Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1119 (2006) 183–187
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Rapid chloride analysis using miniaturised isotachophoresis

Jeff E. Prest *, Sara J. Baldock, Peter R. Fielden, Nicholas J. Goddard, Stephan Mohr, Bernard J. Treves Brown

School of Chemical Engineering and Analytical Science, The University of Manchester, P.O. Box 88, M60 1QD Manchester, UK

Available online 1 December 2005

Abstract

A new design of miniaturised separation device for performing isotachophoresis (ITP) has been produced. The device contains a simple arrangement of channels comprising a single separation channel with a 'double T' injection geometry. The device was produced in poly(methyl methacrylate) and incorporates an on-column conductivity detector. A new electrolyte system was developed to enable the rapid determination of chloride to be made. This electrolyte system uses a leading ion of 3.5 mM nitrate at pH 3.0 with 0.5 mM indium(III) added as a complexing agent. Use of this electrolyte system with the new separation device allowed chloride samples to be analysed in under 100 s, with a limit of detection (LOD) calculated to be 2.2 mg l^{-1}.

Keywords: Isotachophoresis; Miniaturisation; Microdevice; Chip; Chloride analysis; Conductivity detection

1. Introduction

The miniaturisation of analytical systems offers numerous potential benefits such as faster analysis speeds, reduced sample consumption and, particularly through the use of polymeric materials, reduced cost. The relative simplicity of electrophoretic separation systems makes them ideal for miniaturisation. Although isotachophoresis (ITP) is one of the less frequently encountered electroseparation techniques it offers a number of advantages over the more commonly encountered method of capillary zone electrophoresis (CZE). Separation parameters can be easily controlled by varying the electrolyte system used. The concentration of the leading electrolyte governs the (constant) concentration of all of the sample zones, making the technique suitable for both dilute and concentrated samples. The electrolyte system also determines what is being analysed as only those constituents within the sample which have mobilities lower than that of the leading electrolyte and higher than that of the terminating electrolyte will be identified. Thus, miniaturised ITP has been applied to a range of samples such as small inorganic anions in waters [1], amino acids [2] and β-blockers [3]. Devices used for such separations have included cast poly(dimethylsiloxane) PDMS devices with serpentine channels [4], injection moulded polystyrene devices with single a straight separation channel [5], and milled poly(methyl methacrylate) (PMMA) devices with two separation channels [6], bidirectional separation channels [7] and a single channel device with a variable volume injector [8]. One of the most widely used designs is that of hot embossed PMMA device with a coupled-column arrangement originally developed by Graß et al. [9]. This type of device has been used for not only ITP separations but also for two-dimensional ITP–ITP [10], ITP–CZE [1] and CZE–CZE separations [11].

A drawback of the method has traditionally been the difficulty of analysing the important chloride ion. The major limitation in the determination of this species is that it has a high electrophoretic mobility. This makes it difficult to fulfil one of the major requirements of the electrolyte system for isotachophoretic analysis, namely that the leading ion needs to have a higher mobility than any ions of interest. It is in fact common practice to use chloride as the leading ion. One solution to this problem is to use the highly mobile dithionate ion as the leading ion [12]. This approach has been previously used in miniaturised ITP [1]. Unfortunately, this method cannot discriminate between chloride, bromide and iodide ions. Separation of this group of three halides is difficult in aqueous systems because they possess similar mobilities. They can however be separated using isotachophoresis if cadmium(II), a highly toxic species, is employed as a complexing agent [13].

* Corresponding author. Tel.: +44 161 3068900; fax: +44 161 3064896.
E-mail address: j.prest@manchester.ac.uk (J.E. Prest).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.11.030

This paper presents a new design of PMMA microchip for performing miniaturised ITP. The chip has a simple design with a single separation channel with an integrated on-column conductivity detector and a 'double T' injection geometry. Also introduced in this study is a newly formulated electrolyte system which uses complexation with indium(III). This new electrolyte system was used in conjunction with the new design of separation device to allow fast isotachophoretic determinations of chloride samples to be made.

2. Experimental

2.1. Fabrication and instrumentation

The separations performed in this study were carried out using a miniaturised PMMA separation device with an integrated conductivity detector. Fig. 1 shows a schematic view of the device. The main channel in the device from well B to well C is 60 mm long, and the effective separation distance, from the injection point to the conductivity detector is 43 mm. The device incorporates a 'double T' injection geometry in the flow path between wells A and D. All of the channels are 200 μm wide and 300 μm deep. The on-column conductivity detector is formed from a pair of 50 μm diameter platinum wire electrodes (Goodfellow, Huntingdon, UK) arranged in an opposed configuration. Fabrication of the device was done in-house using a previously described direct milling technique [7]. The device was sealed using a piece of 400 μm thick self-adhesive polyester laminate (Ritrama, Monza, Italy).

A PS350 high voltage, 5 kV power supply (Stanford Research Systems, Sunnyvale, CA, USA), configured to supply negative voltages was used to provide the constant currents required to drive the separations. Conductivity detection was achieved using a system built in-house, which uses capacitive coupling to isolate the low voltage detection circuitry from the high separation voltages. This design is based on an oscillating circuit and leads to the output from the detector being in the form of a frequency. As the conductivity increases the frequency decreases, with a near linear relationship over the range of conductivities encountered during isotachophoretic separations. Thus, in this work relative step heights (RSH) were calculated using the following expression:

$$\mathrm{RSH} = \frac{f_{\mathrm{sample}} - f_{\mathrm{LE}}}{f_{\mathrm{TE}} - f_{\mathrm{LE}}}$$

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

Electrolyte and sample movement was made using a gravity feed hydrodynamic fluid transport system. This system comprised a series of reservoirs with associated LFAA1201718H two-way solenoid actuated valves (The Lee Company, Westbrook, CT, USA) to control the flows. The reservoirs of the leading and terminating electrolytes (B and C, respectively in Fig. 1) were constructed using barrels of disposable 20 ml syringes. The sample reservoir (A) was formed using a barrel of a 2 ml disposable syringe. Reservoirs A and B were raised to produce pressures of 2200 Pa and reservoir C to produce a pressure of 2150 Pa. The arrangement used resulted in flow rates of 4.5 μl s^{-1} for the sample and leading electrolytes and 2.6 μl s^{-1} for the terminating electrolyte when the appropriate valves were open to give a flow to waste.

Control of the power supply, fluid transport system and data acquisition from the detector was carried out using a standard PC with LabVIEW software (version 7.1, National Instruments, Austin, TX, USA). The NIDAQ driver (National Instruments), programmed using LabVIEW, was used to control the hardware interfacing which was made using three National Instruments cards. Full details of the instrumentation have been previously described by the authors [14].

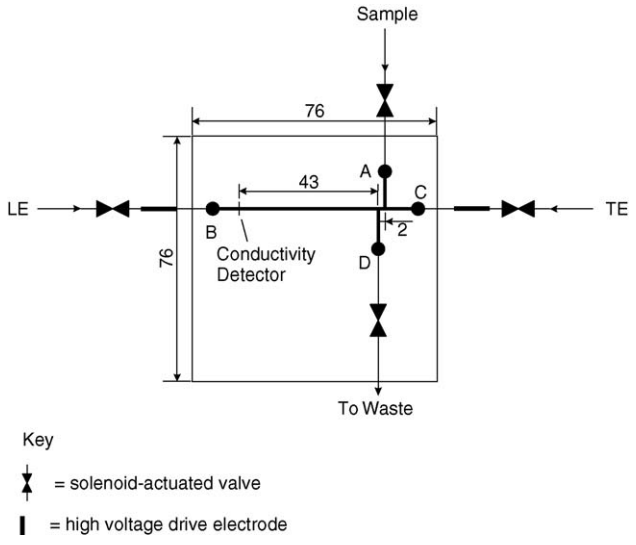

Fig. 1. Schematic diagram of the miniaturised PMMA separation device. All channels are 200 μm wide and 300 μm deep. Letters A, B, C and D refer to the wells into which the inlet/outlet connections to the device are made. Sample enters through A, leading electrolyte through B and terminating electrolyte through C. D exits to waste. Dimensions are shown in mm.

2.2. Separation conditions

In this study, unless otherwise stated, separations were performed using a five-step control program detailed in Table 1. Step 1 flushes the device and fills the separation channel with leading electrolyte. Step 2 loads the terminating electrolyte into the device and step 3 injects the sample in a position between

Table 1
Separation program used to carry out miniaturised isotachophoretic separations

Step	Time (s)	Current (μA)	Valve status			
			A	B	C	D
1	30	0	x	o	x	o
2	1	0	x	x	o	o
3	1	0	o	x	x	o
4	45	30	x	x	x	x
5	1000	7.5	x	x	x	x

Where *x*: closed; o: open.