Chemical Sensor for Detection of Lead Levels in Herbal Medicine

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ABSTRACT. A new tube type Pb$^{2+}$ sensor made from two types of mixtures, namely clay-PbI$_2$ and chitosan-PbI$_2$ were prepared. An electromotive force (EMF) with Ag/AgCl as the reference electrode was used as the output signal. The highest performance of the Pb$^{2+}$ sensor from clay-PbI$_2$ was obtained at Pb$^{2+}$ solutions in HNO$_3$ and pH 3 with sensor sensitivity of 20.67 mV/decade. The highest performance of the Pb$^{2+}$ sensor from chitosan-PbI$_2$ was obtained at Pb$^{2+}$ solutions in demineralized water with sensor sensitivity of 32.49 mV / decade. Application of the two sensors on several commercial herbal samples resulted in an average recovery of 85.62% and 94.10% for sensor from chitosan-PbI$_2$ and clay-PbI$_2$, respectively.

Keywords: herbal medicine; Pb$^{2+}$ sensor; chitosan; clay.

INTRODUCTION

In accordance to the Indonesia Regulation of the Food and Drug Administration No. 32 of 2019, limit of lead metal contamination in herbal medicines is 10 ppm (BPOM RI, 2019). Lead poisoning can cause disturbances in children's intelligence, accumulation in bones, anaemia, blood, soft tissues (kidneys, bone marrow, liver, brain). Theses dangerous effects of lead contamination in the body is the reason for this study (Wai, Mar, Kosaka, & Watanabe, 2017; Rocha & Trujillo, 2019). Metal contamination has been found in many traditional medicines due to non-standard process, unsafe storage and packaging conditions that may lead to poisoning. This high metal contamination can occur due to the environmental degradation on soil, water, and air. The presence of heavy metal contamination can cause health effects for humans depends on the metal-bound body parts. These heavy metals are indigestible and can enter the human body through food, drinking water, or air. Heavy metals that are toxic in the body will cause health issue, in some cases can cause death (Vervolakos, Arseniou, & Samakouri, 2016). Therefore, it is very important to conduct the assessment of Pb levels in herbal medicines on the market.

Several studies have been carried out in the manufacture of electrochemical sensors for Pb$^{2+}$ detection (Bhat, Ijeri, & Srivastava, 2018; Kaur & Aulakh, 2016; Golc, Horvath, Huszthy and Toth 2018; Mausavi, et al., 2018; Shojaei & Zanganeh, 2019). However, most of these studies used synthetic materials which are not easy to find and expensive. A chemical sensor prototype has been developed to detect Pb$^{2+}$ based on chitosan-PbS composites, in which the sensor has a detection limit of 0.032 ppm in the linear concentration range of 0.032-0.332 ppm. (Novitasari, et al., 2016). In order to prepare an easy-to-manufacture Pb$^{2+}$ sensor and can be used repeatedly, it is necessary to modify the design and supporting materials.

Chitosan is a biopolymer that can be applied in various aspects of life because of its special chemical properties. Chitosan dissolves easily in an acidic solution of pH < 6.0, where the -NH$_2$ group will be protonated and forms -NH$_3^+$ (pK$_a$ = 6.3) (Jiménez-Gómez & Cecilia, 2020). The amine group in chitosan is advantageous, because it is easily modified by a cross-linking reaction process, one of which is glutaraldehyde as an efficient crosslinking agent (Akakuru, et. al., 2017; Mulyasuryani, Haryanto, Sulistyarti, & Rumhayati, 2018; Nair, Best & Cameron, 2020). The cross-linked chitosan forms a hydrogel that can be used as a supporting membrane in the development of Pb$^{2+}$ sensors (Novitasari, et al., 2016). Meanwhile, clay is a mineral with the main content of SiO$_2$ and Al$_2$O$_3$, in which the outer surface layer binds the Na$^+$, K$^+$ and NH$_4^+$ ions (Yahaya, Jikan, Badaruzaman, & Adamu, 2017; Garcia-Valles, Alfonso, Martinez, & Roca, 2020). The cations in the outer layer cause the clay to act as a cation exchanger, thus it has a capacitor layer and is conductive (Fatnasi, Soltebeck, & Es-Souni, 2014). Based on such character, the conductive clay can be used as a modifier for carbon paste electrodes for the development of electrochemical sensors (Gómez, Fernandez, Borrás, Mostany, & Scharifker, 2011; Akanji, Arotiba & Nkosi, 2019).

Furthermore, PbI$_2$ can be used as a recognition agent which has a solubility constant of 4.41 x 10$^{-9}$. 

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The low solubility value causes the equilibrium reaction quickly take place and can react with very small concentrations of Pb\(^{2+}\). The change in concentration in the analyte solution causes a shift in the equilibrium which resulted in a potential difference which can be detected by a potentiometer (Mulyasuryani, 2011). In addition, the concentration of the recognition agent affects the equilibrium and ion density in the membrane, which resulted in ion diffusion power and measured cell potential value. The sensitivity of the electrodes is also affected by the pH of the solution, in which the pH of the solution (in this case is the H\(^+\) ion activity) affects the Nernst value of the electrode which is stated in the following equation:

\[
E_{\text{cell}} = K - 0.0296 \log[\text{Pb}^{2+}]
\]

Note: \(E_{\text{cell}}\) is the reading signal (electromotive force, EMF) and \(K\) is the cell constant.

**EXPERIMENTAL SECTION**

**Materials**

Materials used in this research were lead(II) acetate (Sigma Aldrich), lead(III) iodide (Sigma), acetate buffer (PB) solution (pH 4; 0.01M), glutaraldehyde (Sigma Aldrich), acetic acid (Merck), chitosan and clay (local product), nitric acid (Merck), and herbal samples.

Instruments used in this research were mini tubes (5 mm OD, 50 µL), mini Ag/AgCl reference electrode IPPG junction, 4.5mm OD, 52µL (Achema, RE-1S), 0.2 mm carbon electrode (local product), digital multimeter phorex MY-60 (local product), glassy carbon electrode of 0.5 mm (Metrohm RDE.GC50) with electrode shaft, potentiostat galvanostat (Autolab PGSTAT204), pH meter Senz TI-13MO597, Yenaco YNC-OV oven 30L, shaker and general laboratory glassware.

**Membrane Preparation**

Chitosan was firstly treated by mixing 0.4 g of chitosan with 3 mL of glacial acetic acid and 9 mL of distilled water, stirred for overnight. Chitosan-PbI\(_2\) was prepared from 0.1 g of PbI\(_2\), 2 mL of the chitosan, and 0.1% glutaraldehyde (Table 1). The mixture was stirred for 6 hours at room temperature, then a small amount of chitosan-PbI\(_2\) is attached to a small hole at the end of the mini tube (Figure 1).

The clay-PbI\(_2\) membrane was made from a mixture of clay and PbI\(_2\) with the mass ratio according to Table 1. The mixture was added with a few drops of distilled water (demineralized water) so that a thin plate can be formed. The thin plate was then attached to the bottom of the tube which has a small hole (Figure 1).

**Manufacture of Pb\(^{2+}\) sensors**

The Pb\(^{2+}\) sensor was made according to the design in Figure 1. The sensor body was made of a mini glass tube, in which the bottom of the tube was filled with a membrane material, and then it was dried at 60°C for 2 hours. The glass tube was filled with 200 µL 1 M lead(II) acetate as an internal solution. The carbon rod was connected to the cable through the banana jack, then inserted into the tube that already contains the internal solution. Next, the banana jack was glued to the tube so that it was airtight.

**Table 1. Pb\(^{2+}\) sensors prepared in various membrane compositions and sample solutions**

| Sensor | Code Sensor | PbI\(_2\) (g) | Clay (g) | Chitosan (mL) | Glutaraldehyde (mL) | Solution *) |
|--------|-------------|-------------|--------|---------------|---------------------|-------------|
| I      | Clay_PbI\(_2\) 1 | 0.1         | 0.3    | -             | -                   | A, B, C     |
| II     | Clay_PbI\(_2\) 2 | 0.1         | 0.5    | -             | -                   | A, D        |
| III    | Chit_PbI\(_2\) 1 | 0.1         | -      | 2             | 0.05                | A, B, C     |
| IV     | Chit_PbI\(_2\) 2 | 0.1         | -      | 2             | 0.50                | A, D        |

*) Solution A: Pb\(^{2+}\) in demineralized water; Solution B: Pb\(^{2+}\) in HNO\(_3\) pH 4; Solution C: Pb\(^{2+}\) in acetate buffer pH 4; Solution D: Pb\(^{2+}\) in HNO\(_3\) pH 3.

![Figure 1. Design of Pb\(^{2+}\) sensor](image-url)
EMF measurement procedure

A series of Pb\(^{2+}\) solutions with a concentration range of 10\(^{-6}\) to 10\(^{-1}\) M was prepared. The Pb\(^{2+}\) sensor is connected to the negative pole (-) on the digital multimeter, and the positive pole (+) is connected to Ag / AgCl as a reference. Both were put in 5 mL of Pb\(^{2+}\) solution, the EMF value was recorded after two minutes. The linear regression equation is generated from the relationship between log [Pb\(^{2+}\)] and EMF. The sensor sensitivity is the slope of the linear regression equation. Measurement of the sample solution is carried out in the same way as above, where the sample is digested in HNO\(_3\).

RESULTS AND DISCUSSION

The Pb\(^{2+}\) sensor from Clay-Pb\(_2\)

As mentioned earlier in the research method, the Pb\(^{2+}\) sensor made of clay-Pb\(_2\) has two compositions, namely 3:1 and 5:1 in mass ratio of clay:Pb\(_2\). Clay-Pb\(_2\) is a Pb\(^{2+}\) sensor of 3:1 ratio, the sensor measures Pb\(^{2+}\) (A; B; C) solutions in a concentration range of 1 µM - 0.1 M. The results are given in Figure 2. Based on Figure 2, the quantitative relationship between the concentration and the signal is generated in the range 10\(^{-6}\) - 10\(^{-3}\) M for solutions A and B, while for solution C, it is only 10\(^{-5}\) to 10\(^{-4}\) M. The sensitivity of Clay-Pb\(_2\) in each solution is shown in Table 2. Based on Table 2, Clay-Pb\(_2\) is more sensitive at solution A. Because the sensitivity of the Clay-Pb\(_2\) sensor in solution C give the lowest value, the clay-Pb\(_2\) sensor does not used to measure solution C. Not only due to its low sensitivity, the clay-Pb\(_2\) sensor was also easily damaged.

The Clay-Pb\(_2\) sensor is a Pb\(^{2+}\) sensor with a mass ratio of 5:1 (clay: Pb\(_2\)). The sensor measures solutions A and D at concentrations of 10\(^{-6}\) - 10\(^{-3}\) M. The experimental results are shown in Figure 3. Based on Figure 3, the Clay-Pb\(_2\) is more sensitive to solution D because it is more acidic than solution A, hence the solubility equilibrium of Pb\(_2\) on the sensor will be easier. As presented in Table 2, the highest sensitivity is generated by the Clay-Pb\(_2\) sensor, with a sensitivity of 20.67 mV / decade to solution D, in which in theory, the divalent sensor will have a sensitivity of 29.89 mV / decade at 25°C. As of the Clay-Pb\(_2\) sensitivity to solution D, it has value that closer to theoretical. Although the Clay-Pb\(_2\) sensor was used in a high acidity solution, the sensor is not easily damaged (more robust and does not leak) and produces a more stable signal.

The Pb\(^{2+}\) sensor from Chit-Pb\(_2\)

The Chit-Pb\(_2\) and Chit-Pb\(_2\) sensors have a difference in the volume of glutaraldehyde added as crosslinking agent. On the Chit-Pb\(_2\) sensor, the glutaraldehyde 0.1% volume was 0.05 mL. The sensor was used to measure A, B, and C solutions with Pb\(^{2+}\) concentrations of 1 µM - 0.1 M. The experimental results are shown in Figure 4. Sensitivity and concentration range are shown in Table 3. Based on Figure 4, the quantitative concentration range is the same as the Clay-Pb\(_2\) 1 sensor, however for Pb\(^{2+}\) solution in demineralized water, the Chit-Pb\(_2\) sensor is considerably insensitive, this is very different to that of the Clay-Pb\(_2\) sensor. Thus, adjusting the pH of the solution greatly affects the performance of the Clay-Pb\(_2\) sensor.

The Pb\(^{2+}\) sensor from Chit-Pb\(_2\) is a sensor with a glutaraldehyde 0.1% volume of 0.5 mL. The sensor was used to measure the Pb\(^{2+}\) in the A and D solutions at concentrations of 1 µM - 0.1 M.
Table 2. Sensitivity of the Clay_PbI₂₁ and Clay_PbI₂₂ sensors. The sensors measure Pb²⁺ solution in demineralized water (A); in HNO₃ pH 4 (B); in acetate buffer pH 4 (C), and in HNO₃ pH 3 (D).

| Solution     | Clay_PbI₂₁ Concentration range (M) | Sensitivity (mV/decade) | Clay_PbI₂₂ Concentration range (M) | Sensitivity (mV/decade) |
|--------------|------------------------------------|-------------------------|------------------------------------|-------------------------|
| A            | 10⁻⁶ - 10⁻³                         | 7.04                    | 10⁻⁶ - 10⁻³                         | 7.12                    |
| B            | 10⁻⁶ - 10⁻³                         | 6.30                    | -                                  | -                       |
| C            | 10⁻⁵ - 10⁻⁴                         | 5.30                    | -                                  | -                       |
| D            | -                                  | -                       | 10⁻⁶ - 10⁻³                         | 20.67                   |

Figure 3. Correlation curve between log [Pb²⁺] versus EMF, Clay_PbI₂₂ sensor measures Pb²⁺ solution in demineralized water (A); and in HNO₃ pH 3 (D).

Figure 4. Correlation curve between log [Pb²⁺] versus EMF, Chit_PbI₂₁ sensor measures Pb²⁺ solution in demineralized water (A); in HNO₃ pH 4 (B); and in acetate buffer pH 4 (C).
The results of the experiment are presented in Figure 5. The highest Chit_Pbl2_2 sensitivity was produced in solution A (Table 3), which probably due to more glutaraldehyde was added, so that the level chitosan swelling is greater, thus attracts more water. This was then accelerating the equilibrium of Pb\(^{2+}\) ions in solution with PbI\(_2\) on the sensor. A higher concentration of HNO\(_3\) in the solution will accelerate the equilibrium, but on the other hand, a large amount of NO\(_3^-\) can interfere the sensor. Based on the results of the four Pb\(^{2+}\) sensors, to determine the level of Pb\(^{2+}\) dissolved in water, Chit_Pbl2_2 sensor was the best option. However, when applied to solid samples, it requires preparation, which commonly uses HNO\(_3\) as a solvent.

Application of Pb\(^{2+}\) sensor in herbal medicine samples

The herbal sample consisted of two solid samples and one liquid sample. All samples were dissolved in 0.001 M nitric acid (pH = 3). Therefore, the standard curve was made with a solution of Pb\(^{2+}\) in HNO\(_3\) pH 3 in the range of 5 - 25 ppm. The sensors used to determine Pb\(^{2+}\) in herbal sample are Clay_Pbl2_2 and Chit_Pbl2_2. The linear regression equation for the Clay_Pbl2_2 sensor is \(E_{\text{cell}} = 275.84 + 20.281 \log [\text{Pb}^{2+}]\), whereas for the Chit_Pbl2_2 sensor is \(E_{\text{cell}} = 174.52 + 16.51 \log [\text{Pb}^{2+}]\). Recovery was determined by adding 20 ppm of standard Pb\(^{2+}\) solution to the sample solution.

Based on the linear regression equation, with the concentration range of 5 - 25 ppm Pb\(^{2+}\) in HNO\(_3\) pH 3, the sensitivity of the Chit_Pbl2_2 sensor is 16.51 mV / decade, whereas the sensitivity of Clay_Pbl2_2 is 20.67 mV / decade. The average recovery of 20 ppm Pb\(^{2+}\) added to the herbal sample is 85.62% for the Chit_Pbl2_2 sensor and 94.10% for Clay_Pbl2_2. As observed in Table 3, the recovery and accuracy of Clay_Pbl2_2 sensor is higher than that of Chit_Pbl2_2 sensor. In addition, sample measurements were also carried out using differential pulse voltammetry (DPV) method (Table 5). The Pb\(^{2+}\) levels were considerably identical to the results of the potentiometric determination, except for the JD sample.

### Table 3. Sensitivity of the Chit_Pbl2_1 and Chit_Pbl2_2 sensors in a Pb\(^{2+}\) solution in demineralized water without pH adjustment (A); in HNO\(_3\) at pH 4 (B); in acetate buffer pH 4 (C) and in HNO\(_3\) at pH 3 (D)

| Solution   | Chit_Pbl2_1 | Chit_Pbl2_2 |
|------------|-------------|-------------|
|            | Concentration range (M) | Sensitivity (mV/decade) | Concentration range (M) | Sensitivity (mV/decade) |
| A          | 10\(^{-6}\) - 10\(^{-3}\) | 3.24 | 10\(^{-6}\) - 10\(^{-3}\) | 3.24 |
| B          | 10\(^{-6}\) - 10\(^{-3}\) | 7.49 | - | - |
| C          | 10\(^{-6}\) - 10\(^{-4}\) | 7.03 | - | - |
| D          | - | - | 10\(^{-6}\) - 10\(^{-3}\) | 19.25 |
Table 4. Recovery of Pb$^{2+}$ determination by the Clay_Pbl$_2$ (II) and Chit_Pbl$_2$ (IV) sensors, with addition standard of 20 ppm Pb$^{2+}$ solution.

| Sample code | Concentration of Pb$^{2+}$ in sample (ppm) | Concentration of Pb$^{2+}$ in sample + standard (ppm) | Recovery (%) |
|-------------|------------------------------------------|--------------------------------------------------|--------------|
| JD          | 5.4                                      | 24.2                                             | 94.05 ± 1.75 | 85.53 ± 6.07 |
| MKD         | 14.7                                     | 33.3                                             | 92.94 ± 1.21 | 84.98 ± 2.79 |
| PM          | 15.4                                     | 34.4                                             | 95.30 ± 0.27 | 86.34 ± 1.27 |

Table 5. Levels of Pb$^{2+}$ (mg / pack) in the herbal sample determined by the Clay_Pbl$_2$ (sensor II) and the Chit_Pbl$_2$ (sensor IV), compared with the results of the DPV determination.

| Sample code | Volume packing | Concentration of Pb$^{2+}$ (mg/pack) | Sensor II | Sensor IV | DPV |
|-------------|----------------|-------------------------------------|-----------|-----------|-----|
| JD          | 600 mL         |                                     | 8.1       | 6.8       | 7.9 |
| MKD         | 7 g            |                                     | 1.0       | 1.0       | 1.0 |
| PM          | 9 g            |                                     | 1.4       | 1.4       | 1.4 |

CONCLUSIONS

The electrolyte condition of the sample solution affects the performance of both Pb$^{2+}$ sensor from Clay_Pbl$_2$ and Chit_Pbl$_2$ sensors. The highest performance was resulted by the Chit_Pbl$_2$ sensor for Pb$^{2+}$ solution in demineralized water, which is 32.49 mV / decade. Application of the Chit_Pbl$_2$ sensor to herbal medicinal samples dissolved in HNO$_3$ pH 3 had a sensitivity of 16.51 mV / decade in the concentration range of 5-25 ppm, with an average recovery of 85.62%. Meanwhile, the application of the Clay_Pbl$_2$ sensor produces a sensitivity of 20.67 mV/decade with an average recovery of 94.10%.

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