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Carboxyl esterase-alcohol oxidase based biosensor for the aspartame determination

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Abstract

This study attempted to establish an efficient biosensor for the aspartame determination in soft drinks and commercial sweetener tablets. The sensor was a bienzyme system composed of carboxyl esterase and alcohol oxidase, immobilized in gelatin membrane, subsequently combined with the dissolved oxygen electrode. The optimum operational conditions for the enzyme sensor were pH 8.0 and 37 °C. A linear relationship was observed between dissolved oxygen (D.O) consumption and the aspartame concentrations in the range of 5.0×10^{-8} and 4.0×10^{-7} M. One assay could be completed within 10 minutes. In the case of aspartame determination in commercial soft drinks and sweetener tablets by this system, the results were found to be in close agreement with the labeled values provided by manufacturer.

Keywords: Bienzyme sensor; Carboxyl esterase; Alcohol oxidase; Aspartame

1. Introduction

Aspartame (N-L-α-aspartyl-L-phenylalanine methyl ester) is a low-calorie artificial sweetener that is about 200 times sweeter than table sugar. It is composed of three smaller chemicals; two amino acids called aspartic acid, phenylalanine and methanol (Ranney, Operman, Muldoon, & McMahon, 1976). Since phenylalanine can be neurotoxic and can affect the synthesis of inhibitory monoamine neurotransmitters, the phenylalanine in aspartame could conceivably mediate neurologic effects. The neurotoxicity of methanol in primates has also been well documented (Potts, Praglin, Farkas, Orbisan, & Chickering, 1955). Aspartame is widely used in foods, soft drinks and dietary products. Its increased application in food industry has given a new impetus to the development of fast and efficient methods for its determination (Dinçkaya, Çağın, & Telefoncu, 1994; Pereira & Fatibello-Filho, 1998; Wrabel & Wrabel, 1997). Since its discovery, a variety of methods for the determination of this sweetener have been proposed; spectrophotometric procedures for determining aspartame involves different chemical reagents, such as ninhydrine, chloroanilic acid, diethyldithiocarbamate and p-dimethylaminobenzaldehyde. However, these methods are time consuming or do not have the selectivity required for aspartame determination in some commercial samples. Potentiometric methods, and a method involving the titration of aspartame with dinitrofluoro benzene and detection of the equivalence point with a fluoride electrode have also been developed (Fatibello-Filho, Marcolino-Junior, & Pereira, 1999). Gas–liquid chromatography (Furda, Malizia, Kolor, & Vernieri, 1975) and high-performance liquid chromatography (HPLC) (Galetti & Bocchini, 1996; Patianaargson, Chuapradit, & Srisukphonraruk, 2001; Qu, Qi, Liu, & Mou, 1999) have been used for determining aspartame in several samples. However, these methods are costly and require pretreatment of the food samples prior to the chromatographic operation. Various biosensing systems have been developed for aspartame analysis in dietary products such as co-immobilized L-aspartase and carboxypeptidase and immobilized L-aspartase. However, these electrodes are characterized by short lifetimes because of the low stability of the L-aspartase and by

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interference from L-aspartic acid (Fatibello-Filho et al., 1999). On the otherhand, *Bacillus subtillis* based microbial sensor has been proposed but it also suffers serious interferences from glucose and the amino acid constituents of aspartame (Renneberg, Riedel, & Scheller, 1985). Other biosensors based on aspartate aminotransferase-glutamate oxidase (Villarta, Suleiman, & Guilbault, 1995) and co-immobilized α-chymotrypsin and alcohol oxidase (Chou, 1996) with the linear range between 2.0×10^{-4} M–1.5×10^{-3} M and 3.3×10^{-4} M–2.0×10^{-3} M aspartame, respectively. Moreover, a spectrophotometric system including α-chymotrypsin, alcohol oxidase and horseradish peroxidase was proposed by Dinçkaya et al. (1994) and linear range was found to be 3.4×10^{-4} M–3.4×10^{-3} M aspartame. An amino acid analyzer was used to develop a semi-automatic method for the determination of aspartame in soft drinks. However, the necessary time for the analysis of sample ranged from 20 to 60 min (Fatibello-Filho et al., 1999).

In this work, a new biosensor system based on carboxyl esterase and alcohol oxidase has been developed. Aspartame is first cleaved by esterase to L-Asp-L-Phe and methanol. Methanol is then oxidized by alcohol oxidase. Oxygen consumption during the enzymatic reaction was followed by using an oxygenmeter.

2. Materials and methods

2.1. Materials

Carboxyl esterase; Carboxyl-ester hydrolase (EC 3.1.1.1) from porcine liver, Alcohol oxidase (EC 1.1.3.13) from *Candida boidinii*, 225 bloom calf skin gelatin and glutaraldehyde were obtained from Sigma Chem.Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Apparatus

WTW inoLab Oxi Level 2 model dissolved oxygenmeter based on amperometric mode was used for the experiments.

2.3. Electrode preparation

Esterase (2.0 IU), Alcohol oxidase (20 IU) and gelatin (10 mg) were mixed at 38 °C in 250 μl of phosphate buffer (pH 7.5, 50 mM). Then, the solution spread over the dissolved oxygen (D.O) probe membrane and allowed to dry at 4 °C for 1 h. Finally, it was immersed in 2.5% glutaraldehyde in 50 mM phosphate buffer (pH 7.5) for 5 min (Ertaş, Timur, Akyilmaz, & Dinçkaya, 2000).

2.4. Measurements

In order to determine the aspartame concentration, oxygen consumption in enzymatic reaction was measured. By using a thermostatic reaction cell, all the measurements were performed at 37 °C with varying aspartame concentrations in steady-state conditions.

3. Results and discussion

3.1. Enzyme electrode optimization

3.1.1. Effect of pH

The effect of pH on the electrode response between pH 5.0 and 10.0 was investigated. Fig. 1 showed that the highest response was observed in phosphate buffer (50 mM) at pH 8.0.

3.1.2. Effect of temperature

The effect of temperature on the response of the biosensor system was examined in the range of 15–52 °C. As is shown in Fig. 2, maximum sensor response was found at 37 °C. Therefore, this value was accepted as optimum temperature for all subsequent experiments.

3.2. Analytical characteristics

3.2.1. Linear range

A linearity for the biosensor was obtained in concentration range between 0.5×10^{-7} and 0.4×10^{-6} M aspartame in 10 min response time (Fig. 3). At higher concentrations, standard curve showed a deviation from linearity.

3.2.2. Accuracy

The reproducibility of the biosensor was tested for 2.0×10^{-7} M ($n=7$) aspartame concentration and the

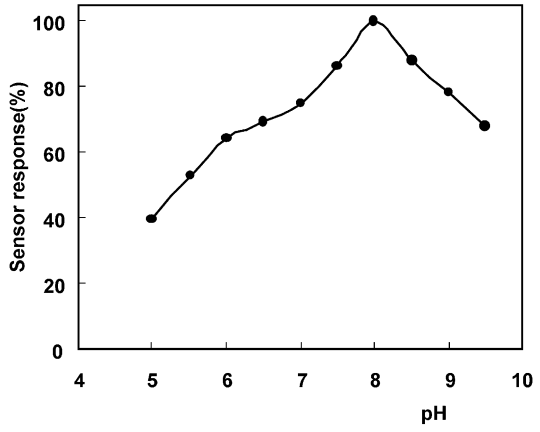

Fig. 1. Optimum pH of carboxyl esterase-alcohol oxidase biosensor (Potassium phosphate buffer, 50 mM: pH 5.0–8.0; Tris–HCl, 50 mM: pH 8.5–9.0; Sodium borate, 50 mM: pH 9.5; 37 °C, Aspartame: 0.2 μM).

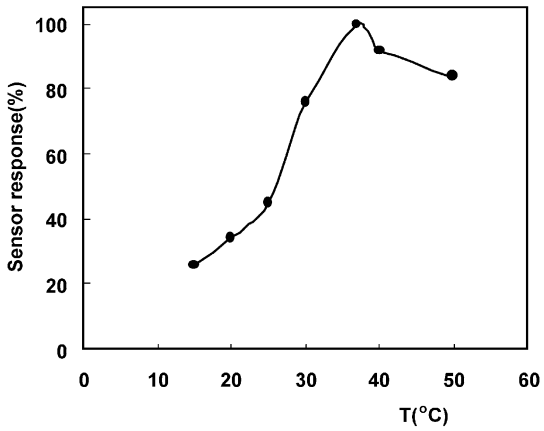

Fig. 2. Optimum temperature of carboxyl esterase-alcohol oxidase biosensor (in potassium phosphate buffer; 50 mM, pH 8.0, Aspartame; 0.2 μM).

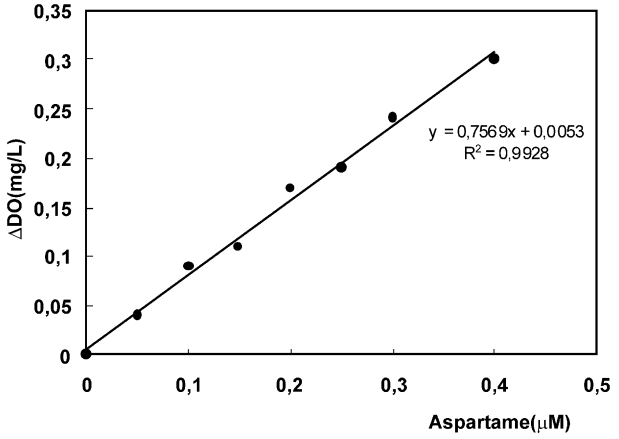

Fig. 3. Calibration graph for the aspartame determination (in potassium phosphate buffer; 50 mM, pH 8.0, 37 °C).

standard deviation (SD) and variation coefficient (cv) were calculated as $\pm 0.049\times10^{-7}$ M and 2.4%, respectively.

3.2.3. Stabilities

The thermal stability experiments showed that after a 9 h period, only 5.9% decrease of the beginning activity of the biosensor was observed at working conditions (phosphate buffer, pH 8.0, 50 mM and 37 °C). During this period, approximately 27 measurements have been made and it could be possible to make more measurements in a longer time period. Furthermore, when the electrode was stored at 4 °C, the system was stable for more than 30 days and 96 assays (three measurements per day) without any detectable activity decrease.

3.3. Aspartame determination in soft drinks and commercial sweetener tablets

The contents of aspartame in real soft drink samples and commercial sweetener tablets were determined.

Table 1
Aspartame detection in various samples[a]

Samples	Label value (g/l)	Aspartame found (g/l)	Recovery (%)
Diet Coca cola	0.24	0.246±0.0131	102.5
Pepsi Light	0.35	0.372±0.0045	106.3
Sweetener tablet (Sanpa-Nutra Sweet-Bilim Drug Industry-Tr)	0.294	0.306±0.0067	104.1

[a] All results are given as value±SD.

Known amount of the samples were used as stock substrate solutions with different dilutions by working buffer and added to the reaction cell after equilibration was occurred and then the change in current was measured. The signals obtained from the samples were recorded and aspartame concentration was calculated by using calibration graph. Each analysis was performed five times and results are given in Table 1. As is seen in Table 1, the results are well agreed with the labeled values provided by manufacturer and nature of the sample didn't affect the analysis. Our system enables us to determine aspartame with an acceptable degree of precision and accuracy in the range of 5.0×10^{-8} M and 4.0×10^{-7} M. Such a development facilitates rapid quality control testing in food industry.

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