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Flame Atomic Absorption Spectrometric Determination of Manganese in Alloys after Preconcentration onto Amberlite XAD-4 Loaded with *Saccharomyces carlsbergensis*

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A sensitive and simple enrichment method using microorganisms as an adsorbent is described for the determination of trace manganese in alloys. Manganese was determined by flame atomic absorption spectrometry after preconcentration onto Amberlite XAD-4 loaded with *Saccharomyces carlsbergensis*. The optimum values of pH, amount of adsorbent, amount of microorganism, eluent type and volume of sample solution were determined for the quantitative recovery (>95%) of manganese. Results have been compared with those obtained without using microorganisms. Under optimum conditions, recoveries were 98 ± 3% with microorganisms and 68 ± 2% without microorganisms for manganese at a 95% confidence level. The limit of detection for manganese was 60 ng/mL and 197 ng/mL with and without microorganisms, respectively. The proposed method was applied to the determination of manganese in ferrosilicon alloy (NBS SRM 59a), aluminum alloy (NBS SRM 85b) and aluminum foil. Manganese was determined with a relative error lower than 5% in all samples.

Key Words: Manganese, preconcentration, flame atomic absorption spectrometry, alloy, *Saccharomyces carlsbergensis*

Introduction

Trace metal ions play important roles in a wide spectrum of areas. The determination of trace metal ions is becoming increasingly important because of the increased interest in environmental samples including water, soil, plants etc. Manganese is an essential micronutrient for organisms and plants. However, it is toxic at higher levels and chronic manganese poisoning affects the central nervous system[1]. Therefore, the determination of trace manganese in environmental samples is also important. Flame atomic absorption

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spectrometry (FAAS) has been widely used for manganese determination in various samples[2–4]. However, the direct determination of trace manganese by FAAS is limited because of insufficient sensitivity and/or matrix interference problems. These problems can be overcome by using preconcentration and separation methods. For this purpose many methods, such as solvent extraction[5], coprecipitation[6], electrodeposition[7], ion exchange[8] and adsorption[9] have been used for the preconcentration of trace metals. In recent years, preconcentration by microorganisms has been widely used[10,11]. The use of microorganisms as a biosorbent for metals has become a good alternative to the other preconcentration methods as regards higher recovery, economic advantages, simplicity and environmental protection. In general, microorganisms have the ability to selectively adsorb a specific element without preconcentrating the matrix[12]. Either living or nonliving microorganisms, such as yeasts, fungi, bacteria and algae are capable of accumulating heavy metals from aqueous solutions by different chemical and biological mechanism[13,14]. Microbial cell products such as metabolites, polysaccharides, and cell wall constituents are effective in metal accumulation. In the literature, biosorption is explained as follows: Biosorption process takes place on the cellular membrane. If there is no biological activity (a dead cell), the metallic species are firstly adsorbed on a cellular membrane and after passing through this membrane they are absorbed into this cell structure[15]. Biomolecules (proteins, polysaccharides and cellulose), which contain sulfates, carboxylates, phosphates etc. in their structure, are responsible for the absorption process. It may be concluded that some processes such as absorption, cationic exchange, chelating, precipitation or crystallization take place in the cellular membranes of the microorganism[16].

Yeasts are considered to be more effective in heavy metal accumulation because of their great tolerance towards metals and their high binding capacity to the cell[13]. Maquieira et al.[17] have explained the specificity of microorganisms. According to their explanation, there are many binding sites on the cell wall of microorganisms, and these have a diversity of properties. Each element may be coordinated with different functional groups. In a mixture of many elements, each of them can form a stable complex with any of the functional groups present in the cell wall of the microorganism. Therefore, by varying pH or elution conditions, selectivity can be obtained and this is well demonstrated in the interference effects where the elements are in relatively high concentrations. Microorganisms have been used as freely suspended cells or as loaded cells on a support. The use of loaded cell systems on a support has many advantages over the use of freely suspended cells. These include a better capability of re-using the biomass, easy separation of cells from the reaction mixture, high biomass loadings and minimal clogging in continuous flow systems. Loaded cell systems can be used in both batch and column experiments.

Nakajima et al.[18] used *Saccharomyces cerevisiae* and some other microorganisms in a comparative study for metal uptake by immobilizing them on calcium alginate gel. Maquiera et al.[17] used *S. cerevisiae* by immobilizing the microorganisms covalently on controlled pore glass (CPG) for trace metal preconcentration. Gencer et al.[19] used a yeast immobilized column fermenter with wood chip packing to ferment synthetic media containing glucose to ethanol. Bağ et al.[13,20] immobilized *S. cerevisiae, A. niger* and *E. coli* separately on sepiolite and used them for the preconcentration of Fe, Ni, Cd, Cu, Zn and Cr.

In this study, manganese was determined in alloys by FAAS after preconcentration onto Amberlite XAD-4, a well - known Amberlite XAD resin, loaded with *Saccharomyces carlsbergensis.* This study is the first in which *S. carlsbergensis* has been used for preconcentration. The results are compared with those obtained with Amberlite XAD-4 unloaded with microorganisms.

Experimental

Apparatus

A Philips PU 9285 model flame atomic absorption spectrometer equipped with deuterium lamp background correction, a hallow cathode lamp (HCL) and an air acetylene burner was used for the determination of the manganese. The instrumental parameters were as follows: wavelength, 279.5 nm; bandpass, 1.0 nm; lamp current, 9.0 mA; fuel flow rate, 1.0 L/min. All pH measurements were performed with a Jenway 3010 model digital pH meter.

Reagents

Doubly distilled deionized water and analytical reagent grade chemicals were used unless otherwise specified. Manganese stock solution (1000 μg/mL) was prepared by dissolving the appropriate amount of manganese(II) sulfate. The working solutions were prepared by dilution from the stock solution. Amberlite XAD-4 (Sigma Chem. 20-40 mesh, 780 m^2/g) was used as a substrate for the immobilization of *S. carlsbergensis*.

Procedures

Preparation of *Saccharomyces carlsbergensis*

S. carlsbergensis was maintained, cultivated, grown and prepared as a dry powder according to a procedure given by Bağ et al.[13] Commercially available Amberlite XAD-4 was prepared as a substrate by washing successively with methanol, water, 1 mol/L HCl and water to remove organic and inorganic contaminants. The immobilization of *S. carlsbergensis* was performed according to the procedure recommended by Bağ et al.[13] First 200 mg of dry yeast powder was mixed with 1 g of Amberlite XAD-4. The mixture was wetted with 2 mL of doubly distilled deionized water and thoroughly mixed. After mixing, the paste was heated in an oven at 80^{o}C for 24 h to dry the mixture. The wetting and drying steps were repeated to maximize the contact between *S. carlsbergensis* and Amberlite XAD-4, thereby improving the immobilization efficiency.

Preparation of the column

Initially 0.3 g of Amberlite XAD-4 loaded with *S. carlsbergensis* was packed in a glass column (10 mm i.d. and 200 mm in length). Before use, approximately 20 mL of 1 mol/L HCl in acetone solution and 20 mL of doubly distilled deionized water were passed through the column in order to condition and clean it. Then the column was conditioned to the studied pH by using HCl and/or NH_3 solution.

Sorption Procedure

An aliquot of a solution (100 mL) containing 30 μg of Mn^{2+} was taken and the pH was adjusted to the desired value with hydrochloric acid or ammonia. The resulting solution was passed through the column at a flow rate adjusted to the desired value determined experimentally. The retained metal ion was then eluted from the adsorbent with 10 mL of 1 mol/L HCl solution in acetone into a small beaker. The eluate was evaporated to about 0.5 mL. It was diluted to 5-10 mL with 1 mol/L HCl. This solution was aspirated

into an air-acetylene flame for manganese determination by FAAS. The Amberlite XAD-4 loaded with *S. carlsbergensis* was used repeatedly after washing with 1 mol/L HCl in acetone solution and distilled water.

The recovery of manganese was calculated from the ratio of the concentration found by FAAS to that calculated theoretically. All experiments for the determination of the optimum conditions (pH, bed height, etc.) were performed according to the general procedure described above.

Dissolution of Samples

The developed method was applied to the determination of manganese in certified ferrosilicon alloy (NBS SRM 59a), aluminum alloy (NBS SRM 85b) and aluminum foil. The certified ferrosilicon alloy sample was dissolved according to the procedure adopted from Acar et al.[21] First 0.3 g of ferrosilicon alloy was weighed accurately into a 250 mL Teflon beaker. In order to moisten the sample a minimal volume of 0.02 mol/L nitric acid was added. Then 5 mL of perchloric acid (72-74%) was added. The beaker was covered with a Teflon cover and heated on a hot-plate at about 120 °C for 2 h. After cooling, the interior surface of the Teflon cover was washed with 5 mL of concentrated nitric acid into the container and reheated on a hot-plate at 150 °C until the acid volume was about 2 mL. The beaker was removed and cooled. After the interior surface of the beaker and the cover had been washed with 6 mL of 0.02 mol/L nitric acid 12 mL of concentrated HF was added. The mixture was heated at 100 °C for 45 min, and then at 150 °C until a complete decomposition of the sample was achieved. The resulting solution was transferred into a 250 mL volumetric flask. The interior of the beaker was washed with 0.02 mol/L nitric acid several times and the final solution was diluted to the mark with doubly distilled deionized water.

Aluminum alloy was dissolved as follows: 0.2 g of sample was weighed and put into a 250 mL beaker and dissolved by adding 5 mL of concentrated hydrochloric acid. The solution was heated on a water bath (at 80 °C) for 30 min to complete the dissolution. The solution was cooled and transferred to a 100 mL volumetric flask and diluted to the mark with water. Then 0.04 g of aluminum foil was dissolved by adding 5 mL of conc. hydrochloric acid and 5 mL of conc. nitric acid and heating. The solution was cooled and diluted to 100 mL.

Result and Discussion

Effect of pH

The retention of manganese on the column containing Amberlite XAD-4 loaded with *S. carlsbergensis* was studied as a function of pH. For that purpose, the pH value of sample solutions was adjusted to a range of 2-10 with HCl or NH_3. Similar experiments were also repeated by using a column containing Amberlite XAD-4 without microorganisms. As shown in Figure 1, the optimum pH of the sample solution is 8 for manganese with or without microorganisms.

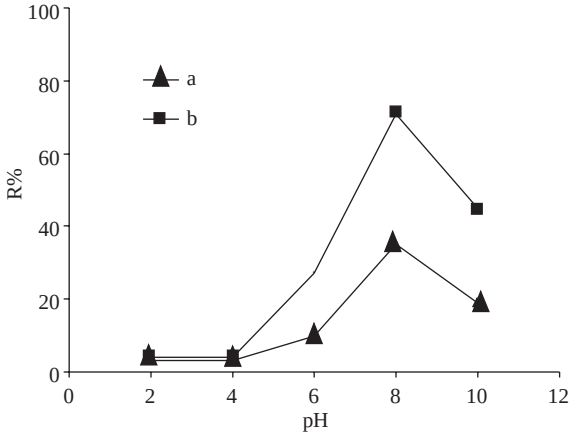

Figure 1. The effect of pH on recovery of Mn on Amberlite XAD-4 loaded with and without microorganism (manganese concentration: 0.3 μg/mL, adsorbent: 0.3 g, sample volume: 100 mL, flow rate: 1 mL/min, elution solution: 10 mL 1 mol/L HCl) in acetone.

a: without microorganisms b: with microorganisms

Effect of the amount of microorganism

In order to examine the effect of the amount of microorganisms, 1 g of Amberlite XAD-4 was mixed with 50, 100, 150, and 200 mg of dried microorganisms, and this mixture was mixed with 2-3 mL of doubly distilled water. This mixture was dried at 105 °C and used as an adsorbent for manganese. By applying the general preconcentration procedure, the recoveries of manganese were determined as a function of the amount of microorganisms. Figure 2 shows that the recovery increases with increasing amounts of microorganisms. A quantitative recovery was obtained for 200 mg of microorganisms. Therefore, 200 mg of dry microorganism in 1 g of Amberlite XAD-4 was used as an adsorbent for subsequent experiments.

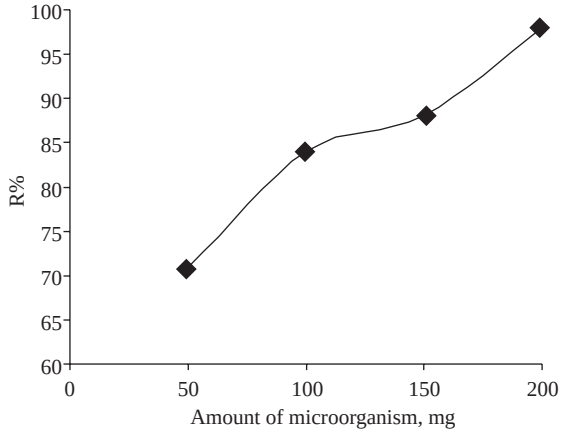

Figure 2. The effect of the amount of microorganism on recovery of manganese (manganese concentration: 0.3 μg/mL, pH: 8, adsorbent: 0.3 g, sample volume: 100 mL, flow rate: 1 mL/min, elution solution: 10 mL 1 mol/L HCl) in acetone.

Effect of the amount of adsorbent (bed height)

The retention of the element studied was examined in relation to the amount of adsorbent, which varied from 100 mg to 500 mg. Above 100 mg of adsorbent, the recovery of manganese gradually increased, but that at about 300 mg of adsorbent it reached plateau (Figure 3).

Therefore, 300 mg of adsorbent was the optimum level for all preconcentration purposes.

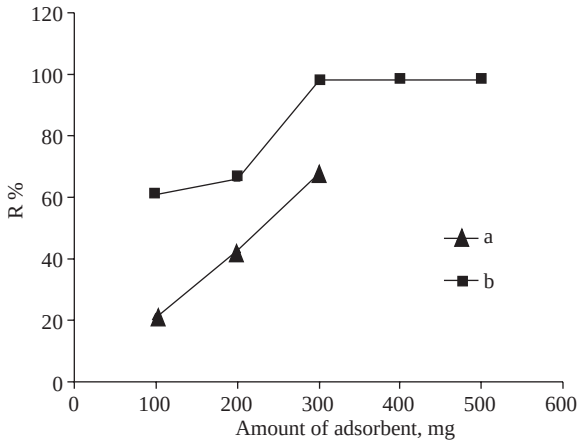

Figure 3. The effect of the amount of adsorbent on recovery of manganese (manganese concentration: 0.3 μg/mL, pH: 8, sample volume: 100 mL, flow rate: 1 mL/min, elution solution: 10 mL 1 mol/L HCl)
a: without microorganisms b: with microorganisms

Effect of the type and volume of elution solutions

The elution studies were performed with 1 mol/L HCl and 1 mol/L HNO_3 in water and in acetone, and mixtures of thereof. As can be seen in Table 1, 10 mL of 1 mol/L HCl in acetone solution was satisfactory for manganese (recovery >95%) with microorganisms.

Table 1. The effect of the type and volume of elution solution on the recovery of manganese (manganese concentration, 0.3 μg/mL; pH, 8; adsorbent, 0.3 g; flow rate, 1 mL/min).

Type of elution solution	Volume (mL)	Concentration (mol/L^{-1})	Recovery[a] (%)	
			With microorganisms	Without microorganisms
HCl	10	1	71	35
HNO_3	10	1	45	22
HCl/HNO_3 (1+3 (v/v))	10	1	72	18
HNO_3 (acetone)	10	1	74	52
HCl (acetone)	10	1	98	68

[a]Mean of 3 determinations.

Effect of flow rates of sample solutions

The retention of an element on an adsorbent depends upon the flow rate of the sample solution. Therefore, the effect of the flow rate of sample solution on the recovery of manganese was investigated under optimum

conditions (pH, eluent type etc.). The solution was passed through the column with the flow rates adjusted in a range of 1-5 mL/min. Figure 4 shows that maximum recovery was obtained with a flow rate of 1 mL/min. By increasing the flow rate, the recovery decreases gradually. Therefore, a flow rate of 1 mL/min was applied for subsequent experiments.

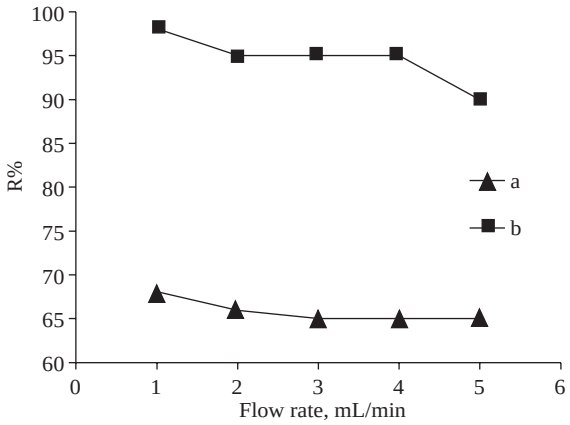

Figure 4. The effect of flow rate of sample solutions on recovery of manganese (manganese concentration: 0.3 μg/mL[1], adsorbent: 0.3 g, sample volume: 100 mL, elution solution: 10 mL 1 mol/L HCl).
a: without microorganisms b: with microorganisms

Effect of the volume of sample solutions

The effect of changes in the volume of sample solution passed through the column on the retention of manganese was also investigated; 100, 250, 500, 750, and 1000 mL of sample solutions containing a fixed amount of manganese (30 μg) corresponding to 0.30, 0.12, 0.06 and 0.03 μg/mL of manganese, respectively, were passed through the column at optimum conditions. Manganese could be recovered quantitatively up to 250 mL of sample solution.

At higher sample volumes, the recoveries decreased gradually with increasing sample volumes (Figure 5). The elution volume was 10 mL and the preconcentration factor was 25 for manganese.

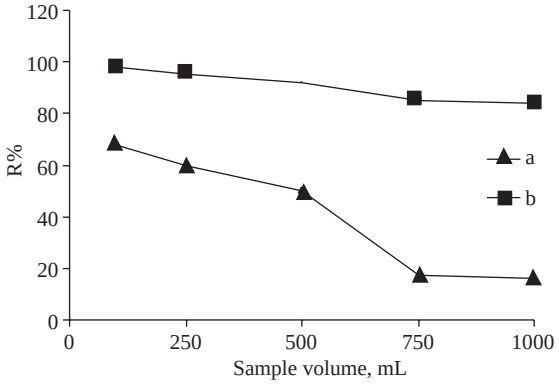

Figure 5. The effect of the volume of sample solutions on recovery of manganese (amount of manganese: 30 μg, pH: 8, flow rate: 1 mL/min, adsorbent: 0.3 g, elution solution: 10 mL 1 mol/L HCl in acetone).
a: without microorganisms b: with microorganisms

Precision of the method

For the precision of the method, the optimum conditions mentioned above were used. For this purpose, successive retention and elution cycles were performed with 100 mL of sample solution containing 30 μg of manganese. Table 2 shows that the recovery of manganese is quantitative (> 95 %) and the relative standard deviation is lower than 5%.

Table 2. Precision of the method[a] (manganese concentration, 0.3 μg/mL; pH, 8; flow rate, 1 mL/min; adsorbent, 0.3 g; elution solution, 10 mL 1 mol/L HCl in acetone).

Element	R $\pm ts/\sqrt{N}$, (%)	RSD (%)
Mn (with microorganisms)	98 ± 3	2.5
Mn (without microorganisms)	68 ± 2	2.4

[a]Mean of 5 determinations at a 95% confidence level

Calibration graph and detection limit

The calibration graph was linear up to 5 μg/mL for manganese. The detection limit (evaluated as the concentration corresponding to 3 times the standard deviation of the blank signal) was 60 ng/mL and 197 ng/mL for manganese, by using the column with and without microorganisms, respectively.

Effect of interfering ions

In order to investigate the effect of the interference of some elements, especially alkaline and alkaline earth elements, and main matrix elements (Fe and Al) of the alloys on the recovery of manganese, this was examined when they existed together in the same medium. The concentration of manganese was fixed at 0.3 μg/mL and the concentration of interfering metal ions was adjusted in a range of 0.5-1000 μg/mL. The results are given in Table 3. This shows that manganese can be determined quantitatively in alloys without interference from aluminum and iron.

The effect of column reuse

To test the long-term stability of the column containing adsorbent, the column was subjected to successive adsorption and desorption cycles by passing 100 mL of metal solutions through it, and then stripping the metals by the appropriate eluent. The procedure was carried out 5 times a day and the next 5 runs were made 1 day later, and so on. The columns were stored in doubly distilled deionized water. The stability and potential recyclability of the column were assessed by monitoring the changes in the recovery of the manganese. As shown in Figure 6, after the runs a small decrease occurred in the recoveries. The column seems to be relatively effective up to 11 runs (R > 90%).

Application

The proposed method has been applied to the determination of manganese in aluminum foil, aluminum based alloy and ferrosilicon based alloy. For this purpose, standard reference materials (NBS SRM 59a and 85b) and aluminum foil were dissolved as described above and the general preconcentration and determination procedure were applied at optimum conditions. Due to the high manganese content of the alloys, in this experiment 0.1 mL of sample solution was diluted to 100 mL to reduce the concentration at the working

range of the method after preconcentration. This diluted solution was preconcentrated as described above. The alloys have a high manganese content and this can be easily determined by using standard FAAS without the need for manganese preconcentration. However, in order to check the accuracy of the method in such matrices these samples were analyzed. For the application of the method to the real sample, 100 mL of the solution of aluminum foil was used directly for preconcentration. The preconcentration procedure was repeated 5 times. The results are summarized in Tables 4 and 5.

Table 3. Effect of other ions on the recovery of manganese (manganese concentration, 0.3 μg/mL; pH, 8; flow rate, 1 mL/min; adsorbent, 0.3 g; elution solution, 10 mL 1 mol/L HCl in acetone).

Interfering ions	Concentration (μg/mL)	Recovery of Mn (%)	
		with microorganisms	without microorganisms
Na^+	–	98	68
	25	96	63
	35	91	60
	50	90	58
	100	89	44
	500	49	18
	1000	45	9
K^+	-	98	68
	20	93	55
	30	85	49
	50	77	41
	100	76	35
	250	32	20
	500	25	16
Ca^{2+}	-	98	68
	10	96	60
	20	85	42
	50	52	17
	100	49	9
	250	36	3
Mg^{2+}	-	98	68
	0.5	98	68
	2.5	63	48
	5	57	27
	10	49	11
	50	11	7
Al^{3+}	-	98	68
	15	98	-
	30	98	-
	60	98	-
	120	98	-
	240	97	-
	360	97	
Fe^{3+}	-	98	68
	10.5	98	-
	21	98	-

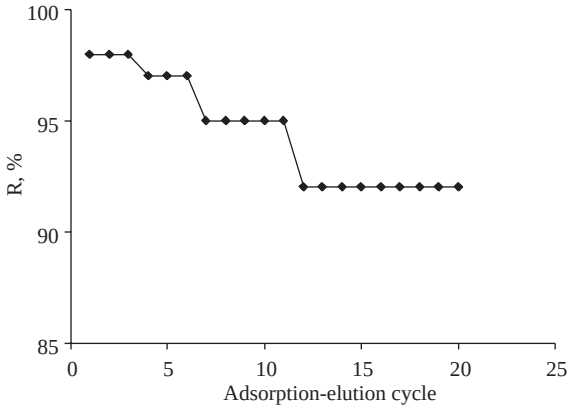

Figure 6. The effect of column reuse on recovery of manganese (manganese concentration: 0.3 μg/mL, pH: 8, flow rate: 1 mL/min, adsorbent: 0.3 g, elution solution: 10 mL 1 mol/L HCl in acetone).

Table 4. Determination of manganese in alloys (pH, 8; adsorbent, 0.3 g; flow rate, 1 mL/min; elution solution, 10 mL 1 mol/L HCl in acetone).

Sample	Concentration (% m/m)		Relative error (%)
	Certified	Found[3] $\bar{x} \pm ts/\sqrt{N}$	
NBS SRM 85b[1]	0.610	0.636 ± 0.004	+3
NBS SRM 59a[2]	0.75	0.74 ± 0.06	−2

[1]The composition of the aluminum based alloy (NBS SRM 85) was Al 93.097%, Mn 0.61%, Si 0.18%, Cu 3.99%, Ni 0.084%, Cr 0.211%, V 0.006%, Ti 0.022%, Ga 0.019%, Fe 0.24%, Pb 0.021%, Mg 1.49% and Zn 0.030% (m/m).

[2]The composition of the ferrosilicon based alloy (NBS SRM 59a) was Al 0.35%, Mn 0.75%, Si 48.10%, Cu 0.052%, Ni 0.033%, Cr 0.080%, Fe 50.05%, C 0.046%, S 0.002%, P 0.016%, Ca 0.042% and B 0.058 % (m/m).

[3]Mean of 5 determinations at a 95% confidence level.

Table 5. Determination of manganese in aluminum foil (pH, 8; adsorbent, 0.3 g; flow rate, 1 mL/min; elution solution, 10 mL 1 mol/L HCl in acetone).

Added (μg g^{-1})	Found (μg g^{-1})^a	Recovery (%)
-	68 ± 6	-
500	540 ± 5	95

$^{a)}$Mean of 5 determinations with standard deviation.

Conclusion

The proposed preconcentration procedure based on adsorption on a microorganism for manganese is simple, sensitive, precise and accurate. The proposed method can be applied to the preconcentration and determination of manganese in some alloys containing mainly aluminum, iron and silicon with a relative error of <5 %. It can be concluded that the use of microorganisms increased the retention of manganese with respect to Amberlite XAD-4 alone. By using microorganisms, higher preconcentration factors were obtained. It can also be concluded that the preconcentration procedure can be applied to the determination of manganese in various samples containing magnesium at lower concentrations only.

Acknowledgments

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