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# Recovery and Refining of Au by Gold-Cyanide Ion Biosorption Using Animal Fibrous Proteins

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## ABSTRACT

Animal fibrous proteins (AFPs) such as egg-shell membrane (ESM), chicken feather (CF), wool, silk, or elastin are an intricate network of stable and water-insoluble fibers with high surface area and are abundant bioresources. Every AFP tested was found to accumulate gold-cyanide ion from aqueous solutions in high yield, depending on pH and some other parameters. Gold-cyanide ion is adsorbed by AFP at low pH range, with maximum binding observed at approx pH 2.0. Under the certain conditions, gold-cyanide ion was accumulated up to 8.6, 7.1, 9.8, 2.4, and 3.9% of dry weight on ESM, CF, wool, silk, and elastin, respectively. In the case of ESM, it was found that ESM removed gold-cyanide ion almost quantitatively and almost all the gold uptake by ESM was easily desorbed with 0.1 *M* NaOH. ESM can be used repeatedly for the process of gold adsorption-desorption. The gold-biosorptive capacity of ESM that was chemically modified with glutaraldehyde was higher than that of control. In column procedure, ESM packed on column removed gold-cyanide ion from the dilute aqueous solution to extremely low concentrations (nondetectable concentration of below 1 ppb).

## INTRODUCTION

Gold is a precious metal and used in not only jewelry but extensively in high technology areas, particularly in computer applications that demand the highest reliability *(1)*. Gold recovery from secondary sources such as electronic scrap *(2)* and waste electroplating solutions is therefore an important technology as well as recovery from primary resources such as leach solutions.

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The recovery of gold from dilute solutions generally involves either zinc-dust precipitation, carbon adsorption, or solvent extraction. Recently recovery process alternatives based on ion-exchange resins have received specific attention *(3)*. However, in most cases, their high costs limit the usage of ion-exchange resins. Recently, some investigations have focused on the gold recovery and mining process by means of biosorbents, such as algae *(4–7)* and microorganisms *(8–12)*. However, few reports appear on the biosorption of gold-cyanide ion.

Cyanidation of gold ores is commonly used for the mobilization of the metal. Although effective, the process poses a number of problems in the recovery of gold.

Recently, we showed that various heavy metals are accumulated in high yield *(13)* by the hen egg-shell membrane (ESM), and the ESM have removed gold from dilute tetrachloroaurate (III) solutions. Also, we found that chicken feather (CF) was promising to use in the removal/recovery of precious metals as well as water pollution control *(14)*.

This study focuses on the accumulation of gold-cyanide ion by animal fibrous proteins (AFPs), such as egg-shell membrane (ESM), chicken feather (CF), wool, silk, or elastin, and outlines some parameters in the potential use of AFP for removing gold from aqueous solution. The refining of the gold-cyanide ion from the electroplating solution and mining operations are also discussed.

## MATERIALS AND METHODS

### Sample Preparation

ESM was mechanically stripped from the shells after immersion of the hen (White leghorn) egg shells, which were collected from a local confectionery, in 0.5 *M* HCl overnight and then further in 0.5 *M* NaOH for 1 h followed by rinsing with distilled deionized water 10 times. Broiler-chicken feather sample was obtained from the waste of a local chicken industry of Japan. The feather was washed twice with water containing detergent by electric washing machine, then washed with deionized-distilled water, and then dried in air at room temperature. Both Corriedale virgin wool and silk samples were obtained from the university stock farm (Tohoku University). The wool was washed twice with water containing detergent by dipping overnight, air-dried at room temperature, defatted with diethyl ether for 48 h, and washed with methanol and then deionized-distilled water. Silk and cotton were washed with methanol, then fully washed with deionized-distilled water. Elastin was acquired as follows. Bovine *ligamentum nuchae* was cleaned of adhering fat, cut into small cubes, and homogenized in 1 *M* NaCl. The precipitates were dilapidated with chloroform-methanol (2:1, v/v), and then washed with ethanol and deionized-distilled water.

All samples were desiccated over phosphorus pentaoxide under reduced pressure at room temperature for over 10 h.

## Chemical Modifications of ESM

Chemical modification reactions of ESM were performed with formaldehyde and glutaraldehyde as follows:

1. Formaldehyde crosslinking followed a modified procedure of Bullock *(15)*. Approximately 250 mg of ESM and 45 mL of aqueous 3.7% (w/v) formaldehyde containing 0.1% (w/v) hydrochloric acid was heated at 100°C for 12 h. The chemically modified ESM sample was washed with distilled water, 0.5% (w/v) sodium carbonate, and finally deionized-distilled water.
2. Glutaraldehyde crosslinking used a modified procedure of Griffith *(16)*. Approximately 250 mg of ESM was reacted with 45 mL of aqueous 1.25% (w/v) glutaraldehyde containing 0.1% (w/v) hydrochloric acid at 100°C for 12 h. The result was washed first with distilled water, then 0.5% (w/v) sodium carbonate, and finally deionized-distilled water.

Both modified samples were dried over phosphorus pentaoxide under reduced pressure over 10 h in a desiccator.

## Chemicals and Metal Solutions

Stock solutions of gold-cyanide ion (18 m*M*) were prepared by dissolving gold (I) cyanide in aqueous 5% (w/v) sodium-cyanide solution. Dilution was done by addition of deionized-distilled water daily as required. All pH adjustments were carried out with 1 *M* HCl and 1 *M* NaOH. For safety precautions, all experiments with cyanide-containing solutions were performed in a hood to avoid exposure to HCN.

## Adsorption Experiments

Two procedures were utilized in experiments reported here. In the batch procedure, approximately the same weight of AFPs (approx 25–35 mg) was precisely weighed, then placed directly into the aqueous solution containing gold-cyanide ion in 40-mL test tubes. To prevent metal contamination during experiments, the test tubes were soaked with 10% (w/v) HCl overnight. Then, they were washed and rinsed with deionized-distilled water. This cleaning procedure was used throughout the experiments. The volume of solution was always 10 mL unless otherwise specified. The pH values of reaction mixtures were adjusted with 1 *M* HCl or 1 *M* NaOH at the start of the experiment and maintained when possible. Solutions were shaken at elimination in a thermostated water bath with shaker at 25°C. After an appropriate time, aliquots of the solution of reaction mixture were analyzed for the remaining metal ions.

In the column procedure, experimental scale of collection of gold was carried out by the crushed ESM powder-packed mini column (diameter 1 cm, length 10 or 20 cm). The slurry of powdered ESM was poured into a column, then pH-adjusted solution containing metal ions was passed through the column, and then effluent was analyzed for the metal ions. The crushed ESM powder was prepared by crushing by Ostar blending crusher. The column-sorption experiments were performed at room temperature.

### Desorption Experiments

ESM was isolated from a gold-ESM reaction mixture of batch method with tweezers and resoaked in an equal volume of solution containing 0.1 *M* NaOH for 1 h at 25°C. The amount of gold liberated from the ESM was determined from the free-gold concentrations measured in the solution.

### Gold Analysis

Gold determinations were performed by atomic-absorption spectrophotometer (AES SAS-727, Seiko) or an inductively coupled plasma mass spectrometer (ELAN 6000 ICP-MS, Perkin Elmer, Norwalk, CT).

Sorption capacity is calculated by $q = (C_0 - C_e)Vlm$; where $q$ is the sorption capacity (mg Au/g sorbent), $C_0$ the initial gold concentration, $C_e$ the residual gold concentration in solution (mg Au/L), $V$ the volume of solution (1), and $m$ the sorbent mass (g).

## RESULTS AND DISCUSSION

Figure 1 shows the kinetics of biosorption of gold-cyanide ion on ESM, CF, wool, silk, and elastin at pH 2.0 and 25°C by the batch method. The AFP-gold system reached the equilibrium plateau corresponding to 100% of the gold-uptake capacity of AFP at a contact time in excess of 3 h. Within 1 h of contact, the biosorption system reached approx 80–100% of the total gold uptake.

The effect of pH and temperature on the uptake of gold-cyanide ion by ESM, CF, and wool was studied in the pH range from 1.0 to 12.0 for 3 h of soaking time at 4–40°C. As shown in Figs. 2, 3, and 4, the adsorption of gold-cyanide ion on AFPs was highly dependent on pH, with maximum adsorption occurring near pH 2.0, below and above which the uptake declined greatly. Thus, the adsorption of gold-cyanide ion by AFPs is markedly affected by the pH of the solution. Also, a slight increase in the gold-cyanide ion biosorptive uptake at pH 2.0 was observed when the temperature decreased from 40 to 4°C. However, differences were not considerable for the temperature-range tested.

To screen AFPs for maximal uptake of gold-cyanide ion, ESM, CF, wool, silk, and elastin were examined. The sorption experiments were

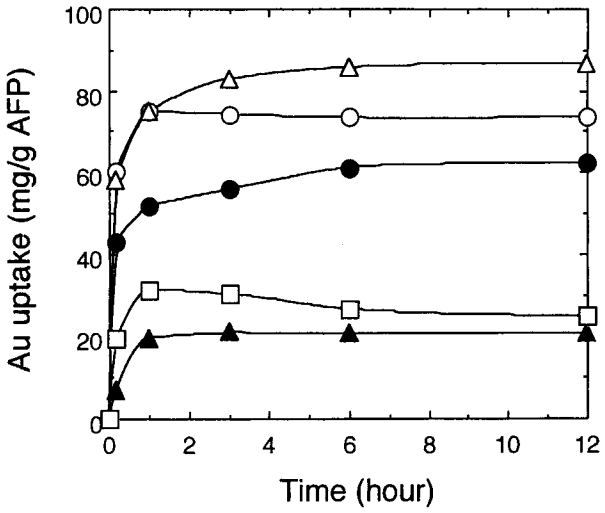


Fig. 1. Kinetics of the biosorption of gold-cyanide ion by ESM (○), CF (●), wool (△), silk (▲) and elastin (□). The sorbents were soaked in 10 mL of a solution (pH 2.0) containing 3.0 m*M* of gold at 25°C.

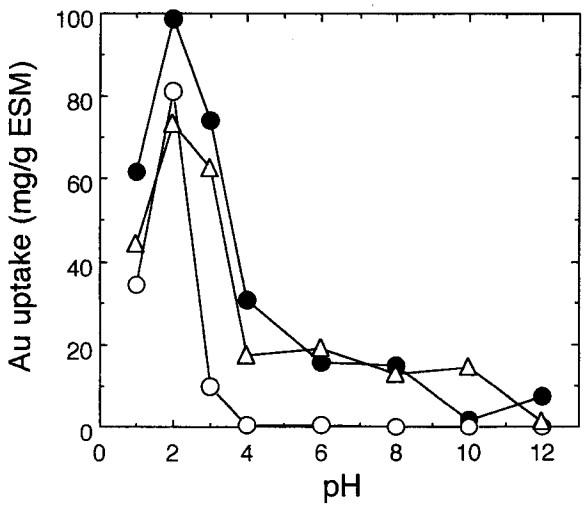


Fig. 2. Effect of pH and temperature on the biosorption of gold-cyanide ion by ESM. The ESM was soaked in 10 mL of a solution containing 3.8 m*M* of gold; (○) at 4°C (refrigerator); (●) at 25°C; (△) at 40°C.

performed in the maximum conditions (3 h of contact time at pH 2.0) at 25°C. As shown in Table 1, the ability to uptake gold-cyanide ion differs with different AFPs. ESM, CF, and wool took up more than 70 mg Au/g AFP dry weight from the solution, which suggested that AFPs have excellent ability for accumulation of gold-cyanide ion. Of the AFP tested, extremely high abilities for gold-cyanide uptake were found in ESM and

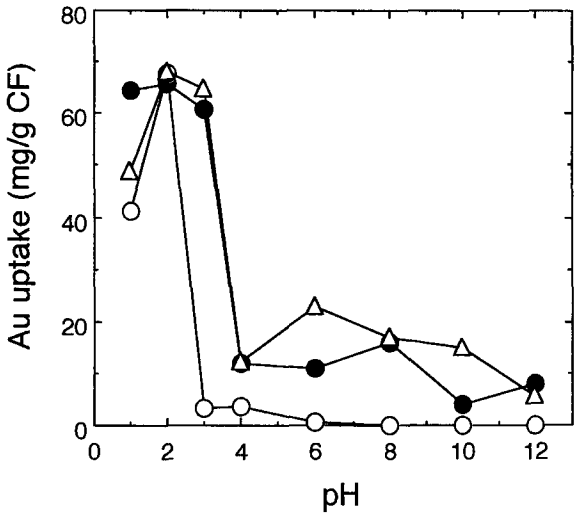


Fig. 3. Effect of pH and temperature on the biosorption of gold-cyanide ion by CF. The CF was soaked in 10 mL of a solution containing 3.8 m*M* of gold: (○) at 4°C (refrigerator); (●) at 25°C; (△) at 40°C.

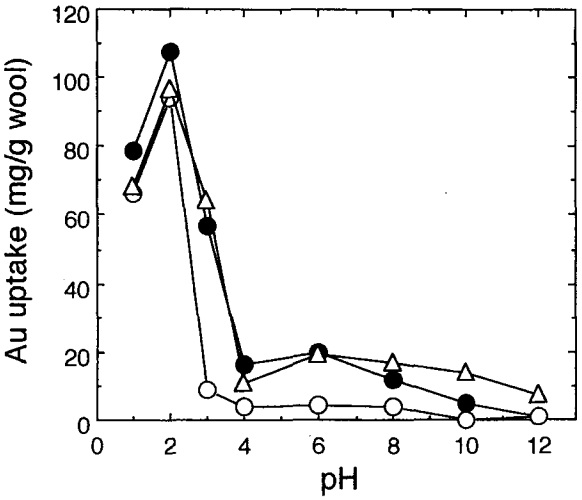


Fig. 4. Effect of pH and temperature on the biosorption of gold-cyanide ion by wool. The wool was soaked in 10 mL of a solution containing 3.8 m*M* of gold; (○) at 4°C (refrigerator); (●) at 25°C; (△) at 40°C.

wool. No uptake phenomenon was found on cotton. As described previously, ESM have a high ability for accumulating gold-cyanide ion. In order to elucidate of recovering of the gold uptake, we examined five cycles of gold adsorption-desorption by ESM. In batch systems, the gold-uptake ESM (Au: 3.0 m*M*, pH: 2.0, contact time: 3 h, temp.: 25°C) was desorbed by 0.1 *M* NaOH solution for 1 h at 25°C. As shown in Fig. 5, ESM recovered

Table 1
Biosorption of Gold-Cyanide Ion by Animal Fibrous Proteins (AFPs) and Cotton. The Sorbents (30 mg dry weight basis) Were Soaked in 10 mL of a Solution Containing 3.4 m*M* of Gold for 3 h at 25°C

| sorbents | Au uptake (mg/ g sorbent) |
|---|---|
| Egg shell membrane | 85.8 |
| Chicken feather | 70.8 |
| Wool | 98.1 |
| Silk | 23.6 |
| Elastin | 38.9 |
| Cotton | 3.3 |

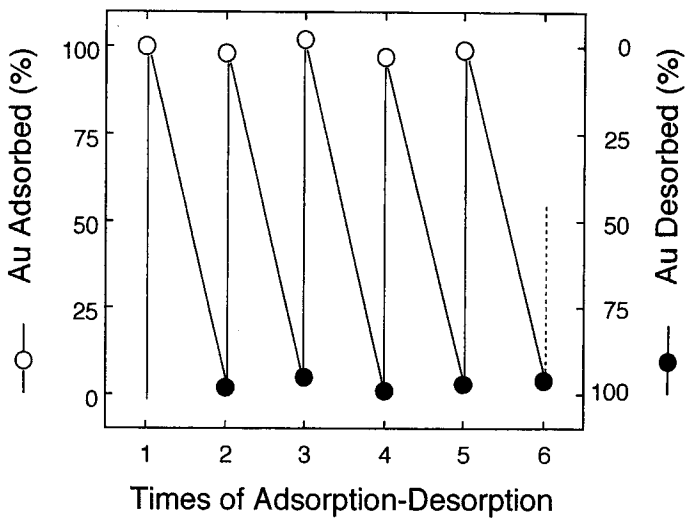


Fig. 5. Repeated test of gold adsorption (○) -desorption (●) by ESM. The gold adsorbed ESM (Au: 3.0 m*M*, pH: 2.0, contact time: 3 h, temp.: 25°C) was desorbed with 10 mL of 0.1 *M* NaOH solution for 1 h at 25°C, expressed as a percentage of the first uptake value (73 mg Au/g ESM).

gold almost quantitatively and almost all the gold uptake was desorbed with 0.1 *M* NaOH through the five cycles tested. The ESM had no damage during five adsorption-desorption cycles. These results show that ESM is very stable and can be used repeatedly.

The adsorption isotherms for gold cyanide ion, plotted in Fig. 6, show the evolution of metal concentration in AFP vs residual metallic concentration in solution. Each isotherm has a similar form, and shows two regions for AFPs: At low concentrations the isotherms are concave, at higher con-

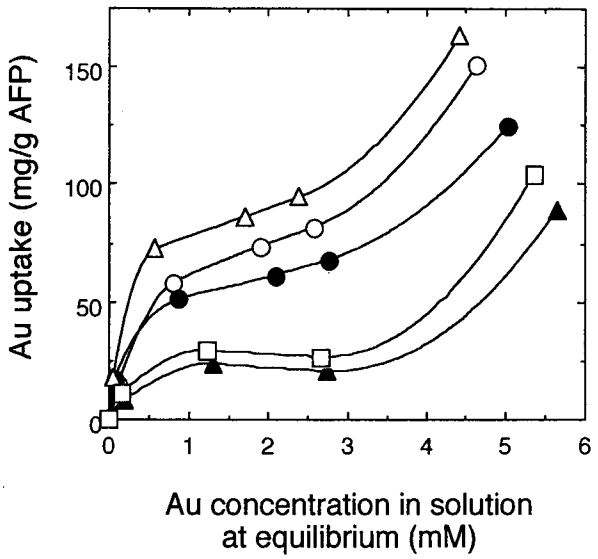


Fig. 6. Gold biosorption isotherms for ESM (○), CF (●), wool (△), silk (▲) and elastin (□). The sorbents were soaked in 10 mL of a solution (pH 2.0) containing from 0.3 to 7.0 m*M* of gold for 3 h at 25°C.

centrations they are convex toward the concentration axis. These isotherms are not fully fitted by two representative adsorption isotherms, Langmuir and Freundlich models. Such adsorption isotherms are called BET (Brunauer-Emmett-Teller) adsorption isotherm, and it is used in case of the formation of multimolecular adsorbed layers *(17)*. The characteristics of the gold-cyanide ion adsorption profile suggest ionic interactions, possibly involving electrostatic interactions between the negatively charged gold-cyanide ion and positively charged AFP ligands at pH 2.0. Moreover, it is suggested that gold-adsorption isotherms for AFPs are convex to the concentration axis at high concentrations, because an interaction among gold-cyanide ions begins to influence the adsorption phenomenon as gold concentration in solution increases.

ESM protein was chemically modified in two different ways in order to increase its gold sorption capacity. The uptake of gold-cyanide ion by native and modified ESM is compared in Table 2. Although the apparent differences on the uptake of gold were hardly discernible between native and formaldehyde-crosslinked ESM, the sorption capacity increased 40% when crosslinked with glutaraldehyde.

A column was used for the removal of metal from dilute aqueous solutions. Figure 7 shows the gold removal by the column packed with ESM (1.5 g) from very low concentrations of aqueous gold-cyanide ion solution (100 ppb). As shown in Fig. 7, high removal efficiency was maintained for initial 100 mL of effluent to unidentified concentration of gold (below 1 ppb). The results indicate that this biosorption process can deal

Table 2
Effect of the Chemical Modifications of ESM on the Biosorption of Gold Cyanide Ion

| Sorbent type | Au uptake (mg/ g ESM) |
|---|---|
| ESM | 73.4 |
| ESM crosslinked with formaldehyde | 70.3 |
| ESM crosslinked with glutaraldehyde | 101.0 |

The modified ESM was soaked in 10 mL of a solution (pH 2.0) containing 3.0 m*M* of gold at 25°C. The chemical modification details were given in the text.

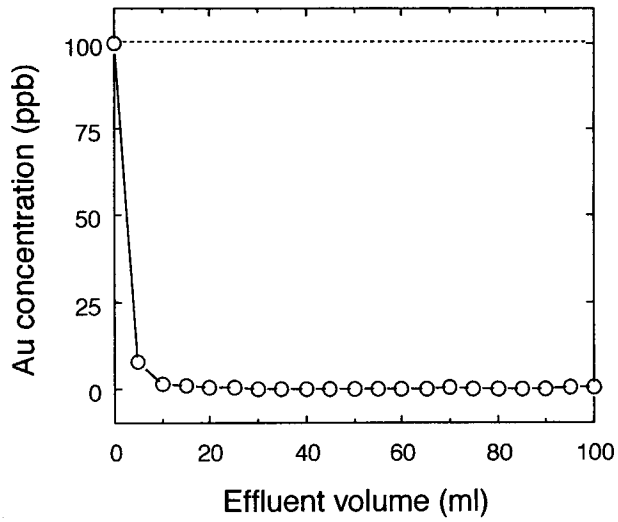


Fig. 7. Gold removal by the column packed with ESM (1.5 g) from a solution (pH 2.0) containing 100 ppb of gold at room temperature. Flow rate was about 50 ml/h.

with low concentration of metallic effluents such as waste-electroplating solutions. Actually, when the metals cyanized solution (25 ppm of gold with 18 ppm of zinc and 12 ppm of copper as co-ions) from gold electroplating of computer contacts was charged on the same ESM column, gold was efficiently removed from the solution to an undetectable concentration of gold (below 10 ppb; data not shown). Cyanidation was carried out by dissolving in aqueous 5% (w/v) sodium-cyanide solution. These experiments suggested that the application of such biosorption columns have a potential for the recovery and refining of gold from secondary sources such as electronic scrap and waste electroplating solutions.

It was found from the results that AFP have a high affinity for the binding gold-cyanide ions. A knowledge of the chemistry of AFP interac-

tions with gold might provide insight into mechanisms for environmental transport and deposition of gold. It appears that the AFP system might be useful in the recovery of gold from industrial waste waters or from direct mining operations. We have shown that ESM can be used for the purpose of removal/recovery of gold. Thus, it may be possible to use the AFP-adsorption system to recover gold complexes from mining dumps or present-day mining operations.

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