Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 573–574 (2006) 75–80
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Optical bio-sniffer for methyl mercaptan in halitosis

Kohji Mitsubayashi [a,*], Takeshi Minamide [b], Kimio Otsuka [a], Hiroyuki Kudo [a], Hirokazu Saito [a]

[a] *Department of Biomedical Devices and Instrumentation, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan*
[b] *Graduate School of Engineering, TOKAI University, 1117 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan*

Received 28 November 2005; received in revised form 19 January 2006; accepted 19 January 2006
Available online 28 February 2006

Abstract

An optical bio-sniffer for methyl mercaptan (MM) one of major odorous chemicals in halitosis (bad breath) was constructed by immobilizing monoamine oxidase type A (MAO-A) onto a tip of a fiber optic oxygen sensor (od: 1.59 mm) with an oxygen sensitive ruthenium organic complex (excitation: 470 nm, fluorescent: 600 nm). A flow cell for circulating buffer solution was applied to rinse and clean the tip of the device like nasal mucosa. In order to amplify the bio-sniffer output, a substrate regeneration cycle caused by coupling MAO-A with L-ascorbic acid (AsA) as reducing reaction with reagent system was applied to the sensor system. After evaluating the sensor characteristics using a gas flow measurement system with a gas generator, the optical bio-sniffer was applied to expired gases from healthy male volunteers for halitosis analysis as a physiological application.

The optical bio-sniffer was applied to detect the oxygen consumption induced by MAO-A enzymatic reaction (and AsA chemical reduction) with gaseous MM application. The bio-sniffer was calibrated against MM vapor from 8.7 to 11500 ppb with correlation coefficient of 0.977, including a MM threshold (200 ppb) of pathologic halitosis and the human sense of smell level 3.5 (10.0 ppb), with good gas-selectivity based on the MAO-A substrate specificity. As the result of the physiological application, the optical bio-sniffer could successfully monitor the MM level change in breath samples during daytime, which is consistent with the previously reported results.

Keywords: Optical bio-sniffer; Halitosis; Methyl mercaptan; Monoamine oxidase; Oxygen sensitive optical fiber

1. Introduction

Halitosis (bad breath) is measured to diagnose dental hygiene in clinical dentistry. Human beings are sensitive to halitosis in others but unable to assess the halitosis in their own breath. The main chemical constituents of oral odorous chemicals are volatile sulfides such as hydrogen sulfide (H_2S) and methyl mercaptan (MM:CH_3SH) [1,2]. ACGIH (The American Conference of Governmental Industrial Hygienists) and the Environment Agency Government of Japan had specified the MM as a typical volatile organic compound (VOC) and malodorous substance. The threshold level of pathologic halitosis has been reported as the concentration of 200 ppb MM [2]. The maximum permissible concentration of gaseous MM in the work place is defined as 5.0 ppm (TLV-TWA: threshold limit value-time weighted average concentration) [3]. However, there are no convenient approaches to measure the MM vapor for evaluating the halitosis. Many types of gas sensors have been investigated and developed. For example, semiconductor type gas sensors were improved the gas selectivity and sensitivity [4-6]. Nevertheless, semiconductor sensors are still inadequate to sense multi substances such as included in expiratory gas, because the sensor outputs the change in electrical conductivity by adsorption of gaseous substances [4-8].

In humans, monoamine oxidase, one of the xenobiotic metabolizing enzymes, has been reported to catalyze the oxidation of monoamine compounds including methyl mercaptan [9-11]. We have been developed and reported several kinds of bioelectronic sniffers (gas-sensor) using metabolizing enzymes for convenient measurements of VOC (volatile chemical compounds) including methyl mercaptan [12-16]. Recently, an oxygen sensor based on an optical fiber was also applied for developing a newly optical sniffer and improving the sensitivity for methyl mercaptan.

* Corresponding author. Tel.: +81 3 5280 8091; fax: +81 3 5280 8094.
E-mail address: m.bdi@tmd.ac.jp (K. Mitsubayashi).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.01.062

In this study, we have constructed an optical bio-sniffer for the measurement of MM as major odorous chemical in bad breath using monoamine oxidase. After the evaluation of the sensor characteristics using a batch measurement system, the optical sniffer was applied to the halitosis analysis as a physiological application.

2. Experimental section

2.1. Construction of an optical bio-sniffer for MM

The structure and photograph of an optical bio-sniffer (and its components) for methyl mercaptan (MM) in the gas phase was shown in Fig. 1. The sniffer device constructed by using an enzyme immobilized membrane, a flow cell and a commercial available oxygen-sensitive optical fiber with a ruthenium-organic complex ([FOXY-R/RTV-Flat (silicone overcoat), Lot No. OX3200], 1/16″ outer diameter, stainless steel tube beveled, Ocean Optics, Inc., FL, USA). The commercial available optical fiber was coated in a sol–gel process with the ruthenium-organic complex that is subjected to an optical quenching (excitation wavelength: 470 nm, fluorescent wavelength: 600 nm) in the presence of oxygen molecules, in both the liquid and gas phases.

Monoamine oxidase type A (MAO-A, E.C.1.4.3.4., 142 nmole min^{-1} mg^{-1} protein, from adult human liver, Gentest corp., MA, USA) was used as the MM recognition material for the halitosis bio-sniffer. For enzyme immobilization, MAO-A was mixed with PVA-SbQ solution (photocrosslinkable polyvinyl alcohol containing stilbazole quaternized; Type: SPP (Styryl Pyridinium Polymer)-H-13 (Bio), Tokyo Gosei Kogyo Co., Tokyo, Japan) in a weight ratio of 1:2, [17,18] to a dialysis membrane (thickness: 15 μm, Part No.157-0144-02., thickness 15 μm, Technicon Chemicals Co., S.A., Oceq, Belgium) spread on a glass plate, and then irradiated with a fluorescent lamp for 30 min in order to photocrosslink the solution and immobilize the enzyme to the dialysis membrane. The MAO-A immobilized membrane was removed from the glass plate and immersed in phosphate buffer (pH 8.5, 100 mmol l^{-1}). In order to prevent enzyme deactivation when not in use, the membrane was stored in buffer below 10 °C.

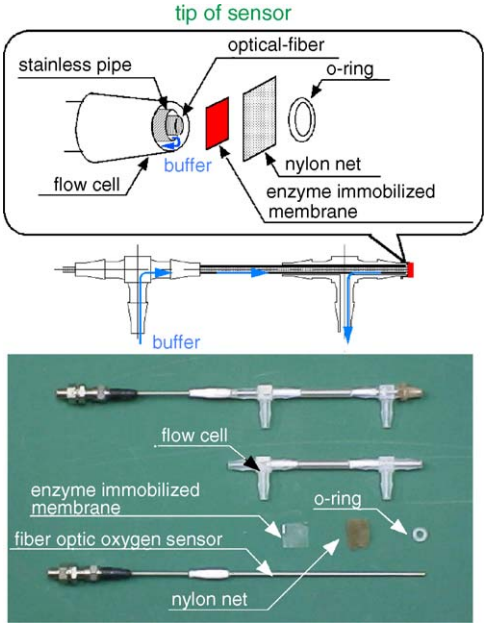

Fig. 1. Structure and photograph of an optical bio-sniffer with a sensor tip cleaning system in the gas phase and the device components (fiber optic oxygen sensor, enzyme membrane, stainless tube, 2 T-tubes, etc.).

The flow cell was constructed by connecting two T-tubes (mini fitting: VFT406, AS ONE Corp., Osaka, Japan) to both sides of a stainless steel pipe (o.d. 2.44 mm, i.d. 1.99 mm) and the inner side edges of the two T-tubes were closed by a sealing tape. The enzyme membrane, which was cut to the required dimensions using a scalpel, was used to close one of the open edges of the flow cell and secured with a supporting nylon mesh net and a rubber O-ring (see the enlargement of Fig. 1). The fiber tip of the optical oxygen sensor was inserted from another open edge to the flow cell and adjusted so as to directly touch the surface of the enzyme membrane (see the enlargement of Fig. 1). Then a gap at another outer edge between the T-tube pipe and fiber-optic oxygen sensor was also closed with the sealing tape. As the figure indicates, buffer solution in the flow cell was flowed into the stainless tube from the middle edge of the root-side T-tube to that one of the tip-side, thus rinsing and cleaning the fiber tip and the enzyme membrane like the nasal mucosa.

2.2. Gas flow measurement system with optical bio-sniffer

Fig. 2 illustrates a batch flow measurement system with 3-port valve connecting to a gas generator for evaluating the characteristics of the optical bio-sniffer and to a sampling bag for the breath analysis. Gas (standard or sample) and phosphate buffer solution could be flowed individually through the flow cell in the system.

A standard substance in the gas phase was supplied from a gas generator (PEMEATER, Type, PD-1B-2, Gastec Corp., Yokohama, Japan), which is a standardized machine approved by the Ministry for Labor and the Environmental Agency in Japan and by the Environmental Protection Agency (EPA) and the National Bureau of Standards (NBS) in the USA for gas calibration purposes. As the Fig. 2 indicates, the sensor tip was inserted into the side hole of a PTFE tube (o.d. 8 mm, i.d. 6 mm) from the gas generator. Some mass-flow controllers (with a needle-bulb regulator, Type: RK1200, Koflok, Tokyo, Japan) were used to control the flow rates of a filtered standard air and of the gases supplied from the gas generator, thus adjusting the concentrations of gaseous substances into the gas compartment of the optical bio-sniffer to be varied with a final flow rate of 100 ml min^{-1}. Phosphate buffer solution (pH 8.5, 100 mmol l^{-1}) in a carrier reservoir, with the temperature maintained at 25 °C, was flowed and circulated to the fiber tip and the enzyme membrane through the flow cell of the optical bio-sniffer with a flow rate of 1.68 ml min^{-1} using a peristaltic pump (Type: MP-3N, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The circulation of phosphate buffer solution was applied to realize a continuous measurement of the gaseous substances by supplying the dissolved oxygen, and by removing the enzyme products