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Optical isopropanol biosensor using NADH-dependent secondary alcohol dehydrogenase (S-ADH)

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ABSTRACT

Isopropanol (IPA) is an important solvent used in industrial activity often found in hospitals as antiseptic alcohol rub. Also, IPA may have the potential to be a biomarker of diabetic ketoacidosis. In this study, an optical biosensor using NADH-dependent secondary alcohol dehydrogenase (S-ADH) for IPA measurement was constructed and evaluated. An ultraviolet light emitting diode (UV-LED, $\lambda = 340$ nm) was employed as the excitation light to excite nicotinamide adenine dinucleotide (NADH). A photomultiplier tube (PMT) was connected to a two-way branch optical fiber for measuring the fluorescence emitted from the NADH. S-ADH was immobilized on the membrane to catalyze IPA to acetone and reduce NAD^+ to be NADH. This IPA biosensor shows highly sensitivity and selectivity, the calibration range is from 500 nmol L^{-1} to 1 mmol L^{-1}. The optimization of buffer pH, temperature, and the enzyme-immobilized method were also evaluated. The detection of IPA in nail related cosmetic using our IPA biosensor was also carried out. The results showed that large amounts of IPA were used in these kinds of cosmetics. This IPA biosensor comes with the advantages of rapid reaction, good reproducibility, and wide dynamic range, and is also expected to use for clinical IPA detections in serum or other medical and health related applications.

1. Introduction

Isopropanol (IPA), also known as 2-propanol or isopropyl alcohol, is a colorless liquid with a bitter taste [1] and is the simplest secondary alcohol. IPA is an important solvent and commonly used in industrial activity such as a washing agent in the process of semiconductor manufacturing [2] and it is often found in the environment like in food additives, a component of furniture cleaner or as a solvent of some cosmetics [3]. Also, IPA is widely used in hospitals as the antiseptic alcohol gel for and disinfectant.

From a medical point of view, IPA rarely exists in the human body or only in very low concentration unless accidental ingestion or exposure to gaseous IPA [4,5]. Recently, several studies reported that IPA could be measured in plasma or urine from ketoacidosis patients, who had not been exposed to or ingested IPA [6,7]. For example, Petersen et al. pointed out that in the 75% of diabetic ketoacidosis (DKA) and 52% of alcoholic ketoacidosis (AKA) postmortems, IPA could be measured in the serum with a concentration of 15.1 ± 13.0 mg/dL and 18.6 ± 12.5 mg/dL [8]. Jessica B. Dwyer reported a chronic alcoholic patient case that IPA could be detected in the serum with a concentration of 6 mg/dl [9]. The researchers postulated that the serum IPA not only came from the ingestion but also may come from the metabolism of acetone by the enzymatic reaction of alcohol dehydrogenase (ADH) in some situations [8–10]. Usually, IPA would be metabolized to acetone in the liver by ADH or cytochrome P-4502E1 [11–13], however, in some situations, ADH will also convert acetone to IPA reversely due to the change of serum pH, excessive acetone or the elevated ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidase nicotinamide adenine dinucleotide (NAD^+) [9,10]. It seems there is potential that blood IPA can be a biomarker of DKA or to be the monitoring indicator for a diabetic because hyperglycemia is one of the most important factors causing DKA. Not only in humans, some studies also found that blood and rumen IPA concentration would increase in the ketotic dairy cows, which is

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an obsession for animal husbandry [14,15]. Therefore, measuring IPA in the biological sample is necessary and valuable.

The conventional blood or urine IPA measurement methods include gas chromatography-flame ionization detector (GC-FID) [16–19] or headspace gas chromatography with flame ionization detection (HS-GC–FID) [7,8,20]. These GC-FID based detection methods are highly sensitive but inconvenient to use, they need complicated pretreatment of samples and need high costs for a large amount of detection. An enzyme-based biochemical sensor provides an alternative candidate method for an accurate, simplified and low cost of IPA measuring. In our previous studies, we have already developed an optical biosensor using ultraviolet light-emitting diode (UV-LED), optical fiber probe, and photomultiplier tube (PMT) for ethanol determination [21]. The measuring principle of this ethanol sensor is based on the enzymatic reaction ADH that can catalyze ethanol to become acetaldehyde and meanwhile reduces co-enzyme NAD^+, which acts as the electron acceptor, to be NADH. NADH comes with the unique optical property that when it absorbs 340 nm UV light, it will release visible fluorescence while NAD^+ does not have same property [22]. Thus, the ethanol concentration is determined according to the fluorescence intensity emitted from the NADH [21,23–25]. This kind of NADH-dependent optical biosensor is convenient to use, low cost and with quick response time. Besides, it is not only can be used for the ethanol determination but also for other compounds according to corresponding enzymes. For example, NADH-dependent secondary-alcohol dehydrogenase (S-ADH) comes with following catalyzing reaction equation:

$$\text{isopropanol} + NAD^+ \overset{S-ADH}{\rightleftarrows} \text{acetone} + NADH \quad (1)$$

S-ADH can catalyze isopropanol to be acetone and utilize NAD^+ as the electron acceptor in alkaline condition. Therefore, an IPA biosensor also can be constructed according to this kind of technology and conceptions.

In this research, we constructed and evaluated a highly sensitive, which was based on the NADH-dependent S-ADH for the determination of IPA in the liquid phase. By the enzymatic reaction of S-ADH, relevant concentrations of NADH would be produced depending on the concentration of IPA. The produced NADH would be excited using a UV-LED system and the fluorescence would be calculated by the PMT. These two components were connected together using a two-way branch optical fiber and equipped with a fiber probe that was fixed with the enzyme-immobilized membrane. Construction and characterization of the IPA biosensor using S-ADH immobilized membrane and UV-LED excitation system would be described first. And then, the measurements of the IPA in nail related cosmetics products were also performed.

2. Experiment

2.1. Reagents and chemicals

β-NADH were bought from Oriental Yeast, Co., Ltd., Japan and dissolved in deionized (DI) distilled water collected from Milli-Q purification system (Millipore, Co., USA) for several concentrations. NAD^+ (β-NAD^+: Oriental Yeast Co., Ltd., Japan) and NADH-dependent secondary alcohol dehydrogenase (S-ADH, EC. 1.1.1.x, 1 unit mg^{-1}, E001) from yeast (Daicel Chiral Technologies, Co., Japan) were prepared in suitable buffer respectively and storage in the refrigerator before use. Hydrophilic polytetrafluoroethylene filter membrane (H-PTFE, pore size: 0.2 μm, porosity: 80%, JGWP14225) employed for S-ADH immobilization was purchased from Millipore, USA. The synthesis method of the polymer using 2-methacryloyloxyethyl phosphorylcholine polymerized with 2-ethylhexyl methacrylate (PMEH) was according to our previous report [26]. Buffer chemicals including di-potassium hydrogen phosphate (K_2HPO_4, 98.0%), potassium di-hydrogen 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 obtained from Wako Pure Chemical Industries, Japan and prepare in DI water.

2.2. Construction of fiber optic NADH measurement system and IPA biosensor

The NADH measurement system was composed of four main components: an UV-LED (UVTOP® BL335, Sensor Electronic Technology, Inc., USA) with power supplier circuit (Yokogawa Electric Corporation, Japan) as the excitation light source, a photomultiplier (PMT, C8502, Hamamatsu Co., Japan) as the fluorescence intensity detector, an optical fiber probe (F1000-900, Ocean Optics Inc. USA) for sample contacting and a two-way branch optical fiber (BIF600-UV/VIS, core size 600 ± 10 μm, Ocean Optics Inc. USA) for assembling. Two pieces of band-pass filters (BPF, λ=340 ± 10 nm and 490 ± 10 nm, Asahi Spectra) were equipped in front of UV-LED and PMT respectively for the purpose of reducing the interference of background noise.

The IPA biosensor was erected by attaching an enzyme-immobilized membrane on the top of fiber probe. The S-ADH enzyme membrane was manufactured by mixing S-ADH (1 mg cm^{-2}) with PMEH solution (10% w/w in ethanol, 10 μl cm^{-2}) uniformly and spread averagely on the cleaned H-PTFE membrane. Then, the moist membrane would be prepared in the refrigerator at 4 °C immediately 3 h for enzyme immobilizing and drying. After that, the enzyme membrane would have over coat with pure PMEH solution (10% w/w in ethanol, 5 μl cm^{-2}) for the purpose of fixing the enzyme stable. The S-ADH immobilized membrane was cut out into 0.5 cm × 0.5 cm and set onto the top of optical fiber probe flat with 3 mm silicone tube (ø=1 mm, As One Co., Japan). In this method, the IPA biosensor was fabricated.

2.3. Measurement of standard NADH and IPA solution

The experimental diagram is schematized in Fig. 1. The optical probe was immersed in the middle of buffer and serial concentrations of standard NADH solution were dropped into the dark cuvette, which contained with 300 μl of pH 8.5 Tris–HCl buffer. The final concentrations of NADH were adjusted from 1 from 1 nmol L^{-1} to 100 mmol L^{-1}. The fluorescence that emitted from NADH would be guided to the PMT through the two-way branch optical fiber and the intensity would be displayed on the computer in real-time. The integration time of fluorescence intensity calculation was set at 1 s and using the unit of count per second (cps).

The optical probe equipped with the enzyme-immobilized membrane was used to determine the standard IPA, and the method was same as NADH measurement. When S-ADH immobilized membrane was in contact with to IPA solution, NAD^+ would be oxidized to NADH as mentioned as equation (1). Various concentrations (10 nmol L^{-1} to 100 mmol L^{-1}) of standard IPA solutions were added into dark cuvette that contained 300 μl of the buffer with 20 mmol L^{-1} of NAD^+. The increase of fluorescence intensity that emitted from NADH was measured by the PMT.

2.4. Optimization of IPA biosensor including reproducibility, buffers pH, temperature, and selectivity

Reproducibility is one of the important indicators for evaluating the ability of a biosensor. The reproducibility of IPA biosensor

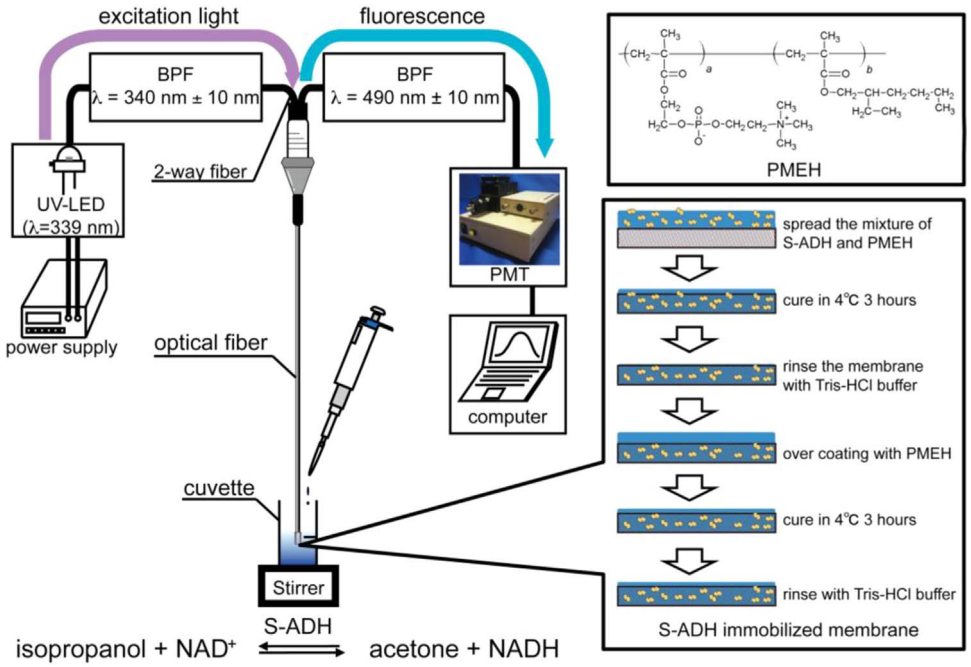

Fig. 1. The composition of optical IPA biosensor including UV-LED, PMT and optical fiber probe with enzyme immobilized membrane. S-ADH was immobilized on a H-PTFE membrane using the PMEH polymer.

was tested by measuring 1 mmol L^{-1} first, and then the optical probe with S-ADH membrane was immersed into 50 ml of cleaning Tris–HCl buffer without NAD^+ for ten minutes to remove the produced NADH and residual IPA on the S-ADH membrane. After washed, the measurement steps were repeated again and for ten cycles. Two kinds of enzyme-immobilized methods were compared, one is as described at graph 2.2 and the other is a similar process but without PMEH over-coating.

To confirm the optimization conditions of the IPA biosensor, several probable interference factors were surveyed. The buffer pH was considered first because it is known that the enzyme kinetic would be altered under different pH conditions and change the reaction velocity. Besides, pH is also one of the factors influences fluorescence. 20 mmol L^{-1} of NAD^+ were prepared in different pH buffers, includes of phosphate buffer (PB, 0.1 mol L^{-1}, pH 5.5, 6.0, 7.0, 7.5 and 8.0), 2-Amino-2-hydroxymethyl-propane-1,3-diol -Hydrochloric acid buffer (Tris–HCl, 0.1 mol L^{-1}, pH 8.0, 8.5, 9.0 and 9.5) and sodium carbonate-bicarbonate buffer (CB, 0.1 mol L^{-1}, pH 9.5, 10.0 and 11.0). Comparing the fluorescence intensity that was estimated at 1 mmol L-1 of IPA carried out the assessments of these pH conditions. The standard NADH (500 μmol L^{-1}) was also measured in several pH buffers as mentioned above.

Temperature is also one of the important influence elements that affect the enzyme kinetics and fluorescence intensity. The black cuvette was put into a water bath tool (bathtool, BT-60, As One Co., Japan), which was used for keeping the temperature. A liquid pump (gear pump, AWT-40W, Tec World Co., Japan) with Teflon tube was connected to a low-temperature bath (CTB-2, As One Co., Japan) that was used to control the temperature of circulation water. A soft thermistor (LS450-T, Osaka Micro Computer Inc. Japan) was inserted into the buffer, which was contained in the cuvette, for monitoring of the temperature. The fluorescence intensity of 500 μmol L^{-1} standard NADH and 1 mmol L^{-1} IPA were measured from 10 °C to 50 °C respectively.

The influence of NAD^+ concentration for the biosensor was also concerned; several NAD^+ concentrations (0.1, 1, 20 and 100 mmol L^{-1}) were prepared in Tris–HCl buffer (0.1 mol L^{-1}, pH 8.5) and were conducted to calibration curve determinate as described at 2.3 paragraph.

For verifying the selectivity of S-ADH, eight kinds of similar chemical structure compounds were measured and compared to standard IPA. The following chemicals were detected: 1-butanol, 2-butanol, 1-propanol, ethanol, acetaldehyde, methanol, 2-butanone and acetone. All of these substances were measured at the concentrations 1 mmol L^{-1}. The experiment of S-ADH selectivity was carried out in optimized condition.

2.5. Measurement of IPA in commercial cosmetic products

IPA is commonly found in the living environment, for example, as the solvent of cosmetics or a component of furniture cleaner. Thus, ten kinds of nail related commercial cosmetic products were detected using our IPA biosensor. The measurement samples need to be pretreated by mixing 50 μg of the sample with 450 μl deionized distilled water. Most samples were not dissolved totally in water due to additives, but it would not interfere our purpose because IPA is soluble in water. The sample mixture was centrifuged 5 min at 10,000 rpm to make an insoluble precipitate and the supernatant was be used for the experiments. The measurement was carried out at optimum condition. 3.03 μl of supernatant was added to the cuvette, which contained 300 μl of the buffer with 20 mmol L of NAD^+. Through this kind of pretreatment method, the original sample has been diluted to one thousand times, and sample 4, 5 and 7 were more diluted to ten thousand times.

These samples were also measured the IPA concentrations using NADH absorbance method. This method utilized the optical property of NADH that it absorbs UV at the peak of 340 nm wavelengths. Serials of 10 μl standard IPA were mixed with 90 μl of Tris–HCl buffer contained 20 mmol L^{-1} NAD^+ and injected into 96-well strip plate (437,915, Clear, Thermo Fisher Scientific Inc., Japan). After 0.5 μl (0.625 mg S-ADH per one μl H_2O) of S-ADH solution were injected into each well, the plate would be shack for 5 min and put into plate reader (SH-1000, CORONA ELECTRIC Co., Ltd, Japan) for detection of absorbance. By this way, the calibration

curve of IPA concentration versus NADH absorbance was obtained. The commercial cosmetics samples were pretreated and diluted as mentioned before and measured the IPA concentration by this method.

3. Results and discussion

3.1. Characterization of the IPA biosensor

Results of NADH measurement are presented in supplementary Fig. S1. Fluorescence intensity increased immediately and reached to steady state in a short time when the standard NADH was dropped into the dark cuvette. This phenomenon revealed that the NADH measurement system had a fast response. 90% response time was within 2 min at 1 mol L^{-1}. The calibration range for NADH detection was from 10 nmol L^{-1} to 10 mmol L^{-1} (R=0.997), and more details are described in the Supplementary information.

For standard IPA solution measurement, Fig. 2 shows the temporal changes of fluorescence intensity to various concentrations of standard IPA (inserted box) and the calibration curve. When standard IPA solution was dropped into the cuvette, the fluorescence intensity increased immediately and reached to the steady state within 5 min. The quick response was confirmed and the fluorescence signal reflected that the enzyme membrane produced NADH through the S-ADH enzymatic reaction of catalyzing IPA to acetone and this biosensor measures the IPA level according to the difference of NADH fluorescence intensity (Δintensity). The dynamic range was confirmed from 500 nmol L^{-1} to 1 mmol L^{-1} with a correlation coefficient of 0.998 for n=5. The calibration curve having a power approximation and can be described as following equation:

$$\Delta\text{intensity(cps)} = 14.09 \times \left(\text{IPA concentration}\left(\text{nmol } L^{-1}\right)\right)^{0.787} \quad (2)$$

Higher concentration of IPA was out of calibration range due to the saturation of enzyme reaction, however, controlling the volume of buffer in the cuvette or appropriate dilution of the sample can resolve it easily.

3.2. Optimization of IPA biosensor including reproducibility, buffers pH, temperature, and selectivity

Fig. 3 highlights reproducibility difference between the two kinds of enzyme-immobilized methods. Obviously, method

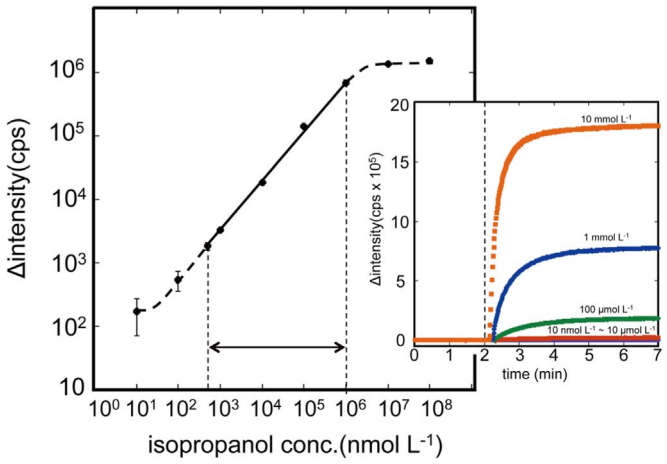

Fig. 2. Calibration curve of IPA biosensor and the temporal change of fluorescents intensity to each concentration. The calibration range is from 500 nmol L^{-1} to 1 mmol L^{-1}.

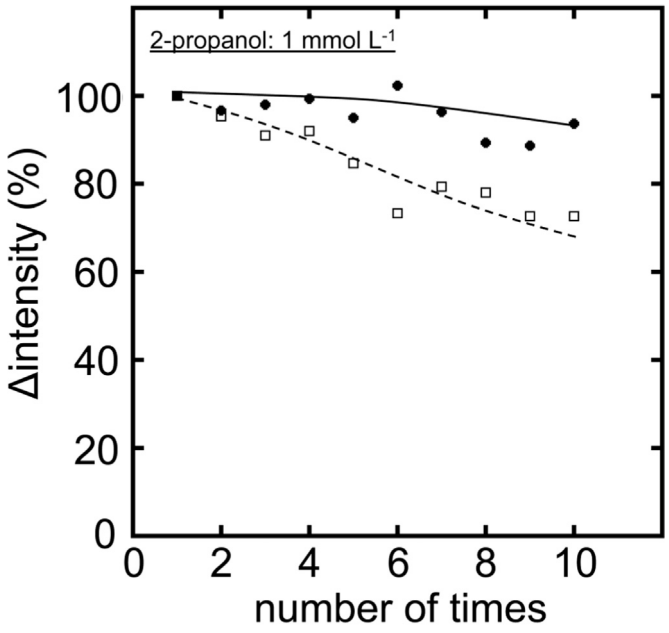

Fig. 3. Comparison of enzyme immobilized methods with and without PMEH over coating by ten times of 1 mmol L^{-1} IPA detection. Improving of the reproducibility by PMEH over coating was confirmed (C.V.=4.62%). (□) enzyme immobilized without over coating. (•) enzyme immobilized with the PMEH over coating.

1 without PMEH over coating could not be reused because the fluorescence signal decreased progressively in every cycle. The more likely explanation rests in that one layer of PMEH could not fix the S-ADH on H-PTFE membrane instable; the enzyme would lose in each reaction step or washing period. Meanwhile, the coefficient variation (C.V.) for method 2, with PMEH over coating, is only 4.62%. This high reproducibility represents that the IPA biosensor is reusable because the S-ADH fixed on the H-PTFE membrane in steady by PMEH over coating.

The optimization measurement conditions for the IPA biosensor have been evaluated including the buffer pH, temperature and the concentration of NAD^+. Regarding of the buffer pH experiments, Δintensity fluorescence of 1 mmol L^{-1} IPA was measured in serious of buffer pH conditions. PB buffer was varied from pH 5.5 to pH 8.0, Tris–HCl buffer was adjusted from pH 8.0 to pH 9.5 and CB buffer was prepared from pH 9.5 to pH 11.0. All of the buffers were 0.1 mmol L^{-1} with 20 mmol L^{-1} of NAD^+. As shown in Fig. 4, Δintensity of fluorescence shows the plateau value from pH 8.5 to pH 9.5. This result indicates that the reaction kinetics of S-ADH associated with pH and the weak alkaline condition is favorable for our biosensor. In the case of strong alkaline pH, the fluorescence signal suddenly decreased due to the degradation of the NAD^+. The NAD^+ solution in the condition of pH 11.0 would become brown color within hours and come with unusually high background noise. Therefore, although pH 9.5 shows highest Δintensity, considering the stability and the storage of NAD^+ [27], the experiment condition was decided at pH 8.5. Fig. S3 presents the result of NADH fluorescence intensity in different pH conditions. It seems probable that the influence of pH on the NADH fluorescence is slight, because no obvious change of fluorescence intensity in different pH conditions was observed. Therefore, it is reasonable to consider that pH influences enzyme kinetic of S-ADH more.

It is known that temperature is one of the important elements for enzyme kinetics and fluorescence emission intensity; the temperatures of IPA biosensor measurement environment were characterized. The detail results are offered in the supplementary information. Theoretically, the IPA biosensor was expected to

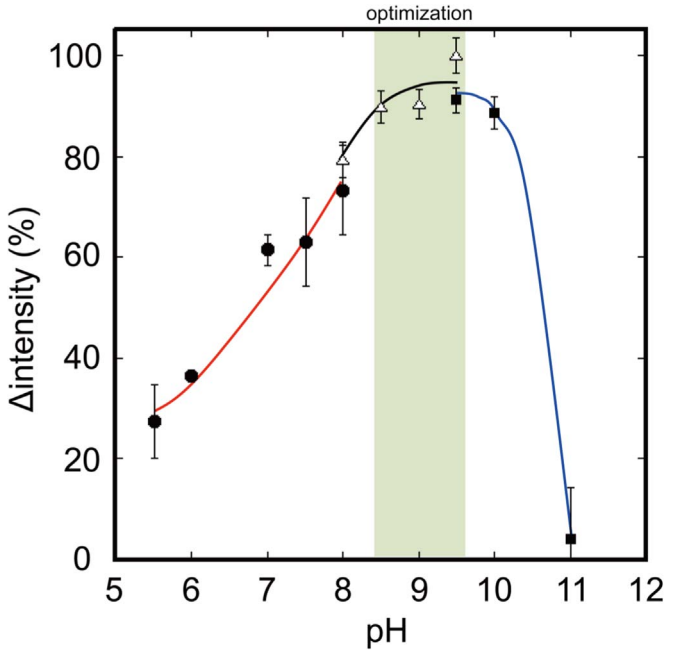

Fig. 4. Characteristic of IPA biosensor in different pH conditions. The plateau was found from pH 8.5 to pH 9.5 and pH 8.5 was decided to all experiments.

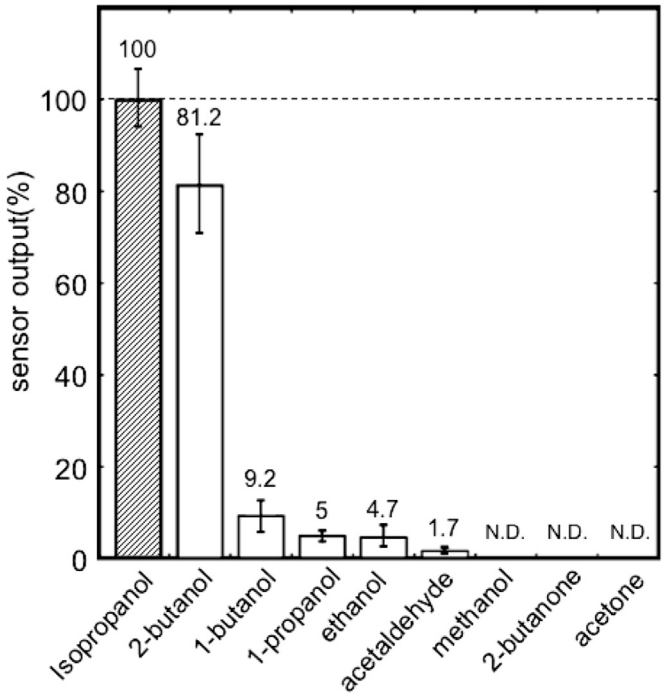

Fig. 5. The selectivity of the S-ADH biosensor. The relative fluorescent intensity of 2-butanol was 81.2% and other chemical substances displayed weak response. All substances were measured in a concentration of 1.0 mmol L^{-1}.

display weak fluorescence signal at a lower temperature and strong intensity at a higher temperature until enzyme denatures. However, the obtained results were totally different: lower temperature with higher signal and higher temperature showed in contrast (Fig. S2). A similar trend was also observed in standard NADH measurement. This result is in line with the study of Zelent et al. They researched about the temperature dependence for fluorescence of NADH and revealed out that the fluorescence intensity increases when temperature decreases [28]. Accordingly, it is likely that the temperature interferes the fluorescence of NADH much more than affects the enzymatic kinetics of S-ADH. Also, this result signalizes the necessary of recalibration when the biosensor works in that temperature dramatic change environment.

Then, the effect of NAD^+ concentration to the calibration range for the IPA biosensor was evaluated. The dynamic range of IPA measurement was from 100 nmol L^{-1} to 10 μmol L^{-1} for 100 μmol L^{-1} of NAD^+, 100 nmol L^{-1} to 100 μmol L^{-1} for 1 mmol L^{-1} of NAD^+ and 500 nmol L^{-1} to 1 mmol L^{-1} for 20 and 100 mmol L^{-1} of NAD^+. The lower limit of detection was found in 0.1 and 1 mmol L^{-1} of NAD^+ and a higher concentration of NAD^+ could be used to measure a wide range of IPA concentrations. One of the potential reasons is that higher concentration of NAD^+ would contribute to the appearance of competitive inhibition and decrease the reaction rate. The optimized concentration of NAD^+ should be chosen according to the experimental purpose. In this study, 20 mmol L^{-1} of NAD^+ was chosen for all experiments.

Fig. 5 illustrates the selectivity of S-ADH. Eight kinds of different species with a similar chemical structure including 2-butanol, 1-butanol, 1-propanol, ethanol and other aldehydes and ketones were employed to compare with IPA. As observed in Fig. 5, 2-butanol presented about 81.2% of fluorescence signal compared to IPA in the same concentration, and the other compounds showed a slight reaction. This high selectivity was attributed to the specific molecular distinguish activity of S-ADH. The observed high reacting of 2-butanol not only indicated that the possible existence of 2-butanol in measured sample should be considered but also presents that this IPA biosensor can be used for 2-butanol determination. Besides, compared to IPA, 2-butanol rare exists in the human body [29]. Therefore, the interference of 2-butanol could be ignored for our purpose of detecting the IPA in the body fluid such as serum, urine or saliva.

3.3. IPA concentration in nail related cosmetics

The results of IPA measurement in the nail related cosmetic are shown in Fig. 6 and the type of the detected cosmetic are listed in Table 1. The observed concentrations of IPA were various; Sample 1, 2, 3, and 6 contained about 0.6 mol L^{-1} of IPA, sample 4, 5, and 7 were observed higher IPA concentrations, and sample 8, cuticle

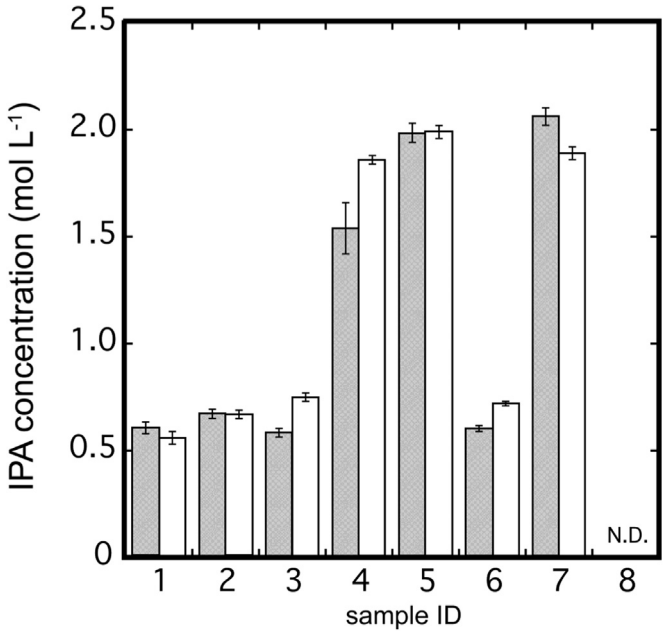

Fig. 6. Measurement results of commercial cosmetics products using the IPA biosensor and NADH absorbance method. Several of IPA concentration was observed in nail related cosmetics and sample 8 'cuticle oil' did not contain any IPA.

Table 1
Information of measurement cosmetic samples.

Sample ID	Type	Usage	Main ingredient
1	Nail base coat	Nail strengthen base of manicure	Ethyl acetate, IPA, butyl acetate, phthalic anhydride, glycols copolymer
2	Nail top coat	Nail manicure protect	Ethyl acetate, IPA, butyl acetate, acetyl methionine, nitrocellulose
3	Nail top coat	Nail art decoration	Ethyl acetate, IPA, butyl acetate, phthalic anhydride, adipic acid
4	Nail hardener	Nail strengthen instant protection	Ethyl acetate, IPA, butyl acetate, acetate butyrate, camphor
5	Nail color	Nail makeup	Ethyl acetate, IPA, butyl acetate, acetyl methionine, camphor, benzoic acid
6	Nail color	Nail makeup	Ethyl acetate, IPA, butyl acetate, citric acid, yellow no.203, titanium oxide
7	Nail coat clearer	Clear fingernails without painting manicure	Ethyl acetate, IPA, butyl acetate, phthalic anhydride, glycols copolymer
8	Cuticle oil	Nourishes softer and supple cuticles	Ethylhexyl palmitate, triethylhexonin, isopropyl palmitate, *Macadamia integrifolia* seed oil

oil, did not contain IPA. Fig. S4 describes the scatter plot of measurement results detected by IPA biosensor and NADH absorbance detection method and displayed high correlation (analyzed by Pearson correlation coefficient statistic method, $R=0.972$, $P<0.001$). It is worth mentioning that the dynamic range of our IPA biosensor was from 500 nmol L^{-1} to 1 mmol L^{-1} NAD^+, which was much wider than NADH absorbance detection method (dynamic range: 100 μmol L^{-1} – 1 mmol L^{-1}).

IPA is a usual ingredient used in nail cosmetic for antifoaming and decreasing the viscosity [30]. It is known that the lungs and skin are both of the IPA absorption paths for the human body; thus, the usage amount and exposure duration should be carefully considered when using these IPA contained cosmetics, especially of nail colors and hair dyes. The assay ability of this IPA biosensor was proven by this real sample detection.

4. Conclusions

In this work, a UV-LED based fiber-optic NADH fluorescence measurement combined with S-ADH immobilized membrane for determination of IPA was developed, and demonstrated. The optimization conditions were characterized including buffer pH, temperature, and concentration of NAD^+. This IPA biosensor performed calibration range from 500 nmol L^{-1} to 1 mmol L^{-1} with high selectivity. Compared to traditional detection methods, rapid response of the biosensor can be applied for large amount measurement in the efficient evaluation. S-ADH was immobilized well on the H-PTFE membrane using PMEH polymer and the protective over coating could sustain the reuse of the biosensor with good reproducibility. The analysis ability of IPA biosensor was investigated by determination of commercial cosmetics and the detected results were similar with NADH absorbance detection method. Real sample measurement not only proved that IPA is widely used in nail related cosmetics but also reminded us to pay attention in usage amount and exposure duration when using these products. This biosensor offers a new tool for IPA measurement and is expected to use in medical fields such as DKA or AKA monitoring. We intend to continue improving this biosensor and apply this sensor concept to determinate other chemical substances.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.06.036.

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