International Journal of Applied Science and Engineering
2012. 10, 4: 307-317

# Preparation of New Sorbent by Functionalization of Cross Linked Chitosan with 4-amino-3-hydroxybenzoic Acid and Its Application for Solid Phase Extraction of Ni(II) and Pb(II) from Environmental Samples and Determination by AAS

K. Seshaiah[a,*], Yapati Harinath[a], Yerukala Suneetha[a], Bukya Ramesh Naik[a], and Min-Chao Wang[b]

[a] *Inorganic and Analytical Chemistry Division, Department of Chemistry, Sri Venkateswara University, Tirupati, India*
[b] *Department of Environmental Engineering and Management, Chaoyang University of Technology, Wufong District, Taichung, Taiwan, R. O. C.*

**Abstract:** Chitosan, a biopolymer was crosslinked with ethylene glycol diglycidyl ether and the modified chitisan was functionalized with 4-amino-3-hydroxybenzoic acid through amide bond. The new sorbent was applied for the solidphase extraction of Ni(II) and Pb(II) from environmental samples prior to determination by flame atomic absorption spectrometry (FAAS). The experimental conditions for functionalization of crosslinked chitosan and separation/pre-concentration of the target metal ions were optimized and the interference of commonly coexisting ions in water samples was examined. The sorption capacity values of functionalized chitosan for Pb and Ni were found to be 95.02 and 96.16 mg $g^{-1}$ respectively. The detection limits of the method were 0.024 μg $L^{-1}$ for Ni and 0.058 μg $L^{-1}$ for Pb, with a pre-concentration factor of 100. The developed method was applied for the determination of two metal ions in real water samples. The method was validated by the analysis of certified reference material of NIST-SRM water sample.

**Keywords:** Solid phase extraction; cross-linked chitosan; 4-amino-3-hydroxybenzoic acid; AAS; environmental samples.

## 1. Introduction

Nickel is a toxic element of wide spread distribution in the environment. It usually, enters water from waste disposals of different industrial processes such as electroplating, batteries, pigments, for paints and ceramics, surgical and dental prostheses, magnetic tapes and computer components, catalysts and also it is emitted to the atmosphere from volcanoes, and windblown dust [1, 2]. Nickel at trace amount may be beneficial as an activator of some enzyme systems, but if the nickel (II) ion intake over the permissible levels results in different types of diseases such as pulmonary fibrosis, lung cancer, renal edema, skin dermatitis and gastrointestinal disorder such as nausea, vomiting and diarrhea [3].

Lead is a toxic element, and it exerts extensive damage to the central and peripheral nervous systems, causing severe memory disability as well as hyperactivity, impulsiveness, learning disabilities and

* Corresponding author; e-mail: seshaiahsvu@yahoo.co.in

Received 20 June 2012
Revised 10 August 2012
Accepted 20 October 2012

aggressive behavior in children [4]. Main sources of lead are the manufacturing of storage batteries, pigments, mining, metal electroplating, painting, coating, smelting, petrochemical, plumbing fuels, photographic materials, matches and explosives [5, 6].

In view of the toxic effects by Ni and Pb, it is important to determine the concentration of these metal ions in water and food samples, the sources through which they enter human body. Direct determination of metal ions in environmental and biological samples that are present at trace level is not possible owing to matrix effect and low concentration of metal ions in a sample [7]. Therefore, a separation and pre-concentration step is necessary prior to its measurement. Among the separation and pre-concentration methods, the solid phase extraction based on solid support modified or functionalized with complexing agents has become the promising method for trace metal analysis. Chitosan resins are considered to be very suitable natural solid supports in functionalization for pre-concentration of trace metal ions because of its unique properties such as high sorption ability, easy derivatization and bio degradability. Chitosan possesses high hydrophilicity which provides faster adsorption rate in aqueous medium [8, 9]. The reactive amino groups in chitosan provide easiness in introducing various moieties for wider applications in solid phase extraction compared to other synthetic polymers [10-14]. Introduction of aromatic and heteroaromatic fragments to the chitosan backbone results in polymers, which can be used as bioactive, sorption, and metal-containing multifunctional materials. The disadvantage of commercially available chitosan was shrinking in acidic solutions. However, this drawback can be overcome by crosslinking the chitosan with ethylene glycol diglycidyl ether (EGDE).

This work is aimed at synthesis of a new sorbent by cross linking chitosan and chemically modifying it by attaching 4-Amino-3-Hydroxybenzoic acid. Studies have been carried out systematically to investigate the application of the new sorbent, 4-amino-3-hydroxybenzoic acid modified chitosan for selective separation and preconcentration of heavy metal ions, Ni(II) and Pb(II) from environmental samples prior to determination by flame atomic absorption spectrometry. The experimental parameters viz. pH of sample and amount of sorbent, type and concentration of eluent for desorption of metals from solid support and flow rate that influence the analysis were optimized.

## 2. Experimental

### 2.1. Apparatus

Atomic absorption spectrometer (Shimadzu AA-6300, Kyoto-Japan,) was used for the determination of metal ions. Microwave digestion (MarsXpress, CEM Corp., United States) was used in the preparation of leafy vegetable samples for metal extraction. A Fourier transform infrared (FT-IR) spectrometer (Thermo-Nicolet FT-IR, Nicolet IR-200, United States) was used in the characterization of ligand and functionalized resin. An Elico (LI-129) pH meter was used for pH measurements. The pH meter was calibrated using standard buffer solutions of pH 4.0 and 9.2.

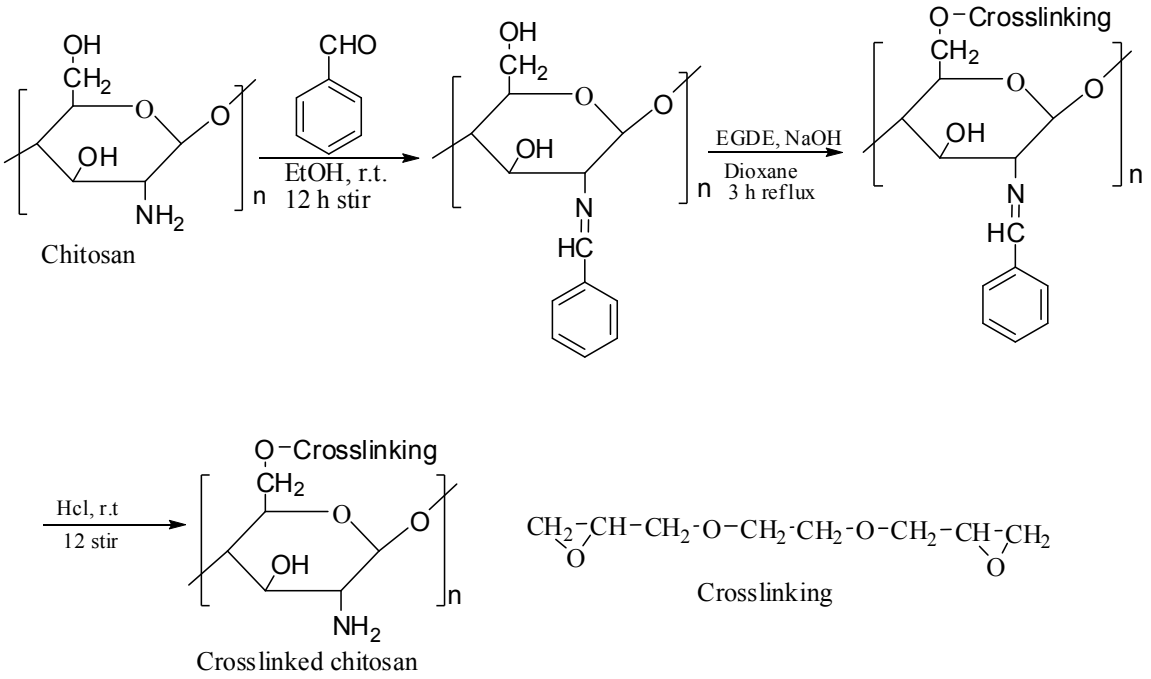
### 2.2. Chemicals and reagents

Stock solutions (1mg/mL) of metal were prepared by dissolving appropriate amounts of analytical grade metal salts, in deionized water acidified with 10ml of the acid. Working solutions of these metal ions were obtained by dilution of the stock solution with doubly distilled water. The pH was

adjusted with 0.1M HCl and NaOH. Acetate and phosphate buffers were used to adjust pH in the ranges 3.0-6.0 and 6.8-8.0 respectively. Chitosan and 4-amino-3-hydroxybenzoic acid was purchased from Aldrich. All the other reagents used were of analytical reagent grade.

### 2.3. Synthesis of cross-linked chitosan (CCTS)

Cross linked chitosan was synthesized in a similar manner to the previous work [15] 20 g of Chitosan particles (100-300 µm diameter) was suspended in 200 mL of ethanol followed by the addition of 80 g benzaldehyde. The mixture was stirred at room temperature for 12 h, to protect amine groups of chitosan as schiff base. After the reaction, the product was filtered and washed three times with ethanol and water to remove un-reacted benzaldehyde. The chitosan derivative in which the amino groups were protected by benzaldehyde was refluxed with 30 g of ethyleneglycoldiglycidylether (EDGE) in 300 mL of dioxane and 40 mL of 1M NaOH for 3 h. The product, cross linked chitosan (CCTS) was filtered and washed three times by stirring with 0.5 M hydrochloric acid solution at room temperature and again filtered and washed with ethanol and water. Schematic representation of synthesis of CCTS is shown in Scheme 1.

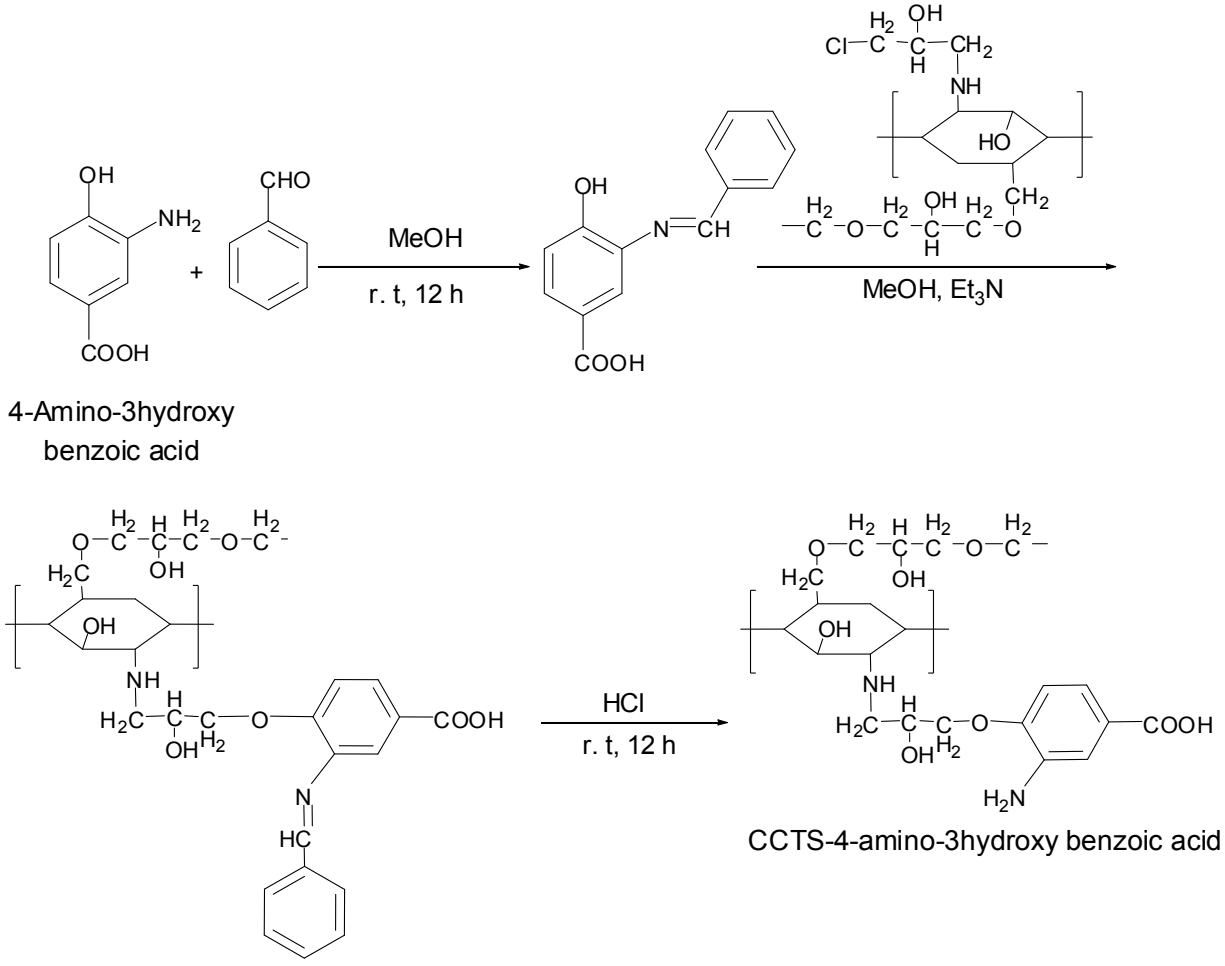



**Scheme 1.** Synthesis of cross-linked chitosan (CCTS)

### 2.4. Functionalization of CCTS with 4-amino-3-hydroxy benzoic acid resin (CCTS-AHBA)

Cross-linked chitosan (5 g) was suspended in a mixture of water (50 mL) and ethanol (50 mL), then chloromethyloxirane (10 g) was added to the suspension, and the mixture was refluxed for 3 hrs. After the reaction was finished, the product was filtered on a filter paper, and washed three times with ethanol and water to remove unreacted chloromethyloxarine. Then the amino group of 4-amino-3-hydroxy benzoic acid moiety was protected by treating 10 gm of

AHBA with 20 gm of benzaldehyde in 20 mL of methanol. The mixture was stirred for 12 hrs at room temp. Then the CCTS with chloromethyloxirane (5 g) and the AHBA protected-amino group were suspended in dioxane (100 mL), and to this suspension, 2 M NaOH (40 mL) was added. The mixture was refluxed for 3 hrs in order to couple hydroxyl-phenolic group of AHBA with chloroterminal of the CCTS. Finally, the protection group (benzaldehyde),which was condensed with amino groups of AHBA, was removed by stirring the product in 100 mL of 0.5 M HCL for 12 hrs at room temp. This procedure was repeated two times. Then the product (CCTS-AHBA) was filtered on the glass filter and washed with methanol and water. The resin was used as a sorbent for separation/ preconcentration of metals from environmental samples. Schematic representation of functionalization of cross linked chitosan with 4-amino 3-hydroxybenzoicacid in shown in Scheme 2.




**Scheme 2.** Functionalization of CCTS with 4-Amino-3-hydroxybenzoic acid

## 2.5. Recommended procedure for preconcentration and determination of metal ions

Cross linked chitosan functionalized with 4-amino 3-hydroxybenzoicacid (0.025 g) was packed in glass column (1.0 cm i.d., length of 10 cm) plugged with a small portion of glass wool at both ends in order to prevent loss of the particles during solution loading. pH of water samples containing Pb(II) and Ni(II) was adjusted to 5.0 and 7.0 respectively, and passed through the column, at a flow rate of 2 mL $min^{-1}$. After

completely passing sample solution the column was washed with double distilled water. The bound metal ions were stripped from the column with 10 mL of 2 M $HNO_3$. The concentration of the metal ions in elutes was determined by atomic absorption spectrometry.

### 2.6. Microwave digestion procedure

Sample preparation of the leafy vegetables was carried out by microwave digestion. Approximately 1 g (dry mass) of leafy vegetables materials were weighed directly into the PTFE vessels, to which 10 mL of concentrated $HNO_3$ was added and the vessels were capped immediately. The digestion programme consisted of a ramp time of 10 min to reach 150 $^0$C and a dwell time of 10 min at 150 $^0$C. The power was 800 W. After the completion of program vessels were cooled, vented and opened and then 2mL of 30% $H_2O_2$ was added and filtered the solutions into 25 mL volumetric flasks and made up with double distilled water. Blanks were prepared by following similar digestion procedure without plant material.

## 3. Results and discussion

### 3.1. Characterization of functionalized chitosan

The FTIR spectrums of CCTS and CCTS-AHBA were shown in Figure 1. The FTIR spectrum of CCTS (Figure 1 (A)), showed bands at 3,456 $cm^{-1}$, 2,918 $cm^{-1}$, 1,658 $cm^{-1}$, 1,553 $cm^{-1}$, 1,377 $cm^{-1}$, 1,076 $cm^{-1}$ due to OH and NH2 stretching, C-H stretching, C=O stretching, N-H bending, C-N stretching of the amino group and C-O-C stretching vibration in the glucopyranose ring. FT-IR spectrum of the CCTS-AHBA (Figure 1 (B)) exhibited absorption bands at 1668$cm^{-1}$, 700 $cm^{-1}$ corresponding to C=O stretching of carboxylic acid and substituted phenyl ring respectively. There is no significant changes occurred for the peaks in the range 920–1200$cm^{-1}$ clearly shows that etheral linkage was not ruptured during functionalization.

### 3.2. Effect of pH on metal pre-concentration

The pH of the sample solution is an important analytical factor for the quantitative recoveries of metal ions by solid phase extraction. The effect of the pH on the pre-concentration of metals on functionalized chitosan was studied by determination of Pb(II) and Ni(II). Each element was preconcentrated in the pH range of 2.0-9.0 and the respective results were shown in Figure 2. The results obtained indicate that the two metal ions could be retained quantitatively by the modified Chitosan at the pH of 5.0 for Pb and 7.0 for Ni.

### 3.3. Effect of amount of resin on pre-concentration of metals

The amount of modified chitosan required for maximum pre-concentration of metals was studied. The quantitative retention of analytes was tested by taking different amounts (0.01-0.05 g) of CCTS-AHBA in the column. It was found that quantitative recovery for the metal ions was observed when 0.025 g of sorbent was used in the column. Therefore 0.025 g of CCTS-AHBA was used for further studies.

### 3.4. Effect of flow rate

The Effect of flow rate on the retention and recovery of metal ions on chitosan functionalized with 4-amino-3-hydroxy benzoicacid was examined by varying flow rate from 1.0-4.0 mL $min^{-1}$ under optimum conditions. At flow rate greater than 2.5 mL

$min^{-1}$, there was decrease in the recovery of metal ion. The reason of this decrease may be due to insufficient contact of metal ions and functionalized resin to equilibrium. Flow rate less than 1.0 mL $min^{-1}$ were not studied to avoid long analysis times.

Therefore flow rate of the elution solution was chosen as 2 mL $min.^{-1}$

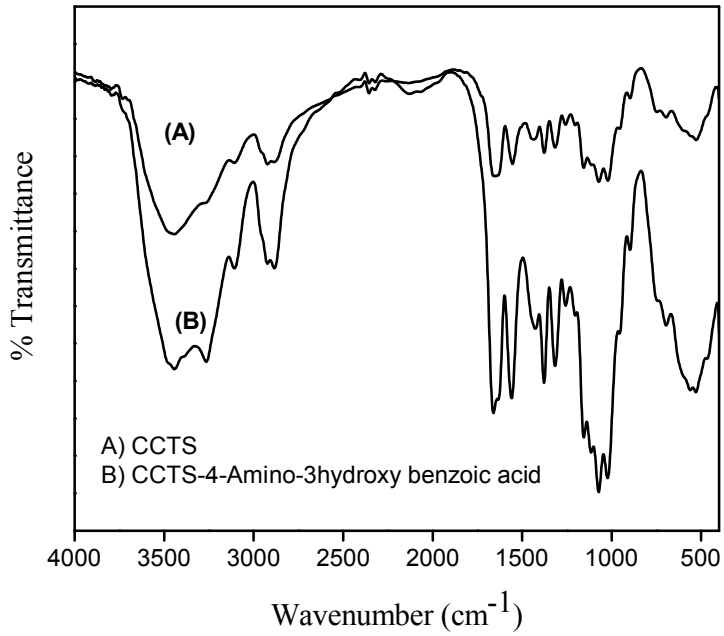



**Figure 1.** FTIR spectrum of CCTS and CCTS functionalized with 4-amino-3-hydroxy benzoicacid

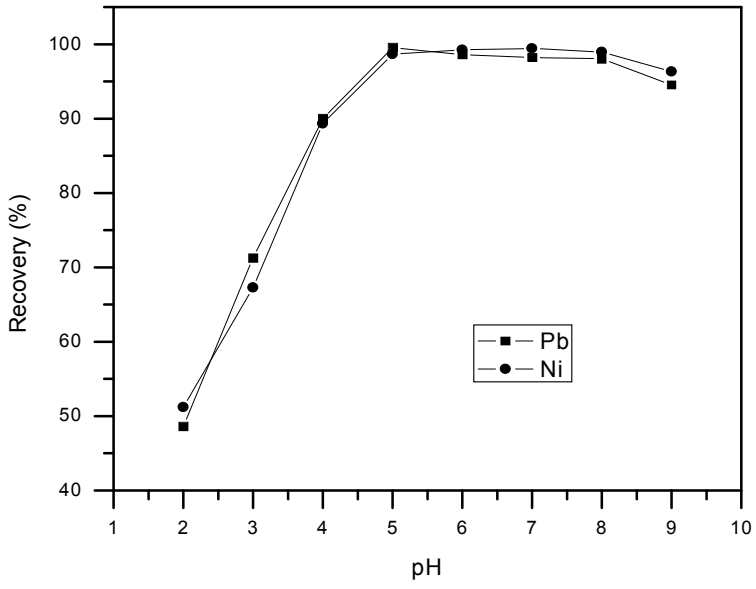



**Figure 2.** Effect of pH on recovery (%) of metal ions

### 3.5. Effect of $HNO_3$ concentration on elution

The effect of $HNO_3$ concentration on elution of metal ions on CCTS-AHBA was studied by carrying out the elution with 0.5-3.0 M $HNO_3$. The results were showed in Figure 3. This data indicate that the highest recoveries for Ni(II) and Pb(II) were obtained when $HNO_3$ concentration was 2 M or more. Hence 2 M $HNO_3$ was chosen as eluent for further experimental studies.

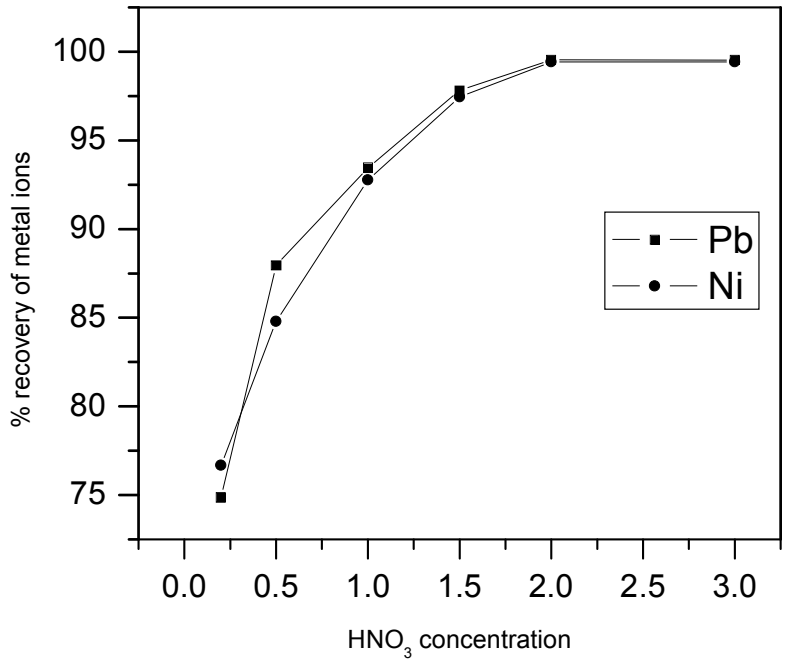



**Figure 3.** Effect of $HNO_3$ concentration on elution of metal ions

### 3.6. Sorption capacity and detection limit

The sorption capacity of CCTS-AHBA was determined by batch method. The resin (0.04g) was saturated with Ni and Pb ion solutions (concentration, 25 μg $mL^{-1}$) by equilibrating the solutions on a mechanical shaker under optimum conditions. The solid matrix was filtered and removed. The concentration of metal ions in the solution was determined by AAS. The Sorption capacities were calculated by mass balance and found 96.16 mg $g^{-1}$ for Ni and 95.02 mg $g^{-1}$ for Pb. The detection limits defined as the concentration of metal ion which gives a signal equivalent to reagent blank signal plus three times the standard deviation of the reagent blank of 5 measurements were found as 0.024 μg $L^{-1}$ for Ni and 0.058 μg $L^{-1}$ for Pb.

### 3.7. Effect of sample volume

In order to investigate the enrichment of trace amounts of Ni and Pb by the functionalized resin, the effect of sample volume on the recovery of metal ions was studied by passing 100 -1000 mL of sample

solution. At the higher volumes the recovery of analytes was not quantitative. The pre-concentration factor for simultaneous pre-concentration and extraction was calculated by the ratio of the highest sample volume for each analytes and the lowest final eluent volume (10 mL). The quantitative recoveries were obtained for sample volume of 1000 mL for Ni and Pb. Therefore pre-concentration factor for Ni (II) and Pb (II) was 100.

### 3.8. Resin stability and reusability

The stability of CCTS-AHBA was studied in acid (1.0-4.0 M $HNO_3$) solution. It was shaken with acid solutions of varying concentrations for 4h and filtered. The solid was washed with distilled water until free from acid, air-dried and its sorption capacity was determined using batch method. The sorption capacity of the acid treated resin was found to be similar (variation<3%) to that of the untreated one. This shows that the present resin can resist to acid concentration up to 4.0 M.

The reusability of resin was tested by sorption of metal ions onto a column loaded with CCTS-AHBA from a solution having a concentration 25 µg $L^{-1}$ at a flow rate of 2.0-3.0 mL $min^{-1}$ and eluting with 1.0 M $HNO_3$ and determined by the recommended procedure. It was found that the sorption capacity after 10 cycles of sorption and desorption did not vary more that 2% for two metal ions. Therefore the resin showed good reusability and stability for the determination of metal ions.

### 3.9. Effect of diverse ions

The effect of diverse ions on the sorption of Ni(II) and Pb(II) by CCTS-AHBA was investigated. The limits of tolerance defined as the amount of diverse ions which caused 2 % error in the recovery of metal ions. The results are presented in Table 1. The results indicate that the common cations and anions present in water samples do not interfere in the analysis of metals under the reported conditions.

**Table 1.** Tolerance limits of matrix ions for determination of Ni (II) and Pb (II) by the proposed method (n=5)

| Matrix ion | Tolerance limit mg $L^{-1}$ | Recovery (%) |
|---|---|---|
| $Na^+$ | 40000 | 98.73 ± 0.27 |
| $K^+$ | 40000 | 98.04 ± 0.21 |
| $Ca^{2+}$ | 30000 | 96.88 ± 0.24 |
| $Mg^{2+}$ | 25000 | 96.26 ± 0.37 |
| $Cl^-$ | 20000 | 97.12 ± 0.22 |
| $NO_3^-$ | 20000 | 96.80 ± 0.21 |
| $CH_3COO^-$ | 10000 | 96.16 ± 0.25 |
| $PO_4^{3-}$ | 20000 | 95.97 ± 0.25 |
| $SO_4^-$ | 20000 | 98.82 ± 0.20 |

### 3.10. Accuracy

The accuracy of the procedure was investigated by determining metal ions in standard reference material (NIST-SRM) 1643 e. The results are presented in Table 2. The results show the quantitative recoveries of >98% for two metal ions with a RSD of below 5%.

**Table 2.** Determination of Ni (II), and Pb (II) in water samples (*n* = 5)

| Metal ion | NIST 1643e water (μg $L^{-1}$) | |
|---|---|---|
| | Certified value | Proposed method |
| Ni(II) | 62.41 | 62.47 ± 0.37 |
| Pb(II) | 19.63 | 19.58±0.03 |

### 3.11. Analytical application

The application of the proposed method for the analysis of real samples was studied by determining Pb(II) and Ni(II) in bore well water and sea water collected from Bay of Bengal (Nellore, A.P., India), and in leafy vegetables like Chukkau (Rumex vesicarius), palakura (Ichnocarpus frutescens), Thotakura (Amaranthus tricolor) collected from agricultural fields nearby Tirupati town. These samples were subjected to dissolution, preconcentration and metal determination using the proposed procedure. Metal concentrations in these samples are presented in Table 3 and Table 4.

**Table 3.** Determination of Ni(II) and Pb(II) in water samples

| Sample | Metal ion | Added | Found | Recovery |
|---|---|---|---|---|
| Borewell water[a] | Ni | 0.0 | 8.50 | - |
| | | 5.0 | 13.48 | 99.6 |
| | Pb | 0.0 | 9.82 | - |
| | | 5.0 | 14.78 | 99.2 |
| Sea water[b] | Ni | 0.0 | 6.76 | - |
| | | 5.0 | 11.78 | 100.4 |
| | Pb | 0.0 | 5.97 | - |
| | | 5.0 | 10.96 | 99.8 |

**Table 4.** Determination of trace metals from leafy vegetables by proposed method (n=4, and concentration of metal ions in μg $g^{-1}$

| Leafy sample | Metal ion (μg g-1) | |
|---|---|---|
| | Ni | Pb |
| Palakura (Ichnocarpus frutescens) | 1.28 ± 0.08 | 1.03 ± 0.04 |
| Thotakura ( Amaranthus tricolor) | 2.72 ± 0.14 | 3.55 ± 0.05 |
| Chukkaku ( Rumex vesicarius) | 2.52 ± 0.10 | 3.86 ± 0.07 |

## 4. Conclusion

The new sorbent, cross linked chitosan functionalized with 4-amino-3-hydroxy benzoic acid was successfully applied for separation and pre-concentration of Pb(II) and Ni(II) from environmental samples and determination by AAS. Preconcentration of Pb(II) and Ni(II) was pH dependent and maximum recovery was achieved at pH 5 for Pb(II) and pH 7 for Ni(II) respectively. The possible interference of commonly found metal ions in water was investigated and found the effect as negligible. Analytical features, such as enrichment factor, and limit of detection are comparable to those presented by methods described in the literature. The presented method is simple, sensitive and suitable for the monitoring of metal ions in real samples.

## References

[ 1] Stoeppler, M. John Wiley, New York 1980. 29.

[ 2] Franco E., Rossetto, John D. Turnbull, Evert Niebore. 1994. Characterization of nickel-induced mutations. *Science of The Total Environment*, 148, 2-3: 201-206

[ 3] Akhtar, N., Iqbal, J., and Iqbal. M. 2004. Removal and recovery of nickel(II) from aqueous solution by loofa sponge-immobilized biomass of Chlorella sorokiniana: characterization studies. *Journal of Hazardous Materials*, 108, 1-2: 85-94.

[ 4] Ornek, A., Ozacar, M., and Sengil. I. A. 2007. Adsorption of lead onto formaldehyde or sulphuric acid treated acorn waste: Equilibrium and kinetic studies. *Biochemical Engineering Journal*, 37, 2: 192-200.

[ 5] Benhima, H., Chiban, M., Sinan, F., Seta, P., and Persin, M. 2008. Removal of lead and cadmium ions from aqueous solution by adsorption onto micro-particles of dry plants. *Colloids and Surfaces B: Biointerfaces*, 61, 10-16.

[ 6] Wu, X. W., Ma, H. W., Yang, J., Wang, F.J., and Li, Z. H. 2012. Adsorption of Pb(II) from aqueous solution by a poly-elemental mesoporous adsorbent. *Applied surface Science*, 258, 14: 5516-5521.

[ 7] Suneetha, Y., Naresh Kumar, B., Harinath, Y., Reddy, D. H. K., and Seshaiah, K. 2012. Functionalization of cross linked chitosan with 2-aminopyridine-3-carboxylic acid for solid phase extraction of cadmium and zinc ions and their determination by atomic absorption spectrometry. *Microchimica Acta*, 176, 1-2: 169-176.

[ 8] Oshita, K., Oshima, M., Gao, Y. H., Lee, K. H., and Motomizu, S. 2002. Adsorption Behavior of Mercury and Precious Metals on Cross-Linked Chitosan and the Removal of Ultratrace Amounts of Mercury in Concentrated Hydrochloric Acid by a Column Treatment with Cross-Linked Chitosan. *Analytical Sciences*, 18, 10: 1121-1125.

[ 9] Gao, Y. H., Oshita K., Lee, K. H., Oshima, M., and Motomizu, S. 2002. Development of column-pretreatment chelating resins for matrix elimination/multi-element determination by inductively coupled plasma-mass spectrometry. *Analyst*, 127, 1713-1719.

[10] Fajardo, A. R., Lopes, L. C., Rubira, A. F., and Muniz E.C. 2012. Development and application of chitosan/poly(vinyl alcohol) films for removal and recovery of Pb(II). *Chemical Engineering Journal*, 183, 253-260.

[11] Ashoka, G. and Fereidoon, S. 2007. Use of chitosan for the removal of metal ion contaminants and proteins from water. *Food chemistry*, 104, 1: 989-996.

[12] Wan Ngah, W. S. and Fatinathan, S.

2010. Adsorption characterization of Pb(II) and Cu(II) ions onto chitosan-tripolyphosphate beads: kinetic, equilibrium and thermodynamic studies. *Journal of Environmental Management*, 91, 4: 958-969.

[13] Repo, E., Warchol, J. K., Kurniawan, T. A., and Sillanpaa, M.E.T. 2010. Adsorption of Co(II) and Ni(II) by EDTA- and/or DTPA-modified chitosan: Kinetic and equilibrium modeling. *Chemical Engineering Journal*, 161, 1-2: 73-82.

[14] Wu, F. C., Tseng, R. L., Juang, R. S., and 2010. A review and experimental verification of using chitosan and its derivatives as adsorbents for selected heavy metals. *Journal of Environmental Management*, 91, 4: 798-806.

[15] Hosoba, M., Oshita, K., Katarina, R. K., Takayanagi, T., Oshima M., and Motomizu, S. 2009. Synthesis of novel chitosan resin possessing histidine moiety and its application to the determination of trace silver by ICP-AES coupled with triplet automated-pretreatment system. *Analytica Chimica Acta*, 639, 1-2: 51-56.