Solid-phase Extraction on Magnetic Multi-walled Carbon Nanotubes Coupled with Flame Atomic Absorption Spectrometry for Determining Lead and Cadmium in Traditional Chinese Medicine

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Summary: In this study, magnetic carbon nanotubes (MCNTs) were prepared by mixing the magnetic particles and multi-walled carbon nanotubes in dispersed solutions. These MCNTs were used as adsorbents of magnetic solid-phase extraction (MSPE). By coupling MSPE with flame atomic absorption spectrometry, a rapid and sensitive method for analyzing lead and cadmium using ammonium pyrrolidine dithiocarbamate as chelating reagent was established. Under optimal conditions, calibration graphs were linear in the range of 10.0–400.0 μg L^{-1} and 10.0–300.0 μg L^{-1} with detection limit of 0.6 μg L^{-1} and 0.5 μg L^{-1} for Pb and Cd, respectively. A good relative standard deviation for determining 300.0 μg L^{-1} of Pb and Cd were 3.8 and 3.4%, respectively. The proposed method was applied to analyze several traditional Chinese medicine samples with satisfactory results.

Introduction

Lead and cadmium are highly toxic and hazardous elements even at trace levels [1]. Thus, determination of lead and cadmium in traditional chinese medicine samples is of clear significance to human health. Various instrumental methods, such as spectrometry, inductively coupled plasma-optical emission spectrometry [2], inductively coupled plasma-mass spectrometry [3], electrothermal atomic absorption spectrometry [4], and flame atomic absorption spectrometry (FAAS) [5] are widely applied for determining trace amounts of Pb and Cd. In these instrumental determinations, low concentrations of analytes and complexity of matrices are the main problems [6]. Therefore, a preconcentration/separation procedure is required, such as liquid-liquid extraction [7], liquid-phase microextraction [8], coprecipitation [9], ion exchange [10], cloud-point extraction [11], or solid-phase extraction (SPE) [12, 13], before instrumental determination of metal ions.

SPE is a routine extraction method for trace levels of contaminants in complex samples. Multi-walled carbon nanotubes (MWCNTs) also exhibited strong sorption properties toward various compounds because of their high surface area and large micropore volume; thus, MWCNTs have been used as SPE adsorbents for the separation and preconcentration of trace analytes [14, 15]. However, some unavoidable difficulties occur when CNTs are applied to extract target compounds from large volumes of liquid samples [16]. When column dynamic extraction mode is used, water samples passing through nanoparticle-packed SPE columns consume much time because of high back pressure. When static batch mode is used, centrifugal separation is inapplicable for large volumes of samples, and filtration will encounter the same problem as that of column dynamic extraction mode [17].

By combining the advantages of MWCNTs and magnetic nanoparticles to fabricate nanosized SPE adsorbents with high surface area, high chemical stability, and good magnetic separability, a new kind of magnetic nanocomposite (MNP) sorbent can be obtained. Recently, Feng *et al.,.* used a simple method to immobilize MWCNT sheets onto magnetic particles by simple adsorption [16]. In their work, fabricated magnetic CNTs without chemical modification were used as magnetic solid-phase extraction (MSPE) adsorbents to successfully extract

PAEs from beverage, environmental water, and perfume samples. Therefore, we extend its application to inorganic analysis, and have obtained a consistent conclusion.

This study aimed to develop a MSPE method for preconcentration and determination of trace amounts of Pb and Cd from traditional chinese medicine samples. These metal ions formed stable complexes with ammonium pyrrolidine dithiocarbamate (APDC), and the formed complexes were adsorbed on $Fe_3O_4@SiO_2$/MWCNTs in a batch extraction procedure. The MNPs were then collected using an external magnetic field. Afterward, the extracted heavy metal ions were washed from the surface of the adsorbent and determined simultaneously using FAAS.

Experiment

A Shimadzu Model AA-6300C atomic absorption spectrometer (Shimadzu, Tokyo, Japan) equipped with hollow cathode lamps for lead and cadmium with a deuterium lamp for background correction, was used. The instrumental parameters were adjusted according to the manufacturer's recommendations. A pH3-3C digital pH meter equipped with a combined glass–calomel electrode (Hangzhou Dongxing Instrument Factory, Hangzhou, China) was used for pH adjustment.

MWCNTs were supplied by Shenzhen Nanotech Port Co. (Shenzhen, China) with >93% purity, diameter between 40 and 60 nm and length of 10-20 μm. Fe_3O_4 nanoparticles were prepared by chemical coprecipitation method [18]. Magnetic microspheres coated with silica layer ($Fe_3O_4@SiO_2$) were synthesized according to previously reported methods [19, 20].

The fabrication process of magnetic MWCNTs was similar to the described procedure of Feng *et al.,* [16]. Briefly, MWCNTs (25.0 mg) and $Fe_3O_4@SiO_2$ (50.0 mg) were dispersed in DMF separately. After combining the two solutions, the MWCNTs and $Fe_3O_4@SiO_2$ were dispersed homogeneously by vortexing vigorously for 1.0 min. Upon mixing, the MWCNTs and magnetic particles assembled to form magnetic MWCNTs. The resulting magnetic MWCNTs were washed with pure water and acetone in sequence, and then resuspended in 5.0 mL of water. The final concentration of the suspension solution was 15.0 mg mL^{-1}.

Liquorice, cassia seed, dahurian angelica root and reed rhizome samples were purchased from medicinal materials market (Baoding, China). Each dried samples was homogenized in a stainless steel blender, and then weighed into a beaker. A total of 15.0 mL of concentrated HNO_3 and 5.0 mL of H_2O_2 (30% w/w) were added, The solution was heated until transparent, and continuously evaporated until near dryness. The dissolved residue in 0.1 mol L^{-1} HNO_3 was diluted with 50.0 mL of deionized water.

The SPE procedure was performed as follows: An aliquot of 50.0 mL of digestion solution or standard solution was prepared. APDC solution (0.5 mL, 2.0 g L^{-1}) was added, and the pH value was adjusted to 4.0 with ammonium acetate buffer solution (0.2 mol L^{-1}). Subsequently, 2.0 mL of the magnetic MWCNTs suspension was added to the solution, and the mixture was sonicated for 2.0 min. After equilibrium, an Nd-Fe-B strong magnet (10 mm × 10 mm × 50 mm) was deposited at the bottom of the bottle and the sorbents were isolated from the solution. After about 1.0 min, the solution became clear and the supernatant was decanted. Finally, 2.0 mol L^{-1} HNO_3 was added to desorb the analytes (washed twice, 1.0 mL each time). The eluent was collected. After filtration through a 0.45 μm membrane, the analyte ions in the eluent were determined by FAAS.

Results and discussion

The hydrophobic chelate of Cd and Pb formed with APDC from the aqueous phase and can easily interact with the CNTs, which increases extraction efficiency of the heavy metal ions [21]. The ligand amount is an important factor for the quantitative retentions of metal ions in SPE techniques [22]. To investigate the optimum amounts of ligand solution on the quantitative recoveries of the analyte ions on MCNTs, volume of APDC solution (2.0 g L^{-1}) was varied from 0.0 -1.0 mL (Fig. 1). The recoveries of the analyte ions increased with increasing volume of added APDC and reached a constant value over 95% with at least 0.4 mL. The recovery values of the analytes were quantitative at the volume of ligand ranging from 0.4–0.6 mL. On this basis, further studies were conducted at an APDC volume of 0.5 mL.
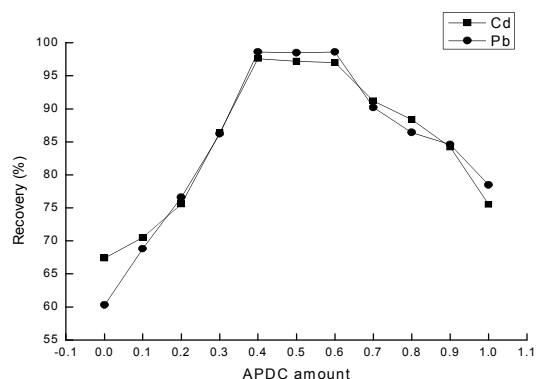
Fig. 1:   Effect of APDC amount on the recoveries of analytes obtained from MSPE-FAAS. Extraction conditions: sample volume, 50.0 mL; pH,4.0; eluent type, 2.0 mol L^{-1} HNO$_3$; eluent volume, 2.0 mL.

pK$_a$ value for APDC is 3.29 [23]. This value is consistent with the quantitative recovery values at the acidic pHs. The influences of pH of the analyte solutions on the recoveries of Pb and Cd were investigated within pH 2.0–10.0. The quantitative recoveries (>95%) for Pb and Cd were within pH 4.0–6.0. At the basic pH values, the recoveries were not quantitative. All subsequent studies were conducted at pH 4.0 with an ammonium acetate buffer solution.
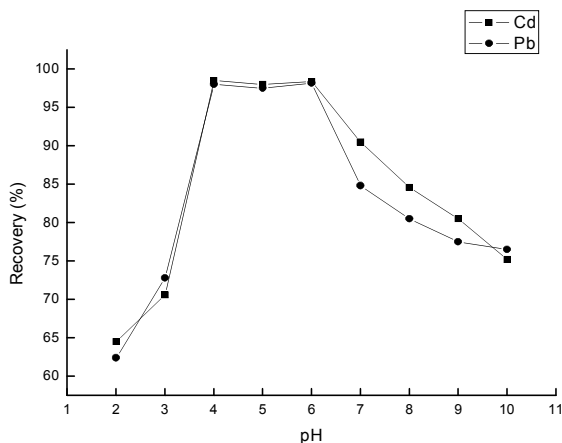

Fig. 2:   Effect of pH on the recoveries of analytes obtained from MSPE-FAAS. Extraction conditions: sample volume, 50.0 mL; APDC solution (0.5 mL, 2.0 g L^{-1}); eluent type, 2.0 mol L^{-1} HNO$_3$; eluent volume, 2.0 mL.

In the present work, the Fe$_3$O$_4$@SiO$_2$/MWCNTs showed an excellent capability to retain the heavy metal ions. Thus, quantitative extraction of the heavy metal ions was achieved using 30.0 mg of the Fe$_3$O$_4$@SiO$_2$/MWCNTs (2.0 mL of the adsorbent suspension, the ratio of Fe$_3$O$_4$/SiO$_2$ NPs and MWCNTs added was kept constant at 2:1). At higher amounts of the adsorbent, the extraction efficiency was almost constant. Therefore, in the subsequent experiments, 2.0 mL of the adsorbent suspension was added to 50.0 mL of the sample solution.

The adsorption of Pb and Cd at pH<4.0 could be incomplete (Fig. 2). Thus, nitric acid at different concentrations and volumes was studied for eluting the retained Pb and Cd from the surface of the adsorbent. A concentration of 2.0 mol L^{-1} nitric acid is required to obtain quantitative elution if the eluent volume is set to 2.0 mL.

The effect of potential ions, in real samples on the recovery of 300.0 μg L^{-1} of each of Pb and Cd standard solution in the presence of various amounts of individual interfering ions was also examined. A given species was considered to interfere if it resulted in a ±5% variation of the FAAS signal. All studied ions (Mn^{2+}, Cr^{3+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and Hg^{2+}) did not affect the absorbance in the MSPE-FAAS system when they are present in 100-fold excess. Higher concentrations of alkali and alkaline earth metals (K$^+$, Na$^+$, Ca^{2+}, and Mg^{2+}) can be tolerated. The above results indicated that the developed method is applicable to the analysis of Pb and Cd in traditional chinese medicine samples.

The limit of detection (LOD) was calculated as the ratio of thrice the standard deviation of the blank signals over the slope of the calibration curve. The LOD values of 0.6 and 0.5 μg L^{-1} were obtained for Pb and Cd, respectively. For a sample volume of 50.0 mL, the calibration graph exhibited linearity over the range of 10.0-400.0 μg L^{-1} for Pb and 10.0-300.0 μg L^{-1} for Cd. The relative standard deviation (RSD) for ten replicate measurements of solutions containing 300.0 μg L^{-1} each of Pb^{2+} and Cd^{2+} was 3.8 and 3.4%, respectively.

The proposed method was applied to determine of Pb and Cd in traditional chinese medicine samples. The results and the recovery for the spiked samples are listed in Table-1. The recoveries for the four spiked samples range within 90.0%–98.5%.

Table-1: Determination of Pb and Cd in traditional chinese medicine samples using MSPE-FAAS method (n=3).

Sample	Spiked (µg L⁻¹)		Found (µg L⁻¹)[a]		Recovery (%)	
	Pb	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**
Liquorice	0.0	0.0	ND[b]	3.6±0.2	–	–
	10.0	10.0	9.0±0.4	13.2±0.6	90.0	96.0
	20.0	20.0	19.3±0.8	23.3±1.2	96.5	98.5
Cassia Seed	0.0	0.0	ND	ND	–	–
	10.0	10.0	9.1±0.4	9.3±0.4	91.0	93.0
	20.0	20.0	19.4±0.8	19.4±0.9	97.0	97.0
Dahurian Angelicaroot	0.0	0.0	ND	6.4±0.3	–	–
	10.0	10.0	9.1±0.4	16.1±0.7	91.0	97.0
	20.0	20.0	19.4±0.8	26.1±1.2	97.0	98.5
Reed Rhizome	0.0	0.0	4.1±0.2	ND	–	–
	10.0	10.0	13.5±0.5	9.3±0.4	94.0	93.0
	20.0	20.0	23.8±1.1	19.5±0.8	98.5	97.5

[a] Mean of three experiments±standard deviation.
[b] Not detected.

Conclusion

A magnetic composite of MWCNTs and $Fe_3O_4@SiO_2$ was prepared by simple adsorption. The performance of magnetic CNTs for MSPE was evaluated by enriching Pb and Cd. Under optimized conditions, a rapid and sensitive method for determining Pb and Cd from traditional chinese medicine samples was established by the coupling of MSPE with FAAS. Compared with traditional SPE method, MSPE-FAAS avoids the time-consuming column passing and filtration operation. This method also shows a significant analytical potential in separating and preconcentrating target analytes from complex samples.

Acknowledgements

References

1. O. Lindqvist, Environmental impact of mercury and other heavy metals, *J. Power Sources*, **57**, 3 (1995).
2. M. Faraji, Y. Yamini, A. Saleh, M. Rezaee, M. Ghambarian and R. Hassani, A nanoparticle-based solid-phase extraction procedure followed by flow injection inductively coupled plasma-optical emission spectrometry to determine some heavy metal ions in water samples, *Anal. Chim. Acta*, **659**, 172 (2010).
3. L. Li, B. Hu, L. B. Xia and Z. Jiang, Determination of trace Cd and Pb in environmental and biological samples by ETV-ICP-MS after single-drop microextraction, *Talanta*, **70**, 468 (2006).
4. D. L. G. Borges, M. A. M. S. da Veiga, V. L. A. Frescura, B. Welz and A. Curtius, Cloud-point extraction for the determination of Cd, Pb and Pd in blood by electrothermal atomic absorption spectrometry, using Ir or Ru as permanent modifiers, *J. Anal. At. Spectrom.,* **18**, 501 (2003) .
5. J. R. Chen and K. C. Teo, Determination of cadmium, copper, lead and zinc in water samples by flame atomic absorption spectrometry after cloud point extraction, *Anal. Chim. Acta*, **450**, 215 (2001).
6. Y. A. Zolotov and N. M. Kuzmin, *Preconcentration of Trace Elements*, Elsevier Press, Amsterdam, p.51 (1990).
7. M. B. Arain, T. G. Kazi, M. K. Jamali , H. I. Afridi, N. Jalbani, R. A. Sarfraz , J. A. Baig, G. A. Kandhro and M. A. Memon, Time saving modified BCR sequential extraction procedure for the fraction of Cd, Cr, Cu, Ni, Pb and Zn in sediment samples of polluted lake, *J. Hazard. Mater.*, **160**, 235(2008).
8. H. M. Jiang and B. Hu, Determination of trace Cd and Pb in natural waters by direct single drop microextraction combined with electrothermal atomic absorption spectrometry, *Microchim. Acta*, **161**, 101(2008).
9. H. W. Chen, J. C. Jin and Y. F. Wang, Flow injection on-line coprecipitation preconcentration system using copper(II) diethyldithiocarbamate as carrier for flame atomic absorption spectrometric determination of cadmium, lead and nickel in environmental samples, *Anal. Chim. Acta*, **353**, 181 (1997).
10. M. Soylak, A. Kars and I. Narin, Coprecipitation of Ni^{2+}, Cd^{2+} and Pb^{2+} for preconcentration in environmental samples prior to flame atomic absorption spectrometric

determinations, *J. Hazard. Mater.*, **159**, 435 (2008).

11. J. L. Manzoori and Gh. Karim-Nezhad, Selective cloud point extraction and preconcentration of trace amounts of silver as a dithizone complex prior to flame atomic absorption spectrometric determination, *Anal. Chim. Acta*, **484**, 155 (2003).

12. M. G. Pereira, E. R. Pereira-Filho, H. Berndt and M. A. Z. Arruda, Determination of cadmium and lead at low levels by using preconcentration at fullerene coupled to thermospray flame furnace atomic absorption spectrometry, *Spectrochim. Acta B,* **59**, 515 (2004).

13. E. Ivanova, H. Berndt and E. Pulvermacher, Air driven on-line separation and preconcentration on a C_{18} column coupled with thermospray flame furnace AAS for the determination of cadmium and lead at μg l^{-1} levels, *J. Anal. At. Spectrom*., **19**, 1507 (2004).

14. Q. X. Zhou, J. P. Xiao, W. D. Wang, G. G. Liu, Q. Z. Shi and J. H. Wang. Determination of atrazine and simazine in environmental water samples using multiwalled carbon nanotubes as the adsorbents for preconcentration prior to high performance liquid chromatography with diode array detector, *Talanta*, **68**, 1309 (2006).

15. P. Liang, Y. Liu and L. Guo, Determination of trace rare earth elements by inductively coupled plasma atomic emission spectrometry after preconcentration with multiwalled carbon nanotubes, *Spectrochim. Acta B*, **60**, 125 (2005).

16. Y. B. Luo, Q. W. Yu, B. F. Yuan and Y. Q. Feng, Fast microextraction of phthalate acid esters from beverage, environmental water and perfume samples by magnetic multi-walled carbon nanotubes, *Talanta*, **90**, 123 (2012).

17. A. Mehdinia, F. Roohi and A. Jabbari, Rapid magnetic solid phase extraction with in situ derivatization of methylmercury in seawater by Fe_3O_4/polyaniline nanoparticle, *J. Chromatogr. A*, **1218**, 4269 (2011).

18. X. L. Zhao, Y. L. Shi, Y. Q. Cai and S. F. Mou, Cetyltrimethylammonium bromide-coated magnetic nanoparticles for the preconcentration of phenolic compounds from environmental water samples, *Environ. Sci. Technol.,* **42**, 1201 (2008).

19. P. Wu, J. Zhu and Z. Xu, Template-assisted synthesis of mesoporous magnetic nanocomposite particles, *Adv. Funct. Mater.*, **14**, 345 (2004).

20. Z. Y. Ma, Y. P. Guan and H. Z. Liu, Superparamagnetic silica nanoparticles with immobilized metal affinity ligands for protein adsorption, *J. Magn. Magn. Mater.*, **301**, 469 (2006).

21. M. Tuzen, K. O. Saygi and M. Soylak, Solid phase extraction of heavy metal ions in environmental samples on multiwalled carbon nanotubes, *J. Hazard. Mater.*, **152**, 632(2008).

22. F. Sabermahani and M. A.Taher, Determination of trace amounts of cadmium and copper by atomic absorption spectrometry after simultaneous extraction and preconcentration using a new water-soluble polyacrylic acid/alumina sorbent, *Microchim. Acta*, **159**, 117(2007).

23. R. R. Scharfe, V. S. Sastri and C. L. Chakrabarti, Stability of metal dithiocarbamate complexes, *Anal. Chem.,* **45**, 413 (1973).