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Biosorption of aluminum on *Pseudomonas aeruginosa* loaded on Chromosorb 106 prior to its graphite furnace atomic absorption spectrometric determination

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Abstract

A biosorption procedure for separation-enrichment of aluminum in environmental samples has been presented in this work. *Pseudomonas aeruginosa* loaded on Chromosorb 106 has been used as biosorbent for that purpose. *P. aeruginosa* is a gram-negative, aerobic rod. The influences of pH of the aqueous solution, eluent type, eluent volume, sample volume, etc. were examined on the quantitative recovery of aluminum in *P. aeruginosa* loaded on Chromosorb 106. The effects of concomitant ions on the recoveries of aluminum were also investigated. The detection limit based on 3 sigma for aluminum is 30 ng L^{-1}. Three certified reference materials (LGC 6010 Hard Drinking Water, NIST-SRM 1568a Rice Flour and NRCC-DORM-2 Dogfish Muscle) were analyzed for the validation of the presented procedure. The proposed procedure was applied to the determination of aluminum in environmental samples including natural water and food samples. The concentration of aluminum in real samples was found at ppb level.

Keywords: Aluminum; *Pseudomonas aeruginosa*; Biosorption; Preconcentration; Atomic absorption spectrometry

1. Introduction

Aluminum (Al) is a non-essential element to which humans are frequently exposed. Aluminum is widespread throughout nature, air, water, plants and consequently in all the food [1,2]. Aluminum is most commonly used in food technology as cans, packaging materials, kitchen utensils, and vessels [1]. Aluminum is also preferred due to its corrosion resistance and easy processing properties [3]. Aluminum accumulation may increase the risks of neurological and bone diseases, e.g., Alzheimer's disease, Parkinson's disease, encephalopathy/dialysis dementia, and osteomalacia [4,5]. Biologically, aluminum is essentially associated to the development and activity of the brain and to nerve conductivity [1,6]. The determination of very low levels of Al has become increasingly very important in environmental and clinical chemistry since its negative roles in the human life [5,7,8]. Because of aluminum accumulation in the tissues of patients with chronic renal failure, also monitoring of aluminum concentration in dialysis fluids has increasing attentions [5]. The diluted dialysis fluids should not contain aluminum concentrations higher than 10 μg L^{-1} [9,10].

The determination of aluminum and other elements at trace levels by the instrumental techniques including graphite and/or flame atomic absorption spectrometry, inductively coupled plasma mass spectrometry is rather difficult due to interfering effect of matrix and low levels of aluminum in environmental samples. Preconcentration/separation procedures could be used to solve these problems for aluminum and other elements [11–16]. The procedures including solvent extraction, cloud point extraction, coprecipitation, ion-exchange, electrodeposition and solid phase extraction have been used for the preconcentration and separation of trace elements [17–23]. Solid phase extraction of heavy metals on biosorbents is also popular topic at these works [24–26]. The biosorption procedure is based on biosorption of the heavy metals and desorption of these metals from the organisms [27–29]. Microorganisms immobilized on natural and synthetic adsorbents have been used for separa-

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tion and preconcentration of heavy metal including aluminum from various media [30–33].

Pseudomonas aeruginosa is a gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae*. *P. aeruginosa* is pathogens of humans [34,35]. It is often preliminarily identified by its pearlescent appearance and grape-like odor in vitro. It is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon utilizing microorganism, causing microbial corrosion [34,35].

The aim of the presented work is to show possible usage of *P. aeruginosa* loaded on Chromosorb 106 as biosorbent for the separation–preconcentration of aluminum at trace levels.

2. Experimental

2.1. Apparatus

A Perkin-Elmer AAnalyst 700 atomic absorption spectrometer equipped with HGA graphite furnace and with deuterium background corrector was used. For graphite furnace measurements, argon was used as inert gas. The operating parameters for working elements were set as recommended by the manufacturer given in Table 1. Pyrolytic-coated graphite tubes (Perkin-Elmer part no. B3 001264) with a platform were used. The samples of 20 μL plus 5 μL of 10,000 mg L^{-1} $Mg(NO_3)_2$ as matrix modifier during the study were injected into the furnace using Perkin-Elmer AS-800 autosampler. The signals were measured as peak areas.

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. Ethos D (Milestone S.r.l., Sorisole, BG, Italy) closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used.

2.2. Reagents and solution

All chemicals used were of analytical reagent grade and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1}) was used for all dilutions. All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1 + 9) and were rinsed with distilled water prior to use. Aluminum standard solution used for calibration was produced by diluting a stock solution of 1000 mg L^{-1} (Sigma Chem. Co. St. Louis, MO, USA). Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Table 1
Instrument settings and analytical conditions for GFAAS determination of aluminum

Wavelength (nm)	309.3
Slit width (nm)	0.7
Instrumental conditions	
Argon flow (mL min^{-1})	250
Heating program temperature °C (ramp time (s), hold time (s))	
Drying 1	100 (5, 20)
Drying 2	140 (15, 15)
Ashing	1700 (10, 20)
Atomization	2500 (0, 5)
Cleaning	2600 (1, 3)

Phosphate buffer solutions ($H_2PO_4^-$/H_3PO_4) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} sodium dihydrogen phosphate and phosphoric acid solutions for pH 2 and 3. Acetate buffer solutions (CH_3COO^-/CH_3COOH) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} acetic acid and 0.1 mol L^{-1} sodium acetate solutions for pH 4. Phosphate buffer solutions ($H_2PO_4^-$/HPO_4^{2-}) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} sodium dihydrogenphosphate and 0.1 mol L^{-1} sodium hydrogen phosphate for pH 5, 6 and 7. Ammonium buffer solutions were prepared by mixing of appropriate amounts of 0.1 mol L^{-1} ammonia and 0.1 mol L^{-1} ammonium chloride solutions for pH 8–9.

2.3. Preparation of biomass

The liquid medium was prepared by mixing 2 g of peptone, 2 g meat extract and 1 g mineral medium (10 g $CaCl_2 \cdot 2H_2O$, 20 g $MgCl_2 \cdot 6H_2O$ and 1 g $MnCl_2 \cdot 4H_2O$) and was dissolved in the 200 mL distilled water, and sterilized at 120 °C for 20 min. To prepare a starter culture, the bacterial strain, *P. aeruginosa* was grown in solid stock medium. It was inoculated into a 10 mL liquid nutrient medium. It was incubated at 30 °C for 24 h. The previously prepared 200 mL sterile liquid mediums were inoculated with 2 mL of the starter culture, and incubated in 10 vials at pH 7.3. The bacterial cultures were kept in continuous shaking at 30 °C. The stationary phases of each 200 mL liquid bacterial cultures were detected by microscopic observations. After reaching stationary phases, 16–24 h of incubation periods, *P. aeruginosa* cell density was 4.0–4.6 at 600 nm, and at this time the bacterial cells were harvested and separated from the media using centrifugation at 7000 rpm for 15 min. The isolated biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried.

Hundred and fifty milligrams of dry and dead *P. aeruginosa* was mixed with 500 mg of Chromosorb 106 [24]. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *P. aeruginosa* and Chromosorb 106, thereby improving the immobilization efficiency. Then, the product obtained was used as biosorbent for the present work.

2.4. Column preparation

A 10 cm in length and 1 cm in diameter column, with a small plug of glass wool, placed on the bottom of the column was used. The column was filled with 650 mg of biosorbent. The bed depth of biosorbent in the column was approximately 2.5 cm. The resin column was prepared by aspirating water slurry of *P. aeruginosa*-loaded on Chromosorb 106 into the glass column. It was conditioned by passing phosphate buffer solution then it was used for separation–preconcentration study. After each

use, the column was washed by passing 10–15 mL of phosphate buffer solution for regeneration of the biosorbent.

2.5. Preconcentration procedure

Forty to fifty millilitres of solution containing 0.20 μg of Al(III) was added to 10 mL of buffer solution. The *P. aeruginosa* loaded on Chromosorb 106 column was preconditioned by passing buffer solution. The buffered metal solution was passed through the column at a flow rate of 5 mL min^{-1}. After passing of this solution completely, the column was rinsed with twice 10 mL of water. The sorbed metal ions on the column were eluted with 8–10 mL portion of 1 mol L^{-1} HCl. The residue is diluted to 10.0 mL with distilled water. The eluent was analyzed for the determinations of aluminum concentrations by graphite furnace atomic absorption spectrometer.

2.6. Application to real samples

LGC 6010 Hard Drinking Water leaves certified reference material, tap from Tokat city, seawater from Black Sea and river water from Greenriver–Tokat analyzed were filtered through a cellulose membrane filter (Millipore) of 0.45 μm pore size. The pH of the samples was adjusted to 6.0. Then procedure given in Section 2.5 was applied to each sample. The levels of aluminum in the samples were determined by graphite furnace AAS.

Digestion conditions for microwave system for the samples were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively [36,37].

Rice Flour (NIST-SRM 1568a) and Dogfish Muscle (NRCC-DORM-2) certified reference materials (250 mg), yogurt (1.0 g), rice (1.0 g) and chicken meat (1.0 g) were digested with 6 mL of HNO_3 (65%), 2 mL of H_2O_2 (30%) in microwave digestion system and diluted to 50 mL with deionized water. A blank digest was carried out in the same way. Then the preconcentration procedure given above was applied to the final solutions.

In order to digest cow milk, red wine produced in Tokat City, beer and whisky, 1.0 mL of each sample was mixed with 1.0 mL of concentrated HNO_3 and 0.5 mL of H_2O_2 in microwave system. After digestion, the volume of the digested sample was made up to 25.0 mL with distilled water. Blanks were prepared in the same way as the sample, but omitting the sample. The separation–preconcentration procedure given above was applied to the samples.

3. Results and discussion

3.1. Influences of pH

A critical parameter in achieving quantitative adsorption and recovery of trace elements on solid phase extraction studies is pH [38–40]. The effect of pH on the adsorption of aluminum on biosorbent was investigated in the pH range of 2–9 by using model solutions containing 0.20 μg of aluminum(III). The adsorbed aluminum on biosorbent was eluted with 1 mol L^{-1} HCl. The results are depicted in Fig. 1. Aluminum ion was quantitatively recovered at the pH range of 6–8. The recoveries for aluminum were not quantitative at the pH value lower than 6 and higher than 8. At the pH values lower than pH 6, due to positive charges on the surface of biomass, the recoveries were not quantitative. At the higher pH values than 8, aluminum hydroxide precipitate was occurred under the optimal working condition; due to this point the recoveries were not quantitative. All further works were carried out at pH 6 by using phosphate buffer.

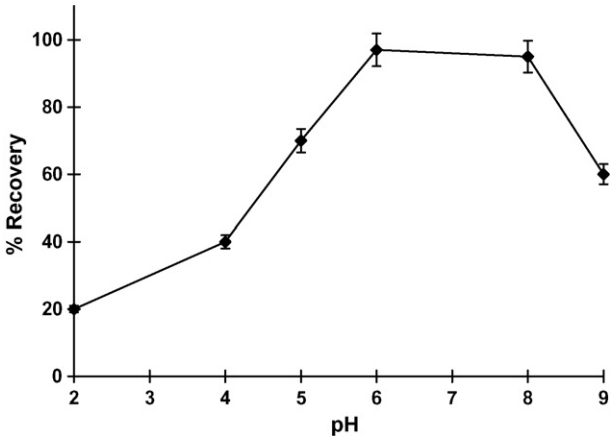

Fig. 1. The pH effects on the recoveries of aluminum ions on biosorbent.

3.2. Eluent type and volume

The influences of various eluents given in Table 2 on desorption of aluminum from *P. aeruginosa* loaded on Chromosorb 106 were also investigated. For these studies, 10 mL of each eluent was used. Aluminum was quantitatively recovered only with 1 mol L^{-1} HCl. The effects of volume of 1 mol L^{-1} HCl as eluent were also investigated in the range of 5.0–10.0 mL. Quantitative recovery values (>95%) were obtained after 8.0 mL of 1 mol L^{-1} HCl. These results are agreed with works in literature [41–43].

3.3. Flow rates

In the solid phase extraction works, flow rates of sample and eluent are two important parameters [43–46]. The influences of the sample and eluent flow rates on the recoveries of Al(III) were investigated in the range of 1.0–10.0 mL min^{-1}. The flow rates were adjusted to use of the stopcock of the column. The retentions for aluminum(III) on *P. aeruginosa* loaded on Chromosorb 106 column were virtually quantitative for sample and eluent flow rates up to 5.0 mL min^{-1}.

Table 2
Influences of various eluent on the desorption of aluminum

Eluent	Recovery (%)
0.5 mol L^{-1} HCl	80 ± 2
1 mol L^{-1} HCl	97 ± 3
0.5 mol L^{-1} HNO_3	70 ± 3
1 mol L^{-1} HNO_3	90 ± 3

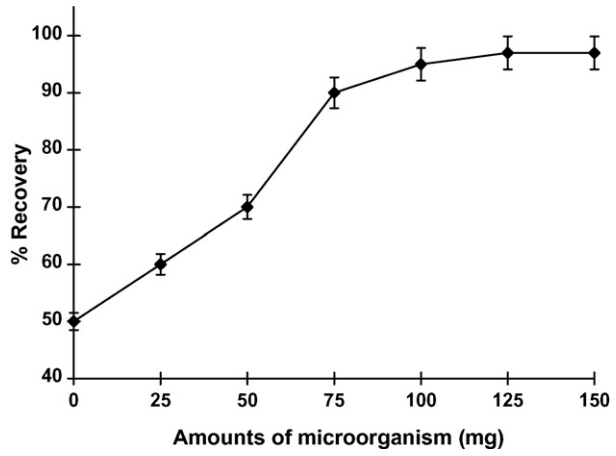

Fig. 2. Amounts of microorganism and recovery of aluminum relations.

3.4. Influences of amounts of microorganisms

The influences of amount of *P. aeruginosa* that loaded on 650 mg of Chromosorb 106 column for the recoveries of Al ions were investigated in the range of 0–150 mg. The results are given in Fig. 2. The recovery value of aluminum without microorganisms was 50%. The recovery values increased with the increasing amounts of microorganism and reach to quantitative value 100 mg of microorganism. All further works 150 mg of microorganism was loaded on to 500 mg of Chromosorb 106. This value is agree with our works in literature [24,47,48] for biosorption of heavy metals.

3.5. Effect of sample volume

The effect of sample volume on the retention behavior of aluminum(III) on biosorbent column were also examined. The recovery values as a function of sample volume were shown in Fig. 3. The recoveries were constant when up to 500 mL of the sample solution was used. At the higher volumes, the recoveries for analytes were decreased. The recoveries of aluminum on biomass were not quantitative probably due to the excess analytes loaded over the column capacity with increasing sample volume above 500 mL. The preconcentration factor is calculated by the ratio of the highest sample volume (500 mL) and the lowest eluent volume (10 mL). The preconcentration factor was 50.

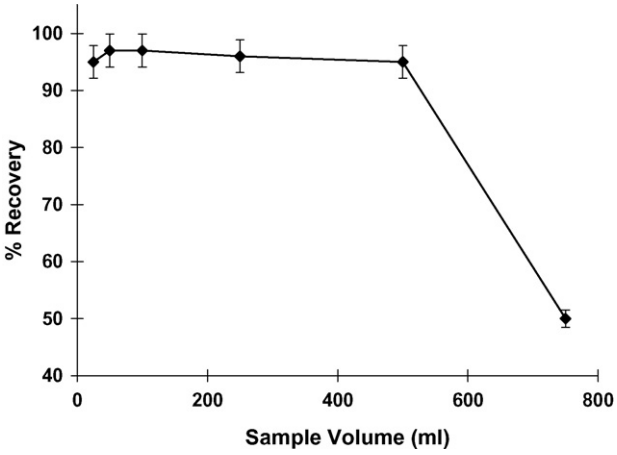

Fig. 3. The influences of sample volume ($N = 3$).

Table 3
Influences of some foreign ions on the recoveries of aluminum ($N = 3$)

Ion	Added as	Concentration (mg L^{-1})	Recovery (%)
Na^+	NaCl	20,000	95 ± 3
K^+	KCl	5,000	96 ± 4
Ca^{2+}	$CaCl_2$	5,000	95 ± 3
Mg^{2+}	$MgCl_2$	5,000	95 ± 3
Cl^-	NaCl	30,000	96 ± 3
F^-	NaF	1,000	99 ± 4
NO_3^-	KNO_3	3,000	95 ± 2
SO_4^{2-}	Na_2SO_4	3,000	96 ± 2
PO_4^{3-}	Na_3PO_4	3,000	96 ± 4
CH_3COO^-	CH_3COONa	3,000	99 ± 2
HCO_3^-	$NaHCO_3$	3,000	97 ± 3
Mn^{2+}	$MnSO_4$	50	96 ± 2
Fe^{3+}	$FeCl_3$	50	98 ± 2
Cu^{2+}	$CuSO_4$	50	96 ± 3
Pb^{2+}	$Pb(NO_3)_2$	50	97 ± 2
Zn^{2+}	$ZnSO_4$	50	96 ± 3
Cr^{3+}	$Cr(NO_3)_3$	50	96 ± 4
Cd^{2+}	$Cd(NO_3)_2$	50	97 ± 3
Ni^{2+}	$NiSO_4$	50	95 ± 2
Co^{2+}	$CoSO_4$	50	98 ± 2

3.6. Influences of concomitants

The effects of possible matrix ions in the environmental samples and some transition metals on the recoveries of aluminum were also examined by adding known concentrations of each ion in a solution containing aluminum(III) and then determining the latter. The results were summarized in Table 3. The tolerated amounts of each ion were the concentration values tested that caused less than 5% the absorbance alteration.

As shown in Table 3, the ions normally present in natural samples do not interfere under the experimental conditions used for the recoveries of aluminum ions at trace levels. Also, some of the transition metals at mg L^{-1} levels had no interference on the recoveries of aluminum ions. This results show that the proposed preconcentration/separation method for aluminum could be applied to the highly saline samples and the samples that contains some transition metals.

3.7. Reuse of biosorbent

The stability and potential regeneration of biosorbent were also examined. The column can be reused after regenerated with 10 mL 1 mol L^{-1} HCl and 10 mL distilled water, respectively, and relatively stable up to 50 runs without appreciable loss. After 50 runs, the recovery values were found below 80%. The biosorbent could be stored for at least 3 months and used repeatedly without any appreciable amount of reagent being lost.

Table 4
The results for tests of addition/recovery for aluminum determination in some real samples (sample volume: 250 mL, final volume: 10 mL ($N=4$))

Added ($\mu g\,L^{-1}$)	Spring water		Acidic dialysis fluid		Basic dialysis fluid	
	Found ($\mu g\,L^{-1}$)	Recovery (%)	Found ($\mu g\,L^{-1}$)	Recovery (%)	Found ($\mu g\,L^{-1}$)	Recovery (%)
–	10 ± 1[a]	–	15 ± 1	–	17 ± 1	–
5	15 ± 2	100 ± 3	19 ± 2	95 ± 2	21 ± 2	95 ± 2
10	19 ± 2	95 ± 2	24 ± 2	96 ± 3	26 ± 2	96 ± 3
20	29 ± 2	97 ± 2	36 ± 2	103 ± 2	36 ± 2	97 ± 2

[a]Standard deviation.

3.8. Accuracy of the results

In order to estimate the accuracy of the procedure, different amounts of aluminum(III) ions were added to spring water from Sivas and; acidic dialysis fluid and basic dialysis fluid. The resulting solutions were submitted to the procedure given in Section 2. The results are shown in Table 4. A good agreement was obtained between the added and measured analyte amounts of aluminum. The recovery values calculated for the added standards were always higher than 95%, thus confirming the accuracy of the procedure and its independence from the matrix effects. These results confirm the validity of the proposed preconcentration method.

3.9. Adsorption capacity

In order to study the adsorptive capacity of biosorbent, batch method was used. To 0.1 g of sorbent was added 50 mL of solution containing 1.0 mg of aluminum(III) ion at pH 6.0. After shaking for 1 h, the mixture was filtered. Ten millilitres of the supernatant solution was diluted to 100 mL and determined by atomic absorption spectrometry. The capacity of biosorbent for analytes was found as 10.5 mg g^{-1}.

3.10. Precision, detection limit and sensitivity

The relative standard deviations for graphite furnace atomic absorption spectrometric determinations for aluminum(III) are lower than 10%. The detection limit of the present work was calculated after application of the preconcentration procedure to blank solutions. The detection limit for aluminum, defined as the concentration equivalent to three times the standard deviation ($N=11$) of the reagent blank were found as 30 ng L^{-1}.

The absolute sensitivity is defined by the mass of an element, which gives a peak absorbance of 0.0044; it was found 16 pg for Al.

3.11. Analysis of the real samples

The validation of the presented procedure is performed by the analysis of three certified reference materials (LGC 6010 Hard Drinking Water, NIST-SRM 1568a Rice Flour and NRCC-DORM-2 Dogfish Muscle). The results are given in Table 5. The certified and observed values for certified reference materials were in good agreement with the certified values of SRM's.

Table 5
The level of aluminum in certified reference materials after application of the presented procedure ($N=4$)

Reference material	Certified value	Our value
LGC 6010 Hard Drinking Water (mg L^{-1})	0.206	0.202 ± 0.010
NIST-SRM 1568a Rice Flour ($\mu g\,g^{-1}$)	4.4	4.5 ± 0.2
NRCC-DORM-2 Dogfish Muscle ($\mu g\,g^{-1}$)	10.9	10.4 ± 0.8

Table 6
The concentration of aluminum in natural water samples and microwave digested food samples ($N=4$)

Sample	Concentration
Tap water ($\mu g\,L^{-1}$)	6.5 ± 0.4
River water ($\mu g\,L^{-1}$)	34 ± 3
Sea water ($\mu g\,L^{-1}$)	15 ± 2
Red wine ($\mu g\,L^{-1}$)	88 ± 3
Beer ($\mu g\,L^{-1}$)	40 ± 2
Whisky ($\mu g\,L^{-1}$)	120 ± 8
Cow milk (mg L^{-1})	1.5 ± 0.1
Yogurt ($\mu g\,g^{-1}$)	2.8 ± 0.2
Chicken meat ($\mu g\,g^{-1}$)	0.60 ± 0.04
Rice ($\mu g\,g^{-1}$)	1.2 ± 0.1

The presented preconcentration procedure was applied to various natural water samples and some microwave digested food samples produced in Turkey. The results are given in Table 6. The concentration of aluminum in real samples were found at ppb level. These levels are lower than the levels of Turkish Authorities for food samples [49].

4. Conclusion

P. aeruginosa loaded on Chromosorb 106 is an effective biosorbent for the separation and preconcentration of aluminum ions in the real samples. It exhibits good chemical stability and reusability. The high stability of the resin permitted hundreds of adsorption-elution process along the studies without a significant decrease in the recoveries. The preconcentration factor is 50. In addition to validating the developed method by successfully analyzing standard reference materials, aluminum content was established in natural waters and food samples.

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