

Penicillium chrysogenum immobilised silica: flame atomic absorption spectrometric Pb determination in industrial effluent, sludge and food samples

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Abstract A solid phase extraction method based on the immobilisation of *Penicillium chrysogenum* on silica was developed for preconcentration and flame atomic absorption spectrometric determination of Pb(II). The immobilised sorbent was packed in a column to optimise the conditions for its quantitative sorption and desorption. Various parameters such as pH, type of eluent, sample and eluent flow rate and influence of others ions were systematically studied. Pb(II) was quantitatively sorbed at pH 4.0 at a flow rate of 2.0 mL min⁻¹ and eluted with 10.0 mL of 1.0 M HCl at 0.2 mL min⁻¹ flow rate. The devised procedure applied for Pb determination in tap water, an industrial effluent and sludge sample, black tea, spinach leaves and green beans (canned food) was reproducible, with a relative standard deviation of 0.8%. The method was validated by a BCR 701 standard lake sediment sample. The limit of detection and limit of quantification for Pb(II) were 7.16 and 23.88 µg L⁻¹, respectively, with an enrichment factor of 100.

Keywords Pb · Solid phase extraction · *Penicillium chrysogenum* · FAAS

Introduction

Pb is released in the environment due to its industrial uses. It is a toxic element, and according to WHO the maximum permissible limit value for industrial discharge is 10 µg L⁻¹ Pb (Sud et al. 2008). Thus, its determination in sludge and industrial effluents has significance for control measures while the analysis of water and food samples for Pb is important due to its harmful effects. Direct analysis of such sample for Pb using flame atomic absorption spectrometry (FAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) exhibits limitations due to the chemical and spectral interferences arising from matrix elements and also lack of adequate sensitivity for its detection at low levels. Hence, various preconcentration methods for Pb determination were devised (Korn et al. 2006) though solid phase extraction (SPE) offers advantages over the existing methods owing to its simplicity, flexibility and availability of large number of sorbents.

A number of solid phase extraction methods have been developed for enrichment and determination of Pb(II). They involved either chemical reactions to prepare the sorbent (Er et al. 2013; Cui et al. 2008; Turan et al. 2012) or physical interaction with a suitable solid support to prepare impregnated sorbents. The reagents most commonly used were spectroscopic reagents used in conjugation with alumina (Mahmoud et al. 2010), Amberlite XAD-4 (Islam et al. 2011) and MCI GEL CHP20P resin (Aydin and Soy lak 2010). A method using C18 disc immobilised with a liquid extractant was also devised (Karve and Rajgor 2007). There is still a considerable interest amongst researchers to prepare new sorbents, and utilising microorganisms is an interesting alternative approach (Turker 2007).

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It was remarked by Wang and Chen (2009) that fungal biomass is a low-cost sorbent. For the separation, removal and preconcentration of Pb(II) various types of fungal species immobilised on different solid supports were studied. Chitosan-immobilised *Rhizopus arrhizus* beads (Lang et al. 2013) and immobilised *Pycnoporus sanguineus* beads (Yus Azila et al. 2008) were reported for Pb(II). Solid phase extraction of Pb(II) was carried out with *Aspergillus fumigatus* (Soylak et al. 2006), *Penicillium italicum* (Mendil et al. 2008) and *Aspergillus niger* (Baytak et al. 2007) immobilised on Diaion HP-2MG resin, Sepabeads SP 70 and silica, respectively. To the best of our knowledge, solid phase extraction and preconcentration of Pb(II) using *Penicillium chrysogenum* have not been reported.

In view of this, a simple method was devised for immobilisation of NaOH-treated *P. chrysogenum* on silica to preconcentrate and determine Pb(II) using FAAS. The solid phase was packed in a column, and the systematic investigations based on the parameters influencing sorption and desorption of Pb(II) were used to devise a method for Pb(II) determination in real samples.

Materials and methods

Instrument

A flame atomic absorption spectrophotometer AAnalyst200 (PerkinElmer, Shelton, CT) equipped with an air-acetylene burner with deuterium arc background correction was used for metal ion determination. The Pb hollow cathode lamp was operated at 10 mA current, and absorbance measured at 217.0 nm with a spectral band width of 0.5 nm. A pH meter Elico LI-127 digital pH meter (Elico India Ltd., Hyderabad, India) with a combined glass electrode was used for measurement of pH. Double-distilled water was produced from LAB-SIL quartz double distiller (LAB-SIL Instruments Pvt. Ltd., Bangalore, India). An inductively coupled plasma atomic emission spectrometry (ARCOS from M/s. Spectro, Germany) was also used for comparison of results.

Chemicals

All chemicals used were of analytical reagent grade. The stock solution containing 1000 mg L⁻¹ of Pb(II) was prepared by dissolving appropriate amount of Pb(NO₃)₂ in slightly acidic double-distilled water. The solution was standardised volumetrically, and working standard solution containing 20 µg mL⁻¹ was prepared by appropriate dilution. Activated silica gel (60–120 mesh) was prepared using conc. HCl (Cui et al. 2008). For culture, all media used were from Himedia Laboratories Pvt. Ltd., Mumbai,

India. A glass column (150 mm × 10 mm, J-sil make, Mumbai, India) was used for packing *P. chrysogenum* immobilised on silica.

Preparation of sorbent and optimisation

The method adopted to prepare the sorbent by immobilisation of *P. chrysogenum* on silica (PCS) by a physical method was similar to the previous report (Karve and Choudhary 2015). In order to preconcentration Pb(II) on this sorbent, a glass column containing glass wool as support was packed with 350 mg of the PCS. Using this column packed with the sorbent, various parameters, i.e. pH, eluent, sample and eluent flow rate, were systematically investigated for Pb(II) sorption and desorption to devise the procedure.

Preconcentration procedure

A sample solution containing 20 µg Pb(II) was adjusted to pH 4.0 using dilute NH₃ and HCl and passed through the column which was also preconditioned to the same pH. Using 10 mL of 1.0 M HCl, Pb(II) was eluted and detected by FAAS and its concentration determined from a calibration curve which was linear within the range 1–4 ppm.

Preparation of industrial effluent, industrial sludge and food samples

The industrial effluent was filtered through a nylon membrane filter (Millipore) of 0.45 µm pore size.

1.0 g of industrial sludge sample was treated with HF and HNO₃ to prepare the sample solution (Karve and Rajgor 2007).

Samples of black tea (10.0 g), spinach leaves (20.0 g) and green beans (canned food) (20.0 g) were obtained by treating with conc. HNO₃ and keeping over night followed by treatment with conc. HClO₄. The resultant solution was evaporated to dryness, and the residue extracted with double-distilled water (Ganjali et al. 2004). Appropriate aliquots from all these samples were then taken for Pb(II) preconcentration as per the procedure described in the section above.

Results and discussion

Effect of pH

The pH of aqueous phase influences metal sorption (Lang et al. 2013; Iram et al. 2015). The ionic interaction of a metal ion with the functional groups present in the fungal cell wall results in its uptake by the biomass. Pb(II) sorption was studied from pH 1.0–6.0 (Fig. 1). Preliminary

studies indicated that only 40% of Pb(II) was sorbed on activated silica and its impregnation was necessary for complete Pb(II) sorption. As the aqueous-phase pH increased, Pb(II) sorption increased and it was quantitative ($98.7 \pm 0.3\%$) at pH 4.0; hence, pH 4.0 was considered as the optimum pH for further studies.

Effect of eluent type

An appropriate choice of eluent is required to recover Pb(II) to reuse the sorbent for the next cycle. Different concentrations of HCl and HNO₃ were studied (Table 1). Quantitative recovery ($98.6 \pm 0.4\%$) could be accomplished with 1.0 M HNO₃ and 1.0 M HCl. Preliminary studies indicated that 1.0 M HNO₃ caused leakage of biomass from the column, thereby reducing its efficiency, and therefore, 1.0 M HCl was chosen as an eluent, and its volume and flow rate were studied for Pb(II) recovery.

The minimum volume of an eluent is essential to achieve a high enrichment factor while the use of higher

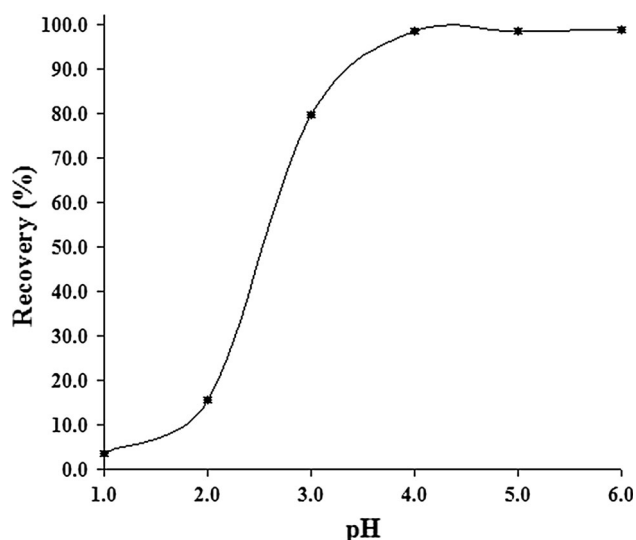


Fig. 1 Effect of pH on sorption of Pb(II) on PCS [aqueous phase: 50 mL containing 20 μg Pb(II), flow rate: 2 mL min⁻¹, eluent: 10 mL, 1.0 M HCl, flow rate = 0.2 mL min⁻¹ ($N = 3$)]

Table 1 Effect of type and concentration of eluent on the recovery of Pb(II)^a ($N = 3$)

Eluents → Conc. [M] ↓	Recovery of Pb(II) (%) ± RSD	
	HCl	HNO ₃
1.0×10^{-1}	19.9 ± 0.6	29.0 ± 0.6
5.0×10^{-1}	76.1 ± 0.8	79.9 ± 0.7
1.0	98.6 ± 0.4	98.7 ± 0.5

RSD relative standard deviation

^a Aqueous condition: 50 mL containing 20 μg Pb(II), pH = 4.0, flow rate = 2.0 mL min⁻¹, eluent: 10 mL, flow rate = 0.2 mL min⁻¹

flow rates ensures rapid elution. Using 5.0 mL of 1.0 M HCl resulted in $64.1 \pm 0.8\%$ Pb(II) recovery while it was quantitative with 10.0 and 15.0 mL of 1.0 M HCl. With 10.0 mL of 1.0 M HCl as eluent and 0.1 and 0.2 mL min⁻¹ flow rates, the recovery was quantitative ($98.7 \pm 0.6\%$ and $98.6 \pm 0.4\%$, respectively) and it decreased to $78.5 \pm 0.7\%$ at 0.5 mL min⁻¹ flow rate. Therefore, 10.0 mL of 1.0 M HCl at a flow rate of 0.2 mL min⁻¹ was used for all subsequent studies.

Flow rate of sample solution

The flow rate of sample solution has considerable influence in solid phase extraction, as high flow rates diminish the contact between the analyte and sorbent. Pb(II) sorption was studied at a sample flow rate of 1.0–4.0 mL min⁻¹, and it was quantitatively sorbed from 1.0 to 3.0 mL min⁻¹ sample flow rate (Fig. 2). A sample flow rate of 2.0 mL min⁻¹ was used in all studies as flow rate >3.0 mL min⁻¹ resulted in decreased Pb(II) sorption.

Effect of foreign ions

The influence of other ions in sample solution during sorption of Pb(II) was studied at the aqueous-phase pH 4.0 and using a flow rate 2.0 mL min⁻¹ (Table 2). The tolerance limit was set as the maximum amount of the foreign ion that causes an approximately $\pm 2\%$ error in the quantitative recovery of Pb(II). Several ions showed the tolerance limit ratio of 1:5 with Pb(II).

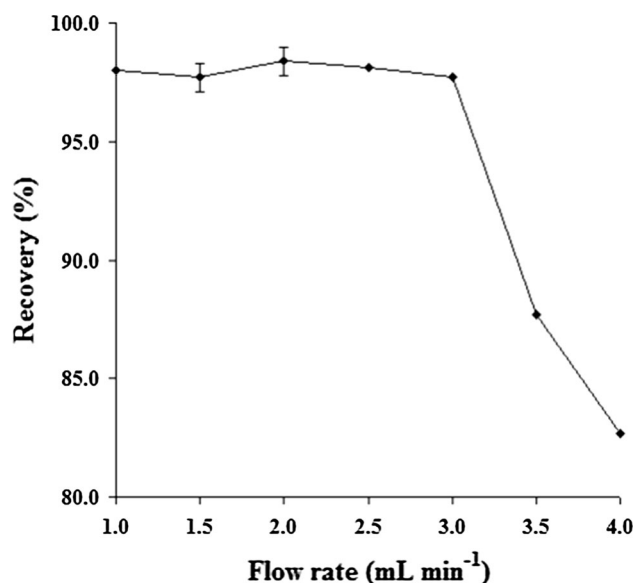


Fig. 2 Effect of flow rate on Pb(II) sorption on PCS [aqueous phase: 50 mL containing 20 μg Pb(II), pH = 4.0, eluent: 10 mL, 1.0 M HCl, flow rate = 0.2 mL min⁻¹ ($N = 3$)]



Table 2 Tolerance limit for coexisting ions in sorption of 20 µg Pb(II)^a (*N* = 3)

Foreign ion	Tolerance limit (µg)	Recovery (%) ± RSD
Na ⁺	1500	98.8 ± 0.6
K ⁺	1500	98.5 ± 0.6
Ca ²⁺	100	98.7 ± 0.6
Mg ²⁺	100	99.1 ± 0.6
Cr ³⁺	40	98.3 ± 0.8
Mn ²⁺	100	98.7 ± 0.7
Co ²⁺	100	98.7 ± 0.7
Ni ²⁺	100	99.1 ± 0.7
Cd ²⁺	100	98.7 ± 0.7
Zn ²⁺	100	98.7 ± 0.6
Cu ²⁺	100	98.7 ± 0.7
Fe ³⁺	200	98.2 ± 0.4

RSD relative standard deviation

^a Aqueous condition: 50 mL containing 20 µg Pb(II), pH = 4.0, flow rate = 2.0 mL min⁻¹, eluent: 10 mL, 1.0 M HCl, flow rate = 0.2 mL min⁻¹

Table 3 Determination of Pb(II) in tap water sample^a (*N* = 3)

Sample	Added (µg)	Found (µg)	Recovery (%) ± RSD
Tap water	0	N.D	–
	5	4.92	98.5 ± 0.2
	10	9.84	98.6 ± 0.3
	20	19.68	98.5 ± 0.2

RSD relative standard deviation, N.D not detected

^a Aqueous phase: 50 mL tap water, pH = 4.0, flow rate = 2.0 mL min⁻¹, eluent: 10 mL, 1.0 M HCl, flow rate = 0.2 mL min⁻¹

Analytical performances

The preconcentration factor for Pb(II) was determined by passing a sample solution (25–1000 mL) containing

20 µg Pb(II) at pH 4.0 through the column. Its recovery was quantitative even up to 1000 mL. As the eluent volume was 10 mL, a preconcentration factor of 100 was achieved. The limit of detection (LOD) is defined as three times the standard deviations of the blank, and definition of limit of quantification (LOQ) is ten times the standard deviations of the blank for Pb(II), and they were found to be 7.16 and 23.88 µg L⁻¹, respectively. The relative standard deviation (RSD) was 0.8%.

Application to certified sample

A sample solution using 1.750 g of Lake sediment reference material (BCR-701) was prepared (Pueyo et al. 2001), and Pb(II) determined in an aliquot using the devised procedure. The amount of Pb(II) was 142.6 ± 1.2 µg/g (*N* = 3) as against the certified value of 143.0 ± 6.0 µg/g indicating good agreement with the standard value.

Application to real samples

A tap water sample was spiked with different amounts of Pb(II) and determined using the general procedure (Table 3). These results suggest that the method can be used for tap water analysis.

Sample solutions of industrial effluent and sludge sample and some food products were prepared as described in the section related to application and were applied for determination of Pb(II) using the devised procedure.

The results of analysis (Table 4) of real samples were compared with those obtained by ICP-AES to evaluate Pb(II) recovery. The method was thus useful for analysis of complex materials with good sensitivity and reproducibility.

The LOD and preconcentration factors and applications of the devised procedure were comparable with other solid phase extraction procedures (Table 5).

Table 4 Determination of Pb(II) in various real sample^a (*N* = 3)

Sample	Recovery (µg g ⁻¹)		Recovery (%) ± RSD
	Present method	ICP-AES result	
Tea	25.21	25.58	98.6 ± 0.2
Spinach leaves	14.90	15.10	98.5 ± 0.1
Green bean (canned food)	5.96	6.05	98.5 ± 0.1
Industrial sludge	58.08	58.90	98.6 ± 0.2
Industrial effluent [†]	2.04	2.07	98.6 ± 0.3

RSD relative standard deviation

^a Aqueous phase: 20 mL real sample solution, pH = 4.0, flow rate = 2.0 mL min⁻¹, eluent: 10 mL, 1.0 M HCl, flow rate = 0.2 mL min⁻¹

[†] µg mL⁻¹

Table 5 Comparison of the proposed method with some solid phase extraction methods for Pb(II)

System (method for modification of sorbent)	LOD ($\mu\text{g L}^{-1}$)	PF	Applications	Ref.
Silica gel/ofloxacin (C)	0.13	100	SRM GBW 08301 river sediment, balsam pear leaves, pig liver, river and lake water	Cui et al. (2008)
Poly(2-thiozylmethacrylamide-co-divinyl-co-2-acrylamido-2-methyl-1-propanesulfonic acid)	1.07	150	TMDA-70 Fortified lake water, SPS-WW1 Batch 111-Wastewater, RM8704 Buffalo river sediment, GBW07605 Tea, well water, river water, street sediment and tea	Turan et al. (2012)
Alumina/1-nitroso-2-naphthol (C)	–	200	Waste water and tap water	Mahmoud et al. (2010)
MCI GEL CHP20P resin/2-nitroso-1-naphthol (I)	0.28	20	CRM BCR-144R Sewage Sludge (domestic origin), BCR-141R Calcareous Loam Soil, NIST 1568a Rice Flour and NIST 1577b Bovine Liver, spring and tap water	Aydin and Soylak (2010)
Sepabeads SP 70/ <i>Penicillium italicum</i> (I)	2.70	25	SRM IAEA 336 Lichen & NIST 1573a tomato leaves, natural waters, cultivated mushroom, lichen, moss, refined table salt	Mendil et al. (2008)
Activated carbon/2-((2-aminoethylamino)methyl)phenol (C)	0.16	100	SRM GBW 08501 peach leaf, tuckahoe, tap and lake water	Hu et al. (2010)
Silica/ <i>Penicillium chrysogenum</i> (I)	7.16	100	BCR-701 lake sediment, tap water, industrial effluent, industrial sludge, spinach leaves, canned food, black tea	This work

I impregnation, C chemical modification, LOD limit of detection, PF preconcentration factor

Conclusion

Penicillium chrysogenum immobilised on silica as solid phase offers a simple, efficient and cost-effective method for Pb(II) enrichment. Under the optimised conditions, Pb(II) was quantitatively sorbed at pH 4.0 using a 2.0 mL min^{-1} flow rate. The recovery of Pb(II) was easy using 1.0 M HCl at 0.2 mL min^{-1} flow rate, enabling the reuse of the sorbent. The devised procedure is useful to detect Pb contamination in environmental samples and can be used for treatment of industrial waste water.

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