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

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25

26 **Abstract**

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

42 advantages of rapid reaction, good reproducibility, and wide dynamic range, and
43 is also expected to use for clinical IPA detections in serum or other medical and
44 health related applications.

45

46

47 **1. Introduction**

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

55 From a medical point of view, IPA rarely exists in the human body or
56 only in very low concentration unless accidental ingestion or exposure to
57 gaseous IPA[4, 5]. Recently, several studies reported that IPA could be
58 measured in plasma or urine from ketoacidosis patients, who had not been
59 exposed to or ingested IPA[6, 7]. For example, Petersen et al pointed out that in
60 the 75% of diabetic ketoacidosis (DKA) and 52% of alcoholic ketoacidosis (AKA)
61 postmortems, IPA could be measured in the serum with a concentration of
62 15.1 ± 13.0 mg/dL and 18.6 ± 12.5 mg/dL[8]. Jessica B. Dwyer reported a chronic

63 alcoholic patient case that IPA could be detected in the serum with a
64 concentration of 6 mg/dl[9]. The researchers postulated that the serum IPA not
65 only came from the ingestion but also may come from the metabolism of acetone
66 by the enzymatic reaction of alcohol dehydrogenase (ADH) in some
67 situations[8-10]. Usually, IPA would be metabolized to acetone in the liver by
68 ADH or cytochrome P-4502E1 [11-13], however, in some situations, ADH will
69 also convert acetone to IPA reversely due to the change of serum pH, excessive
70 acetone or the elevated ratio of reduced nicotinamide adenine dinucleotide
71 (NADH) to oxidase nicotinamide adenine dinucleotide (NAD⁺)[9, 10]. It seems
72 there is potential that blood IPA can be a biomarker of DKA or to be the
73 monitoring indicator for a diabetic because hyperglycemia is one of the most
74 important factors causing DKA. Not only in humans, some studies also found
75 that blood and rumen IPA concentration would increase in the ketotic dairy cows,
76 which is an obsession for animal husbandry [14, 15]. Therefore, measuring IPA
77 in the biological sample is necessary and valuable.

78 The conventional blood or urine IPA measurement methods include gas

79 chromatography-flame ionization detector (GC-FID)[16-19] or headspace gas
80 chromatography with flame ionization detection (HS-GC-FID)[7, 8, 20]. These
81 GC-FID based detection methods are highly sensitive but inconvenient to use,
82 they need complicated pretreatment of samples and need high costs for a large
83 amount of detection. An enzyme-based biochemical sensor provides an
84 alternative candidate method for an accurate, simplified and low cost of IPA
85 measuring. In our previous studies, we have already developed an optical
86 biosensor using ultraviolet light-emitting diode (UV-LED), optical fiber probe, and
87 photomultiplier tube (PMT) for ethanol determination[21]. The measuring
88 principle of this ethanol sensor is based on the enzymatic reaction ADH that can
89 catalyze ethanol to become acetaldehyde and meanwhile reduces co-enzyme
90 NAD^+ , which acts as the electron acceptor, to be NADH. NADH comes with the
91 unique optical property that when it absorbs 340 nm UV light, it will release
92 visible fluorescence while NAD^+ does not have same property[22]. Thus, the
93 ethanol concentration is determined according to the fluorescence intensity
94 emitted from the NADH[21, 23-25]. This kind of NADH-dependent optical

95 biosensor is convenient to use, low cost and with quick response time. Besides,
96 it is not only can be used for the ethanol determination but also for other
97 compounds according to corresponding enzymes. For example,
98 NADH-dependent secondary-alcohol dehydrogenase (S-ADH) comes with
99 following catalyzing reaction equation:

100

102

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

106 In this research, we constructed and evaluated a highly sensitive, which
107 was based on the NADH-dependent S-ADH for the determination of IPA in the
108 liquid phase. By the enzymatic reaction of S-ADH, relevant concentrations of
109 NADH would be produced depending on the concentration of IPA. The produced
110 NADH would be excited using a UV-LED system and the fluorescence would be

111 calculated by the PMT. These two components were connected together using a
112 two-way branch optical fiber and equipped with a fiber probe that was fixed with
113 the enzyme-immobilized membrane. Construction and characterization of the
114 IPA biosensor using S-ADH immobilized membrane and UV-LED excitation
115 system would be described first. And then, the measurements of the IPA in nail
116 related cosmetics products were also performed.

117

118 **2. Experiment**

119 **2.1 Reagents and chemicals**

120 β -NADH were bought from Oriental Yeast, Co., Ltd., Japan and
121 dissolved in deionized (DI) distilled water collected from Milli-Q purification
122 system (Millipore, Co., USA) for several concentrations. NAD⁺ (β -NAD⁺: Oriental
123 Yeast Co., Ltd., Japan)) and NADH-dependent secondary alcohol
124 dehydrogenase (S-ADH, EC. 1.1.1.x, 1 unit mg⁻¹, E001) from yeast (Daicel
125 Chiral Technologies, Co., Japan) were prepared in suitable buffer respectively
126 and storage in the refrigerator before use. Hydrophilic polytetrafluoroethene filter

127 membrane (H-PTFE, pore size: 0.2 μm , porosity: 80%, JGWP14225) employed
128 for S-ADH immobilization was purchased from Millipore, USA. The synthesis
129 method of the polymer using 2-methacryloyloxyethyl phosphorylcholine
130 polymerized with 2-ethylhexyl methacrylate (PMEH) was according to our
131 previous report [26]. Buffer chemicals including di-potassium hydrogen
132 phosphate (K_2HPO_4 , 98.0%), potassium di-hydrogen phosphate (KH_2PO_4 , 98%),
133 2-Amino-2-hydroxymethyl-1,3-propanediol (Tris, 99.9%, Biochemistry grade),
134 Hydrochloric Acid (HCl , 1 mol L^{-1}), sodium hydrogen carbonate (NaHCO_3 , 99.5%,
135 JIS special grade), sodium carbonate (Na_2CO_3 , 99.8%, JIS special grade) and
136 standard isopropanol (99.7%, JIS special grade) solution were obtained from
137 Wako Pure Chemical Industries, Japan and prepare in DI water.

138

139 **2.2 Construction of fiber optic NADH measurement system and IPA** 140 **biosensor**

141 The NADH measurement system was composed of four main
142 components: an UV-LED (UVTOP[®] BL335, Sensor Electronic Technology, Inc.,

143 USA) with power supplier circuit (Yokogawa Electric Corporation, Japan) as the
144 excitation light source, a photomultiplier (PMT, C8502, Hamamatsu Co., Japan)
145 as the fluorescence intensity detector, an optical fiber probe (F1000-900, Ocean
146 Optics Inc. USA) for sample contacting and a two-way branch optical fiber
147 (BIF600-UV/VIS, core size $600 \pm 10 \mu\text{m}$, Ocean Optics Inc. USA) for assembling.
148 Two pieces of band-pass filters (BPF, $\lambda=340 \pm 10 \text{ nm}$ and $490 \pm 10 \text{ nm}$, Asahi
149 Spectra) were equipped in front of UV-LED and PMT respectively for the
150 purpose of reducing the interference of background noise.

151 The IPA biosensor was erected by attaching an enzyme-immobilized
152 membrane on the top of fiber probe. The S-ADH enzyme membrane was
153 manufactured by mixing S-ADH (1 mg cm^{-2}) with PME solution (10% w/w in
154 ethanol, $10 \mu\text{l cm}^{-2}$) uniformly and spread averagely on the cleaned H-PTFE
155 membrane. Then, the moist membrane would be prepared in the refrigerator at 4
156 °C immediately 3 hours for enzyme immobilizing and drying. After that, the
157 enzyme membrane would have over coat with pure PME solution (10% w/w in
158 ethanol, $5 \mu\text{l cm}^{-2}$) for the purpose of fixing the enzyme stable. The S-ADH

159 immobilized membrane was cut out into 0.5 cm × 0.5 cm and set onto the top of
160 optical fiber probe flat with 3 mm silicone tube ($\varnothing = 1$ mm, As One Co., Japan). In
161 this method, the IPA biosensor was fabricated.

162

163 **2.3 Measurement of standard NADH and IPA solution**

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

173 The optical probe equipped with the enzyme-immobilized membrane was used
174 to determine the standard IPA, and the method was same as NADH

175 measurement. When S-ADH immobilized membrane was in contact with to IPA
176 solution, NAD^+ would be oxidized to NADH as mentioned as equation (1).
177 Various concentrations (10 nmol L^{-1} to 100 mmol L^{-1}) of standard IPA solutions
178 were added into dark cuvette that contained $300 \mu\text{l}$ of the buffer with 20 mmol L^{-1}
179 of NAD^+ . The increase of fluorescence intensity that emitted from NADH was
180 measured by the PMT.

181

182 **2.4 Optimization of IPA biosensor including reproducibility, buffers pH,**
183 **temperature, and selectivity**

184 Reproducibility is one of the important indicators for evaluating the
185 ability of a biosensor. The reproducibility of IPA biosensor was tested by
186 measuring 1 mmol L^{-1} first, and then the optical probe with S-ADH membrane
187 was immersed into 50 ml of cleaning Tris-HCl buffer without NAD^+ for ten
188 minutes to remove the produced NADH and residual IPA on the S-ADH
189 membrane. After washed, the measurement steps were repeated again and for
190 ten cycles. Two kinds of enzyme-immobilized methods were compared, one is

191 as described at graph 2.2 and the other is a similar process but without PME
192 over-coating.

193 To confirm the optimization conditions of the IPA biosensor, several
194 probable interference factors were surveyed. The buffer pH was considered first
195 because it is known that the enzyme kinetic would be altered under different pH
196 conditions and change the reaction velocity. Besides, pH is also one of the
197 factors influences fluorescence. 20 mmol L⁻¹ of NAD⁺ were prepared in different
198 pH buffers, includes of phosphate buffer (PB, 0.1 mol L⁻¹, pH 5.5, 6.0, 7.0, 7.5
199 and 8.0), 2-Amino-2-hydroxymethyl-propane-1,3-diol -Hydrochloric acid buffer
200 (Tris-HCl, 0.1 mol L⁻¹, pH 8.0, 8.5, 9.0 and 9.5) and sodium
201 carbonate-bicarbonate buffer (CB, 0.1 mol L⁻¹, pH 9.5, 10 and 11). Comparing
202 the fluorescence intensity that was estimated at 1 mmol L⁻¹ of IPA carried out
203 the assessments of these pH conditions. The standard NADH (500 μmol L⁻¹)
204 was also measured in several pH buffers as mentioned above.

205 Temperature is also one of the important influence elements that affect
206 the enzyme kinetics and fluorescence intensity. The black cuvette was put into a

207 water bath tool (bathtool, BT-60, As One Co., Japan), which was used for
208 keeping the temperature. A liquid pump (gear pump, AWT-40W, Tec World Co.,
209 Japan) with Teflon tube was connected to a low-temperature bath (CTB-2, As
210 One Co., Japan) that was used to control the temperature of circulation water. A
211 soft thermistor (LS450-T, Osaka Micro Computer Inc. Japan) was inserted into
212 the buffer, which was contained in the cuvette, for monitoring of the temperature.
213 The fluorescence intensity of $500 \mu\text{mol L}^{-1}$ standard NADH and 1 mmol L^{-1} IPA
214 were measured from 10°C to 50°C respectively.

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

219 For verifying the selectivity of S-ADH, eight kinds of similar chemical
220 structure compounds were measured and compared to standard IPA. The
221 following chemicals were detected: 1-butanol, 2-butanol, 1-propanol, ethanol,
222 acetaldehyde, methanol, 2-butanone and acetone. All of these substances were

223 measured at the concentrations 1 mmol L⁻¹. The experiment of S-ADH selectivity
224 was carried out in optimized condition.

225

226 **2.5 Measurement of IPA in commercial cosmetic products**

227 IPA is commonly found in the living environment, for example, as the
228 solvent of cosmetics or a component of furniture cleaner. Thus, ten kinds of nail
229 related commercial cosmetic products were detected using our IPA
230 biosensor. The measurement samples need to be pretreated by mixing 50 µg of
231 the sample with 450 µl deionized distilled water. Most samples were not
232 dissolved totally in water due to additives, but it would not interfere our purpose
233 because IPA is soluble in water. The sample mixture was centrifuged 5 minutes
234 at 10000 rpm to make an insoluble precipitate and the supernatant was be used
235 for the experiments. The measurement was carried out at optimum condition.
236 3.03 µl of supernatant was added to the cuvette, which contained 300 µl of the
237 buffer with 20 mmol L of NAD⁺. Through this kind of pretreatment method, the
238 original sample has been diluted to one thousand times, and sample 4, 5 and 7

239 were more diluted to ten thousand times.

240 These samples were also measured the IPA concentrations using
241 NADH absorbance method. This method utilized the optical property of NADH
242 that it absorbs UV at the peak of 340 nm wavelengths. Serials of 10 μ l standard
243 IPA were mixed with 90 μ l of Tris-HCl buffer contained 20 mmol L⁻¹ NAD⁺ and
244 injected into 96-well strip plate (437915, Clear, Thermo Fisher Scientific Inc.,
245 Japan). After 0.5 μ l (0.625 mg S-ADH per one μ l H₂O) of S-ADH solution were
246 injected into each well, the plate would be shack for 5 minutes and put into plate
247 reader (SH-1000, CORONA ELECTRIC Co., Ltd, Japan) for detection of
248 absorbance. By this way, the calibration curve of IPA concentration versus
249 NADH absorbance was obtained. The commercial cosmetics samples were
250 pretreated and diluted as mentioned before and measured the IPA concentration
251 by this method.

252

253 **3 Results and discussion**

254 **3.1 Characterization of the IPA biosensor**

255 Results of NADH measurement are presented in supplementary Fig.
256 S1. Fluorescence intensity increased immediately and reached to steady state in
257 a short time when the standard NADH was dropped into the dark cuvette. This
258 phenomenon revealed that the NADH measurement system had a fast response.
259 90% response time was within 2 minutes at 1 mol L⁻¹. The calibration range for
260 NADH detection was from 10 nmol L⁻¹ to 10 mmol L⁻¹ (R = 0.997), and more
261 details are described in the supplementary information.

262 For standard IPA solution measurement, Fig. 2 shows the temporal
263 changes of fluorescence intensity to various concentrations of standard IPA
264 (inserted box) and the calibration curve. When standard IPA solution was
265 dropped into the cuvette, the fluorescence intensity increased immediately and
266 reached to the steady state within 5 minutes. The quick response was confirmed
267 and the fluorescence signal reflected that the enzyme membrane produced
268 NADH through the S-ADH enzymatic reaction of catalyzing IPA to acetone and

269 this biosensor measures the IPA level according to the difference of NADH
270 fluorescence intensity (Δ intensity). The dynamic range was confirmed from 500
271 nmol L⁻¹ to 1 mmol L⁻¹ with a correlation coefficient of 0.998 for n = 5. The
272 calibration curve having a power approximation and can be described as
273 following equation:

$$274 \quad \Delta\text{intensity(cps)} = 14.09 \times (\text{IPA concentration(nmol L}^{-1}\text{)})^{0.787} \quad (2)$$

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

278

279 **3.2 Optimization of IPA biosensor including reproducibility, buffers pH,** 280 **temperature, and selectivity**

281 Fig. 3 highlights reproducibility difference between the two kinds of
282 enzyme-immobilized methods. Obviously, method 1 without PMEHL over coating
283 could not be reused because the fluorescence signal decreased progressively in
284 every cycle. The more likely explanation rests in that one layer of PMEHL could

285 not fix the S-ADH on H-PTFE membrane instable; the enzyme would lose in
286 each reaction step or washing period. Meanwhile, the coefficient variation (C.V.)
287 for method 2, with PME-H over coating, is only 4.62%. This high reproducibility
288 represents that the IPA biosensor is reusable because the S-ADH fixed on the
289 H-PTFE membrane in steady by PME-H over coating.

290 The optimization measurement conditions for the IPA biosensor have
291 been evaluated including the buffer pH, temperature and the concentration of
292 NAD^+ . Regarding of the buffer pH experiments, Δ intensity fluorescence of 1
293 mmol L^{-1} IPA was measured in serious of buffer pH conditions. PB buffer was
294 varied from pH 5.5 to pH 8.0, Tris-HCl buffer was adjusted from pH 8.0 to pH 9.5
295 and CB buffer was prepared from pH 9.5 to pH 11. All of the buffers were 0.1
296 mmol L^{-1} with 20 mmol L^{-1} of NAD^+ . As shown in Fig. 4, Δ intensity of
297 fluorescence shows the plateau value from pH 8.5 to pH 9.5. This result
298 indicates that the reaction kinetics of S-ADH associated with pH and the weak
299 alkaline condition is favorable for our biosensor. In the case of strong alkaline pH,
300 the fluorescence signal suddenly decreased due to the degradation of the NAD^+ .

301 The NAD^+ solution in the condition of pH 11 would become brown color within
302 hours and come with unusually high background noise. Therefore, although pH
303 9.5 shows highest Δ intensity, considering the stability and the storage of
304 NAD^+ [27], the experiment condition was decided at pH 8.5. Fig. S3 presents the
305 result of NADH fluorescence intensity in different pH conditions. It seems
306 probable that the influence of pH on the NADH fluorescence is slight, because
307 no obvious change of fluorescence intensity in different pH conditions was
308 observed. Therefore, it is reasonable to consider that pH influences enzyme
309 kinetic of S-ADH more

310 It is known that temperature is one of the important elements for
311 enzyme kinetics and fluorescence emission intensity; the temperatures of IPA
312 biosensor measurement environment were characterized. The detail results are
313 offered in the supplementary information. Theoretically, the IPA biosensor was
314 expected to display weak fluorescence signal at a lower temperature and strong
315 intensity at a higher temperature until enzyme denatures. However, the obtained
316 results were totally different: lower temperature with higher signal and higher

317 temperature showed in contrast (Fig. S2). A similar trend was also observed in
318 standard NADH measurement. This result is in line with the study of Zelent et al.
319 They researched about the temperature dependence for fluorescence of NADH
320 and revealed out that the fluorescence intensity increases when temperature
321 decreases[28]. Accordingly, it is likely that the temperature interferes the
322 fluorescence of NADH much more than affects the enzymatic kinetics of S-ADH.
323 Also, this result signalizes the necessary of recalibration when the biosensor
324 works in that temperature dramatic change environment.

325 Then, the effect of NAD^+ concentration to the calibration range for the
326 IPA biosensor was evaluated. The dynamic range of IPA measurement was from
327 100 nmol L^{-1} to $10 \text{ } \mu\text{mol L}^{-1}$ for $100 \text{ } \mu\text{mol L}^{-1}$ of NAD^+ , 100 nmol L^{-1} to $100 \text{ } \mu\text{mol}$
328 L^{-1} for 1 mmol L^{-1} of NAD^+ and 500 nmol L^{-1} to 1 mmol L^{-1} for 20 and 100 mmol
329 L^{-1} of NAD^+ . The lower limit of detection was found in 0.1 and 1 mmol L^{-1} of NAD^+
330 and a higher concentration of NAD^+ could be used to measure a wide range of
331 IPA concentrations. One of the potential reasons is that higher concentration of
332 NAD^+ would contribute to the appearance of competitive inhibition and decrease

333 the reaction rate. The optimized concentration of NAD^+ should be chosen
334 according to the experimental purpose. In this study, 20 mmol L^{-1} of NAD^+ was
335 chosen for all experiments.

336 Fig. 5 illustrates the selectivity of S-ADH. Eight kinds of different
337 species with a similar chemical structure including 2-butanol, 1-butanol,
338 1-propanol, ethanol and other aldehydes and ketones were employed to
339 compare with IPA. As observed in Fig. 5, 2-butanol presented about 81.2% of
340 fluorescence signal compared to IPA in the same concentration, and the other
341 compounds showed a slight reaction. This high selectivity was attributed to the
342 specific molecular distinguish activity of S-ADH. The observed high reacting of
343 2-butanol not only indicated that the possible existence of 2-butanol in measured
344 sample should be considered but also presents that this IPA biosensor can be
345 used for 2-butanol determination. Besides, compared to IPA, 2-butanol rare
346 exists in the human body[29]. Therefore, the interference of 2-butanol could be
347 ignored for our purpose of detecting the IPA in the body fluid such as serum,
348 urine or saliva.

349

350 **3.3 IPA concentration in nail related cosmetics**

351 The results of IPA measurement in the nail related cosmetic are shown
352 in Fig. 6 and the type of the detected cosmetic are listed in Table 1. The
353 observed concentrations of IPA were various; Sample 1, 2, 3, and 6 contained
354 about 0.6 mol L⁻¹ of IPA, sample 4, 5, and 7 were observed higher IPA
355 concentrations, and sample 8, cuticle oil, did not contain IPA. Fig. S4 describes
356 the scatter plot of measurement results detected by IPA biosensor and NADH
357 absorbance detection method and displayed high correlation (analyzed by
358 Pearson correlation coefficient statistic method, $R = 0.972$, $P < 0.001$). It is worth
359 mentioning that the dynamic range of our IPA biosensor was from 500 nmol L⁻¹ to
360 1 mmol L⁻¹ NAD⁺, which was much wider than NADH absorbance detection
361 method (dynamic range: 100 μ mol L⁻¹ to 1 mmol L⁻¹).

362 IPA is a usual ingredient used in nail cosmetic for antifoaming and
363 decreasing the viscosity[30]. It is known that the lungs and skin are both of the
364 IPA absorption paths for the human body; thus, the usage amount and exposure

365 duration should be carefully considered when using these IPA contained
366 cosmetics, especially of nail colors and hair dyes. The assay ability of this IPA
367 biosensor was proven by this real sample detection.

368

369 **4. Conclusions**

370 In this work, a UV-LED based fiber-optic NADH fluorescence
371 measurement combined with S-ADH immobilized membrane for determination
372 of IPA was developed, and demonstrated. The optimization conditions were
373 characterized including buffer pH, temperature, and concentration of NAD⁺. This
374 IPA biosensor performed calibration range from 500 nmol L⁻¹ to 1 mmol L⁻¹ with
375 high selectivity. Compared to traditional detection methods, rapid response of
376 the biosensor can be applied for large amount measurement in the efficient
377 evaluation. S-ADH was immobilized well on the H-PTFE membrane using PME
378 polymer and the protective over coating could sustain the reuse of the biosensor
379 with good reproducibility. The analysis ability of IPA biosensor was investigated
380 by determination of commercial cosmetics and the detected results were similar
381 with NADH absorbance detection method. Real sample measurement not only
382 proved that IPA is widely used in nail related cosmetics but also reminded us to
383 pay attention in usage amount and exposure duration when using these
384 products. This biosensor offers a new tool for IPA measurement and is expected

385 to use in medical fields such as DKA or AKA monitoring. We intend to continue
386 improving this biosensor and apply this sensor concept to determinate other
387 chemical substances.

388

389

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482

483 **Figure captions:**

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

487

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

491

492 Figure 3. Comparison of enzyme immobilized methods with and without PMEHE
493 over coating by ten times of 1 mmol L^{-1} IPA detection. Improving of the
494 reproducibility by PMEHE over coating was confirmed (C.V. = 4.62%). ($\square$)
495 enzyme immobilized without over coating. ($\bullet$) enzyme immobilized with the
496 PMEHE over coating.

497

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

500

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

504

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

509

510 **Figures:**

511

512

513

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

529 **Table:**

Table1

Information of measurement cosmetic samples

sample ID	type	usage	main ingredient
1	nail base coat	nail strengthen base of manicure	ethyl acetate, <u>IPA</u> , butyl acetate, phthalic anhydride, glycols copolymer
2	nail top coat	nail manicure protect	ethyl acetate, <u>IPA</u> , butyl acetate, acetyl methionine, nitrocellulose
3	nail top coat	nail art decoration	ethyl acetate, <u>IPA</u> , butyl acetate, phthalic anhydride, adipid acid
4	nail hardener	nail strengthen instant protection	ethyl acetate, <u>IPA</u> , butyl acetate, acetate butyrate, camphor
5	nail color	nail makeup	ethyl acetate, <u>IPA</u> , butyl acetate, acetyl methionine, camphor, benzoic acid
6	nail color	nail makeup	ethyl acetate, <u>IPA</u> , butyl acetate, citric acid, yellow no.203, titanium oxide
7	nail coat clearer	clear fingernails without painting manicure	ethyl acetate, <u>IPA</u> , butyl acetate, phthalic anhydride, glycols copolymer
8	cuticle oil	nourishies softer and supple cuticles	ethylhexyl palmitate, triethylhexonin, isopropyl ppalimate, macadama interifolia seed oil

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GRAPHICAL ABSTRACT