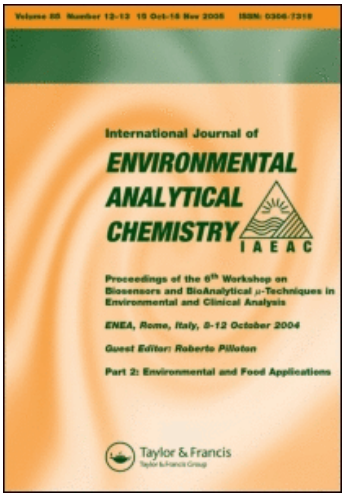

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Bioelectronic sniffers for formaldehyde in the gas phase

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Two kinds of enzyme electrodes were constructed by immobilizing ALDH or FALDH into a Pt-electrode deposited onto a hydrophilic-polytetrafluoroethylene membrane. A bioelectronic sniffer device for formaldehyde vapor was fabricated by incorporating the enzyme electrode into the reaction unit with both gas- and liquid-compartments separated by a diaphragm membrane. The sensitive area of the electrode was placed onto the diaphragm membrane in the liquid compartment. Gaseous substances in the gas-compartment could diffuse to the electrode through the diaphragm. The amperometric current of the sniffer-device with ALDH and FALDH increased by applying formaldehyde vapor, thus obtaining the calibration range of 10–2000 ppb, including the maximum permitted concentrations (80 ppb) and the human detection limit (410 ppb). In comparison with the outputs obtained by applying other gaseous substances, the sniffer-device with biocatalysts, especially FALDH, indicated high gas-selectivity for formaldehyde vapor.

Keywords: Bioelectronic sniffer; Formaldehyde vapor; Aldehyde dehydrogenase; Gas selectivity

1. Introduction

Formaldehyde is one of the harmful volatile organic compounds (VOCs), and has been reported to induce Sick-house Syndrome, especially at the hermetic house [1]. The maximum permitted concentration of formaldehyde vapor as defined by WHO and the Ministry of Health and Welfare in Japan is 80 ppb [2], that is lower than the detection limit (410 ppb) of the human sense of smell [3].

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Therefore, a way is required for measuring formaldehyde vapor with high sensitivity and selectivity from environmental and medical points of view. Many types of gas sensors have been investigated and developed. Considerable effort has been directed toward improving the gas selectivity and sensitivity of semiconductor-type gas sensors [4–7]. At present semiconductor sensors are still inadequate for sensing formaldehyde vapor in the residential atmosphere, including various kinds of chemical substances such as dietary odorants, body scents, detergent vapors and solvents from building materials, because the sensor response is based only on changes in electrical conductivity of the device following adsorption of gaseous substances [8–12]. Although an improvement in semiconductor sensors has been reported [13], it is still far from the selectivity achievable using biological recognition systems such as enzymes.

For the measurement of formaldehyde in the liquid phase, biosensors have been extensively researched. A formaldehyde biosensor for the liquid phase has also been investigated and applied widely for the measurement of formaldehyde concentration [13–16]. Aldehyde dehydrogenase (ALDH) and formaldehyde dehydrogenase (FALDH) are commonly used in the construction of formaldehyde biosensors with diaphorase and electrochemical mediator [15, 16].

In the previous issues [17–19], we reported some biochemical gas sensors, called 'bioelectronic sniffers', for ethanol, trimethylamine, methyl mercaptan, acetaldehyde, etc. In this work, we have constructed two kinds of bioelectronic sniffer using the enzyme catalytic reaction for measurement of gaseous formaldehyde. The performance of the sniffer is evaluated, such as sensitivity, calibration behavior and selectivity, and compared with the human sense of smell.

2. Experimental

2.1 *Construction of a bioelectronic sniffer for formaldehyde vapor*

Figure 1 illustrates the structure of the bioelectronic sniffer for formaldehyde vapor. The sensor consisted of an enzyme electrode, a homemade reaction unit with two compartments for liquid and gas phases, and a porous diaphragm membrane separating these compartments.

The enzyme electrode was constructed using a Pt-deposited electrode (figure 2). Pt-layer (3000 Å) was formed by sputtering (CFS-4ES-231, Shibaura Engineerring Works Co., Ltd.) onto one side of a hydrophilic polytetrafluoroethylene (H-PTFE) membrane (JGWP14225, thickness = 80 μm, pore size = 0.2 μm, Nihon Millipore Ltd., Tokyo, Japan). This electrode membrane offers chemical stability, strength, and flexibility. As previously reported [20], the Pt-deposited membrane retained its flexibility after vacuum deposition.

The electrode membrane was cut using a scalpel into 6 mm wide strips (50 mm in length). In order to isolate a sensitive area at the center, the electrode membrane was covered with two heat-weldable polyethylene films (thickness 80 μm) with a circular hole (diameter, 4 mm) in a sandwich configuration, and isolated electrically by a heat sealing machine (SURE Sealer, NL-201P, Ishizaki Electricity Manufacturing Co., Ltd., Tokyo, Japan) except for the circular hole and both edges of the electrode membrane. The strip membrane was thus separated into three discrete areas: sensitive area, lead area and electrical terminal area.

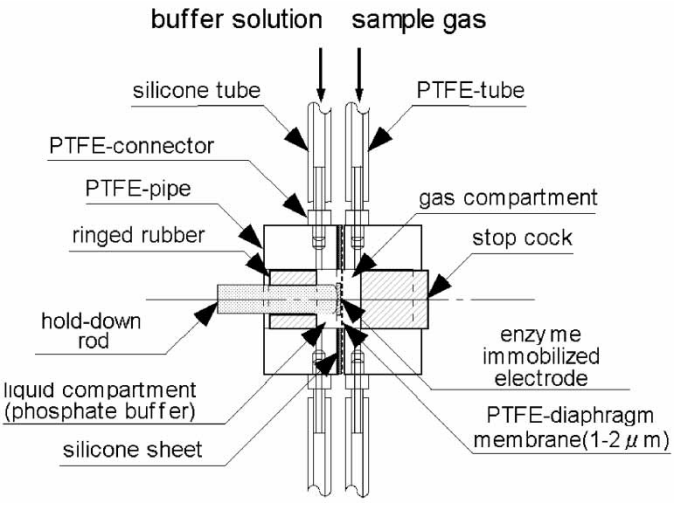

Figure 1. Structure of a bioelectronic sniffer for formaldehyde vapor, with a reaction unit with both gas and liquid phase compartments.

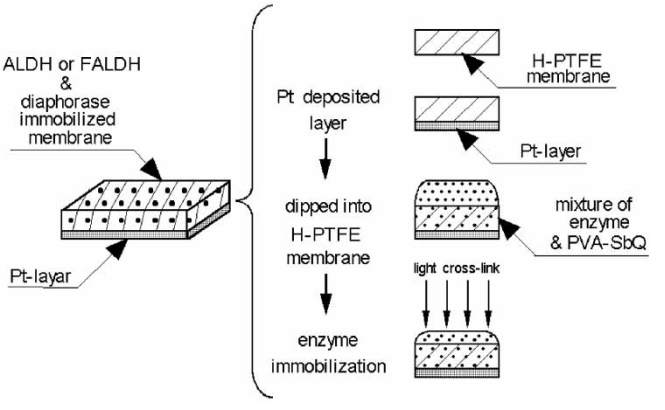

Figure 2. Fabrication process of an enzyme immobilized electrode with a Pt-deposited PTFE membrane using PVA-SbQ.

Two types of the bio-sniffer were constructed by immobilizing diaphorase (D5540, EC 1.8.1.4, 5–20 units mg^{-1}, from *Clostridium kluyveri*, Sigma Chemical Co., St. Louis, MO, USA) and aldehyde dehydrogenase (ALDH, EC 1.2.1.5, 171832, 20 units mg^{-1}, from *Yeast*, Boehringer Mannheim, France) or formaldehyde dehydrogenase (FALDH, EC 1.2.1.1, 1 unit mg^{-1}, solid, from *Pseudomonas* sp., Funakoshi Co., Ltd., Tokyo, Japan) into the sensitive region of the gold-coated membrane.

Two kinds of enzyme (diaphorase and ALDH or FALDH) were mixed with distilled water and photo-cross-linkable poly(vinyl alcohol) having stilbazolium groups (PVA-SbQ (Stilbazole Quaternized); PVA-SbQ, SPP-H-13 (Bio), Toyo Gosei Kogyo Co., Ltd., Tokyo, Japan) in a weight ratio of 1 : 2 : 60. PVA-SbQ (developed at the Research Institute for Polymers and Textiles, the Japanese Ministry of International

Trade and Industry) is a biocompatible, non-hazardous material that has been assessed by a range of toxicity tests (oral, eye and skin surface). The enzyme/PVA-SbQ mixture was placed onto the non-deposited side of the sensitive area and spread over the surface of the membrane until it had permeated (observed as a darkening of the membrane). The membrane strips were then placed in the dark at room temperature for 1 h to allow for complete permeation, then irradiated with a fluorescent light for 30 min [21]. The device was immediately rinsed in phosphate buffer solution (50 mmol L^{-1}, pH 7.5) and any mixture on the surface of the electrode was removed. It peels away from the surface and could be easily removed by gentle rubbing because the mixture swells when wetted. The membrane electrode was stored below 10°C until required.

Next, the reaction unit was constructed using the PTFE materials. Two hollow PTFE tubes (o.d.: 40 mm, i.d.: 20 mm) were cut to a length of 20 mm and the surfaces of the tubes were polished. Two 6 mm diameter tapped holes were drilled across the entire diameter of each tube and four PTFE tube connectors (o.d.: 6 mm, i.d.: 2 mm) were screwed into the outside of all of the tapped holes. A porous PTFE membrane (PTFE membrane, A-135: pore size 20–30 μm, thickness 0.13 mm, ZITEX Co. Ltd., Nagano, Japan) and the enzyme electrode were sandwiched between the two tube blocks, which were then held firmly together using a mechanical clamp. In this way, the PTFE membrane acted as a separating diaphragm between the two hollow compartments of the tubes. The enzyme immobilized side was faced to the diaphragm membrane.

A cylindrical rubber stop cock (o.d.: 20.5 mm) was inserted into the large hole of one of the tubes, thus forming the gas compartment of the reaction unit. The volume of the compartment was adjusted to 0.5 mL. A rubber O-ring was placed around the cylindrical rod (o.d.: 20.5 mm) and inserted into the hole of the other tube, thus producing the liquid compartment of the reaction unit. The sensitive area of the enzyme electrode was immersed in the liquid compartment filled with phosphate buffer (50 mmol L^{-1}, pH 7.5) and adjusted so as to directly touch the surface of the diaphragm membrane by pushing by the rod (see figure 1).

Figure 3 indicates enzyme and electrochemical reactions for detecting formaldehyde. Formaldehyde is dehydrogenated by ALDH or FALDH using oxidized NAD as electron acceptor. Then NADH is dehydrogenated by diaphorase using potassium ferricyanide (1.0 mmol L^{-1}) as an electrochemical mediator, thus obtaining the oxidizing current of its reduction form at the Pt-electrode [15, 16].

Gas and phosphate buffer solution including the oxidized NAD and mediator could be flowed individually through each inlet tube connector to the gas and liquid compartments of the reaction unit, respectively. Gaseous substances in the gas compartment could diffuse through into the liquid compartment of the reaction unit through the PTFE diaphragm membrane.

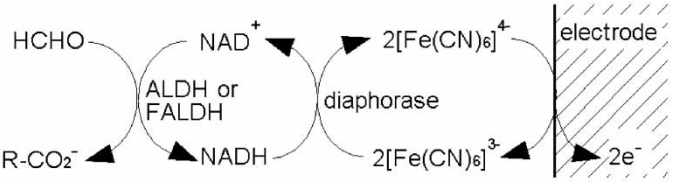

Figure 3. Principal reactions for detecting formaldehyde using aldehyde (or formaldehyde) dehydrogenase and diaphorase.

2.2 *Gas flow measurement system*

The bio-sniffer was used in a batch flow measurement system (figure 4). A standard substance in the gas phase was supplied from a gas generator (Permeater, 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, with a diffusion tube (D-01 and D-20, Gastec Corp., Yokohama, Japan).

Two mass flow controllers with a needle-bulb regulator (Type: RK1200, Koflok, Tokyo, Japan) were used to adjust the flow rates of a filtered standard air and the gases supplied from the gas generator, thus permitting the concentrations of gaseous substances into the gas compartment of the bio-optical sniffer to be varied with a final flow rate of 200 mL min^{-1}.

Phosphate buffer solution (pH 7.5, 50 mmol L^{-1}) in a carrier reservoir with the temperature maintained was flowed and circulated to the sensitive area and the enzyme membrane through the reaction unit of the bio-sniffer with a flow rate of 0.69 mL min^{-1} using a peristaltic pump (SJ-1211L, ATTO Corp., Tokyo, Japan).

A computer controlled potentiostat (Potentiostat, Model 1112, BAS Inc., Tokyo, Japan) was electrically connected to the edge of the Pt-deposited membrane. Pt-wire (o.d.: 0.5 mm) as a counter electrode was inserted into the buffer tube connected to the liquid compartment. A fixed voltage of 81 mV *versus* Pt-wire was applied to the Pt working electrode coated on the H-PTFE membrane [15, 16]. The output current was monitored graphically on a continuous computer display and saved on hard disk for later analysis.

Other gaseous substances (acetaldehyde, methanol, ethanol, benzene, acetone) were also analysed in order to investigate the selectivity and to compare the ALDH and FALDH immobilized devices.

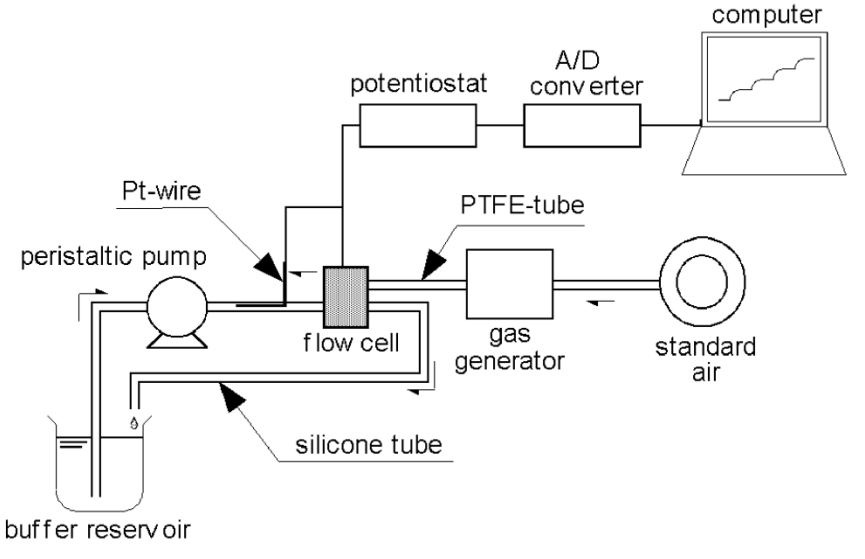

Figure 4. Schematic diagram of the gas-flow measurement system for the bioelectronic sniffer.

3. Results and discussion

3.1 *Evaluation of the bioelectronic sniffer*

Prior to formaldehyde measurement in the gas phase, the effect of NADH concentration on the diaphorase and electrochemical reactions with a Pt-deposited electrode is shows in order to obtain the optimal concentration of the oxidized NAD.

Figure 5 shows the relationship between the concentration of NADH and the output current of the diaphorase immobilized electrode. As the figure indicates, the oxidation current is increased with increasing the NADH concentration. Since there was little enhancement of the sensor output for the NADH concentration above $3\,\mathrm{mmol\,L^{-1}}$, the oxidized NAD of $1\,\mathrm{mmol\,L^{-1}}$ was generally used in all experiments for the measurement of formaldehyde vapor as reported previously [15, 16].

The calibration curves of the bio-sniffer immobilized with ALDH (open squares) and FALDH (filled circles) for formaldehyde vapor is shown in figure 6. As the figure indicates, the sensor outputs of both devices were related to the concentration of formaldehyde in the gas phase over the range 10.0–60.0 ppb (ALDH, correlation coefficient of 0.984) and 40–1000 ppb (FALDH, correlation coefficient of 0.999), respectively, deduced from exponential regression analysis of the log–log plot by a method of least squares according to the equations:

ALDH sniffer

$$\text{output current (nA)} = 0.16 \times [\text{gaseous formaldehyde (ppb)}]^{1.16}$$

FALDH sniffer

$$\text{output current (nA)} = 0.08 \times [\text{gaseous formaldehyde (ppb)}]^{1.30}$$

The lower detection limits of both devices are lower than the maximum permissible concentration of formaldehyde vapor in the residential house (80 ppb, left-hand arrowhead in the figure) and formaldehyde detection limit for the human sense of smell (410 ppb, right-hand arrowhead) [3].

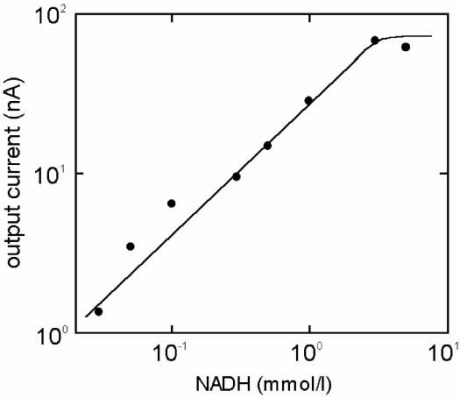

Figure 5. Effect of NADH concentration on the diaphorase and electrochemical reactions with Pt-deposited electrode.

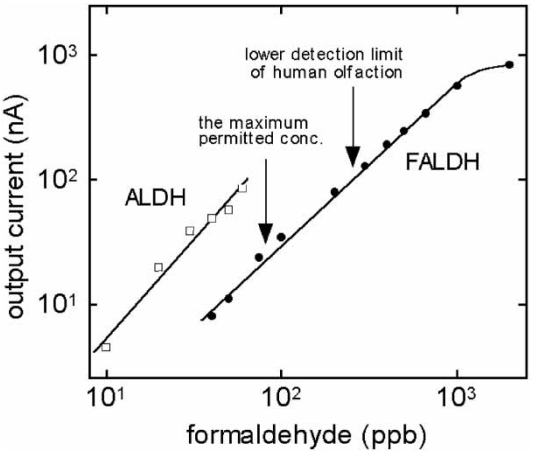

Figure 6. Calibration curves of the bioelectronic sniffer immobilized with ALDH (open squares) and FALDH (filled circles) for formaldehyde vapor. (Left-hand arrowhead: the maximum permitted concentration (80 ppm), Right-hand arrowhead: lower detection limit of human olfaction (410 ppm)).

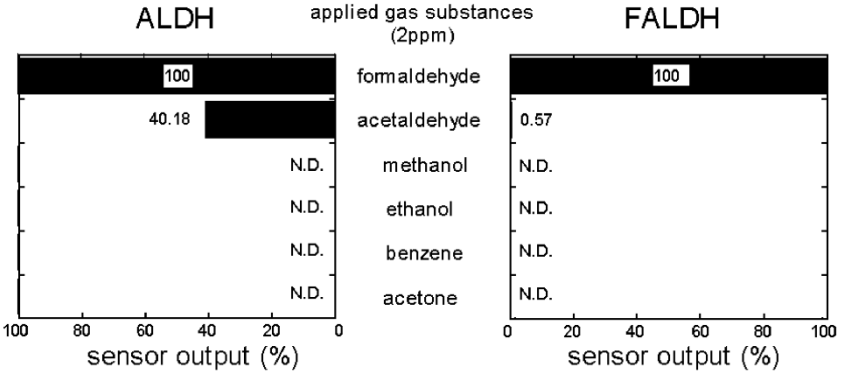

Figure 7. Comparison of the gas-selectivity between the ALDH and FALDH immobilized sniffer devices using various kinds of gaseous substances (2 ppm; formaldehyde, acetaldehyde, methanol, ethanol, benzene and acetone).

The sensitivity of the ALDH sniffer is better than that of FALDH. The result is consistent with the differences of enzyme activities between ALDH (20 units mg^{-1}) and FALDH (1 unit mg^{-1}) as described above.

3.2 *Gas selectivity of the bio-sniffers with ALDH and FALDH*

The selectivity of both the bio-sniffers with ALDH and FALDH for several gas substances (2 ppm; formaldehyde, acetaldehyde, methanol, ethanol, benzene and acetone) is shown in figure 7. The ALDH bio-sniffer responded to acetaldehyde vapor but both the devices gave no response to all the chemicals other than formaldehyde and acetaldehyde because of the substrate specificities of ALDH and FALDH.

Based on the results, two kinds of the bio-sniffer devices for formaldehyde have the different characteristics. Namely, the ALDH and FALDH sniffer devices are considered to be useful for detecting with high sensitivity and selectivity, respectively.

The buffer flow was effective not only for supplying co-enzyme and mediator for the enzyme and electrochemical reactions, but also for removing formaldehyde which diffuses through the diaphragm membrane into the liquid compartment and the enzyme membrane and enzymatic products as reported previously [17].

4. Conclusions

The bioelectronic sniffer for formaldehyde with ALDH or FALDH has a simple construction and low detection limit, covering the concentration range encountered in the formaldehyde permitted concentration in the residential house (80 ppm) and the sensing range of smell in humans (410 ppm). The sniffer devices, especially FALDH, indicated highly gas-selectivity for formaldehyde vapor. We are currently involved in applying the bio-sniffer to the environmental analysis and the results will be reported in the near future.

The bio-sniffer can also be used for detection of other substances in the gas phase simply by changing the biomolecule (enzyme, microorganism, organelle, etc.) used in constructing the sensor. This would make the biosensor of value in many different areas of not only environmental monitoring, but also health and safety monitoring and clinical analysis.

Acknowledgements

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