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Use of matrix-modifier in the Determination of traces of Mo, Co, Cu, Mn and Ni in *Chromatium vinosum* by Zeeman atomic absorption spectrometry

Efrosini Piperaki*, Joachim Erfkamp, Ekkehard Diemann, and Achim Müller

Faculty of Chemistry, University of Bielefeld, P. O. Box 8640, D-4800 Bielefeld, Federal Republic of Germany

Summary. The use of the matrix-modifying tissue-solubilizer didodecyldimethylammonium hydroxide in the Zeeman-AAS determination of traces of Mo, Co, Cu, Mn and Ni in cells of *Chromatium vinosum* is described. The correlation to data obtained by deproteinization or wet digestion is convincing ($r > 0.99$), and the time needed for sample preparation is reduced from about an hour to a few minutes.

Key words: Zeeman atomic absorption spectrometry — Tissue-solubilizer — Matrix-modifier — Trace metal determination — *Chromatium vinosum*

Introduction

Trace element analysis by atomic absorption spectroscopy (AAS) is of great significance for science, especially for biochemistry and bioinorganic chemistry concerning research on active sites of important metallo-enzymes like the bacterial nitrogenase systems (Mo- and V-containing nitrogenases; Müller and Newton 1983) or hydrogenases. An Mo-nitrogenase and an Ni-containing hydrogenase are present in the purple sulfur bacterium *Chromatium vinosum*.

Atomic absorption spectrometry is the method chosen here for metal determination in biological samples; however, it is liable to various interference effects. To overcome these effects, in many papers methods have been reported including liquid-liquid extraction (Jan et al. 1978; Eady et al. 1984; Jin et al. 1985; Green et al. 1984; Ward 1979), ion exchange (Smits and van Grieken 1981, Riley and Taylor 1968) and coprecipitation (Hudnik et al. 1978) but those methods are time-consuming and not free from error.

Matrix-modification, which achieves suppression of the interference, has been reported for some elements (Tominaga et al. 1982; Stein and Umland 1986; Ekanem et al. 1986; Game et al. 1986; Ioannou and Piperaki 1986) in different biological samples.

In this work we describe a direct Zeeman-AAS method for the determination of Mo, Co, Cu, Mn and Ni in the purple sulfur bacterium *C. vinosum* with a simple and rapid modification of the matrix using a tissue-solubilizer (didodecyldimethylammonium hydroxide). The results were compared with those obtained after protein precipitation and after wet digestion.

Work in progress showed that the modifier used could also be applied in the case of other biological samples (e.g. blood, human saliva) for the analysis of various metals (e.g. Cr, Cd, Pb).

Materials and methods

Zeeman AAS. An Hitachi model 170-70 Zeeman-effect graphite furnace AAS, was used. The light sources were supplied by a single-element (Mo) and a multielement (Co, Cu, Mn, Ni) hollow-cathode lamp. The sample solution was injected into the pyrolytic graphite tube (Hitachi 170-5101) with an Eppendorf micropipet.

Reagents. The matrix-modifier didodecyldimethylammonium hydroxide (Serva 22262, 25% v/v in *n*-propanol) was prepared daily. Standard metal solutions (Fixanal) containing 10 g/l of the metal were used. Dilute solutions were prepared daily by appropriate dilution with high-quality doubly deionised water. The acids used were of *suprapure* quality (Merck). All glassware used was rinsed with dilute nitric acid and then rinsed at least ten times with water.

* *Present address:* Laboratory of Analytical Chemistry, Solonos 104, GR-10680 Athens, Greece

Offprint requests to: A. Müller

Table 1. Parameters for temperature programme on Hitachi 170-70 Zeeman-AAS for various trace metals

Element	Dry		Ash		Atomize			
	Time (s)	Current (A)	Time (s)	Current (A)	Time (s)	Current (A)	Carrier gas flow (l/min)	Wavelength λ (nm)
Mo	10	30	40	145	8	310	0.0	313.3
Co	10	22	40	40	5	310	0.05	240.9
Cu	10	22	40	50	5	310	0.05	324.9
Mn	10	22	30	38	5	280	0.05	279.8
Ni	10	22	40	80	5	310	0.0	231.9

Table 2. Analytical recovery data (each value is the mean of three determinations)

Element	Matrix-modifier			Deproteinization			Wet digestion		
	Standard added (µg/L)	Measured (µg/L)	Recovered (%)	Standard added (µg/L)	Measured (µg/L)	Recovered (%)	Standard added (µg/L)	Measured (µg/L)	Recovered (%)
Mo	0	52.3	—	0	51.3	—			
	30	77.7	84.7	50	89.7	77.0			
	50	99.2	93.8	100	166.7	115.5			
	75	122.5	93.6	150	205.1	102.6			
	100	153.2	100.9	200	243.6	96.1			
	125	172.4	96.1						
Mean			93.8			97.8			
Co	0	231.1	—	0	219.3	—	0	217.4	—
	200	428.9	98.9	200	429.8	105.3	200	434.8	108.8
	400	570.5	85.0	400	622.8	100.8	400	608.7	97.8
	600	828.9	99.7	600	833.3	100.2	600	782.6	94.2
				800	1026	100.9	800	1044	103.2
Mean			94.5			102.3			101.0
Cu	0	493.5	—	0	431.3	—	0	517.3	—
	200	689.3	97.9	200	606.1	87.4	200	698.0	90.4
	400	915.3	105.4	400	842.8	102.9	400	965.5	112.0
	600	1085	98.6	600	1012	96.8	600	1149	105.3
	800	1299	100.7	800	1209	97.2	800	1303	98.3
Mean			100.6			96.1			101.5
Mn	0	202.6	—	0	221.7	—	0	211.8	—
	50	270.9	97.5	100	314.0	92.3	100	309.0	97.2
	100	319.6	97.4	200	438.4	108.3	200	451.1	119.6
	150	370.9	99.1	400	637.3	104.0	400	645.6	108.4
	200	447.8	112.8	600	878.9	109.5	600	806.0	99.0
				800	1006	98.0	800	1005	99.1
Mean			101.7			102.4			104.7
Ni	0	393.0	—	0	402.8	—	0	407.4	—
	200	601.1	104.0	200	601.8	99.5	200	592.6	92.8
	400	763.4	92.6	400	880.2	199.3	400	777.8	92.6
	600	936.0	90.5	600	967.0	94.0	600	1000	98.8
	800	1179	98.3	800	1210	100.9	800	1222	101.9
Mean			96.3			103.4			96.5

Growth of cells of C. vinosum. Cells of the purple sulfur bacterium *Chromatium vinosum* DSM 180 (ATCC 17899) were grown at 20° C and illuminated with an apparatus containing four fluorescent tubes in screw-capped culture bottles filled with Pfennig's medium for purple sulfur bacteria (DSM 1977). Anaerobiosis was maintained according to the Hungate technique (Hungate 1969): 1000-ml starting cultures were used as inoculation of 20-l batch cultures which were grown to an absorbance of 2. After harvesting the cells by centrifugation ($3000 \times g$, 20 min), they were washed by repeated resuspension in the buffer solution of the medium and centrifugation. The cells were stored in the buffer solution at 4° C under nitrogen atmosphere.

Matrix-modification method. In a series of Eppendorf microtubes (1.5 ml), 0.1 ml of the cell sample with the appropriate amount of metal (final concentration 30–125 ppb for Mo and 200–800 ppb for Co, Cu, Mn and Ni) with 0.1 ml of the modifier reagent, for Mo determination, and 0.9 ml for the other metals, were transferred. After the mixture had been stirred vigorously (stirrer Heidolph Reax 2000–2400/min), 20 µl were injected into the graphite tube. The standard addition method was used.

Deproteinization method. An aliquot (0.1 ml) of the cell sample and 0.9 ml 3 M HNO_3 were transferred into a 10-ml centrifuge tube, stirred, heated in a boiling-water bath for 15 min and centrifuged ($2000 \times g$, 10 min). The metals were determined in the supernatant solution by the standard addition method. Analytical recovery experiments were performed by addition of fixed amounts of metal standards in a series of centrifuge tubes containing 0.1 ml of the cell sample and 0.9 ml 3 M HNO_3, heating at least 30 min in the boiling-water bath (to control the complete protein precipitation) and proceeding as described above.

Wet-digestion method. Measurements were also made after treatment of the cells with a mixture of conc. HNO_3/H_2SO_4 for 1 h. The sequential dry-ash-atomize programme of the Zeeman-effect AAS was optimized for each metal separately and the peak absorbance was determined (Table 1).

Estimation of protein content of the cells. The protein content of the cell suspension was determined after extraction of the photosynthetic pigments (treatment with a mixture of acetone/ethanol 7:1) using a modified micro-biuret assay (Schmidt et al. 1963).

Results

Analytical recovery results obtained with the matrix-modification method, the deproteinization method and the wet-digestion method are listed in Table 2. The precision was determined for each element in the sample as the relative standard deviation when no metal was added, with the matrix-modification method and after precipitation of the proteins. The detection limit was taken as three times the standard deviation. The results are as shown in Table 3. The final metal content of the *C. vinosum* cell suspension, related to the protein content, is given in Table 4.

Table 3. Standard deviation and detection limit for various elements in samples of *Chromatium vinosum*

Element	*n*	Coefficient of variation, CV (%)		Detection limit (µg/L)
		Deproteinization	Matrix modifier	
Mo	9	±5.2	±3.0	5.0
Co	12	±7.0	±2.2	37.0
Cu	9	±7.1	±5.5	32.0
Mn	7	±7.4	±2.6	22.0
Ni	7	±4.7	±3.5	40.0

Table 4. Final concentrations of trace metals in *Chromatium vinosum* cells

Element	Concentration in 100 mg protein/mL cell suspension (ppm)
Mo	0.104 ± 0.003
Co	2.31 ± 0.1
Cu	4.94 ± 0.3
Mn	2.03 ± 0.1
Ni	3.93 ± 0.1

The tissue-solubilizer, added as matrix-modifying agent for the metal analysis in biological samples, homogenized the organic matrix, resulting in the cells breaking rapidly at the ashing step; thus, prior to the the atomization step, the metal in the sample and the added metal are in the same form. If this is not so, systematic errors cannot be avoided, even if the standard addition method is used. Dilution of the sample with water or modification of the matrix with NH_4NO_3 had no success in such complicated samples as these biological ones. The proposed method is excellent and correlates well with the other two ($r \geqslant 0.99$ in all cases). It is simple, rapid and minimizes contamination problems.

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References

Deutsche Sammlung von Mikroorganismen (1977) Catalogue of strains GSF Report M339, Gesellschaft für Strahlenforschung, 2nd Edition

Eady RE, Robson RL (1984) Characteristics of N_2 fixation in Mo-limited batch and continuous cultures of *Azotobacter vinelandii.* Biochem J 224:853–862

Ekanem EJ, Barnard CLR, Ottaway JM, Fell GS (1986) Improved determination of cadmium in blood by flame atomic fluorescence spectrometry. J Anal At Spectrom 1:349–353

Game I, Balabanoff L, Valdebenito R, Vivaldi L (1986) Use of matrix modifier and L'vov platform in the determination of copper in pooled human saliva by electrothermal atomic absorption spectrometry. Analyst 111:1139–1141

Green RJ, Asher CJ (1984) Measurement of submicrogram amounts of nickel in plant material by electrothermal atomic absorption spectroscopy. Analyst 109:503–505

Hudnik V, Gomiscek S, Gorenc B (1978) The determination of trace metals in mineral waters Part I: atomic absorption spectrometric determination of Cd, Co, Cr, Cu, Ni and Pb by electrothermal atomization after concentration by coprecipitation. Anal Chim Acta 98:39–46

Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. Methods Microbiol 3B:117–132

Ioannou P, Piperaki EA (1986) Kinetic fluorometric determination of aluminium in serum. Clin Chem 32:1481–1483

Jan TK, Young DR (1978) Determination of microgram amounts of some transition metals in sea water by methyl isobutyl ketone-nitric acid successive extraction and flameless atomic absorption spectrophotometry. Anal Chem 50:1250–1253

Jin L-Zh, Ni ZhM (1985) Determination of Nickel in urine and other biological samples by graphite furnace atomic absorption spectrometry. Fresenius Z Anal Chem 321:72–76

Müller A, Newton WE (1983) Nitrogen fixation — the chemical-biochemical-genetic interface. Plenum Press, New York

Riley JP, Taylor D (1968) Chelating resins for the concentration of trace elements from sea water and their analytical use in conjunction with atomic absorption spectrophotometry. Anal Chim Acta 40:479–485

Schmidt K, Liannen-Jensen S, Schlegel HG (1963) Die Carotinoide der Thiorhodaceae. Arch Mikrobiol 46:117–126

Smits J, Grieken R van (1981) Enrichment of trace anions from water with 2,2′-diaminodiethylamine-cellulose filters. Anal Chim Acta 123:9–17

Stein K, Umland F (1986) Spurenbestimmung von Blei, Cadmium und Mangan in Honigen und Zuckern. Fresenius Z Anal Chem 323:176–177

Tominaga M, Umezaki Y (1982) Comparison of ascorbic acid and related compounds as interference suppressors in electrothermal atomic absorption spectrometry. Anal Chim Acta 139:279–285

Ward NI, Stephens R, Ryan DE (1979) Comparison of three analytical methods for the determination of trace elements in whole blood. Anal Chim Acta 110:9–19

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