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# A New Biomaterial, Hen Egg Shell Membrane, to Eliminate Heavy Metal Ion from Their Dilute Waste Solution

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## ABSTRACT

The egg shell membrane (ESM) is an intricate lattice network of stable and water-insoluble fibers with high surface area. ESM accumulates and eliminates various heavy metal ions from dilute aqueous solution with high affinity and in short contact time, depending on pH and characteristics of the individual ion. Under certain conditions, the level of precious ions, Au, Pt, and Pd accumulation approaches 55, 25, and 22% of dry wt of ESM, respectively. Also uranium uptake 30% of that of ESM. Experiments suggested that ESM is promising to use for the purpose of removal/recovery of metals and water pollution control.

**Index Entries:** Egg shell membrane; metal ion; biosorption.

## INTRODUCTION

Almost all biominerals, including avian egg shells, have one common feature. They are composed of an inorganic (mineralized) phase, and an organic part consisting of proteins. The hen egg shell membrane (ESM) is an intricate lattice network of fibers and is suggested to be the part where

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calcium deposition takes place. Although the precise role of ESM on the mineralization of egg shell is not very clear, the insoluble protein would appear to be essential for mineralization to occur (*1–3*). From the fact of ESM mineralization, we considered that ESM would accumulate various types of inorganic ions.

It has been well reported that certain types of biomaterials, such as microbials (*4,5*) and seaweeds (*6–8*) possess a potential to sequester and accumulate inorganic ions in aqueous solutions. Metal concentration by biomaterials from a solution is termed biosorption, and industrial application of these are so-called "biosorbents." We found ESM protein eliminated various heavy metal ions from their dilute aqueous solutions in high yield and in short contact time, depending on pH and characteristics of the individual metal ion. ESM protein can be used repeatedly in the sorption-desorption for the accumulation of most heavy metals. ESM is therefore promising to use for removal/recovery of metals and water pollution control. This might also be pertinent to effluents containing radioactive metals. Biosorption is generally considered to be a rapid physical/chemical process and not expensive. The following positive considerations for this process using ESM can be taken into account:

1. Abundant resource (byproduct of egg industry);
2. Highly stable and water-insoluble;
3. Very high surface area with homogeniety and allows metal ions, as well as extremely large organic nutrient crosslinked protein molecule, to pass freely through; and
4. Egg shell consisted with calcium carbonate may not be obstacle for the process.

It is recognized that two ESMs surround the hen egg, a thick outer membrane attached to the shell and a thin inner membrane. Each of these membranes is composed of a network of fibers (*9*). The shell is attached to the outer of these membranes by the mammillae, which are roughly hemispherical knobs composed of large spherulite crystal of calcite (*2,10*). ESM contains crosslinking amino acids, such as desmosine and a large amount of cystine, suggesting that ESM consists of keratin- or elastin-like protein (*11,12*). In the recent report, collagen-like proteins were detected in the ESM (*9*). However, the structural proteins of ESM are still not well characterized.

This paper outlines some parameters in the potential use of a new biomaterial, ESM, for sequestering and accumulating heavy metal from aqueous solution.

## MATERIALS AND METHODS

ESM was mechanically stripped from the shells after immersion of the hen (White leghorn) egg shells, which were collected from local con-

fectionery, in 1% HCl overnight followed by rinsing with distilled water, and then desiccated over phosphorus pentaoxide under reduced pressure at room temperature for over 10 h. Approximately the same area (3 × 3 cm) of scraps of ESM (about 30 mg) were precisely weighed, then contact to the aqueous solution of individual metal ion. ESM was placed directly into the aqueous solutions containing various metal ions in 40-mL test tubes. The volume of solution was always 20 mL unless otherwise specified. Solution were shaken at 60 rpm in a thermostated water bath with shaker at 25°C for 3 h. Initial concentration of metal solution was always $3.0 \times 10^{-3}M$ unless otherwise specified. Metal ion solutions were prepared by dissolving metal salts or complex in distilled deionized water, then pH adjusted by addition of 2% HCl. Measurement of pH of solutions were done by glass electrode pH meter type F-12 (Horiba Inc., Kyoto, Japan). The effect of other ions present in the solution on the biosorptive desired metal uptake was examined using binary ion systems with different initial concentration of the coions. All the reactions were terminated by the separation of ESM from the solution with tweezers and then ESM was washed with the metal-ion free aqueous solution of the same pH.

The kinetics of palladium biosorption by ESM was investigated in a series of contact test tubes at the initial concentration of $3.0 \times 10^{-3}M$ at 25°C and at the desired pH. After desired durations of contact, the reactions were terminated, then the residual palladium concentrations were determined, and the result was the time-dependent palladium uptake profile. The biosorptive uptake capacity was determined as a function of residual metals concentration or by measurement of dry weight of ESM complexed with metals. Metal ion analysis was performed by using inductively coupled plasma atomic spectrometry (ICP AES: SPS 1200A, Seiko Instrument Inc., Tokyo, Japan).

For scanning electron microscopic (SEM) observation the egg shell was coated with a thin layer of gold and examined with a scanning electron microscope (Hitachi Model A-1300, Hitachi, Japan) at 15 kV of the acceleration voltage.

## RESULTS

Ultrastructural examination with SEM provided the evidence of physical structure of ESM as shown in Fig. 1. ESM is an intricate lattice network of protein fibers that has numerous organic knobs. The fiber diameter is estimated to be in the range of 1–3 μm.

ESM exhibited a certain degree of biosorption of all metal ions tested except $Na^+$ and $K^+$. These include $Na^+$, $K^+$, $Ni^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ag^+$, $Hg^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $U^{6+}$ (as $UO_2^{2+}$), $La^{3+}$, $Nd^{3+}$, $Pr^{3+}$, $Gd^{3+}$, $Ce^{3+}$, $Sm^{3+}$, $Eu^{3+}$, $V^{4+}$ (as $VO^{2+}$), $Au^{3+}$ (as $AuCl^{4-}$), $Pt^{2+}$ (as $PtCl_4^{2-}$), and $Pd^{2+}$ (data for all not shown). The metal ions not specified were added as sulfate, nitrate, or chloride salts. Figure 2 demonstrates the pH dependence

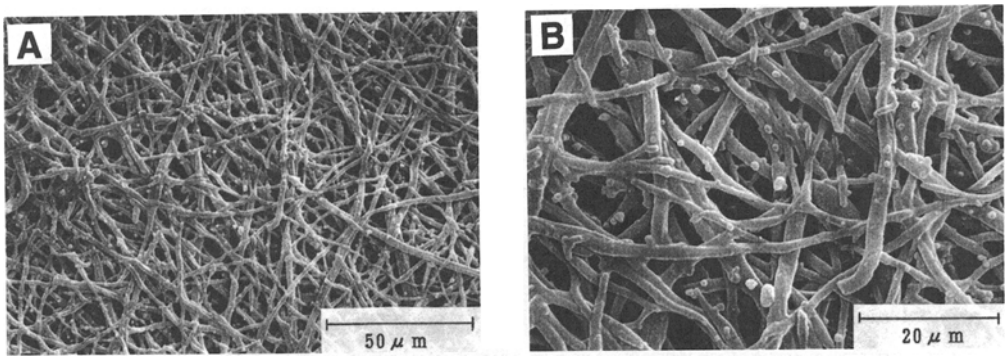


Fig. 1. SEM photographed display the structure of egg shell membrane stripped from the shell. (**A**) 1000 ×, (**B**) 2500 ×.

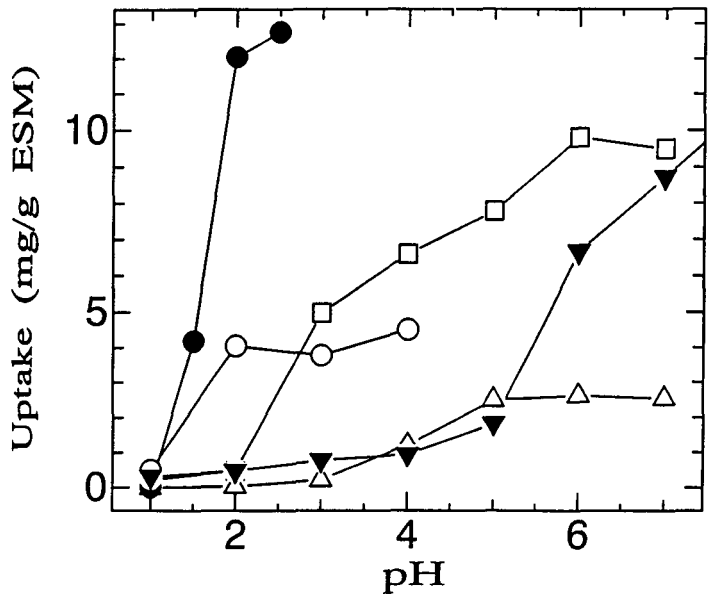


Fig. 2. Effect of pH on adsorption of common metal ions by ESM. (●) $Fe^{3+}$ (as $Fe_2(SO_4)_3$); (○) $Fe^{2+}$ (as $FeSO_4$); (□) $Cu^{2+}$ (as $CuSO_4$); (▼) $Zn^{2+}$ (as $ZnSO_4$); (△) $Ca^{2+}$ (as $CaCl_2$). Experimental details were given in the text.

of common metal ion uptake to ESM. The maximum uptake values for different common metal ions were ranging from a few mg/g ESM ($Ca^{2+}$) to 13 mg/g ESM ($Fe^{3+}$) and were dependent on pH.

In the preliminary determination of adequate contact time for the given experiments using $Cu^{2+}$, different time intervals ranging from 1 min to 24 h were examined. It was found that uptake was very fast and more than 95% of the copper uptake takes place within less than 10 min of contact with ESM. And also in the preliminary experiment of reproducibility using $Cu^{2+}$, 15 times determination of uptake values for 12 h contact time

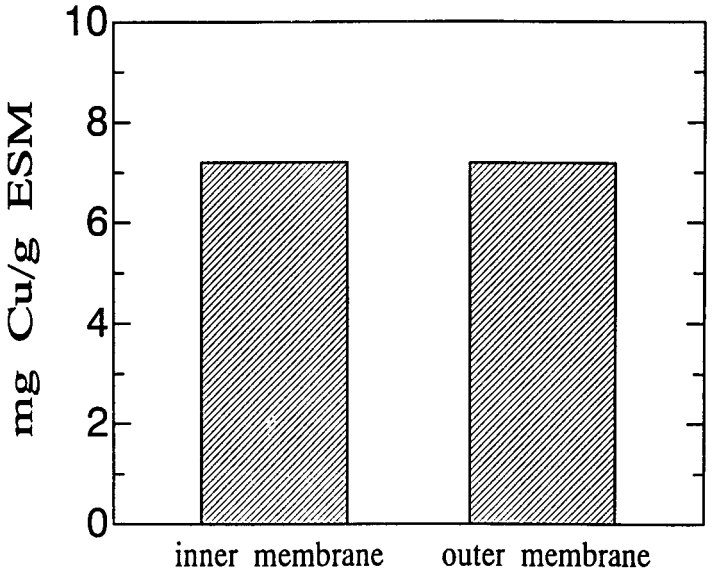


Fig. 3. Uptake of $Cu^{2+}$ to two different ESM, inner membrane and outer membrane. Experimental details were given in the text.

at pH 5.0 shows satisfactorily reproducible data (not shown). Figure 3 shows the effect of ESM layer of occlusion of $Cu^{2+}$, at pH 5.0 for 12 h contact time. There was no different uptake value between inner and outer membrane.

Figures 4 and 5 show the pH dependence of uptake to ESM of Ag, Zn, Co, and Cd, and lanthanoids, respectively. With the exception of $Ag^{+}$, $Fe^{3+}$, and $Cu^{2+}$, all of these metal ions are more weakly bound at lower pH and there are significant differences in behavior. For example at pH 3.0, nearly 90% of the $Ag^{+}$ is bound under the conditions of the experiment, whereas only less than 10% of the $Zn^{2+}$ is bound. This variation would enable one to perform selective elution of bound metal ions from ESM by judicious choice of pH. Lanthanoids show the same uptake profile for different pH as shown in Fig. 5.

Figure 6 shows the pH dependence of uptake to ESM of precious elements, Au, Pt, and Pd. These metal ions are bound to the ESM much more strongly than any other metal ion tested. Another unique aspect in the binding of $Au^{3+}$, $Pt^{2+}$, and $Pd^{2+}$ is the high metal binding capacity of the ESM for these ions, e.g., under certain conditions the ESM has a binding capacity for gold that is at least 55% of the dry wt of ESM (about 2.5 mmol of gold/g of dry wt ESM), for platinum that is at least 25%, and for palladium that is at least 22%. In contrast to the behavior of most metal ions under these conditions, the binding of precious elements is not completely reversed by lowering the pH of solution. Actually the biosorption of all of these precious elements to ESM takes place under the condition of 2% HCl solution (data not shown). However, exposure of metal-bound ESM to 2% HCl solution is effective in stripping these metal ions from ESM.

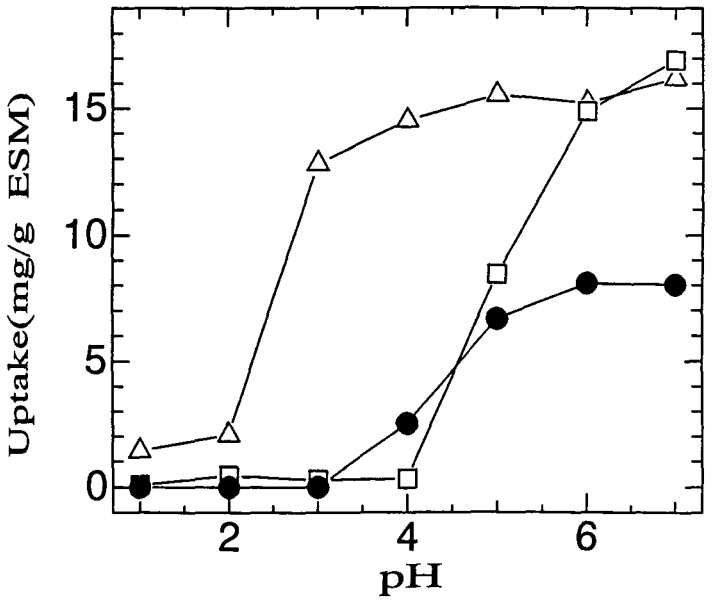


Fig. 4. Effect of pH on uptake of $Ag^{+}$ as $Ag_2SO_4$ (△), $Co^{2+}$ as $CoCl_2$ (●), and $Cd^{2+}$ as $CdSO_4$ (□).

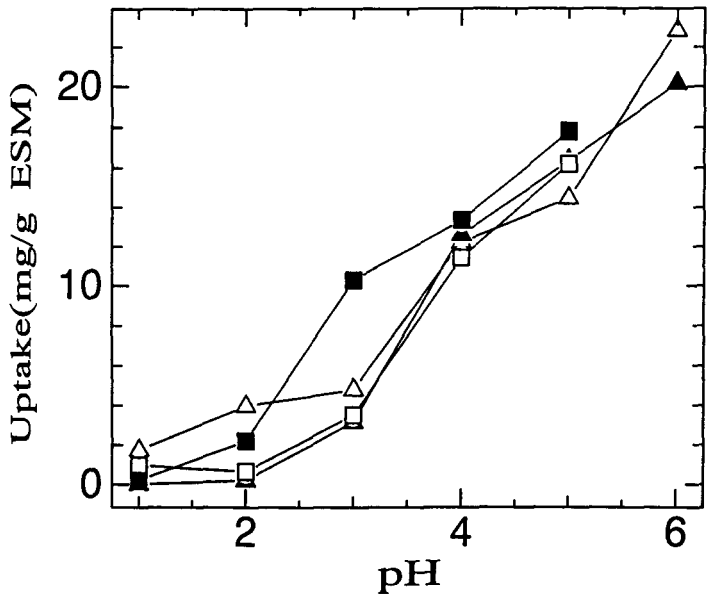


Fig. 5. Effect of pH on uptake of lanthanoids as nitrate. (■) $Sm^{3+}$; (□) $Pr^{3+}$ (▲) $Nd^{3+}$; (△) $Gd^{3+}$.

For example, roughly 80% of Au loaded at pH 4.0 to ESM was reversed to the 2% HCl solution. Under the condition of 2% HCl at room temperature, ESM was stable and can be used repeatedly. Interestingly, Pd is not bound to ESM at higher pH solution over pH 6.0, as shown in Fig. 5.

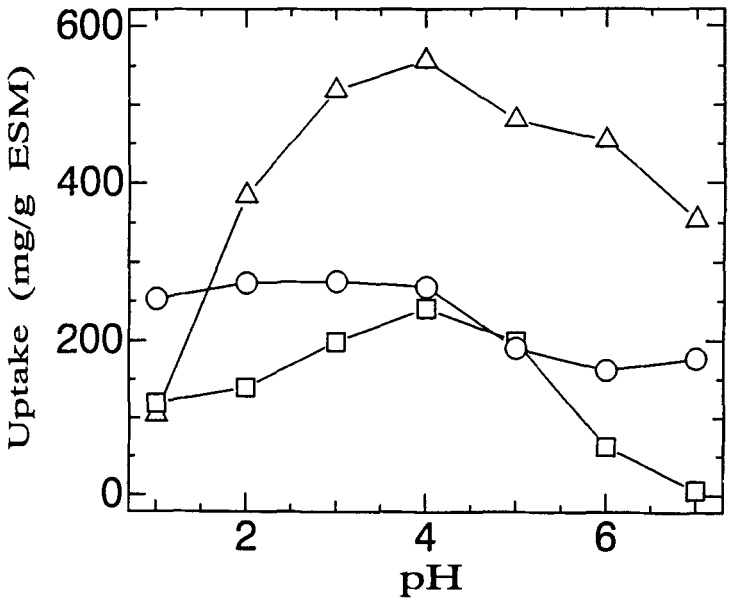


Fig. 6. Effect of pH on uptake of precious metal ions to EMS. (△) $Au^{3+}$ (as $AuCl_4^-$); (○) $Pt^{2+}$ (as $PtCl_2^{2-}$); (□) $Pd^{2+}$ (as $PdCl_2$).

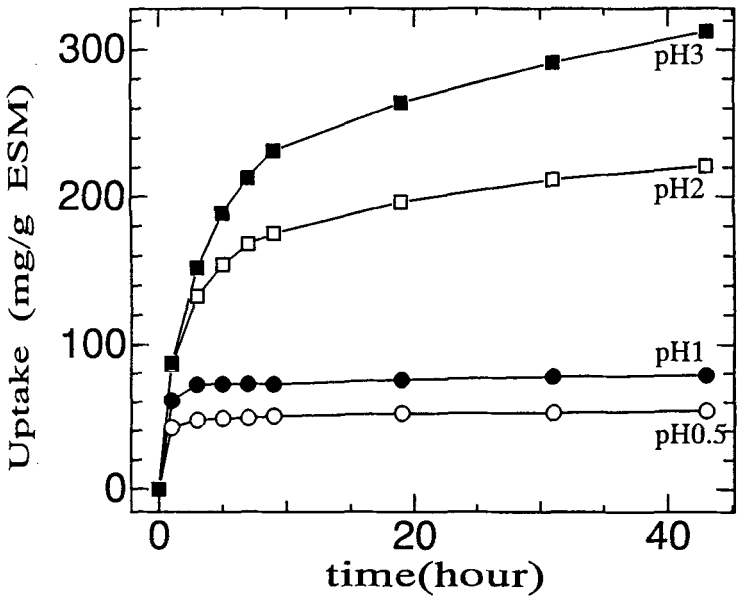


Fig. 7. Kinetics of $Pd^{2+}$ uptake at different pH as $PdCl_2$. Experimental details were given in the text.

Figure 7 illustrated that during the initial 45 h of shaking of ESM with the palladium ion solution at different pH values of 0.5, 1.0, 2.0, and 3.0, uptake appeared to be relatively fast. It was established that more than 80% the palladium uptake takes place within less than 5 h of contact with

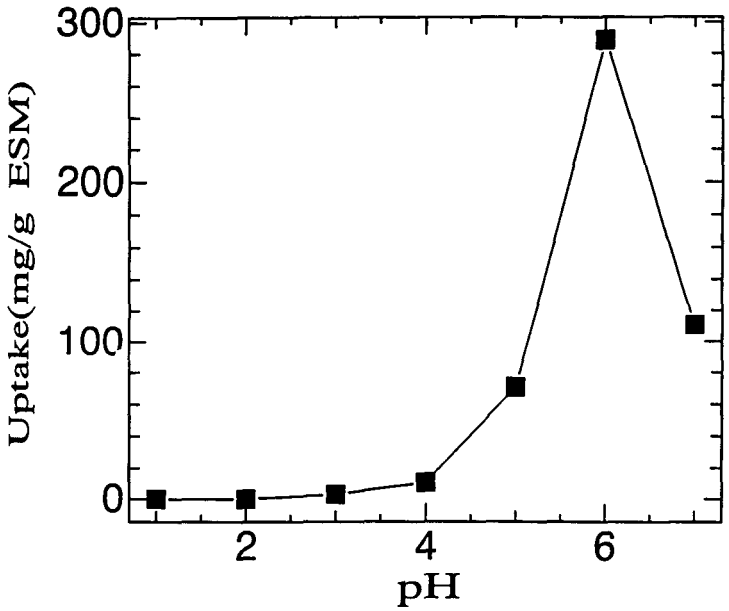


Fig. 8. Effect of pH on uptake of $U^{6+}$ as $UO_2(CH_3COO)_2$.

ESM. It is likely that this period represented surface binding by adsorption to binding site on the ESM. The rate of uptake decreased over subsequent time, with little further uptake up to a period of 45 h of shaking. The effect on palladium uptake of coexisting metal ions in the solution was examined. $Cu^{2+}$ and $Ni^{2+}$ were added to the $3.0 \times 10^{-3}M$ palladium solution at pH values of 1.0, 2.0, and 3.0 for five times concentration of co-ions. It was observed that at all pH, no uptake of co-ions takes place and no significant change in uptake rate and capacity of palladium was observed in the presence of the coexisting metal ions (data not shown).

Figure 8 shows the pH effects on the uptake of $UO_2^{2+}$ to ESM. Surprisingly, ESM plays very unique behavior for uranium to have the outstandingly high binding capacity: i.e., under pH 6.0 ESM has binding capacity that is at least 30% of dry wt of ESM. And the binding of uranium is completely reversible by lowering with respect to the pH at 4.0. From this behavior, we show that ESM could be applicable to the removal and/or recovery of radioactive metals from the effluents containing these metals.

ESM as biosorbent is mainly composed of protein and some biological organic such as saccharides, lipids and some inorganic component occurring naturally such as salts of metal cations (*13*). The identity and function of the protein components of the double membrane is not completely understood. It is supported the involvement of collagen-like connective tissue protein network in the ESMs. And ESM fibers were observed coated with a layer of flocculent ruthenium red-staining material suggestive of glycoproteins or proteoglycans that were intimately associated with the fibers (*9*). The precise role of these structure is not known.

The identities of the functional groups responsible for metal ion binding to ESM are unknown at this time. There are some unique groups containing in ESM protein, that is aldehyde, Shiff base, quaternary pyridinium, secondary amine. On the other hands, there is extremely high concentration of cystine residue compared to that of other protein such as collagen and elastin suggest that high proportion of sulfhydryl and disulfide groups are composed to ESM protein (*11*). These groups may be groups responsible for precious metal ions binding. Further experiments are in progress to characterize the location of binding sites and the mechanisms of binding of the precious metals, as well as for other metals.

It has been proposed to prohibit or strictly control the discharge of hazardous heavy metals into environment. Conventional techniques for metal recovery and eliminate from effluents prior to discharge include precipitation, ion-exchange, electrolysis, evaporative distillation, liquid-liquid extraction and solvent extraction. Unfortunately, these techniques are likely to become increasingly expensive. Whatever the nature of ESM, this essential point can be noted: ESM has an unusually high affinity for the binding of precious metal ions and uranium. This process may be useful in the area of water pollution control and waste-water treatment, and the process may also have direct application in the mining. One must point out that egg shell consisted with calcium carbonate was not obstacle for the effect on biosorption.

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