Biosens Bioelectron 1990;5:379–95.
[16] Albery WJ, Cass AEG, Shu ZX. Inhibited enzyme electrodes Part 3: Inhibited enzyme electrodes. A sensor for low levels of H_2S and HCN. Biosens Bioelectron 1990;5:397–413.
[17] Yunhui Y, Minghui Y, Hua W, Jianhui J, Guoli S, Ruqin Y. An amperomertic horseradish peroxidase inhibition biosensor based on Cysteamine self-assembled monolayer for the determination of sulfides. Sens Actuators B 2004;102:162–8.
[18] Lijun L, Zhichun C, Shaoming Y, Xin J, Xianfu L. A novel inhibition biosensor constructed by layer-by-layer technique based on biospecific affinity for the determination of sulfide. Sens Actuators B 2008;129:218–24.
[19] Shahidi Pour Savizi I, Kariminia HR, Ghadiri M, Roosta-Azad R. Amperometric sulfide detection using *Coprinus cinereus* peroxidase immobilized on screen printed electrode in an enzyme inhibition based biosensor. Biosens Bioelectron 2012;35:297–301.
[20] Kariminia HR, Ghadiri M, Roosta-Azad R. Spectrophotometric determination of sulfide based on peroxidase inhibition by detection of purpurogallin formation. Ecotoxicol Environ Saf 2013;91:117–21.
[21] Santagostini L, Gullotti M, De Gioia L, Fantucci P, Franzini E, Marchesini A, et al. Probing the location of the substrate binding site of Ascorbate oxidase near type 1 copper: an investigation through spectroscopic, inhibition and docking studies. Int J Biochem Cell Biol 2004;36:881–92.
[22] Rekha K, Gouda MD, Thakur MS, Karanth NG. Ascorbate oxidase based amperometric biosensor for organophosphorous pesticide monitoring. Biosens Bioelectron 2000;15:499–502.
[23] Matsumoto K, Yamada K, Osajima K. Ascorbate electrode for determination of ascorbic acid in food. Anal Chem 1981;53:1974–9.
[24] Tomita IN, Manzoli A, Fertonani FL, Yamanaka H. Amperometric biosensor for ascorbic acid. Eclética Quím 2005;30:37–43.
[25] Uchiyama S, Umetsu Y. Concentration-step amperometric sensor of l-ascorbic acid using cucumber juice. Anal Chim Acta 1991;255:53–7.
[26] Mascini M, Iannello M, Palleschi G. Enzyme electrodes with improved mechanical and analytical characteristics obtained by binding enzymes to nylon nets. Anal Chim Acta 1983;146:135–48.
[27] Weuster-Botz D. Experimental design for fermentation media development: statistical design or global random search. Biosci Bioeng 2000;90:473–83.
[28] Wen G, Zheng J, Zhao C, Shuang S, Dong C, Choi MFM. A microbial biosensing system for monitoring methane. Enzyme Microbial Technol 2008;43:257–60.
[29] Mirzaei M, Amoabediny GH, Yazdian F, Sheikhpour M, Ebrahimi E, Ebrahimi HB. An immobilized *Thiobacillus thioparus* biosensing system for monitoring sulfide hydrogen; optimized parameters in a bioreactor. Process Biochem 2014;49:380–5.