Copper-Ion Adsorption and Gold-Ion Reduction by Polyphenols Prepared by the Enzymatic Reaction of Horseradish Peroxidase

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ABSTRACT: Polyphenols were synthesized via a horseradish peroxidase reaction from phenol, catechol, and pyrogallol for use as copper-ion adsorbents. The molecular weights of the polyphenols ranged from about 1000 to 3000 g/mol. The hydroxyl group contents in the polyphenols from phenol, catechol, and pyrogallol were 5.9, 4.0, and 0.94 mol/kg, respectively, as determined by titration. The saturation binding capacity for copper ions of the polyphenols from phenol, catechol, and pyrogallol were calculated to be 1.44, 0.88, and 0.22 mol/kg, respectively, at pH 4.5. Copper ions were efficiently adsorbed via an ion-exchange interaction by the synthesized polyphenols with vicinal hydroxyl groups, and those polyphenols could be applied to metal adsorption. Gold ions were selectively reduced by the phenol group in polypyrogallol in acid media to form gold particles. The reduced gold particles were eluted with a solution of $1.0M$ thiourea plus $0.5M$ HCl. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 247–252, 2010

Key words: adsorption; bioengineering; enzymes; ion exchangers

INTRODUCTION

Polyphenols are bulky biopolymers often found in fruits such as grapes and persimmon. The basic monomer structure of polyphenol comprises a benzoic acid ring and a hydroxyl group, with the latter being capable of effectively binding proteins. In food chemistry, polyphenols have been widely studied as a functional food because they are known to behave as antioxidants and so on.[1,2]

In hydrometallurgy, metal ions in solution are typically adsorbed and reduced by polyphenol-containing adsorbents. Polyphenol in tannin compounds has a strong reduction power for metal ions. Nakano and coworkers[3,4] reported that Cr(VI) and Pd(II) could be reduced to Cr(III) and Pd(0), respectively, by Mimosa (Wattle) tannin gel (a type of polyphenol). Inoue et al.[5] used polyphenol contained within persimmon peel for selective gold adsorption. The adsorbed gold ions were reduced to fine gold particles via a redox reaction between polyphenol and the gold ions; this confirmed that polyphenol could be a powerful adsorbent for the recovery of gold ions. In these reactions, the polyphenol was used directly for metal adsorption without molecular design; that is, the fruit materials containing various kinds of polyphenol were simply crosslinked via a condensation reaction with sulfuric acid. It seems that synthesis of designed polyphenols would be likely to give materials with improved metal adsorptivity.

Polymerization catalyzed by enzymes has attracted much attention as a new methodology for polymer syntheses. It is believed that enzymatic polymerization will provide new polymeric materials that are difficult to obtain by conventional methods.[6] Peroxidase is renowned for inducing the oxidative polymerization of phenol derivatives under mild reaction conditions. Substrates of phenol and monomers, such as 4-phenylphenol,[7] bisphenol,[8] phenylalanine,[9] and tyrosine derivatives,[10] were polymerized via the peroxidase reaction to form fascinating and controlled polymer materials. The polymerization of functional monomers to give polyphenols provides a suitable platform to monitor their potential as highly efficient metal adsorbents.

It is apparent from the performance of biomass waste that the efficiency of metal adsorption is dependent on the hydroxyl groups in polyphenol. The design of an artificial polyphenol would improve the metal adsorbent; the location of the appropriate position of hydroxyl groups at a high density enhances the metal-adsorption performance. The enzymatic reaction is a powerful technique for preparing polyphenols, along with the selection of

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the substrate under mild conditions. The novelty in this study was the polymerization of polyphenol from various phenollike substrates for use in metal adsorption. The substrate structure is a key to polymerizing polyphenol and to adsorbing metal ions.

In this study, phenol, catechol, and pyrogallol were polymerized via the enzymatic reaction of horseradish peroxidase (HRP) to examine the effect of the number of the hydroxyl groups in the phenolic monomers on the copper adsorption. By using copper ion as the metal ion, we could examine the binding site of copper with the hydroxyl groups of phenol derivatives in polyphenols via intermolecular or intramolecular interaction. The pH dependence and isotherms for the adsorption of copper ion by the polyphenols were determined in batch mode to elucidate the copper-ion binding sites. The phenol groups in polyphenol can also reduce gold ions in acid media. The selectivity to gold ions by polypyrogallol in an acid solution was examined.

Scheme 1 Reaction scheme of the polyphenol polymerization.

and room temperature, respectively. The standard used was polystyrene. The hydroxyl group content of the polyphenols was determined by titrimetry. The morphology of the polyphenol surfaces was examined by scanning electron microscopy (JSM-5200, JEOL, Japan).

Copper adsorption by the polyphenols

For the pH dependence study, 0.1 mM metal solutions were individually prepared with various pH values. The pH was adjusted with 0.1M 2-[4-(2-hydroxyethyl-)-1-piperazinyl] ethane sulfonic acid and 0.1M HCl. A 15-mL aliquot of each test solution was shaken with 15 mg of polyphenol in a stoppered flask at 303 K for 24 h. To determine the saturation binding capacity for copper ions, the concentration of copper ions was changed from 0.1 to 10 mM. In the kinetics experiments, the initial concentration of copper ions for polycatechol and polypyrogallol was set at 5.0 mM. The amount of polyphenols added, the reaction temperature, and the pH were set at 15 mg, 303 K, and 4.5, respectively. The concentrations of metal ion before and after adsorption were determined with a Shimadzu model AA-6650 atomic absorption spectrophotometer (Japan). The data were used to calculate the percentage adsorption of copper and the amount of copper adsorbed via eqs. (1) and (2), respectively:

$$\text{Adsorption of Cu(II)}(\%) = 100(C_0 - C_{eq})/C_0 \quad (1)$$

where C_0 is the initial concentration of metal ion and C_{eq} is the equilibrium concentration after adsorption.

$$q = (\text{Amount of copper ion adsorbed})/$$
$$(\text{Weight of polyphenol added}) \quad (2)$$

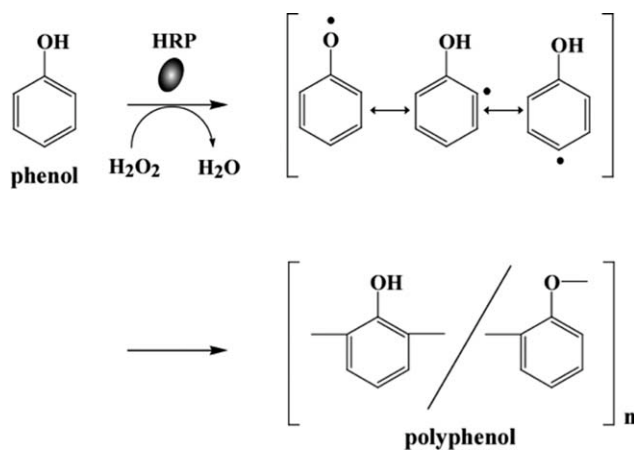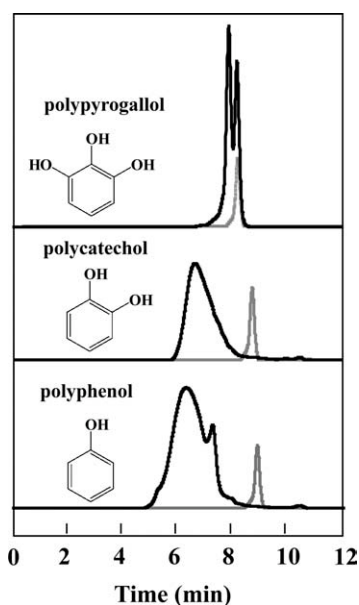where q is the amount of copper ion adsorbed (mol/kg).

EXPERIMENTAL

Materials

Catechol and pyrogallol were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Phenol was purchased from Sigma-Aldrich Co., USA. HRP (NXKUL) was obtained from Tokyo Kasei Kogyo Co., Japan. Hydrogen peroxide was purchased from Wako Chemical Industries Co., Japan. Analytical-grade HAuCl$_4$·4H$_2$O was used to prepare test solutions of the gold. The other reagents were analytical grade or higher.

Polymerization of the phenol derivatives via an enzymatic reaction (Scheme 1)

The phenolic monomer (10 mmol) and HRP (3 mg) were mixed in a methanol/phosphate buffer (1 : 1 volume ratio, 0.01M, pH 6.0). Hydrogen peroxide (5%, 6.8 mL) was added to initiate the polymerization, and the mixture was heated for 24 h at 303 K. The resulting precipitate was filtered, washed with water, and dried *in vacuo.* The diameter of the polyphenol particles was within 300 μm.

The spectra of the synthesized polyphenols by Fourier transform infrared spectroscopy were obtained with a Jasco FT/IR-410 spectrometer (Japan) with the KBr method. The molecular weight distributions were determined by size exclusion chromatography. The size exclusion chromatography setup comprised a Waters 515 high performance liquid chromatography (HPLC) pump and a Waters UV 2487 dual-absorbance detector with a Styragel HR 1 (Waters, USA) column. The eluent, flow rate, and column temperature were tetrahydrofuran, 1.0 mL/min,

Figure 1 Size exclusion chromatography of polyphenols. The gray line indicates the monomers' chromatograms.

Reduction of gold ions in the HCl solution

The adsorption and reduction behavior of polypyrogallol was tested batchwise. A metal solution (2 mM) was prepared in 0.5M hydrochloric acid. The metal solution (10 mL) was mixed together with 15 mg of the gel and shaken for 24 h at 30°C to attain equilibrium. The concentration of metal ions was determined by Shimadzu model AA-6650 atomic absorption spectrophotometer (Japan).

Elution of the metal ions

After the adsorption of copper ions, 15 mg of polycatechol or polypyrogallol was mixed with an HCl solution at various concentrations. After 24 h, the solution was filtered, and the copper concentration in the filtrate was determined.

To elute the reduced gold, gold ions (2.5 mM) in a 0.5M HCl solution were mixed with polypyrogallol (15 mg) to obtain the reduced gold particles. Polypyrogallol, including the reduced gold particles, was mixed with a 1.0M thiourea solution (0.5M HCl) for a prescribed time. After filtration, the concentration of gold ions in the filtered solution was determined with the Shimadzu model AA-6650 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

#### Characterization of the polyphenols

The molecular weight of the polyphenols was determined by size exclusion chromatography, as shown in Figure 1. For polypyrogallol, two sharp peaks at

TABLE I
**Average Molecular Weights and Densities of the Hydroxyl Groups**

	Molecular weight (g/mol)	Amount of OH groups (mol/kg)
Polypyrogallol	15,500	5.9
Polycatechol	2,000	4.0
Polyphenol	3,000	0.94

7.9 and 8.2 min were obtained; the latter was attributed to the monomer. The peaks for polyphenol and polycatechol at 6.2 min were broadened, and the molecular weights of polyphenol and polycatechol were approximately 2000 and 3000 g/mol, respectively. The molecular weights of the polyphenols were strongly related to the reaction media. In this study, a 1 : 1 mixture of methanol and phosphate buffer solution was used as the solvent for the polymerization. If the methanol ratio in the medium was increased, the peak in the chromatogram was sharper because of the solubility of the polyphenol.

In the Fourier transform infrared spectra, the band at 3400 cm^{-1} indicated the residual (unreacted) hydroxyl groups of the phenol in the polyphenols. The bands at 1240, 1610, and 1190 cm^{-1} corresponded to the modes associated with C—O—C, benzene, and C—OH, respectively, and indicated successful polyphenol synthesis with HRP. The molecular weights and hydroxyl group contents of the polyphenols are summarized in Table I. With increasing number of hydroxyl groups in the phenol derivatives, the hydroxyl group content of the synthesized polyphenols increased. The hydroxyl group contents in phenol, catechol, and pyrogallol were 10.6, 18.2, and 23.8 mol/kg. The hydroxyl group content in polypyrogallol, for example, was found to be 5.9 mol/kg (Table I); this indicated that 17.9 mol/kg of hydroxyl groups were used for the polymerization to form ether bonds between the monomers. The morphology of the polyphenols was observed by scanning electron microscopy. It was apparent (see Fig. 2) that the surfaces of the polyphenols were porous.
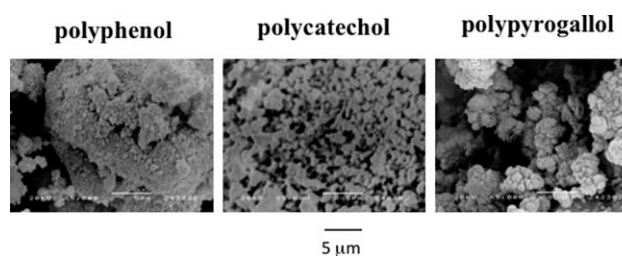
Figure 2 SEM images of the polyphenols.
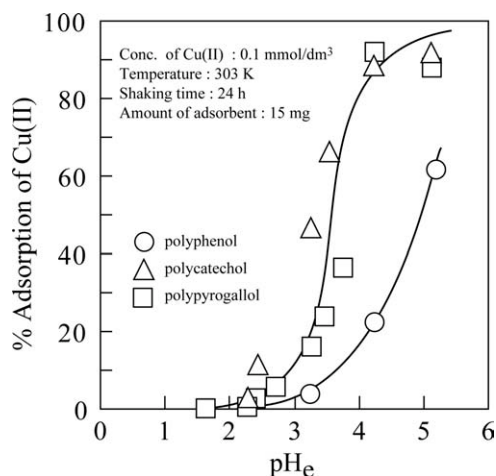
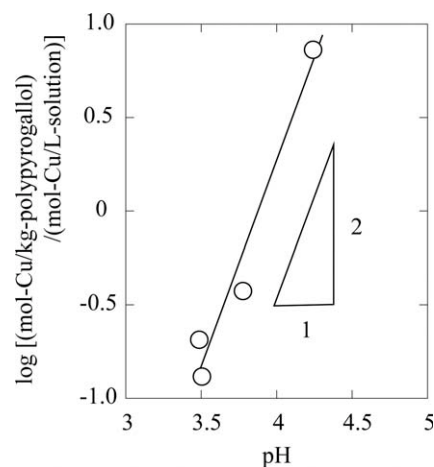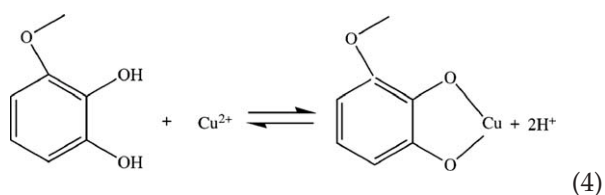**Figure 3** pH dependence of the copper-ion adsorption by polyphenols.

Adsorption of copper ions by the polyphenols

The percentage adsorption of copper ions by the polyphenols as a function of pH is shown in Figure 3. For each polyphenol, the percentage adsorption of copper ions increased with increasing pH; this indicated that adsorption occurred via an ion-exchange reaction. The pH dependence of copper-ion adsorption overlapped for polypyrogallol and polycatechol and was significantly different from the case of polyphenol because polypyrogallol and polycatechol had a much higher affinity than polyphenol for copper ions.

Copper adsorption by polypyrogallol, as shown in Figure 3, was analyzed to understand the adsorption mechanism. The distribution ratio was defined as follows:

$$D[(\text{mol of Cu/kg of polypyrogallol})/$$
$$(\text{mol of Cu/L of solution})] =$$
$$(\text{Amount of copper ion adsorbed})/$$
$$(\text{Copper concentration in solution}) \quad (3)$$

The logarithm of D as a function of pH for copper adsorption to polypyrogallol is shown in Figure 4. The slope of the straight line was calculated to be 2 from pH 3.5 to 4.2; this indicated that the copper ion was adsorbed to the polypyrogallol to release the two protons. Namely, the adsorption mechanism could be described by the following equation:


(4)

Copper ions are suggested to have formed a complex with the two hydroxyl groups in a pyrogallol unit in polypyrogallol or with one hydroxyl group in two pyrogallol units.

The time course of copper adsorption is shown in Figure 5. Polyphenol showed a much smaller copper adsorptivity than the other polyphenols. Polypyrogallol and polycatechol adsorbed copper ions at similar rates. The polyphenol polymerized from the substrate with the greater number of hydroxyl groups represented the higher adsorption rate of copper ions.

Adsorption isotherms for copper ions are shown in Figure 6. The isotherm adsorption of copper ions to the polyphenols were fitted to the following Langmuir equation:

$$C_{eq}/q = C_{eq}/q_m + 1/(bq_m) \quad (5)$$

Figure 4 Log D of the copper ion adsorbed on polypyrogallol versus pH.
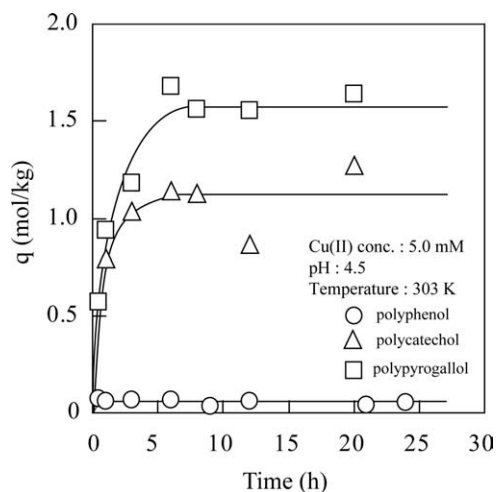
Figure 5 Time course curves of copper adsorption by the polyphenols.
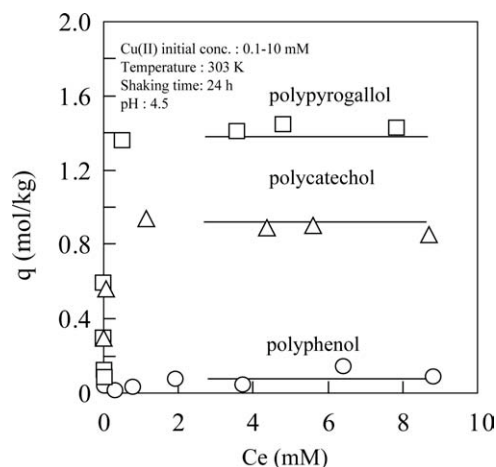
Figure 6 Isotherm curves of copper-ion adsorption by polyphenols.

Figure 7 Selective recovery of gold ions by polypyrogallol in a $0.5M$ HCl solution.

where C_{eq}, q_m, q, and b are the concentration of copper ions at equilibrium, the saturation capacity of copper ions, the amount of copper adsorbed, and the Langmuir constant. The results are summarized in Table II. The saturation binding capacities were in the following order: polyphenol, polycatechol, and polypyrogallol. The saturation binding capacity by polypyrogallol was calculated to be 1.44 mol/kg. The Langmuir constant indicates the affinity between the metal ions and the adsorbent. The Langmuir constant of polycatechol and polypyrogallol were 15.22 and 11.06 L/kg, respectively, which suggested that their adsorption behaviors were the same. The highest saturation binding capacity of polypyrogallol was due to the high functional group density of the hydroxyl groups. The adsorption capacities of the polyphenols were smaller to the hydroxyl group contents in Table I; this showed that not all of the hydroxyl groups functioned as adsorption sites for copper. It was clear that vicinal hydroxyl groups in the polyphenols were required to adsorb copper ions via an ion-exchange interaction. During polymerization, the hydroxyl groups in the phenol derivatives were polymerized, not to be possibly used for the adsorption site.

There are many articles reporting the use of biomass waste for metal adsorption. Tannin, a kind of polyphenol, was used for metal adsorption.[11] Copper ions were adsorbed to a polyphenol compound contained in biomass.[12] The general adsorption mechanism of metal ions is via an ion-exchange interaction and coordination binding. Copper adsorption by a polyphenol polymerized from an enzymatic reaction also occurred via an ion-exchange interaction. The enzymatic reaction enabled us to polymerize the polyphenol at the phenol group selectively. Changes in the reaction conditions induced various structures of the polyphenols. The design of the substrate for the recognition of the metal ions for polymerization via an enzymatic reaction has the potential for the preparation of metal adsorbents.

Selective reduction of gold ions in the HCl solution

Polypyrogallol is a kind of polyphenol and has the power to reduce gold ions to gold particles. The adsorption selectivity pattern of the grape gel for Au(III), Cu(II), Pd(II), Pt(IV), and Zn(II) at a concentration of $0.5M$ of hydrochloric acid solution was studied batchwise with a metal solution containing 0.2 mmol of each metal ion. The results observed in the case of the individual metal solution is depicted in Figure 7, where a distinct selectivity was observed for Au(III) and the gel was found to exhibit only weak extractability for the other metal ions. The

TABLE II
Summary of the Adsorption Binding Capacity and Langmuir Constant Values

	Adsorption binding capacity (mol/kg)	Langmuir constant (L/kg)	R^2 value
Polyphenol	0.22	3.92	0.996
Polycatechol	0.88	15.22	0.998
Polypyrogallol	1.44	11.06	0.999

TABLE III
Elution Percentage of Copper Ions by the HCl Solution

Concentration of HCl (M)	Polycatechol	Polypyrogallol
0.25	14	48
1	17	27
2	67	33
3	42	54
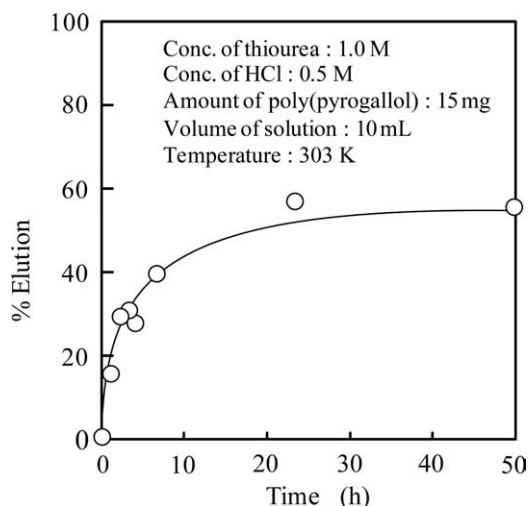
Figure 8 Time course curve of the gold particle elution by thiourea plus an HCl solution.

gold particles were observed by optical microscopy, the same as in Parajuli et al.'s[5] study. This indicated that the gold ions were selectively reduced to gold particles by the phenol groups in polypyrogallol.

Elution of the copper ions and reduced gold particles

To determine the capacity of the polyphenols for repeated usage as adsorbents, the elution of the copper ions from the polyphenols was examined. The percentage elution with various HCl concentrations is shown in Table III. With increasing HCl concentration, the percentage elution of polycatechol increased, whereas that of polypyrogallol was roughly constant in the range 30–50%. The implication was that, especially for polypyrogallol, the irreversible adsorption of copper ions occurred.

Reduced gold particles were eluted with a solution of thiourea plus HCl. The elution percentage as a function of reaction time is shown in Figure 8. With increasing concentration of thiourea, the elution percentage of gold ions increased up to 60%;

this demonstrated that the gold reduced by polypyrogallol was easily recovered.

CONCLUSIONS

Phenol and its derivatives, catechol and pyrogallol, were polymerized via the enzymatic reaction of HRP for copper adsorption. Increasing the hydroxyl group content in the phenolic monomer increased the rate of adsorption and the saturation binding capacity for copper ions. The presence of vicinal hydroxyl groups in the polyphenols was required for efficient capture of the copper ions. The gold ions were selectively reduced by polypyrogallol in an HCl solution to form gold particles. The reduced gold particles were easily eluted by a solution of thiourea plus HCl. Polyphenols designed to recognize metal ions could be polymerized by enzymatic reaction by the selection of appropriate substrates.

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