DOI: 10.1007/s10967-007-7006-5 *Journal of Radioanalytical and Nuclear Chemistry, Vol. 275, No.2 (2008) 423–426*

# Preconcentration of gold by *Mimusops elengi* seed proteins

**D. Nayak,[1] K. M. Hazra,[2] S. Laskar,[2] S. Lahiri[1]***

[1] *Chemical Sciences Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata-700 064, India*
[2] *Natural Product Laboratory, Department of Chemistry, The University of Burdwan, Burdwan 713 104, India*

(Received April 23, 2007)

The present study has been performed to preconcentrate gold using the proteins extracted from *Mimusops elengi* Linn. (Family: Sapotaceae) seed by radiometric technique using $^{198}$Au. Effects of buffer and pH dependence on the binding affinity of the gold have also been examined. It has been found that the binding of gold with *M. elengi* protein neither depends on the addition of buffer nor the composition (phosphate/citrate) of buffer. The adsorption of gold is also independent of pH of the solution. To verify the gold-protein interaction, inter-comparisons have been made between four different approaches, (1) notably extraction with anion-exchange resin Amberlite IRA 400, (2) trichloroacetic acid (TCA) precipitation, (3) isoelectric precipitation and (4) dialysis of protein after incubation with gold. Good agreement has been observed for all the cases. Binding of gold have been studied with three different concentrations of gold, 1, 10 and 50 ppm spiked with $^{198}$Au.

## Introduction

Several separation techniques are available for preconcentration of scanty distributed metals. These include precipitation, co-precipitation, electrolysis, liquid-liquid extraction and solid-liquid extraction. Conventional method of recovery of gold involves several well defined techniques such as cementation, solvent extraction, etc. These studies have been performed for the recovery of gold from HCl solutions using various extractants like basic amines, organophosphorous compounds and extractants containing sulphur as donor atom.[1,2] Recently, attention has been devoted to the use of bio-reagents to accumulate or preconcentrate different metal ions because of their ability to absorb ions from solution. Proteins derived from various natural sources also act as potential agent for metal separation as well as preconcentration, through its selective metal binding affinity. Abundance of the materials and their selectivity make them powerful tools for analytical measurements. The sorption of element species by biological substrates involves less amount of chemicals as well as they offer interesting technological applications in the recovery of precious metals.

Earlier, bio-sorption of gold has been reported in a fungal isolate, *Cladosporium cladosporioides*.[3] A few examples have shown that some gold complexes can be quantitatively separated from solution by a pure strain of algae *Chlorella vulgaris*.[4,5] Earlier it has been found that the *Rhizoclonium riparium* a member of Chlorophyceae can accumulate gold from solution of wide pH range.[6] The two members of bacillariophyceae, *Nitzschia obtusa* and *Navicula minima,* are also efficient for pre-concentration of gold from solution with maximum 1 mg·kg$^{-1}$ gold concentration.[7] However, to the best of our knowledge until now seed protein has not been utilized as bio-reagent to preconcentrate gold or any other metal.

The aim of the present study was to find the efficacy of *Mimusops elengi* seed protein as a bio-reagent for the extraction of gold. Bakul (*Mimusops elengi* Linn.) belonging to the family Sapotaceae, grows wild and is also cultivated for its ornamental appearance and fragrant flowers throughout India. It is mainly used as valuable wood for building purposes, bridges, boats, cars and also used as folk medicines.[8,9] Although the plant is widely cultivated under Indian natural forestry program, a large amount of seed is thrown away, even though it contains appreciable amount of nitrogen and protein. We have used $^{198}$Au as tracer of gold to study the analytical use of this seed protein for preconcentration of trace amount of gold. The use of spiked radionuclides to understand the efficacy of protein in metal binding process is an efficient method as it estimates the accurate amount of metal binding at very low concentration and also the detection of metal becomes much easier than by the conventional methods. As there are very few reports on the metal protein interaction with radionuclides, it has been felt that intercomparison among various approaches can validate the method of investigation. In the present study, metal protein interaction and extent of gold accumulation by the seed protein have been investigated using four different approaches: (1) adsorption of unbound gold using anion-exchange resin, (2) trichloroacetic acid (TCA) precipitation, (3) isoelectric precipitation and (4) dialysis of protein after incubation with gold radionuclides.

## Experimental

### *Collection and isolation of M. elengi seed protein*

*M. elengi* seeds were collected from the Forest Department, Burdwan, W. Bengal, India, in the middle

* E-mail: susanta.lahiri@saha.ac.in

*0236–5731/USD 20.00*

*Akadémiai Kiadó, Budapest*
*Springer, Dordrecht*

of January 2003. Finely powered bakul (*M. elengi*) seeds were deoiled with petroleum ether at 40–60 °C for 60 hours, washed twice with chloroform-methanol mixture (3 : 1) and air dried. Protein extraction from deoiled seed was carried out at room temperature for 30 minutes using distilled water (50 : 1, v/w) at various pHs between 2 and 12. The respective pH levels were maintained throughout the experiments.[10–12] Protein was dialyzed against 0.01M phosphate buffer (pH 7.0) for 48 hours at 4 °C and freeze dried. A schematic diagram for the extraction of protein from *M. elengi* seed is presented in Fig. 1.

Molecular weight of the protein was determined by gel filtration technique. For this purpose protein was extracted with 0.1M NaCl solution at pH 8.0. Four peaks were obtained by gel filtration using Sephadex G-200 column (2.5×45 $cm^2$) at 20 °C.[13] The molecular weights of these components corresponding to the four peaks, i.e., A, B, C, D (Table 1) were determined from a linear curve that was calibrated using reference protein standards (BSA, ovalbumin, pepsin and lysozyme).[14] The isoelectric point of the protein was found pH 4.

*Preparation of spiked gold solution*

The $^{198}Au$ was obtained from the Board of Radiation and Isotope Technology (BRIT), Mumbai, India in the form of chloroauric acid solution. Different concentrations of gold solutions (1 ppm, 10 ppm and 50 ppm) were prepared by dissolving pure gold foil in aqua regia and spiked with $^{198}Au$. Finally each solution were evaporated to dryness and taken in 0.01M HCl. The amount of gold adsorbed was measured by monitoring 411.8 keV photopeak of $^{198}Au$ ($T_{1/2} = 2.693$ d) by γ-spectrometry with the help of an HPGe detector coupled with a PC based multi-channel analyzer (Gamma Fast) and having a detector (Baltic Scientific) resolution of 2.0 keV at 1.33 MeV.

*Adsorption of gold to the protein*

In order to study the binding of metal ions with the protein four different methods were applied. Protein solution of concentration $4 \cdot 10^{-6}$M (considering average molecular weight) was prepared by dissolving the protein in distilled water. The pH of the stock protein solution was 7.3. Measured amount of protein solution was incubated with a measured amount of $^{198}Au$ tracer solution for 40 minutes at room temperature (20 °C). Dependence of $^{198}Au$ radiotracer binding with protein on pH was studied separately with 0.01M tri-sodium citrate and 0.01M phosphate buffer at four different pH levels such as 7.1, 7.2, 7.4 and 7.7. In each case equal volume of protein solution was mixed with equal volume of buffer solution and incubated for 40 minutes with same amount of $^{198}Au$ tracer solution. After incubation 0.1 g of Amberlite IRA 400, an anionic-exchanger was added to each solution, shook for 10 minutes, then centrifuged and settled (Method I). A fraction of supernatant was removed and assayed for $^{198}Au$ by means of HPGe detector. Appropriate decay corrections have been made in each set of data. Only the free gold ions would bind to Amberlite and the gold bound to protein will remain in the solution. Effect of phosphate buffer at pH 7.4 was studied in other three methods.

In Method II, after incubation of gold with protein, 1% trichloroacetic acid (TCA) was added to the above solution, and kept for 1 hour for complete precipitation of proteins from the solution. It was expected that the metals bound with protein would be precipitated and only free metals would remain in the solution. After precipitation solution was centrifuged followed by ultra filtration using Millipore syringe filter of pore size of 0.2 μm. The filtrates were assayed for gold by γ-spectrometry.

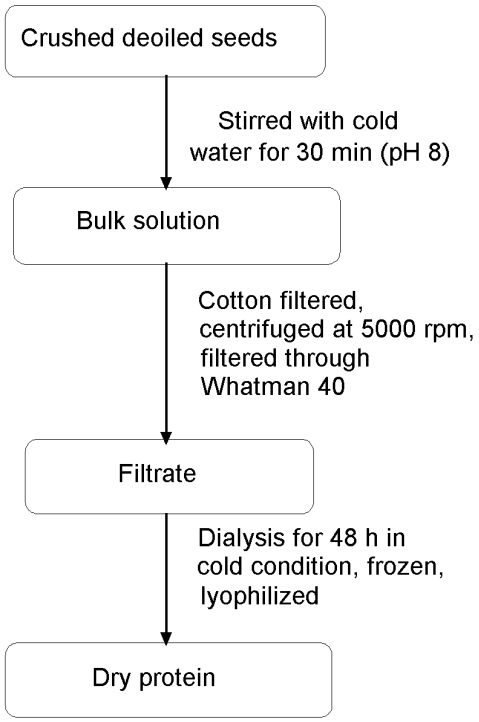


*Fig. 1.* Schematic diagram of extraction of *M. elengi* seed protein

*Table 1.* Relative amount of isolated protein fractions from the deoiled seeds of *M. elengi* fractionated by gel filtration with Sephadex-D-200

| Protein fraction | Molecular weight determined by gel-filtration curve | Relative amount, % |
|---|---|---|
| Fraction A | 355000 | 67.55 |
| Fraction B | 42700 | 1.32 |
| Fraction C | 31600 | 2.32 |
| Fraction D | 21800 | 28.81 |

Average molecular weight: 247380 Daltons.

In Method III, 1M HCl was used to precipitate the proteins and metal binding was assayed like Method II.

For dialysis (Method IV), the protein solution was incubated with gold solution in a dialysis sac (pore size 12 kDa MWCO) for 40 minutes. Immediately after the addition of gold solution, count of the dialysis sac was taken. Dialysis was started against cold Millipore water. Gamma-spectra of the dialysis sac were taken every 1 hour time intervals and compared to that of the first one. Only metals bound with the protein remained in the dialysis sac and free metals were diffused to the water outside the sac. Appropriate decay corrections have been made in each set of data.

To study the effect of concentration of gold on its binding with protein the other three sets of experiments were carried out without addition of buffer. Protein solution of same concentration was incubated with 1 ppm, 10 ppm and 50 ppm gold solutions spiked with $^{198}$Au at room temperature (20 °C). Adsorption of gold was measured by four methods as mentioned above.

## Results and discussion

Protein content of the deoiled seeds was found to be 18.13%. The solubility profile showed that it is more soluble in alkaline pH than acidic one. Amino acid analysis of the seed protein revealed the presence of sixteen amino acids, of which nine were essential (Table 2). Isolated *M. elengi* seed protein was rich in aspartic acid, glutamic acid, glycine, valine, leucine and arginine, but low in methionine.[14]

First the interaction of protein with trace amount of $^{198}$Au was studied. The anion-exchanger Amberlite IRA 400 can adsorb quantitatively $AuCl_4^-$ ions from a gold solution. Thus adequate amount of Amberlite IRA 400 was added to the protein solution after incubation with $^{198}$Au tracer so that free $AuCl_4^-$ ions present in the solution will be adsorbed by the resin and the supernatant solution would indicate only the amount of $^{198}$Au bound with protein (Method I). It was found that the gold adsorption was almost pH independent, with a little fluctuation. In the presence of citrate buffer, the adsorption percentage of gold with *M. elengi* seed protein is slightly less than that of phosphate buffer in the pH range 7.1 to 7.4 (Fig. 2). However, the difference is insignificant and it can be concluded that the gold adsorption neither depends on the addition of buffer nor the composition (phosphate/citrate) of buffer.

Effect of phosphate buffer was studied by other three methods at pH 7.4. The results of inter-comparison of these four approaches have been shown in Fig. 3. It has been observed that Methods II, III, IV, were in good agreement for binding of $^{198}$Au radionuclide (99% maximum) with the seed protein. In Method I apparently less binding of gold with protein was observed than that of other three methods. This may be due to the fact that ion-exchange resin is capable of breaking the metal protein linkage, which in turn shows the less binding of gold than the actual one. Also it is noteworthy to mention that the isoelectric point of the seed protein (pH 4) was less than the experimental pH. Therefore, the net surface charge of the protein will be negative, and part of the protein may have been bound to the resin along with free gold ions. However, the gold binding with the seed protein was not affected in presence of phosphate buffer.

Three different concentrations of gold solutions (1 ppm, 10 ppm and 50 ppm) were used in absence of buffer to understand the role of substrate concentration on the adsorption process. As Methods II, III, IV corroborate each other; the role of substrate concentration has been checked only by dialysis method (Method IV). Figure 4 represents adsorption percentage of $^{198}$Au in protein obtained through dialysis with varying concentration of gold solutions. Binding of gold with protein increases with increasing gold concentration up to 50 $mg \cdot kg^{-1}$ gold concentration. Therefore, the amount of protein taken in this experiment is capable of preconcentrate gold even from 50 ppm gold solution.

*Table 2.* Amino acid composition of isolated *M. elengi* seed protein

| Amino acid | g/16 g nitrogen[15] |
|---|---|
| Aspartic acid | 10.38 |
| Threonine* | 5.46 |
| Serine | 6.72 |
| Glutamic acid | 14.83 |
| Proline | 6.00 |
| Glycine | 9.77 |
| Alanine | 8.82 |
| Valine* | 7.03 |
| Methionine* | 0.81 |
| Isoleucine* | 4.46 |
| Leucine* | 8.06 |
| Tyrosine | 2.83 |
| Phenylalanine* | 2.99 |
| Histidine* | 2.10 |
| Lysine* | 3.57 |
| Arginine* | 6.12 |

*Essential amino acids.

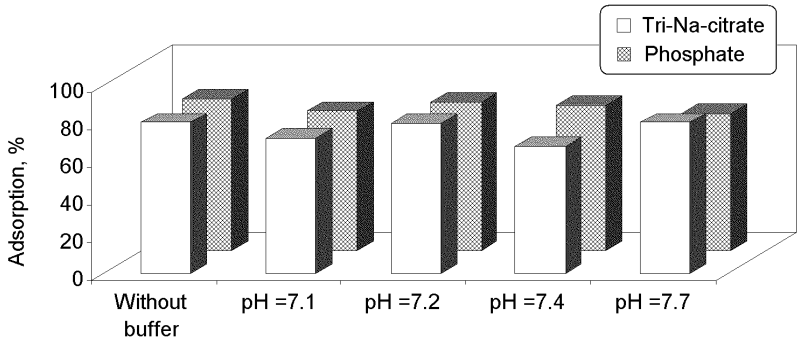


*Fig. 2.* Adsorption percentage of $^{198}$Au tracer in protein with tri-sodium citrate and phosphate buffer of various pH

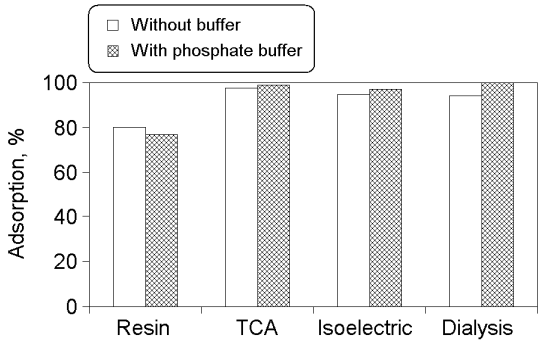


*Fig. 3.* Comparison of results on $^{198}$Au tracer binding in protein with and without phosphate buffer at pH 7.4 obtained through different approaches

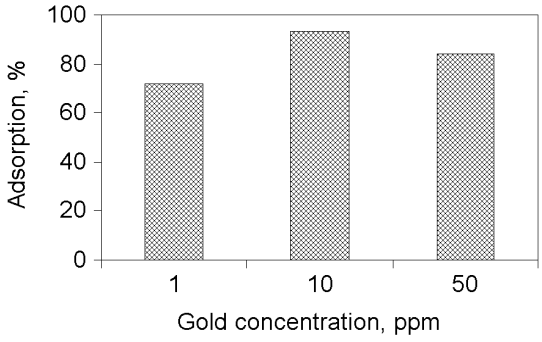


*Fig. 4.* Adsorption percentage of $^{198}$Au in protein with various concentration of gold solutions in absence of buffer obtained through dialysis

## Conclusions

From the present experiment it can be concluded that the protein derived from the *Mimusops elengi* seed efficiently accumulates gold and may be useful bio-reagent for preconcentration of gold from natural solution. The net charge of the protein is important for binding of the metals. It can be said from the amino acid composition of *Mimusops elengi* seed protein (Table 2) that the active binding sites of this protein are mainly $COO^-$ and $NH_3^+$ groups. Gold remains in solution as $AuCl_4^-$ ions, which may bind with the $NH_3^+$ group. The various active binding sites of protein like $NH_3^+$, imidazole nitrogen of histidine, etc., are responsible for efficient accumulation of gold. However, further investigation is required to identify the exact binding sites to which gold is attached. The potentiality of natural resource to preconcentrate precious metals will help to exclude the use of hazardous chemicals in similar process and is a step toward green chemistry. Use of radioactive tracers is an efficient method to quickly scan the potentiality of different bio-reagents for using them as prospective alternative to hazardous chemicals.

## References

1. N. R. DAS, S. BANERJEE, K. CHATTERJEE, S. LAHIRI, Radiochim. Acta, 83 (1998) 39.
2. N. R. DAS, S. BANERJEE, K. CHATTERJEE, S. LAHIRI, Appl. Radiation Isotopes, 50 (1999) 643.
3. A. V. PETHKAR, K. M. PAKNIKAR, J. Biotechnol., 63 (1998) 121.
4. M. HOSEA, B. GREENE, R. MCPHERSON, M. HENZI, M. D. ALEXANDER, D. W. DARNALL, Inorg. Chim. Acta, 123 (1986) 161.
5. B. GREENE, M. HOSEA, R. MCPHERSON, M. HENZI, M. D. ALEXANDER, D. W. DARNALL, Environ. Sci. Technol., 20 (1986) 627.
6. D. NAYAK, M. NAG, S. BANERJEE, R. PAL, S. LASKAR, S. LAHIRI, J. Radioanal. Nucl. Chem., 268 (2006) 337.
7. N. CHAKRABORTY, R. PAL, A. RAMASWAMI, D. NAYAK, S. LAHIRI, J. Radioanal. Nucl. Chem., 270 (2006) 645.
8. L. D. KAPOOR, in: C. R. C. Handbook of Ayurvedic Medicinal Plants, CRC Press, New York, 1990, p. 232.
9. Editorial Committee, CSIR; The Useful Plants of India, Publication and Information Directorate, CSIR, New Delhi, India, 1986, p. 375.
10. B. BASAK, U. K. BHATTACHARRYA, A. SINHABABU, S. LASKAR, Appl. Biochem. Biotechnol., 49 (1994) 281.
11. M. J. GUERRA, Y. K. PARK, J. Am. Oil Chem. Soc., 52 (1975) 73.
12. S. LASKAR, A. SINHABABU, S. THAKUR, B. BASAK, J. Am. Lab., 30 (1998) 22.
13. J. WHITAKER, Anal. Chem., 35 (1963) 1950.
14. K. M. HAZRA, S. LASKAR, Adv. Food Sci., 27 (2005) 116.
15. AOAC Official Methods of the Analysis of the AOAC, Association of Official Analytical Chemists, 12th ed., Washington D.C. 1975, p. 744.