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Short communication

Biomolecule–metal interactions: Applications in extraction and separation techniques

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ABSTRACT

We have isolated and characterized an alkaloid, piperine and a protein arachin, from black pepper and groundnut respectively. Interactions of these compounds with various metal ions in trace scale from different domains of the periodic table were studied using radiometric method. It is revealed that piperine has high selectivity and specificity towards gold. The protein arachin shows high specificity towards mercury only. The high selectivity of these two bioreagents for the specific metal ions Au and Hg show that the bioreagents have considerable potential to replace the synthetic chemicals and sometime they are superior to synthetic one.

1. Introduction

The philosophy of green chemistry enforces mandatory minimization of the use of hazardous synthetic chemicals. Many bioreagents have shown promising ability for metal preconcentration and separation. Some of our earlier studies indicate that natural reagents are powerful and potential candidates in the field of analytical chemistry. For example, an environment friendly radiochemical methodology for separation of no-carrier-added (nca) thallium radionuclide from nca lead and nca mercury radionuclides was developed using algae as bioreagent. [1]. Experiments were carried out with different algal genera from different taxonomical groups (e.g., green alga, *Oedogonium cardiacum*; blue green alga, *Spirulina subsala*; and red alga, *Catenella repens*) to examine their usefulness in removing different radionuclides from 'tracer packet of heavy metals' [2]. It has been found that at basic pH cyanophycean members are most suitable for heavy metal biosorption [3]. Similarly, *Saccharomyces cerevisiae*, common Baker's yeast has been found as highly selective reagent for extraction of ^{152}Eu [4].

However, the main disadvantages of using direct biological species are (i) seasonal availability, (ii) difficult and time consuming to maintain the culture medium and (iii) reproducibility of biological species. Therefore in the second stage of our green chemistry programme, we have used bioreagents like protein, alkaloid or some other moieties derived from various biological species [5–6].

In this work we present the efficacy of two naturally occurring reagents, e.g., piperine and arachin in extraction and preconcentration processes of metal ions. Piperine is an alkaloid found naturally in *Piper nigrum* L. (Family: Piperaceae). In our earlier work, we found that piperine is a potential reagent for extraction of gold [6]. In this work we report the efficacy of this alkaloid as extracting agent in other domains of the periodic table.

Similarly, proteins derived from various natural sources may act as potential agent for metal separation as well as preconcentration, through its selective metal binding affinity. Groundnut, *Arachis hypogaea*, is abundant all over the world. It is rich in protein content, arachin being the major protein in groundnut. Arachin is well characterized with a molecular weight of about 33 kDa [7]. Considering the possible metal binding sites containing sulphur and nitrogen [8], interaction studies of this protein with different metals have been carried out.

Both of these studies arouses an interesting question whether naturally occurring substances can really replace synthetic chemicals in terms of selectivity and specificity. This attempt is our humble approach to rewrite some chemical processes with greener alternatives even with some affordable compromise with respect to yield and purity.

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2. Experimental

Triethylamine and $CaCl_2$ were procured from Merck (Darmstadt, Germany) and Merck (Mumbai, India) respectively.

2.1. Extraction and purification of piperine

Isolation of piperine was carried out as per the method described by Ikan [9]. Piperine was purified by repeated crystallization from benzene and petroleum ether (b.p. 60–80 °C). The purity of the crystals was checked by TLC, UV–visible spectroscopy and melting point verification [6].

2.2. Isolation of the arachin fraction of protein from groundnut

Blanched groundnuts were refluxed in light petroleum for 1 h and filtered in order to expel the oil. The solid residue was extracted with 0.1% sodium hydroxide solution. The solution of sodium proteionate was filtered off and the protein was precipitated by addition of dilute sulphuric acid until the isoelectric point (pH 5) was reached. The protein was allowed to settle, filtered and washed free from salt and then dried.

Dried protein was shaken with 2% aqueous sodium chloride to give a solution of conarachin. The residue was extracted with 10% aqueous sodium chloride to give a solution of arachin and an insoluble residue [10]. 1 M $CaCl_2$ was added to the crude arachin extract when oily precipitate was collected and re-dissolved in 0.025 M EDTA in 10% (w/v) NaCl, the pH being maintained at ~8 by drop wise addition of triethylamine [11]. The solution was dialysed against the solvent twice followed by dialysis against water and lyophilized. Then the protein was subjected to electrophoresis. This protein solution was lyophilized to obtain solid protein for further experiments.

2.3. Procurement and production of radionuclides

The radioisotopes ^{59}Fe ($T_{1/2}$ = 44.47 d), ^{60}Co ($T_{1/2}$ = 5.27 y), ^{134}Cs ($T_{1/2}$ = 30 y) and $^{152,154}Eu$ ($T_{1/2}$ = 12.5 y) were obtained from the Board of Radiation and Isotope Technology, BRIT (Mumbai, India). Weighed amounts of sodium arsenate (Na_2HAsO_4, $7H_2O$) and sodium arsenite ($NaAsO_2$) salts were irradiated with thermal neutron in the CIRUS reactor, BARC, Mumbai, with a neutron flux 9×10^{12} cm^{-2} s^{-1} to produce ^{76}As ($T_{1/2}$ = 1.087 d) through ^{75}As (n, γ) reaction in the corresponding matrix. ^{51}Cr ($T_{1/2}$ = 27.7 d) tracer was obtained by neutron activation of $Cr(NO_3)_3$ with a flux of 10^{11} cm^{-2} s^{-1} for 6 h.

Some of the radionuclides were produced using a 12 MV pelletron facility at BARC-TIFR Pelletron, Mumbai. A gold foil of thickness 18 mg/cm^2 was irradiated with collimated 48 MeV beam of $^{7}Li^{3+}$ to produce $^{199-201}Tl$, $^{199-200}Pb$, ^{197}Hg in the gold matrix.

The product radionuclides were detected with their corresponding γ-energy peaks. The irradiated gold foil was dissolved and spiked with ^{198}Au. This solution was then used for sorption studies of $^{199-201}Tl$, $^{197,199-201}Pb$, ^{197}Hg and bulk gold onto piperine. Gamma spectroscopic analysis was done with the help of an HPGe detector having a resolution of 2.0 keV at 1.33 MeV for studying the metal protein interaction with these aforesaid radionuclides.

2.4. Accumulation study with piperine

Sorption was examined in batch mode, with 0.1 g of piperine taken in HCl solution containing measured amount of radionuclide solution. The pH of the final solution was maintained by addition of dilute HCl or NH_3. A 2 mL fraction of the supernatant liquid was removed, and assayed for radionuclides by means of gamma spectrometry. Studies of metal uptake with varying weights of piperine were also performed.

2.5. Interaction of radioisotopes with the protein

Results of gel electrophoresis revealed that Arachin is composed of different molecular weight fractions, of which we have separated the 21 kDa fraction and used in our experiments (Precision Plus Protein Standard by BIO-RAD was used as marker lane). Fig. 1 shows the result of gel electrophoresis. A single band in the molecular weight region of 21,000 Da confirms the separation of this subunit of arachin in pure form.

Its interactions with various metal ions from different domains of the periodic table were studied using radiometric method. 0.1 g of the solid protein obtained after lyophilization was dissolved in 4 mL of 0.1 M acetate buffer. This protein solution was incubated with 100 μL of active solution containing the radioisotope solution in 10^{-5} M HCl medium for 30 min at room temperature (20 °C). The protein–radioisotope mixture was dialyzed against triple distilled water for 7 h. Immediately after the addition of the active solution, count of the dialysis sack (a cellulose sack) was taken. Dialysis was started against deionized water. γ-ray spectra of the dialysis sack containing the protein and active solution was obtained after 1 h time intervals. Only metals bound with the protein remained in the dialysis sac and free metal was diffused to the water outside the sack.

To confirm the metal–protein interactions, three other methods were applied including ion exchange, trichloroacetic acid (TCA) precipitation and HCl precipitation. To the radioisotope incubated protein solution, 0.1 g of Dowex 50 (1 × 8, 20–50 mesh), a cation exchanger, was added and was shaken for 10 min, then centrifuged and settled. A fraction of supernatant was removed and assayed for radionuclides by means of HPGe detector. It is assumed that only the free ions will bind to Dowex-50 and the metal bound to protein will remain in the solution. Among the other two methods, after incubation, 1% (TCA) was added to the radionuclidic solution, and kept for 1 h for complete precipitation of proteins from the solution. It was expected that the metals bound with protein would be pre-

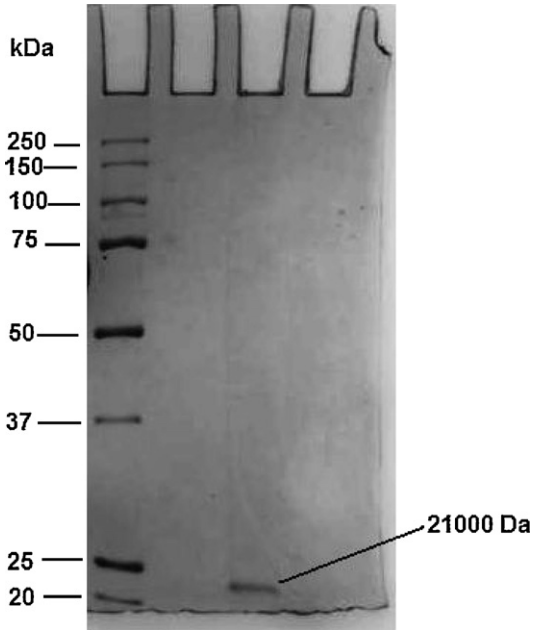

Fig. 1. The SDS-PAGE after gel electrophoresis showing the marker lane (left) and the purified 21 kDa molecular weight fraction of arachin (right).