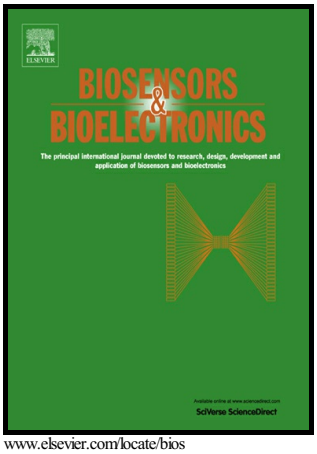

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Bio-sniffer (gas-phase biosensor) with secondary alcohol dehydrogenase (S-ADH) for determination of isopropanol in exhaled air as a potential volatile biomarker

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Keywords:

isopropanol vapor; biosensor; NADH; secondary alcohol dehydrogenase; volatile organic compounds; gas sensor

Abstract

Exhaled breath analysis has attracted lots of researchers attention in the past decades due to its advantages such as its non-invasive property and the possibility of continuous monitoring. In addition, several volatile organic compounds in breath have been identified as biomarkers for some diseases. Particularly, studies have pointed out that concentration of isopropanol (IPA) in exhaled air might relate with certain illnesses such as liver disease, chronic obstructive pulmonary (COPD), and lung cancer. In this study, a highly sensitive and selective biochemical gas sensor (bio-sniffer) for the breath IPA concentration determination was constructed and optimized. This bio-sniffer measures the concentration of IPA according to the fluorescence intensity of oxidized nicotinamide adenine dinucleotide (NADH), which was produced by an enzymatic reaction of secondary alcohol dehydrogenase (S-ADH). The NADH detection system employed an UV–LED as the excitation light, and a highly sensitive photomultiplier tube (PMT) as a fluorescence intensity detector. A gas-sensing region was developed using an optical fiber probe equipped with a

flow-cell and enzyme immobilized membrane, and connected to the NADH measurement system. The calibration range of the IPA bio-sniffer was confirmed from 1 ppb to 9060 ppb that was comparable to other IPA analysis methods. The results of the analysis of breath IPA concentration in healthy subjects using the bio-sniffer showed a mean concentration of 16.0 ppb, which was similar to other studies. These results have demonstrated that this highly sensitive and selective bio-sniffer could be used to measure the IPA in exhaled air, and it is expected to apply for breath IPA research and investigation of biomarkers for clinical diagnosis.

Introduction

Over the last few decades, researchers have increased their interests in analyzing human breath because the exhaled air contains a variety of bio-information, such as traces of eating, signals of the status of the body's metabolism, biological clocks and signs of some diseases (Cao and Duan 2006; Risby and Solga 2006; Sinues et al. 2013). Additionally, compared to blood examination, non-invasive property of the breath measuring not only can reduce the uncomfortable feeling of a patient but also provides a continuous pattern for monitoring the changing of the human body. Researchers have identified more than two hundred volatile organic compounds (VOCs) in the composition of breath (Pauling et al. 1971), and some of them have the potential to be biomarkers for indicating of certain diseases (Cheepsattayakorn and Cheepsattayakorn 2013; Konvalina and Haick 2014). For example, nitric oxide is one of the well-known indicators for asthma (Kharitonov et al. 1994), and a higher concentration of breath acetone, which is related to lipid metabolism, was observed in patient with diabetes mellitus (Reyes-Reyes et al. 2015;

Tassopou.Cn et al. 1969). Searching biomarkers for cancer screening in an early stage is always an important issue in the health care industry. Recently, several researchers have found that the isopropanol (IPA) concentrations in the breath may relate with some illnesses. For instance, Hanouneh et al. discovered that the breath IPA concentration of patients with liver disease was higher than in healthy people (Hanouneh et al. 2014), Phillips et al. used breath IPA level to predict breast cancer (Phillips et al. 2006), and Cikach et al. observed an increasing trend of IPA concentration in the breath of patients with pulmonary arterial hypertension.(Cikach et al. 2014). Several other studies have also demonstrated that lung cancer patients might exhale a greater concentration of IPA (Buszewski et al. 2012a; Rudnicka et al. 2011; Rudnicka et al. 2014; Ulanowska et al. 2011; Wehinger et al. 2007). Not only the breath IPA, some studies also pointed out that the blood IPA concentration would increase in ketoacidosis patients (Bailey 1990; Palmiere et al. 2012). However, most studies mentioned above analyzed dozens of substances in the breath at the same time. Not much research exists that concentrates specifically on isopropanol in the

breath and its implications for clinical diagnosis. A comprehensive investigation of the relationship between a status of human health and the exhaled IPA is necessary and extremely valuable.

Currently, direct breath IPA analysis methods include gas chromatography-mass spectrometry (GC-MS) (Rudnicka et al. 2014), GC-MS with time-of-flight mass spectrometry (GC-TOF-MS or GC-MS x TPF-MS) (Rudnicka et al. 2011), proton transfer reaction-MS (PTR–MS) (Wehinger et al. 2007), solid-phase microextraction with mass spectrometry (SPME-MS) (Kischkel et al. 2010), and selected ion flow tube-MS (SIFT-MS) (Cikach et al. 2014; Hanouneh et al. 2014) are used. These techniques, usually with high sensitivity, can detect targets at a very low concentration, but there still exists some deficiencies, including difficulties in continuous breath monitoring and inconvenience of a large amount of detection due to the complicated operations and the high cost of instruments. In addition, most of these analyzers identify the target molecules by mass-to-charge ratio (*m/z*); it will cause confusion when the substances display the same *m/z* fragment. Other reported IPA detectors like

ZnO–CdO composites (Cai et al. 2014) or a transmission sensor (Elosua et al. 2013) may be used more easily than GC related methods. But their sensitivity, usually at the ppm (parts per million) level, was insufficient for breath measurement because the breath IPA mostly exists at ppb (parts per billion) concentrations.

A biochemical gas-sensor exploiting advantages of specific enzymatic reactions could be a good candidate for the determination of VOCs in exhaled breath. Nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase enzyme is one of the appropriate choices for developing a biosensor. This kind of enzymes can normally divide hydrogen molecules from a target substance, and meanwhile, transferring electrons to a coenzyme which is usually NAD (Ying 2008). The reduction form of NAD (NADH) has a unique fluorescence property releasing visible fluorescence when it is exposed to 340 nm wavelength ultraviolet (UV) light (Kudo et al. 2009). However, oxidation form of NAD (NAD^+) does not have this similar feature. Therefore, utilizing the NAD-dependent enzymatic reaction and the NADH optical property could construct a sensor for

measuring a concentration of the enzyme reacting substance. Previously, our groups developed a bio-sniffer for measuring of formaldehyde (FA) and acetone concentration in a gas phase (Kudo et al. 2012; Ye et al. 2015). The FA bio-sniffer combined a UV light-emitting diode (UV-LED) and a photomultiplier tube (PMT) with a two-way branch optical fiber for NADH fluorescence measurements, and employed the formaldehyde dehydrogenase to distinguish and catalyze FA. One of the difficulties of constructing an enzyme-based gas sensor is that this kind of NADH catalytic reaction prefers to react in a liquid medium, which can cause a dilemma for the enzyme to contact with the coenzyme and gas substance at the same time. Our FA biosensor overcomes this problem by using an optical fiber equipped with a specialized flow cell and a formaldehyde dehydrogenase immobilized membrane as a diaphragm. Circulated NAD^+ contained buffer through the flow channel permitted the enzyme to interact with NAD^+ and FA gas simultaneously. Furthermore, designing of the flow cell also allowed the bio-sniffer to continuously monitor instead of single point detect because the circulated buffer could remove the

produced NADH and immediately supply fresh NAD^+ to the sensing region.

NAD-dependent secondary alcohol dehydrogenase (S-ADH) is an enzyme that can catalyze secondary alcohols to ketones, and the main substrate is IPA. The enzymatic reaction of the S-ADH is presented at the following equation:

$$\text{Isopropanol} + NAD^+ \underset{\text{pH 6}}{\overset{\text{S-ADH, pH 8}}{\rightleftharpoons}} \text{acetone} + NADH \quad (1)$$

In a weak alkaline condition, S-ADH prefers to catalyze IPA to acetone and simultaneously produces proportional NADH. Thus, according to the fluorescence intensity of NADH, the concentration of the IPA could be calculated. Recently, we have developed an optical biosensor utilizing the enzymatic reaction of S-ADH as described in the equation (1) for determination of the IPA concentration in the liquid phase (Chien et al. 2016). The composition of this biosensor is similar to the bio-sniffer including the portion of UV-LED, PMT and optical fibers. But this IPA biosensor can only perform in liquid phase detection because it lacks of the flow cell equipment. The results of this liquid phase IPA

biosensor demonstrate that measuring the concentration of the IPA by increasing of NADH fluorescence intensity is feasible. Therefore, by combining the technique of bio-sniffer and this IPA biosensor, it is possible to construct a new bio-sniffer to detect the IPA in the gas phase.

The aims of this study include: (1) to construct an bio-sniffer for IPA vapor measurements using a S-ADH immobilized membrane, a high power UV-LED, a high sensitivity PMT, and an optical fiber with flow-cell, (2) to investigate and optimize the factors that may interfere the performance of the bio-sniffer, and (3) to apply the bio-sniffer to analyze the IPA concentration in the exhaled breath supplied by healthy subjects.

2. Materials and methods

2.1 Standards and reagents

Dipotassium hydrogen phosphate (K_2HPO_4, 98%), potassium dihydrogen phosphate (KH_2PO_4, 98%), 2-Amino-2-hydroxymethyl-1,3-propanediol (Tris, 99.9%, Biochemistry grade), Hydrochloric Acid (HCl, 1 mol L^{-1}), sodium hydrogen carbonate ($NaHCO_4$, 99.5%, JIS special grade), sodium carbonate (Na_2CO_4, 99.8%, JIS special grade) and standard isopropanol (99.7%, JIS special grade) solution were purchased from Wako Pure Chemical Industries, Japan. β-NAD^+ were bought from Oriental Yeast, Co., Ltd., Japan. NADH-dependent secondary alcohol dehydrogenase (S-ADH, EC. 1.1.1.x, 1 unit mg^{-1}, E001) from yeast was obtained from Daicel Chiral Technologies, Co., Japan. All agents are analytical grade and no further purification is needed before used. Deionized (DI) distilled water collected from Milli-Q purification system (Millipore, Co., USA) was used for all buffer solution. Hydrophilic polytetrafluoroethene filter membrane (H-PTFE, pore size: 0.2 μm, porosity: 80%, JGWP14225) employed for S-ADH immobilization was bought from

Millipore, USA. The synthesis method of the polymer using 2-methacryloyloxyethyl phosphorylcholine polymerized with 2-ethylhexyl methacrylate (PMEH) was reported in our previous study (Kudo et al. 2009).

2.2 Construction of the IPA bio-sniffer

The IPA bio-sniffer comprised a photon detection unit, an excitation light source, a bifurcated optical fiber, an optical fiber probe equipped with cylindrical flow cell and an enzyme immobilized membrane. The NADH excitation equipment contains a high power UV-LED (UVTOP® BL335, Sensor Electronic Technology, Inc., USA), which could emit the UV-light with the central wavelength at 340 nm, and a controllable power supplier (GS200, Yokogawa Electric Corporation, Japan). A PMT (C8855, Hamamatsu corporation, Japan) was employed as the NADH fluorescence intensity detector. These two components were connected to the two ends of bifurcated optical fiber (core size 600 ± 10 μm, BIF600-UV/VIS, Ocean Optics Inc. USA) and the common end was assembled with an optical fiber probe (F1000-ANGLE90, Ocean Optics Inc.

USA). Between the excitation unit and the bifurcated fiber, a band-pass filter (BPF, λ=340 ± 10 nm, Asahi Spectra Co., Ltd., Japan) was embedded for purifying the excitation light, and another BPF (λ=490 ± 10 nm) was installed in front of the detection site to reduce the interference of background noise.

The IPA sensing region was manufactured by equipping a flow-cell on the top end of fiber probe and attaching with a S-ADH immobilized membrane. The cylindrical flow cell was constructed by combining a silicone (Φ2 = mm) tube, which was etched with microflow channels, and a polymethylmethacrylate (PMMA) tube (Φ4 mm). The optical fiber probe was inserted into the central of the flow cell as shown in inserted box of fig.1. In this design, the NAD^+ (1 mmol L^{-1}) contained the Tris-HCl buffer (0.1 mol L^{-1}) was first pumped into the entrance flow channel and streamed through the space that between the cell and enzyme membrane, then withdraw from the other flow channel. The processes of the enzyme membrane include adding the S-ADH (1.25 unit cm^{-2}) powder into PMEH solution (10%, 2.5 μl cm^{-2}), mixing homogenously, then spreading the mixture carefully on an H-PTFE membrane. The wet membrane was dried for 3

hours in a refrigerator at 4℃, and was kept it in the refrigerator until experiment. An elastic silicone O-ring was used to fix the S-ADH membrane onto the top of the flow cell.

2.3 Characterization of the IPA bio-sniffers

As presented in fig.1, an air compressor (SLP-15EBD, Anest Iwata Co., Ltd., Japan) connected with activate carbon filter was used to supply ultra-clean air and was linked to a gas generator (PD-1B-2, Gastec, Co., Ltd., Japan), which was employed to provide the standard IPA vapor. Both pure air and IPA vapor were delivered through the Teflon tubes, and the flow-rate of both was adjusted at 200 ml min^{-1} by a mass flow controller (RK1200, Koflok Inc., Japan). A switch valve in front of the flow controller was used to select a gas sample (air or standard IPA vapor) applied to the sensing region of the bio-sniffer. The stream of buffer in the flow-cell kept the S-ADH membrane moist, supplied fresh NAD^+, and eliminated the reaction products. When S-ADH contacted with the IPA vapor, NADH was produced immediately, and the increment in the fluorescence

intensity was measured and recorded by the PMT. At the same time, circulated buffer removed the NADH and brought new NAD^+. Thus, the working principle of the bio-sniffer was established on the balance between the producing and eliminating rate of NADH.

Following steps carried out to characterize the IPA bio-sniffer: The clean air was flowed into the Teflon tube first, and then, switched the valve to let the IPA vapor be supplied to the bio-sniffer. Finally, switched the valve again to stop the supply of the IPA vapor, and flowed the clean air. 0.5 ppb to 54 ppm of standard IPA vapor was detected to determine the calibration range.

Method evaluating the reproducibility of the IPA bio-sniffer is as follows: First, the clean air was flowed for five minutes, and then 108 ppb of IPA was measured for five minutes. Finally, clean air was flowed again for five minutes. This cycle was repeated for ten times, and the coefficient of variation (C.V.) was calculated.

2.4 Effect of pH, buffer flow rate, enzyme units and the selectivity

For optimizing the detection conditions, several influence factors including buffer pH, buffer flow rate, and immobilized-enzyme units were evaluated. Considering enzyme kinetics, effect of buffer pH was investigated firstly. 1 mmol L^{-1} of NAD^{+} was prepared in phosphate buffer (PB, 0.1 mol L^{-1}, pH 5.0, 6.0, 7.0, 7.5 and 8.0), Tris-HCl buffer (0.1 mol L^{-1}, pH 8.0, 8.5, and 9.0), and carbonate-bicarbonate buffer (CB, 0.1 mol L^{-1}, pH 9.0, 9.5, 10.0, and 11.0). The relative signal response of IPA bio-sniffer using these buffers was measured by detecting 108 ppb of standard IPA vapor.

A flow rate of circulated buffer not only influenced the signal intensity as mentioned in section 2.3 but also interfered the response rate of the bio-sniffer. A liquid chromatography pump (SP-21-32, FLOM Co. Ltd., Japan) was used to control the flow rate of the buffer solution, and the buffer was circulated at a speed of 0.5 ml min^{-1} first, and progressively increased to 3.0 ml min^{-1} at the interval of 0.5 ml min^{-1}. The measured IPA concentration was set at 340 ppb, and the evaluated items include the change in intensity of the fluorescence and time taking for 90% of response.

In order to improve the signal intensity and reduce the consumption of enzyme, the unit of immobilized enzyme on the H-PTFE membrane was determined. The amount of immobilized S-ADH was examined from 0.25 unit cm^{-2} to 2 unit cm^{-2}, and the change in intensity was measured at 340 ppb of IPA vapor.

For the purpose of confirming the selectivity of the S-ADH, ten kinds of substances that have similar chemical structure to IPA were compared. They were: 2-butanol (racemic), (S)-(+)-2-butanol, (R)-(-)-2-butanol, 1-propanol, ethanol, 1-butanol, methanol, acetone, 2-buanone, and acetaldehyde. All of these gasses were measured at 600 ppb, and obtained change in fluorescence intensities were compared to the standard IPA gas at the same concentration.

2.5 Measuring the breath IPA from healthy volunteers

The IPA bio-sniffer was applied to determine the IPA concentration in the breath of healthy people for verifying its analytical ability after measurement conditions were optimized. All of the volunteers participated to this study

complied with following requirements: (1) No strenuous exercise within 24 hours, (2) no smoking within an hour, (3) no alcoholic beverage drinking within 24 hours, and (4) without illness. The exhaled breath collection method (Bikov et al. 2013) is described as follows: The volunteers took a rest until the breath became smooth and quiet. Then, the subjects were asked to do a deep inhalation through the nose and hold the breath for 15 seconds. The first 3 seconds of the exhaling air were abandoned, and the rest of the breath was collected using a 2-liter gas-sampling bag (2-9981-03, As one Co., ltd., Japan). This was because the first 3 seconds of exhaled breath was supposed to be the dead space air, and they might affect the result of some breath substance analysis such as nitric oxide (Paredi et al. 1998). The collected sample was connected to an air pump and supplied to the bio-sniffer for analyzing. The sample breath was delivered at a flow rate of 200 ml min^{-1} as the standard gas was measured. This experiment was authorized by the Human Investigations Committee of Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, authorization code: 2015-06, acting up to Declaration of Helsinki. The

differences of the breath IPA concentration between the stratified groups (genders and ages) were analyzed by student t-test. A significant difference was defined as the p-value less than 0.05.

3 Results and Discussion

3.1 Characteristics of the Acetone bio-sniffer

This bio-sniffer utilized the S-ADH to transform the concentration of IPA to an optical signal, which presented as the change in the NADH fluorescence intensity (Δintensity, unit: count). The calibration curve and the typical fluorescence response of the IPA bio-sniffer were presented in Fig. 2. When the bio-sniffer contacted to the IPA vapor, the fluorescence intensity increased immediately and reached a steady state within two minutes. After that, the fluorescence intensity recovered to its initial value when supply of the IPA vapor was stopped. These phenomena demonstrated that NADH increased on the enzyme membrane during the catalytic reaction of the S-ADH, the steady state presented that there was a balance between the production and elimination rate of NADH that caused by the S-ADH and the buffer flow respectively. The time that bio-sniffer needed for reaching to 90% of the steady state was around 40 to 90 seconds depending on the detected IPA concentrations. The change in intensity between the steady state and the background value was calculated and

plotted versus the IPA concentration (double logarithmic scale). The bio-sniffer displayed high sensitivity and wide calibration range. The calibration curve using power approximation curve fit for 1 to 9060 ppb determination was confirmed and the correlation coefficient was 0.997. It can be described by the following equation:

$$\Delta\text{intensity (counts)} = 2108.1 \times (\text{IPA concentration ppb})^{0.793} \quad (2)$$

The sensitivity of the bio-sniffer was estimated to be 1277.8 counts ppb^{-1} in the linear range of 1–350 ppb and the limit of detection (LOD, 3 times of blank standard deviation) was determined as 0.75 ppb. The calibration range of the bio-sniffer is comparable to or more than other reported IPA detection methods such as GC-MS combined analyzers (31.9–319 ppb) (Rudnicka et al. 2014), SPME-GC-MS methods (1.6–159.65 ppb) (Rudnicka et al. 2011), and cataluminescence sensor (60–600 ppb) (Wu et al. 2011). Furthermore, this bio-sniffer could be used for human exhaled air analysis because the calibration range includes the reported breath IPA concentration in health people and some diseases. For example, Claire Turner et al. investigated total propanol in breath

(which is believed to be mostly IPA) of healthy people and observed a range of 0 to 135 ppb with the mean value of 22 ppb (Turner et al. 2006). Concentration of exhaled IPA in patients with lung cancer was found from a range of 81.2 to 329 ppb with the mean value of 159 ppb in Sabine Kischkel's study (Kischkel et al. 2010). In addition, Joanna Rudnicka et al. also observed that chronic obstructive pulmonary (COPD) patients presented the breath IPA concentrations from 167 to 900 ppb with a median value of 523 ppb (Rudnicka et al. 2014).

The influence of the buffer solution flow rate was evaluated and optimized. The lower flow rate showed a higher signal but a longer time to reach a steady state, and it also required more time to recover to the initial value when supply of IPA gas was stopped. This demonstrated that the lower flow rate could provide well sensitivity but with poor response speed. On the other hand, the higher flow rate had opposite effects. Taking these aspects into account, we selected 1.5 ml min^{-1} as the condition in the subsequent experiments. The detailed result is shown in Fig. S1.

The IPA bio-sniffer also displayed high reproducibility. As shown in Fig. 3,

the bio-sniffer was performed for ten successive 108 ppb IPA vapor measurements and showed the coefficient of variation value (C.V.) of 2.59% (n = 10). This reproducibility of the bio-sniffer presented that the S-ADH was maintained stably on the H-PTFE membrane by the PMEH, and it also indicated that the bio-sniffer could continuously measure within a reasonable timeframe.

3.2 Effects of pH, immobilized enzyme units and the selectivity of S-ADH

For optimizing the performance and the analysis ability of the IPA bio-sniffer, several considerable factors, which might affect the fluorescence signals and the stability of the sniffer were investigated and evaluated. The pH value of the circulated buffer solution was investigated first because it directly influenced the enzyme kinetic of the S-ADH, fluorescence of NADH, and the stability of the bio-sniffer. Change in fluorescence intensity of the 108 ppb IPA detecting using various pH buffer solutions was presented in Fig.S2. A plateau of the related change in intensity was observed from pH 7.5 to pH 9.0, which implied that this pH range was appropriate for the bio-sniffer. There are two

possible considerations for this result: one is that the weak alkaline condition is suitable for the enzymatic activity of the S-ADH; another is that the NADH expresses higher fluorescence in this condition. According to our previous study, there is almost no correlation between the fluorescence intensity of NADH and the pH value (Chien et al. 2016). Therefore, it is reasonable to assume that the enzyme kinetic of S-ADH caused this result. Also, after taking the stability of the NAD^+ into consideration, pH 8.5 was selected for the experiments condition (Anderson and Anderson 1963).

The S-ADH unit, which was immobilized on an H-PTFE membrane, was also studied for optimizing the signal intensity and avoiding the unnecessary consumption of the enzyme. As shown in Fig. 4, the fluorescent signals, which were measured at a concentration of 340 ppb IPA, increased with the amounts of immobilized S-ADH, and a saturation of change in intensity was observed when the immobilized unit was more than 1.25 unit cm^{-2}. Consequently, immobilized 1.25 unit cm^{-2} of the S-ADH on an H-PTFE membrane as the sensing region of the bio-sniffer was chosen.

The selectivity is one of the important requirements for determining a sensor or an analysis method for human breath monitoring. Ten kinds of volatile organic compounds, which have similar chemical structure to the IPA, were detected at 600 ppb, and the fluorescence signals were compared to the standard IPA vapor at the same concentration. Fig. 5 displays the result of S-ADH selectivity evaluation. The bio-sniffer slightly responded to primary alcohols such as ethanol, 1-propanol, and 1-butanol, and almost not reacted to acetaldehyde and some ketones. Although S-ADH showed relatively high responses to racemic 2-butanol and (S)-(+)-2-butanol (58% and 88.9% respectively), it would not interfere our purpose of human breath analysis due to the rare existence of two substances in the human exhaled breath or body tissues (Buszewski et al. 2012b; Libardoni et al. 2006).

According to these results, various conditions were researched and optimized, and the effective performance of the bio-sniffer was established.

3.3 Analysis of IPA in breath

The IPA concentration in the breath from a healthy human was investigated using developed IPA bio-sniffer. In total, we measured 67 breath samples from 46 healthy volunteers. Fig. S3 displays the fluorescence responses when using the IPA bio-sniffer to analyze the human breath sample, it was very similar to the standard IPA measuring. Two of the breath IPA detection results (subject A: 20.7 ppb, and subject B: 6.0 ppb) are shown as a demonstration. The summary of a healthy population investigation is expressed in table 1. For all the volunteers, the measured breath ranged from 2.1 ppb to 54.4 ppb, and the mean ± standard deviation concentration was 16.1 ± 11.9 ppb. 5 samples did not be calibrated due to their signals was out of the calibration range. This obtained a result is similar to other studies (Conkle et al. 1975; Kischkel et al. 2010; Rudnicka et al. 2011; Turner et al. 2006). We also stratified the collected results into four groups according to the gender and the age. Student t-test was used to examine the difference between groups. The mean concentrations of each group were similar at around 16.0 ppb. Additionally, no significant difference was observed between male and female, and age below

and above 25 years old. Accordingly, the results of this experiment suggested that the IPA bio-sniffer enables to analyze the human exhaled breath, and this bio-sniffer is expected to be used for investigating about the breath biomarkers in lung-related diseases or monitoring of some illness.

4. Conclusion

In conclusion, a highly sensitive and selective gas-phase bio-sniffer using an enzymatic reaction of S-ADH for analysis of the IPA in the exhaled air was developed and optimized. A Y-shaped two-way optical fiber was used to integrate a UV-LED and a PMT to compose the main part of the bio-sniffer. An optical fiber probe equipped with a flow cell and an enzyme-immobilized membrane constructed a gas-sensing region. Designing of the circulated buffer solution allows the sniffer to measure continuously because it not only maintained the concentration of the NAD^+ but also removed the reacted products. The calibration range of the bio-sniffer was from 1 ppb to 9060 ppb, which is comparable to other reported IPA analyzed methods such as GC-MS associated instruments, and this calibration range also includes concentrations of breath IPA in healthy people and people who have a disease. Besides, the quick reaction and high reproducibility demonstrated the potential for real-time breath monitoring. The breath analysis from a healthy population showed a mean breath IPA concentration existed around 16.0 ppb, and no statistical

difference was observed between gender and age groups. In our latest breath investigation, an elevated trend of breath IPA was observed in diabetes patients. The detail results and discussion will be reported in the near future. This novel bio-sniffer can be a good candidate for IPA measurement and may bring some benefits for related breath research. It is also expected to find application as a non-invasive analytical instrument for clinical uses such as scanning of breath biomarkers.

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Figure captions

Figure 1.

Schematic diagram of the IPA bio-sniffer. A flow rate of the delivered gas (compressed air or standard IPA) was adjusted by a mass flow controller (set at 200 ml min^{-1}). The inserted box illustrates the sectional view of the flow cell. NAD^+ contained Tris-HCl buffer was circulated in the flow channel. S-ADH immobilized membrane was attached on the flow cell using a silicon O-ring.

Figure 2.

Calibration curve of the IPA bio-sniffer. The calibration ranged was confirmed from 1 ppb to 9060 ppb with R^2 = 0.994. The inserted box described the typical responses of the bio-sniffer. The measurement cycles were carried out by 2 min of pure air, 10 min of standard IPA and 10 min of pure air again.

Figure 3.

Reproducibility of the IPA bio-sniffer. The C.V. value was 2.59% for ten times of 108 ppb IPA cyclic measurements.

Figure 4.

Optimization of the immobilized enzyme units on a H-PTFE membrane. By measuring 340 ppb of IPA vapor, the saturation of the ∆intensity was observed after 1.25 unit cm^{-2}, and this amounts of S-ADH was used for the other experiments.

Figure 5.

Selectivity of the S-ADH evaluating by bio-sniffer. (S)-(+)-2-butanol and 2-butanol (racemic) displayed 89% and 58% of responses. The IPA bio-sniffer responded slightly to the ethanol, some primary alcohols, and other substances.

Table 1. Breath isopropanol (ppb) in a health population

	n*	measured range	mean ± SD**
total subjects	67(5)	2.1 - 54.4	16.1 ± 11.9
male	41(4)	2.1 - 42.1	15.9 ± 11.7
female	26(1)	4.1 - 54.4	16.5 ± 12.4
until 25 years old	28(2)	2.4 - 54.4	16.3 ± 13.5
above 25 years old	49(3)	2.1 - 42.6	16.0 ± 10.8

*Total n (Nono detcted n)

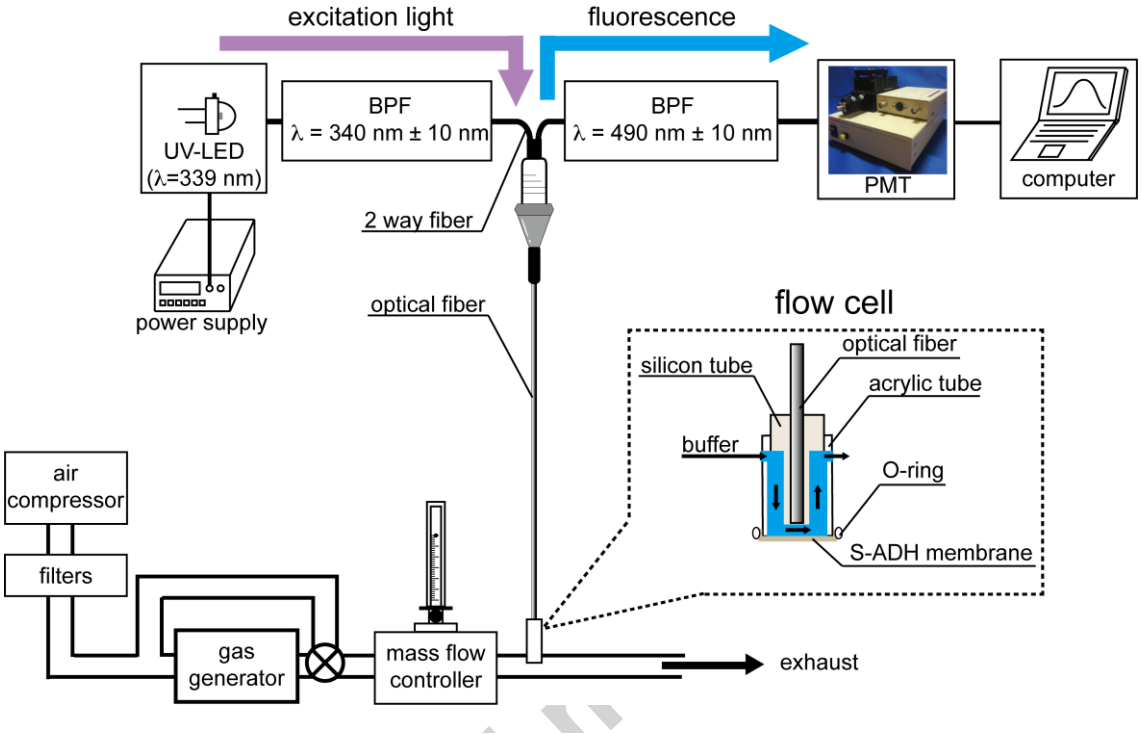
**no statistical differences was found in each groups

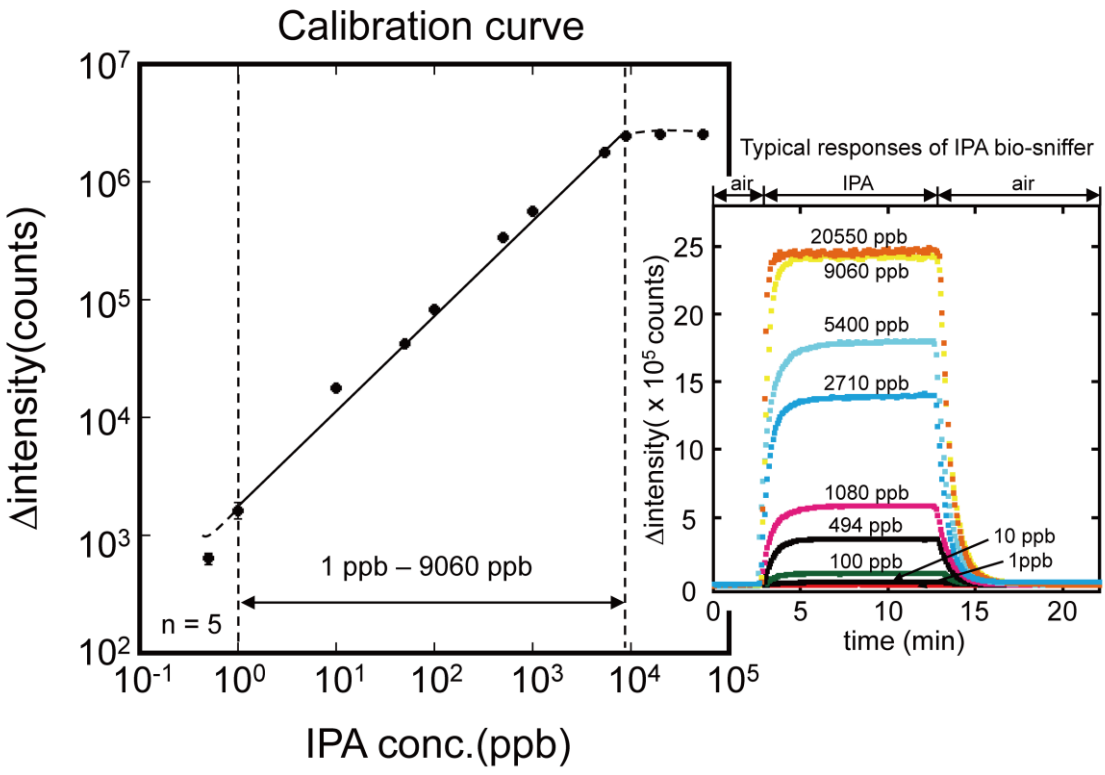
Highlights:

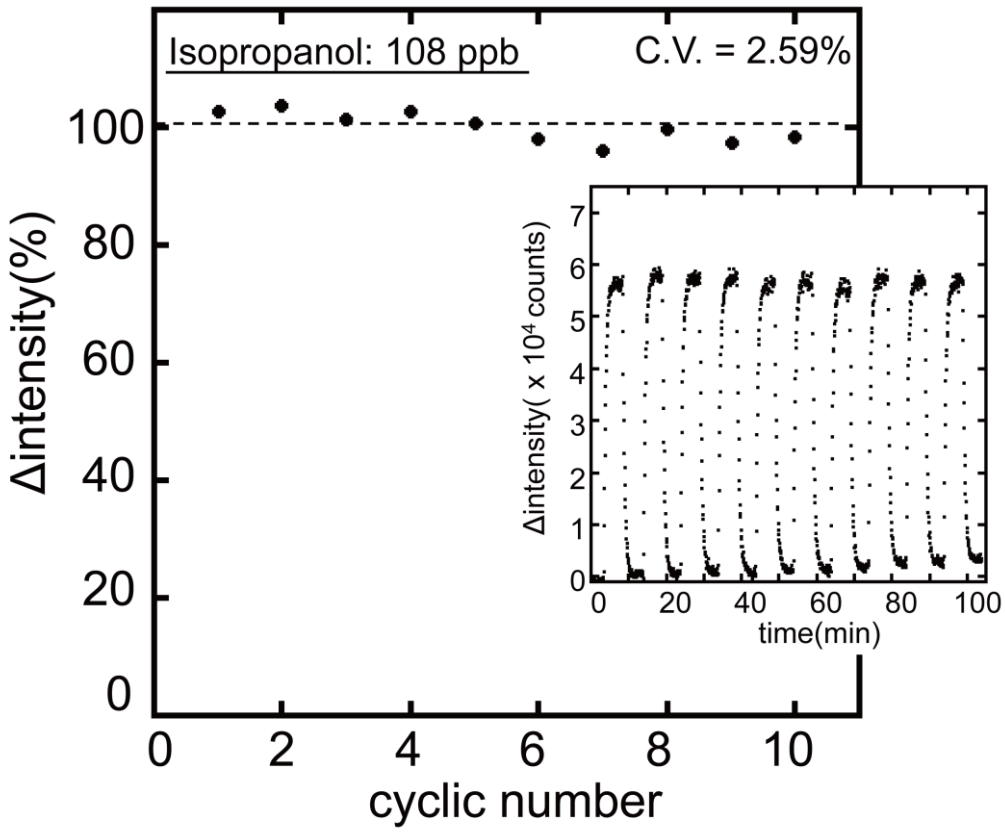
- An isopropanol bio-sniffer based on secondary alcohol dehydrogenase
- The Bio-sniffer measured IPA gas using the fluorescence intensity of NADH

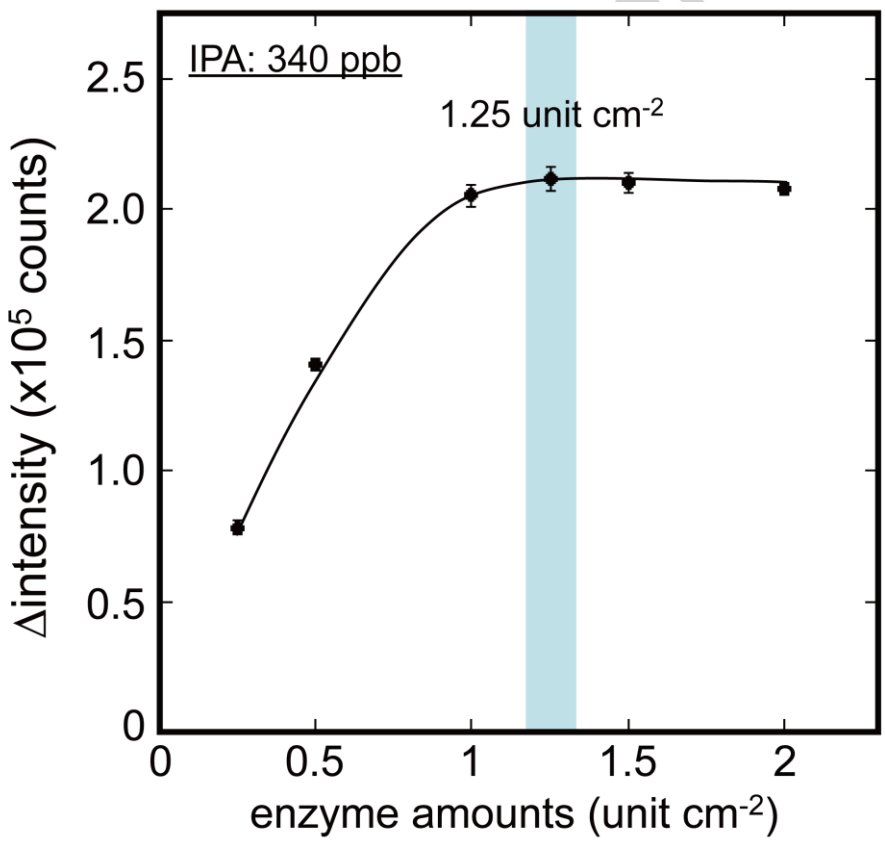
- This IPA bio-sniffer possessed wide calibration range and high sensitivity

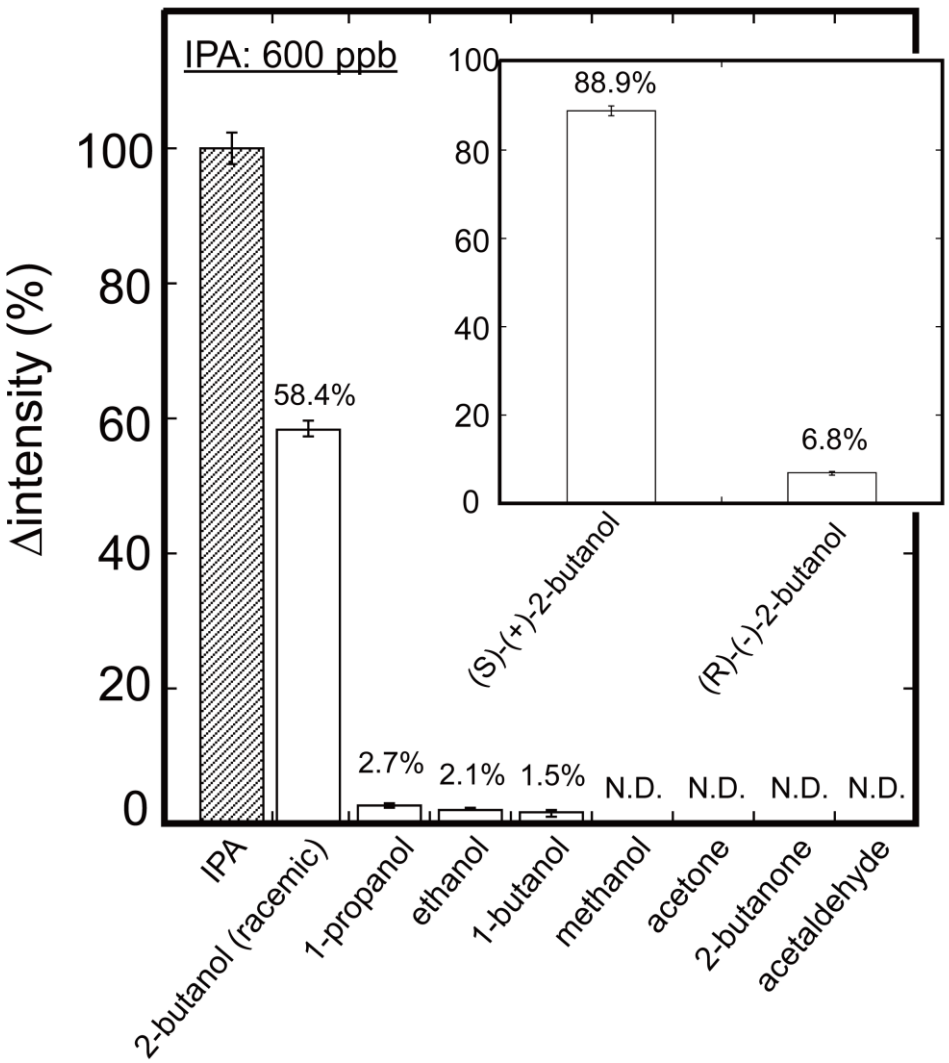
- Breath samples from healthy volunteers were measured by the IPA bio-sniffer






Isopropanol: 108 ppb
C.V. = 2.59%
Δintensity(%)
cyclic number
Δintensity(x 10⁴ counts)
time(min)


IPA: 340 ppb
1.25 unit cm-2
Δintensity (x10⁵ counts)
enzyme amounts (unit cm-2)


IPA: 600 ppb
Δintensity (%)
100
80
60
40
20
0
58.4%
2.7%
2.1%
1.5%
N.D.
N.D.
N.D.
N.D.
IPA
2-butanol (racemic)
1-propanol
ethanol
1-butanol
methanol
acetone
2-butanone
acetaldehyde
88.9%
6.8%
(S)-(+)-2-butanol
(R)-(-)-2-butanol