



Development of Biosensors for Ethanol Gas Detection

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ABSTRACT

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This work developed a biosensor for the measurement of ethanol gas in the air. The biosensors were synthesized by mixing signal layer materials containing SiO₂ and polyimide (PI) substrates using the enzyme Alcohol Dehydrogenase (ADH) and coenzyme Nicotinamide Adenine Dinucleotide (NAD⁺) as a biosensor. The electrodes were coated on biosensors by DC magnetron sputtering method for test the response performance of the developed biosensors. The ADH/NAD⁺ was immobilized on the Ag electrode by Glutaric dialdehyde 25 wt. % cross-linking procedure. It was found that, alcohol biosensors can be exhibited sensing ethanol gas at even low concentrations from 300 ppb to very high concentrations up to 1900 ppm, response time 3 s, recovery times 1-2 minutes and good sensitivity. The SiO₂ substrate has excellent, which provides significant advantages for wearable electronic device that compact, easy to use and reduce direct contact with alcoholics. The alcohol biosensors can adoption in next generation to other electronic devices, because easy to integrate, such as a module alcohol biosensor with wireless or the fabrication of the RCL circuit. Furthermore, the alcohol biosensors based on SiO₂/Ag/ADH, PI/Ag/ADH is artificial intelligence strategy for stable practical wearable electronic devices.

1. INTRODUCTION

Blood alcohol determination plays an important role in laboratory medicine and forensic medicine for several reasons [1, 2]. Driving under the influence of alcohol represents a major safety concern due to the synergistic or additive effect of these substances of abuse. Cause in traffic accident such as car or motorcycle accident. Hence, rapid road-site testing of these substances is highly desired to reduce risks of fatal accidents [3]. Corresponding with the road traffic laws in Thailand and many countries, which stipulate that blood alcohol concentration 200 ppm violation of the Act. Artificial intelligence in practical capable strategy of physics, chemistry and biology sensing and diversity sensing have broadened the scope of various sensing applications to wearable electronics, especially for the wireless monitoring [4]. For example, the MQ-3 gas sensor module for detecting alcohol. MQ-3 Semiconductor sensor for alcohol gas has good sensitivity to alcohol in wide range and has advantages such as long lifespan. It is with low cost and suitable for various applications of detecting alcohol at different concentration and simple drive circuit. It is widely used in domestic alcohol gas alarm, industrial alcohol gas alarm and portable alcohol detector [5].

Nowadays, there are many methods are being used for alcohol measurement, such as spectrometric analysis and chromatographic analysis or breathalyzer, where the alcohol concentration or refractivity is detected [6, 7]. However, these methods are time-consuming and complex to perform laborious sample pre-treatment. In addition, expensive analytical apparatuses are necessary [8]. Including

breathalyzer analysis methods the results can be analyzed by inhaling the breath alcohol tester through the alcohol mouthpiece (mouthpiece plastic) [9]. Direct contact between the drinker and the detector, and it is necessary to constantly change the alcohol mouthpiece. Thus, there is an increasing requirement for rapid, accurate and inexpensive methods for blood alcohol determination [10]. Alcohol biosensors is another interesting and suitable alternative, because biosensors have biological substances such as enzyme used as a detector for the substance to be measured, and the choice of enzyme alcohol biosensors will give a specific response to the substance to be measured (ethanol) [11]. We are selected enzyme ADH immobilized on electrode. The resulting enzyme electrode exhibits excellent electrocatalysis for the oxidation of reduced NAD⁺ [12]. Enzyme dehydrogenase catalyzes oxidation in the conditions of oxygen as a co-substrate (in smooth chemical conditions). This approach based on chemical bonding of the cofactor (which was checked by infrared spectroscopy method) led to good performances in terms of long-term stability of the electrochemical response [13]. Thus, avoiding interferences due to the presence of oxidizable substances in samples [14-16]. We decided to use 2 materials such as Silicon dioxide and PI for structure thin film. 1. Silicon that have properties metal oxide semiconductors are widely used as sensor materials because of many advantages, high sensitivity, fast response, recovery, and low detection limits over other semiconductors [17-20]. Including have a large surface area. The porous structure improves gas absorption efficiency, because the high surface-to-volume ratio [21, 22]. 2. PI is regarded as high-performance

polymers because of their excellent chemical, thermal and irradiation stability, high mechanical strength and reliable electrical insulation. Nowadays there are state-of-the-art microelectronic applications [23, 24]. PI has found applications as enzyme immobilization membrane, due to their good chemical stability and low reactivity [25-28]. Moreover, we selected Ag used as electrodes for the fabricated to alcohol biosensors and module alcohol biosensors for wearable electronics devices. Ag it has excellent electrical conductivity, excellent repeatability and durability [29-32]. Flexing endurance and are broadly used in flexible electrodes and flexible sensors [33]. Thus, Ag is suitable for use as electrodes.

Therefore, the researcher is interested in developing Biosensors for the detection of ethanol gas for wearable electronic devices. Reduce direct contact with drinkers, high sensitivity, selectivity, rapid response and stability. Inexpensive compared to breath alcohol monitors. Then, we are selected MQ-3 gas sensor module used to it as prototype equipment due to inexpensive. Fabrication module alcohol biosensor for wearable electronic devices. 2 materials such as SiO₂ and PI use to Structure of thin film. Enzyme ADH and coenzyme NAD⁺ was used as biosensors, immobilization on Ag electrode by DC magnetron sputtering method. The efficiency of response alcohol biosensors developed was tested. Alcohol biosensors module and module alcohol biosensors for wearable electronic devices compare with MQ-3 gas sensor module prototype equipment. Furthermore, the technology has been applied to the development of sensors. Next generations, in this work, can be apply or connect to other wearable electronic devices, or used for further analysis of substances.

2. EXPERIMENTAL

2.1 Synthesis thin film by DC magnetron sputtering

2.1.1 Preparation signal layer structure

5-mm thick SiO₂ and PI substrate was ultrasonic cleaner with acetone and deionized water using times 10 minutes. After that 5-mm SiO₂, PI was take dried under nitrogen flow at temperature 250°C for 4 h for clean the structure by used Tube furnace.

2.1.2 Synthesis thin film

Formation a pattern of Ag electrode by DC magnetron sputtering methods, it consists of a DC power supply, Magnetron Sputtering Gun inside the vacuum and magnetron sputtering gun cooling system. After that preparation thin film in the vacuum chamber at base pressure 2.1 mT under Ar gas atmosphere Ar 99.99%, flow rating 23.3±0.1 cm³ /min and total working pressure 50 mT. Constant frequency DC high voltage power supplies 17.24 kHz adjust the current/potential at the cathode terminals while sputtering, to find the right conditions. The signal layer structure is far from the sputtering target 5.0 cm, during sputtering there is no heating, while doing it takes 15 minutes. Which is the optimum time condition for thin film preparation.

2.2 Alcohol biosensor process

2.2.1 Chemicals

Alcohol Dehydrogenase from *Saccharomyces cerevisiae* ≥ 300 units/mg protein were obtained from Sigma chemical

company, and beta-Nicotinamide Adenine Dinucleotide hydrate 98+% (from Germany) used as received. Glutaric dialdehyde 25 wt. % solution in water obtained from Belgium, was diluted to 2.5 wt. % with deionized water. Albumine bovine serum for biochemistry additional reagent IGSS protease was obtained from United States of America. After that, preparation of the phosphate buffer solution by prepared with 0.1 mol/L Na₂HPO₄ and 0.1 mol/L NaH₂PO₄. Next process, all solutions was made up with deionized water. The ADH stock solution were preparation with 100 μL phosphate buffer solution (pH 7.5) and stored at 4°C.

2.2.2 Biosensor process

ADH/NAD⁺ was immobilized on the Ag electrode by glutaric dialdehyde/Albumine bovine serum cross-linking procedure. Enzymes solution was obtained by dissolving 3 mg of ADH and 6 mg of NAD⁺ in 100 μL of 0.1 mol/L (pH 7.5) phosphate buffer solution. ADH and NAD⁺ mixed with vigorous stirring, containing 8 mg Albumine bovine serum. And then the glutaric dialdehyde (2.5 wt. %) was dilute to 2.5% wt, 1.9% wt up to a final concentration of a 0.9% wt. with deionized water. The Glutaric dialdehyde 2.5% wt. which is a bifunctional cross-linking agent was added to the ADH/NAD⁺ solution. In the process, the solution was completely mixed. Next steps, the Ag electrodes was immobilized on ADH solution by used micropipettes size 10-100 μl, 10±20 μl enzyme solution was immobilized on them. This procedure, we can take repeated two or three times. After that, the enzyme electrodes was placed in a desiccator for sometimes to the gel formation. The resulting enzyme electrodes as receptor (defined as SiO₂/Ag/ADH, PI/Ag/ADH) and kept dry enzyme structure in phosphate buffer solution (pH 7.5) in refrigerator at 4°C 5 h before use.

2.3 Analytical method

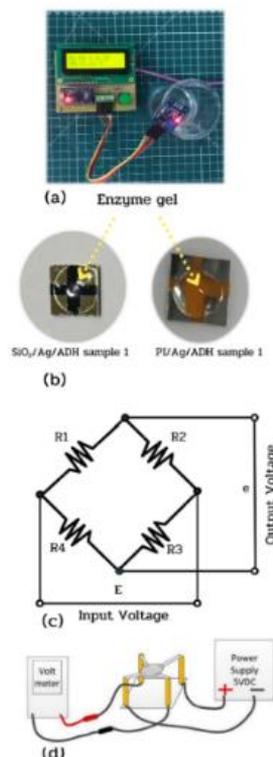


Figure 1. The ethanol gas test

Figure 1 shows the ethanol gas test (a) shown the Ethanol gas response to the MQ-3 alcohol biosensor module. (b) developed signal layer structure material, in figure for the structure of SiO₂/Ag/ADH sample 1 and PI/Ag/ADH sample 1. (c) shown the sensor bridge circuit (d) ethanol gas response test of Module Alcohol biosensor for wearable electronic devices.

3. RESULT AND DISCUSSION

3.1 XRD analysis

The X-ray diffraction pattern of Ag thin film deposition on SiO₂ and PI substrates shown in Figures 2, 3. The X-ray diffraction patterns of SiO₂ were found at an angle equal 38.3 and 44.3 degree. The X-ray diffraction pattern of SiO₂ is considered which uses the reference position from the ICDD standard number 00-047-1300, was found SiO₂ had a plane equal (211), (202), (212), (203), (302) and (114), respectively. The Ag thin film can be observed and the peak namely (111), (200) according to ICDD number card (00-004-0783) cubic type by plane (111) is Preferred Orientation. It can be said that the thin film coated on SiO₂ material is Ag thin film. As for the Ag thin films coated on the PI material, X-ray diffraction and plane diffraction patterns of the Ag thin films were observed in the same way, there are angles equal 38.3 and 44.3 degree. Crystallite size and lattice constant of main peak (111) are shown in Figure 4, were calculate the lattice constant of the Ag thin film prepared for installation from Eq. (1):

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}} \quad (1)$$

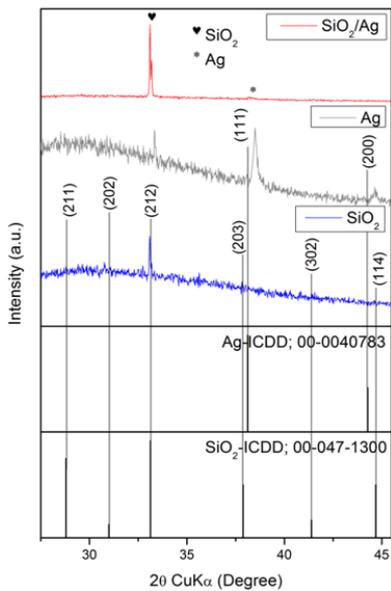


Figure 2. Comparison XRD pattern of thin film Ag based on SiO₂ at deposition times 15 minutes

The grain size was calculated by Scherrer equation from Eq. (2):

$$L = \frac{k\lambda}{\beta \cos \theta} \quad (2)$$

where, k is constant safe factor 0.9 for Face-centered cubic structure, λ is X-ray diffraction length $CuK\alpha=1.5406 \text{ \AA}$, β is full width half maximum intensity (FWHM), θ is the Bragg angle and compare to standard as shown in Table 1.

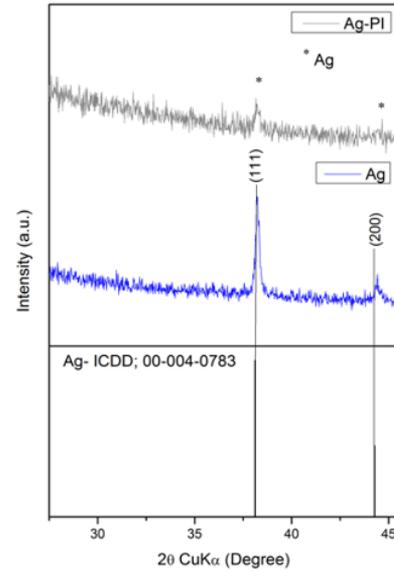


Figure 3. Comparison XRD pattern of thin film Ag based on PI at deposition times 15 minutes

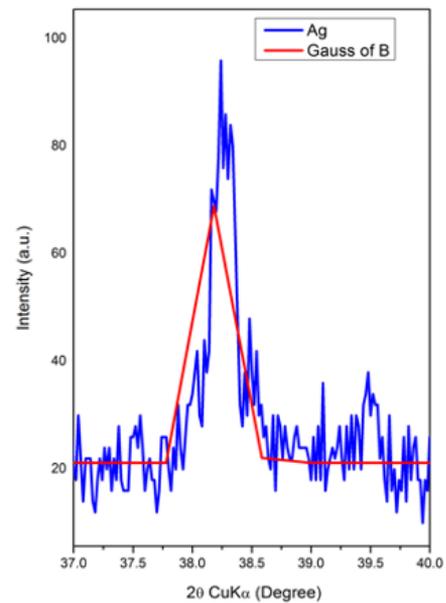


Figure 4. Equiangular X-ray diffraction pattern at an angle equal 2θ to 38.1164 degree

Table 1. A standard and calculated comparison of Ag thin film crystal structure

Standard (ICDD; 00-004-0783)				Ag thin film synthetic				
Peak position (2 theta)	D-spacing (Å)	(hkl)	Lattice constant (Å)	Peak position (2 theta)	D-spacing (Å)	FWHM (Å)	Crystallite size D (nm)	Lattice constant (Å)
38.116	2.359	111	4.086	38.261	2.350	0.262	31.985	4.072

3.2 SEM analysis

Microstructure, the SEM characterization showed the surface morphology of Ag thin film deposited on SiO₂ and PI substrate at different magnification 5.000x and 10.000x shown in Figure 5, Figure 5 (a) (b) shown Ag thin film on SiO₂ and PI at magnifications 5.000x and Figure 5 (c) (d) shown Ag thin film on SiO₂ and PI at magnifications 10.000x. The morphology of Ag thin films with a thickness of 1 μm coated on SiO₂ and PI materials has a smooth surface. Ag thin films coated on SiO₂ materials have a smoother and more graven surface than Ag thin films coated on PI materials, are under the same sputtering conditions.

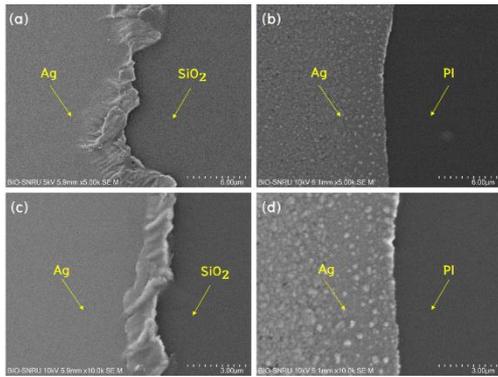


Figure 5. The SEM image of Ag thin film deposited on SiO₂ and PI substrate at magnifications 5.000x and 10.000 x

3.3 Sensing performance of Module Alcohol biosensors

Module alcohol biosensors for wearable electronic devices, as illustrated in Figure 6. Figure 6 (a) (b) is Module alcohol biosensor based on SiO₂/Ag/ADH sample 1, Figure 6 (c) (d) is Module alcohol biosensor based on SiO₂/Ag/ADH sample 2, Figure 6 (e) (f) is Module alcohol biosensor based on PI/Ag/ADH sample 1 and Figure 6 (g) (h) is Module alcohol biosensor based on PI/Ag/ADH sample 2. Module alcohol biosensors it can be detected to different gasses to ethanol are under conditions and general environment. All tests are under standard test condition at 25°C±2°C; 65%±5%RH of alcohol and 31°C±2°C; 66%±5%RH of alcohol in the general environment, respectively. Nevertheless, Module alcohol biosensor based on SiO₂/Ag/ADH and PI/Ag/ADH sample 2 tasted by Digital Multimeter Sefram series 7332 brand. In addition, Module alcohol biosensor based on SiO₂/Ag/ADH, PI/Ag/ADH sample 1 used a DC power supply to test by supplying 5 V to them, and then compare with resistance before and after exposure molecule ethanol gas in the air.

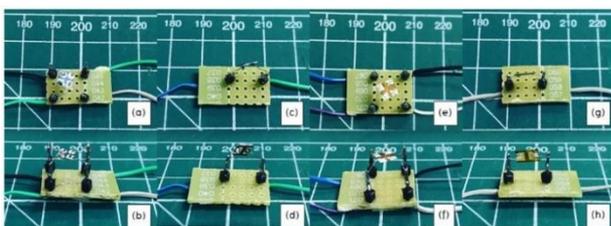


Figure 6. Module alcohol biosensors for wearable electronic devices developed

Electrical Resistivity (RS/RO) measurement of Module alcohol biosensors for wearable electronic devices, as

illustrated in Figures 7, 8. The value ordinate means resistance (R) ratio of the sensor (RS/RO), abscissa is concentration of gasses. RS means resistance in different gasses, RO means resistance of sensor in 5, 10, 15, 20, 25, 30, 35 and 40% ethanol concentrations, respectively. Good instrument performance must be within an RS/RO value in the dynamic rang range of 0.001 to 0.999. If the value of ethanol in the air is more than the sensor can detect, the sensor will read a value 0, and if the sensor reads an RS/RO value greater than 1 that mean the volume of ethanol in the air that the sensor can detect in low concentration.

Experimental result it was found that, Module alcohol biosensor based on SiO₂/Ag/ADH sample 1 has an RS/RO range between 0.191 to 22.09 are under conditions and 0.461 to 2.609 in the general environment. Module alcohol biosensor based on SiO₂/Ag/ADH sample 2 has an RS/RO range 1.7 to 12.49 are under conditions and 5.56 to 11.66 in the general environment. Next, Module alcohol biosensor based on PI/Ag/ADH sample 1 has an RS/RO range 1.786 to 11.622 are under conditions, 5.964 to 10.928 in the general environment and Module alcohol biosensor based on PI/Ag/ADH sample 2 has an RS/RO range 4.426 to 20.81 are under conditions and 0.85 to 5.794 in the general environment.

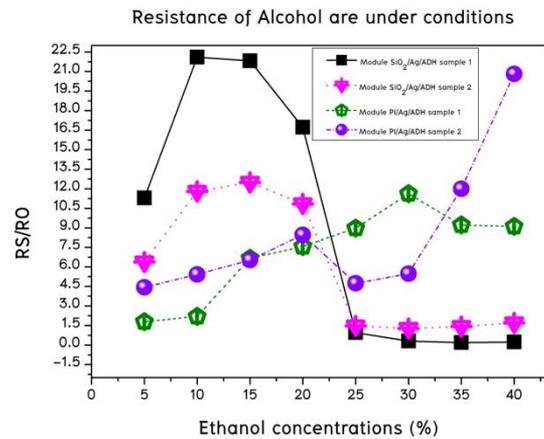


Figure 7. The typical sensitivity characteristics of the module alcohol biosensors are under conditions at temperature 25°C

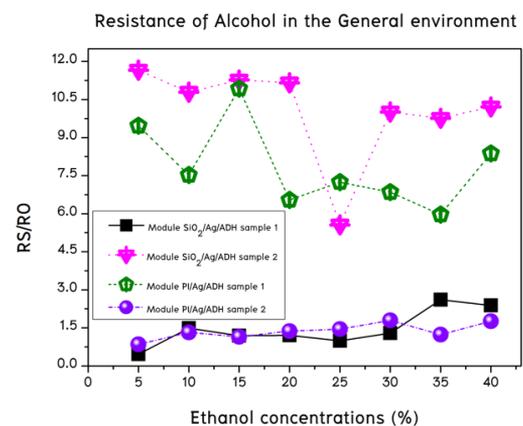


Figure 8. The typical sensitivity characteristics of the module alcohol biosensors in the general environment

Figure 7 can be seen that the alcohol concentration ranges from 5% to 20%, Module alcohol biosensor based on PI/Ag/ADH sample 1, 2 better responses to ethanol gas more than Module alcohol biosensor based on SiO₂/Ag/ADH sample 1, 2. Module alcohol biosensor SiO₂/Ag/ADH sample

1, 2 is more responsive to ethanol gas at the alcohol concentration range of 25% to 40% are under conditions.

It can be seen that the Module alcohol biosensors for wearable electronic devices based on SiO₂/Ag/ADH sample 1 and PI/Ag/ADH sample 2 better response to ethanol gas more than Module alcohol biosensor based on SiO₂/Ag/ADH sample 2 and Module alcohol biosensor based on PI/Ag/ADH sample 1 in all concentrations of alcohol are under general environment (Figure 8). The results of the intensive experiments confirmed the efficiency of the effective material selection and inexpensive, that PI material is cheaper compared to SiO₂.

From the above information, it can be concluded that, Module alcohol biosensors for wearable electronic devices can response to ethanol gas in the air by estimated RS/RO value has changed. Next steps, we will modifications Module alcohol biosensors for electronic devices by fabrication with MQ-3 gas sensor module used as an equipment prototype for used sensors for detecting ethanol gas in type compact and easy to use. We selected MQ-3 gas sensor module as an equipment prototype because cheap cost around 60 baths in Thai and just equal 2 USD.

3.4 Sensing performance of MQ-3 Alcohol biosensors module developed

Wearable platforms are an artificial intelligent interest in this generation, after are made module alcohol biosensors, recent advance have been made by incorporating MQ-3 gas sensor module equipment prototype, as illustrated in Figure 9 and shows structure of them. Figure 9 (a) (b) are MQ-3 gas sensor module shown the internal structure which compound Al₂O₃/Au/SnO₂, Figure 9 (c) (d) are MQ-3 alcohol biosensor module shown the internal structure which compound SiO₂/Ag/ADH sample 1, Figure 9 (e) (f) are MQ-3 alcohol biosensor module shown the internal structure which compound PI/Ag/ADH sample 1 and Figure 9 (g) (h) is MQ-3 alcohol biosensor module shown the internal structure which compound PI/Ag/ADH sample 2, respectively.

Accordingly, we demonstrated wirelessly operable MQ-3 alcohol biosensors module with MQ-3 gas sensor module by supplying 5 V DC power supply. Rapid response to ethanol gas in both (RS/RO) value of the MQ-3 gas sensor module with MQ-3 alcohol biosensors module developed shown in Figures 10, 11. We used MQ-3 gas sensor module is reference and calibrations with MQ-3 alcohol biosensors module developed. The MQ-3 alcohol biosensors module developed are test under standard condition test at 25°C±2°C; 65%±5%RH of alcohol and 31°C±2°C; 65%±5%RH, 31°C±2°C; 66%±5%RH, 29°C±2°C; 66%±5%RH and 27°C±2°C; 65%±5%RH of alcohol in the general environment.

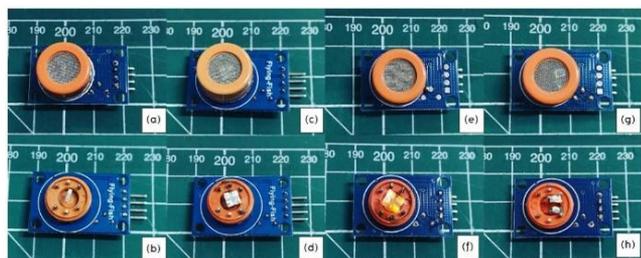


Figure 9. Structure of MQ-3 gas sensor module with MQ-3 Alcohol biosensors module developed

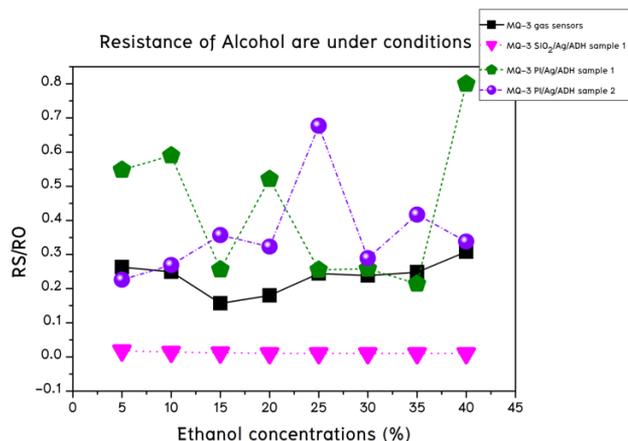


Figure 10. The typical sensitivity characteristics of the MQ-3 gas sensor module with MQ-3 alcohol biosensors module developed are under conditions temperature at 25°C

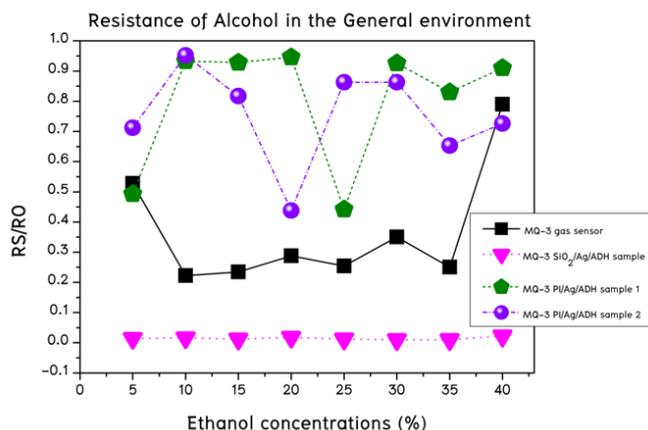


Figure 11. The typical sensitivity characteristics of the MQ-3 gas sensor module with MQ-3 alcohol biosensors module developed in the general environment

When exposure to ethanol gas MQ-3 alcohol biosensors module as illustrated in Figures 10, 11, RS/RO data will be change when enzymatic oxidation of ethanol gas in the air. ADH defined as receptor, the RS/RO value shown a value of 1, which means no ethanol molecules in the air. It was found that, firstly MQ-3 gas sensor module equipment prototype has an RS/RO value in the range 0.18 to 0.308 and 0.222 to 0.79. Secondly, MQ-3 alcohol biosensor based on SiO₂/Ag/ADH sample 1 has an RS/RO value in the range 0.014 to 0.01 and 0.012 to 0.022. Thirdly, MQ-3 alcohol biosensor based on PI/Ag/ADH sample 1 has an RS/RO value in the range 0.214 to 0.59 and 0.442 to 0.946. And last MQ-3 alcohol biosensor based on PI/Ag/ADH sample 2 has an RS/RO value in the range 0.226 to 0.677 and 0.4383 to 0.952 are tests under standard conditions and general environment. Figures 10, 11 show response efficiency to ethanol gas of biosensors. In conclusion, MQ-3 alcohol biosensors based on PI/Ag/ADH sample 1, 2 response better to ethanol gas more than MQ-3 gas sensor module equipment prototype and MQ-3 alcohol biosensors based on SiO₂/Ag/ADH sample 1 are under conditions. In general environment, it was found that MQ-3 alcohol biosensor based on PI/Ag/ADH sample 1, 2 responses better to ethanol gas more than MQ-3 gas sensor module equipment prototype and MQ-3 alcohol biosensors based on SiO₂/Ag/ADH sample 1 also even under general environment.

3.5 Power of Sensitivity (Ps) body of the MQ-3 Alcohol biosensors module developed

Ps of MQ-3 Alcohol biosensors module developed with the MQ-3 gas sensor module are under conditions at $\leq 0.6(31\Omega \pm 3\Omega)$ $25^\circ\text{C} \pm 2^\circ\text{C}$; $65\% \pm 5\% \text{RH}$ of alcohol and under general environment at $\leq 0.6(31\Omega \pm 3\Omega)$ $31^\circ\text{C} \pm 2^\circ\text{C}$; $65\% \pm 5\% \text{RH}$, $31^\circ\text{C} \pm 2^\circ\text{C}$; $66\% \pm 5\% \text{RH}$, $29^\circ\text{C} \pm 2^\circ\text{C}$; $66\% \pm 5\% \text{RH}$ and $27^\circ\text{C} \pm 2^\circ\text{C}$; $65\% \pm 5\% \text{RH}$. As illustrated in Figures 12, 13 were calculate by Eq. (3):

$$Ps = Vc^2 \times Rs / (Rs + RL)^2 \quad (3)$$

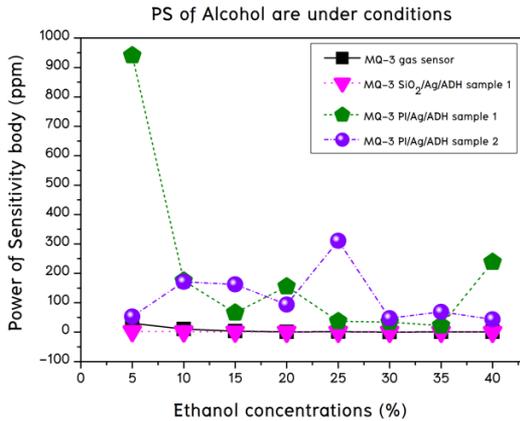


Figure 12. Ps body of the MQ-3 alcohol biosensors module developed with MQ-3 gas sensor module exposure to ethanol are under conditions temperature at 25°C

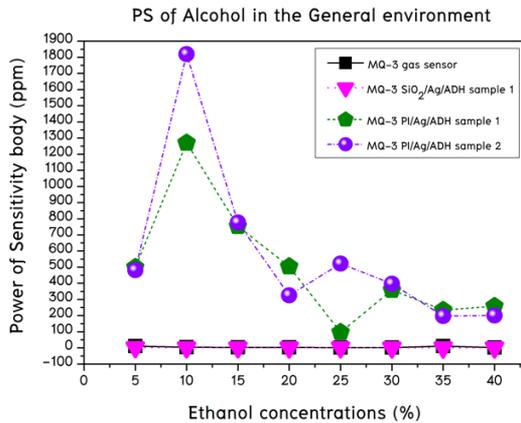


Figure 13. Ps body of the MQ-3 alcohol biosensors module developed with MQ-3 gas sensor module exposure to ethanol gas in general environment

Figures 12, 13 show the Ps body of the MQ-3 alcohol biosensors module developed with MQ-3 gas sensor module exposure to ethanol gas. It was found that, are under conditions the MQ-3 gas sensor module can detected ethanol gas dynamic range 1 to 30 ppm and 1 to 10 ppm in the general environment. As illustrated in Figures 12, 13. Our MQ-3 alcohol biosensors module it can be detected ethanol gas in the air in dynamic range 309 ppb to 941 ppm by MQ-3 alcohol biosensor based on $\text{SiO}_2/\text{Ag}/\text{ADH}$ sample 1 it can be detected at 309 ppb to 3 ppm, MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 1 it can be detected at 23 to 941 ppm and MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 2 it can be detected at 44 to 311 ppm, as illustrated in Figure 12. In general environment, Our MQ-3 alcohol biosensors module it can be detected ethanol gas in dynamic range 309 ppb to 1900 ppm.

The MQ-3 alcohol biosensor based on $\text{SiO}_2/\text{Ag}/\text{ADH}$ sample 1 it can be detected at 309 ppb to 3 ppm, MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 1 it can be detected at 97 to 1270 ppm and MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 2 it can be detected at 197 to 1819 ppm. Thus, the MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 1, 2 can detected ethanol gas over a wider range of alcohol concentrations and suitable the most.

The Diagram showing the detector operation when the receptor detects ethanol gas of the MQ-3 alcohol biosensors module developed, coupled enzymatic oxidation of ethanol gas in the air as illustrated in Figure 14.

ENZYMATIC OXIDATION

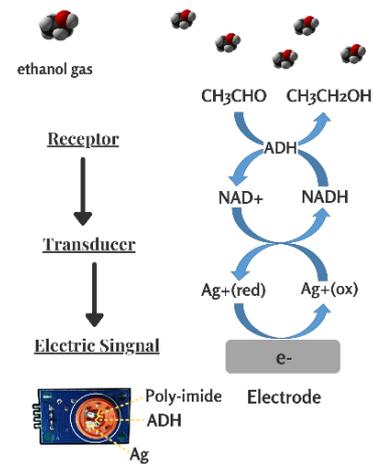


Figure 14. Enzymatic oxidation of ethanol gas in the air

Sensitivity curve of the VRL means the loop voltage (VC) is used to detect the voltage VRL on load resistance. Sensitivity curve of the VRL indicates the detection capability of ethanol gas inversely proportional to the sensor's electrical power supply, by the material being the translator of the electrode and the dependent variable, temperature and humidity as a control translator, as illustrated in Figures 15, 16. Figures 15, 16(a) shown the sensitivity curve of the VRL of MQ-3 gas sensor module, Figures 15, 16(b) are MQ-3 alcohol biosensor based on $\text{SiO}_2/\text{Ag}/\text{ADH}$ sample 1, Figures 15, 16(c) are MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 1 and Figures 15, 16(d) are MQ-3 alcohol biosensor based on $\text{PI}/\text{Ag}/\text{ADH}$ sample 2 are test under standard conditions and general environment, Figures 15, 16. The graph shows that VRL value has changed the way electrochemical transistor when the receptor can sensing ethanol gas and recovered in the absence of ethanol gas in the air during 1-2 minutes.

Figures 15, 16 show sensitivity curve of the VRL in alcohol with different concentrations. Sensitivity curve of the VRL demonstrates the ability to detect ethanol gas inversely proportional to the sensor's electrical power supply. First, the MQ-3 gas sensor module equipment prototype VRL voltage is used to power the sensor with an average value of 2.999 V and 2.633 V, (Figures 15, 16(a)) when comparison between VRL voltage and Sensitivity to ethanol gas, it was found that, MQ-3 gas sensor module equipment prototype it uses moderate electrical power and can detected ethanol gas at a low concentration range. Second, the MQ-3 alcohol biosensors based on $\text{SiO}_2/\text{Ag}/\text{ADH}$ sample 1 VRL voltage is used to power the sensor with an average value of 4.92 V equal (Figures 15, 16(b)), it is very electrically powered and detects

ethanol gas at a very small range of concentrations in the ppb scale. Third, the MQ-3 alcohol biosensors based on PI/Ag/ADH sample 1 VRL voltage is used to power the sensor with an average value of 0.439 V and 0.200 V (Figures 15, 16(c)), it consumes little electricity and can detected ethanol gas at a range of concentrations from the low concentration to the high concentration range and the MQ-3 alcohol biosensors based on PI/Ag/ADH sample 2 VRL voltage is used to power the sensor with an average value of 0.466 V and 0.198 V (Figures 15, 16(d)), it consumes little electricity and can

detected ethanol gas at a range of concentrations from the low concentration to the high also as the MQ-3 alcohol biosensors based on PI/Ag/ADH sample 1, all test are under standard conditions and general environment. Finally, it can be concluded that, MQ-3 alcohol biosensors based on PI/Ag/ADH sample 1, 2 it uses less VRL voltage to power than MQ-3 gas sensor module equipment prototype and MQ-3 alcohol biosensors based on SiO₂/Ag/ADH sample 1 calculate 2% and 4% respectively. The ability to save energy is a key feature of the artificial intelligence platform.

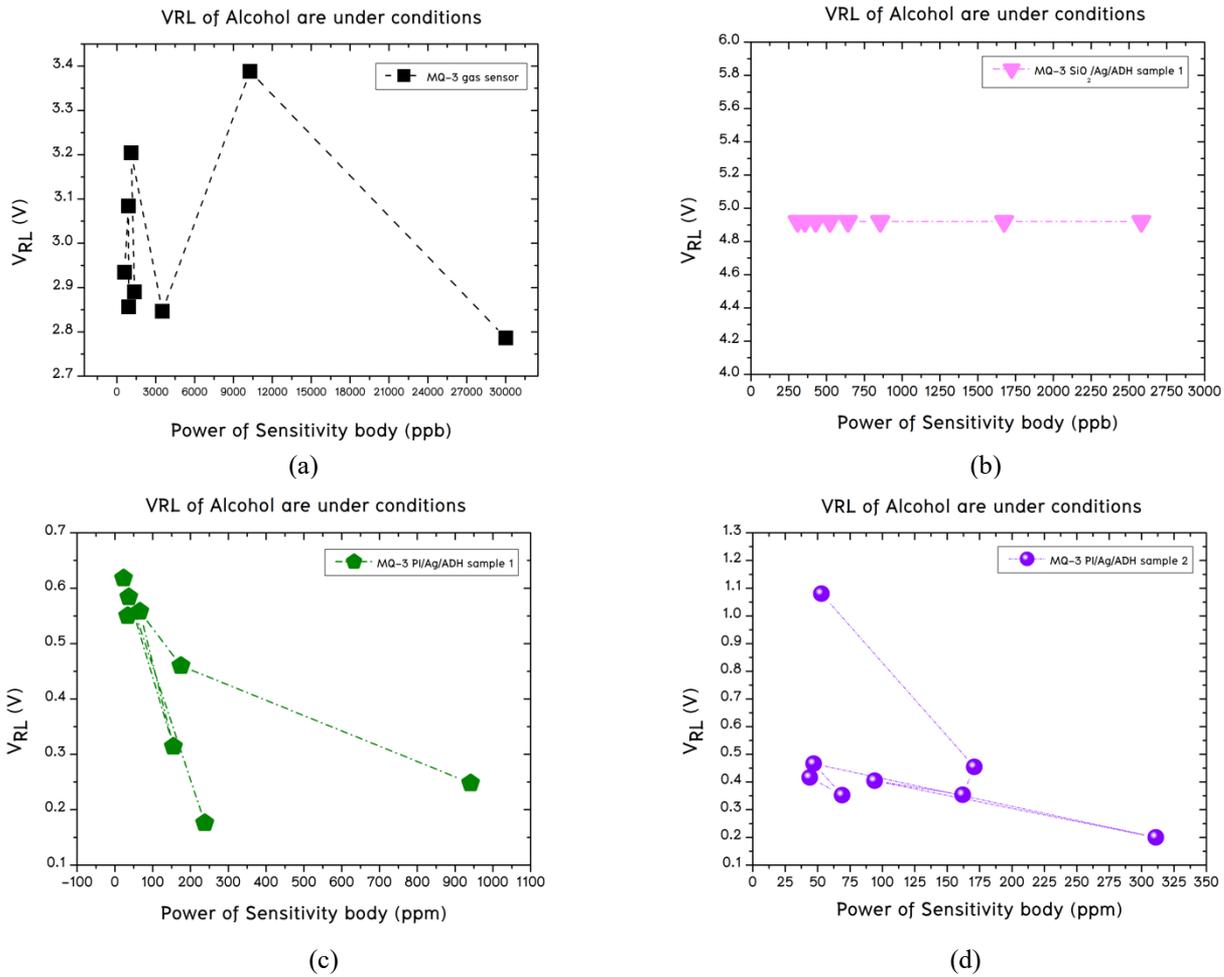
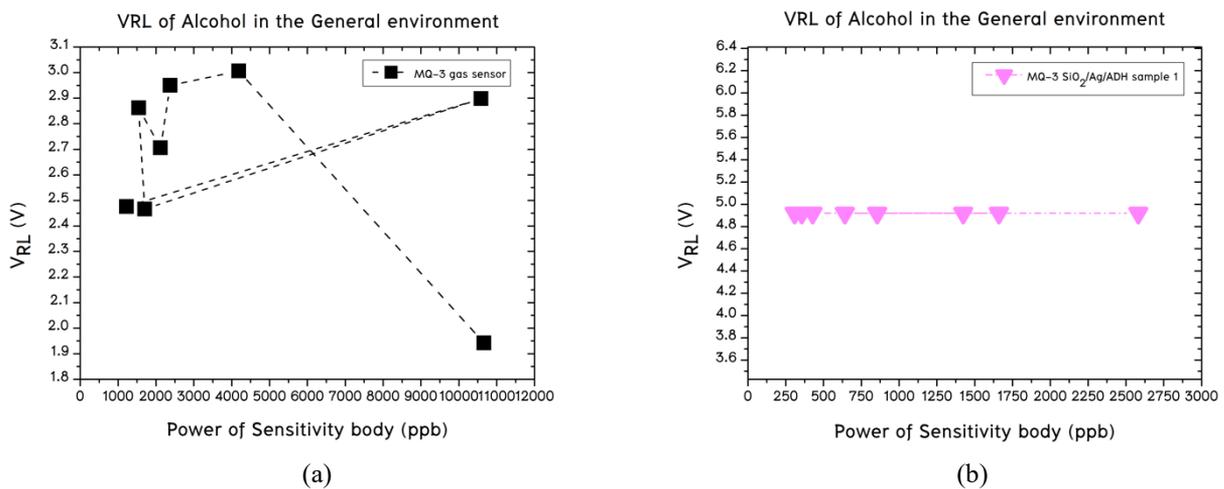
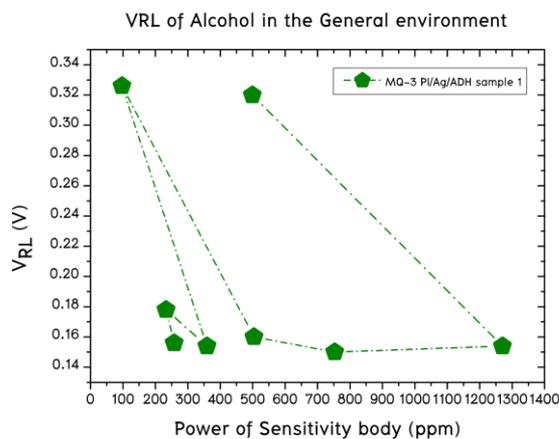
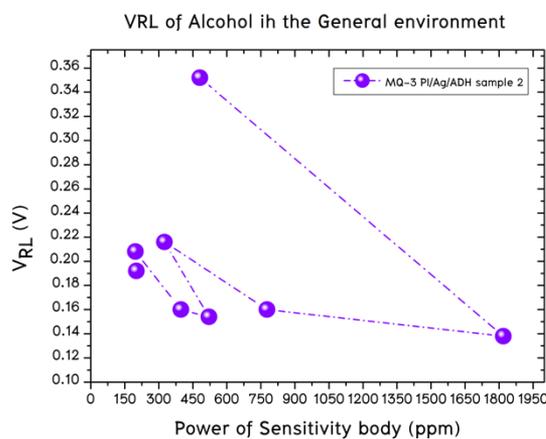


Figure 15. Sensitivity Curve of the VRL in Alcohol with different concentrations are under standard conditions





(c)



(d)

Figure 16. Sensitivity curve of the VRL in alcohol with different concentrations in the general environment

4. CONCLUSIONS

We demonstrated the first Alcohol biosensor made with MQ-3 gas sensor module based on SiO₂ and PI substrates is an electrochemical transistor integrated with the enzyme ADH and coenzyme NAD⁺. The alcohol biosensors are easy to fabricate using DC magnetron sputtering techniques, made on an inexpensive, easy to integrate. We showed that the alcohol biosensors detection ethanol gas in the air, calibration with the MQ-3 gas sensor module equipment prototype. It can be concluded from the experimental results, our MQ-3 alcohol biosensors based on PI/Ag/ADH sample 1, 2 can detect ethanol gas from the range of low concentration to the range of high concentration. Detects ethanol gas in a wider range compared with MQ-3 gas sensor module equipment prototype and MQ-3 alcohol biosensors based on SiO₂/Ag/ADH sample 1. In next generation, module alcohol biosensors can adoption to technology in sensor development and applied for further analysis of other substances.

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REFERENCES

[1] Rothschild, M.L., Mastin, B., Willer, T.W. (2006). Reducing alcohol impaired driving crashes through the use of social marketing. *Accident Analysis & Prevention*, 38(6): 18-30. <http://dx.doi.org/10.1016/j.aap.2006.05.010>

[2] Dula, C.S., Dwyer, W.O., Leverng, G. (2007). Policing the drunk driver: measuring law enforcement involvement in reducing alcohol-impaired driving. *Journal of Safety Research*, 38(3): 67-72. <https://doi.org/10.1016/j.jsr.2006.10.007>

[3] Mishra, K.R., Sempionatto, R.J., Li, Z., et al. (2020). Simultaneous detection of salivary Δ^9 -tetrahydrocannabinol and alcohol using a Wearable Electrochemical Ring Sensor. *Talanta*, 211: 120757. <http://dx.doi.org/10.1016/j.talanta.2020.120757>

[4] Kim, S.Y., Kim, J., Cheong, W.H., et al. (2018). Alcohol gas sensor capable of wireless detection using In₂O₃/Pt nanoparticles and Ag nanowires. *Sensors and Actuators B: Chemical*, 259: 825-832. <https://doi.org/10.1016/j.snb.2017.12.139>

[5] Winsen, Z. (2022). SENSORS. <http://www.winsensor.com/contract.html>, accessed on Apr. 13, 2022.

[6] Kitagawa, Y., Kitabatake, K., Suda, M., et al. (1991). Amperometric detection of alcohol in beer using a flow cell and immobilized alcohol dehydrogenase. *Anal Chem.*, 63(20): 2391-2393. <http://dx.doi.org/10.1021/ac00020a036>

[7] Blanke, R.V., Decker, W.J. (1987). Analysis of toxic substance. In: Tietz NW, editor. *Fundamentals of clinical chemistry*. Philadelphia, PA. WB Saunders Co, 869-905.

[8] Vijayakumar, A.R., Csöregi, E., Heller, A., Gorton, L. (1996). Alcohol biosensors based on coupled oxidase-peroxidase systems. *Anal Chim Acta*, 327(3): 223-234. [https://doi.org/10.1016/0003-2670\(96\)00093-1](https://doi.org/10.1016/0003-2670(96)00093-1)

[9] Chinvejitwanich, W., Panuvej, C., Issaravanich, S. (1998). Measurement the level of Blood Alcohol Concentration after drinking alcoholic beverages. *Journal of Health Systems Research*, 6(2): 106-116. <https://kb.hsri.or.th/dspace/bitstream/handle/11228/1098/jv6n2-2.pdf?sequence=1&isAllowed=y>.

[10] Gülce, H., Gülce, A., Kavanoz, M., Coskun, H., Yildiz, A. (2002). A new amperometric enzyme electrode for alcohol determination. *Biosensor Bioelectron*, 17(6-7): 517-521. [http://dx.doi.org/10.1016/S0956-5663\(02\)00008-8](http://dx.doi.org/10.1016/S0956-5663(02)00008-8)

[11] Kuswandi, B., Irmawati, T., Hidayat, A.M., Jayus, J., Ahmad, M. (2014). A simple visual ethanol biosensor based on alcohol oxidase immobilized onto polyaniline film for halal verification of fermented beverage samples. *Sensors*, 14(2): 2135-2149. <http://dx.doi.org/10.3390/s140202135>

[12] Cai, C.X., Xue, K.H., Zhou, Y.M., Yang, H. (1997). Amperometric biosensor for ethanol based on immobilization of alcohol dehydrogenase on a nickel hexacyanoferrate modified microband gold electrode. *Talanta*, 44(3): 339-347. [http://doi.org/10.1016/S0039-9140\(96\)02049-8](http://doi.org/10.1016/S0039-9140(96)02049-8)

[13] Wang, Z., Etienne, M., Quiles, F., Kohring, G.W., Walcarius, A. (2012). Durable cofactor immobilization

- in sol-gel bio-composite thin films for reagentless biosensors and bioreactors using dehydrogenases. *Biosensors and Bioelectronics*, 32(15): 111-117. <http://doi.org/10.1016/j.bios.2011.11.043>
- [14] Mullor, S.G., Cabezedo, M.S., Ordieres, A.J., Ruiz, B.L. (1996). Alcohol biosensor based on alcohol dehydrogenase and Meldola blue immobilized into a carbon paste electrode. *Talanta*, 43(5): 779-784. [http://doi.org/10.1016/0039-9140\(95\)01802-6](http://doi.org/10.1016/0039-9140(95)01802-6)
- [15] Santos, A.S., Freire, R.S., Kubota, L.T. (2003). Highly stable amperometric biosensor for ethanol based on Meldola's blue adsorbed on silica gel modified with niobium oxide. *J Electroanal Chem*, 547(2): 135-142. [http://dx.doi.org/10.1016/S0022-0728\(03\)00186-4](http://dx.doi.org/10.1016/S0022-0728(03)00186-4)
- [16] Sprules, S.D., Hartley, I.C., Wedge, R., Hart, J.P., Pittson, R. (1996). A disposable reagentless screen-printed amperometric biosensor for the measurement of alcohol in beverages. *Anal Chim Acta*, 329(3): 215-221. [https://doi.org/10.1016/0003-2670\(96\)00121-3](https://doi.org/10.1016/0003-2670(96)00121-3)
- [17] Kolmakov, A., Klenov, D.O., Lilach, Y., Stemmer, S., Moskovits, M. (2005). Enhanced gas sensing by individual SnO₂ nanowires and nanobelts functionalized with pd catalyst particles. *Nano Lett.*, 5(4): 667-673. <https://doi.org/10.1021/nl050082v>
- [18] Jin, C.H., Park, S.H., Kim, H.S., Lee, C. (2012). Ultrasensitive multiple networked Ga₂O₃-core/ZnO-shell nanorod gas sensors. *Sens Actuators B*, 161(1): 223-228. <https://doi.org/10.1016/j.snb.2011.10.023>
- [19] Park, S.H., An, S.Y., Ko, H.S., Jin, C.H., Lee, C. (2012). Synthesis of nanograined ZnO nanowires and their enhanced gas sensing properties. *ACS Appl Mater Interfaces*, 4(7): 3650-3656. <https://doi.org/10.1021/am300741r>
- [20] Kwon, Y.J., Kim, H.S., Lee, S.M., Chin, I.J., Seong, T.Y., Lee, W.I. (2012). Enhanced ethanol sensing properties of TiO₂ nanotube sensors. *Sens Actuators B*, 173: 441-446. <https://doi.org/10.1016/j.snb.2012.07.062>
- [21] Sun, Y.F., Liu, S.B., Meng, F.L., Liu, J.Y., Jin, Z., Kong, L.T., Liu, J.H. (2012). Metal oxide nanostructures and their gas sensing properties: A review. *Sensors*, 12(3): 2610-2631. <http://dx.doi.org/10.3390/s120302610>
- [22] Kim, B.Y., Cho, J.S., Yoon, J.W., et al. (2016). Extremely sensitive ethanol sensor using Pt-doped SnO₂ hollow nanospheres prepared by Kirkendall diffusion. *Sens Actuators B Chem*, 234: 353-360. <http://dx.doi.org/10.1016/j.snb.2016.05.002>
- [23] Tsai, M.H., Huang, S.L. (2006). Synthesis and Characteristics of polyimide/siloxane hybrid films for reliability adhesion. *Surface and Coatings Technology*, 200(10), 3297-3302. <https://doi.org/10.1016/j.surfcoat.2005.07.030>
- [24] Qi, H.X., Wang, X.L., Zhu, T.S., Li, J., Xiong, L., Liu, F. (2019). Low dielectric poly (imide siloxane) films enabled by a well-defined disiloxane-linked alkyl diamine. *American Chemical Society*, 4(26): 22143-22151. <https://doi.org/10.1021/acsomega.9b03302>
- [25] Ghaemy, M., Nasab, S.M.A. (2010). Synthesis and identification of organosoluble polyimides: Thermal, photophysical and chemiluminescence properties. *Polym. J.*, 42: 648-656. <https://doi.org/10.1038/pj.2010.57>
- [26] Chou, W.Y., Kuo, C.W., Chang, C.W., Yeh, B.L., Chang, M.H. (2010). Tuning surface properties in photosensitive polyimide. Material design for high performance organic thin-film transistors. *J. Mater. Chem.*, 20(26): 5474-5480. <http://doi.org/5474-5480>. 10.1039/c0jm00196a
- [27] Jiang, L.Z., Liu, J.G., Wu, D.Z., Li, H.Q., Jin, R.G. (2006). A methodology for the preparation of nanoporous polyimide films with low dielectric constants. *Thin Solid Films*, 510(1-2): 241-246. <https://doi.org/10.1016/j.tsf.2005.12.216>
- [28] Jin, X.Z., Ishii, H. (2005). Novel positive-type photosensitive polyimide with low dielectric constant. *J. Appl. Polym. Sci.*, 98(1): 15-21. <https://doi.org/10.1002/app.21994>
- [29] Kim, J., Kim, M., Lee, M.S., et al. (2017). Wearable smart sensor systems integrated on soft contact lenses for wireless ocular diagnostics. *Nat Commun.*, 8: 14997. <http://dx.doi.org/10.1038/ncomms14997>
- [30] Park, J., Kim, J., Kim, K., et al. (2016). Wearable wireless gas sensors using highly stretchable and transparent structures of nanowires and graphene. *Nanoscale*, 8(20): 10591-10597. <http://dx.doi.org/10.1039/C6NR01468B>
- [31] Choi, S., Park, J., Hyun, W., et al. (2015). Stretchable heater using ligand-exchanged silver nanowire nanocomposite for wearable articular thermotherapy. *ACS Nano*, 9(6): 6626-6633. <http://dx.doi.org/10.1021/acsnano.5b02790>
- [32] Lee, M.S., Lee, K., Kim, S.Y., et al. (2013). High-performance transparent and stretchable electrodes using graphene-metal nanowire hybrid structures. *Nano Lett*, 13(6): 2814-2821. <http://dx.doi.org/10.1021/nl401070p>
- [33] Li, H.F., Ding, G.F., Yang, Z.Q. (2019). A high sensitive flexible pressure sensor designed by silver nanowires embedded in polyimide (AgNW-PI). *Micromachines*, 10(3): 206. <https://doi.org/10.3390/mi10030206>

NOMENCLATURE

SiO ₂ /Ag/ADH	Silicon Dioxide Silver Alcohol Dehydrogenase
PI/Ag/ADH	Poly-imide Silver Alcohol Dehydrogenase

Greek symbols

α	thermal diffusivity, m ² . s ⁻¹
β	thermal expansion coefficient, K ⁻¹
θ	dimensionless temperature
λ	wavelength

Subscripts

SiO ₂	silicon dioxide
PI	poly-imide
Ag	silver
ADH	alcohol dehydrogenase
NAD ⁺	nicotinamide adenine dinucleotide
PBS	phosphate buffer solution