Sequential Injection Analysis for Determination of Lead(II) using Extract of *Caesalpinia Pulcherrima* as a Natural Reagent

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Abstract. The lead(II)-contaminated aquatic environment may endanger humans and living organism. The maximum tolerable limit of lead(II) in river water is 1 mg/L. Therefore, the development of a sensitive and green analytical method to determine lead(II) is required. In this work, we developed a Sequential Injection Analysis (SIA) system equipped with a valve-mixing and a flow-based UV detector for highly sensitive detection of lead(II). In order to deal with the green chemistry system, the environmentally friendly natural reagent is prepared from the extract of *Caesalpinia pulcherrima* instead of a commercial synthetic reagent. The extract of *Caesalpinia pulcherrima* contains several flavonoid compounds which can bind lead(II) ion to form an orange complex compound at a wavelength of 672.4 nm. Various parameters affecting the sensitivity of the proposed method, which includes pH, reaction time, the flow rate to the detector, and stability of *Caesalpinia pulcherrima* extract, were investigated in detail. The optimum conditions for a complex formation of Pb-*Caesalpinia pulcherrima* extract were found at pH 4 along with a reaction time of 10s, and the product flow rate to the detector was 200 µL/s. Under the optimum conditions, a linear calibration graph can be constructed in the lead(II) concentration range of 0-800 ppb with a detection limit of 8.9 ppb. The proposed method is successfully applied to determine lead(II) in industrial wastewater samples with satisfied results.

Keywords: Lead, sequential injection analysis, *Caesalpinia pulcherrima*, extraction, natural reagent

1. Introduction

An industrial process produces greatly various waste depending on type and size of an industry, supervision of industrial processes, degree of water use, and degree of treating existing wastewater. In addition to solid waste (garbage), liquid waste is also pollution material which can directly and indirectly enter aquatic system. In industrial waste, very dangerous pollutants often exist such as heavy metals [1]. Beside polluting water, heavy metals will also remain at the bottom of water and have residence time up to thousands of years. They are also concentrated into the body of living organisms by bioaccumulation and biomagnification through several pathways, namely respiratory tract, food channels, and skin.

Lead/Pb (II) is one of the toxic and dangerous heavy metals, which is found as a pollutant and tend to disturb aquatic ecosystems [1]. The presence of lead (II) which enters the ecosystem can be a pollutant source because of its high toxicity [2]. Acute lead poisoning in humans causes severe damage to kidneys, liver, brain, reproductive system, and central nervous system, to death [3]. Lead which enters waters can come from chemical industry waste, printing industry, industries which produce metal and paint.
According to Indonesian government regulation no. 82 of 2001 concerning the management of water quality and control of water pollution, the threshold of lead (II) level in waters for agricultural, industrial and hydropower activities is 1 mg / L [4].

Determining lead (II) levels has been carried out by atomic absorption spectrophotometry (AAS) and Inductively coupled plasma optical emission spectrometry (ICP-OES) [2,5] and visible spectrophotometry methods [6,7]. However, these methods require a long time analysis, have low sensitivity, use large amounts of reagents, and produce a lot of waste. Another method of determining lead (II) is by an automatic online sequential injection analysis (SIA) [8] and flow injection analysis (FIA) [9]. These methods are very convenience due to easy combination with many kinds of detection system. However, the use of synthetic chemical reagents in the aforementioned methods can cause environmental problems. Therefore, it is necessary to develop another method which has high level of sensitivity, needs not a lot of reagents, can be used for quick and automatic analysis, and is environmentally friendly.

Sequential Injection Analysis (SIA) is a computer-controlled system consisting of a syringe pump, holding coil, multiposition valve, and detector. Its main characteristic is the sequential application of a little solution volume which leads to the holding coil and then the reversal of flow for the formation of mixing zone between sample and reagents. The reaction product is then sent to a detector for analysis. The detector is equipped with a flow cell. An advantage of using SIA is that it produces only a small amount of waste due to the decreased use of reagent solutions and samples, complete automation and control using software, simple adaptation to different methods without significant changes [10].

The Green Chemistry concept develops analytical methods in terms of reducing hazards, avoiding solvents which damage the environment, minimizing substances or using reagents, and reducing waste. Then, the uses of natural reagents or natural compounds from plants are as alternative reagents, rather than using toxic synthetic reagents so that the absence of toxic chemical emissions is one of the alternative procedures to achieve environmentally friendly methods of analysis [11,12].

This study applied the concept of Green Chemistry, and we tried several extracts of natural sources i.e. carrots, leaves and flowers of peacock plants (Caesalpinia pulcherrima) with and without drying process through maceration and boiling procedures using ethanol and water as solvents. However, from several reagents which have been prepared, those which can form complexes between ligand compounds of plant extract with lead (II) indicated by a change in color were only extracts from flowers of the peacock plant (Caesalpinia pulcherrima). Therefore, the developed SIA system for determination of lead (II) in this work employed the peacock flower extract as the natural reagent since it is easily available, inexpensive, harmless or non-toxic, environmentally friendly, and can form stable complex with metal ion.

2. Experimental

2.1. Instrumentations and materials

The main instrument used in this study was a Laboratory-made SIA system consisting of a syringe pump (SP; Hamilton, Reno, Nevada USA) with a volume of 2.5 mL, an eight-port selection valve (SL; Hamilton, Reno, Nevada, USA), UV-Vis 1601 / Shimadzu spectrophotometer equipped with a flow cell, the holding coil (PTFE tubing 1.8 mm i.d with the volume of 2.5 mL), a capillary tube (PTFE, 0.75 mm i.d) for flowing all solution. All systems were computer-controlled using MPV LITE 2.0 software prepared by Visual Basic Program. Other apparatuses include a pH meter (Horiba), a non-metallic knife for cutting peacock flowers, and some equipment commonly used for chemical analysis.

The chemicals used in this study were pro analysis (pa) grades purchased from Merck (Indonesia) which include \( \text{Pb(NO}_3\text{)}_2 \), concentrated \( \text{HNO}_3 \) (70%), ethanol (96%). The stock solution of 100 mg/L Lead(II) was prepared by dissolving 0.0159 g of \( \text{Pb(NO}_3\text{)}_2 \) in 100 mL of distilled water. For preparation of a calibration curve, this stock solution was diluted with certain volume of distilled water to obtain the desired concentration. Wastewater samples were taken from Tambak Oso river water, PT Intan.
Ustrix's liquid waste, and Brantas river water. Peacock flowers were harvested in Malang Area (East Java, Indonesia).

2.2. Preparation of the natural reagent from peacock flowers

The natural reagent was made by extracting active compounds (ligands) from peacock flowers through maceration method. For this purpose, 40 g of fresh peacock flowers, which had been cut into small pieces by a non-metallic knife, was weighed and then immersed in 100 mL of 96% ethanol in a dark closed container for 24 h. After filtered, the resulted extract solution was stored in a dark glass bottle. The stability test of reagents from peacock flower extract was carried out by measuring the readable absorbance with UV-Visible Spectrophotometry and observing the color changes which occurred in the solution every day. Measurements were carried out for 14 days.

2.3. Determining the maximum wavelength of peacock flower extract and lead(II)-natural reagent complex

The maximum wavelength of a peacock flower extract was carried out by measurement of its absorbance spectrophotometrically in the range of 500-800 nm. The highest absorbance peak at a particular wavelength showed the maximum wavelength of a peacock flower extract as the natural reagent. Determining wavelength of the lead(II)-peacock flower extract was carried out by a similar method. In this work, 1 ppm lead(II) solution was reacted with a peacock flower extract and its absorbance was measured in the range of 500-800 nm (visible area).

2.4. Preparation and measurement of wastewater samples by sequential injection analysis (SIA)

![SIA Manifold for lead (II) determination. SP: syringe pump, SV: syringe valve, HC: holding coil, MT: mixing tip, SL: selection valve, distilled water (1), waste (2), standard lead (II)/samples (3), peacock flower extract reagent (4), and HNO$_3$ 0.1 M (5).](image)

**Figure 1.** SIA Manifold for lead (II) determination. SP: syringe pump, SV: syringe valve, HC: holding coil, MT: mixing tip, SL: selection valve, distilled water (1), waste (2), standard lead (II)/samples (3), peacock flower extract reagent (4), and HNO$_3$ 0.1 M (5).

2.4.1. Washing all lines and detectors.

Before performing measurements, the entire line on the SIA device must be washed to remove such impurities or other residual solutions which were still present in all PTFE tubing lines as the following procedures: the syringe valve (SV) was set at the in-position, and then the syringe pump (SP) was set to aspirate 2500 μL of distilled water with a flow rate of 100 μL/s. After set the SV to the out-position, the SP would dispense distilled water to the holding coil. Furthermore, the distilled water in the holding coil
(HC) was flowed to all ports / lines to be used (i.e. port 1 to 8), each of which was 300 μL with a flow rate of 50 μL/s. In the port 1 (Mixing Tip/MT), distilled water is flowed and taken back 1000 μL with a flow rate 100 μL/s. The excess of distilled water was flowed to the waste in the port 7; whereas, in the port 2 it had been connected to the detector so that the line at this port as well as the detector could be washed.

2.4.2. Measurement of lead (II) concentration in wastewater samples.
Determination of lead (II) in wastewater samples was carried out as the following procedures: the SV was in the out-position, and the SP was set to aspirate 50 μL of lead (II) solution/ sample solution via the port 3 with a flow rate of 50 μL/s towards the HC. Then, the reagent solution of the peacock flower extract (150 μL) was aspirated into the HC via the port 4 at a flow rate of 50 μL/s. Both solutions were dispensed to the mixing tip (port 1) of the selection valve (SL) with a speed of 50 μL/s for 10 s to allow the homogeneous reaction between lead (II) and the reagent. Then, the reaction product was returned to the HC. The SV was set at in-position and the SP was set to aspirate 1800 μL of distilled water at a speed of 150 μL/s. After the SV was reset to the out-position, all solutions in the HC were dispensed towards the detector via port 2 with a flow rate of 100 μL/s. The absorbance of the reaction product was then detected at a wavelength of 672.4 nm and all data were recorded in the computer.

2.5. Preparation of wastewater samples
The first sample was taken from Tambak Oso River water, located on Jalan Rungkut Industri Raya, Rungkut Menanggal, Gunung Anyar, Surabaya. It is located in the industrial area of PT Surabaya Industri Estate Rungkut (SIER) with a sampling coordinate point of 7°19'48.5 "S 112°45'27.8" E. The second sample came from wastewater from PT. Ustrix Diamond. PT Intan Ustrix is a manufacturing company engaged in the manufacturing of cardboard boxes located on Jalan Raya Roomo Meduran, Roomo, Gresik Regency. The third sample was obtained from the Brantas river water located at Jalan Brawijaya, Kauman, Klojen, Malang City with a sampling coordinate point of 7°58'43, 2 "S 112°37'52, 4" E. The sampling time was carried out in April 2018. After sampled, all wastewater samples were treated by adding 1 mL of concentrated HNO₃ for every 100 mL sample, and filtered by using a 0.45 μL filter paper which was then poured in a clean polyethylene bottle. All samples were stored in the refrigerator prior to analysis.

3. Result and Discussion
The principle of determining lead (II) in this work is the formation of an orange complex of lead (II)-peacock flower extract (in ethanol) which is detected at 672.4 nm. In this study, we performed an optimization of chemical parameter (pH of the complex) and physical parameters (reaction time and flow rate of reaction product towards the detector) to obtain highest sensitivity of the proposed method. In addition, the stability of peacock flower extract as a natural complexing reagent was also studied.

3.1. Stability of Peacock Flower Extract as a Natural Reagent
The stability test of peacock flower extract was conducted to find out how long the peacock flower extract can be used as a natural reagent. Since mostly natural reagents are less stable or resistant to changes in environmental conditions, volatile, and easily oxidized, it is necessary to test the stability of the peacock flower extract by measuring the readable absorbance by using Visible Spectrophotometry at a wavelength of 663.2 nm and to observe discoloration which occurs in the solution every day for 14 days. To maintain the Peacock Flower extract in a long time, the given treatment is storing the extract in a dark bottle to avoid light; thus, it can reduce the rate of degradation reaction. The extract is stored in a tightly closed container so that it is not quickly oxidized by the presence of oxygen. The test results on the stability of the peacock flower extract as a natural reagent were shown in Figure 2.
Figure 2. Relationship between observation time (days) to the absorbance of the peacock flower extract at a wavelength of 663.2 nm.

The stability test results of Peacock flower reagents as the complexing agent for 14 days showed that on day 1 to 10 the absorbance of the peacock flower extract tended to be stable. Whereas, on the 11th to 14th day there was a decrease in absorbance because it was possible to decompose the active compounds contained in the reagent. While observing colors every day, there was no significant color change from the original color (clear brown). This showed that the peacock flower extract reagent is stable for 10 days.

3.2. Effect of pH

The optimization of pH was carried out at a variation of pH 2-4.5 by adding 0.1 M HNO₃ to certain volume in the SIA system via port 5 (see Figure 1) after Lead(II) solution and reagent were reacted as shown in Figure 3. This procedure is very important to determine the most stable complex formation between lead (II) and the Peacock flower.

Figure 3. Effect of pH on the complex formation of lead (II)-peacock flower extract. Operational conditions: Lead(II): 1 ppm with volume of 60 µL, reagent/ peacock flower extract volume: 60 µL, reaction time: 10 s, and flow rate: 200 µL/s.

As given in Figure 3, it showed that there was an increase in absorbance from pH 2 to 4, and then a decrease at pH 4.5. Since at pH 2-3.5 the complex formation of lead (II)-peacock flower extract was not occurred completely and at pH 4.5 lead (II) solution was estimated to begin hydrolyzed into Pb(OH)⁺,
pH 4, which showed the highest absorbance, was selected as the optimum condition and used for further experiment.

3.3. Effect of reaction time
The optimum reaction time was studied from 5 to 20 s after mixing lead (II) solution with the peacock flower extract in the mixing tip (MT). This study is required to find out how long it took to react between lead (II) solution and reagent the peacock flower extract to form complex compounds completely and to form a stable complex.

![Figure 4](image.png)

**Figure 4.** Effect of reaction time on the absorbance of lead (II)-peacock flowers extract complex. Operational conditions are similar as in Figure 3 except for reaction times.

As shown in Figure 4, it was found that there was an increase in absorbance from 5 s to 10 s. This result indicated that the longer the contact time between the sample and the reagent was, the more the formation of complex would be. However, the absorbances tend to stable/constant from 10 s to 15 s, indicating the formation of complex had been stable. Therefore, the reaction time of 10 s was selected for further experiment as the optimized reaction time.

3.4. Effect of flow rate

![Figure 5](image.png)

**Figure 5.** Effect of flow rate on the absorbance of lead (II)-peacock flowers extract complex. Operational conditions are similar as in Figure 3 except for the flow rates.

Optimization of the flow rate of the reaction product towards the detector was carried out at various flow rates of 100, 125, 150, 175, and 200 µL/s as given in Figure 5. Flow rate optimization is necessary to determine the detector's ability to detect absorption of solutions. At certain flow rates sometimes the
detector was unable to read the overall absorption results due to certain factors, thus affecting the measurement results. The flow rate also affected the speed analysis time and the shape of SIA peaks produced.

Figure 6 Peak profile of lead (II)-peacock flowers extract complex in determining the optimum flow rate. Operational conditions are similar as in Figure 3 except for the flow rates.

Figure 5 showed the faster the flow rate was, the more the absorbance would increase. It was because at faster flow rate, the mixture of the solution had not been dispersed with distilled water. Thus, there was no effect from the carrier which could cause a decrease in absorbance. On the other hand, when slower flow rate occurred, there would be a large dispersion between a mixture of the peacock flower extract with the carrier solution (distilled water) which caused a decrease in the absorbance. The resulted peak profiles as displayed by the detector were presented in Figure 6.

As shown in Figure 6, the slower the flow rate was, the wider the formed peak became and tailings would be formed. It happened since at slow flow rate, the flow of solution was also slow. Therefore, it took longer to reach the detector. In addition, the dispersion of solutions with carrier solution (water) could also cause peak widening. Conversely, when the flow rate was faster, the peak formed would be more pointed and not be widened as the time needed for the solution to reach the detector was rapid and there was no dispersion of solution with the carrier solution. Therefore, the peak produced was tapered and not widened. When more than 200 µL/s flow rate was carried out, a very large back pressure would occur and caused the PTFE capillary tubing connection which linked the selection valve to the detector to be disconnected. Thus, the optimum flow rate used was 200 µL/s since at this flow rate the highest absorbance value was obtained, the produced peak was pointed, and no tailings were formed.

| Parameters      | Range studied | Optimum Condition |
|-----------------|---------------|-------------------|
| pH              | 2 – 4.5       | 4                 |
| Reaction time   | 5 – 20 s      | 20 s              |
| Flow rate       | 100 - 200 µL/s| 200 µL/s          |
3.5. Calibration Curve
The calibration curve was constructed from 0 to 0.8 ppm of lead (II) standard solution which was reacted with the peacock flower extract under optimum condition shown in Table 1. As presented in Figure 7, excellent linearity of $R^2 = 0.9959$ could be obtained. From the standard deviation of measurement of 10 blank solution, the detection limit (LOD) was found to be 8.96 ppb, showing excellent sensitivity of the proposed SIA method for determination of lead (II) using the peacock flower extract as the natural reagent.

![Figure 7](image)

**Figure 7.** The calibration curve for determination of lead (II) using the peacock flower extract as the natural reagent. Operational conditions are similar as in Figure 3 except for lead (II) concentrations

3.6. Application to Lead (II) determination in Wastewater Samples
In this study, the proposed SIA system by employing the natural reagent was applied to the determination of lead (II) in wastewater samples originating from Tambak Oso river water (SEJ industrial area, Surabaya) (sample A), PT. Diamond Ustrix (sample B) and Brantas river water (Splendid animal market area, Malang) (sample C). The analytical results were shown in Table 2.

| Samples     | Pb(II) added/ ppm | Pb(II) found/ ppm | Recovery (%) |
|-------------|-------------------|-------------------|--------------|
| Sample A    | -                 | 0.1772±0.0158*    | -            |
|             | 0.05              | 0.2116            | 91           |
| Sample B    | -                 | 0.0832±0.0075*    | -            |
|             | 0.05              | 0.1246            | 90           |
| Sample C    | -                 | 0.7342±0.0485*    | -            |
|             | 0.05              | 0.7696            | 98           |

* Standard deviation of 3 measurements

As shown in Table 2, the concentration levels of lead (II) in river water (samples A and C) were higher than those contained in industrial wastewater. It was possible since in river water there were wastes from various industries intermingled with each other. Each industrial waste contained a number of lead(II) in varying levels. In addition to industrial waste, it was possible that the river had also been
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polluted by domestic waste or environmental influences which had affected the heavy metal content of lead (II) in it. In accordance with Indonesian government regulations no. 82 of 2001 concerning management of water quality and control of water pollution, the threshold of lead (II) concentration in wastewater equals to 1 ppm. Therefore, the three samples are still in acceptable level.

Validation tests were conducted to show that the proposed SIA method could provide accurate and reliable results. Based on Table 2 it revealed that the analysis results of wastewater samples showed high accuracy since the recovery was close to 100%. Additionally, our proposed SIA method using the natural reagent are showing several advantages such as more environmental friendly, uses a small amount of reagent (60 μL), has high sensitivity, and fast analysis time (50 samples/h).

4. Conclusions
Peacock flower extract can be used as the natural complexing reagent which has good stability for 10 days. The SIA method using the natural reagents can be applied to the determination of lead(II) in wastewater with accurate results.

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