Preconcentration and Determination of Trace Cu, Pb, and Zn in Biological Samples by Combining Carbon Nanofibers (CNFs) as a Solid Phase Extraction Adsorbent with ICP-MS

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INTRODUCTION

Trace elements play an important biochemical and physiological role in human growth, development, health and disease (1,2) because they participate in a wide variety of metabolic processes, including the synthesis or degradation of carbohydrates, lipids, proteins, and nucleic acids. Copper (Cu) has great influence on arterial strength (3), lead (Pb) is classified as a prevalent toxic metal, which constitutes a major environmental health problem (4), and zinc (Zn), although considered essential, can also produce toxic effects when its intake is excessively elevated (5). Therefore, sensitive, reproducible, and accurate analytical methods are required for the determination of trace elements in environmental and biological samples.

Inductively coupled plasma mass spectrometry (ICP-MS) (6,7), atomic absorption spectrometry (AAS) (8,9), and inductively coupled plasma atomic emission spectrometry (ICP-AES) (10,11) still represent the most important routine methods for the determination of trace elements in environmental and biological samples. Despite the great improvement in the power of detection of these instruments, direct determination of trace elements at extremely low concentrations by modern analytical techniques is, in many cases, difficult. This is not only due to the insufficient sensitivity of the meth-

ABSTRACT

A procedure for the determination of trace Cu, Pb, and Zn in biological samples has been developed using inductively coupled plasma mass spectrometry (ICP-MS) and carbon nanofibers (CNFs) as a solid phase extraction adsorbent. The effect of various experimental parameters, such as pH, sample flow rate and volume, elution solution and interfering ions on the retention of the studied ions, have been investigated systematically. The experimental results showed that Cu, Pb, and Zn were quantitatively sorbed on a microcolumn containing CNFs in the range of pH 7.0–9.0 and then eluted completely with 1.0 mol mL⁻¹ HNO₃. The detection limits (DL) for Cu, Pb, and Zn were 0.031 ng mL⁻¹, 0.027 ng mL⁻¹ and 0.062 ng mL⁻¹, respectively. The relative standard deviations (RSD) were less than 5.0% ($c=1.0$ ng mL⁻¹, $n=9$). The proposed method was applied to the determination of trace Cu, Pb, and Zn in a mussel certified reference material (GBW 08571) with satisfactory results.

ods, but also due to matrix interferences. Therefore, the use of separation and/or preconcentration procedures is often necessary before the determination step (12–18). Among them, solid phase extraction has become increasingly popular because of its major advantages which include (i) simplicity of operation, (ii) high concentration factor, (iii) rapid phase separation, and (iv) the ability to combine with different detection techniques (19,

20). Being an important factor in separation and preconcentration, the sorbent material for solid-phase extraction determines the sensitivity and selectivity of the analytical method. Thus, the development of a new adsorbent material with high selectivity and sensitivity is forever of interest to analysts.

In recent years, nanometer-size material has drawn growing attention in the analytical sciences because of its special properties. Some nanometer-size substances (such as multi-walled carbon nanotubes, nanometer-sized zirconium oxide, FeC nanoparticles, nanometer-size silicon dioxide and titanium dioxide nanotubes) have been successfully used as solid-phase extractions for the preconcentration/separation of metal and non-metal ions as well as the adsorption of organic compounds (21–27). It is well known that carbon nanofibers (CNFs), as an interesting member of carbon materials, provides the very attractive features of having small size, large specific surface area, excellent mechanical strength, high chemical stability, and unique electrical properties, which make them a promising candidate as adsorbents (28). In addition, their surface properties can be modified with chemical treatment to satisfy some special needs (29). CNFs have been found to be an ideal adsorbent for hydrogen storage (30). However, to the best of our knowledge, studies on the application of CNFs in trace elemental analysis have received little attention so far.

The present work is aimed at exploring the adsorptive potential

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of CNFs for the preconcentration of trace metal elements using the column method. A new method using a microcolumn packed with CNFs coupled to ICP-MS is described for the preconcentration/separation and determination of trace elements in biological samples. The proposed method was applied to the determination of Cu, Pb, and Zn in a certified reference material of mussel (GBW 08571) with satisfactory results.

EXPERIMENTAL

Instrumentation

An X-7 ICP-MS system (Thermo Elemental Corporation, USA) with a concentric nebulizer (Meinhard®) was used for the analyte determinations. The optimum operating conditions of the ICP-MS are given in Table I.

A HL-2 peristaltic pump (Shanghai Qingpu Huxi Instrument Factory, P.R. China) coupled to a self-made polytetrafluoroethylene (PTFE) microcolumn (20 mm × 3.0 mm i.d.), packed with CNFs, was used for the preconcentration/separation process. A minimum length PTFE tubing with an i.d. of 0.5 mm was used for all connections.

The pH values of the solution were measured with a pH meter (Thermo Orion Corporation, USA) supplied with a combined electrode. An ETHOS T microwave digestion device (Milestone, Italy) was used for sample digestion. This device is pressure- and temperature-controlled, so that on reaching maximum pressure and temperature the microwave energy input is restricted automatically.

Standard Solutions and Reagents

The stock standard solutions (1.0 mg mL⁻¹) of Cu, Pb, and Zn were obtained from the National Analysis Center of Iron & Steel (Beijing, P.R. China). The working solu-

tions were prepared from dilutions of the stock solutions. Quantification of the elemental concentration was performed using external calibration with aqueous standard solutions using ¹¹⁵In as the internal standard. All reagents used were ultrapure or at least of analytical grade. High purity deionized water (18.2 MΩ) obtained from a Milli-Q™ A10 system (Millipore Corporation, USA) was used throughout this work. NH₃·H₂O and NH₄Cl were used to adjust the pH values of the sample solutions. The CNFs were kindly provided by Shenyang Metal Institute of Chinese Academy (Shenyang, P.R. China). Before use, the CNFs were oxidized with concentrated HNO₃ as described in the literature (31). The treated CNFs were dried at 100 °C and stored for future use.

Column Preparation

The microcolumn was prepared by placing 20 mg of the treated CNFs into an empty PTFE column (20 mm × 3.0 mm i.d.) using a packing method. To avoid loss of CNFs when the sample solution passes through the microcolumn, a small amount of quartz wool was used to plug both ends of the microcolumn. The microcolumn was then connected to a peristaltic pump to form the preconcentration system. Before use, 0.5 mol mL⁻¹

HNO₃ and high purity deionized water were passed through the microcolumn in order to clean and condition it. Then, the column was conditioned to the desired pH value with the solution mixture of NH₃·H₂O and NH₄Cl.

Sample Pretreatment

A sample portion (0.0500 g of mussel) was accurately weighed into 100-mL Teflon® vessels, and then 6.0 mL concentrated HNO₃ and 2.0 mL HClO₄ were added, respectively. After about 5 minutes, when the first vigorous reaction had taken place, the digestion vessels were closed and placed into the microwave oven. The samples were digested in the microwave oven at 180 °C (ramp, 10 minutes; hold, 15 minutes) at 1.0 kW power. When the digestion programs were completed, the vessels were cooled to room temperature. The vessels were then opened carefully, and a clear acid phase was observed. The clear acid phase from the microwave digestion was transferred to another vessel, and then heated on a hotplate at 140 °C to evaporate excess reagents. After reaching near dryness, the residues were dissolved with 0.1 mol L⁻¹ HNO₃ and diluted to the desired volume. Procedural blanks were prepared in the same way.

TABLE I
ICP-MS Operating Conditions

Plasma power	1300 W
Plasma argon flow rate	14.5 L min ⁻¹
Auxiliary argon flow rate	0.77 L min ⁻¹
Nebulizer argon flow rate	0.95 L min ⁻¹
Sampler orifice (nickel)	1.1 mm
Skimmer orifice (nickel)	0.7 mm
Acquisition mode	Peak jumping
Number of sweep	100
Dwell time	10ms
Acquisition time	40 s
Number of measurements per peak	3
Isotopes	⁶³ Cu, ²⁰⁸ Pb, ⁶⁶ Zn, and ¹¹⁵ In

General Procedure

Sample solutions were prepared by appropriate dilution of the stock solutions and adjusted to the desired pH with the solution mixture of $\text{NH}_3 \cdot \text{H}_2\text{O}$ and NH_4Cl used previously. The obtained solution was passed through the microcolumn with a peristaltic pump at the desired flow rate. Afterwards, the retained metal ions were eluted with 2.0 mL of 1.0 mol L^{-1} HNO_3 . The analytes in the effluents were determined by ICP-MS. The column could be used repeatedly (about 40 adsorption-elution cycles) after regeneration with 1.0 mol L^{-1} HNO_3 and deionized water.

RESULTS AND DISCUSSION

Surface Functionalization of CNFs

CNFs, as a new carbon material, have attracted great attention in applications such as gas adsorbent and catalyst support owing to a large specific surface, high chemical stability, and unique mechanical properties. The untreated CNFs, however, have a non-polar nature, a low surface energy and, therefore, are usually unable to form a strong adhesive force between their surface and the polar

substances (32). Fortunately, some investigations showed that the treatment of CNFs with oxidized acids can introduce many functional groups such as carboxyl ($-\text{COOH}$), hydroxyl ($-\text{OH}$), and carbonyl ($>\text{C}=\text{O}$) on the surface of CNFs (31). The oxygen-containing groups attached on the surfaces of CNFs can improve their adsorption capability of ions in solution. In this work, CNFs were treated with concentrated nitric acid as described in the literature (31).

Effect of pH on Adsorption

Due to the oxidation treatment with concentrated HNO_3 , some acidic groups could form on the surface of the CNFs. Thus, the adsorption of metal ions strongly depends on the pH. For this reason, the retention of the studied ions on the column containing CNFs was studied as a function of pH. The pH values of the solutions were adjusted ranging from 1.0–10 with $\text{NH}_3 \cdot \text{H}_2\text{O}$ and NH_4Cl . The adsorption percentage was calculated based on the difference between the amounts of the metal ions in the starting sample and the solution from the column. The effects of pH value on the analyte recoveries are shown in Figure 1. It can be seen from Figure 1 that the adsorption of the studied ions was quantitative (>90%) in part of the pH range of

7.0–9.0. Thus, a pH of 8.0 was used in this work.

Effect of Eluent Concentration and Volume

The choice of eluent is very important for the successful coupling of a microcolumn preconcentration system to ICP-MS. From Figure 1 it can be seen that the adsorption of the analytes on the CNFs is at $\text{pH} < 2.0$. For this reason, various concentrations of HNO_3 were studied for the desorption of the retained analytes from the microcolumn. The results in Table II show that 1.0 mol L^{-1} HNO_3 was sufficient for the quantitative elution with good recoveries (>90%).

In addition, the effect of eluent volume on analyte recoveries was studied by keeping the HNO_3 concentration of 1.0 mol L^{-1} (Table III). It was found that quantitative recoveries (>90%) were obtained with 2.0 mL of 1.0 mol L^{-1} HNO_3 . Thus, a 2.0-mL sampling volume was used in this work.

Influence of Sample Flow Rate

The sample flow rate through the column packed with CNFs is a very important parameter since it affects the retention of the cations on the adsorbent and the length of a complete analysis. The effect of sample flow rate on the adsorption percentage of the analytes was investigated by passing 20 mL of sample solution through the micro-

Fig. 1. Effect of pH on recovery of the analytes from the CNFs. Cu^{2+} and Zn^{2+} : 5.0 ng mL^{-1} ; Pb^{2+} : 2.0 ng mL^{-1} ; sample volume: 20 mL.

TABLE II
Eluent Efficiency (%)
for Removing the Analytes
From the CNFs

Eluent ^a (HNO_3 , mol L^{-1})	Recovery (%)		
	Cu^{2+}	Pb^{2+}	Zn^{2+}
0.1	35.2	42.5	37.9
0.3	48.4	54.3	44.8
0.5	80.6	84.6	78.6
1.0	96.4	98.2	94.8
1.5	95.8	94.7	97.4
2.0	97.2	96.8	98.3

^a Eluent volume: 2.0 mL.

TABLE III
Effect of Eluent Volume on
Recovery of Analytes

Volume (mL)	Recovery (%)		
	Cu^{2+}	Pb^{2+}	Zn^{2+}
0.5	45.3	39.3	37.2
1.0	74.1	52.6	61.3
1.5	95.4	89.4	91.8
2.0	97.6	94.7	96.1
2.5	98.2	96.2	94.7
3.0	93.7	95.3	93.9

column at a peristaltic pump flow rate between 0.2 and 2.0 mL min⁻¹. It can be seen in Figure 2 that with a flow rate up to 1.0 mL min⁻¹ there is no obvious effect on the analyte recoveries, which at optimum conditions are above 90%. Accordingly, all subsequent experiments were performed using the flow rate of 1.0 mL min⁻¹.

Effect of Sample Solution Volume

In order to explore the possibility of enriching low concentrations of the analytes from a large sample volume, the effect of sample volume on the adsorption of the analytes was studied. For this purpose,

50, 100, 200, 250, and 300 mL of the sample solutions containing 10 ng of the analytes were passed through the microcolumn at a 1.0 mL min⁻¹ flow rate. The results from Figure 3 show that quantitative recoveries (>90%) were obtained for sample volumes up to 200 mL. In this study, because the elution volume was 2.0 mL, an enrichment factor of 100-fold was obtained for the studied ions.

Adsorption Capacity

Adsorption capacity, as an important parameter for the evaluation of adsorbents, was investigated by a method provided in the literature (33). For this purpose, 20-mL aliquots of a series of concentra-

tions (0.4–2.4 µg mL⁻¹) were adjusted to the appropriate pH, then pre-concentrated and eluted. The amount of metal ions adsorbed at each concentration level was determined by ICP-MS. Breakthrough curves were obtained by plotting the metal ion concentrations (µg mL⁻¹) versus the milligrams of metal ions adsorbed on per gram of adsorbent (Figure 4). The adsorption capacities calculated from the breakthrough curve were 1.9, 1.6, and 1.5 mg g⁻¹ for Cu²⁺, Pb²⁺, and Zn²⁺, respectively.

Effect of Interfering Ions

The effects of potential interfering ions in biological and environmental samples on the preconcentration and determination of the analytes were examined. The tolerance limit of coexisting ions is defined as the largest amount making the recovery of the analyte less than 90%. In these experiments, the interfering ion concentrations were varied, whereas the concentrations of Cu²⁺, Pb²⁺, and Zn²⁺ were kept at 5.0 ng mL⁻¹ with a 20.0-mL sample volume. The effects of interfering ions on the recoveries of the analytes are presented in Table IV. It can be seen that the major cations and anions were not found to interfere within the range of their amounts determined.

Fig. 2. Effect of sample flow rate on recovery of the analytes from the CNFs. pH: 8.0; Cu²⁺ and Zn²⁺: 5.0 ng mL⁻¹; Pb²⁺: 2.0 ng mL⁻¹; sample volume: 20 mL.

Fig. 3. Effect of sample volume on recovery of the analytes from the CNFs. pH: 8.0; sample flow rate: 1.0 mL min⁻¹.

Fig. 4. Breakthrough curve of the analytes from the CNFs. pH: 8.0; sample volume: 20 mL.

TABLE IV
Effects of Coexisting Ions

Coexisting Ion	Concentration Ratio ^a
Na ⁺ , K ⁺	20,000
Ca ²⁺ , Mg ²⁺	10,000
Al ³⁺ , Fe ³⁺	250
SiO ₃ ²⁻ , SO ₄ ²⁻ , PO ₄ ³⁻	5000

^a Concentration ratio: Foreign ion/determined ions.

Column Reuse

In order to examine the long-term stability of CNFs, they were subjected to successive adsorption and desorption cycles by passing 20 mL of the solutions containing the analytes through the column. The stability and potential regeneration of the column were assessed by monitoring the changes in the recoveries of the analytes. The column can be reused after regeneration with 10 mL of 1.0 mol mL⁻¹ HNO₃ and 20 mL deionized water, and is stable up to 40 adsorption-elution cycles without obvious decrease in the adsorption capacity and the recoveries of the analytes.

Detection Limits and Precisions

Under the optimized conditions, the calibration graphs were linear from 0.5 to 100 ng mL⁻¹ with a correlation coefficient better than 0.9995. The detection limits of this method for Cu, Pb, and Zn, based on three times the standard deviation of 9 runs of the blank solution, were found to be 0.031, 0.027, and 0.062 ng mL⁻¹ with an enrichment factor of 100, respectively. The relative standard deviations (RSD) of this method, obtained for the nine determinations of 1.0 ng mL⁻¹ of Cu, Pb, and Zn, were 4.2%, 3.6%, and 2.8%, respectively.

TABLE V
Analytical Results of the Analytes in SRM GBW 08571 Mussel

Element	Added (μg g ⁻¹)	Found ^a (μg g ⁻¹)	Certified (μg g ⁻¹)	Recovery (%)
Cu	0	8.1 ± 0.5	7.7 ± 0.9	-
	5.0	12.9 ± 0.9	-	96.0
Pb	0	1.91 ± 0.1	1.96 ± 0.09	-
	1.0	2.89 ± 0.17	-	98.0
Zn	0	145 ± 6	138 ± 9	-
	50	196 ± 8	-	102

^a Mean value ± standard deviation, n=3.

Sample Analysis

In order to establish the validity of the proposed method, the mussel reference material GBW 08571 (obtained from Langfang, P.R. China) was analyzed. The obtained results are summarized in Table V. As can be seen, the determined values were in good agreement with the certified values, and the recoveries of the studied ions were reasonable for trace analysis, ranging from 96–102%.

CONCLUSION

The adsorption behavior of the metal ions on the modified carbon nanofibers (CNFs) has been investigated systematically. A simple, rapid, and reliable method was developed for the preconcentration of the metal ions Cu, Pb, and Zn in biological samples by using carbon nanofibers treated with concentrated HNO₃ as a solid phase extraction sorbent for ICP-MS determination. The analytes retained on the CNFs can be easily desorbed and no carryover is observed in the next analysis. An enrichment factor of 100 was achieved. In summary, the obtained experimental results have demonstrated that CNFs have great potential as an adsorbent for the preconcentration of trace/ultra-trace metal ions.

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REFERENCES

1. B. Huang, S. Lin, S. Chen, G. Zhou, F. Yin, and Z. Lou, *Biol. Trace Element Res.* 29, 133 (1991).
2. A. Aharoni, B. Tesler, Y. Paltiel, J. Tal, Z. Dori, and M. Sharf, *Am. J. Clin. Nutr.* 55, 104 (1992).
3. F. Timur, B. F. Senkal, O. Kaplan, G. Kaya, C. Ozcan, N. M. Karaaslan, and M. Yaman, *At. Spectrosc.* 30(6), 191 (2009).
4. O. Kaplan and M. Yaman, *At. Spectrosc.* 30(1), 1 (2009).
5. S. Chen, D. Lu, and Z. Hu, *J. of AOAC Intern.* 90(1), 258 (2007).
6. N.N. Meeravali and S. Jiang, *At. Spectrosc.* 31(4), 111 (2010).
7. M. Yin and B. Li, *Spectrochim. Acta Part B* 53, 1447 (1998).
8. B. Pilar, M. Antonio, M. Jorge, K. Tiina, and B. Adela, *At. Spectrosc.* 21(1), 5 (2000).
9. A.R. Domingo, R. Lidia, M. Tatiana, A. Juan, and C. Pedro, *Intern. J. Environ. Anal. Chem.* 83(4), 327 (2003).

10. K. Sreenivasa Rao, T. Balaji, T. Prasada Rao, Y. Babu, and G. R. K. Naidu, *Spectrochim. Acta Part B* 57, 1333 (2002).
11. Q. Zhou, X. Zhao, G. Xie, and J. Xiao, *At. Spectrosc.* 29(4), 145 (2008).
12. S. Chen, *Metall. Anal.* 20(3), 7 (2006).
13. F. Fujimori, T. Hayashi, and H. Haraguchi, *Fresenius J. Anal. Chem.* 363, 277 (1999).
14. A.G. Coedo, T.D. López, and F. Alguacil, *Anal. Chim. Acta* 315 (3), 331 (1995).
15. E.R. Unsworth, J.M. Cook, and S.J. Hill, *Anal. Chim. Acta* 442, 141 (2001).
16. A.A. Menegário and M.F. Giné, *Spectrochim. Acta Part B* 55(4), 355 (2000).
17. S.N. Willie and R.E. Sturgeon, *Spectrochim. Acta, Part B*, 56, 1707 (2001).
18. N.M.M. Coelho, L.M. Coelho, and E.S. de Lima, *Talanta* 66(4), 818 (2005).
19. A. V. Lemos, L. S. G. Teixeira, M. D. A. Bezerra, A. C. S. Costa, J. T. Castro, L. A. M. Cardoso, D. S. D. Jesus, E. S. Santos, P. X. Baliza, and L. N. Santos, *Appl. Spectrosc. Rev.* 43, 303 (2008).
20. V. Camel, *Spectrochim. Acta Part B* 58, 1177 (2003).
21. W. Yiwei, J. Yinyan, H. Deyan, W. Fang, and Z. Junxia, *Microchim. Acta* 159(3-4), 333 (2007).
22. R. Yinzhe, F. Zhefeng, and W. Jianying, *Microchim. Acta* 158(3-4), 227 (2007).
23. W. Hong, W. Hongyu, H. Baoping, D. Baixiang, L. Jusheng, and T. Jiuying, *Microchim. Acta* 166(1-2), 41 (2009).
24. S. Chen, X. Zhan, D. Lu, M. Yang, and C. Liu, *At. Spectrosc.* 29(4), 124 (2008).
25. Y. Cui, X. Chang, Y. Zhai, X. Zhu, H. Zheng, and N. Lian, *Microchem. J.* 83, 35(2006).
26. Q. Zhou, X. Zhao, G. Xie, and J. Xiao, *At. Spectrosc.* 29(4), 145 (2008).
27. Y. Cai, G. Jiang, J. Liu, and Q. Zhou, *Anal. Chem.* 75, 2517 (2003).
28. D. Eva, O. Saldador, and V. Aurelio, *J. Coll. Inter. Sci.* 305(1), 7 (2007).
29. M. L. Toebe, J. M. P. van Heeswijk, J. H. Bitter, A. J. Van Dillen, and K. P. de Jong, *Carbon* 42, 307 (2004).
30. A. Chambers, C. Park, R.T.K. Baker, and N.M. Rodriguez, *Phys. Chem.* 102B, 4253 (1998).
31. P.V. Lakshminarayanan, H. Toghiani, and C.U. Pittman Jr., *Carbon* 42, 2433 (2004).
32. H. Hubert, X. Ai, S. Haiber, M. Heintze, V. Brüser, E. Pasch, W. Brandle, and G. Marginean, *Spectrochim. Acta Part B* 57, 1601 (2002).
33. A. Maqieira, H.A.M. Elmahadi, and R. Puchades, *Anal. Chem.* 66, 3632 (1994).