# CHAPTER III
# EXPERIMENTAL

## 3.1 Materials and Chemicals

| | Company |
|---|---|
| • Mercaptoundecanoic acid (11MUA), 95% | Aldrich |
| • Mercaptooctanoic acid (8MOA), 95% | Aldrich |
| • Mercaptohexanoic acid (6MOA), 90% | Aldrich |
| • Mercaptopropionic acid (3MPA), 99% | Aldrich |
| • Ethanol ($C_2H_5OH$), 99.5% | Acros |
| • N-hydroxysuccinimide (NHS), 98% | Aldrich |
| • N-(3-dimethylamnopropyl)-N-ethylcarbodiimide hydrochloride (EDS), 98% | Sigma-Aldrich |
| • Potassium Chloride (KCl), >99% | Fisher Scientific |
| • Potassium Ferricyanide ($K_3Fe(CN)_6$), >99% | Fisher Scientific |
| • Potassium Ferricyanide Trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$), >99% | Fisher Scientific |
| • Phosphate Buffer pH 7, 7.2, 8 | Fisher Scientific |
| • Acetate Buffer | Ricca Chemical |
| • Distilled water | |
| • Claudin 4 Recombinant Protein | Novusbio |
| • Claudin 4 Antibody | Novusbio |
| • Gold electrode C220BT | Dropsens |

## 3.2 Equipment

| | |
|---|---|
| • Gamry reference 600 potentiostat | Gamry instruments |

## 3.3 Software

- Electrochemical Impedance Spectroscopy
- Cyclic Voltammetry
- Echem Analysis

## 3.4 Methodology

### 3.4.1 Gold Electrode Cleaning

The gold electrode was sonicated in ethanol for 10 minutes, and then rinsed with distilled water and dried under air. Then the gold electrode was cleaned using 8 cycles of cyclic voltammetry, with Gamry Framework in a 1 mL acetate buffer and 10 mL distilled water mixture as an electrolyte. The potential range was -0.6 to 0.6 V with a scan rate 100 mV/s. Then gold electrode was rinsed with ethanol and dried under air flow.

### 3.4.2 Functionalization Process

The pretreated electrode was immersed in 11-mercaptoundecanoic acid (11 MUA) of 0.1 mM in ethanol solution for 3 h in order to form a self-assembled monolayer (SAM). The alkyl chain length of an alkanethiol was changed to the 8 MOA, 6 MHA, 3 MPA in order to compare the effects of SAM's height on sensing efficiency, in particular, electron transfer rate. After 3 h, the substrate was rinsed with ethanol and distilled water in order to remove the unbounded thiols. CV and EIS were carried out in order to evaluate blocking behavior of SAM layer.

### 3.4.3 Electrochemical Characterization

A three-electrode electrochemical cell consisting of a gold electrode (0.1257 $cm^2$) as the working electrode, a silver electrode as a reference electrode, and a platinum electrode as a counter electrode was used for electrochemical characterization of SAMs in order to evaluate the SAM's blocking behavior against electron transfer on the modified surface.

*3.4.3.1 Cyclic Voltammetric Measurement (CV)*

Cyclic voltammetric measurement was carried out in a 0.05 M phosphate buffer solution (pH 7) mixed with 0.1 M potassium chloride, 5 mM potassium ferricyanide and 5 mM potassium ferricyanide trihydrate. The electric otential range was -0.6 to 0.6 V with a scan rate 100 mV/s.

*3.4.3.2 Electrochemical Impedance Spectroscopy (EIS)*

The impedance measurement was carried out using an AC signal of 5 mV amplitude at the formal potential of the redox couple at a wide frequency range of 1 Hz to 100,000 Hz. The electrolytic solution contained 0.1 M potassium chloride, 5 mM potassium ferricyanide and 5 mM potassium ferricyanide trihydrate in 0.05 M phosphate buffer at pH 7.

3.4.4 Activation Process

0.2 M N-hydroxysuccinimide (NHS) and 0.05 M N-(3-dimethylamnopropyl)-N-ethylcarbodiimide hydrochloride (EDC) were dropped onto the surface of the thiol-modified gold electrode for 10 minutes then rinsed with distilled water and dried under air. A co-addition of EDC and NHS can facilitate the formation of an active NHS ester to bind antibodies on the SAM.

3.4.5 Antibody Attachment

The thiol-modified gold electrode surface was cover with 40 µl Claudin 4 Antibodies for 1 hr. The excess antibodies were removed from the electrode surface by rinsing with PBS pH 7.2. Then the antibody-modified electrode was treated with ethanolamine solution for 10 minutes, and then rinsed by PBS and dried under air, to prevent the unreacted and non-specific sites. Then, the antibody-modified gold electrode was evaluated using EIS. The solution of 0.05 M phosphate buffer (pH 7) was used as an electrolyte mixed with 0.1 M potassium chloride, 5 mM potassium ferricyanide and 5 mM potassium ferricyanide trihydrate.

3.4.6 Antigen Detection

0.5 μg/ml antigen solution was injected over the antibody-modified gold electrode for 10 minutes then rinsed with PBS and dried under air. After that, EIS was used for detection of antigen in a 0.05 M phosphate buffer solution at pH 7 containing 0.1 M potassium chloride, 5 mM potassium ferricyanide and 5 mM potassium ferricyanide trihydrate as an electrolyte. The electrochemical biosensor was tested by changing the concentration of antigen from 0.5μg/ml to 1μg/ml and 2μg/ml.