ELSEVIER

Journal of Chromatography A, 706 (1995) 167–173

JOURNAL OF CHROMATOGRAPHY A

Determination of Cd, Co, Cu, Fe, Mn, Ni and Zn in coral skeletons by chelation ion chromatography

William Shotyk*, Ina Immenhauser-Potthast

Geological Institute, University of Berne, Baltzerstrasse 1, CH-3012 Berne, Switzerland

Abstract

Cadmium, Co, Cu, Fe, Mn, Ni and Zn incorporated in the aragonitic skeletons of corals (*Porites*) were analyzed by chelation ion chromatography (CIC). A 500-mg amount of bleached, oven-dried coral powder was dissolved in 1 ml of 14.4 *M* HNO_3 and buffered at pH 5.4 ± 0.1 using 50 ml of 2 *M* ammonium acetate. A 30-g amount of sample was concentrated on-line by pumping the solution through a Dionex MetPac CC-1 chelating concentrator column at a rate of 3 ml/min, and the MetPac was rinsed with 2 *M* ammonium acetate to elute alkaline earth metals to waste. The transition metals were eluted on a second concentrator column (Dionex TMC-1) using 2 *M* HNO_3, and the TMC-1 was then converted to a salt form using 0.1 *M* NH_4NO_3 and switched on-line with the analytical column, the Dionex IonPac CS-5. The metals were then eluted from the separator column using 6 m*M* pyridine–2,6-dicarboxylic acid–0.4 *M* NaOH; the pH of this eluent was 4.4. The separated metals were, after they had left the column, complexed by 0.5 m*M* 4-(2-pyridylazo)resorcinol, and the absorbance was measured in a Dionex ultraviolet–visible detector at 520 nm. The use of two separator columns in series improved the metal separation. This is especially important for Ni and Co because coral samples typically contained an order of magnitude more Zn than Ni or Co. With two columns in series, Ni, Co and Zn were baseline-separated even when a 30-g sample containing 5 ppb Ni, 5 ppb Co and 100 ppb Zn was concentrated. The absolute sensitivity of the instrument was approximately 10 ng Cd. Although Cd was successfully determined in several corals using CIC, most coral samples contain on the order 1 ng/g Cd or less and, thus, the quantitative measurement of Cd in most corals by CIC would require tens of grams of coral powder. Unfortunately, it is not possible to obtain such large quantities of coral material from annual bands. The chelation system may be useful, however, to pre-concentrate coral digests for Cd analyses using more sensitive methods of detection (e.g. graphite furnace atomic absorption spectrometry or inductively coupled plasma mass spectrometry).

1. Introduction

Man-made pollution is an increasing problem in tropical marine environments. Heavy loads of sediments may be lethal to corals and lesser quantities may inhibit coral growth, cause changes in growth forms or alter the species composition of reef-building organisms [1]. The fringing reef ecosystem of Mauritius (Indian Ocean) is degenerating because of coral diseases, widespread eutrophication and algal growth in some parts of the reef and degradation of lagoons. An increase in the number of sea urchins, a reduction in coral vitality and a decrease in specimen variation can be observed [2]. A geochemical study was undertaken using the widespread recent scleractinian coral genus

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00104-2

Porites to find possible reasons for reef degradation.

Anthropogenic inputs of sewage could be responsible for the degradation of the corals, caused by nutrients or toxic heavy metals [3,4]. There are several possible sources of pollution that could affect the coastal environment of Mauritius, e.g., waste dump sites, sugar cane industry and sewage from towns, hotels and private houses on the beach. In addition there is an industrial region in Port Louis Harbour. Nutrients, pesticides and heavy metals may be washed out during the rainy season and transported into the lagoon by rivers, canals and ground water.

A wide range of trace metals have been determined in the calcium carbonate (aragonite) skeletons of reef-corals [5]. Pb and Cd in corals, for example, are well known indicators of anthropogenic activity [6,7]. In contrast, Mn is an indicator of detrital inputs [8,9]. There have been reports of Pb measurements in acid digests of coral skeletons directly, without preconcentrating the solutions, using anodic stripping voltammetry [10]. However, in most cases, especially with respect to ultratrace metals such as Cd and Pb, some kind of preconcentration step is required before the coral digests can be analyzed. For example, St. John [11] concentrated Cd, Co, Cu, Fe, Ni, Pb and Zn using ammonium pyrrolidine dithiocarbamate (APDC) followed by extraction into methyl isobutyl ketone (MIBK). Other investigators [7–9,12] used a very similar approach.

To evaluate the possible role of heavy metals in degrading the reef ecosystem at Mauritius, an effort was made to measure the concentrations of Cd, Co, Cu, Fe, Mn, Ni and Zn in acid-dissolved skeletons of massive colonial corals of the genus *Porites*. The principle objective of this report is to evaluate the chelation ion chromatography (CIC) method for measuring these metals in the aragonitic coral skeletons. The CIC system combines an on-line chelation preconcentration step with the analytical chromatography for the simultaneous determination of these metals [13,14].

2. Experimental

2.1. Sampling and preparation of corals

Massive, hermatypic, colonial coralla of the scleractinian coral genus *Porites* LINK were used in this study. *Porites solida* and *Porites lutea*, both recent species, were chosen because of their widespread occurrence in all parts and all depths of the reef complex. Samples were taken in the autumn of 1990 and the summer of 1991 from the lagoon and the outer reef slope in relation to various possible sources of pollution. Samples from unaffected areas were taken as control samples to determine background concentrations.

After sampling, the corals were washed and cleaned several times in water and a desinfectant (Sagrotan) and dried in the sun. For transport they were well wrapped in clean paper. Longitudinal cuts of the central part of the colonies were made parallel to the growth of the coral using a rock cutting saw (type Cutler-Hammer). These cuts were X-rayed to examine growth rate and structure. Parts of these coral slices were cut into small pieces with a fretsaw. For each sample a new saw blade was used to avoid metal contamination by abrasion. After sawing, the skeleton pieces were washed in 18 MΩ deionized water, blown dry with compressed air and cleaned ultrasonically in PTFE beakers containing deionized water. The corals were bleached in 30% H_2O_2 (Merck Suprapur) to remove tissues and organic substances. Finally, the corals were leached in deionized H_2O and dried at 90–105°C. Special care was taken to use clean preparation procedures and appropriate materials for labware [12,15]. All plastic ware was cleaned with soap and hot water, rinsed in deionized water, put in a 10% HNO_3 bath (made from Merck Suprapur HNO_3) for several days and finally washed four times with deionized water before use. The clean coral pieces were powdered using an agate mortar and pestle in a laminar air flow cabinet and stored in clean plastic jars.

2.2. Preparation of acid digests

Samples (500 mg) of oven-dried coral powder were placed in clean, acid-washed PTFE bottles and dissolved in 1 ml of 14.4 *M* HNO_3 (Merck Suprapur). The solutions were buffered at pH 5.4 ± 0.1 using 52.5 g of 2 *M* ammonium acetate to give a final volume of sample solution of 50 ml. All of the reagents used were trace-metal grade (Merck Suprapur HNO_3, CH_3COOH, NH_4OH) and all solutions were made up in 18 MΩ deionized water. Ammonium acetate was made from Merck Suprapur CH_3COOH and NH_4OH, and ammonium nitrate was made from Merck Suprapur NH_4OH and HNO_3.

2.3. Chelation ion chromatography

CIC was performed using a Dionex 4500i IC system. The recommended configuration, preparation, operation and applications of this system are described in detail elsewhere [16]. We evaluated two configurations: a single CS-5 separator column and two CS-5 separator columns in series to improve peak separation (see below).

Samples were concentrated on-line by pumping the solution through a MetPac CC-1 chelating concentrator column. This column contains a macroporous iminodiacetate chelating resin; the capacity, selectivity and retention characteristics of this column are described elsewhere [16]. The sample solutions were pumped through this column at a rate of 3 ml/min. The coefficient of variation of the mass of solution concentrated by the sample pump was approximately 1%. The MetPac was then rinsed with high-purity 2 *M* ammonium acetate to remove alkaline earth metals (which were eluted to waste). The transition metals were then eluted to a second concentrator column (TMC-1) using 2 *M* Suprapur HNO_3. The TMC-1 contains a fully sulfonated cation-exchange resin as interface between the high-capacity chelation concentrator column (MetPac CC-1) and the low-capacity analytical column (IonPac CS-5). Before the TMC-1 could be switched on-line with the analytical stream, it had to be converted from the H^+ form to the NH_4^+ form; this was accomplished using 0.1 *M* NH_4NO_3 (pH 3.5). Following this step, the TMC-1 was switched on-line with the analytical column, the IonPac CS-5. The concentrated metals were then eluted with 6 m*M* pyridine–2,6-dicarboxylic acid (PDCA) in 0.4 *M* NaOH; the pH of this eluent was 4.4. After the separated metals left the column they were complexed by 0.5 m*M* 4-(2-pyridylazo)resorcinol (PAR), a metallochromic indicator. Absorbance was measured in a Dionex UV–VIS detector at 520 nm.

3. Results

3.1. Metal analyses of corals

Single separator column

Using a single separator column, Fe, Cu, Zn and Mn could be readily quantified by concentrating a 15-g digest (Fig. 1a). Using this approach, Ni could also be determined in most of the samples. However, in some samples Ni was below the limit of detection. Concentrating more sample to increase the Ni signal did not solve this problem because the Zn peak eventually overlapped that of Ni. A second disadvantage of this arrangement was that Co could not be quantified because it was masked by Zn (Fig. 1a). The Zn-to-Co ratio in corals was typically 10:1 at least; thus, Co could not be determined using a single separator column, especially in contaminated samples containing elevated concentrations of Zn. A third problem with this configuration was that Cd could not clearly be separated from Co [17]. Cadmium concentrations in these materials were much lower than those of Co; thus, it was not possible to measure Cd under these conditions.

Two separator columns in series

Using two separator columns in series with the same eluent improved the metal separation (Fig. 1b). The main advantage of using two columns was the clear separation of Ni, Zn and Co. Coral samples typically contain an order of magnitude

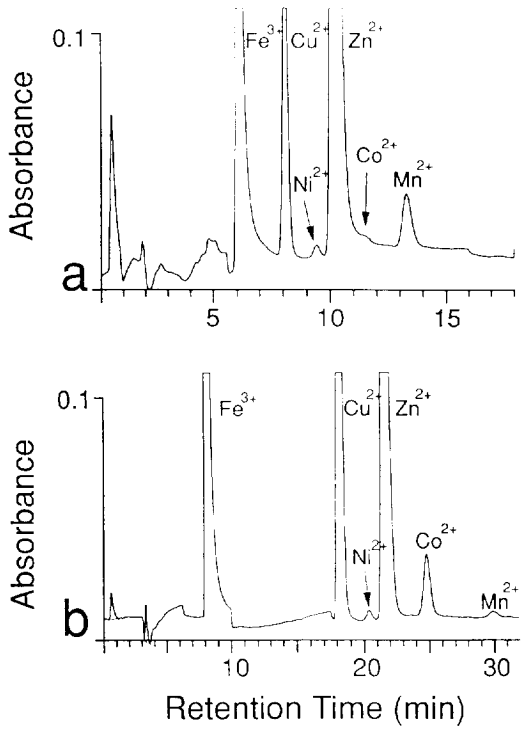

Fig. 1. (a) 6 m*M* PDCA eluent with one CS-5 separator column, 15 g of coral digest (sample No. 9005) concentrated. Notice that Co appears as a shoulder on Zn. Concentrations (ng/g) are as follows: 14.9 Fe, 2.8 Cu, 0.5 Ni, 12.1 Zn and 1.5 Mn. (b) 6 m*M* PDCA eluent with two CS-5 separator columns in series, 30 g of coral digest (sample No. 9187) concentrated. Notice that Co is now clearly separated from Zn. Concentrations (ng/g) are as follows: 24.4 Fe, 21.8 Cu, 1.5 Ni, 19.5 Zn, 2.2 Co and 0.9 Mn.

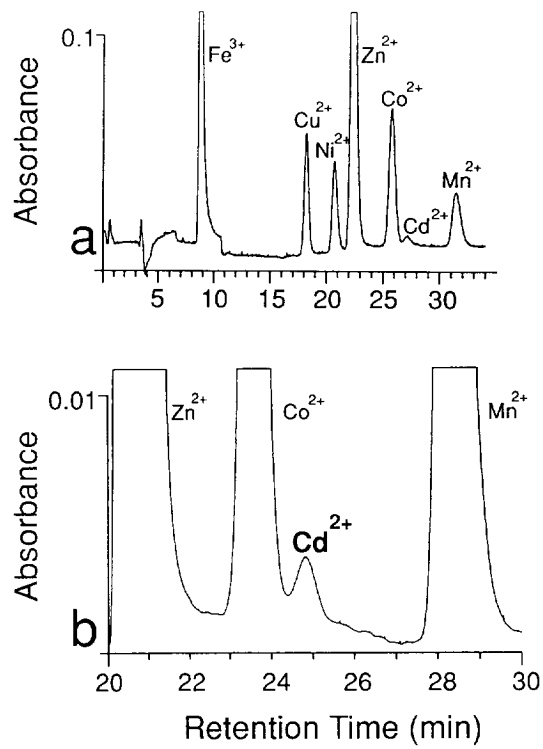

Fig. 2. (a) Chromatogram of National Institute of Science and Technology Standard Reference Material 1643c "Trace Metals in Water". Again, 6 m*M* PDCA eluent was used with two CS-5 separator columns in series, 30 g concentrated. Note the Cd peak with a retention time of approximately 27 min. Concentrations (ng/g) are as follows: 16.2 Fe, 3.3 Cu, 9.9 Ni, 10.3 Zn, 3.3 Co, 1.7 Cd and 4.3 Mn. (b) Expanded view of part of the chromatogram of an acid digest of a coral sample (9127) showing a Cd peak (1.1 ng/g Cd); this corresponds to 110 ng/g in the solid coral.

more Zn than Ni or Co. While concentrating 15 g of sample was sufficient for measuring Ni in many samples, it was not for measuring Co. With two columns in series, however, even when 30-g samples were concentrated, Ni and Co were still clearly separated from Zn (Fig. 1b). Nickel, Co, and Zn were baseline-separated even when a 30-g sample containing 5 ng/g Ni, 5 ng/g Co and 100 ng/g Zn was concentrated. The principle disadvantage of using two separator columns is the increased time required per analysis.

Determination of Cd

Using two separator columns in series, Cd was clearly separated from Co and Zn (Fig. 2a and b). To identify a Cd peak readily, a peak area of approximately 3000 counts · s (30 mAU · s) was required. A plot of the absolute quantity of Cd concentrated versus measured peak area (Fig. 3) yielded the response function which could be used to calculate the amount of Cd needed to produce a peak of this size. The linear regression equation indicates that 10 ng of Cd must be concentrated in order to obtain a measurable peak: this quantity of Cd is the absolute detection limit of the method. Thus, when a 30-g digest (obtained by dissolving a 500-mg sample) is concentrated, a minimum detection limit of approximately 0.3 ng/g Cd can be achieved. Using the CIC method described here, Cd was measured in two coral samples. However, Cd

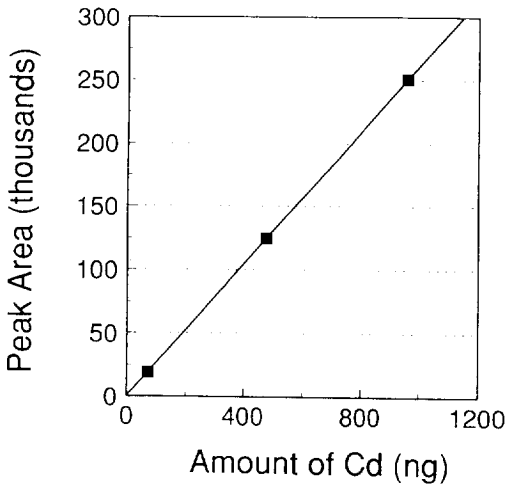

Fig. 3. Measured area of Cd peaks as a function of absolute quantity of Cd concentrated from water samples. For a Cd peak to be readily identifiable, a peak area of approximately 3000 counts · s (30 mAU · s) is required. The linear regression ($y = 263x + 261$, $r^2 = 0.9999$) indicates that approximately 10 ng of Cd must be concentrated in order to obtain a peak area of this magnitude: this quantity of Cd is the absolute detection limit of the method.

was not detected in the majority of the corals. In other words, it was not possible to determine the 'background' Cd concentrations in the corals. While it may be possible to improve the detection limit by concentrating more sample, some tens of grams of solid material would probably be needed. Not only is it impossible to obtain such large quantities of coral material from annual bands, but concentrating such large quantities of material would also concentrate Zn and Co which might eventually mask the Cd peak. The detection limit for Cd is, therefore, generally inadequate for measuring Cd in coral samples from annual bands. The chelation concentration system may be useful, however, to preconcentrate coral digests and eliminate the matrix for Cd analyses by graphite furnace atomic absorption spectrometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) [18,19].

Effect of $CaCO_3$ matrix

In order to evaluate the effect of the $CaCO_3$ matrix on the metal measurements, solid $CaCO_3$ powder (Merck Suprapur) was used to prepare a series of calibration standards. A 100-mg quantity of $CaCO_3$ was dissolved in 2 ml of HNO_3. Appropriate volumes of individual 1000-ppm metal standards and 2 ml of HNO_3 were added to ensure stability, and the standards were diluted to 50 ml. Prior to analysis, these standards were buffered to pH 5.4 ± 0.1 by adding 52.5 g of 2 *M* ammonium acetate which diluted them to 100 ml.

The influence of the $CaCO_3$ matrix is seen in the extreme example given in Fig. 4 which shows a calibration curve for Mn in pure water compared with Mn in a $CaCO_3$ background. The effect of matrix suppression on measured peak area for the other metals ranges from approximately 5 to 20%.

Determination of Pb

Using an eluent consisting of 4 m*M* PDCA, 0.4 *M* NaOH, 2 m*M* Na_2SO_4 and 15 m*M* NaCl (also pH 4.4), Pb was clearly separated from the other peaks (Fig. 5). However, the CIC method was not sufficiently sensitive to determine Pb at the low concentrations present in these corals. If unlimited amounts of sample material were available, Pb could have been measured in the coral digests by concentrating for a sufficiently long time. However, as noted above, the amount of material available from annual bands was

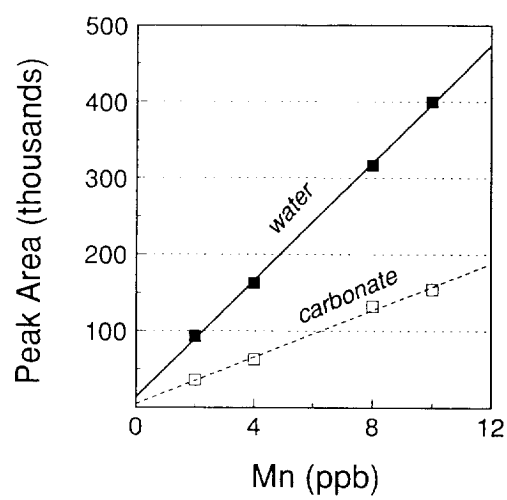

Fig. 4. Measured area of Mn peaks as a function of Mn concentration in solution. Solid squares correspond to Mn concentrations in a water matrix, open squares to Mn concentrations in a $CaCO_3$ matrix.

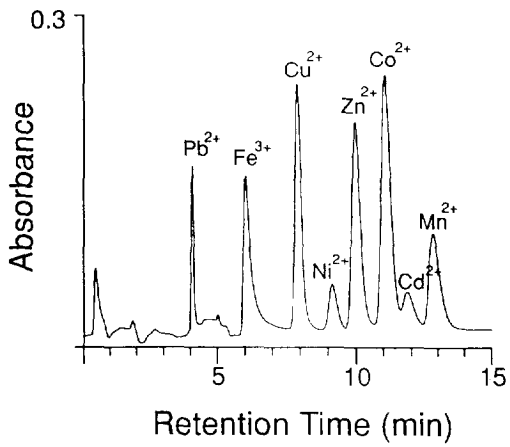

Fig. 5. Chromatogram of trace metals in water. A 10-g amount of solution containing 36 ng/g of each metal was concentrated. Eluent used was 4 m*M* PDCA, 0.4 *M* NaOH, 2 m*M* Na_2SO_4 and 15 m*M* NaCl (also pH 4.4). Note the Pb peak at approximately 4 min.

limited. Again, the CIC method may be useful to preconcentrate the coral digests and eliminate the matrix for Pb analyses using more sensitive detection methods (GFAAS or ICP-MS).

4. Conclusions

CIC was used for the simultaneous determination of Cd, Co, Cu, Fe, Mn, Ni and Zn in acid digests of coral samples. With a single separator column the method was suitable for the quantitative measurement of Fe, Cu, Zn and Mn. The use of two separator columns in series greatly improved peak separation and allowed the quantitative determination of Ni and Co, in addition to the other metals. Even in samples containing Zn/Ni and Zn/Co ratios greater than 20 or more, Ni and Co were clearly separated from Zn. Although the method is relatively slow (10 min for concentrating the sample and almost 40 min for analytical chromatography), the sensitivity is comparable to that of GFAAS for Cu, Fe, and Zn and better than GFAAS for Co, Ni and Mn.

For 30-g digests made up using 500-mg samples of coral powder, the detection limit for Cd was approximately 0.3 ng/g. Using the CIC method, however, Cd was measured only in two samples. While it may be possible to measure lower Cd concentrations by concentrating more sample, some tens of grams of solid material might be needed. It is not possible to obtain such large quantities of coral material from annual bands. The detection limit for Cd, therefore, is generally inadequate for measuring Cd in such samples. The chelation concentration system may be useful, however, to preconcentrate coral digests and eliminate the matrix for Cd analyses by GFAAS or ICP-MS.

Acknowledgements

This work is a summary of part of the Ph.D. Thesis by I.I.-P., supervised by Dr. J. Geister. We are indebted to Prof. A. Matter of this institute for generously providing all of the required laboratory facilities. Additional financial support from the Canton of Berne (SEVA Lottofonds) and the Swiss National Science Foundation (Grants 21-30207.90 and 21-30207.92) is also sincerely appreciated. René Trost (now at Dionex Switzerland) and Stefan Brand of Henry A. Sarasin (Basel, Switzerland) provided expert technical support with all aspects of ion chromatography.

References

[1] R.A. Pastorok and G.R. Bilyard, *Mar. Ecol. Prog. Ser.*, 21 (1985) 175.
[2] L. Hottinger, N. Muller, J. Muller and P. Vasseur, *Etude des Ecosystemes Littoraux de Maurice*, Rapport No. 1, Université d'Aix-Marseille I et III, Université de Maurice, 1990, 56 pp.
[3] J. Muller et al., *Etude des Ecosystemes Littoraux de Maurice*, Rapport No. 5, Université d'Aix-Marseille I et III, Université de Maurice, 1991.
[4] F. Gendre, *Thèse*, Faculté de Sciences, Institut de Géologie, Neuchâtel, 1992.
[5] L.S. Howard and B.E. Brown, *Oceanogr. Mar. Biol. Ann. Rev.*, 22 (1984) 195.
[6] G.T. Shen, *Ph.D. Thesis*, Massachusetts Institute of Technology, Cambridge, MA, 1986.

[7] G.T. Shen, E.A. Boyle and D.W. Lea, *Nature*, 328 (1987) 794.
[8] L.J. Linn, M.L. Delayney and E.R.M. Druffel, *Geochim. Cosmochim. Acta*, 54 (1990) 387.
[9] G.T. Shen, T.M. Campbell, R.B. Dunbar, G.M. Wellington, M.W. Colgan and P.W. Glynn, *Coral Reefs*, 10 (1991) 91.
[10] R.E. Dodge and T.R. Gilbert, *Mar. Biol.*, 82 (1984) 9.
[11] B.E. St. John, *Proc. 2nd Int. Coral Reef Symp.*, 2 (1974) 461.
[12] G.T. Shen and E.A. Boyle, *Chem. Geol.*, 67 (1988) 47.
[13] A. Siriraks, H.M. Kingston and J.M. Riviello, *Anal. Chem.*, 62 (1990) 1185.
[14] N. Cardellicchio, S. Cavalli and J.M. Riviello, *J. Chromatogr.*, 640 (1993) 207.
[15] C.F. Boutron, *Fresenius J. Anal. Chem.*, 337 (1990) 482.
[16] Dionex, *Technical Note* No. 25, 1992, 20 pp.
[17] W. Shotyk, I. Potthast and J. Geister, *Proc. 9th Int. Conf. Heavy Metals Environ.*, 2 (1993) 37.
[18] D.W. Boomer, M.J. Powell and J. Hipfner, *Talanta*, 37 (1990) 127.
[19] L.W. McLaren, J.W.H. Lam, S.S. Berman, K. Akatsuka and M.A. Azeredo, *J. Anal. Atom. Spectrom.*, 8 (1993) 279.