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Development of a Fluorescent Sensor Based on Resazurin and Hydrotalcite for the Determination of Ethanol in Alcoholic Beverages

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Abstract

In this study, a fluorescent ethanol sensor is developed to determine the ethanol concentration in the liquid phase. The sensor is developed using a complex of resazurin (RA)/resorufin (RO) and a hydrotalcite (HT) catalyst in a sol-gel matrix of methyltrimethoxysilane (MTMS) to produce a fluorescent ethanol-sensing membrane (RA/RO*HT membrane). The operation mechanism of the RA/RO*HT membrane is based on (i) the oxidation of ethanol to acetaldehyde and (ii) the reduction of RA to RO, through electron flows followed by EtOH ↔ HT ↔ RA/RO ↔ EtOH interactions. These possible redox reactions can lead to an increased fluorescence intensity of the RA/RO*HT membrane as the ethanol concentration increases. The RA/RO*HT membrane shows a linear detection range of 1–20 vol.% EtOH with limit of detection (LOD) of 0.178%. Additionally, the RA/RO*HT membrane has high sensitivity and accuracy for determining the alcohol content in several Korean alcoholic beverages.

Keywords: Ethanol determination, Resazurin, Resorufin, Fluorescent ethanol sensor, Alcoholic beverages

1. INTRODUCTION

Ethanol is a chemical compound that is widely used in many applications, including hygienic solutions in biomedicine, solvents for materials in chemical industries and cosmetics, fuel for vehicles, and as a main ingredient in the food industry. Therefore, diverse ethanol sensors have been developed to fulfill the requirements of different fields. In general, ethanol sensors can be classified into gas or liquid sensors. Various types of ethanol gas sensors have been developed because of the recent interest in using green energy, such as ethanol, to replace gasoline [1], wherein semiconductor materials are generally used for the fabrication of ethanol gas sensors because of the change in the electrical conductivity of materials following the adsorption of ethanol gas at high temperatures [2-4]. On the other hand, a wide variety of ethanol liquid sensors have been developed, thereby enabling the direct or indirect measurement of ethanol in the liquid phase via various methods, such as chromatography [5], photometry [6-9], and electrochemistry [10,11]. Generally, photometric and electrochemical methods determine the ethanol concentration based on the oxygen consumption of the ethanol oxidation reaction or the change in the concentration of the final product (e.g., hydrogen peroxide).

The first optical sensor for ethanol was developed in the 1950s. The operation principle of this sensor was based on the redox reaction of ethanol with $K_2Cr_2O_7$ solution resulting in a color change in $K_2Cr_2O_7$ from orange to green via the reduction of Cr^{6+} to Cr^{3+}, and the ethanol concentration was calculated from the absorbance value of a sample at λ = 600 nm [7]. Ethanol sensors that belong to the photometric sensor group have been actively developed over the past two decades. Most ethanol sensors employ the enzyme alcohol oxidase (AOX), which indirectly measures the concentration of ethanol in the liquid phase [12]. Herein, the change in oxygen consumption during the oxidation reaction of ethanol is expressed as the change in the fluorescence intensity of oxygen-sensitive dyes captured in oxygen-sensing membranes, and ethanol concentrations were ultimately calculated from the fluorescence intensities [8]. The production of hydrogen peroxide by the oxidation of ethanol is controlled using various colored substances for ethanol detection [13-15]. However, few studies have been conducted on optical ethanol sensors that do not use AOX, and only a few have demonstrated the potential of optical materials for ethanol sensors. Simon et al. [9] used two types of dyes (malachite and amino-N-methylphthalimide) to detect ethanol based on the polarity of different ethanol solutions.

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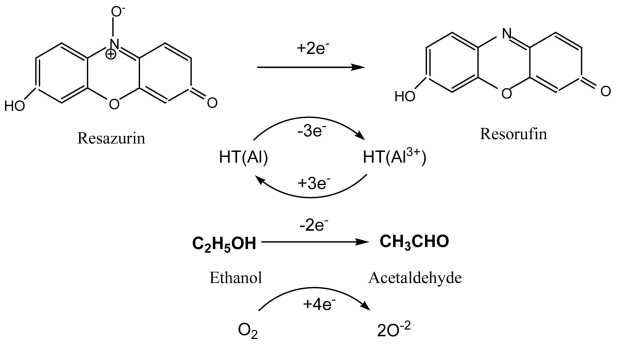


Scheme 1. HT catalysis in the oxidation reaction of ethanol to acetaldehyde and the reduction reaction of resazurin (RA) to resorufin (RO) through the electron donation and electron acceptance of HT

Takahashi et al. [16] measured the ethanol concentration using a combination of rhodamine 6G and anion-exchange clay.

From this perspective, a fluorescent ethanol-sensing membrane was developed for direct determination of ethanol in the liquid phase. An ethanol-sensing membrane was fabricated using a combination of resazurin (RA), a fluorescent dye, and hydrotalcite (HT), a catalyst, in the supporting material of a sol-gel matrix of methyltrimethoxysilane (MTMS). In this combination, HT catalyzes the reduction and oxidation of resazurin (RA) to resorufin (RO) in an acidic medium and of ethanol to acetaldehyde, respectively, through electron donation or acceptance (Scheme 1). The interaction of these reactions through electron flow increases the fluorescence intensity of the ethanol-sensing membrane (i.e., the RA/RO*HT membrane) when exposed to higher ethanol concentrations. Additionally, the RA/RO complex is sensitive to the polarity of several short chain alcohols. Therefore, increasing the ethanol concentration decreased the polarity of the ethanol solution and increased the fluorescence intensity of the RA/RO*HT membrane. The RA/RO*HT membrane was used to determine the ethanol concentration in certain Korean alcoholic beverages.

2. EXPERIMENTAL

2.1 Materials

Ethanol, methanol, iso-propanol, resazurin (RE), methyltrimethoxysilane (MTMS), hydrotalcite (HT), L-ascorbic acid, tartaric acid, malic acid, lactic acid, acetic acid, succinic acid, glucose, fructose, and saccharose were purchased from Sigma-Aldrich Chemical Co. (Seoul, South Korea). Other chemicals, such as hydrochloric acid, sodium hydroxide, and sodium phosphate (mono- and di-basic), were of analytical grade and used without further purification.

2.2 Preparation of the RA/RO*HT membrane

First, 10 mM RA (30 μL) in ethanol was absorbed in 10 mg HT and aged at room temperature for 24 h. Sol-gel of MTMS was prepared by mixing 0.2 mL MTMS with 0.8 mL ethanol and 20 μL concentrated HCl. The sol-gel was aged at room temperature for approximately 24 h for polymerization. Subsequently, the sol-gel (1 mL) was mixed with the RA/HT combination (~69 μg RA/10 mg HT), then immediately coated on PET film in the area of 42.25 cm^2 and dried at room temperature for several hours before being allowed to dry at 60°C for 12 h. The membrane was washed by soaking in distilled water for 15 min, and no dye leakage was observed.

2.3 Measurements of ethanol solutions

The RA/RO*HT membrane was cut and pasted onto the bottom of each well of a 24-well microtiter plate. The membrane was cleaned by soaking it in distilled water for 4 h prior to being used for ethanol determination. Upon cleaning, the membrane was exposed to different concentrations of ethanol (0–20 vol%) for 10 min and the fluorescence intensity was measured using a multifunctional fluorescence microtiter plate reader (Safire^2, Tecan Austria GmbH, Austria). Data were collected from the fluorescence intensity at an emission wavelength of 570 nm (λ_{em}=570 nm) and an excitation wavelength of 500 nm (λ_{ex}=500 nm) for the RA/RO*HT membrane. The limit of detection (LOD) was based on the ratio of the standard deviation (SD) of the blank samples to the slope of the calibration curve (SI) as follows:

$$LOD = 3.3*SD/SI$$

The reversibility of the RA/RO*HT membrane was measured in 10% ethanol and distilled water. The membrane was first exposed to distilled water and then to a 10% ethanol solution before being repeated. A microtiter plate reader was used for fluorescence measurement over a period (10 min) with an interval of 30 s during measurement.

Subsequently, the effects of pH and temperature on the ethanol measurements were investigated. The RA/RO*HT membrane was exposed to 10% ethanol solution in the range from pH 2.0 to pH 8.0. Additionally, the RA/RO*HT membrane was tested with different temperatures (20, 25, 30, 33, and 35°C) in the ethanol concentration range from 0 to 10%.

The long-term stability of the RA/RO*HT membrane was evaluated by comparing the sensitivity of the RA/RO*HT membrane at initial and after a certain time of use. The effects of common beverage components on the RA/RO*HT membrane were investigated by adding individual components to a 10% ethanol solution. More specifically, 2 g/L glucose, 2 g/L fructose, 2 g/L saccharose, 0.15 g/L ascorbic acid, 1.8 g/L malic acid, 1.2 g/L lactic acid, 0.6 g/L acetic acid, 2.4 g/L tartaric acid, and 1.2 g/L succinic acid.

2.4 Ethanol determination in Korean alcoholic beverages

Based on the linear detection range of the RA/RO*HT membrane for the ethanol concentration, three types of Korean alcoholic beverages with different ethanol concentrations were chosen for this study, i.e., beer (4.5%), makgeolli (6%), and soju (16.9%). Samples of beer and makgeolli were pretreated before measurement to reduce interference from organic compounds in alcoholic beverages through filtration with a filter of 0.45 μm pore size and pH adjustment to pH 6. Additionally, carbon nanoparticles reduced the color of the beer.

3. RESULTS AND DISCUSSIONS

3.1 Choice of materials

Resazurin (RA) is a phenoxazine dye widely used in biology [17]. It can participate in redox reactions in biological processes as an oxidation reagent and is converted to the resorufin form (RO) with a pink color that differs from the blue color of RA in the initial samples. RO has a high fluorescence intensity, making it suitable for use in optical devices. As shown in Fig. 1(a), RA shows maximum emission wavelengths (λ_{em}) of 580 nm and 625 nm in distilled water (above, left). These two emission wavelengths can vary approximately ±10 nm based on the solvents used and the measurement conditions, e.g., λ_{em}=590 m and 635 m in ethanol (Fig. 1(a), above, right). Moreover, they can also be shifted to shorter emission wavelengths (i.e., 570 and 615 nm for RA in distilled water) using another fluorescence spectrophotometer (Fig. 1(a), below, left), and RO has a maximum emission wavelength of 570 nm. The properties of the ethanol-sensing membrane were studied on a 24-well microtiter plate, and emission wavelengths of 570 and 615 nm were chosen to collect the fluorescence data.

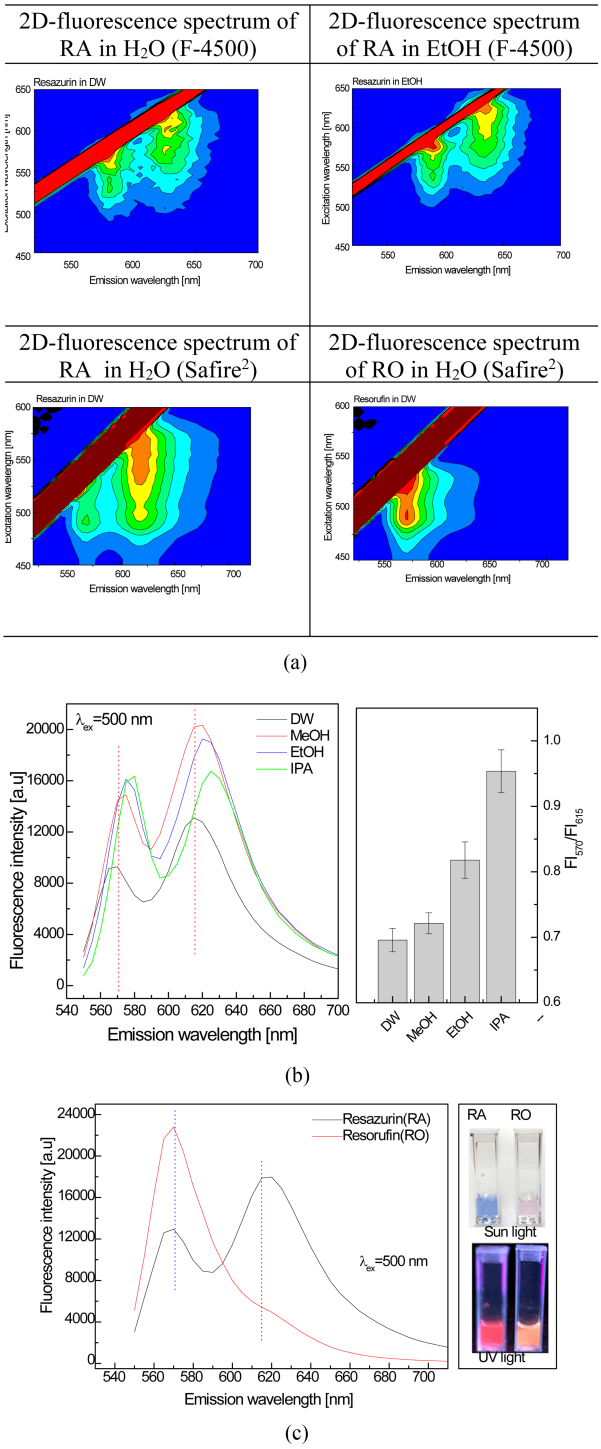


Fig. 1. (a) (above) 2D-fluorescence spectra of 0.1 mM RA(resazurin) in distilled water (DW) and absolute ethanol (EtOH) measured by a fluorescence spectrometer (F-4500), and (below) 2D- fluorescence spectra of 0.1 mM RA and RO (resorufin) in DW, measured using a fluorescence microtiter plate reader (Safire^2), (b) response of 0.1 mM RA in short chain alcohols with different polarities (E_T^N: MeOH = 0.762, EtOH = 0.654, IPA = 0.546) and (c) fluorescence emission spectra of RA and RO, and their photos under sunlight and UV light at 365 nm (power of 8 W)

Based on the emission wavelengths of RA and RO, the ratio of the fluorescence intensities at λ_{em} =570 nm and 615 nm must be considered to evaluate the conversion of RA to RO in different environments. In addition to its capability in redox reactions, RA is sensitive to the polarity of several short chain alcohols (Fig. 1(b)). In this study, 0.1 mM RA was exposed to various solutions (water, methanol, ethanol, and isopropanol) with different polarities. The relative polarity (E_T^N) of the solutions decreased in the order: 1> 0.762> 0.654> 0.546, corresponding to distilled water (DW)> methanol (MeOH)> ethanol (EtOH)> isopropanol (IPA). As depicted in Fig. 1(b), the ratio of the fluorescence intensities at λ_{em}=570 and 615 nm (FI_{570}/FI_{615}) increased as the polarity of the solutions decreased. This was attributed to the conversion of RA to RO in solvents with different polarities and the reorientation of solvent molecules by RO [18,19]. Additionally, the pH of the solvents can explain the varying fluorescence intensities of these two bands. The decrease in the intensity of the band at 615 nm and the increase in the intensity of the band at 570 nm are caused by the protonation of the N-oxide group of RA at lower pH [17]. Therefore, the sensitivity of the RA/RO complex to the polarity of short chain alcohols and the pH of the solvents contributed to the sensitivity of the ethanol-sensing membrane in this study.

As shown in Fig. 1(c), the fluorescence emission wavelength (λ_{em} = 615 nm) of RA was shifted to λ_{em} = 570 nm when RA was reduced to RO. The change in the fluorescence emission spectrum of RA before and after reduction is clear under normal sunlight and UV light, as shown in the photos in Fig. 1(c).

The sol-gel matrix of MTMS was selected to support the indicator dye because of its high stability and good capture into the matrix. The polymerization and condensation of MTMS can produce Si-O-Si-CH3 bonds that render the MTMS membrane hydrophobic [20]. Additionally, we used MTMS, which has been shown to be an excellent supporting material for fluorescent dyes, in our previous studies for the fabrication of sensing membranes [21].

HT is a rhombohedral hydrotalcite with the chemical formula $Mg_6Al_2(OH)_{16}CO_3.4H_2O$. It is well-dispersed in polymer solutions and is commonly used as an anion-exchange clay. Aluminum (Al or Al^{3+}) in HT plays an important role in reducing resazurin (RA) to resorufin (RO) and in the oxidation of ethanol by electron donation or acceptance, respectively, as shown in Scheme 1.

3.2 Properties of the RA/RO*HT membrane

Membranes produced from the sol-gel of silanes are generally thin and smooth. The SEM and AFM images in Fig. 2 show that the MTMS membrane was extremely smooth with a surface mean roughness (Ra) of 0.24 nm and a root mean square roughness (Rq) of 0.298 nm. However, the surface of the RA/RO*HT membrane containing the RA/RO complex and HT in MTMS was rougher than that of the MTMS membrane alone, with Ra was 1.81 nm and Rq was 2.85 nm. Fig. 2 shows the strong fluorescence emission of the RA/RO*HT membrane under UV irradiation.

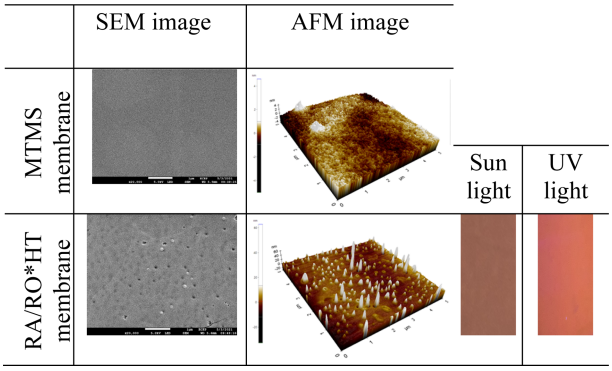
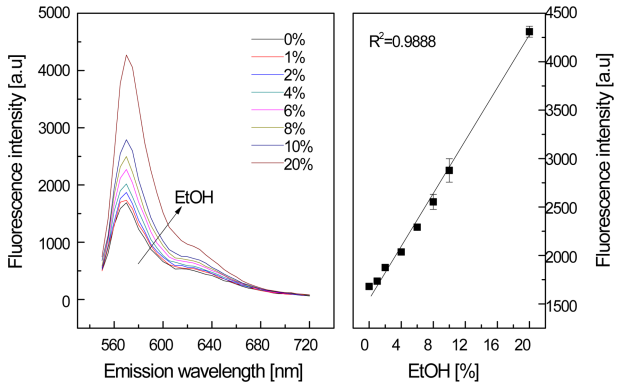


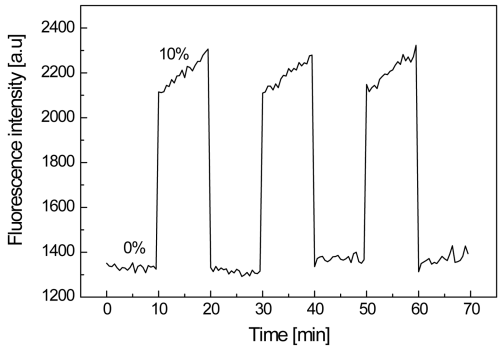
Fig. 2. SEM and AFM images of the sol-gel membrane (MTMS) and RA/RO*HT membrane, and photos of the RA/RO*HT membrane under sunlight and UV light.

3.3 Characterization of the RA/RO*HT membrane

When RA and HT were captured in the MTMS sol-gel membrane, the reduction of RA occurred immediately. This is because of the acidity of the MTMS sol-gel, which is a good medium for the reduction of RA during HT catalysis. Therefore, the emission wavelength of RO at λ_{em} = 570 nm appears prominently compared to the emission wavelength of RA at λ_{em} = 615 nm in terms of the fluorescence intensity (Fig. 3(a) (left)). As depicted in Fig. 3(a) (left), the fluorescence intensity of the RA/RO complex at λ_{em} = 570 nm increased significantly with the concentrations of ethanol solution. This good response of the RA/RO*HT membrane to an ethanol solution is presented using a calibration curve with a linear detection range of 1%–20% ethanol (Fig. 3(a) (right)) and a limit of detection (LOD) of 0.178%. Compared to the results of Simon et al. [9], the RA/RO*HT membrane proposed in this study shows a better response than the malachite membrane used in Simon's research in terms of the limit of detection. In fact, the RA/RO*HT membrane also had high sensitivity to ethanol concentrations of over 20%, but the lifetime of the membrane could be shortened owing to the loss of the RA/RO complex. Therefore, we chose an ethanol measurement range of 1–20% to maintain the long lifetime of the



(a)



(b)

Fig. 3. (a) Response of the RA/RO*HT membrane at different concentrations of ethanol and a calibration curve for ethanol detection at λ_{ex} = 500 nm and λ_{em} = 570 nm and (b) the rre-peatability of the RA/RO*HT membrane exposing repeatedly at 0% and 10% ethanol (λ_{ex} = 500 nm and λ_{em}= 570 nm)

ethanol-sensing membrane. Compared with other ethanol sensors using the enzyme AOX, such as those described by Barsan et al. [11] and Patel et al. [10], this RA/RO*HT membrane showed a wider ethanol detection range than the electrode biosensors. This indicates that the RA/RO*HT membrane can be used to measure the concentration of ethanol in real alcohol samples without dilution.

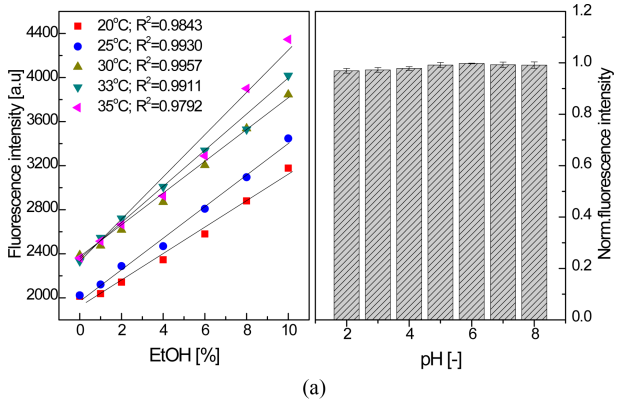
The RA/RO*HT membrane exhibits high sensitivity and fast reversibility when repeatedly exposed to distilled water (0% ethanol) and 10% ethanol (Fig. 3(b)). The repeatability was stable after each measurement cycle, as the relative standard deviation (RSD) was exceedingly small, that is, 2.1% in 0% ethanol and 0.4% in 10% ethanol. Fig. 3(b) also indicates that 95% of the fluorescence intensity was obtained in a short time (approximately 30 s – 1 min), but approximately 10 min was required to reach saturation at an ethanol concentration of 10%. Meanwhile, the return time from 10% ethanol to the starting point (0% ethanol) was short time (1 2 min).

In this study, alcoholic beverages in the food industry are the main targets for the use of RA/RO*HT membranes in real applications. Therefore, pH and temperature are crucial parameters that must be investigated to adjust the conditions suitable for ethanol measurement. The fluorescence intensity of the RA/RO*HT membrane increased as the temperature increased from 20°C to 25°C at all ethanol concentrations in the range of 1 – 10% (Fig. 4(a) (left)). In the temperature range of 30 – 35°C, the fluorescence intensity of the RA/RO*HT membrane was higher than that in the temperature range of 20 – 25°C. However, the fluorescence intensity of the membrane at λ_{em} = 570 nm did not increase significantly with increasing temperature in the ethanol concentration range from 1% to 6% in this temperature range (30 – 35°C).

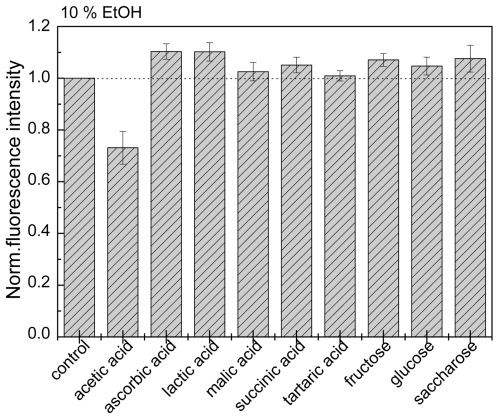
The fluorescence intensity of the RA/RO*HT membrane increased only at ethanol concentrations greater than 6%. According to Flamigni et al., [18], the fluorescence quenching of RO at low temperatures is owing to solute-solvent interactions between the negative partial charges localized on the two end oxygen atoms of RO and the solvent. Shielding of the oxygen negative charges reduces the interaction between RO and bulk alcohol.

The pH of a solution is often measured during the fermentation of alcoholic drinks. Low pH typically occurs in fermented solutions during the production of alcoholic beverages. The fluorescence intensity of the RA/RO*HT membrane decreased slightly in the ethanol solution at a low pH (from pH 2 to 4) (Fig. 4(a) (right)). Higher fluorescence intensities were observed in ethanol solutions with pH > 4. In low-pH solutions, the low fluorescence intensity is explained by the formation of RO agglomerates [18]. Protonation results in the formation of a neutral form of resorufin, which can lead to aggregation. However, in this study, the RA/RO complex was captured in the MTMS sol-gel matrix, minimizing the possibility of dye agglomeration in acidic solutions. It can be concluded that the effects of pH on the RA/RO*HT membrane were insignificant within the pH range of 2–8, but pH 6 was chosen for the preparation of the standard samples.

The operating mechanism of the RA/RO*HT membrane is based on the interaction between the oxidation of ethanol and the reduction of resazurin (RA) to resorufin (RO) during the catalysis of HT through electron donation and acceptance. Additionally, the sensitivity of the RA/RO*HT membrane to the polarity of short chain alcohols is a crucial reason for its operation. Many oxidizing compounds are present in beverages and can affect the ethanol measurement of ethanol-sensing membranes. This could be attributed to the presence of acids, such as tartaric acid and



(a)



(b)

Fig. 4. (a) (left) Response of the RA/RO*HT membrane at different temperatures of 20°C – 35°C in the ethanol concentration range of 1 - 10%; and (right) at 10% EtOH in the pH range of 2.0 – 8.0 and (b) Responses of the RA/RO*HT membrane at 10% ethanol in the presence of various acids such as 0.6 g/L acetic acid, 0.15 g/L ascorbic acid, 1.2 g/L lactic acid, 1.8 g/L malic acid, 1.2 g/L succinic acid, 2.4 g/L tartaric acid, and 2 g/L of different sugars such as glucose, fructose, and saccharose

ascorbic acid, in alcoholic beverages. Therefore, some acids that arose during processing or were produced as final products were tested using the RA/RO*HT membrane.

The acid concentration used in this study was based on the typical amount of each acid in the beverage. The RA/RO*HT membrane was stable in the presence of a given amount of acid (Fig. 4(b)). Compared to the control sample, the recovery of the RA/RO*HT membrane is 110.3% for 0.15 g/L ascorbic acid, 110.2% for 1.2 g/L lactic acid, 102.5% for 1.8 g/L malic acid, 105.1% for 1.2 g/L succinic acid, and 100.9% for 2.4 g/L tartaric acid. However, the fluorescence intensity of the RA/RO*HT membrane decreased significantly at 0.6 g/L acetic acid (73.1% recovery). This can be attributed to the relationship between acetaldehyde produced from the oxidation of ethanol and acetic acid. Acetaldehyde is

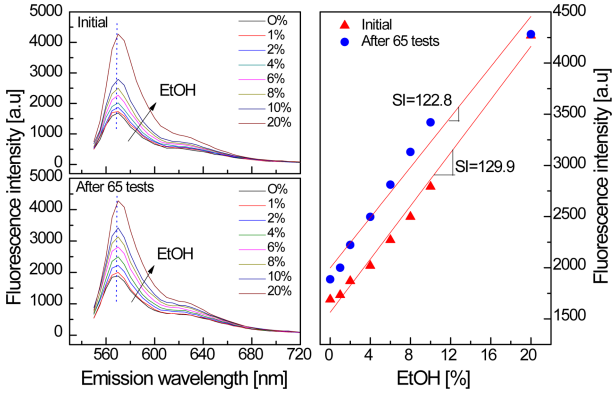


Fig. 5. Lifetime of RA/RO*HT membrane after 65 consecutive measurements of ethanol samples

commonly used as a precursor to acetic acid; therefore, the presence of acetic acid with amount of 0.6 g/L inhibited the oxidation of ethanol to acetaldehyde, which destroyed the electron flow of the reactions shown in Scheme 1 and resulted in a decrease in the fluorescence intensity of the RA/RO*HT membrane. Thus, with minimal effect from most common interferences, the RA/RO*HT membrane can be used in the food industry and other fields (e.g., the environment).

In addition to the effects of various acids, the amount of sugar contained in the beverage is usually very high (the total sugar amount varies in the range of 1–8 g/L); therefore, the amount of sugar may affect the measurement of the ethanol-sensing membrane. Large amounts of sugar affect the motion of the alcohol in the solution and reduce the contact between ethanol and the RA/RO*HT membrane. The presence of 2 g/L of each type of sugar did not significantly affect the response of the RA/RO*HT membrane, with recovery percentages of 104.7%, 107.1%, and 107.5% for glucose, fructose, and saccharose, respectively (Fig. 4(b)).

The RA/RO*HT membrane was stable and could be used for continuous analysis of approximately 65 samples. Fig. 5 shows that, after 65 consecutive measurements, the slope index (SI = 122.8) of the RA/RO*HT membrane was not significantly different from that of the RA/RO*HT membrane (SI = 129.9). There were no chemical bonds between the sol-gel MTMS and the RA/RO complex, but the RA/RO*HT membrane could be used to measure many samples without dye leakage. This can be attributable to the absorption of the RA/RO complex in the HT layer, as HT is an anion exchange clay and RO exists in the anion form, which leads to a tight bond of RA/RO in the layers of HT. We attempted to conjugate the RA/RO complex and HT with other supporting polymers (e.g., ethyl cellulose) through chemical

Table 1. Comparison of some ethanol sensors used for the determination of ethanol in liquid phase

Detection method	Linear detection range	Limit of detection	Response time	Long-term stability	Ref.
Photometry (color change from redox reactions of $K_2Cr_2O_7$ and EtOH)	1 - 20%	0.5%	20 min	-	[6]
Enzyme + fluorescence dye (fluorescence quenching by oxygen amount)	0.29 - 5.2%	-	1.5 min	-	[8]
Fluorescence dye (sensitivity to polarity of EtOH solution)	0 - 50%	0.6%	<5 min	5 week	[9]
Enzyme + electrodes (potentionmetry)	0.0058 - 1.75%	-	12 sec	12 h	[10]
Semiconductor nanomaterials + electrode (voltammetry)	5.8×10^{-7} - 0.58%	3.48×10^{-7} %	10 sec	-	[22]
Metallic fluorescent probe (aggregation of Cu nanocluster by EtOH)	0 – 50%	0.1%	<1 min	-	[23]
Solid-sate fluorescence of phenol derivatives	0 – 100%	1%	30 min	-	[24]
Fluorescence dye (sensitivity to oxidation of EtOH)	1 - 2 %	0.178%	30–60 sec	65 test	This study

binding; however, this bonding reduced the sensitivity of the RA/RO complex to the ethanol solution, resulting in a lower response of the RA/RO*HT membrane (data not shown).

Table 1 lists the ethanol sensors used for determining the ethanol in the liquid phase. Some analytical parameters were compared with those of the RA/RO*HT membrane developed in this study. The RA/RO*HT membrane exhibited good analytical performance, specifically in terms of high sensitivity, wide detection range, fast response time, and long-term stability.

3.4 Application of the RA/RO*HT membrane

To evaluate the accuracy of the ethanol sensor for practical applications, it must be tested using real samples. An RA/RO*HT membrane was used to determine the ethanol content of three Korean alcoholic beverages: beer (4.5%), makgeolli (6%), and soju (16.9%).

According to the equation collected from the linear calibration curve of standard ethanol solutions (y=396.14*x – 623.95 with R^2=0.991) with the RA/RO*HT membrane and the data obtained from measuring samples of Korean alcoholic beverages (Fig. 6), the ethanol concentrations of three real samples are presented in the table in Fig. 6. In the table, the accuracy of the RA/RO*HT membrane is compared with the alcohol content provided by the producers. The data in the table in Fig. 6 show that the RA/RO*HT membrane has a high sensitivity and accuracy for real samples.

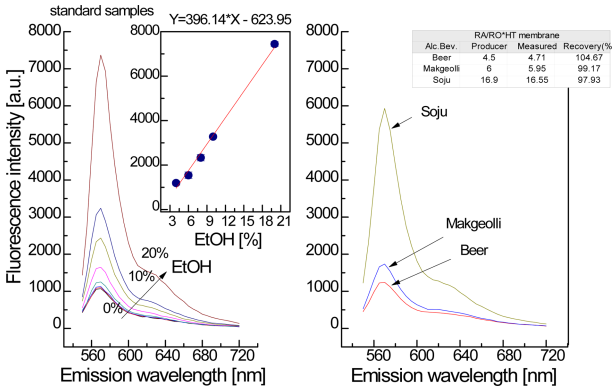


Fig. 6. Response and recovery of RA/RO*HT membranes from several Korean alcoholic beverages

4. CONCLUSION

A fluorescent ethanol-sensing membrane based on the combination of the RA/RO complex with HT in the sol-gel matrix of MTMS was successfully developed to accurately determine the ethanol concentrations in the liquid phase. Herein, the interaction between the reduction of RA/RO and the oxidation of ethanol under HT catalysis influenced the fluorescence intensity of the RA/RO*HT membrane (i.e., the fluorescence intensity increased with increasing ethanol concentration). The RA/RO*HT membrane exhibited a linear detection range of 1–20 vol.% with a low limit of detection (LOD) of 0.178% ethanol. The RA/RO*HT membrane was stable, with high reversibility within 10 min and continuous measurements over approximately 65 tests.

The RA/RO*HT membrane showed a good response to the ethanol content of Korean alcoholic beverages with high accuracy and fast recovery, and is a reliable sensor for determining the concentrations of ethanol in real samples without dilution.

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