Electrochemical Detection of Alcohol using Enzyme Sensor with Chromatography Paper and its Potential Application as *halal* Sensor

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ABSTRACT

This research is a preliminary study aimed to develop a `*halal* sensor` that can detect non-halal ingredients in food such as alcohol and pork meat. Sample solutions containing ethanol, alcohol oxidase, horseradish peroxidase, and electron mediator is dropped onto chromatography paper on top of carbon paste electrode. The relation between ethanol concentrations and open circuit potential value is studied. Despite using the paper, one are able to see that the electrode potential is dependant on the ethanol concentration and the relationship between these two parameters concurred with Nernst equation. The use of chromatography paper can produce a low cost and possible miniaturization of sensor.

Keywords: ethanol, nernst equation, enzymatic sensor, chromatography paper, potentiometric

1 INTRODUCTION

Culture, religion, and health are among the few factors that affect people around the world to consume their food daily. Muslims, for example, depend heavily on the *halal* status of their food [1]. *Halal* is a term used to describe everything, including food that is permissible according to Islamic law [2]. The Islamic law forbids Muslims from consuming food that contains porcine derivatives and alcohol, among others [1-3]. The term *halal* is not only limited to the food ingredients but also considers the absence of non-halal species mixed during packaging or storage process [2]. As these criteria are difficult to be decided by consumers alone, Muslims countries usually use a special label or certificate called halal label (Fig. 1) on food packaging or restaurants [2]. However, this label is still scarcely available in non-Muslim countries and there is no globally recognized halal label as yet [3]. Therefore, Muslims in these countries for study or travel purposes always find it difficult to determine which food is *halal* for them. Although there were many previous researches on halal authentication in general, seldom do they aim for common people in their daily usage [4-5].

This research is a preliminary study aimed to develop a `*halal* sensor` especially for the people mentioned above. One of the major factors to decide the halal status of food is whether alcohol is included in the ingredient. As the first step to realizing a portable `*halal* sensor`, this paper discusses the detection of ethanol using enzymatic sensor with chromatography paper on top of carbon paste electrode. The relation between ethanol concentrations and electrode potentials is studied.

Figure 1 : Official halal label widely used in Malaysia and is issued by the Department of Islamic Development Malaysia (JAKIM)[2].

2 MATERIALS AND METHODS

2.1 Electrode structure

The whole structure of our electrode is illustrated in Fig. 2. All electrodes used in this experiment are prepared in the laboratory using commercially available materials including carbon paste powder and silver-silver chloride (Ag/AgCl) ink. Working electrode (WE) and counter electrode (CE) are made from carbon paste while reference electrode (RE) is made from Ag/AgCl ink. The diameter of all three electrode surfaces are approximately 1.2mm and the distances between all three electrode surfaces are kept approximately 1.0~2.0mm. As shown in the figure, chromatography paper (ChrPr) is sandwiched between polytetrafluoroethylene (PTFE) plate, on top of the electrodes, and polyvinyl chloride (PVC) plate during measurement. We used Whatman 1Chr chromatography paper throughout the experiments. The PVC plate is used to apply pressure on top of the electrode to keep the paper in place and to eliminate the possibility of an air gap to exist between the paper and the electrode surfaces. Chromatography paper is used to contain reagents and the sample solution. Moreover, in a real situation, dropping sample solution directly onto the flat surface of the electrode without the paper make it prone to spilling. Usage of the paper can help prevent this, thus, make it easy for any users to handle the sensor. We also chose paper because of its affordability and the possibility of making the sensor

disposable. In future, enzymes and mediators will be soaked into the paper and left to dry beforehand. Sample solution containing only the substrates to be measured such as ethanol will be dropped during measurement.

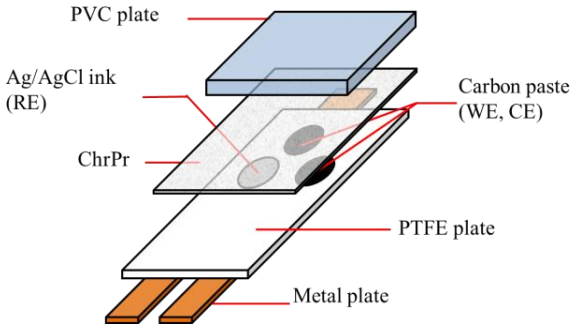

Figure 2 : The whole electrode structure.

2.2 Chemicals

Our ethanol detection scheme is based on a previous report [9]. Ethanol with different concentrations are mixed with solutions containing enzymes and mediator and are kept in refrigerator(4℃) for more than 12 hours before experiments. The sample solution includes 2.0U/mL alcohol oxidase (AOD), 2.0 U/mL horseradish peroxidase (HRP), 50mM $K_4[Fe(CN)_6]$ (Ferro), 100mM phosphate buffer solution (PBS) with pH 7.0 apart from the ethanol. The AOD, derived from *Pichea Pastoris* (yeast), is purchased from MP Biomedicals, while the HRP is purchased from Wako Pure Chemical Industries, Ltd.. The chemical reaction between ethanol and enzymes is shown in Fig. 3.

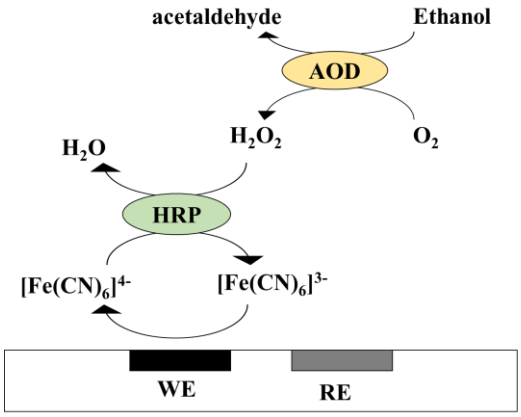

Figure 3: Chemical reaction of ethanol with enzymes and mediator.

2.3 Measurement

In this paper, potentiometric measurement is used as the main indicator of relationship between ethanol concentration and electrode potential. Potential difference between WE and RE is measured with ALS/CH Instruments`s electrochemical analyzer, model 610DR. Open circuit potential vs. time (OCPT) measurement method is used to measure the electrode potentials. OCPT does not apply any potential or electrical current onto the electrodes, thus the electrode potential observed is determined by concentration ratio of Ferro and $K_3[Fe(CN)_6]$ (Ferri) alone. Electrode potential value is plotted against the Ferro and Ferri concentrations or against the ethanol concentrations, using Nernst equation ;

$$E = E^{\circ} + \frac{RT}{nF}\ln\left(\frac{C_{ox}}{C_{red}}\right), \quad (1)$$

where E [V] is the measured electrode potential, E^o [V] is standard electrode potential, R [J/K] is gas constant, T [K] is temperature, n is the number of electrons transferred, F is Faraday`s constant, C_{ox} [M] is concentration of Ferri, and C_{red} [M] is concentration of Ferro. All measurements were conducted at room temperature, 25 ℃. Sample solution is dropped from one end of chromatography paper while pressure is applied on top of the PVC plate as illustrated in Fig. 4. Pressure is applied using apparatus as shown in Fig. 5(b).

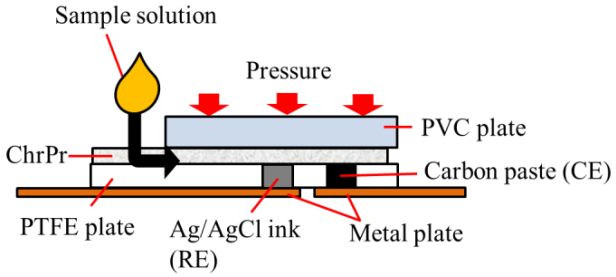

Figure 4: Side view of electrode during experiment.

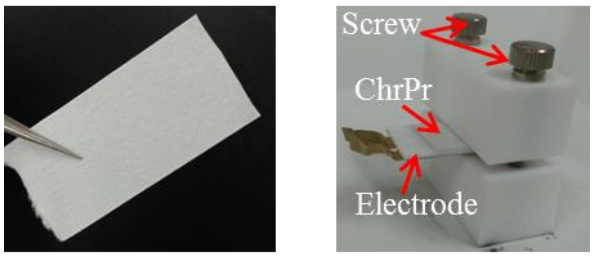

Figure 5: (a) A piece of chromatography paper used with the electrodes; (b) Set up of electrode during experiment. Screw is used to apply pressure on top of electrode.

3 RESULTS

Fig. 6(a) shows relationship between electrode potential, E and ethanol concentration, C_{Eth} in natural logarithm. Note that significantly different potential value is observed for each ethanol concentration 0.001, 0.002, 0.005, 0.01, 0.05, and 0.1% (v/v). Sample solutions that include only the redox couple Ferri and Ferro but not the enzymes or ethanol are also tested using the same experimental method to produce the results in Fig. 6(b). Gradient value of the graphs indicates the (RT/nF) value in Eq. 1 while the y-intercept value indicates the concentration ratio of Ferri and Ferro. The gradient value of Fig. 6(a), 0.0261, is comparatively equal to the gradient value of Fig. 6(b), 0.0232, which also shows that the results follow Nernst equation. However, the standard electrode potentials indicated by the y-intercept value are different between the two graphs. A plausible explanation for this is the different chemical substances included in the two different sample solutions.

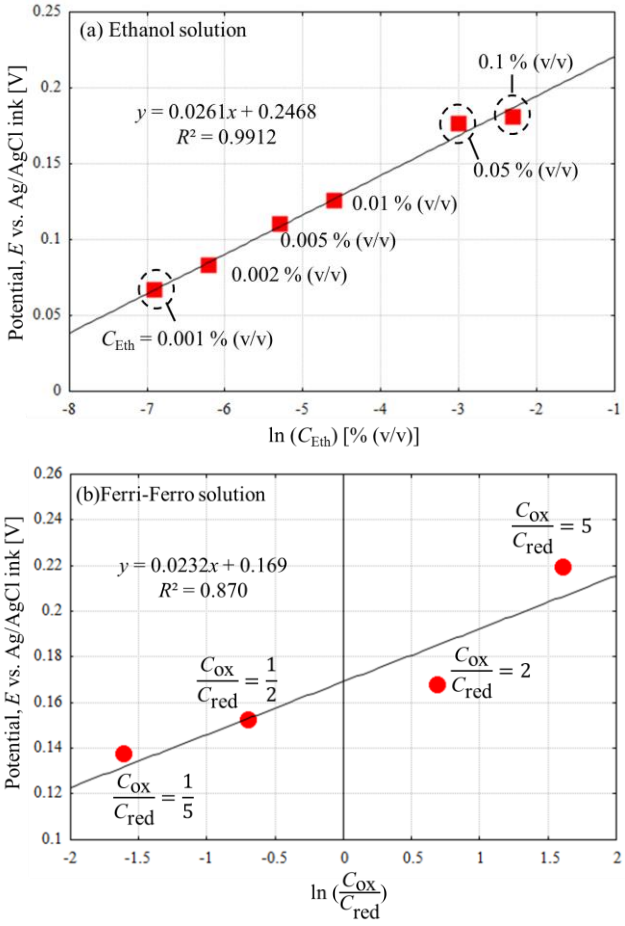

Figure 6: (a) E versus log natural of C_{Eth} ; (b) E versus log natural of concentration ratio of Ferri (C_{ox}) and Ferro (C_{red}).

4 DISCUSSIONS

As shown in Fig. 6(a), the measured electrode potential value is linearly proportional to the log natural of ethanol concentrations. Note that although one can still differentiate the potential value of ethanol concentration at as low as 0.001% (v/v), the difference becomes smaller as the ethanol concentration become larger. For example, the difference of potential values between 0.005% (v/v) and 0.01% (v/v) was approximately 15 mV whereas between 0.05% (v/v) and 0.1% (v/v) it was approximately 5mV. However, in view of alcohol concentration limit for *halal* identification, potential value that can differentiate ethanol concentration less or more than 0.1% (v/v) can be suggested. This is because although some countries have an upper limit of more than 0.1% (v/v) such as Indonesia(1.0% (v/v)), or Thailand(5.0% (v/v)), halal certifying authorities in most countries generally allow alcohol content in food and beverages of up to 0.1% (v/v) [6-7]. This shows that as our sensor can detect ethanol concentrations of up to 0.1% (v/v), it can be used in most situations. However, increasing that detection limit can serve a wider base of consumers.

Another challenge to realize this `*halal* sensor` is to add the function of detecting ingredients derived from pork. There is a considerable amount of researches and papers published about pork identification or different meat species identification [4-5, 8, 10]. Although we have used enzymatic sensor using chromatography paper in this paper for ethanol detection, the exactly same method might be difficult to apply for pork detection because of the complexity in identifying the origin of a specific meat species, especially in highly processed food [10]. Chen & Hsieh (2000) developed an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody to reliably detect pork in various commercial meat products [10]. M. van der Spiegel et al. (2012) also mentioned the use of carbon to nitrogen ratio (C/N) as a potential measurement to distinguish vegetable or animal origin of ingredients in food products [11]. Differentiation of vegetable and animal can be useful for *halal* detection in countries where ritually slaughtered meats are also hardly attainable, besides the use of pork meat and alcohol in many food products and premises. However, work is needed of a method to assimilate those measurement techniques to be used with chromatography paper. Usage of chromatography paper can eliminate the need of special techniques to bound enzymes onto electrode surfaces and thus can help realize a low cost `*halal* sensor` that is disposable and also small enough to be portable.

5 CONCLUSION

We have shown that electrode potential values are dependent on ethanol concentration. Our future research will be focused on soaking the enzymes and mediators beforehand into chromatography paper and to drop only the ethanol for measurement. Work is also required to incorporate techniques using chromatography paper for porcine derivatives detection to realize this `*halal* sensor`.

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