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## DETERMINATION OF HEAVY METALS BY ISOTACHOPHORESIS

F. M. EVERAERTS\*, Th. P. E. M. VERHEGGEN, J. C. REIJENGA and G. V. A. ABEN

*Laboratory of Instrumental Analysis, University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)*

and

P. GEBAUER and P. BOČEK

*Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno (Czechoslovakia)*

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### SUMMARY

The suitability of isotachophoresis for the analysis of metals in, *e.g.*, environmental samples was studied. In a cationic operational system the heavy metals Fe, Cu, Ni, Cd, Co, Zn, Pb and Mn were simultaneously determined. The separation was achieved through complex formation with one of the counter ions, hydroxyisobutyric acid. The other counter ion was acetic acid, the leading ion was 0.02 *M* potassium or sodium (pH 4.1) and the terminator was  $H^+$ . The analysis time was 15 min at 60  $\mu A$  in a 0.2 mm I.D. capillary. Aqueous samples containing ppm and ppb amounts were enriched on a cation exchanger with an extremely low affinity for sodium (Chelex 100). Good recovery, linearity, precision and accuracy were obtained even down to the ppb\* range. Although the sensitivity of the method is not greater than that of some of the more established methods for the individual metals, a great advantage of isotachophoresis is the simultaneous determination of the metals, with equal response factors. An example is given of the determination of metals, including aluminium, in serum.

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### INTRODUCTION

Almost all available analytical methods have been applied to the determination of heavy metals in environmental samples, and several have become so-called established methods, often with dedicated instrumentation. Atomic-absorption and -emission spectrometry in all their varieties have been called such established methods, having high sample throughputs with detection limits in the ppb range or even lower. Such spectrometric methods make use of physical properties of atoms, preferably independent of their chemical environment (molecular structure, valency, matrix). This independence, if achieved, provides a powerful advantage over physical separation methods, where the sample throughput is inherently lower.

When a true comparison between spectrometric and physical separation

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\* Throughout the article the American billion ( $10^9$ ) is meant.

methods is made, a number of additional aspects should also be considered: the sample must undergo clean-up and/or pre-concentration prior to analysis by either method, and the difference in the total analysis time is consequently less extreme than it seems; simultaneous determination of a number of metals is achieved more easily and cheaply, with physical separation methods; physical separation methods use multi-purpose equipment and small changes in the operating conditions make them suitable for a wide range of other applications; one must always be aware of unexpected interferences in the final results. With a physical separation method, interferences are more easily recognized, mainly because of the greater information content of the signals originating from the determination.

This paper describes the use of the physical separation method isotachopheresis for the determination of heavy metals in various samples. The aim was to determine the suitability of this technique for heavy metal separations. The separation of a large number of metals by isotachopheresis has been reported previously<sup>1</sup> and new insights into additional parameters for selectivity, such as choice of solvent<sup>2</sup> and complexation<sup>3</sup>, have further extended its possibilities, which is best illustrated by the simultaneous determination of 14 lanthanides, reported in 1981<sup>4</sup>.

## EXPERIMENTAL

The experiments were carried out in equipment developed and built by Everaerts *et al.*<sup>1</sup>. The separation compartment was a PTFE capillary (*ca.* 250 × 0.2 mm I.D.). UV absorption at 254 nm and a.c. conductivity were used for detection.

In order to achieve sufficient selectivity and to maintain a buffering capacity, a combination of hydroxyisobutyric acid (HIBA) and acetic acid was necessary as a leading electrolyte buffer. Nakatsuka *et al.*<sup>4</sup> investigated the influence of pH and concentration of both acids on the selectivity for lanthanides. More recently, Hirokawa *et al.*<sup>5</sup> made a more detailed study of the mechanism by which ligands such as HIBA affect the effective mobility of a number of bivalent cations. The aim of their investigation was to determine the coordination numbers and stability constants of the metal–oxycarboxylic acid complexes.

Our operational conditions (pH, ionic strength and HIBA concentration, see Table I) were optimized for the determination of bivalent Fe, Cu, Ni, Cd, Zn, Pb and Mn in practical samples. A leading ion concentration of 0.02 *M* was necessary to achieve a sufficient interaction between the sample ions and HIBA. Fe(III) and

TABLE I  
OPERATIONAL SYSTEM FOR HEAVY METAL ANALYSIS BY ISOTACHOPHORESIS

No further additives were needed.

Parameter	Leading electrolyte	Terminating electrolyte
Cation	Na or K	H <sup>+</sup>
Concentration	0.02 <i>M</i>	<i>ca.</i> 0.005 <i>M</i>
Counter ions	HIBA to pH 5.0 Acetic acid to pH 4.1	Acetate

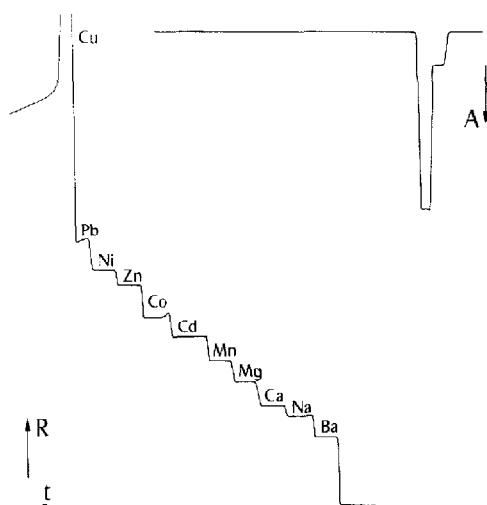


Fig. 1. Isotachopheretic analysis of a standard mixture carried out in the operational system in Table I. The amount injected was *ca.* 1 nmol each. Only Pb and Cu have UV absorption at 254 nm.

Hg(II) did not interfere in the determination because of insufficient effective mobility.

The order of mobility of the metals is determined mainly by the complex stability constant, as reported in the literature<sup>6</sup>, but was also verified by analysing the metals separately. Fig. 1 shows the analysis of a standard mixture of Ba, Na, Ca, Mg, Mn, Cd, Co, Zn, Ni, Pb and Cu. UV absorption is shown by Cu and Pb. Linear calibration graphs were obtained for all metals in the range 0.5–4 nmol, with correlation coefficients of 0.999 or better. In addition, it was shown that a joint calibration graph could be constructed for nine bivalent metals (Fig. 2) with a correlation coefficient of 0.992 in the range mentioned. The minimum detectable amount is *ca.* 100 pmol. The minimum detectable concentration is also determined by the amount in-

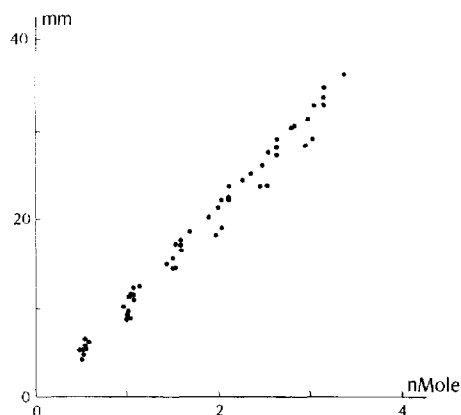


Fig. 2. Joint calibration graph for Ba, Mg, Mn, Cd, Co, Zn, Ni, Pb and Cu in the range 0.5–4 nmol. The correlation coefficient was 0.992 ( $n = 56$ ). Leading ion, potassium.

jected, which is usually between 1 and 10  $\mu\text{l}$ , depending on the composition of the sample. For a molecular weight of 100, this corresponds to a detection limit of 1–10 ppm without sample pre-treatment.

Sample pre-concentration is performed with a cation exchanger, Chelex 100 (Bio-Rad Labs., Richmond, CA, U.S.A.), which has a high affinity, especially for heavy metals, compared with sodium. The general procedure is as follows: regeneration with 2 bed volumes of 1 *N* hydrochloric acid, 5 bed volumes of water, 2 bed volumes of 1 *N* sodium hydroxide and 5 bed volumes of water; application of sample, buffered between pH 4 and 6; rinsing with 0.01 *N* hydrochloric acid; eluting with 1 *N* hydrochloric acid; evaporating the eluate to dryness; and dissolving the residue in water.

The order of affinity of the metals to the cation exchanger in chloride media is approximately<sup>7</sup>  $\text{Cu} \gg \text{Pb} > \text{Al} > \text{Ni} > \text{Zn} > \text{Co} > \text{Cd} > \text{Fe} > \text{Mn} > \text{Ba} > \text{Ca} \gg \text{Na}$ . Because of this, K, Na and  $\text{NH}_4$  do not interfere.

The amount of rinsing liquid is important, particularly for metals such as Co, Cd and Fe. Recovery studies indicated that 2 bed volumes are acceptable. The concentration factor achieved is given by the ratio of the amount of sample applied to the amount of water into which the residue is dissolved. The latter can be *ca.* 200  $\mu\text{l}$  if the eluate is evaporated in a conical flask of *ca.* 10 ml. The amount of sample applied can be chosen up to 1 l for environmental samples, resulting in a concentration factor of 1000 or more with detection limits in the lower ppb range. For physiological samples, sample amounts in the millilitre range can be analysed. The resulting concentration factor is *ca.* 50 with detection limits in the sub-ppm range.

## RESULTS AND DISCUSSION

When concentrating samples by the method described, special precautions may be necessary in order to avoid interferences. A blank determination was therefore performed by concentrating 1 l of deionized water taken from a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). This water was used for the preparation of standard solutions and the operational system. The resulting residue was dissolved in 2 ml of water and consequently the concentration factor was 500. The blank contained only 10 ppb of Al, probably originating from the glassware used. The procedure was then applied to the analysis of river water, the concentration factor being 500. The results, shown in Fig. 3, indicate that ppb amounts can easily be determined: 15 ppb of Mn, 10 ppb of Zn, 60 ppb of Ni, 5 ppb of Cu and 10 ppb of Al. In view of the results of the blank determination, only the value for Al is doubtful. In addition, a UV-absorbing zone of Fe(II) is observed. As the Fe(III) complex migrates outside the leading-terminating electrolyte mobility range, the Fe(II) zone cannot be related to the total iron content.

In the analysis of, *e.g.*, environmental samples, high concentrations of other metals may be present. Because of the properties of the ion exchanger, an excess of sodium will not interfere, so that the method may also be used for, *e.g.*, seawater<sup>8</sup>. Another metal that can occur in excess is calcium and in isotachopheresis too much calcium, migrating in front of the heavy metals, will also limit the amount of sample that can be injected, in the same way as sodium. The affinity of calcium for Chelex 100 is not negligible, resulting in the presence of calcium in the enriched sample. The

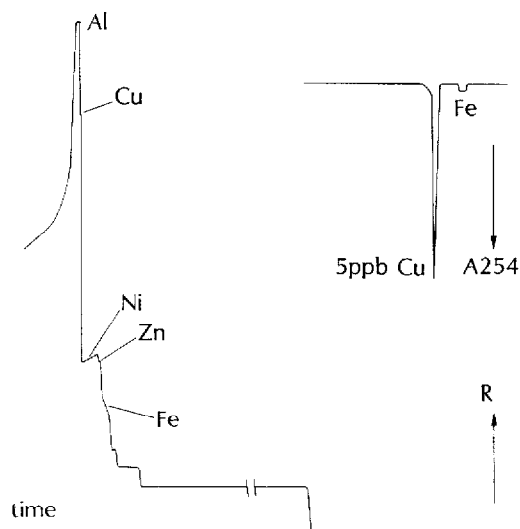


Fig. 3. Analysis of river water (Dommel, Eindhoven, The Netherlands), 1  $\mu$ l after 500-fold pre-concentration. The concentrations were 10 ppb of Zn, 60 ppb of Ni, 5 ppb of Cu and 10 ppb of Al. Leading ion, sodium.

rinsing procedure is critical in this respect. Nevertheless, a significant excess of calcium can be seen in the river water analysis in Fig. 3.

With the Chelex 100 procedure described, the minimum detectable concentration depends mainly on the volume of sample available. For physiological samples other than urine, these are usually millilitre amounts. Consequently, the minimum detectable concentration in, *e.g.*, serum is in the sub-ppm range. An example of the

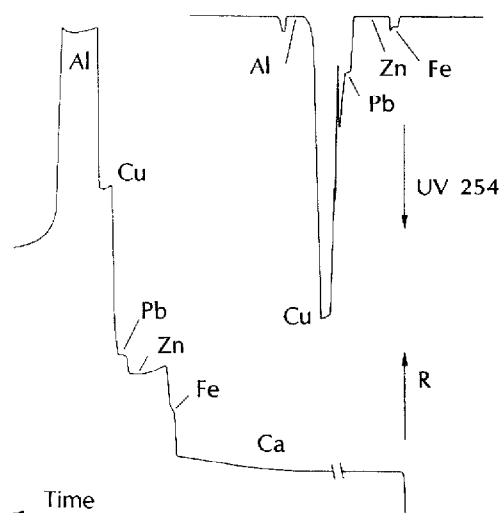


Fig. 4 Analysis of a pooled normal serum, 0.8  $\mu$ l after a 40-fold pre-concentration. The serum was first denatured with trichloroacetic acid. The concentrations were 4 ppm of Zn, 2 ppm of Pb, 1.5 ppm of Cu and 0.4 ppm of Al. Leading ion, sodium.

analysis of a pooled normal serum is shown in Fig. 4. Here 2 ml of serum was denatured with a 15% solution of trichloroacetic acid and diluted in 25 ml of deionized water, to ensure that protein-bound metals were also determined. From this preliminary experiment, satisfactory results were obtained for Al. Future experiments will be carried out to verify whether the denaturation was successful for all metals. The residue finally obtained was dissolved in 50  $\mu$ l of water, resulting in a 40-fold concentration step. Of this solution 0.8  $\mu$ l was injected, equivalent to 32  $\mu$ l of serum.

In addition to the heavy metals, the method seems to be highly suitable for the determination of Al in *e.g.* serum of uraemic patients and dialysis fluids. With established methods the matrix effects disturb the analytical result. The determination of Al in human bone is under investigation, the study being carried out in cooperation with the Catharina Hospital, Eindhoven, The Netherlands.

The suitability of the method for the analysis of water, sewage, sludge and plant material is at present under investigation.

## CONCLUSIONS

The isotachophoretic procedure described is suitable for the simultaneous determination of heavy metals in environmental samples and serum. In addition, the determination of aluminium in physiological samples is achieved with detection limits in the ppb range.

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