Cross linked Sago Starch Phosphate as a Bioadsorbent for the Heavy Metal Pb(II)

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Abstract. A cross linked sago starch phosphate (SgP) was successfully synthesized from sago starch (Sg) and a mixture of Na₂HPO₄-NaH₂PO₄ in an acidic solution. The synthesized SgP was then used as a bioadsorbent of Pb(II) in an in vitro simulation of the human digestion system. The optimized synthesis condition was reached at an acidic pH 6, at a temperature of 30° C, with a reaction time of 10 minutes, and a mixing rate of 100 rpm. Analysis of Sg and SgP using XRD did not show major differences. An absorption band with a wave number of 2345 cm⁻¹ was observed on the IR spectra of SgP and is a characteristic of a phosphate diester bonding (RO-PO₃⁻R'), indicating a successful cross-linking process. It was found that the adsorption of Pb(II) by SgP follows the second order kinetics and Langmuir equation with adsorption capacity of 23.67 mg/g. It was also found that SgP is resistant to digestive enzymes and changes in pH and temperature, making it suitable for use as a bioadsorbent of Pb(II) under varied conditions.

Keywords. bioadsorbent, cross linked sago starch phosphate, heavy metal Pb(II), sago starch

1. Introduction

Starch is a natural polymer and a highly available biomass. Starch is easily modified to improve its properties and uses [1]. One modification that has been done is through the formation of cross-linked phosphate to give a starch with better properties, for example, to help maintain the granule integrity and to make a starch paste more resistant toward retrogradation, high temperature, and low pH. Cross-linked starch phosphate (CSP) can be synthesized from starch with amylase content above 25% [2]. Sago starch (Sg) is known to have an amylase content between 24 and 31% and is highly available in Indonesia with annual production at 12 to 24 million tons [3]. Therefore, Sg is a good candidate to be modified into CSP.

For Industry, CSP is usually synthesized under basic conditions, with POCl₃ or a salt mixture of trisodiumtrimetaphosphate and trisodiumtripolyphosphate as the source of the phosphate in basic solution [4]. POCl₃ is toxic and can pollute the environment. While the phosphate salt mixture produces of very low degree of substitution value. Synthesis of CSP under acidic conditions has been reported with reaction condition of pH 6.5 using mixture of Na₂HPO₄ and NaH₂PO₄ as the source of phosphate [5]. The presence of phosphate groups makes it possible for it to be used to absorb Hg(II) ion in a simulated digestive system.
Here we report an optimized condition to synthesize cross-linked sago starch phosphate (SgP) under acidic conditions. The obtained SgP was then used to absorb Pb(II) ions in a simulated digestive system. Like with Hg(II) ions, phosphate has a high affinity to interact with Pb(II) ions owing to their soft acid base property. Pb is ranked as the seventh most dangerous element for human health as it can be accumulated and cause damage to the nerve system, and obstruct the formation of red blood cells [6]. People can be exposed to Pb through contact with lead containing material such as batteries, paint, and fuel. Before entering and causing damage to the metabolic system, lead should be removed by all means. One way to do this is by the absorption process using a biocompatible absorbent [7]. SgP is a biocompatible material and potentially could be used as bioadsorbent due to its ligand effect, negative partial charge, coordination number, and geometry of cross linked phosphate groups. SgP has an advantage as an adsorbent as it is resistant to physical, chemical, and biological interference [8].

2. Materials

Sago starch, brand Alini, was obtained from a local grocery store and it was used without further treatment. Other materials were used as obtained without further purification such as HCl (Merck), NaOH (Merck), NaH2PO4.H2O (Fisher), Na2HPO4.2H2O (Fisher), (NH4)6Mo7O24 (AnalaR), NH4VO3 (Merck), KH2PO4 (Fisher), Na2CO3.10H2O (Merck), HNO3 (Merck), Pb(NO3)2 (Merck), distilled water, and α-amilase enzyme (Novozymes).

All substrates and products were analyzed using UV-Vis, atomic absorption, and FT-IR spectrophotometers. Their morphology was observed using scanning electron microscope (SEM) and X-ray diffractometer (XRD).

3. Methods

Synthesis of SgP: modified from method in previous report [5]. Reaction conditions of pH, temperature, stirring rate and reaction time were varied in a factorial experimental design. Sg was dispersed in 100 mL of a 3:2 mixture of 0.1 M Na2HPO4 and of 0.1 M NaH2PO4 (3:2) solutions to a Sg concentration of 35%. The suspension pH was adjusted by adding drops of HCl 0.01 M or NaOH 0.01 M, to give a variation of pH at 6, 6.5, and 7. The suspension was then heated at temperatures of 25, 30, 35, 40, 45, and 50 °C, and was mixed with mixing rate of 50, 100, 200, 300, 400, and 450 rpm for 10, 15, 20, 30, 45, and 60 minutes. The suspension was then allowed to precipitate. The precipitate was filtered and dried at 80 ± 5 °C for 6 hours. The obtained SgP flour was kept under vacuum. The calculations to determine the optimal conditions were done using the application Design-Expert 7.0.0. The degree of substitution phosphate (DSP) value was done using the colourimetric method at wavelength of 435 nm. The DSP value was calculated using the equation below

\[ \text{DSP} = \frac{162P}{3100 - 103P} \]

\[ P = \text{percentage phosphate in the SgP} \]

Determination of adsorption capacity of Pb(II) ion by SgP [9]. As much as 0.2 g of SgP was added into a 50 mL solution of 200 ppm Pb(II) ions. The mixture was stirred at 150 rpm at a temperature of 30 °C for 1 hour, while keeping the pH constant at pH 3.00. The mixture was filtered, and the final concentration of Pb(II) ion was determined using Atomic Adsorption Spectrophotometer (AAS) with the equation

\[ Q = \frac{(C_i - C_f)VW}{W} \]

\[ Q = \text{adsorption capacity (mg/g)} \]

\[ C_i = \text{initial concentration of Pb(II) ion} \]

\[ C_f = \text{final concentration of Pb(II) ion} \]

\[ V = \text{volume of solution (mL)} \]

\[ W = \text{mass of SgP (g)} \]
Kinetic study of the Pb(II) ion adsorption by SgP. The amount of 0.2 g SgP was added to a 50 mL solution of 200 ppm Pb(II) ions. The mixture was stirred at a rate of 150 rpm at a temperature of 30 °C and initial pH of 3.00. The reaction time was varied from 0 to 150 minutes. Afterwards, the mixture was filtered. The final concentration of the filtrate was determined using AAS. Interaction between molecules was analyzed with kinetics equations for zero, first, and second order reactions [9].

Equilibrium studies for the adsorption of Pb(II) ion by SgP [10] (Romengga et al. 2012). The amount of 0.2 g SgP was added to 50 mL solutions with 0 to 300 ppm Pb(II) ions. The mixtures were stirred for 1 hour at a rate of 150 rpm at a temperature of 30 °C and initial pH of 3.00. Afterwards, the mixture was filtered. The final concentration of the filtrate was determined using AAS. The adsorption equilibrium was analyzed using Langmuir and Freundlich equations [9].

Resistency test. The amount of 0.5 g of Sg and SgP were soaked in solutions with pH 1.50 for 3 hours; pH 5.80 for 2 hours; pH 6.80 for 2 hours; and pH 8.60 for 1 hour. The temperature of the solutions was kept at 37 °C. The unsoluble Sg and SgP were filtered away and were regarded as resistant mass. The resistant mass was then applied for the adsorption of Pb(II) ion. The filtrate was tested for reducing sugar using Benedict reagent [9].

In vitro human digestive system Bioadsorption of Pb(II) by SgP. The resistant mass of SgP was suspended in a 50 mL solution of 200 ppm Pb(II) ions. The pH of solution was kept at pH 1.50 for 3 hours; pH 6.80 for 2 hours with the addition of 500 IU α-amylase enzyme; pH 5.80 for 2 hours; and pH 8.60 for 1 hour. The suspension was stirred at 300 rpm at temperature of 38 °C. The suspension was then filtered. The residue was dissolved in a 25 mL solution of 0.1 M HNO₃ at room temperature for 1 hour. The HNO₃ solution was then analyzed using AAS to determine the amount of adsorbed Pb(II) ions [9].

4. Results and Discussion
Sg and synthesized SgP have a similar morphology with average granule size of 11.1 and 12.4 μm, respected, as shown on Figure 1. This similar size before and after phosphorylation suggests the morphology of the starch did not change significantly. The slightly bigger size of the SgP compared to the Sg might due to reagent diffusion to the area of the starch polymer with high amylose content, breaking the intermolecular forces, and causing the granules to expand [10]. Phosphate cross linking occurs through esterification between –PO₄³⁻ form phosphoric acid, and outward facing hydrophilic –OH groups at the position of C-3 and C-6 of the starch monomer [11].
The DSp value is used as a parameter to determine the success of SgP synthesis. The DSp value is greatly influenced by the external surroundings during the synthesis process. The highest DSp value of 0.089 was reached at pH 6.00, temperature of 30°C, and rate of mixing at 100 rpm, for 10 minutes. This value is quite different than the reaction conditions previously reported which was run at pH 6.50, temperature at 40°C, and rate of mixing at 300 rpm, for 20 minutes [5]. pH and temperature of reaction must be controlled as such to prevent the hydrolysis of starch, that can reduce the amount of amylose available for phosphorylation [12]. Furthermore, a longer reaction time can cause a reverse reaction, where the formed phosphate ester bonding can detach and lower the DSp value.

![Figure 2](image.png)

**Figure 2.** FTIR spectrum of Sg (red) and SgP (blue)

Sg and SgP also have a similar FTIR spectrum, as shown on Figure 2. The difference between the two is the appearance of a new absorption band at 2345 cm⁻¹, which corresponds to phosphate diester bonding (RO–PO₃–R’), and signals the formation of a new bonding after crosslinking process was completed. Two other important peaks are at 1250 and 997 cm⁻¹, showing the presence of P=O and C-O-P bonding [5].

The diffraction pattern of Sg and SgP are also similar as shown by the peaks at 15°, 17°, and 23°, a characteristic of starch crystal structure (Figure 3). New peaks appeared on the diffractogram of SgP, at 46°, 47°, and 49°, suggested that those new peaks are derived from the –PO₃⁻ functional groups [13]. Both diffractograms show the amorphous nature of the starch. The low DSp value shows that the majority of the SgP still acts similarly to the original starch, and the phosphorilated starch is not detected using XRD, while the presence of the phosphate can be seen in the appearance of a new absorption band in the FTIR spectrum of SgP. These results show that the phosphorylation did not change the bulk characteristics of the starch.
Adsorption kinetics of Pb(II) by SgP. SgP has the ability to absorb Pb(II) ion due to the presence of multidentate coordination sites on phosphate groups. Functional groups that are responsible for the complexation process of Pb(II) ion are P=O, P—O, and –OH. The adsorption process must be done at pH 3 to ensure the solubility of the lead sample in water. pH plays an important role as it influences the surface charge of the adsorbent, ionization degree of functional groups of the adsorbent, and the specification of metal ions to be adsorbed [14]. The adsorption of Pb(II) ion by SgP is following second order kinetics as shown by best fitting line on Figure 4, an indication of a chemisorptions process by sharing or exchanging electrons during complexation of the metal ions with the adsorbent [15].

The possible mechanism for the adsorption of Pb(II) ion by SgP was studied using Langmuir and Freundlich isotherm equations. The highest regression correlation was observed for the calculated data using the Langmuir isotherm model (>99 %). The Langmuir isotherm model assumes that the adsorption
occurs via monolayer adsorption and that the adsorbent consist of finite numbers of equivalent adsorption sites. The Langmuir model also indicates that the adsorbents do not interact with each other [15].

![Figure 5. adsorption isotherm (left) and capacity (right) of Pb(II) ions by SgP](image)

The adsorption capacity of Pb(II) ions by SgP was 23.67 mg/g as shown by Figure 5. This value is higher than adsorption capacity of chitosan-coated sand with value of just 12.32 mg/g [16]. However, the adsorption capacity for Pb(II) ions is less than that for Hg(II) ion studied by using SgP with value of 104.63 mg/g [10]. That SgP adsorbs Hg(II) ions better than Pb(II) ions might due to the smaller size of Hg(II) ions resulting in easier contact with the adsorbent. Sg did not show a capability to absorb Pb(II) ions.

**In Vitro Bioadsorption of Pb(II) ions by SgP.** SgP could be used like other cross-linked starches that are used in thickening food such as soups, gravies, sauces, baby foods, fruit fillings, and puddings [12]. Hence, SgP has the potential to be used orally to adsorb toxic Pb(II) ions before they enter the metabolic system. This initial study of the bioadsorption of Pb(II) ions was done in a simulated digestive system to determine if SgP has good resistance to hydrolysis by the temperature and pH of digestive organs.

The mass of a certain amount of Sg and SgP was observed before and after soaking in a digestive simulated solution at designated pH and time at the temperature of 37 °C. The mass of Sg and SgP before and after soaking was found to be unchanged or slightly changed (Table 1), showing that none or very little of the starches was hydrolyzed under testing conditions. This result was confirmed by Benedict test for reducing sugars to indicate the presence of the hydrolysis process. The Benedict test showed a negative result for reducing sugars in the solution after the soaking process of Sg and SgP. This result showed that the cross linked starch, SgP, has good resistance toward digestive temperature and pH.

The in vitro test was done on 4 different pH values mimicking the variation of pH in the human digestive system. Testing at pH 1.50 for 3 hours is similar to the conditions of digestion in the stomach, at pH 6.80 with addition of 500 IU α-amylase enzyme for 2 hours mimicking the situation with pancreatic α-amylase, at pH 5.80 for 2 hours corresponds to the maltase secretion in the small intestine, and at pH 8.60 for 1 hour corresponds to the organic phosphate hydrolysis in the small intestine. The solubility of Pb at different pH was also tested. It was found that Pb completely dissolved at pH 1.50, and precipitated at the higher pH values. However, upon addition of SgP, the precipitate was redissolved as the pH of solution dropped. SgP is thought to be acidic as the final product of the synthesis did not undergo a washing process.
Table 1. The resistant mass of Sg and SgP

| Sample | pH  | Initial Mass (g) | Final mass (g) |
|--------|-----|----------------|---------------|
| Sg     | 1.50| 0.5003         | 0.3027        |
|        | 5.80| 0.5009         | 0.4321        |
|        | 6.20| 0.5019         | 0.4081        |
|        | 8.60| 0.5022         | 0.4702        |
| SgP    | 1.50| 0.5018         | 0.4631        |
|        | 5.80| 0.5007         | 0.4192        |
|        | 6.20| 0.5012         | 0.4163        |
|        | 8.60| 0.5004         | 0.4673        |

Figure 6 shows that the percent of adsorption varied at different pH values. The smallest percent adsorption occurred at pH 1.5. At lower pH, there is competition between Pb$^{2+}$ and H$^+$ ions. The smaller H$^+$ is believed to be easier at interacting with SgP [17]. The highest percent adsorption was reached at pH 6.8 and 8.6. The ability of SgP to adsorb Pb(II) ions at pH 6.8 and after the addition of $\alpha$-amylase enzyme showed its resistance toward the activities of digestive enzymes. It should be understood that under the experimental conditions using 50 mL Pb(II) ions with a concentration of 200 ppm, where before the addition of SgP, Pb(II) ions will precipitate at pH 6.2 reducing the Pb(II) ions concentration in the solution. Initial AAS analysis of the solutions at pH 6.8 and 8.6 before the addition of SgP showed that the Pb(II) ions concentration of both solutions reduced to around 133 ppm, indicating that about 67 ppm Pb(II) ions had precipitated. However, it was observed that solution pH was reduced after SgP was added, indicating the acidic nature of the SgP. The AAS results in Figure 6 show that 174.33 ppm and 185.79 ppm worth of Pb(II) ions were absorbed by the SgP in the pH 6.8 and pH 8.6 solutions, respectively. These results show that a portion of the Pb(II) that had precipitated was redissolved and increased the amount of Pb(II) ions that were absorbed by the SgP. This apparent acidic character of the SgP still needs further analysis. Perhaps some of the phosphoric acid used for phosphorilation is still present in the SgP.
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