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A Simple and Novel Method to Reduce Heavy Metal Contamination from Aqueous Solutions and Herbal Extracts

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Abstract

Objectives: Alternate methods and new technology that can effectively remove heavy metals from aqueous solutions is increased as a result of the industrial revolution. Such methods find immense use in reduction of heavy metals in herbal extracts making it safe for human consumption.

Methods: A simple, rapid, and novel method has been developed and validated for the removal of metal ions from aqueous solutions and also from herbal extracts using Primary Secondary Amine (PSA). The performance of the proposed method was examined for various parameters for comparable efficiencies such as ratio of PSA to the heavy metal concentration, temperature of reaction and time required for optimal removal of the heavy metal by PSA. The application of this method to remove different types of heavy metals at a time to generate a safe herbal extract can be considered as one of the new promising approaches.

Results: The method of using PSA to remove metal ions from solutions appears robust since it is not affected by temperature and is simple and easy to perform.

Conclusions: Removal of heavy metal pollution still represents a big challenge for ecologists hence cost-effective methods to have potential industrial applications are lucrative and has good prospects in industrial use. It is concluded that this method can be successfully applied for the removal of metal ions from contaminated water samples and from herbal extracts.

Keywords: Primary secondary amine; Heavy metal; Lead; Herbal extracts; Waste water.

Introduction

Heavy metal contamination in herbal medicines is also a global threat to human beings especially at levels above known threshold concentrations. Some of the well-known heavy metals that may be naturally found in the environment include Arsenic, lead, cadmium, mercury. These heavy metals or toxic elements are often at higher levels that could be due to industrial uses and pollution. Toxicity associated with use of Ayurvedic products due to metal content is an increasingly recognized potential public health problem [1]. The study revealed that lead was found in 65% of 252 Ayurvedic medicine samples with mercury and arsenic found in 38 and 32% of samples, respectively. Almost half of samples containing mercury, 36% of samples containing lead and 39% of samples containing arsenic had concentrations of those metals per pill that exceeded, up to several thousand times, the recommended daily intake values for pharmaceutical impurities.

Similarly, detection of heavy metals in wastewater is reported. Some of the human interventions such as like electroplating industry, tanning industry, textile industry etc release residual heavy metals and such heavy metal contaminated wastewater finds its way into the environment, causing a major threat to

human health and the ecosystem. Currently, methods for heavy metal ions removal, such as Electrocoagulation (EC), adsorption using synthetic and natural adsorbents, magnetic field implementation, advanced oxidation processes, membranes, etc. are reported [2]. Several researchers have widely used cryogels as a unique bio-adsorbent for heavy metals removal from wastewaters [3]. To address this challenge, we attempted to look at an alternate method for reducing lead content in standard solutions using the property of adsorption by Primary Secondary Amines (PSA) wherein we have optimized various parameters to arrive at the optimal conditions for efficient reduction of the heavy metal content from solutions using PSA and the results are described in this paper.

Primary and Secondary Amine (PSA) is one of the most powerful solid-phase extraction adsorbents for clean-up of food matrices and multi-pesticide residue analysis [4]. The efficiency of removal of polar pesticides requires water and solvents like toluene, hexane, acetone are known to affect adsorption of pesticides from samples to PSA [4]. There are several reported uses of PSA. These include removal of seven indicator polychlorinated biphenyls in water samples by gas chromatography [5], monitoring of Bisphenols (BPs) in feed as a part of a necessary process for the security of food of animal origin [6], removal sugars and lipids, organic acids, and some pigments [7]. PSA has also been found as the most effective sorbent for removal of various matrices and significantly reducing matrix-enhancement effect making it a valuable reagent for LC-MS analysis.

In this paper, we disclose a novel observation of binding of heavy metal ions with PSA and we looked into this property of PSA in detail and the results are described in this article.

Materials and methods

The *Salacia chinensis* extract was prepared as described before [8] while the *Glycyrrhiza glabra* extract (Batch no:- HN/LE/202402092) was procured from Herbo Nutra Extract Pvt. Ltd, UP, India. PSA was procured from Thermo Scientific, USA (80020-416-100). *Carica papaya* leaf extract (Batch no:- PP/PLE/2310009) and *Cichorium intybus* seeds extract (Batch no:- KCI/501) were procured from Kisalaya Herbals Ltd, MP, India and Prakrutees Organics Pvt. Ltd, Karnataka, India respectively. All other chemicals and reagents were of analytical grade, unless mentioned otherwise.

Preparation of heavy metal solutions and estimation method

Stock solutions of the Heavy Metals (HMs) were prepared by using Mercury chloride, cadmium chloride, and lead nitrate of the respective metals to attain maximum solubility of the metal. The stock solutions were prepared with 1 ppm concentration of respective metal in Milli-Q grade deionized water by compensating for the salt/nonmetallic component (Lead nitrate 16.10 mg in 100ml distilled water followed by 100 fold dilution in distilled water; Cadmium chloride:- 18.08 mg in 100 ml distilled water followed by 10 fold dilution in distilled water; Mercury chloride:- 13.51 mg in 100 ml distilled water further diluted 1:10 in distilled water). All the solutions were stored at 4°C, until further use.

ICP-MS assessments are currently being performed since it is cost-effective and is sensitive with detection limits in a solvent are between 0.01 and 1 ppm [9]. Since high lead contents in lead extracts are variable ranging from 1 ppm to 1000 ppm in different medicinal plant extracts [10], we opted out for this method for all HM estimations. Standard metal solutions for the metal bio sorption analysis were prepared by adding 1.0 ml stock solution to 100 ml of the media giving a final concentration of 1 ppm. Such a standard solution of 1 ppm solution of lead nitrate subjected to various experimental variations and the experimental details are given below. ICP-MS analysis was carried out in an instrument of Agilent Technologies (Model: 7850 ICP-MS) following standard procedures.

Effect of temperature of incubation of PSA with metal solution

Ten ml of 1 ppm of lead solution was taken in a 15 ml centrifuge tube and 0.5 g PSA was added. The tube was swirled for 2 min and allowed to stand at various temperatures like cold (2-8° C), RT (30° C) after which the contents were centrifuged at 5000 rpm for 10 min and the supernatant was subjected to lead analysis. Suitable controls without PSA were run simultaneously.

Effect of time of contact between PSA and lead solution

Ten ml of 1 ppm lead solution was reacted with 0.5 g PSA, swirled for 2 min and keep at ambient temperature in static condition for various time intervals (10 min, 30 min and 120 min). The contents were centrifuged at 5000 rpm for 10 min to allow settlement of insoluble PSA and the supernatant was sent for residual lead analysis.

Effect of different concentration of PSA on lead removal efficiency in a solution

Ten ml of 1 ppm lead solution was treated with various concentrations of PSA namely 0.1, 0.5 g, 1 and 2 g, swirled for 2 min and incubated at ambient temperature in static condition for 30 min. The solutions were later centrifuge at 5000 rpm for 10 min and the supernatant was subjected to lead analysis. Suitable controls without PSA were run simultaneously.

Effect of PSA in reducing mercury and cobalt content from aqueous solutions

20 ml of 10 ppm solution of mercury chloride was treated with 0.5 gm PSA, mixed by swirling for 2 min. The solution was incubated at ambient Temperature in static condition for 30 min after which it was centrifuged at 5000 rpm for 10 min and the supernatant sent for mercury content by ICPMS. Similar protocol was followed for 10 ppm solution of cobalt chloride.

Effect of use of PSA on lead removal from herbal extracts

Since PSA is insoluble in water, to examine its potential to remove heavy metal concentration like lead in herbal extracts, we had to choose herbal extracts that are prepared in water and are soluble in water. For this experiment, we chose two extracts- *Carica papaya* and *Cichorium intybus* water extracts that was procured commercially. Briefly, 10 g of the extract was re-suspended in 100 ml of distilled water. This solution was divided into two parts. The first 50 ml was kept as control while the other 50 ml was subjected to 1 g PSA treatment. Both the treatment and control flasks were kept in static condition for 30 min, after which they were subjected to centrifugation for 10 min at 5000 rpm. The supernatant solution was dried on a Rota evaporator and then examined for lead content by ICP-MS.

Effect of lead removal efficiency in a methanolic solution by PSA

Ten ml of 1 ppm lead solution was prepared in methanol and treated with 1 g PSA, swirled for 2 min and kept at ambient tem-

perature in static condition for 30 min. The solutions were later centrifuged at 5000 rpm for 10 min and the supernatant was subjected to lead analysis. Suitable controls without PSA were run simultaneously.

Spike studies in herbal extracts of *Salacia Chinensis* and *Glycyrrhiza Glabra*

Twenty grams of extracts of *Salacia chinensis* and *Glycyrrhiza glabra* extracts were mixed with 200 ml of 1 ppm of standard lead solution. Such a solution was equally divided into two different flasks, each containing 100 ml of the solution. To the test Flask, 1 gram of PSA was added, mixed thoroughly while the control Flask did not contain PSA. Both the flasks were kept in static condition for approximately 30 minutes. This step ensures sufficient interaction time between the extract, lead solution, and PSA (in the test flask). After the incubation period, both the solutions were centrifuged at 5000 rpm for 10 min. The supernatant from both flasks were collected and concentrated individually using a rotatory evaporator. This step involves evaporating the solvent under reduced pressure, leaving behind the concentrated extract. Finally, dry the concentrated solution to obtain the dry residue.

Active constituent's estimation

To ensure that the treatment with PSA does not affect the content of active constituents, we estimated the content of mangiferin in *Salacia chinensis* extract before and after PSA treatment [11] and total polyphenol content in *Glycyyrrhiza glabra* extract by the method of Matić et al. [12].

HPLC Chromatographic conditions

HPLC analysis was performed using a Waters Alliance e2695 HPLC system consisting of an intelligent pump with a high pressure mixer, auto sampling injection valve equipped with 1 mL loop, and a PDA detector 2998. The compounds were separated on the Phenomenex Luna C18, 250 x 4.6 mm and 5 µm column with a mobile phase consisting of solvent A (Orthophosphoric buffer pH 2.0) and solvent B (acetonitrile). Orthophosphoric acid was prepared by adding 1 ml of Orthophosphoric acid in 1000 ml of Milli Q water.

A linear isocratic composition of OPA (A) and Acetonitrile (B) (85:15 v/v) was developed at a flow rate of 1 ml/min. The injection volume was kept as 20 µl and the detection wavelength was set to 261 nm for Mangiferin detection using the PDA detector and quantified based on peak area and comparision with the standard curve. HPLC functioned at column oven temperature of 35°C and the sample temperature of 15°C. Data were analyzed on a computer equipped with Chromeleon software. Before HPLC run, both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through 0.45 µl filter paper.

Determination of Total Polyphenol content

The total polyphenol content (TPC) was determined by spectrophotometry, using gallic acid as a standard. Briefly, 1 mL of the diluted sample extract was transferred in duplicates to separate tubes containing 5 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g material. The concentration
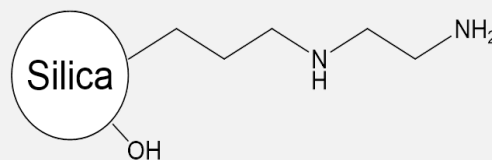

Figure 1: Structure of PSA. Note both primary amine & secondary amine separated by two carbon alkyl chain as main functional groups with silicate moiety at one terminal separated by three carbon alkyl chain from secondary amine group.
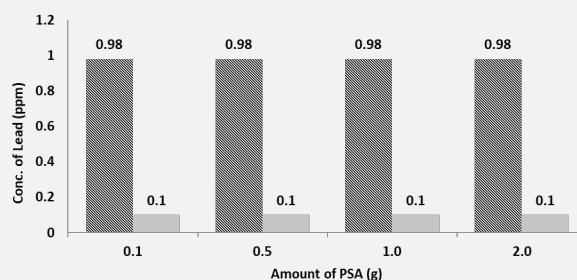

Figure 2: Effect of different ratio of PSA to lead solution in efficiency of lead removal. Note >90% reduction in lead content with merely 0.1% PSA in the experimental conditions described.
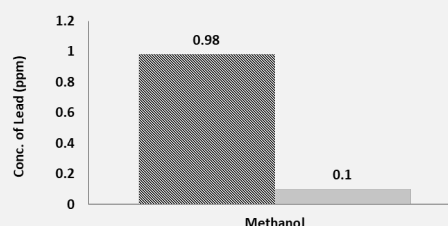
Figure 3: Effect of time of contact of PSA and lead on the efficiency of reduction in lead content. A minimum of 10 minutes appears to be sufficient even for >90% reduction in lead content.
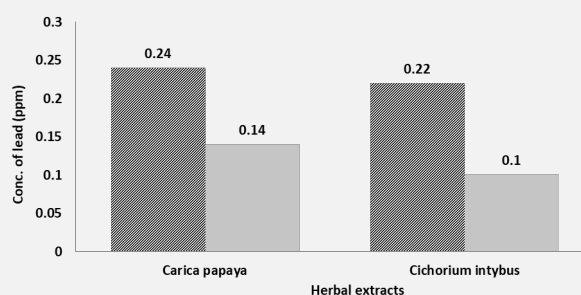
Figure 4: Effect of diluent medium on lead reduction using PSA. Note >90% reduction in lead content from a lead solution made in methanol.

of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 µg/mL.

Results

Figure 1 depicts the structure of PSA where the terminal bonded amine groups of Primary Secondary Amine (PSA) contain one primary amine group and one secondary amine group. Table 1 depicts the effect of temperature on the efficiency of lead content reduction using PSA at 0.1% concentration. It is clear from this table that the reduction in lead content from the samples is similar at all temperatures. The time of contact of PSA along with the lead solution show clearly that a minimum of 10 minutes is sufficient to reduce the lead content by >90% (Table 2). The experiment on variations of ratio of PSA versus lead solution indicate that PSA at a minimal concentration of 0.1% is able to reduce the lead concentration by >90% (Figure 2). The efficiency of reduction of different metal ion like cadmium and mercury indicates that PSA is capable of reducing the content of these metal ions with equal efficiency by >99.9% (Table 3). Figure 3 shows the effect of PSA in reducing lead concentration when methanol is used as the diluent while Figure 4 shows the efficiency of lead removal from two randomly selected extracts with almost 50% reduction from *Carica papaya* and *Cichorium intybus* extracts after PSA treatment. Table 5 shows that the spiked lead of 1 ppm in herbal extracts of *Salacia chinensis* and *Glycyyrhiza glabra* after PSA treatment does show > 2 logs reduction in the lead content without affecting the concentration of the active constituents like mangiferin in *Salacia chinensis* and *Glycyyrhiza glabra* extracts (Figures S1A & S1B).

The mangiferin content in control sample and treated sample was 2.36% and 2.21% respectively.

The TPC of control sample of Glycyrrhiza glabra extract was found 7.0 ± 0.2% while the PSA treated sample showed a TPC of 6.85 ± 0.2%. These results indicate that PSA treatment does not affect the content of the active constituents in the extracts tested in this study.

Discussion

Heavy metals are the natural components of the Earth's crust and are difficult to be degraded. Since these enter the human body through food, water, and air, they are a major health concern at global level [13].

Primary Secondary Amine (PSA) has been found as the most effective sorbent for removal of various matrices and significantly reducing matrix- enhancement effect. It is reported for removal of fatty acids, sugars from samples [14] proteins from meat samples [15]. The effective removal of heavy metal ions with PSA disclosed in this paper is hitherto unreported and appears to be an attractive and cost-effective agent for reducing heavy metal contamination in various sources.

Currently, PSA has number of other potential applications. These include pesticide residue analysis in vegetables [16], analysis of trifloxystrobin and CGA 321113 (trifloxystrobin metabolite) in dragon fruit, star fruit and watermelon, target analysis of over 100 pesticides and contaminants in a complex feed matrix [17], food analysis [18] etc. In 2013, zirconia-based sorbents (Z-Sep) were introduced by Sigma-Aldrich and known as a superior alternative to PSA, with its ability to remove fatty and pigmented compounds and still retain a good recovery of analytes [19]. However, when PSA alone was used as the clean-up material, significant loss of active constituents has

Table 1: Effect of reaction temperature on lead content before and after PSA treatment.

S.N.	Experimental Parameter	Lead content before PSA treatment	Lead content after PSA treatment
1	1 ppm lead solution incubation with PSA at 2-8°C	0.97 ppm	<0.1 ppm
2	1 ppm lead solution incubation with PSA at 37°C	0.97 ppm	<0.1 ppm
3	1 ppm lead solution incubation with PSA at 80°C	0.97 ppm	<0.1 ppm

Table 2: Effect of time of contact on efficiency of lead binding by PSA.

S.N.	Experimental Parameter	Lead content before PSA treatment (ppm)	Lead content after PSA treatment (ppm)
1	Incubation time of 10 min in static condition	0.97	<0.1
2	Incubation time of 30 min in static condition	0.97	<0.1
3	Incubation time of 120 min in static condition	0.97	<0.1

Table 3: Effect of PSA on reduction of cadmium and mercury content from standard solutions.

S.N.	Metal ion solution	Mercury content in ppm (control)	Content after PSA treatment in ppm
1	10 ppm Mercury chloride	>12.50	<0.05
2	10 ppm Cadmium Chloride	>10.05	<1.25

Table 4: Effect of PSA on reduction of lead content from Herbal extracts.

Sr. No.	Experimental Parameters	Lead content in ppm (Control)	Lead content after PSA treatment (ppm)
1	Papaya leaf extract	0.24	0.14
2	Cichorium extract	0.22	< 0.1

Table 5: Effect of lead spike studies in *Glycyrrhiza glabra* and *Salacia chinensis* extracts.

Particular	Initial lead content in ppm (Control)	Lead content after PSA treatment (ppm)
20 g Glycyrrhiza glabra extract with 200 ml of 1 ppm lead solution	6.75	4.4
20 g Salacia chinensis extract with 200 ml of 1 ppm lead solution	28.99	11.98

been observed in dragon fruit (47.1%), star fruit (57.4%) and watermelon (31.7%) [20]. Since we have not seen any loss of active constituents in herbal extracts upon treatment with PSA for lead reduction (mangiferin content in *Salacia chinensis* and polyphenol content in *Glycyrrhiza glabra*), we believe this novel agent will find its utility in reducing such toxic elements in food and herbal industry. We believe with some optimization in ex-

perimental conditions one could achieve higher reduction in the metal ion concentration.

The uses of toluene, hexane and/or acetone in the elution severely reduce the capacity of PSA for removal of fatty acids [4] and water is essential in the elution for full recoveries of the polar pesticides. Also, methanol is the solvent of choice for extraction of majority of herbal extracts [21] and is the preferred solvent for LC-MS analysis, we tested the efficiency of PSA in removing lead from a lead solution in methanol and the results indicate no effect of methanol in lead removing efficiency of PSA, which is interesting.

Significant intake of elements like lead, mercury, cadmium, causes severe health disorders, since these metal ions interfere with regular metabolic functions. Hence, it is logical to limit exposure of these elements to humans. The observations of reduction of more than 99.9% of cadmium and mercury by PSA assume importance from clinical perspective. Herbal medication industry also needs to be regulated appropriately to introduce strict quality control to avoid health hazards and to improve public health. In some pharmaceutical groups, the share of herbal medicinal products is even higher; for example, about 70% of drugs used to treat cardiovascular diseases are plant-based [22]. Buettner et al. [23] that found a 10% increase in lead concentration in the blood of females who took herbal dietary supplements for a month compared the control group. Similar results were obtained in epidemiological studies conducted in Taiwan [24]. Sakharkar [25] reported lead positing in subjects taking herbal medications. Therefore, the present study aimed at systematizing and analyzing information on the content of HMs in medicinal plant materials and herbal medicinal products. Since this product is not classified as dangerous according to Directive 67/548/EEC and its amendments, and is a stable constituent that has no hazardous effects, we believe this sorbent could find its wide utility.

Conclusion

In summary, primary secondary amine could effectively reduce metal ion concentration by a simple treatment from aqueous solutions. The method is universally applicable and could be a potential reagent for removal or reduction of heavy metal ions from waste waters, herbal extracts and drinking water.

Declarations

Authors contributions: Sriram Padmanabhan conceived and supervised the study; Sriram Padmanabhan designed the experiments while Somesh Shintre and Sanman Kolhe performed the experiments; Shankar Vidhate and Sanman analyzed the data; Sriram Padmanabhan wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

**Conflict of interest:** The authors report there are no competing interests to declare.

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Supplementary Figures
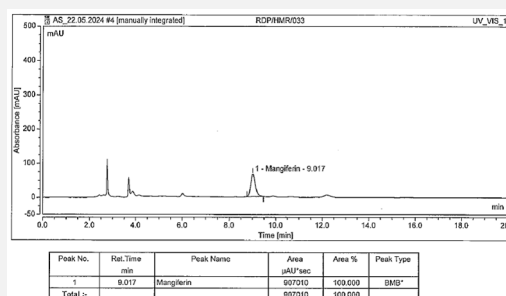
Supp Figure 1A: HPLC chromatogram of mangiferin from Salacia chinensis extract before PSA treatment.
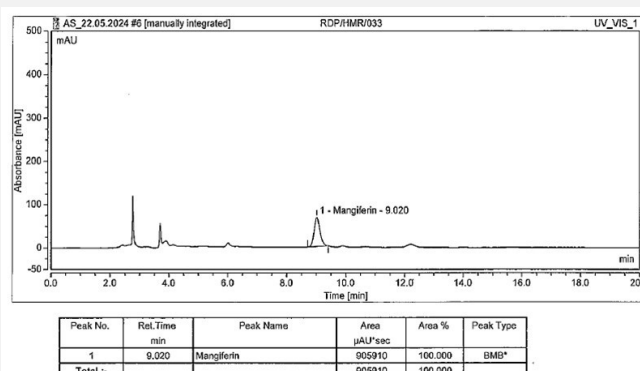
Supp Figure 1B: HPLC chromatogram of mangiferin from *Salacia chinensis* extract after PSA treatment.