Application of nano electrode Ag/AgCl on potentiometric sensor based on molecularly imprinted polymer (MIP) to verify caffeine

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Abstract. Caffeine of Molecularly Imprinted Polymer (MIP) has been synthesized by the cooling-heating method and to obtain sensor material to detect caffeine. The caffeine is verified through a potentiometric method based on galvanic cells involving anodes and cathodes. MIP of caffeine that has been made function as a cathode or working electrode and the modified of the Ag/AgCl reference electrode that made by reducing the AgCl membrane to nano size (44.45 nm) function as an anode. Then the modified Ag/AgCl reference electrode performance test is compared to the standard AgCl reference electrode. The result has shown the modified Ag/AgCl reference electrode produces linearity of the calibration curve in testing caffeine solutions with a concentration range of 0.1 ppm to 5.0 ppm. Based on the result of the modified Ag/AgCl reference electrode’s test range, we got the linearity coefficient value of 0.9993, with a slope of 0.9693 and an intercept of 0.1306. Generated accuracy calculation resulted in a value of 98.85% and a precision of 0.951%. The result of the lower limit of detection (LoD) was at a concentration of 0.2 ppm and the limit of quantification (LoQ) at a concentration of 0.4 ppm. Thus, it concluded that the modified Ag/AgCl reference electrode has the same or equivalent capability as a standard electrode.

1. Introduction
Caffeine (1,3,7-trimethylxanthine) is the natural chemical stimulant and psychoactive that the most widely consumed. The sources of caffeine naturally come from leaves, coffee, tea, cocoa, kola nuts, and cacao beans. The caffeine compound acts as a natural pesticide for plants [1, 2]. Caffeine is a kind of methylxanthine alkaloid, which has the function of refreshment and alleviates lethargy. However, long term or high doses consumed of caffeine inflict adverse reactions such as addiction, neuroticism, muscle twitching, and anxiety. Although, lack of evidence to prove. The recommended of the maximum doses is no more than 400 mg/day [3, 5]. However, the effects of consuming caffeine in a long time or high doses have been debated.

Nowadays the research for verifying caffeine is actively investigated to replace several methods that usually expensive, time-consuming, and complicated such as HPLC/UV detection, spectrophotometry,
chromatography, and biosensing [6, 8] The development of electrochemical methods for caffeine detection has success reported. These include using Molecularly Imprinted Polymer (MIP) [9, 10]. Molecular Imprinting (MI) is a simple technique to verify caffeine [11]. The molecular Imprinting technique is a new and developing technique in testing target molecules that can produce solid polymers through the polymerization process. Polymers produced from the MI technique are called cavity polymers or Molecularly Imprinted Polymers (MIP) by cooling heating method. The resulting cavities function to recognize molecules of the same size, structure, and physical and chemical properties as the template or test target [12, 14]. MIP has high sensitivity, selectivity, and activation [15]. The resulting MIP can function as a sensor material in many fields, such as to detect chemical and biological elements [16, 18] food, chromatography, medicines, and others [19, 20]. In recent decades the application of MIP has had the potential for development in analytical chemistry and electrochemical analysis techniques [21]. The ability of sensitivity in MIP can be improved by reducing it to a nanometer scale [22]. Progress in the nanomaterial field has opened up new opportunities for numerous applications in medicine and others [23].

MIP Caffeine was a success made and combines with electrochemical methods. One of the most widely used components in the electrochemical analysis is the Ag/AgCl electrode and function as anode [24]. While MIP caffeine function as a cathode in the electrochemical cell series. The experiment used a potentiometric method validation technique to verify caffeine. The potentiometric sensors are representative of an equilibrium electrochemical process at membranes in solid, liquid, or condensed phases. The generated voltage is an electromotive force that is dependent on the analyte activity and described by Nernst’s equation [25, 26].

In this study, a comparison test is performed using a standard Ag/AgCl reference electrode, compared with a modified Ag/AgCl reference electrode. The results show that the modified Ag/AgCl reference electrode has the ability, which can be said to be equivalent to the standard Ag/AgCl reference electrode.

2. Experiment
2.1. Preparation
This research was accomplished in several stages; the first stage in this study was the manufacture of a modified Ag/AgCl reference electrode, secondly making the MIP caffeine membrane which functions as a working electrode, and the process of making a caffeine test solution (ppm).

2.1.1. Modified electrode
The modified Ag/AgCl reference electrode was successfully made by modifying the AgCl membrane size. Through the reaction process of the AgNO3 compound with NaCl to obtain solid AgCl powder (the reaction is as in Equation 1).

\[
AgNO_3 + NaCl \rightarrow AgCl + NaNO_3
\]  
(1)

Equation 1 shows the reaction for the formation of AgCl by reacting AgNO3 and NaCl. The results of the reduction of AgCl solids are at the nanoscale (44.45 nm).

2.1.2. MIP caffeine
MIP caffeine was produced following the procedure: 0.025-gram Caffeine, Chloroform 2.01 mL, Monomer (MAA) 0.3 mL, Cross-linker (EDMA) 0.525 mL, BPO 0.07 gram by the colling-heating synthesis method.

2.1.3. Sample solutions
The sample solutions of caffeine have been made by dilution methods (in ppm). The process starts by making the standard solution in the amount of 100 ppm caffeine. Then re-dilute for each blank concentration 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, 0.5 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm.
2.1.4. Internal Solutions
Meanwhile internal sample solutions consist of chloride acid (HCl) as solvent, which is an electrolyte solution; certain substance that if we dilute it in water will produce a solution that can generate electric current and classified to strong electrolyte solution, with 0.1 N concentration that is putted into vessel in the amount of 0.2 mL and added with 0.2 mL caffeine sample solution.

2.2. Experiment
The experiment used a potentiometric method validation technique to verify caffeine. In the electrochemical potentiometric circuit, MIP Caffeine enabled to anode or working electrode and Ag/AgCl reference electrode enabled to the cathode. The measurement of the experiment used IV-Meter.

![Diagram of Experiment](image)

**Figure 1.** Schema of experiment

In the experiments, silver (Ag) is used to increase the absorption of anions because of its easy-to-release electrons and reduced their properties. Silver (Ag) is used as a reference electrode [27]. This produces a reaction,

$$\text{AgCl} + e^- \rightarrow \text{Ag}^+ + \text{Cl}^-$$

Equation 2. The Ag/AgCl Reference electrode provides Cl\(^-\) ion responses by the following electrochemical equation [21].

3. Result and discussion
The Ag/AgCl reference electrode was successfully made by modifying the size of the AgCl membrane. The calculation of the particle size of the Ag/AgCl membrane is obtained from Figure 1. And the parameters presented in Table 1.
Figure 2. AgCl membrane diffagogram

Table 1. Peak Value of AgCl Membrane XRD Result Graph

| Sample | $2\theta$ | $d$ (Å) | FWHM (deg) | FWHM (rad) | Cristal Size (nm) |
|--------|-----------|---------|------------|------------|-------------------|
| AgCl   | 32,288    | 2,7703  | 0,817      | 0,003262   | 44.45             |

The crystal size or membrane AgCl can be determined by using the Debye Scherer equation

$$\lambda \cos \theta = \frac{K \lambda}{B \cos \theta}$$

(3)

Where $d$ is the Scherer constant (0.9), $\lambda$ is the x-ray wavelength (1.54060 Å), $B$ is the Full Width at Half Maximum (FWHM) and $\theta$ is the diffraction peak. Based on the Debye Scherer equation, the crystal size is known, resulting in a crystal size of 44.45 nm, which indicates that the AgCl crystals made meet the nanoscale requirements. Based on the Debye Scherer equation, the crystal size is 44.25 nm. This shows the AgCl membrane that is on nanoscale requirements [29]. Nano AgCl is using as the main material for charging the modified Ag/AgCl reference electrode.

3.1. Test Performances

Testing of modified Ag/AgCl reference electrodes against demi water (demineralized water) and producing a standard electrode potential value of 0.22 V. While the test results of standard Ag/AgCl reference electrodes produce a value of 0.24 V.

3.2. Test Sample Experiment

The test was performed to compare the detection capability of the Modified Ag/AgCl reference electrode and the standard electrode. Test results for caffeine samples in the concentration range 0.1 to 5.0 ppm are presented in Table 2.
Table 2. Result of Experiment

| No | Caffeine concentration (ppm) | Average voltage (mV) |
|----|-------------------------------|----------------------|
|    |                               | Modified Ag/AgCl Reference Electrode | Standard Ag/AgCl Reference Electrode |
| 1  | 0.1                           | 0.133                | 0.133                |
| 2  | 0.2                           | 0.266                | 0.233                |
| 3  | 0.3                           | 0.366                | 0.366                |
| 4  | 0.4                           | 0.533                | 0.5                  |
| 5  | 0.5                           | 0.666                | 0.6                  |
| 6  | 1.0                           | 1.133                | 1.066                |
| 7  | 2.0                           | 2.066                | 1.966                |
| 8  | 3.0                           | 3.066                | 2.966                |
| 9  | 4.0                           | 3.933                | 3.866                |
| 10 | 5.0                           | 4.933                | 4.833                |

The caffeine membrane MIP testing experiment produced a chemical reaction that took place spontaneously. In the test solution and internal solution, the MIP membrane caffeine accumulates under acidic conditions (the number of H\(^+\) ions increases). H\(^+\) ions from the test solution (caffeine solution in ppm) will flow into the internal solution (cathode) through the salt bridge. The phenomenon of flowing H\(^+\) ions from the anode to the cathode (internal solution) is caused because the internal solution has a smaller volume than the test solution. Meanwhile, Cl\(^-\), which is an anion; negatively charged ions with the symbol (H\(^-\)) will flow to the anode. This occurs because in anions the number of electrons is more than protons and produces a negative net charge caused by negatively charged electrons and positively charged protons. When the caffeine concentration is low, ions from caffeine will occupy the cavity of the MIP membrane so that the measured cell potential is also low. According to the test, it is known that when the concentration of caffeine is low, ions from caffeine will occupy the MIP membrane cavity, so the potential of the measuring cell is also low. Increasing the concentration of caffeine will gradually increase the activity of the working electrode ions [14]. The results obtained by the graph of voltage linearity to caffeine concentration on a concentration test scale of 0.1 ppm - 0.5 ppm and 1.0 ppm – 5.0 ppm, shown in Figure 3.

![Figure 3. The Curve of standard and Modified Ag/AgCl Reference Electrode](image_url)
The results of the linearity graph show the good of yield of the performance. The modified electrode generates a coefficient of linearity ($R^2$) 0.9993 and in proportion to the standard electrode with the value of ($R^2$) 0.9996. This score is above the minimum requirement of the yield coefficient of correlation ($R^2$) $> 0.997$ [30]. Complete data on the test results are presented in Table 3.

**Table 3.** Comparison of Testing

| Reference Electrode | $R^2$ | $A$  | $B$    | %RSD | LoD (ppm) | LoQ (ppm) |
|---------------------|-------|------|--------|------|-----------|-----------|
| Modified Electrode  | 0.9993| 0.9693| 0.1036 | 0.951%| 0.2       | 0.2       |
| Standard Electrode  | 0.9996| 0.9505| 0.0846 | 0.958%| 0.4       | 0.4       |

According to Table 3, it is known that the modified electrode has a better for the slope ($b$) and intercept ($a$) values. Meanwhile, the value of %RSD for modified electrode and standard electrode almost equivalent. The Limit of Detection (LoD) and Limit of Quantification (LoQ) have the same values that each 0.2 ppm and 0.4 ppm. These results indicate that MIP is a potential technique for the development of the material sensor in the future [31].

4. **Conclusion**

The scope and limitation of the testing caffeine solution in the range scale 0.1 ppm until 5.0 ppm. The comparison of the modified Ag/AgCl reference electrode with the Ag/AgCl standard indicates that the sensitivity and response of the modified Ag/AgCl reference electrode are very good. The experiment yield coefficient of linearity is ($R^2$) 0.9993, the slope ($a$) 0.9693, the intercept ($b$), precision (%RSD) 0.951%, and (LoD) at a concentration of 0.2 ppm, and (LoQ) at a concentration of 0.4 ppm. The result indicates that MIP Methods potential to verify caffeine.

5. **Reference**

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