

## The composition of maja leaf extract (*Aegle marmelos*) and silica gel for urea biosensor

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**Abstract.** In this work, we used Indonesian-naturalized plant, Maja (*Aegle marmelos*) leaf extract instead of urease enzyme for urea sensor. Maja leaf extract, silica gel, and paraffin were mixed until homogenous, and then pasted to the tip of the silver wire, which was tightly covered by glass tube to assemble a silica gel-Maja leaf extract modified silver electrode. Cyclic voltammetry showed that cathodic and anodic current signals were observed at 0.4 V and 0.625 V, respectively. The optimum composition of silica and Maja leaf extract were also investigated. The best response was observed at the paste composition ratio of silica gel and paraffin of 3:2, with 0.05 gr total weight and 0.03 gr Maja leaf extract.

**Keywords:** silica gel, urea biosensor, Maja leaf extract, urea, *Aegle marmelos*

### 1. Introduction

Urea plays an important role in many biological processes. Human body produces 20 to 30 grams of urea each day [1]. The detection of urea is important in clinical diagnosis. Various methods to detect urease enzyme were established, including optical methods [2] and colorimetry methods [3]. However, some of these methods need a complicated instrument, relatively expensive process, less sensitive in detecting urea, and unavailability of the portable equipment. Therefore, in this research, another alternative method is used, that is electrochemical nonenzymatic biosensor using the Maja leaf extract (*Aegle marmelos*).

Coarse extract from Maja leaf (*Aegle marmelos*) have been used since ancient period to cure various illnesses like asthma, inflammation of mucous membrane, acute bronchitis, and as antidiabetic formula [4]. Elements contained in the concentrated extract of Maja leaf and ethanol are zinc, chromium, iron, selenium, magnesium, copper, and phosphor [5]. One of these elements is expected to be able to decompose urea, following the urease enzyme mechanism. Method used in this research is electrochemical technique using silica gel and Maja (*Aegle marmelos*) extract modified silver electrode. Electrochemical performance of the electrode and optimum condition for glucose biosensor also studied in this research.



## 2. Materials and methods

### 2.1 Equipments

The equipments used in this research were measuring pipette, Ohaus® analytical scale, Thermo Scientific® oven, Oakton® pH meter, hot plate, micropipette, spatula, 25 mL measuring flask, watch glass, glass bottle, *ultrasonic bath*, a set of evaporation equipment, a set of distillation equipment, silver wire (1 mm diameter and 50 mm length) and Autolab Metrohm® potentiostat type AUT84948 with three electrodes system. The three electrode cells were silica gel/Maja leaf extract modified electrode for working electrode, Ag|AgCl (KCl 3M) for reference electrode, and platinum wire for auxiliary electrode.

### 2.2 Materials

Maja leaves were taken from the tree which was cultivated in Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Indonesia. Silica, glucose, urea, and ethanol p.a wet were purchased from sigma aldrich. Paraffin was purchased from Bratachem. Silicon carbide sandpaper grade 1200, N<sub>2</sub> gas, and HVS paper were obtained from local market. Demineralized water was used for dilution in all experiment.

### 2.3 Preparation of Maja leaf extract

Maja leafs were dried for 5 days at room temperature. The 150 g dried Maja leaf was extracted in 1 L ethanol using maceration technique for 72 hours. The remaining Maja leaf was discarded. The Maja extract solution was evaporated using rotavapor at temperature of 60°C until no more solvent is evaporated (about 3 hours). The residue is Maja leaf extract. This Maja leaf extract was kept in the refrigerator to maintain the activity of the Maja leaf extract. This Maja leaf extract will be used as the electrode active material for further experiments.

### 2.4 Preparation of silver electrode

Silver wire with purity of 99.97% was used as electrode. The diameter is 1 mm with 5 cm length. The electrode was subsequently washed with 70% ethanol and aqua DM. Then it was dried at room temperature. The electrode was cleaned using ultrasonic apparatus with operational time of 20 minutes, and then dried at room temperature.

### 2.5 Preparation of silica gel /Maja leaf extract modified silver electrode

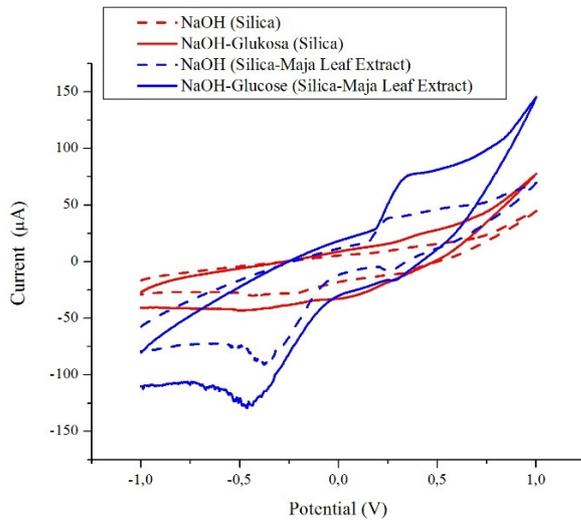
Silica gel, solid paraffin, and Maja leaf (*Aegle marmelos*) extract were mixed with certain variation of mass. The mixture was stirred inside a watch glass until a paste-like mixture is formed. Then, the paste was inserted manually into the glass of the electrode using spatula until homogenous. Finally, the surface of the electrode was polished until smooth and flat using a piece of sand paper.

### 2.6 Characterization of silica gel/Maja leaf modified silver electrode

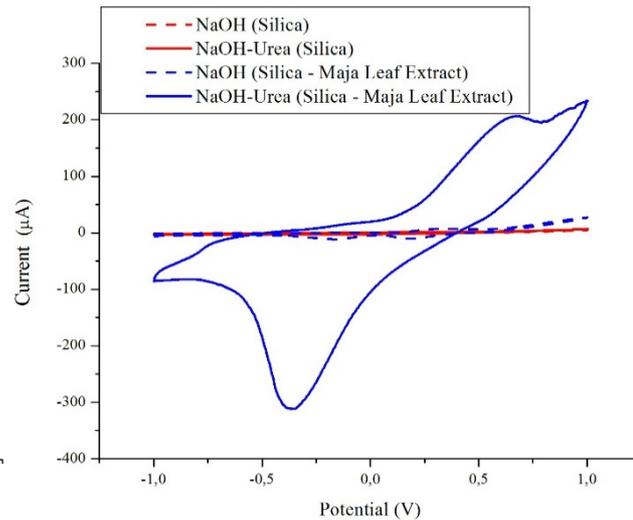
The silver electrode modified with silica gel/Maja leaf extract paste as in the section 1.5 was tested on glucose and urea solution. The test was performed by comparing the silica gel/Maja leaf modified silver electrode with silica gel modified silver electrode in both solutions. The concentrations of both solutions were 5 µM at potential of -1 V to +1 V with a scan rate of 100 mV/seconds.

## 3. Results and discussion

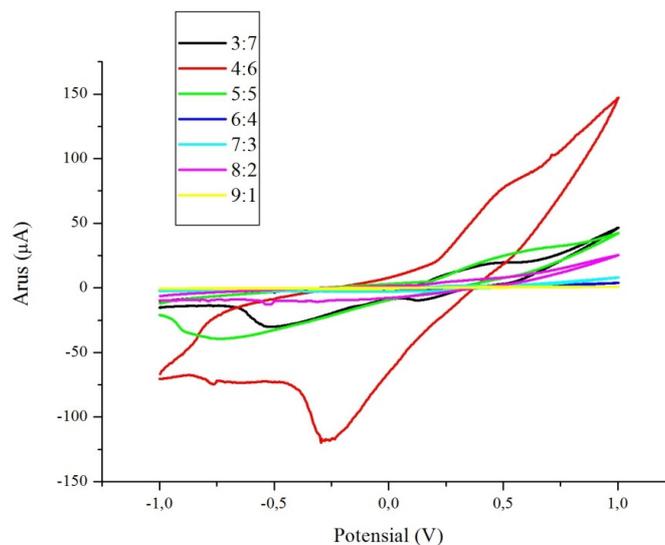
Figure 1 and figure 2 show the response of the silica and silica/Maja leaf extract modified electrodes on glucose and urea, respectively. It shows that the silica/Maja leaf extract modified electrodes give a good response for urea but not for glucose. The comparison of both voltammograms of silica and silica/Maja leaf extract modified electrode can be seen in figure 3.



**Figure 1.** Voltammogram of silica gel and silica gel/Maja leaf extract modified electrode in glucose solution at pH 13



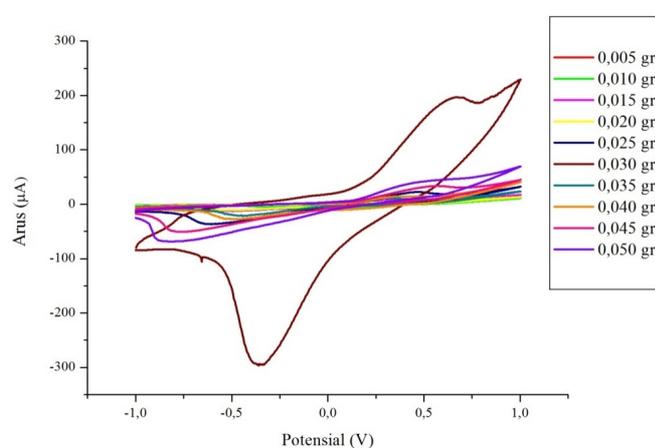
**Figure 2.** Voltammogram of silica gel and silica gel/Maja leaf extract modified electrode in urea solution at pH 13



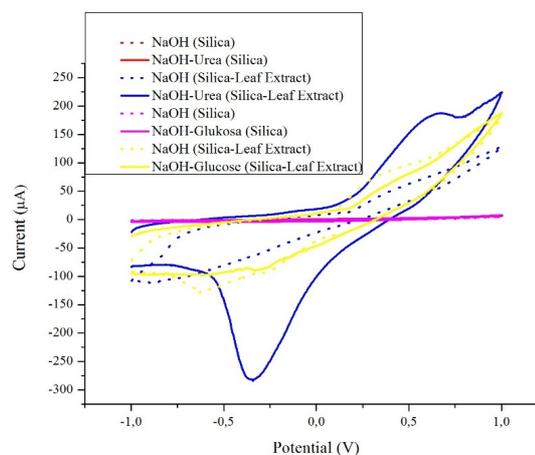
**Figure 3.** Voltammogram of silica gel and paraffin with variations of pasta composition in urea solution

Based on the voltammogram in figure 3, the best compositions are 0.03 grams silica and 0.02 grams paraffin. From the anodic and cathodic peak current, it can be seen that the peak is higher compared to other compositions. The more silica and the less paraffin composition generated a shifting in potential in the detection of urea. While, the less silica composition and the more paraffin cause the disappearance of anodic and cathodic peak current. This is because the redox process does not take place due to the composition of paraffin is blocking the process.

Based on the voltammogram results in figure 4 the best composition of Maja leaf extract (*Aegle Marmelos*) is 0.03 gram. More than 0.03 grams of the composition causes a potential shifting of the signals. This is because a high content of Maja leaf extract which consists of various compounds will



**Figure 4.** Voltammogram of silica gel and paraffin with variations of leaf extract Maja (*Aegle Marmelos*) composition in urea solution



**Figure 5.** Voltammogram of silica gel and silica gel/Maja leaf extract modified electrode in glucose and urea solution at pH 13

affect the redox process at the electrode surface. In this test, silver electrode was modified with Maja leaf extract and silica gel in paste form. Silica gel has semiconductor property, so it could assist the process of electron transfer, thus the measured electrical current is increased. This matrix change was expected to be able to increase the redox response in electrocatalytic activity of Maja leaf. The test was done by comparing the electrode that only modified with silica gel and the electrode that modified with silica gel and Maja leaf extract. Performance of both electrodes were observed by electrical current responses generated from the blank and tested solution with the same concentration of  $5 \mu\text{M}$  by cyclic voltammetry technique at the range potential of  $-1 \text{ V}$  to  $+1 \text{ V}$  and scan rate of  $100 \text{ mV/second}$ . This test was performed at pH 13 by using  $0.1 \text{ M NaOH}$  solution. The obtained voltammogram is shown in figure 5 for all measurement. The detection of glucose using silica gel modified silver electrode can be seen as purple solid line (with the blank in purple dotted line). The generated current is very low in comparison with other electrode. The detection of glucose using silica/Maja leaf extract modified silver electrode can be seen as yellow solid line (the blank is yellow dotted line). The signal current is higher than glucose detection using silica gel modified silver electrode. In the case of urea detection using silica gel modified silver electrode, it can be seen as brown solid line with the blank in brown dotted line. The current response of this electrode on urea is similar to glucose. No significant current is generated. Conversely, the detection of urea using silica/Maja leaf extract modified silver electrode gives a significantly different voltammogram. The voltammogram shows the highest anodic and cathodic peak current response. The voltammogram shows that the cathodic and anodic current signals are  $0.4 \text{ V}$  and  $0.625 \text{ V}$ , respectively. The voltammogram also shows that the electrode indicates a good selectivity for urea in the present of glucose. Meanwhile silica modified silver electrode shows no peak at anodic and cathodic current.

#### 4. Conclusions

The good result was obtained on silver electrode modified with silica gel and Maja leaf extract in the detection of urea. The voltammogram showed that this electrode was succeeded to be applied on both anodic and cathodic current, indicated by the generated higher peak compared to the blank solutions. Meanwhile, the silver electrode that modified only with silica gel did not show any higher peak at anodic and cathodic currents than its blank solution. Urea detection response showed a relatively high current without interference current from the glucose. Maja leaf extract which was used as the main active component, acted as catalyst of redox reaction against urea.

## References

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