Synthesis and characterization of Cu$^{2+}$ imprinted polymer-tannin extract from mango leaf (Mangifera indica L.) for selective separation of Cu$^{2+}$

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Abstract. Tannins are secondary metabolites in Mango (Mangifera indica L.). This compound has a cluster of polyphenols as functional ligands for chelating Cu(II). Cu(II)-imprinted polymer-tannin (Cu-IP-tannin) was successfully synthesized by polymerization of phenol and formaldehyde with the complex of Cu(II)-tannins in acidic medium. Non-imprinted polymer (NIP) was similarly prepared without the presence of Cu(II) ions. The polymers were characterized by Scanning electron microscopy energy dispersive x-ray spectroscopy (SEM-EDS) and Fourier transform infra-red (FTIR). Cu(II) ions were completely removed by leaching the dried and powder of imprinted polymer with HNO$_3$ 0.5 M. The optimum pH of maximum adsorption of Cu(II)-IP-tannin was 7.0 for 120 minutes of contact time. The adsorption of Cu(II)-IP-tannin followed the Freundlich isotherm model with a maximum adsorption capacity of 99.08 mg/g. The relative values of the selectivity factor ($\alpha_r$) of Cu(II)/Ni(II), Cu(II)/Pb(II) and Cu(II)/Fe(II) on a single metal ion selectivity test were 23.89, 55.71 and 26.25, respectively.

Keywords: copper, tannins, ion imprinted polymer, selective separation, mango (Mangifera indica L.)

1. Introduction
Pollution of heavy metals like copper (Cu) especially in the waters brought attention of many parties due to its accumulation in organisms, toxicity and long-term effects. Solid phase extraction currently has been widely used for the separation of metal ions before quantitative analysis [1-3]. This technique was developed by the use of ion exchangers or chelating agent absorbent [4]. The previous research reported tannins as an effective functional ligand used for chelating agent for metal Cu(II) [5-6].

Absorbent selectivity can be improved by Ion Imprinted Polymer (IIP) [7]. In addition, IIP has a high affinity, a good selectivity, an easy preparation, and economical. IIP can be prepared from the reaction between templates, functional monomer/ligand binding agents, crosslinking, and initiator [8].

In this research, we describe the synthesis of a new ion imprinted polymer based on tannins from mango leaf as a chelating agent and metals ion, Cu(II), as a template. The complex is subsequently crosslinked with phenol and formaldehyde using sulfuric acid as a catalyst and initiator. The synthesized polymer was then tested for their adsorption ability in various pH and contact times. The maximum adsorption capacity, reusability and adsorption isotherm were also determined. In addition, the effect of interfering cations (Ni$^{2+}$, Pb$^{2+}$, and Fe$^{2+}$) was investigated to study the IIPs selectivity. The main object of this research is to
investigate the selective analysis of Cu(II) in the presence of competing species by IIP synthesized using natural chelating agent.

2. Experimental

2.1. Materials
All chemicals used in this research were mango leaves (Mangifera indica L.) (Jakarta, Indonesia); Phenol ≥ 99.99 % (Merck), formaldehyde 37 % (Merck), Na₂CO₃, dichloromethane, HNO₃ 65 % (Merck), H₂SO₄ 97 % (Merck), acetic anhydride, chloroform, n-hexane, ethyl acetate (EtOAc), NaOH 99 % (Merck), FeCl₃.6H₂O (Merck), Wagner’s reagent, iodine, KI, HCl 37 % (Merck), double distilled water, and nitrogen gas with high purity. Analytical grade Pb(NO₃)₂, Cu(NO₃)₂.3H₂O, Ni(NO₃)₂.6H₂O, Fe(NO₃)₃.9H₂O were purchased from Merck.

2.2. Instrumentation
The polymer was specified by Fourier Transform Infra-red (FT-IR) spectroscopy (Prestige 21 Shimadzu). Scanning Electron Microscope (SEM) was used to obtain the surface morphology of synthesized polymer, Energy Dispersive X-ray Spectroscopy (EDS) to characterize the composition of atoms in polymers before and after leaching, Spectrophotometer UV-Vis Shimadzu 2460 to study the complex of metal-tannins and Atomic Absorption Spectroscopy (AAS) Shimadzu AA 6800 to analyze metal ions.

2.3. Isolation of tannin from Mango leaf (Mangifera indica L.)
5 kg of wet mango leaves were dried under the sunlight for 3-7 days. The dried leaves were mashed to get the powder of mango leaves. 1 kg powder of dried mango leaves were macerated three times with 5 L of distilled water for 24 h at room temperature. The extract was filtered using Whatman filter paper. The filtrates were extracted in dichloromethane (4:1) for 5 min. The aqueous fraction was then dried by freeze dryer.

2.4. Phytochemical screening
The phytochemical analysis was carried out on water extract using standard procedure to identify the constituents as described by Harborne [9]. Wagner’s reagent was used to identify alkaloids (formation of brownish precipitate). Tannins identification was tested by two drops of 2 % FeCl₃. Blue dark color and precipitate indicated the presence of hydrolysable tannins [9]. 0.5 mL extract was diluted in 5 mL distilled water and heated in water bath. Formation of froth indicated the presence of saponins [9]. 0.5 mL extract was added to the mixture of 0.5 mL chloroform and 0.5 mL acetic anhydride. The reddish brown coloration showed positive result for terpenoids [9]. Whereas, the presence of steroid was marked by the color change from violet to blue or green [9]. Flavonoids were identified by adding 1 mL extract in 0.1 M NaOH (1:1) followed by the drop wise addition of 10 % HCl. A yellow solution that turned colorless indicates the presence of flavonoids [9].

2.5. Synthesis Cu-IP-tannin
The tannin extract was complexed with Cu(II) ions (1:1) in demineralized water as solvent. The solution was stirred for 120 min at room temperature. Then, phenol (50 mmol), HCHO (50 mmol), and 50 mL H₂SO₄ 0.2 M 50 mmol were added to the mixture. The mixture was stirred until homogeneous and purged with nitrogen gas for 10 minutes to release the dissolved oxygen. Polymerization was performed in a temperature-controlled water bath reflux at 65 °C for 5 hours. The resulting product was washed with demineralized water repeatedly to get a neutral pH. The entrapped Cu(II) ions were leached by stirring the polymer in HNO₃ 0.5 M for 27 h in batch experiments. The Cu(II) contents of the filtrate were determined by AAS. The polymer was then washed with demineralized water and dried at 50 °C for 24 h. The procedure of non-impregnated polymers synthesis was similar with the procedure of imprinted polymer synthesis without Cu(II) as the template.

2.6. Copper adsorption study
Table 1. Phytochemical Screening of water extract of the Mango Leaf

| Test          | Result |
|--------------|--------|
| Flavonoid    | +      |
| Terpenoid    | -      |
| Steroid      | -      |
| Tannins      | +      |
| Saponins     | -      |
| Alkaloid     | +      |

Notes: (+) presence, (-) absence

50 mg of imprinted polymer was added into 10 mL of Cu(II) solution at various concentrations of 10, 20, 100, 250, 500 and 1000 mg/L with adjusted pH (2–12) at room temperature for several times (30–150 min) in stirred batch experiments. The remaining copper concentration in the solution was measured by AAS. The adsorption properties were investigated by Langmuir isotherm using equation (1) and Freundlich isotherm using equation (2) [10–11].

$$q_e = \frac{1}{q_m K_e} + \frac{c_e}{q_m}$$

(1)

where $q_e$ is the amount of adsorbed Cu(II) in imprinted polymer (μmol/g), $C_e$ is the equilibrium concentration of Cu(II) ions (μg/mL), $q_m$ is the Langmuir constant associated to adsorption capacity, and $K_e$ is the Langmuir constant associated to the energy of adsorption.

$$\log \frac{q}{q_m} = \log K + \frac{1}{n} \log C$$

(2)

where $K$ and $n$ are Freundlich constants attributed to adsorption capacity and adsorption intensity.

2.7. Selectivity experiments

50 mg of Cu-IP-Tannin and NIP were added into mixed metal solutions with metal concentration of 5 mg/L Cu(II), Pb(II), Ni(II) and Fe(II). All selected competitor ions have similar charge with Cu(II). The experiments were occurred at room temperature with optimum pH and contact time. The amount of each metal was calculated from the initial and equilibrium solution concentration analyzed by AAS. The adsorption capacity, distribution ratio, relative selectivity factor of Cu(II) in regards to Pb(II), Ni(II) and Fe(II) were calculated using equation (3) [10]:

$$Q = \frac{(C_0 - C_e) V}{W}, \quad D = \frac{Q}{C_e}, \quad \alpha = \frac{D_{Cu}}{D_{M}}$$

(3)

where $Q =$ adsorption capacity (g/g), $C_0 =$ initial concentration of each metal (g/L), $C_e =$ equilibrium concentration of each metal (g/L), $V =$ volume of ion metal solution (L), $W =$ mass of imprinted polymer (g), $D =$ the distribution ration (mL/g) and $\alpha =$ relative selectivity factor of Cu(II) and other metals.

3. Results and discussion

3.1. Phytochemical analysis

The presence of flavonoid, terpenoid, steroid, tannins, saponins and alkaloid in water extract of mango leaf was observed by phytochemical screening following the protocol described in Harborne [9]. In table 1, the result shows that tannins were successfully isolated from mango leaf. This compound contained flavonoid and alkaloid that was indicated by the fading color from yellow to colorless solution [9]. The result also showed the brownish precipitate that indicates the presence of alkaloid [9]. It is suggested that the detected alkaloids can be due to the alkaloid compound binding to the tannin compound.
3.2. Characterization study
The FTIR spectra of isolated tannin compound showed the characteristic of stretching vibration of –OH at 3326 cm$^{-1}$, the typical C=O and C-O ester stretching bands at 1611 cm$^{-1}$ and 1310 cm$^{-1}$ respectively. FTIR spectra of NIP and IIP show similar backbones due to their identical components (figure 1). The strong absorption peak at 1611 cm$^{-1}$ is specified for the stretching vibration of C=O and a peak at 1310 cm$^{-1}$ attributed to C-O. The typical sulphonic acid absorption band S=O at 1096 cm$^{-1}$ appeared in imprinted polymer. The shifting of –OH peak to lower wavenumber was observed in IIP due to intermolecular H-bonding [12]. The intensity of –OH stretching band of imprinted polymer was lower compared to that of tannin. Those peaks confirmed that tannin has been copolymerized with phenol-formaldehyde in H$\text{SO}_4$ solution. Furthermore, the similarities of FTIR spectra of IIP leached and unleached indicated that the polymer network was not affected by the leaching process.

The morphology of Cu-IP tannin after leaching was characterized by Scanning Electron Microscopy (SEM). Cu-IP-Tannin leached has formed a circular mold as shown in figure 2. It indicated that all of Cu metal in Cu-IP-Tannin leached has released all and left a mold. EDS (Energy Dispersive X-Ray Spectroscopy) characterization of Cu-IP-Tannin leached and Cu-IP-Tannin unleached was performed to ensure that all of Cu ions metal in the Cu-IP-Tannin process was released. There are some elements of Cu on Cu-IP-Tannin before leaching can be seen in figure 3. In addition to Cu elements, there are also some
Figure 3. EDS Spectrum of Cu-IP-Tannin unleached (a) and Cu-IP-tannin leached (b)

Figure 4. Effect of pH on the adsorption of Cu(II) on imprinted polymer; 50 mg of IIP and NIP; 5 mg/L Cu(II); room temperature

elements of C, S, Na and O from tannins and crosslinkers. There is no Cu ion on Cu-IP-Tannin leached as shown in figure 3. It is indicated that Cu-ions on Cu-IP-Tannin leached have removed.

3.3. Effect of pH
The pH influence on Cu(II) ions adsorption onto the synthesized polymer (IIP and NIP) was observed in batch experiments by altering pH of Cu(II) solution (5 mg/L) from pH 2 to 12. Figure 4 shows similar properties of Cu(II) adsorption on IIP and NIP. Copper ions adsorption increase with increasing pH from 2 to 7 and decrease at higher pH (figure 4). At the acidic atmosphere (pH 2 and 3) there was a competition between Cu- ions and H+ ions to occupy the template formed in the polymer matrix. In addition, H+ ions have a greater affinity for binding ligands than Cu- ions. The solubility of Cu(II) ions is governed by hydroxide concentration and precipitated as copper hydroxide at pH > 7. This also depends on the Cu(II) ions concentration in the medium [13]. Hydroxide ions have a high affinity to Cu- ions, so that Cu- ions prefer to bind with OH- ions in solution compared to adsorbents, which have a specific cavity to Cu- ions. This condition leads to the fact that the optimum adsorption of Cu(II) was reached at pH = 7.

3.4. Equilibrium adsorption time
The equilibrium adsorption time of Cu(II) ions from aqueous solution onto the imprinted polymer was investigated. Based on the figure, at the contact time of 30, 60, 90, and 120 minutes, the adsorption percent increased and tended to be constant at 150 min (figure 5). The interaction of Cu-IIP with Cu(II) could strengthen the ion-dipole interaction. The equilibrium contact time was obtained at 120 minutes with Cu absorption of 92 %. This equilibrium adsorption time was influenced by the complexation rate such as affinity of Cu(II) ions and template groups in polymer structure [13].
3.5. Adsorption capacity

The adsorption profile of Cu(II) in the concentration range 0–1000 mg/L on to imprinted polymer was investigated by batch experiments. 50 mg of synthesized polymer was added to 10 mL Cu(II) solution at optimum pH (pH 7) and equilibrium contact time (120 min). The number of Cu(II) ions adsorbed per unit mass of synthesized polymer escalated with the increase of the initial Cu(II) concentration (figure 6). The initial Cu (II) concentration was escalated to reach saturation (adsorption capacity values). The maximum capacity of synthesized polymer was 99.08 mg/g of Cu-IIP and 99.6 mg/g of NIP. These results indicate that Cu-IIP has as high adsorption capacity as NIP. However IIP has fixed functional group due to imprinting effect, while the functional monomers are randomly distributed in polymer matrix of NIP [14].

The adsorption isotherm was studied to understand the mechanism of adsorption of Cu²⁺ metal ions by Cu-IP-Tannin and NIP, so that it can be known how the molecular distribution was adsorbed between the liquid phase and the solid phase. The type of adsorption isotherm, which often used in the determining of the adsorption isotherm pattern of an adsorbent, is the Langmuir in equation (1) and Freundlich in equation (2). It can be seen in figure 7 that Freundlich model is more suitable in the fitting of isothermal data adsorption of Cu-IP-tannin, while Langmuir model does not have good linearity. The adsorption profile of NIP towards Cu(II) ions follows Langmuir adsorption model ($R^2 = 0.9826$). Meanwhile, the plot of log $Q_e$ vs log $C_e$ exhibits that the adsorption profile of NIP does not obey the Freundlich isotherm adsorption model in the concentration range studied (figure 7). These results show that Cu-IP-tannin has heterogeneous binding sites [10]. The adsorption chamber holds more than one molecular layer and not all adsorbed molecules come into contact with the surface of the adsorbent (multilayer) surface. Langmuir's adsorption isotherm model assumed that the adsorption process of NIP occurs only on the certain homogeneous sites. It is suggested that Cu-IP-tannin has both specific binding sites and unspecific binding sites while NIP only has unspecific binding sites. The specific binding sites are related to the selectivity of adsorbent due to better separation process [15].
3.6. Selectivity study
The adsorption of Cu(II) in the presence of competing divalent ion Pb(II), Fe(II), Ni(II) onto the surface of IIP was studied to be compared with that of NIP. The adsorption of competing ions was lower than Cu(II) adsorption (figure 8). It can be influenced by the size and shape of the cavity sites and affinity of ligand towards imprint metal ion [16]. The relative values of the selectivity factor ($\alpha_r$) of Cu (II)/Ni (II), Cu (II)/Pb (II) and Cu (II)/Fe (II) on a single metal ion selectivity test were 23.89, 55.71, and 26.25, respectively. This result indicated that the synthesized Cu-IP-Tannin was selective to those metals ($\alpha_r > 1$). Therefore, Cu(II) could be detected by the existence of Ni(II), Pb(II) and Fe(II), while NIP had the $\alpha_r$ value of $< 1$.

![Figure 7](image_url)  
**Figure 7.** Langmuir and Freundlich Isotherm model of (a) Cu-IP-Tannin and (b) NIP

![Figure 8](image_url)  
**Figure 8.** Competitive adsorption of Cu(II) and other metal ions; 5 mg/L of Cu(II), Pb(II), Fe(II), and Ni(II); 50 mg synthesized polymer; room temperature
3.7. Reusability

To study the reusability of imprinted polymer, five identical measurements were performed using 50 mg prepared polymer in 10 mL Cu(II) 5 mg/L H₂SO₄ 0.5 M was used for Cu(II) elution. From the calculation results, %RSD (Relative Standard Deviation) for Cu-IP-Tannin was 0.52 %, while CV (Coefficient Variation) Horwitz for Cu-IIP was 1.02 %. The precision was good, if RSD value was lower than CV Horwitz [17]. It can be concluded that Cu-IIP has good reusability in 5 repetitions analysis.

4. Conclusions

A new Cu-imprinted sorbent based on natural chelating agent (tannins) was developed by polymerization of phenol-formaldehyde as crosslinkers in acidic medium using sulfuric acid for selective adsorption of Cu ions. The imprinted polymer has high adsorption capacity towards Cu(II) ions. The Langmuir-Freundlich model results showed that Cu-IP-tannins has heterogeneous surface. In comparison with NIP, Cu-IP-tannins also showed high selectivity towards Cu(II) in the presence of competing metal ions. Furthermore, the Cu-IP-Tannin can be reused for five times analysis.

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