Use of miniaturised isotachophoresis on a planar polymer chip to analyse transition metal ions in solutions from metal processing plants

Jeff E. Prest*, Sara J. Baldock, Peter R. Fielden, Nicholas J. Goddard,
Kristallia Kalimeri, Bernard J. Treves Brown, Michele Zgraggen

Department of Instrumentation and Analytical Science, UMIST, P.O. Box 88, Manchester M60 1QD, UK

Abstract

An electrolyte system, using malic acid as a complexing agent, has been developed to allow the determination of transition metal cations using miniaturised isotachophoresis. The method allowed the simultaneous determination of Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} to be made without interference from other common ions. Limits of detection were calculated to be in the range $0.5–1.0\,mg\,l^{-1}$ for Mn^{2+}, Cr^{3+} Co^{2+} and Zn^{2+} and $2.0\,mg\,l^{-1}$ for Fe^{2+} and $4.7\,mg\,l^{-1}$ for Ni^{2+}. The successful analysis of five industrial samples, containing a range of these metal ions, obtained from metal processing plants were achieved in under 13 min. The separations were performed on a poly(methyl methacrylate) chip with integrated platinum wire conductivity detection electrodes.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Isotachophoresis; Chip technology; Miniaturisation; Instrumentation; Metal cations

1. Introduction

The analysis of transition metal ions is an important area of analytical science both for research and industrial applications. Within the industrial sector there exists a requirement to perform these analyses in a variety of metal processing plants including electroplating and galvanising works. In such plants, methods of determining the identity and concentration of transition metal ions are needed for a range of applications such as monitoring process streams, effluent discharges and quality control. Current practice generally sees the use of liquid chromatography and atomic absorption spectroscopy (AAS) for performing these analyses. However, the use of electrophoretic separation techniques offer alternative, lower cost methods, which could be used for these applications. Electrophoretic methods have also proven to be suitable for miniaturisation as they combine high analytical performance with relatively simple instrumentation. Thus, smaller instruments can be produced allowing analysis to be performed at the point of sampling within the plant, and also possibly on line.

Isotachophoresis (ITP) is a separation method well suited to the analysis of small inorganic ions and therefore presents a possible method for separating and analysing metal ions. The technique has been previously employed to analyse a wide range of metal ions, including those of alkali [1], alkaline earth [2], lanthanide [3] and transition metals. Transition metals have been analysed as both cations and anions in aqueous electrolyte systems using a range of complexing agents including lactic acid [4] tartaric acid [5] and ethylenediaminetetraacetic acid [6] and also in non-aqueous electrolyte systems [7]. However, to allow the widest range of ions to be separated, complexation with 2-hydroxyisobutyric acid (HIBA) has proven to be particularly effective, allowing up to 21 metals to be analysed simultaneously [8].

* Corresponding author. Fax: +44 161 200 4896.
  E-mail address: jprest@umist.ac.uk (J.E. Prest).

In addition to allowing smaller, more portable instruments to be produced, performing separations on a miniaturised scale offers a number of other potential benefits over conventional scale systems. These benefits include improved analytical performance and significant reductions in instrumentation costs, particularly if polymeric materials are used to fabricate the separation devices. Thus, in recent years, a significant amount of work has been done on the design and application of miniaturised separation devices. ITP has proven to be a particularly suitable technique for miniaturisation for a number of reasons. These include the preconcentration effect which occurs during the separations, thus assisting the determination of dilute samples, and the amenability of the method to using electrochemical detectors, which can be readily miniaturised unlike some other systems such as laser-induced fluorescence (LIF). Consequently miniaturised ITP has been successfully applied to a range of anionic separations including those of inorganic arsenic [9] and selenium [10] species, organic acids and small ions in wines [11] and small ions in waters [12]. In all of these applications poly(methyl methacrylate) (PMMA) devices with conductivity detection were used.

Despite the fact that ITP can be successfully performed in a miniaturised format and has proven to be a suitable technique for separating metal cations, few miniaturised ITP metal separations have been reported. This group has previously reported the separation of lithium and lanthanide metals, on a poly(dimethylsiloxane) (PDMS) device [13], small metal cations, using bidirectional ITP on a PMMA device [14], and a range of alkaline earth, transition and lanthanide metals on an injection moulded polystyrene device [15]. In all of these systems, conductivity detection was used. However, more work in this area has been done using other electrophoretic techniques such as capillary zone electrophoresis (CE) and micellar electrokinetic chromatography (MEKC). One of the earliest applications of miniaturised CE was the separation of three metals: Zn^{2+}, Cd^{2+} and Al^{3+}, using a glass device with LIF detection by Jacobson et al. [16]. Subsequently, the number of reported investigations involving separations of metal ions has been small compared to those concerned with bioanalytical separations. A glass device incorporating LIF detection was used by Kutter et al. for the separation of Ca^{2+} and Mg^{2+} [17]. Several groups have analysed mixtures of three alkali metals: K^+, Li^+ and Na^+. These separations have been used to demonstrate the use of particular devices for cationic separations as this sample represents one of the few metal mixtures that can easily be separated without the need for complex electrolyte systems. Such mixtures have been analysed using both glass [18–20] and PMMA [21] devices with conductivity detection. Small ions were also the subject of an investigation by Wang et al. that analysed ions likely to be found in explosive residues, which included K^+ and Na^+, on a PMMA device with conductivity detection [22]. Other research has involved the separation of a small number of transition metals to show the possibility of using chemiluminescence detection with glass [23] and poly(dimethylsiloxane) (PDMS) [24] devices and analysis of a range of samples containing alkali, alkaline earth and transition metals to demonstrate conductivity detection in a glass device [25]. Recently however, Collins and co-worker have investigated the analysis of a wide range of metal ions including transition metals [26], lanthanide metals [27] and uranium [28]. Separations of up to six ions: Cd^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+} and Ni^{2+}, have been achieved using non-aqueous electrolytes in glass devices with UV–vis detection [26]. This group of workers have also applied MEKC to the separation of transition metals [29]. Using this technique separations of seven ions: Co^{2+}, V^{3+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Cd^{2+}, were possible on a glass device with UV–vis detection.

This paper presents a new electrolyte system using malic acid as a complexing agent to allow the separation of a group of transition metals: Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}, expected to be found in process streams at metal processing plants. This method has been used to achieve miniaturised ITP separations of a model mixture containing these ions, and has also been applied to the separation of a range of industrial samples containing various different mixtures of these ions. Thus, the first known use of miniaturised ITP for the analysis of transition metals in industrial process solutions is presented.

2. Experimental

2.1. Instrumentation

The miniaturised ITP separation device used in this work was fabricated using a direct milling technique, which has been previously described [14]. The device consisted of a 78 mm long, 78 mm wide, 6 mm thick PMMA block sealed using a sheet of 98 mm long, 78 mm wide 400 μm thick self adhesive polyester laminate (Plastic Art, Manchester, UK). A schematic of the layout of the device produced is shown in Fig. 1. The flow channel from well B to the bifurcation point is 200 μm wide and 300 μm deep whereas all other channels are 300 μm wide and 300 μm deep. The distance from the injection cross to the bifurcation point is 57 mm, and from the bifurcation point to conductivity detector CD 1 is 44 mm. This was the flow path used for all of the separations performed in this work. An additional conductivity detector, CD 2, is located 5 mm from the bifurcation point in the direction of well E, although this was not used during this investigation. Both detectors comprise of a pair of opposed, on-column, 75 μm diameter platinum wire (Aldrich, Gillingham, UK) electrodes.

The constant currents required to perform the separations were provided by a PS350 5000V-25W high-voltage power supply (Stanford Research Systems, Sunnyvale, CA, USA), configured to supply positive voltages. Conductivity detection was performed using a laboratory-built dual-channel system, which uses capacitive coupling to ensure isolation of the low-voltage detection circuitry from the high voltages used to drive the separations. Transport of electrolytes and sam-

ples was achieved using a gravity feed hydrodynamic fluid transport system which was controlled by means of a series of LFAA1201718H two-way solenoid actuated valves (The Lee Company, Westbrook, CT, USA) positioned as indicated in Fig. 1.

Control of the sample transport system, high voltage power supply and conductivity detector was achieved using LabVIEW software (version 6.1) (National Instruments, Austin, TX, USA), running under the Windows XP operating system (Microsoft, Redmond, WA, USA) on a standard personal computer. The hardware interface is achieved using three National Instruments cards controlled using the NIDAQ driver (National Instruments) and programmed using LabVIEW code. The cards used were a PCI-GPIB board for the power supply, a PCI-6601 timing and digital input/output board for the detector and a PCI-6503 digital input/output card for the fluid transport system. A further LabVIEW program which filters the data and identifies zone boundaries was employed for data processing. This program was used to extract the quantitative information (zone lengths) and qualitative information [relative step heights (RSHs)] from the isotachopherograms obtained. In this work the RSHs were calculated using the following expression:

$$RSH = \frac{f_S - f_{LE}}{f_{TE} - f_{LE}}$$

where f_{LE} = frequency of the response produced by the leading electrolyte (Hz); f_S = frequency of the response produced by the sample (Hz); f_{TE} = frequency of the response produced by the terminating electrolyte (Hz).
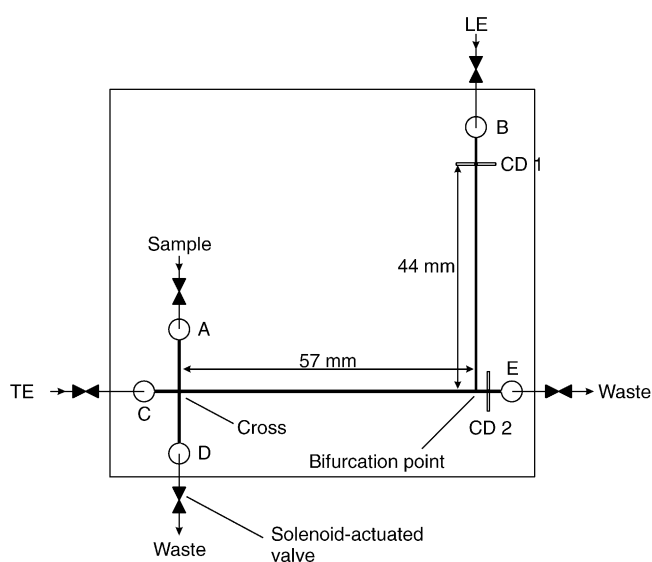

Fig. 1. Schematic diagram of the miniaturised poly(methyl methacrylate) (PMMA) separation device used for isotachophoretic metal separations. The channel from the bifurcation point to well B is 200 μm wide and 300 μm deep, whereas all other channels are 300 μm wide and 300 μm deep. Letters A, B, C, D and E refer to inlet/outlet connectors to the device and associated valves. CD 1 and CD 2 are conductivity detectors. LE and TE refer to leading and terminating electrolytes, respectively.

Zone boundaries are identified by taking a derivative of the filtered data and using a standard algorithm which fits a quadratic polynomial curve to determine the maxima. An elliptic filter was used as it has the same phase response for all frequencies thereby minimising the effect on zone lengths. Four parameters control the resulting output: filter cut-off frequency, filter order, minimum peak height and peak width. The settings used in this work allow the program to determine widths of greater than 0.4 s and changes of heights of greater than 20 Hz.

Inductively coupled plasma mass spectrometry (ICP-MS) analyses were performed using an ELAN DRC II ICP-MS instrument (Perkin-Elmer, Boston, MA, USA).

2.2. Separation conditions

Unless otherwise stated the miniaturised separations performed in this investigation were achieved using the control program shown in Table 1. The first two steps flush the device and fill the separation channels with leading electrolyte. The timing of this step is set to ensure that all traces of previously analysed samples are fully removed from the system before performing subsequent separations. Step 3 positions the terminating electrolyte so that the sample will be sandwiched between the leading and terminating electrolytes. Steps 4 and 5 control the amount of sample injected. The flow path used in step 5 means that the length of the channel between the cross and bifurcation point is used an injection loop with a volume of 5.1 μl. The ITP separations are performed with an initial constant current of 35 μA applied between wells B and C, step 6. The separation current is reduced to 10 μA in step 7 to allow improved detection of dilute samples. In this work such conditions allow the detection of zones with lengths greater than 1 s, the time taken to cross the detector based on the measured migration velocity of 7.30×10^{-5} m s^{-1} [± 2.4% relative standard deviation (R.S.D.) based on five replicate measurements]. During the separation all valves are closed to prevent hydrodynamic flow. The separation system is therefore of the less commonly encountered hydrodynamically closed variety. Further details of this mode of operation can be found in the recent review by Kaniansky et al. [30].

Table 1
Program used to control the fluid transport system and high voltage power supply

Step	Time (s)	Current (μA)	Valve status				
			A	B	C	D	E
1	20	0	■	□	■	■	□
2	20	0	■	□	■	□	■
3	1	0	■	■	□	□	■
4	0.5	0	□	■	■	□	■
5	0.3	0	□	■	■	■	□
6	300	35	■	■	■	■	■
7	1000	10	■	■	■	■	■

■ = closed; □ = open.

Table 2
Composition of electrolyte systems used to separate mixtures of metal ions using isotachophoresis

Electrolyte system	HIB	MAL
Leading electrolyte	10 mM NaOH	10 mM NaOH
Complexing agent	7 mM HIBA	2.5 mM Malic acid
pH Buffer	Propionic acid	Propionic acid
pH	5.2	4.9
Additive	1 mg ml^{-1} HEC	1 mg ml^{-1} HEC
Terminating electrolyte	10 mM TBAOH	10 mM TBAOH

HEC: hydroxyethylcellulose; HIBA: 2-hydroxyisobutyric acid; TBA: tetra-butylammonium.

2.3. Chemicals

The composition of the electrolyte systems used in this work is shown in Table 2. All chemicals were supplied by Aldrich unless otherwise stated. Electrolytes were produced using sodium hydroxide (1.0 M volumetric standard), propionic acid (>99.5%), L-malic acid (99%, Acros, Loughborough, UK), 2-hydroxyisobutyric acid (HIBA) (98%), hydroxyethylcellulose (HEC) (molecular mass ca. 250 000) and tetrabutylammonium hydroxide (1.0 M aqueous solution). Stock sample solutions, 1000 mg l^{-1}, were produced using the following salts: ammonium iron(II) sulphate hexahydrate (99%), cadmium acetate dihydrate (98%), calcium chloride dihydrate (>99%), chromium(III) chloride hexahydrate (97.5%), cobalt(II) chloride hexahydrate (98%), copper(II) chloride dihydrate (>99% Riedel-de Haën, Gillingham, UK), magnesium chloride hexahydrate (99%), manganese(II) chloride tetrahydrate (98%), nickel(II) nitrate hexahydrate (>99%, BDH, Poole, UK) and zinc chloride (>98%). Lead samples were produced using 1000 mg l^{-1} atomic absorption standard solution (lead nitrate in 0.5 M nitric acid) (BDH). All solutions were prepared using >18 MΩ water (Elga Maxima Ultra Pure, Vivendi Water Systems, High Wycombe, UK). The iron(II) sample stock solution was made up in 0.1 M sulphuric acid (95%, Prolabo, Poole, UK) to improve sample stability.

3. Results and discussion

3.1. Electrolyte system

This work required the development of an electrolyte system that could be used for an application to identify and quantify the transition metals likely to be present in process streams within metal processing plants. The particular emphasis of the application involved the following group of metal ions: Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}. On the basis of previous findings initial efforts were focussed towards the use of an electrolyte system using HIBA as the complexing agent. However, although the use of HIBA has proven useful for the analysis of mixtures of metal ions, to achieve the required level of complexation high concentrations of the complexing species are required [31]. To achieve

such high concentrations of HIBA, relatively high leading ion concentrations, in the range 20–30 mM are required. However, the use of high concentration leading ions increases separation times and inhibits the analysis of very dilute samples. Therefore, investigations were made into the possibility of using HIBA at lower concentrations with a 10 mM leading ion concentration to separate transition metal cations. These involved performing separations with Co^{2+}, Ni^{2+} and Zn^{2+}, three ions which are known to be difficult to separate, using HIBA concentrations in the range 0–10 mM. Analysis of the RSHs obtained revealed the largest difference in step heights for these ions was achieved using a HIBA concentration of 7 mM.

Initial separations were thus performed using electrolyte system HIB, the composition of which is shown in Table 2. In this work tetrabutylammonium (TBA) was used as the terminating electrolyte rather than the more commonly encountered use of H^+ for this purpose. This change increased the conductivity of this electrolyte and thus reduced the likelihood of bubble formation occurring during separations. An additional benefit was that the TBA produced sharper, more defined, steps which allowed easier data processing. Unfortunately, the results obtained using this electrolyte system were not ideal, as can be seen in Fig. 2a, which shows an isotachopherogram of a separation of 10 mg l^{-1} of Co^{2+}, Zn^{2+} and Ni^{2+}. Although the Co^{2+} was quite well resolved from the other two ions, the resolution between Zn^{2+} and Ni^{2+} was poor. Additionally, it was not possible to separate Co^{2+}, Fe^{2+} and Cr^{3+} with this system.

The use of a stronger complexing agent than HIBA was thus investigated. Previously, tartaric acid has been successfully used by Hirokawa et al. to separate a range of metal ions
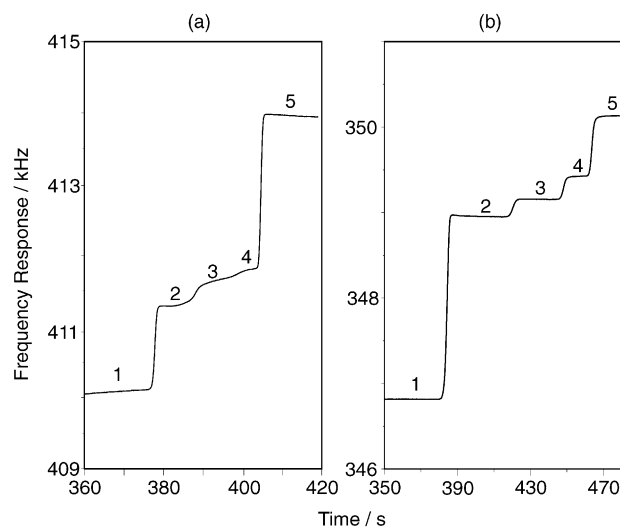

Fig. 2. Isotachopherograms obtained with samples containing 10 mg l^{-1} of Co^{2+}, Zn^{2+} and Ni^{2+} using electrolyte system (a) HIB (Table 2); (b) MAL (Table 2). Separation (b) was achieved using the separation program shown in Table 1; separation (a) was achieved with a similar program except the duration of step 6 was 250 s and the current used in step 7 was 20 μA. 1, Na^+; 2, Co^{2+}; 3, Zn^{2+}; 4, Ni^{2+}; 5, TBA^+.

[5]. However, one feature of complexation with tartaric acid is that it retards the mobility of zinc(II) to a greater extent than that of nickel(II). Without complexation zinc(II) exhibits a slightly higher mobility than nickel(II). Thus, it was thought that a clearer separation between these two species would be more easily achieved using a complexing agent that retarded the nickel(II) to a greater extent than the zinc(II). Tabulated data for the formation constants ($\log K_1$) of zinc(II) and nickel(II) malate complexes, 2.93 and 3.17, respectively [32], revealed malic acid to be a possible candidate to fulfil this aim.

It was found that with malic acid much lower concentrations of complexing agent were sufficient to enable separations of the metal ions of interest in this work. This is illustrated by the separation shown in Fig. 2b which is of a similar sample to that shown in Fig. 2a. In this case, 2.5 mM malic acid was used as the complexing agent. Fig. 2b shows a much clearer separation, with excellent resolution between both Co^{2+} and Zn^{2+}, and Zn^{2+} and Ni^{2+}. Thus, electrolyte system MAL, the full composition of which is shown in Table 2, was used in the remainder of this investigation.

Indeed the electrolyte system proved useful for separating the six ions of particular interest to this study: Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}. An example of an isotachopherogram obtained with a model mixture containing 7.5 mg l^{-1} of each of these species is shown in Fig. 3. This figure shows a good separation of all of these ions, which represent a mixture which has previously proven to be difficult to separate using electrophoretic techniques. It is believed that this is the first successful separation of this range of transition metals using isotachophoresis. This mixture has also proven difficult to separate using other electrophoretic methods, although it has been reported using CE by Kubáň et al., albeit with poor resolution between Zn^{2+} and Ni^{2+} peaks [33].
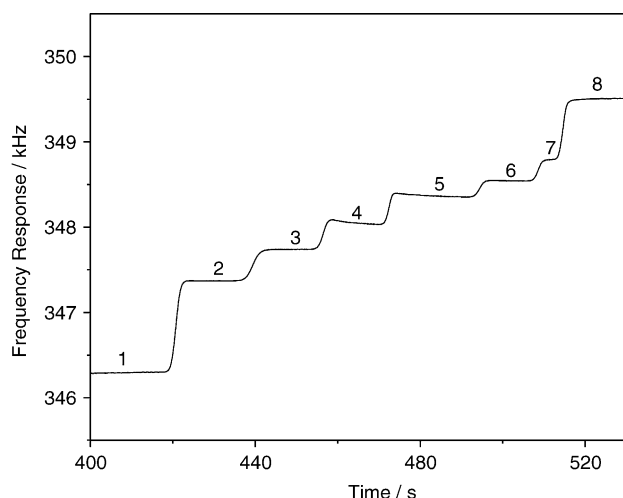
The time taken to achieve the separation was approximately 520 s. This time includes the time taken to flush the chip, load the electrolytes and inject the sample, so the actual separation time was about 480 s (8 min). The separation time offers a significant improvement on those possible using capillary scale separations, which often take 20–60 min to achieve for complex mixtures. However, some faster metal separations have been reported with miniaturised CE, including the separation of three cations in 20 s [16], and indeed miniaturised ITP separations of anions have been achieved in 210 s [10]. Part of the reason for the slower separations achieved here than with anionic miniaturised ITP is the choice of electrolyte system. The use of sodium for this purpose slows down the separation somewhat. The use of a faster leading ion such as potassium or ammonium would therefore reduce the separation time. However, the use of the sodium leading ion eliminates the problems of sodium contamination causing interference with the analysis which is seen as a more desirable feature than faster separations. If analysis time was critical the currents used in both steps 6 and 7 in the separation program could be increased. Yet some care needs to be exercised over increasing the current in step 6, because whilst no decrease in separation resolution will result from such a change [34], the formation of bubbles could occur due to increased Joule heating. The disadvantage of raising the current in step 7 is that this will reduce the ability of the system to analyse dilute samples.

Little interference from other species was expected as can be seen from Table 3 which shows the RSH values obtained with a number of common metal ions with the MAL electrolyte system. The only species found to migrate within the range of metals of interest was cadmium, and the RSH of this species was sufficiently different for it not to interfere with the determination of manganese(II) or chromium(III). Unfortunately, the method cannot be used for determining copper(II) as the effective mobility is retarded so much that this species is slower than the TBA terminating ion used in this work. Further investigation revealed the copper(II) is so slow that it cannot be readily determined isotachophoretically

Fig. 3. Miniaturised isotachophoretic separation of a model mixture containing 7.5 mg l^{-1} of Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}, using electrolyte system MAL shown in Table 2. 1, Na^+; 2, Mn^{2+}; 3, Cr^{3+}; 4, Fe^{2+}; 5, Co^{2+}; 6, Zn^{2+}; 7, Ni^{2+}; 8, TBA^+.

Table 3
Relative step heights of metal ions analysed using the MAL electrolyte system

Ion	Model samples			Industrial samples		
	RSH	R.S.D. (%)	n	RSH	R.S.D. (%)	n
Ca^{2+}	0.103	16.8	24	0.108	20.8	8
Mg^{2+}	0.171	11.4	4	–	–	–
Mn^{2+}	0.337	8.7	22	0.325	10.7	10
Cd^{2+}	0.416	1.4	3	–	–	–
Cr^{3+}	0.468	3.3	22	–	–	–
Fe^{2+}	0.535	1.8	23	0.525	5.5	7
Co^{2+}	0.639	2.9	25	–	–	–
Zn^{2+}	0.704	1.5	25	0.713	3.5	25
Ni^{2+}	0.796	1.2	36	0.787	2.4	15
Pb^{2+}	0.921	0.8	3	–	–	–
Cu^{2+}	>1		3	–	–	–

n: Number of runs; RSH: relative step height.

using the leading electrolyte employed here, as no suitable terminating ion can be used. This is because the effective mobility of the ion is slower than that of H^+. It should be noted that although there appears to be a reasonable difference in the step heights of calcium and magnesium, with calculated RSH values of 0.103 and 0.171, respectively, these ions could not be separated from one another.

3.2. Qualitation and quantitation

Good reproducibility was observed in the RSH measurements for most of the ions investigated in this study. With a range of model samples, over a timescale of three months, R.S.D. values of between 1.2% and 3.3% were obtained for the RSHs of Cd^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}. However, as can be seen in Table 3 the reproducibilities were not so good for Mn^{2+}, Mg^{2+} and Ca^{2+}. Reduced reproducibility for samples yielding low steps is thought to be due to a systematic error related to the conductivity detector used in this work because it is a feature which has been previously observed with other samples [9]. For individual samples with consecutive determinations even better reproducibility was obtained. For example, during the separations performed to produce calibration graphs, which involved four duplicate runs, many of the samples yielded R.S.D.s of less than 1%, with the best results obtained with 25 mg l^{-1} nickel(II) which yielded a RSH of 0.795 (± 0.2 %). However, even with individual samples the reproducibility of the calcium RSH was worse than with the other species.

Calibration graphs were produced for the six transition metals of interest, and also for calcium which was known to be present at a significant concentration in one of the industrial solutions to be analysed. Full details of the equations of these graphs, obtained using weighted linear regression are shown in Table 4. Good linearity was noted, with correlation coefficients greater than 0.990, obtained for all the species except iron(II), over the concentration range 1–50 mg l^{-1} (5–50 mg l^{-1} for Ni^{2+}). A slightly lower level of linearity, with a correlation coefficient of 0.965, was obtained for iron(II). This was thought to be due to a small amount of oxidation to iron(III) in the lower concentration (and hence less acidic) calibration samples.

Limits of detection (LODs) were calculated based on the method recommended by the Royal Society of Chemistry (UK) of the blank signal plus three times the standard deviation of this value [35]. The LOD represents the limit at which qualitative information can be obtained. Limits of quantitation (LOQs), the level above which precise determinations can be made, have also been calculated using the method suggested by the American Chemical Society of the blank signal plus ten times the standard deviation of this value [36]. However, in ITP the blank response is 0, which is not a realistic value, so in this work an estimated blank, taken as the intercept of the calibration equation, or 0 if this was a negative value, plus three times the standard deviation for this value, was used. For Ca^{2+}, Mn^{2+}, Cr^{3+}, Co^{2+} and Zn^{2+} the LODs were calculated to be in the range 0.5–1.0 mg l^{-1}. Higher figures were exhibited by Fe^{2+}, 2.0 mg l^{-1} and Ni^{2+}, especially, 4.7 mg l^{-1}. The higher figure for iron(II) was likely to be due to the same sample stability problems as previously mentioned, which reduced the zone lengths observed with the lower concentration calibration samples. The reason for the high value with nickel(II) was thought to be due to the ion forming an unstable isotachophoretic zone. Two different types of unstable zones can occur in isotachophoretic separations, tailing zones and bleeding zones. The former can normally be discerned by the presence of peaks at the beginning of the step following that formed by the species yielding the unstable zone. The absence of such a feature in this work means that it is likely that the nickel has formed a bleeding zone. In this type of unstable zone, loss of analyte occurs due to the formation of a complex which does not dissociate during the separation due to slow kinetics. Such zones have been previously investigated in detail by Gebauer and Boček [37]. They found that the rate of loss of the analysed substance was constant and independent of concentration. Thus, quantitative measurements can still be made on nickel(II) provided the analysis times for the particular samples being analysed match those of the separations used in producing the calibration curve.

The calculated LOQs for all of the species are more than adequate for the proposed application which involves the analysis of process streams. However, the LODs are too high for monitoring effluent emissions. Such discharges need to comply with regulations like those conceived by the Paris Commission (PARCOM) [38], which include concentration limits of 0.5 mg l^{-1} for chromium (total), nickel(II) and zinc(II) in waste streams from metal plating industries.

Table 4
Weighted linear regression equations for the metal ion calibration curves

	LOD (mg l^{-1})	LOQ (mg l^{-1})	a ± S.D. (s)	b ± S.D. (s l mg^{-1})	r	n	Concentration range (mg l^{-1})
Ca(II)	0.5	1.8	1.61 ± 0.36	2.01 ± 0.09	0.999	6	1–50
Mn(II)	0.8	2.6	3.30 ± 1.00	3.88 ± 0.21	0.993	6	1–50
Cr(III)	0.5	1.7	3.08 ± 0.67	3.82 ± 0.24	0.999	6	1–50
Fe(II)	2.0	6.7	1.17 ± 1.05	1.55 ± 0.27	0.965	6	1–50
Co(II)	1.0	3.4	0.47 ± 1.42	4.19 ± 0.36	0.990	6	1–50
Zn(II)	0.7	1.6	−0.95 ± 0.37	2.89 ± 0.13	0.999	6	1–50
Ni(II)	4.7	7.6	−9.76 ± 1.16	2.81 ± 0.12	0.998	5	5–50

The equations have the form $y = bx + a$. a: intercept; b: slope; LOD: limit of detection (see text for definition); LOQ: limit of quantitation (see text for definition); n: number of data points (four replications performed at each); r: correlation coefficient; S.D.: standard deviation.

For most of the ions analysed in this work such limits of detection should be achievable by slight amendments to the design of the separation device, such as allowing a larger injection volume. However, a very low LOD for nickel(II) will not be possible with the malic acid containing electrolyte system, MAL, due to the problem of this ion forming an unstable zone.

3.3. Analysis of industrial samples

The use of the miniaturised ITP separation device together with electrolyte system MAL was applied to the analysis of five industrial samples, nos. 1–5, obtained from two metal processing plants located in mainland Europe. The samples were obtained from different process streams within the plants and thus consisted of significantly different concentrations of metal ions. In appearance samples 1 and 2 were green solutions, sample 3 was a black solution and samples 4 and 5 were colourless. The pH values of the solutions 1–5 were 2.5, 2.3, 0.7, 1.8 and 2.3, respectively. Prior to analysis all solutions were filtered through 0.45 μm pore size polyethersulphone syringe filters (Gelman Sciences, Ann Arbor, MI, USA). Samples were diluted as appropriate so that the concentrations of the ions being determined corresponded to the range over which the calibrations were performed. Thus, sample 5 was used without dilution whereas samples 1–4 were diluted with >18 MΩ water in the ratios 1:25, 1:150, 1:250 and 1:50, respectively. Fig. 4 shows examples of isotachopherograms obtained with two of the samples, 2 and 5. It can be seen that little additional noise was encountered with these samples compared to the model separations. The analysis times for the industrial samples were generally longer than for the model mixtures. This was due to the presence of species in these samples which possessed high effective mo-

bilities and migrated with the leading ion, thus lengthening the zone of this species. The identities, and concentrations, of such species were not provided by the metal processing companies, but were likely to include Na^+, as well as possibly K^+ or NH_4^+. As the different samples contained different amounts of such species and were also diluted to different amounts, analysis times thus varied, ranging from 500 s for sample 4 up to 750 s for sample 2.

Good agreement in RSHs between the model samples and industrial samples was observed as can be seen by comparing the results shown in Table 3, although there was a slight reduction in reproducibility. Statistical analysis, by means of a Student's *t*-test, on the two sets of RSH data yielded *t* values significantly under the critical *t* values for all of the ions. This indicates that statistically there was no difference in the RSHs obtained and thus highly likely that the species identified in the analyses of the industrial process streams are the same as those attained with the model samples. Again, the most reproducible results were obtained with zinc(II) and nickel(II), with calcium, in contrast, having particularly poor results. Like the results for the model samples, these RSH values are based on a number of different samples. With the industrial samples this particularly shows the robustness of the method since unlike the model solutions the industrial samples contain various other, unknown, ions, the presence of which could have affected the RSH values. With individual samples better reproducibility was achieved than that shown in Table 3, with for example sample 5 yielding a RSH for zinc of 0.709 (± 0.4%), based on 5 determinations.

Good reproducibilities in the zone lengths, with R.S.D.s in the range 1–6%, based on 5 determinations, were achieved with most of the samples. However, noticeably poorer reproducibilities were observed for all the ions in sample 3 and for nickel(II) and zinc(II) in sample 5. With sample 5 the problems with these two ions are probably related to the length of the zinc(II) zone. This zone was sufficiently short that it did not give a well defined step. When such a step is in close proximity to a much longer step, as was the case here with the subsequent nickel(II) zone, the data analysis program can have difficulties in accurately determining the zone boundary between the two zones, which is likely to have caused the poor reproducibilities of these ions. The problems with sample 3 were thought to be due to unknown component(s) in this solution. With this sample some bubble formation was observed during the separations and this is likely to be the reason why all three of the ions determined showed R.S.D.s of 14–17% on the measured zone lengths.

The compositions of the five samples were determined using miniaturised ITP and these results are shown in Table 5. This table also states the concentrations of the ions as provided by the metal processing companies. Although it is known that these concentrations were determined using AAS no information regarding the errors associated with these measurements was forthcoming. The exact ages of the samples are also unknown although they were believed to be approximately 1 year old, with the AAS determinations made
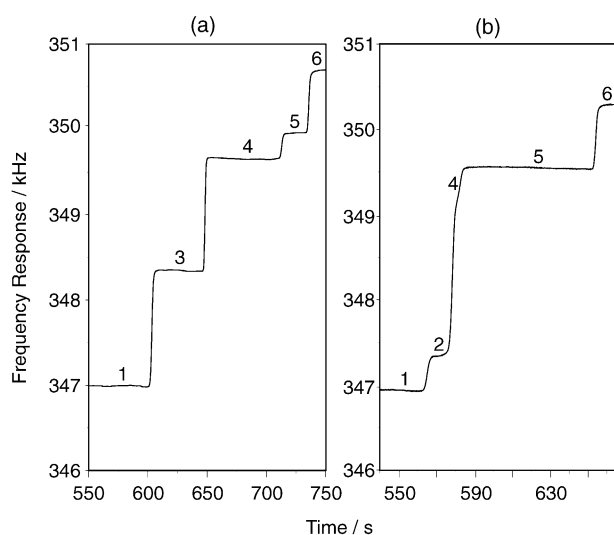

Fig. 4. Isotachopherograms of miniaturised separations of industrial samples attained using electrolyte system MAL shown in Table 2. (a) 1 in 150 dilution of sample 2; (b) undiluted sample 5. 1, Na^+; 2, Ca^{2+}; 3, Mn^{2+}; 4, Zn^{2+}; 5, Ni^{2+}; 6, TBA^+.

when the samples were fresh. Therefore, both age and uncertainty in the quoted concentrations of the samples are factors likely to influence the recoveries achieved using miniaturised ITP. Additionally, some of the variability in the recoveries can also be attributed to features of the different sample solutions. Table 5 also includes the nickel content of all of the samples with exception of sample 3, and the manganese content of sample 2 as determined using ICP-MS. Calcium could not be determined using ICP-MS due to spectral interference whereas the method was thought unsuitable for zinc analysis in the samples due to previously reported matrix suppression effects [39].

In sample 2 the low nickel(II) recovery is probably due to the long analysis time. As can be seen in Fig. 4a the nickel(II) zone was detected after more than 700 s, which was later than in any of the other industrial samples and more importantly the calibration separations. Therefore, this means that the amount of nickel(II) lost due to the stability of the nickel(II)-malate complexes will be higher than expected. Thus, the calculated nickel(II) concentration is probably not an accurate reflection of the amount of nickel(II) present in the original which will be higher than the figure of 1700 mg l^{-1} deter-

mined here. It is likely that the reason for the slowness of the separations involving sample 2 is due to the presence of high concentrations of unknown fast ions in this particular sample.

Poor recoveries with sample 3 were expected even before analysis due to the condition of the sample. Initially it was thought that this was a black solution, but filtering revealed that it was actually a yellow liquid which contained a significant amount of black precipitate. The fact that the solution was yellow suggested that iron(III), originally absent from the solution, was present. Addition of potassium thiocyanate to the sample gave further evidence to this as the characteristic red $[Fe(SCN)(H_2O)_5]^{2+}$ complex was observed. Hence, oxidation to iron(III) will account for some of the reduction in the iron(II) levels found. Iron(III) cannot be determined using the ITP method described in this work as the species will exhibit a very low effective mobility due to hydrolysis. Also some of the metal ions were likely to be contained in the black precipitate and thus would not be found through analysis by ITP. It should be noted that this sample could not be analysed using ICP-MS.

The high recovery of zinc(II) in sample 5 could be the result of the difficulty in determining the zone lengths men-

Table 5
Concentrations of metal ions in industrial samples as determined by miniaturised isotachophoresis

Sample	Ion	Concentration					
		ITP		Stated[a]		ICP-MS	
		mg l^{-1}	R.S.D. (%)	mg l^{-1}	Recovery[b] (%)	mg l^{-1}	Recovery[b] (%)
1	Ca^{2+}	–	–	–	–	–	–
	Mn^{2+}	1.3	15.3	1.5	87	–	–
	Fe^{2+}	–	–	–	–	–	–
	Zn^{2+}	1300	4.6	1100	118	–	–
	Ni^{2+}	1100	3.5	1200	92	1200	92
2	Ca^{2+}	–	–	–	–	–	–
	Mn^{2+}	1600	3.1	4000	40	2300	70
	Fe^{2+}	–	–	–	–	–	–
	Zn^{2+}	3400	4.1	3100	109	–	–
	Ni^{2+}	1700	3.0	2400	71	2400	71
3	Ca^{2+}	–	–	–	–	–	–
	Mn^{2+}	230	25.2	570	40	–	–
	Fe^{2+}	14100	17.0	19500	72	–	–
	Zn^{2+}	2400	4.2	4000	60	–	–
	Ni^{2+}	–	–	14	0	–	–
4	Ca^{2+}	–	–	–	–	–	–
	Mn^{2+}	–	–	–	–	–	–
	Fe^{2+}	–	–	–	–	–	–
	Zn^{2+}	620	4.1	760	82	–	–
	Ni^{2+}	–	–	–	–	3	0
5	Ca^{2+}	6.0	0.3	nd[c]	na[d]	–	–
	Mn^{2+}	–	–	–	–	–	–
	Fe^{2+}	–	–	–	–	–	–
	Zn^{2+}	2.0	0.2	0.5	400	–	–
	Ni^{2+}	28	3.1	28	100	25	112

[a] Concentration information provided by companies supplying samples – obtained using atomic absorption spectroscopy.
[b] Recovery using miniaturised ITP compared to other method.
[c] nd: concentration not determined although presence of species was stated.
[d] na: not applicable.

tioned earlier. No data is known with this sample as to the level of recovery of calcium. This was because although the presence of this ion in the process solution was noted its concentration was not, because unless present in very high concentrations this species was of little interest to the metal processing company. However, as mentioned earlier, this electrolyte system is not ideal for analysing calcium due to the possibility of co-migration with magnesium, and thus over estimation of the concentration present. To the best of our knowledge no magnesium was present in this sample.

It can be seen that in samples 2 and 3 the manganese(II) recovery was much lower than expected when compared to the quoted manganese contents obtained using AAS. Part of the problem with sample 3 may be due to the precipitation mentioned above and also due to the fact that the actual amount detected was below the LOQ. Yet even taking these factors into account the recovery of manganese(II) was significantly lower than those for both iron(II) and zinc(II). No obvious reason for these low recoveries is apparent because it would not be expected that manganese(II) would exhibit lower levels of stability in the original process solutions than the other transition metals [40]. With sample 2 a much higher recovery of manganese(II) was noted when a comparison was made with the ICP-MS results. Thus, it seems likely that the original manganese concentration supplied by the company was too high. As sample 3 is from the same processing plant therefore it is likely that the quoted manganese content is also too high. The level of recovery compared to the ICP-MS result suggests that sample 2 may contain further manganese species other than manganese(II) which would be found by this method (and also AAS) but not ITP which is species specific.

The purpose of performing the separations on the industrial samples was primarily to identify the components present so that this knowledge could then be used to implement appropriate clean up procedures. The two companies employ a range of different clean up methods for the various metal ions. For some of these ions knowing the concentration present is not as important as identifying the particular ion since the same clean up method is used regardless of the concentration present. However, for nickel(II) and zinc(II), two different clean up procedures are used depending on the concentrations present. In this case, therefore, the concentration does need to be known especially as the system used for more dilute samples can be damaged by the presence of concentrated samples. The level of performance achieved with the system is more than adequate to meet these requirements which involve being able to determine concentrations with R.S.D.s of better than 20%.

Although the required application is mainly concerned with the macro components present in the samples, miniaturised ITP allowed for many of the micro components to be identified. Thus, in sample 3 manganese(II) could be separated from iron(II) and in sample 5 zinc(II) could be separated from nickel(II). In this latter case it thought a complete separation was achieved despite the problems arising from the zinc(II) zone shape. This separation would not be possible with other electrolyte systems, such as the HIB system employed earlier in this work, as the greater than ten times difference in concentrations between the ions would lead to the zinc(II) co-migrating with the nickel(II). In sample 1 the concentration differences were so large that the manganese(II) could not be determined simultaneously with the nickel(II) and zinc(II). However, because manganese(II) migrates ahead of these other ions it could be analysed separately by reanalysing the original process solution directly without dilution. The amount of manganese(II) in this sample was found to be under the LOQ hence, like the situation in sample 3, the relatively large error in the determined concentration. For species which migrate behind one or more of the macro sample constituents such a procedure could not realistically be applied as the separation times would be prohibitive. Therefore, the nickel(II) in sample 3 could not be determined. Another micro component which could not be determined was cobalt(II), which was thought to be present at concentrations of under $1\,\mathrm{mg\,l^{-1}}$ (the LOD) in all samples except 4. No chromium(III) was thought to be present in the actual solutions investigated but this species together with higher concentrations of cobalt(II) can be present in other process streams within the metal processing plants.

4. Conclusions

The use of a PMMA separation device with an integrated conductivity detector allowed miniaturised ITP separations of a range of metal ions to be made. A separation method was developed which used a leading electrolyte containing malic acid as a complexing agent to enable the separation of transition metal cations. Using the method the separation of Mn^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}, a mixture which is difficult to separate using electrophoretic methods, was achieved. The method was also successfully applied in an application involving the analysis of industrial process streams obtained from two metal processing plants. Determinations of the major components present in the industrial samples, the primary aim of the project, were readily achieved. In some instances the method even allowed for the simultaneous determination of macro and micro sample constituents. However, some microconstituents were present at such low levels, concentrations of less than 1×10^{-3} those of the macroconstituents, that they could not be determined simultaneously using the separation system used in this work. The levels of performance in terms of detection limits, reproducibility and analysis time were more than adequate for the application investigated. Thus, it can be concluded that miniaturised ITP can be used to monitor the composition of process streams. The current separation system can be readily used for analysing 4–6 samples per hour, which if it can be incorporated with a system for performing sample dilutions could form the basis of an automated on-line analysis systems that would allow the

exploitation of miniaturisation technology and its associated benefits to be fully realised.

Acknowledgements

References

[1] M. Tazaki, M. Takagi, K. Ueno, Chem. Lett. (1982) 639.
[2] T. Hirokawa, Y. Kiso, J. Chromatogr. 248 (1982) 341.
[3] I. Nukatsuka, M. Taga, H. Yoshida, J. Chromatogr. 205 (1981) 95.
[4] P. Gebauer, P. Boček, J. Chromatogr. 242 (1982) 245.
[5] T. Hirokawa, W. Xia, K. Nakamura, I. Tanaka, F. Nishiyama, Y. Kiso, B. Gaš, J. Vacík, J. Chromatogr. A 663 (1994) 245.
[6] P. Gebauer, P. Boček, M. Deml, J. Janák, J. Chromatogr. 199 (1980) 81.
[7] Y. Hirama, H. Yoshida, J. Chromatogr. 322 (1985) 139.
[8] J.E. Prest, P.R. Fielden, Anal. Comm. 36 (1999) 333.
[9] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, J. Chromatogr. A 990 (2003) 325.
[10] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, Anal. Bioanal. Chem. 376 (2003) 78.
[11] M. Masár, D. Kaniansky, R. Bodor, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 167.
[12] R. Bodor, V. Madajová, D. Kaniansky, M. Masár, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 155.
[13] J.E. Prest, S.J. Baldock, B.J. Treves Brown, P.R. Fielden, Analyst 126 (2001) 433.
[14] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, Analyst 127 (2002) 1413.
[15] S.J. Baldock, P.R. Fielden, N.J. Goddard, J.E. Prest, B.J. Treves Brown, J. Chromatogr. A 990 (2003) 11.
[16] S.C. Jacobson, A.W. Moore, J.M. Ramsey, Anal. Chem. 67 (1995) 2059.
[17] J.P. Kutter, R.S. Ramsey, S.C. Jacobson, J.M. Ramsey, J. Microcol. Sep. 10 (1998) 313.
[18] R.M. Guijt, E. Baltussen, G. van der Steen, R.B.M. Schasfoort, S. Schlautmann, H.A.H. Billiet, J. Frank, G.W.K. van Dedem, A. van den Berg, Electrophoresis 22 (2001) 235.
[19] F. Laugere, R.M. Guijt, J. Bastemeijer, G. van der Steen, A. Berthold, E. Baltussen, P. Sarro, G.W.K. van Dedem, M. Vellekoop, A. Bossche, Anal. Chem. 75 (2003) 306.
[20] J. Lichtenberg, N.F. de Rooij, E. Verpoorte, Electrophoresis 23 (2002) 3769.
[21] M. Pumera, J. Wang, F. Opekar, I. Jelínek, J. Feldman, H. Löwe, S. Hardt, Anal. Chem. 74 (2002) 1968.
[22] J. Wang, M. Pumera, G. Collins, F. Opekar, I. Jelínek, Analyst 127 (2002) 719.
[23] K. Tsukagoshi, M. Hashimoto, K. Nakajima, A. Arai, Anal. Sci. 16 (2000) 1111.
[24] B-F. Liu, M. Ozaki, Y. Utsumi, T. Hattori, S. Terabe, Anal. Chem. 75 (2003) 36.
[25] J. Tanyanyiwa, P.C. Hauser, Anal. Chem. 74 (2002) 6378.
[26] G. Deng, G.E. Collins, J. Chromatogr. A 989 (2003) 311.
[27] G.E. Collins, Q. Lu, Anal. Chim. Acta 436 (2001) 181.
[28] G.E. Collins, Q. Lu, Sens. Actuators B 76 (2001) 244.
[29] Q. Lu, G.E. Collins, Analyst 126 (2001) 429.
[30] D. Kaniansky, M. Masár, R. Bodor, M. Žúborová, E. Ölvecká, M. Jöhnck, B. Stanislawski, Electrophoresis 24 (2003) 2208.
[31] F.M. Everaerts, Th.P.E.M. Verheggen, J.C. Reijenga, G.V.A. Aben, P. Gebauer, P. Boček, J. Chromatogr. 320 (1985) 263.
[32] L.G. Sillén, A.E. Martell (Eds.), Stability Constants of Metal-Ion Complexes, Supplement No. 1, Chemical Society, London, 1971.
[33] P. Kubáň, P. Kubáň, V. Kubáň, Electrophoresis 24 (2003) 1397.
[34] F.E.P. Mikkers, F.M. Everaerts, J.A.F. Peek, J. Chromatogr. 168 (1979) 293.
[35] Analytical Methods Committee, Analyst 112 (1987) 199.
[36] American Chemical Society Subcommittee on Environmental Analytical Chemistry, Anal. Chem. 52 (1980) 2242.
[37] P. Gebauer, P. Boček, J. Chromatogr. 299 (1984) 321.
[38] PARCOM Recommendations 92/4, 1992.
[39] M. Gerotto, E. Dell'andrea, A. Bortoli, M. Marchiori, M. Palonta, A. Troncon, Microchem. J. 51 (1995) 73.
[40] A.E. Smith, Analyst 98 (1973) 65.