Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1042 (2004) 181–188
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Novel variable volume injector for performing sample introduction in a miniaturised isotachophoresis device

S.J. Baldock [a,*], P.R. Fielden [a], N.J. Goddard [a], H.R. Kretschmer [b], J.E. Prest [a], B.J. Treves Brown [a]

[a] *Department of Instrumentation and Analytical Science (DIAS), UMIST, P.O. Box 88, Manchester M60 1QD, UK*
[b] *Zentralabteilung Technik, Siemens AG, 13623 Berlin, Germany*

Received 8 March 2004; received in revised form 19 May 2004; accepted 19 May 2004

Abstract

A microdevice design furnished with a novel sample injector, capable of delivering variable volume samples, for miniaturised isotachophoretic separations is presented. Micromachining by direct milling was used to realise two flow channel network designs on poly(methyl methacrylate) chips. Both designs comprised a wide bore sample channel interfaced, via a short connection channel, to a narrow bore separation channel. Superior injection performance was observed with a connection channel angled at 45° to the separation channel compared to a device using a channel angled at 90°. Automated delivery of electrolytes to the microdevice was demonstrated with both hydrostatic pumping and syringe pumps; both gave reproducible sample injection. A range of different sampling strategies were investigated. Isotachophoretic separations of model analytes (metal ions and an anionic dye) demonstrated the potential of the device. Separations of ten metal cations were achieved in under 475 s.

Keywords: Isotachophoresis; Miniaturization; Injection methods; Instrumentation; Chip technology

1. Introduction

Fully integrated analysis devices require precise and flexible sample injection systems and automated sample delivery. Several different injector geometries for electroseparation devices have been investigated. Injector geometry in the earliest miniaturised electroseparation devices took the form of T intersections [1,2], where variable sample volumes could be electrokinetically migrated from a side channel into the separation channel by timed switching of applied potentials. These simple T injector chips were found to suffer from sample leakage, in addition to preferentially injecting higher mobility sample components. Therefore, this approach was quickly abandoned in favour of volumetrically defined injectors such as cross [3,4] or double-T injectors [5,6]. At first these designs were used in the 'floating' sample introduction method, where voltages are only applied to the sample/sample waste channels, leaving the buffer/separation channels to float during injection and vice versa for the separation stage. To circumvent the leakage exhibited by this mode of operation and to inject well defined sample plugs pinched [7] and gated [8] sample introduction protocols were implemented. In the pinched method, sample is drawn transversely across the intersection using an applied potential and is confined in the intersection volume by the application of additional (pinch) voltages to the buffer/separation channel arms. In the separation stage, back voltages are normally applied to the sample/sample waste channel to prevent leakage into the separation channel. Long sampling times are often required to obtain a representative sample. The gated injection strategy brings the two streams of buffer and sample together at a cross intersection so that they both undergo a 90° turn. The potentials of the four channel arms are controlled to prevent unwanted mixing of the streams. Injection of sample into the continuously flowing buffer is effected by floating the potential of the sample and sample waste reservoirs. Although very fast injections can be achieved with this method it suffers from electrokinetic sampling bias. It is

* Corresponding author. Tel.: +44 161 200 8900; fax: +44 161 200 4896.
E-mail address: sara.baldock@umist.ac.uk (S.J. Baldock).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.062

now common practice to apply controlling potentials during injection and several investigative studies have been published. Ramsey and co-workers have used computer simulations to optimise the voltage parameters for pinched [9,10] and gated [10] injections. Other studies have focused on the sample bias caused by these methods; for example, Ramsey and co-workers looked at pinched injections [11–13], Slentz et al. [14] have considered the sampling bias due to the differences in analyte turning radii in gated injections, and Shultz-Lockyear et al. [15] have comprehensively examined the electrokinetic injection behaviour of double-T injectors.

More complex injection geometries have also been proposed and evaluated. Several reports [16–18] investigated a double-cross architecture for sample introduction. In this design the sample stream is focussed through both cross intersections. This confines the sample to a more streamlined band in the second cross, where injection into the separation channel takes place. The improved sample plug distribution in the separation channel was reported to give an enhanced separation performance [17]. Fu et al. [19] designed a multi-T injector with six channel arms which was capable of operating in cross, double-T and triple-T modes to enable electrokinetic injection of three different sample volumes with appropriate manipulations of the applied electric fields.

In order to eliminate the effects of electrokinetic injection bias Zhang and Manz [20] designed cross and T intersections which had sample channels that were narrower than the separation channel, so that no electrokinetic leakage control was required during the injection stage (for the T) and the separation stage (for the cross), thus also simplifying the high-voltage (HV) control hardware required. Higher separation efficiencies were reported with these designs compared to a conventional cross with uniform channel cross sections.

Hydrodynamic injection has been considered as a method to reduce electrokinetic bias. For example, Solignac and Gijs [21] applied a pressure pulse to the sample inlet, using a flexible membrane, whilst performing electrokinetic injection in a cross intersection device in an effort to reduce the electrokinetic bias. Backofen et al. [22] modified the gated injection procedure by using a higher sample reservoir volume to drive sample into the separation capillary whilst the gating voltages were turned off. Bai et al. [23] proposed pressure pinched injections using syringe pumps for microflow devices and proposed that the method would be suitable for injection in capillary zone electrophoresis (CZE) chips.

Several workers have looked at methods for achieving more efficient sample introduction into microchannels. Fang et al. [24] devised a flow-through reservoir to enable sample change without disturbing the chip setup. Sample was introduced into the bottom of the reservoir from below the chip and was allowed to overflow from the sample reservoir along a waste disposal trough, thereby maintaining a constant liquid level in the reservoir. From this reservoir, injection into the separation channel was achieved by means of a cross intersection. However, manual filling and adjustment of the buffer reservoirs was still necessary. Other reports have looked at integrating wide bore sample conduits with narrow electrophoretic separation channels. Attiya et al. [25] interfaced a 1 mm wide sample channel, which enabled sample introduction at a rate of up to 1 ml/min, without disturbing the solutions within a 36–275 μm wide separation channel network. Samples were drawn through the channels under vacuum and the injection volume was defined by a double-T injector. However, as floating electrokinetic injection was used, some leakage was observed into the separation channel necessitating extra flushing steps to obtain representative samples. Lin et al. [26] coupled a 3 mm wide sampling channel to a network of 75 μm wide separation channels. Hydrodynamic sample splitting (to reduce the sample volume to the nanoliter range), followed by gated injection through a cross intersection, was used to deliver samples into the separation capillary.

Comparitively few reports, compared to those for CZE separations, have been published on the use of miniaturised electroseparation devices for performing isotachophoresis (ITP). Most of the devices reported for ITP applications have used volumetrically defined injection methods to effect sample introduction. Although some of the early devices used glass [27,28] most of the miniaturised ITP separations have been carried out in plastic devices, since these materials exhibit lower levels of electroosmotic flow. The authors have presented polymeric devices fabricated from poly(dimethylsiloxane) (PDMS) and injection moulded plastics [poly(methyl methacrylate) (PMMA) polystyrene and Zeonor] incorporating cross and double-T injectors for electrokinetic and pressure based sample introduction [29–31]. In 2002, Wainright et al. [32] reported the use of ACLARA BioSciences LabCards for isotachophoretic pre-concentration of fluorescently labelled ACLARA eTags (reporter molecules) for cell lysate analysis followed by CZE analysis. Injection in the devices was accomplished either by using a 2 cm long section of channel or by using an injection/stacking channel of length 16 mm with a manual replacement of sample by the terminating electrolyte (TE) buffer.

In 2003, the authors reported a two-dimentional (2D) ITP device [33] micromachined in PMMA with three sets of integrated platinum conductivity electrodes. The device comprised two perpendicular channels (a straight 300 μm wide pre-separation column incorporating a cross-injector and a 200 μm wide analytical column) joined via a T intersection. The device had two possible injection strategies: either the cross intersection (27 nl) could be used to effect delivery of high concentration samples into the first column, or low concentration samples could be delivered to the second column by using the fixed volume (5.1 μl) of the pre-separation column. This device has been employed for separations of inorganic arsenic species [33], inorganic selenium species [34] and ascorbate in photographic developer solutions [35]. However, the majority of reported miniaturised ITP separations have been performed on hot embossed PMMA mi-

crodevices with integrated, thin film, sputtered conductivity detection electrodes. In 2001, two initial designs for coupled column separation devices were presented both consisting of four channel compartments (two sample loops of differing volumes, a pre-separation and an analytical column) and three planar buffer reservoirs. The two devices both have a channel depth of 200 μm but differ in volumes and layout. The volumes of the large sample loop, small sample loop, pre-separation column and analytical column were 15, 1.2, 1.2 and 1.2 μl, respectively, for one device and 9.2, 0.96, 1.2 and 1.7 μl for the other [36]. These devices have been used for performing ITP separations of model mixes of organic [36–39] and inorganic/organic anions [40], as well as tryptophan enantiomers [41] and proteins [37]. ITP separations of anions in various sample matrices have also been reported, e.g. water [42], wine [37,43] and food and cosmetics [44]. In addition, separations of three of these sample matrices have been performed using coupled ITP–CZE (water [42,45,46], food and cosmetics [44,45]). ITP–CZE separations have also been reported of selenoamino acids [47,48] and valproic acid in serum [49]. In 2002, a single-column hot embossed chip was reported; this incorporated two sample injection channels (4500 and 500 nl) and a two-section separation column (5700 and 2800 nl) [45]. Although this device has mainly been used for CZE separations [50–52], its use for ITP analysis of wine samples has recently been reported [45]. Most of the separations published using these devices have used the small sample loop for sample injection, the exceptions being the analysis of anions present in water samples where high sample loading of dilute samples was required.

This paper presents the fabrication and performance characterization of a novel variable volume injector for sample delivery in miniaturised isotachophoresis devices. When coupled with either a gravity-fed or syringe pump-based fluid handling system this injector enabled electrolytes and samples to be rapidly and accurately delivered to the chip with the minimum of human intervention.

2. Method

2.1. Microdevice details

The designs of the two novel ITP injectors are given in Fig. 1a and b [53]. Both designs comprise a large-bore sample flow conduit coupled to a narrow-bore separation channel via a short connection channel, through which the sample can be injected from the sample loop into the separation channel. Design one has a sample flow channel of width 1 mm and a depth of 1 mm, a separation channel of width 300 μm and a depth of 200 μm. The two channels are joined by a 300 μm long, 200 μm deep connection channel angled at 90°. Design two has a sample stream channel of width 500 μm and a depth of 500 μm, with a 200 μm wide, 100 μm deep separation channel, which tapers down to 100 μm wide in the area of the detection electrodes. The connection channel is 1 mm long, 100 μm deep and angled at 45°.

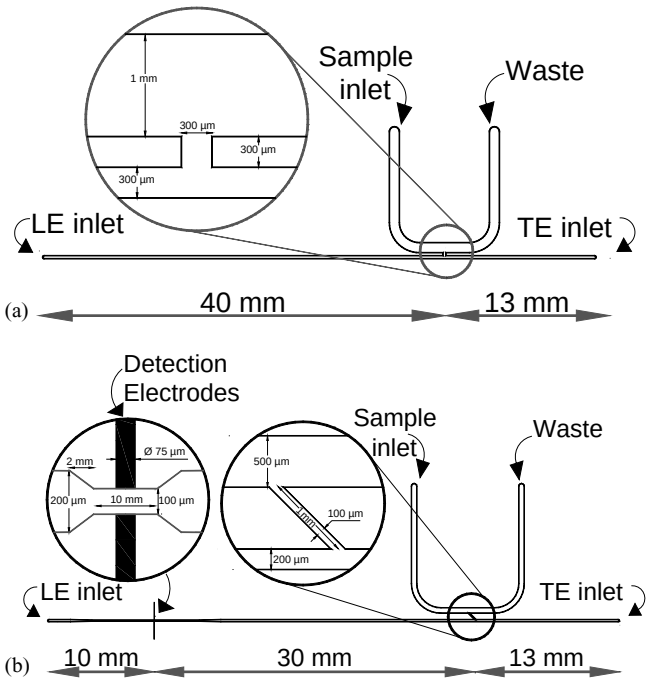

Fig. 1. (a) Design one. The sample inlet/waste channel has dimensions of 1 mm wide by 1 mm deep. The separation channel is 300 μm wide by 200 μm deep. (b) Design two. The sample inlet/waste channel has dimensions of 500 μm wide by 500 μm deep. The separation channel is 200 μm wide (except at the detection electrode area where it tapers to 100 μm wide) and is 100 μm deep. The detection electrodes were formed from diametrically opposed sections of platinum wire (75 μm diameter). LE and TE are the leading and terminating electrolytes, respectively.

Fig. 1 also depicts the fluidic setup for the three inlet channels; leading electrolyte (LE), terminating electrolyte (TE), sample (S) and the one outlet channel; waste (W), used to carry out ITP separations on the devices.

2.2. Microdevice fabrication

The ITP microdevices were fabricated in PMMA sheets (78 mm × 78 mm × 6 mm) using a micromachining procedure. Solid models of the required designs were produced using Mechanical Desktop v5.0 (Autodesk, San Rafael, CA, USA). The models were then converted to toolpaths for a CNC milling machine (CAT3D M6, Datron Technology, Milton Keynes, UK) using EdgeCAM v6.75 (Pathtrace Engineering Systems, Reading, UK). The procedures for forming the flow channels in the two devices were similar. However, device 2 required a preliminary step to form the electrodes. The electrodes were made by milling a groove, with access holes on either end, perpendicular to the intended channel orientation. Platinum wire (75 μm diameter, Aldrich, Gillingham, UK) was then threaded through this groove and the access holes and sealed in place with a UV curable adhesive (Norland Optical Adhesive 61, Norland Products, Cranbury, NJ, USA). A 400 W UV light source was applied for

240 s with the wire side of the chip orientated towards the source followed by 120 s with the device turned over to effect setting of the adhesive. The flow channels were milled into the PMMA sheet; in device 2 the channel bisected the groove containing the platinum wire and hence formed a pair of opposed detection electrodes flush with the sides of the channel. Small pilot holes of 0.89 mm diameter were drilled at the end of each channel arm to allow the subsequent manual drilling and tapping of bosses for MINSTAC 062 Lee fittings (Lee Products, Gerrards Cross, UK) which was carried out following the manufacturers guidelines. To form a sealed device, polyester self adhesive laminate (Plastic Art, Manchester, UK) was bonded to the bottom of the chip.

2.3. Instrumentation for ITP microdevice operation

The instrumentation consisted of a high-voltage programmable power supply unit (PSU), a conductivity detector and either a gravity fed or a syringe pump based fluid handling system, all of which were located within a HV safety enclosure and were controlled by a personal computer running programs written in LabVIEW v6.1 (National Instruments, Austin, TX, USA). The control system used four National Instruments cards. A PCI-6503 controlled the valves through a DIO-24MX relay box (Goldchip, Wimbourne, UK). A PCI-6713E provided the PSU voltage control and valve timing clock. A PCI-6023E enabled current monitoring of the PSU for constant current mode operation. A PCI-6602 acquired data from the conductivity detector. The PSU was either a four-way 1.5 kV constant voltage PSU, described in ref. [54], or a six-way PSU, described in ref. [31], which can supply up to 4 kV in constant voltage mode or 150 μA in constant current mode. The detector was the capacitatively coupled astable oscillator reported in ref. [55]. The gravity fed fluid handling system consisted of a component board fitted with four solenoid valves (model no. LFVA1210120H, Lee Products) and three solution reservoirs (formed from 20 ml syringe barrels). The syringe pump fluid handling system comprised three individually addressable, high precision, stepper motor driven, computer programmable syringe pumps equipped with four-way distribution valves (VersaPump 6 Model No. 54022, Kloehn Europe, Bondaduz, Switzerland). The pumps were capable of 48 000 steps per stroke. Hence, when fitted with 250 μl syringes (Kloehn) the system could dispense electrolytes and samples in increments of 5.2 nl. The syringe pumps were interfaced to the computer via RS-232 and controlled with ASCII string commands. Narrow-bore PTFE tubing (0.032 in. i.d., Lee Products; 1 in. = 2.54 cm) was used to connect the microdevice to the pumps, valves and solution reservoirs. Sections of stainless steel tubing (1 in./16 in. o.d., Supelco, Poole, UK), ∼1 cm long, were incorporated within the tubing for delivery of the separation voltages. Data analysis on the conductivity detection traces was performed using a LabVIEW program which picks out the ITP zone boundaries and the most stable conductivities. The program applies an elliptic filter to the data and then determines the maxima and minima of the derivative using a standard quadratic curve fitting algorithm. Four parameters control the resulting output: filter cut-off frequency, filter order, minimum peak height and peak width.

2.4. Chemicals

For the anionic dye separations, the LE was 0.01 M HCl (0.1 M HPCE grade, Fluka, Gillingham, UK) adjusted to pH 6 with L-histidine (>99%, Sigma, Gillingham, UK). Hydroxyethylcellulose (HEC) 0.1% (w/v) (molecular mass ca. 250 000, Aldrich, Gillingham, UK) was added to suppress any electroosmotic flow (EOF). The TE was 0.01 M 2-N-morpholinoethanesulfonic acid (MES) (>99.5%, Sigma) adjusted to pH 6 with histidine. The anionic dye sample was prepared from a 0.01 M stock solution of amaranth (Aldrich). For the metal cation separations, the LE was 0.02 M NaOH (1 M volumetric standard, Aldrich) with 0.015 M 2-hydroxyisobutyric acid (HIBA) (+98%, Aldrich) added as a complexing agent and adjusted to pH 4.95 using propionic acid (99.6% Sigma). The EOF suppressor was 0.05% (w/v) HEC. The TE was 0.01 M DL-carnitine hydrochloride (+98%, Sigma). The multi-component mixtures of metal ions were prepared from stock solutions (0.05 M) of individual metals using the following salts: calcium chloride dihydrate (+99%, Aldrich), manganese chloride tetrahydrate (+98%, Aldrich), magnesium chloride hexahydrate (99%, Aldrich), cobalt chloride hexahydrate (98%, Aldrich), nickel chloride hexahydrate (98%, BDH), zinc chloride (+98%, Aldrich), lanthanum chloride heptahydrate (99.9%, Aldrich), neodymium chloride hexahydrate (99.9%, Aldrich), gadolinium chloride hexahydrate (99.9%, Aldrich) and copper chloride dihydrate (+99%, Riedel-de Haën, Gillingham, UK). The copper solution used for the syringe pump investigation was prepared from a 1000 ppm atomic spectroscopy standard (Fluka). All solutions were prepared using >18 MΩ water (Elga Maxima Ultra Pure, Vivendi, High Wycombe, UK).

3. Results

The designs, shown in Fig. 1a and b, were evaluated for their suitability for performing reproducible injections for miniaturised ITP separations.

3.1. Evaluation of ITP injector design 1, 90° angled injector

The chip was connected to the gravity-fed fluid handling system and several sample introduction protocols were evaluated using amaranth, an anionic dye, as a model analyte. As this design was not equipped with detection electrodes the sample zone lengths were measured with a microscope and graticule. Pressure-induced sample injections were in-

vestigated using the sampling protocol given in Table 1, and varying the difference in height of the sample and electrolyte reservoirs. Using a six point pressure differential range of 34–172 Nm^{-2} between the sample reservoir and the (lower) LE reservoir enabled sample zone volumes of 248–467 nl to be injected. A linear relationship between the pressure difference ($x\,Nm^{-2}$) and sample zone length (y mm) was obtained ($y = 0.021x + 4.53$, $r = 0.9681$, $n = 5$), where r is the correlation coefficient and n is the number of replicates. Using the analysis protocol given in Table 1, and a pressure differential of 103 Nm^{-2} between the sample and LE reservoir, five amaranth solutions of varying concentration (4×10^{-3} to 1×10^{-2} M) were analysed. A linear response between sample concentration (x M) and sample zone length (y mm) was found over this concentration range ($y = 538x - 0.135$, $r = 0.993$, $n = 5$).

3.2. Evaluation of ITP injector design 2, 45° angled injector

This design (see Fig. 1b) had a longer sample injection channel (1 mm compared to 300 μm) angled at 45° to the other channels. This modification was introduced to reduce bleeding of the sample zones which was often observed along the channel walls with the first injector design, as shown in Fig. 2a. The width and depth of the sample introduction channel were both modified from 1 mm to 500 μm in order to reduce sample consumption and decrease the time required to flush out the sample loop when changing samples. The separation channels were also changed to enhance performance: the width and depth were both decreased from 300 to 200 μm and 200 to 100 μm, respectively. This design modification led to better-resolved zones being injected, as can be seen in Fig. 2b. The spatial resolution of the conductivity detector was enhanced by tapering the channel in the region of the conductivity electrodes. The incorporation of the conductivity detector in this device enabled the ITP performance of the microdevices to be more fully investigated. An ITP electrolyte system for separating metal cations, previously reported by the authors [56], was used to evaluate the microdevice design. ITP separations of metal ion solutions, using the settings given in Table 2 and a pressure differential of 103 Nm^{-2} between the sample and LE reservoir, determined that 10 metal ions could be separated on the 30 mm long separation channel in 475 s at a current of 20 μA, and that nine were resolved within 360 s at 30 μA. Fig. 3 presents typical isotachopherograms. Zone identification in the mixtures was based on the relative step heights (RSHs) obtained from single component runs (RSH is the height difference between the LE and the sample response divided by the height of the LE to TE response). Step heights were found to

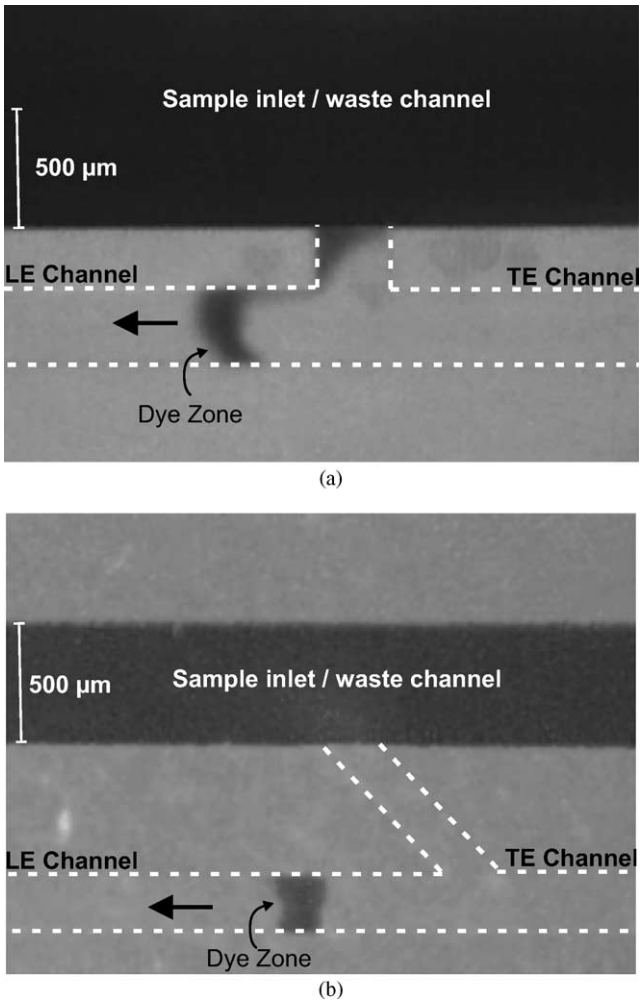

Fig. 2. ITP zone formation of an anionic dye in the two ITP injector devices. (a) A zone shortly after injection using the ITP injector with a 90° angle, the bleeding of the sample zone can be seen along the channel wall. (b) An ITP zone injected with the 45° angled injector, which was effective in reducing tailing.

Table 1
Valve and power supply settings for evaluation of microdevice 1

Step	Time (s)	Valve and PSU settings			
		LE	Waste	TE	Sample
(1) Electrolyte setup	3	●	●	●	▲
(2) Sample setup	3	▲	●	▲	●
(3) Sample injection	0.5	●	▲	▲	●
(4) Separation stage	130	▲, + 1000 V	▲	▲, Gnd	▲

▲, Valve closed; ●, valve open; Gnd, electrode at ground.

Table 2
Valve and power supply settings for evaluation of microdevice 2

Step	Time (s)	Valve and PSU settings			
		LE	Waste	TE	Sample
(1) Electrolyte setup	40	●	●	●	▲
(2) Sample setup	5	▲	●	▲	●
(3) Sample injection	2	●	▲	▲	●
(4) Separation stage	500	▲, Gnd	▲	▲, $I = 20$ or 30 μA	▲

▲, Valve closed; ●, valve open; Gnd, electrode at ground.

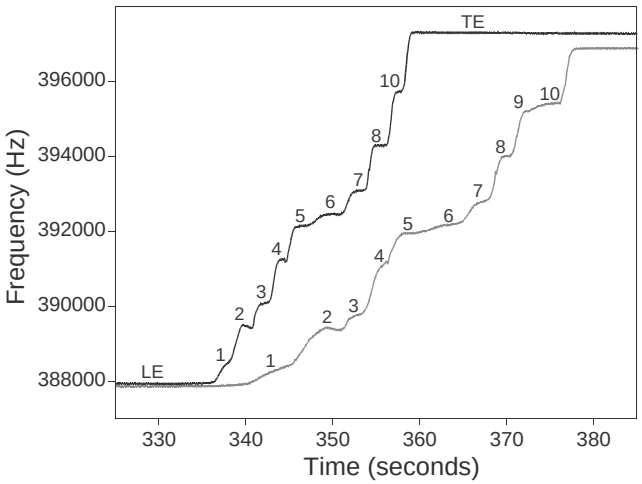

Fig. 3. Isotachopherograms of two metal ion mixtures. (1) Ca(II), (2) Mg(II), (3) Mn(II), (4) Co (II), (5) Ni(II), (6) Zn(II), (7) La(III), (8) Nd (III), (9) Gd(III), (10) Cu(II). LE, 0.02M NaOH with HIBA (0.015 M), pH 4.9 (propionic acid), 0.05% (w/v) HEC. TE, 0.01 M Carnitine hydrochloride. The nine component mixture contains 8×10^{-4} M of each metal ($I = 30\,\mu$A). The 10 component mixture contains 4×10^{-4} M of Co(II), La(III), Nd(II), 1.2×10^{-3} M of Ca(II) and 8×10^{-4} M of the other components ($I = 20\,\mu$A). NB: The ten component trace has been shifted by −95 s for comparison purposes. Fluid setup conditions as given in Table 2. The sample injection stage was 2 s (∼65 nl).

Table 3
Analysis of RSHs of ITP separations of metal cations

Metal ion	RSH ± S.D.	*n*
Ca(II)	0.055 ± 0.007	16
Mg(II)	0.154 ± 0.009	16
Mn(II)	0.221 ± 0.011	16
Co(II)	0.346 ± 0.010	16
Ni(II)	0.443 ± 0.007	16
Zn(II)	0.475 ± 0.006	16
La(III)	0.542 ± 0.008	16
Nd(III)	0.675 ± 0.004	5
Gd(III)	0.814 ± 0.002	5
Cu(II)	0.830 ± 0.006	16

RSH; Relative step height, S.D.; Standard deviation, *n*; number of replicates.

be reproducible and Table 3 lists the values obtained. Similar ITP studies of metal ion separations on a capillary scale laboratory instrument (ItaChrom EA101, J&M Analytische Messung und Regeltechnik, Aalen, Germany), fitted with a pre-separation column (90 mm × 0.8 mm i.d.), an analytical column (160 mm × 0.3 mm i.d.) and a 30 μl injection loop, showed that up to 6 metal ions could be separated in the pre-separation column in 14 min [57]. To separate more ions required the use of the second column and consequently resulted in longer analysis times. A separation time of 43 min,

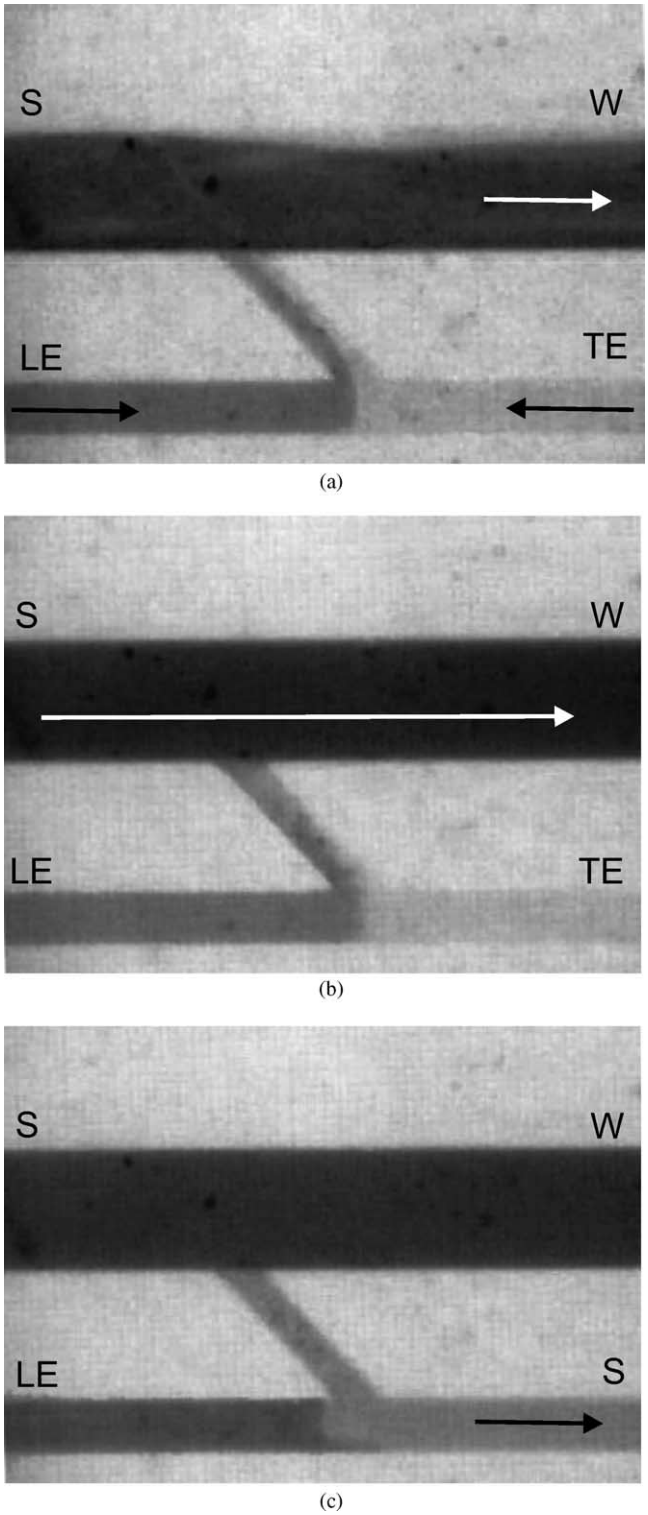

Fig. 4 Photographs of the ITP injector showing the fluid flows in the three channel sections. Where S is the sample stream, LE and TE are the leading and terminating electrolytes respectively and W is the waste outlet stream. (a) Initial electrolyte setup stage: the two electrolytes (here the LE is represented by a coloured dye and the TE is colourless) are allowed to flow through the separation channel to waste. A clean boundary was observed to form between the two electrolytes in the separation channel. (b) Sample loop filling stage: the sample stream (represented by another dye) is allowed to flow through the larger bore sampling channel to waste. The LE/TE boundary in the separation channel was not perturbed by this sampling setup stage. (c) Sample injection stage: the sample is drawn into the separation channel by displacing the TE backwards, a distinct LE/sample boundary is formed at the injector. Thus the sample is inserted between the two electrolytes and the separation stage can commence.

and sample pre-dilution (1:8) to compensate for the larger injection volume of the instrument, was required to analyse the same ten component solution on the instrument. The resolution of two pairs of metal ions Ca(II)/Mg(II) and Ni(II)/Zn(II) was poor compared to the microdevice separation, with RSHs of 0.030/0.039 and 0.356/0.372 being obtained for the two pairs, respectively. However, samples containing >10 metal ion components have also been analysed on this instrument, with a modified electrolyte system (LE 0.03 M NaOH) that was optimised for this purpose, a maximum of 21 were found, by the authors, to be separable, though a separation time of over an hour was required [56].

3.3. Evaluation of syringe pump based system for use with the ITP injector device

Although the gravity fed system was found to be suitable for the delivery and injection of fluids to the microdevice, it required the levels of solutions in the reservoirs to be maintained at the appropriate heights to obtain reproducible filling and injection behaviour. Therefore, in order to minimise the user intervention required and to simplify and speed up operational procedure, a fluid handling system based on the Kloehn Versa 6 syringe pump was introduced. A flow rate of 13 μl/s was found to be the maximum which could be employed without causing leakage from the tubing connections of the microdevice. A typical syringe pump program consisted of the following steps. (1) Fill syringes; LE, TE and sample syringes are filled from storage reservoirs; (2) Fill chip; LE and TE are dispensed through the separation channel to waste. (3) Fill sample loop; sample is loaded through the sample channel to waste. (4) Injection; sample is injected between the LE and TE by aspirating TE from the separation channel so that sample is drawn from the sample loop and displaces the TE backwards; by varying the amount of TE withdrawn from the microdevice different sample volumes can be drawn into the separation channel. Fig. 4 shows a demonstration of a sample introduction sequence using the syringe pump-based fluidic system. Dyes were used to represent the three fluid streams involved in a chip filling procedure. Fig. 4a shows the chip filling stage. The two electrolytes fill the separation channel, forming a distinct boundary between the two liquids (here represented by a dyed and a colourless stream), and pass through the sample loop to waste. The larger width and depth of the sample channel causes the two streams, as they flow towards the waste, to undergo mixing in this channel. Fig. 4b shows the sample loop filling stage, the sample flows smoothly through the sample channel to waste and does not perturb the electrolytes in the separation channel. Fig. 4c shows the sample injection stage, where the TE is aspirated from the separation channel in the direction shown by the arrow, drawing sample into the separation channel without disturbing the LE, thus the LE-sample-TE sandwich necessary for performing ITP separations is formed. Sample solutions were drawn backward so as to maximise the length of the separation channel available before detection.

Using a 5 ppm copper solution, the ability of the syringe pump system to inject variable sample volumes was investigated. The program steps were: chip fill, 208 μl of both LE and TE were dispensed; sample loop fill, 208 μl was dispensed; sample injection, six different sample aspiration volumes (2.08–8.33 μl) were injected. Separations were performed at 30 μA and a linear relationship between aspirated sample volume (x μl) and zone length (y s) was observed ($y = 0.398x + 0.145$, $r = 0.994$, $n = 4$).

Using the same initial syringe pump program, but with an injector aspiration volume of 0.26 μl seven copper samples with concentrations ranging from 10 to 200 ppm were analysed. ITP zone lengths were determined and a linear response between zone length (y s) and concentration (x ppm) ($y = 0.0311x + 1.35$, $r = 0.995$, $n = 4$) was obtained over this range. Using an identical chip filling procedure but with a larger sample aspiration setting, 2.08 μl, four samples, with concentrations ranging from 1 to 10 ppm, were analysed. A linear response between zone length (y s) and sample concentration (x ppm) ($y = 0.243x + 1.48$, $r = 0.983$, $n = 4$) was found over this range. Unfortunately this data series could not be extended to even lower concentrations as the laminate sealing of the microdevice failed, although it should be noted that this device had produced reliable performance for over 540 separations. A more robust sealing method is currently being sought.

4. Conclusion

Two different injector designs for ITP microdevices were fabricated and tested. An injection channel angled at 45° was found to significantly improve the resolution of injected zones. The flow characteristics of the design are such that no pinch voltages are required to prevent leakage so that only a separation voltage need be applied thereby simplifying any power supply hardware requirements. Variable volume injection of samples was readily achieved via a number of sample introduction protocols. The use of a syringe pump based fluid handling system enabled the accurate delivery of electrolytes and samples to the microdevice with the minimum of user intervention. The utility of the microdevice for analysing samples of varying concentrations was illustrated via separations of copper solution over the range of 1–200 ppm.

Acknowledgements

Financial support was provided by the Engineering and Physical Research Council (EPSRC, UK), the European Union (via Framework 5, project no. G1RD-CT2000-00408,

Project Acronym: MEWAPREV) and the Medical Research Council (MRC, UK) via the North West Science Review.

References

[1] A. Manz, D.J. Harrison, E.M.J. Verpoorte, J.C. Fettinger, A. Paulus, H. Lüdi, H.M. Widmer, J. Chromatogr. 593 (1992) 253.
[2] K. Seiler, D.J. Harrison, A. Manz, Anal. Chem. 65 (1993) 1481.
[3] D.J. Harrison, P.G. Glavina, A. Manz, Sens. Actuators B. 10 (1993) 107.
[4] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science (Washington, DC) 261 (1993) 895.
[5] C.S. Effenhauser, A. Manz, H.M. Widmer, Anal. Chem. 65 (1993) 2637.
[6] C.S. Effenhauser, A. Paulus, A. Manz, H.M. Widmer, Anal. Chem. 66 (1994) 2949.
[7] S.C. Jacobson, R. Hergenröder, L.B. Koutny, R.J. Warmack, J.M. Ramsey, Anal. Chem. 66 (1994) 1107.
[8] S.C. Jacobson, L.B. Koutny, R. Hergenröder, A.W. Moore, J.M. Ramsey, Anal. Chem. 66 (1994) 3472.
[9] S.V. Ermakov, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 70 (1998) 4494.
[10] S.V. Ermakov, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 72 (2000) 3512.
[11] S.C. Jacobson, J.M. Ramsey, Anal. Chem. 69 (1997) 3212.
[12] J.P. Alarie, S.C. Jacobson, C.T. Culbertson, J.M. Ramsey, Electrophoresis 21 (2000) 100.
[13] J.P. Alarie, S.C. Jacobson, J.M. Ramsey, Electrophoresis 22 (2001) 312.
[14] B.E. Slentz, N.A. Penner, F. Regnier, Anal. Chem. 74 (2002) 4835.
[15] L.L. Shultz-Lockyear, C.L. Colyer, Z.H. Fan, K.I. Roy, J.D. Harrison, Electrophoresis 20 (1999) 529.
[16] R.J. Yang, L.M. Fu, G.-B. Lee, J. Sep. Sci. 25 (2002) 996.
[17] L.M. Fu, R.J. Yang, G.-B. Lee, Anal. Chem. 75 (2003) 1905.
[18] M. Deshpande, K.B. Greiner, J. West, J.R. Gilbert, L. Bousse, A. Minalla, in: A. van den Berg, W. Olthius, P. Bergveld (Eds.), Micro Total Analysis Systems 2000, Kluwer, Enschede, The Netherlands, 2000, p. 339.
[19] L.M. Fu, R.J. Yang, G.B. Lee, H.H. Liu, Anal. Chem. 74 (2002) 5084.
[20] C.X. Zhang, A. Manz, Anal. Chem. 73 (2001) 2656.
[21] D. Solignac, M.A.M. Gijs, Anal. Chem. 75 (2003) 1652.
[22] U. Backofen, F.-M. Matysik, C.E. Lunte, Anal. Chem. 74 (2002) 4054.
[23] X. Bai, H.J. Lee, J.S. Rossier, F. Reymond, H. Schafer, M. Wossner, H.H. Girault, Lab on a Chip 2 (2002) 4549.
[24] O. Fang, G.M. Xu, Z.L. Fang, Anal. Chem. 74 (2002) 1223.
[25] S. Attiya, A.B. Jemere, T. Tang, G. Fitzpatrick, K. Seiler, N. Chiem, D.J. Harrison, Electrophoresis 22 (2001) 318.
[26] Y.-H. Lin, G.-B. Lee, C.-W. Li, G.-R. Huang, S.-H. Chen, J. Chromatogr. A 937 (2001) 115.
[27] P.A. Walker, M.D. Morris, M.A. Burns, B.N. Johnson, Anal. Chem. 70 (1998) 3766.
[28] S.J. Baldock, N.J. Goddard, P.F. Fielden, in: Proceedings of MICRO.tec 2000, VDE Verlag, Hannover, 2000, p. 615.
[29] P.R. Fielden, S.J. Baldock, N.J. Goddard, J.E. Prest, B.J. Treves Brown, in: Proceedings of the 24th International Symposium on Capillary Chromatography and Electrophoresis, http://www.meetingabstracts.com, Las Vegas, Nevada, 2001.
[30] P.R. Fielden, S.J. Baldock, N.J. Goddard, L. Morrison, J.E. Prest, B.J. Treves Brown, M. Zgraggen, in: D.J. Bornhop, D.A. Dunn, R.P. Mariella, C.J. Murphy, D.V. Nicolau, M. Palmer, R. Raghavachari (Eds.), Proceedings of SPIE, Biomedical Nanotechnology Architectures and Applications, vol. 4626, SPIE, Bellingham, WA, 2002, p. 429.
[31] S.J. Baldock, P.R. Fielden, N.J. Goddard, J.E. Prest, B.J. Treves Brown, J. Chromatogr. A 990 (2003) 11.
[32] A. Wainright, S.J. Williams, G. Ciambrone, Q. Xue, J. Wei, D. Harris, J. Chromatogr. A 979 (2002) 69.
[33] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, J. Chromatogr. A 990 (2003) 325.
[34] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, Anal. Bioanal. Chem. 376 (2003) 78.
[35] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, Analyst 128 (2003) 1131.
[36] B. Graß, A. Neyer, M. Jöhnck, D. Siepe, F. Eisenbeiß, G. Weber, R. Hergenröder, Sens. Actuators B. Chem. 72 (2001) 249.
[37] B. Graß, G. Weber, A. Neyer, M. Schilling, R. Hergenröder, Spectrochim. Acta B 57 (2002) 1575.
[38] M. Masár, M. Žúborová, J. Bielčíková, D. Kaniansky, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 101.
[39] B. Graß, D. Siepe, A. Neyer, R. Hergenröder, Fresenius J. Anal. Chem. 371 (2001) 228.
[40] D. Kaniansky, M. Masár, J. Bielčiková, F. Iványi, F. Eisenbeiß, B. Stanislawski, B. Graß, A. Neyer, M. Jöhnck, Anal. Chem. 72 (2000) 3596.
[41] E. Ölvecká, M. Masár, D. Kaniansky, M. Jöhnck, B. Stanislawski, Electrophoresis 22 (2001) 3347.
[42] R. Bodor, V. Madajová, D. Kaniansky, M. Masár, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 155.
[43] M. Masár, D. Kaniansky, R. Bodor, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 167.
[44] R. Bodor, M. Žúborová, E. Ölvecká, V. Madajová, M. Masár, D. Kaniansky, B. Stanislawski, J. Sep. Sci. 24 (2001) 802.
[45] D. Kaniansky, M. Masár, R. Bodor, M. Žúborová, E. Ölvecká, M. Jöhnck, B. Stanislawski, Electrophoresis 24 (2003) 2208.
[46] R. Bodor, D. Kaniansky, M. Masár, K. Silleová, B. Stanislawski, Electrophoresis 23 (2002) 3630.
[47] B. Graß, R. Hergenröder, A. Neyer, D. Siepe, in: Proceedings of the 24th International Symposium on Capillary Chromatography and Electrophoresis, http://www.meetingabstracts.com, Las Vegas, NV, 2001.
[48] B. Graß, R. Hergenröder, A. Neyer, D. Siepe, J. Sep. Sci. 25 (2002) 135.
[49] E. Ölvecká, M. Koníkova, N. Grobuschek, D. Kaniansky, B. Stanislawski, J. Sep. Sci. 26 (2003) 693.
[50] M. Žúborová, M. Masár, D. Kaniansky, M. Jöhnck, B. Stanislawski, Electrophoresis 23 (2002) 774.
[51] M. Žúborová, Z. Demianová, D. Kaniansky, M. Masár, B. Stanislawski, J. Chromatogr. A 990 (2003) 179.
[52] M. Masár, M. Žúborová, D. Kaniansky, B. Stanislawski, J. Sep. Sci. 26 (2003) 647.
[53] S.J. Baldock, P.R. Fielden, H.-R. Kretschmer, B.J. Treves Brown, Analysis Device for Electrophoretic Separation Assays, EP1380835A1, 2004.
[54] J.E. Prest, S.J. Baldock, P.R. Fielden, N.J. Goddard, B.J. Treves Brown, Analyst 127 (2002) 1413.
[55] D.-I. Vaireanu, P.R. Fielden, B.J. Treves Brown, Meas. Sci. Technol. 11 (2000) 244.
[56] J.E. Prest, P.R. Fielden, Anal. Comm. 36 (1999) 333.
[57] J.E. Prest, Ph.D. Thesis, UMIST, Manchester, 2000.