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Single electrode conductivity detection for electrophoretic separation systems

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Abstract

Various types of detectors have been utilised in electrophoretic separation systems, but conductivity detection provides the obvious choice for detecting ionic substances. This paper describes a new design of conductivity detector utilising a single, on column, sensing electrode. This new design is seen as offering the twin advantages of much higher resolution, than has previously been possible, and simple construction. The detector has been evaluated using separations of sodium and potassium in capillary isotachophoretic and miniaturised planar polymer isotachophoretic separation systems. Good linearity has been found and the limit of detection of sodium calculated to be 0.43 m*M*.

Keywords: Conductivity detection; Detection, Electrophoresis; Isotachophoresis; Instrumentation; Sodium; Potassium; Metal cations

1. Introduction

Various types of detectors have been used with electrophoretic separation systems but by far the most widely used have been UV absorbance detectors and conductivity detectors. For ionic samples the latter type is the obvious choice. These are universal detectors because conductivity is a bulk property based on the individual ionic mobilities. Conductivity detection can be undertaken using either a d.c. method, utilising the driving current of the separation and often referred to as potential gradient detection, or an a.c. method. The latter method uses external driving currents, applied between the sensing electrodes, in the range of 1–10 μA [1], which need to be fully isolated from the high separation field.

Conductivity detectors that have been designed for use in electrophoretic separation systems can be grouped within three types; on-column, end-column or contactless. Of these the on-column type has been most widely used.

On-column detectors are those where the sensing electrodes are placed in direct contact with the samples being detected, which can lead to unwanted electrode reactions and irreversible fouling of the electrode surface. In this type of detector the electrodes are positioned along the separation capillary in either an adjacent or diametric arrangement. The earliest design of conductivity cell for isotachophoresis/electrophoresis, such as that designed by Everaerts and Verheggen [2], used disc electrodes made of platinum, usually, gold or graphite. Later designs used loops of platinum wire welded into the capillary walls [3,4]. Casting methods of construction have also been used for the construction of two

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and three electrode detection cells using Insulcast 510 (Permagil Industries, Plainview, NY, USA) and polyester resin, respectively [5,6]. More recent innovations have seen Huang et al. [7] design a detector which utilises a CO_2 laser to make 40 μm holes in the capillary walls for the electrodes to be positioned in.

End-column detectors were developed in 1991 by Huang et al. [8,9], and have since been commercially produced [10]. End column detectors also have electrodes in direct contact with the species being measured, although in the commercial design the electrodes are easily accessible, but are of a simpler design than on-column ones in that only one electrode is placed in the capillary, the other generally being placed in an electrolyte reservoir.

Contactless detectors were designed, by Gaš et al. [11] and later improved by Vacík et al. [12], to overcome completely the problem of fouling and unwanted electrode reactions. However, the resolution is poorer than with direct contact systems. The detector works by passing an alternating high frequency signal across the separation capillary (cell). Its distribution being determined by the permittivity, permeability and conductivity of the medium occupying the cell.

Described here is a new design for a conductivity detector for use in electrophoretic separation systems which utilises only a single sensing electrode. This design offers simple construction and improved resolution over those currently in use, especially if microelectrodes are used. The design has been evaluated by carrying out isotachophoretic separations.

2. Experimental

Single electrode detectors have been produced for use both in capillary scale and miniaturised separation systems.

The capillary scale instrument utilised was designed and constructed in the laboratory. A schematic of the basic system is shown in Fig. 1. Unless otherwise stated, 0.8 mm I.D., 1.6 mm O.D. fluorinated ethylene–propylene co-polymer (FEP) tubing (Omnifit, Cambridge, UK) was used throughout. The reservoirs consist of 100 ml plastic coated bottles with three-way connectors in their tops (Omnifit). Any unused ports in these connectors are sealed up with epoxy adhesive. Valves V_1, V_2, V_3, V_4 and V_5 are standard four-port 90° valves (Omnifit). The internal volume of one of these valves (V_4), which is 18.3 μl, is used as the sample injection volume. Connector C_1 is a standard four-way connector (Omnifit). To prevent hydrodynamic flow through the system a ceramic frit (Russell, Auchtermuchty, UK) is placed at the entrance to the counter electrode reservoir.

The following procedure was used to operate the capillary scale separation system, loading being achieved using a positive pressure based system.

Firstly the separation capillary is filled with leading electrolyte. To do this V_3, V_4 and V_5 are set to give a flow path from the leading electrolyte reservoir to the counter electrode reservoir. V_1 and V_2 are set to prevent pressure being applied to the terminating electrolyte and sample reservoirs.

Once this has been achieved the terminating electrolyte is loaded into the system. This is achieved by setting V_3 and V_4 to give a flow path from the terminating electrolyte reservoir to the waste reservoir. The setting of V_1 is changed to allow pressure into the terminating electrolyte reservoir.

To load the sample V_4 is set so that the sample will flow from the sample reservoir to the waste reservoir, without going through the separation capillary. V_2 is set so that pressure is applied to the sample reservoir and V_1 is put back into its initial position so that pressure is not applied to the terminating electrolyte reservoir.

Finally to carry out a separation, V_4 is changed to give a flow path from the terminating electrolyte reservoir through the separation capillary to the counter electrode reservoir. During separation V_1 and V_2 are set as they are whilst filling the system with leading electrolyte.

In the capillary scale system the sensing electrode was constructed from a piece of 127 μm diameter platinum wire (Aldrich, Gillingham, UK) placed across the separation capillary. The electrode is positioned 3 cm along an 18 cm separation capillary (this being sufficiently far enough along for the separations to have been completed) which is made of 0.5 mm I.D., 1.6 mm O.D. FEP tubing (Omnifit). A small hole was made in the capillary wall using

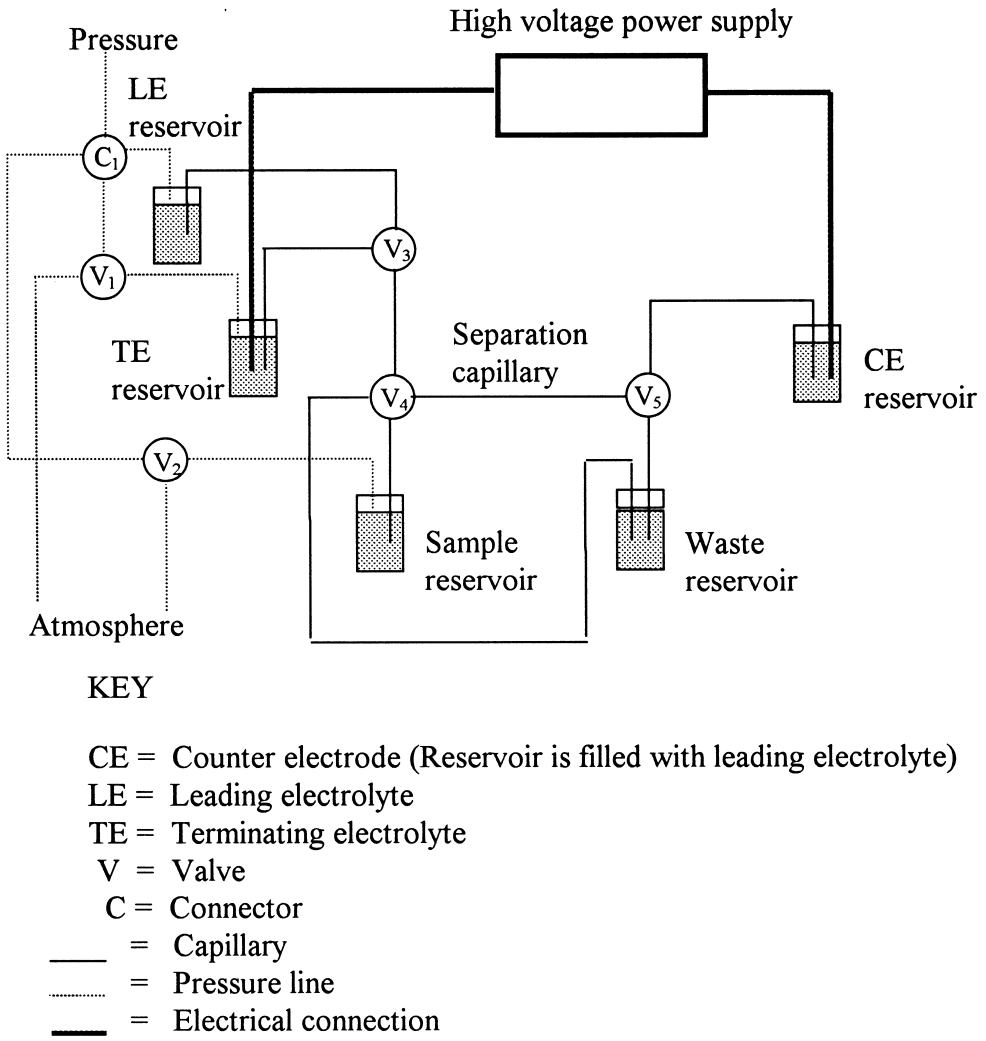

Fig. 1. Schematic of the laboratory built capillary isotachophoretic separation system.

the tip of a sewing needle; the electrode was pushed through this hole and secured in place using epoxy adhesive. To make the system more durable the separation capillary was potted in a white plastic box with Araldite epoxy encapsulant CY1301GB (Ciba–Geigy, supplied by Farnell, Leeds, UK). Another detector has been constructed, but not yet tested which has a 25 μm platinum–iridium (90:10) electrode (Goodfellow, Cambridge, UK) positioned in the same position as the above. This latter detector should allow even higher resolution to be achieved.

The miniaturised separation device, a full description of which can be found in the paper by Baldock et al. [13], is formed of two pieces of silicone rubber elastomer Sylgard 184 (Dow Corning, BDH, Poole, UK). One of these pieces is cast moulded with the pattern of the separation channel using a copper template and the other is a plain piece of polymer. The two pieces of polymer are adhered together to form the flow channel containing chip. A schematic of the device is shown in Fig. 2. The detection electrode is a piece of 25 μm platinum–iridium wire placed between the two pieces of polymer before they are fully bonded. The separation channel is 56

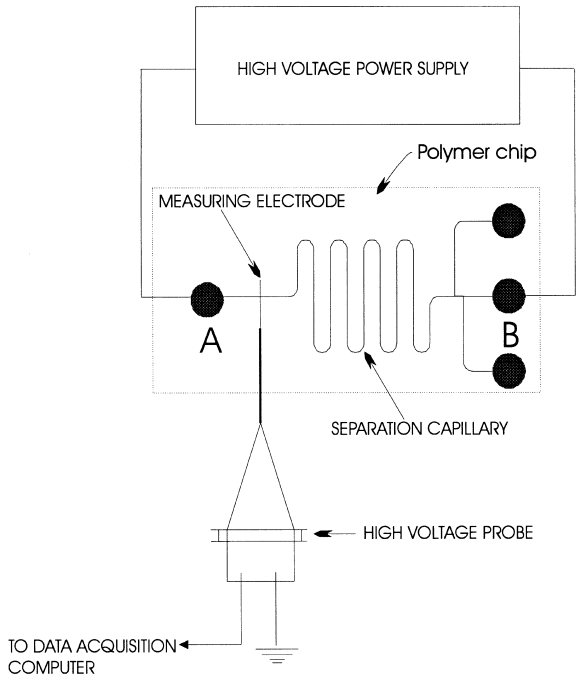

Fig. 2. Schematic of a miniaturised planar polymer isotachophoretic separation system.

μm deep and 324 μm wide at half depth. Total length of the separation channel is 120 mm. The detection electrode is positioned 5 mm along the separation channel from well A.

To use the miniaturised separation system the separation channel and well A are filled with leading electrolyte by syringe. Samples are then placed in well B using an eppendorf pipette. Terminating electrolyte is then added to this well using a syringe.

In both situations a Glassman EH10R10.0-22 (Glassman, Whitehouse Station, NJ, USA) high voltage power supply was used to drive the separations. For safety reasons all of the high voltage work was carried out within an instrument enclosure equipped with a safety interlock. The results were acquired by connecting a HV15HF high voltage probe (Universal Probes, Roydon, UK) to the measuring electrode. The output of this probe was collected using a LAB PC+ data acquisition card (National Instruments, Austin, TX, USA) mounted in a PC and controlled using LabVIEW, version 4.0 (National Instruments).

2.1. Chemicals

All separations were carried out using a leading electrolyte of 10 m*M* HCl with 0.1% (w/w) hydroxyethylcellulose (HEC) added to suppress electroosmotic flow. The terminating electrolyte used was 10 m*M* carnitine hydrochloride. This unbuffered electrolyte system was used for evaluation purposes due to its ease of preparation. However, it should be noted that this electrolyte system does restrict the number of metal cations which can be separated. Sodium samples were made using atomic absorption standard solutions and potassium samples using potassium hydroxide volumetric standard solution. All the chemicals used were of ultrapure grade and were used without further purification. They were all obtained from Aldrich with the exception of the carnitine hydrochloride which was obtained from Sigma (Gillingham, UK).

3. Results and discussion

Fig. 3 shows an isotachopherogram obtained for a 0.44 m*M* sodium sample using a separation current of 75 μA with start and finish potentials of 0.89 and 1.48 kV, respectively. From this figure the main difference between the output of this detector and that of more commonly used conductivity detectors in isotachophoretic separations can be clearly seen. Whereas normally each substance produces a step, with this type of detector, which is simply measuring the potential at a point, each substance produces a zone of a different gradient.

In this instance the change in gradient is very apparent. This clarity of this feature is helped by the fact that there is a large difference between the conductivities, and hence gradients, of the leading and terminating electrolytes. However, for other species more subtle gradient changes can occur. Additionally the gradients for each component will be dependent on the composition of the sample. For example, the terminating electrolyte will produce a certain (maximum) gradient when a blank is run. When a sample is run the terminating electrolyte will produce a less steep gradient because there will be a lower drop in potential across it. This is due to there now being a larger drop in potential across the

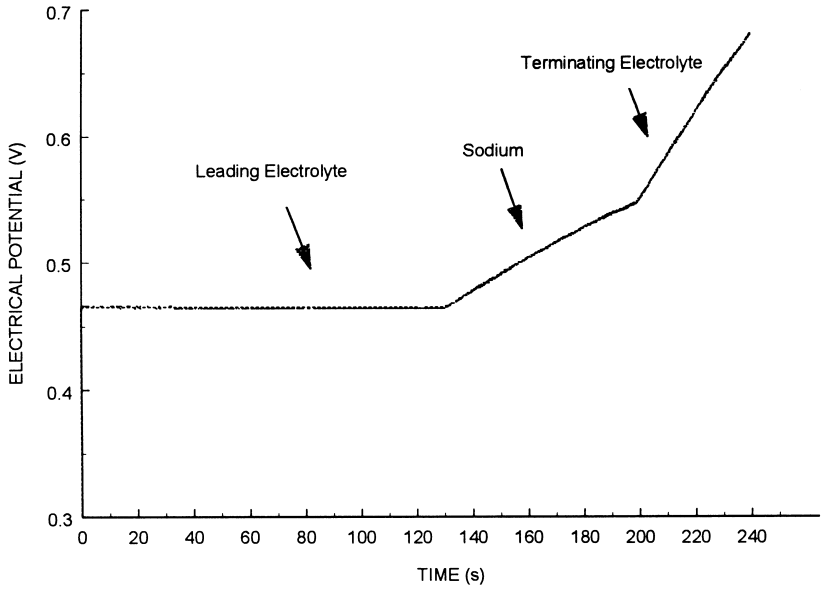

Fig. 3. Isotachopherogram obtained for a 0.44 m*M* sample of sodium using the laboratory built capillary scale separation system.

leading electrolyte and sample than across the leading electrolyte on its own due to the sample having a lower conductivity than the leading electrolyte. A mathematical model is in preparation which will allow the gradient for the various components of a sample to be calculated to aid identification of species.

A standard calibration curve for a series of five sodium samples, of concentrations ranging from 0.44 to 4.35 m*M*, and a blank has been calculated. This calibration curve exhibits good linearity, a correlation coefficient of 0.99 being obtained. Linear regression analysis provides values for the slope and intercept of 57.9 ± 3.6 and 44.3 ± 8.8, respectively, with the errors shown being the 95% confidence limits. The limit of detection of sodium using this detector has been calculated to be 0.43 m*M*. In this work the limit of detection has been taken as the concentration giving a signal equal to the intercept plus three times the standard deviation of the intercept. It should be noted that there is a sizeable amount of sodium in the blank solution, the 95% confidence limit for the concentration being calculated, using standard additions to be 0.77 ± 0.19 m*M*, hence the high value of the intercept in the regression equation.

Fig. 4 shows the isotachopherogram obtained for the separation of a 2.52 m*M* potassium and 2.17 m*M* sodium sample using a miniaturised planar polymer separation device. This separation was carried out using a constant voltage of 1.0 kV with start and finish currents of 84 and 47 μA, respectively. This figure shows steps associated with different components. These are a result of the sample component zones being longer than the length between the measuring electrode and well A. In such a situation the resistance along the separation channel between the measuring electrode and well A will be constant and that between well B and the measuring electrode approximately constant. Thus, the potentials across these lengths of the separation channel will be constant meaning the potential being measured by the detector will be constant and its output a horizontal line.

Calibration of the miniaturised separation device will be carried out once the construction of a new power supply unit has been completed. This unit which will allow the potential control required to allow the electrokinetic injection of samples. This improvement will overcome the problems of introducing reproducible sample volumes into the system. At present although reproducible volumes of sample are placed into well B the amount of sample entering the separation channel can vary. This is due to this

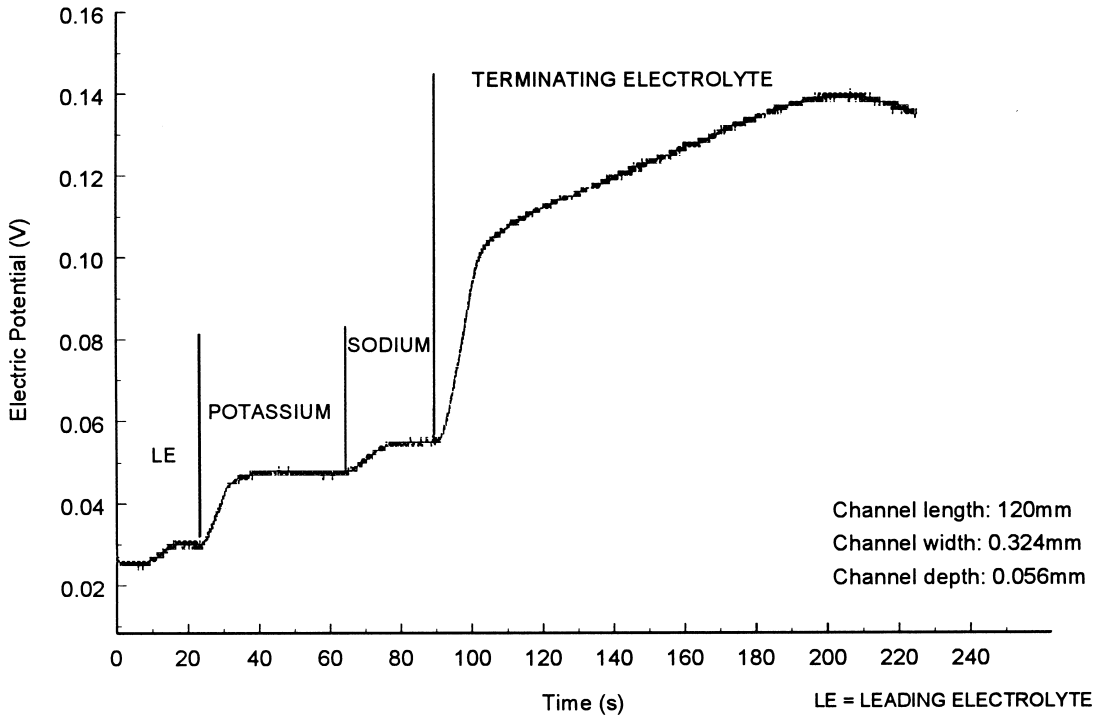

Fig. 4. Isotachopherogram obtained for a separation of a 2.52 m*M* potassium and 2.17 m*M* sodium sample using a miniaturised separation device.

volume being dependent on a number of factors such as how much of the leading electrolyte enters well B when the system is filled and the position of the sample within the well.

4. Conclusions

This preliminary work with the single electrode detector shows that it can be used to separate simple metal samples and has great potential for use with electrophoretic separation systems, especially once the mathematical model is available to aid qualitative determination. To produce the best possible results when the detector is used with isotachophoretic separation systems an electrolyte system with as large as possible difference in the conductivities of the leading and terminating electrolyte systems. The detector seems especially suited for use with the miniaturised planar separation systems where the ease of construction is a particular advantage.

Acknowledgements

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References

[1] F.M. Everaerts, J.L. Becker, Th.P.E.M. Verheggen, Isotachophoresis: Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[2] F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 73 (1972) 193.
[3] S. Stankoviansky, P. Čičmanec, D. Kaniansky, J. Chromatogr. 106 (1975) 131.
[4] D. Kaniansky, M. Koval', S. Stankoviansky, J. Chromatogr. 267 (1983) 67.
[5] B.J. Wanders, T.A.A.M. van de Goor, F.M. Everaerts, J. Chromatogr. A 652 (1993) 291.
[6] F. Foret, M. Deml, V. Kahle, P. Boček, Electrophoresis 7 (1986) 430.
[7] X.H. Huang, T.K.J. Pang, M.J. Gordon, R.N. Zare, Anal. Chem. 59 (1987) 2747.

[8] X.H. Huang, R.N. Zare, S. Sloss, A.G. Ewing, Anal. Chem. 63 (1991) 189.
[9] X. Huang, R.N. Zare, Anal. Chem. 63 (1991) 2193.
[10] C. Haber, W.R. Jones, J. Soglia, M.A. Surve, M. McGlynn, A. Caplan, J.R. Reineck, C. Krstanovic, J. Cap. Electrophoresis 3 (1996) 1.
[11] B. Gaš, M. Demjaněnko, J. Vacík, J. Chromatogr. 192 (1980) 253.
[12] J. Vacík, J. Zuska, I. Muselasová, J. Chromatogr. 320 (1985) 233.
[13] S.J. Baldock, N. Bektaş, P.R. Fielden, N.J. Goddard, L.W. Pickering, J.E. Prest, R.D. Snook, B.J. Treves Brown, D.I. Vaireanu, in: D.J. Harrison, A. van den Berg (Eds.), Micro Total Analysis Systems 1998, Kluwer, Dordrecht, 1998, p. 359.