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SIMPLE CELL FOR CONDUCTIMETRIC DETECTION IN CAPILLARY ISOTACHOPHORESIS

D. KANIANSKY*
Institute of Chemistry, Komenský University, Mlynská Dolina CH-2, 842 15 Bratislava (Czechoslovakia)
M. KOVAL'
Department of Analytical Chemistry, Faculty of Science, Komenský University, 842 15 Bratislava (Czechoslovakia)
and
S. STANKOVIANSKY
Institute of Chemistry, Komenský University, Mlynská Dolina CH-2, 842 15 Bratislava (Czechoslovakia)
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SUMMARY

A procedure for the manufacture of conductivity detection cells for use in capillary isotachophoresis is described in detail. This procedure is a simple method for welding the measuring electrodes of the cell directly into the wall of a capillary tube of an internal diameter as small as 0.1 mm. When the detection cell is mounted into a tube made of a chemically inert plastic material, analyses in both aqueous and non-aqueous solutions can be carried out.

INTRODUCTION

Considerable efforts have been devoted to the development of conductivity and/or potential gradient detectors suitable for the detection of the zones in capillary isotachophoresis (ITP). These universal detectors, having a high resolving power, are mainly used with the measuring electrodes which are in direct contact with the solution to be measured[1–15]. As the conductivities (or resistivities) and/or potential gradients are measured under the applied driving current, the cell used for the measurement and the measuring electronics must fulfill certain requirements: (a) the thickness of the measuring electrodes should not exceed *ca.* 0.01 mm, otherwise the bipolar nature of the electrodes can lead to undesirable electrochemical reactions[2,11]; (b) the surface of the electrodes must be smooth and clean; (c) the material of which the measuring electrodes are made is also important and Pt–Ir alloys give the best results[11]; (d) leak currents through the measuring electrodes must be prevented to a high degree (see ref. 11, p. 188).

A contactless type of conductivity detector suitable for capillary ITP was developed recently[16] to avoid some disturbing phenomena that can occur when contact

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conductivity detection is used. Unfortunately, this promising detection technique seems to be applicable only to dilute solutions with a resolving power slightly poorer than that achieved by a well designed contact conductivity detector[11]. Consequently, the pH range in which the separations can be carried out is more restricted. Lower concentrations in the zones are also undesirable for the simultaneous use of conductivity and UV–photometric detectors as the signal-to-noise ratio of the latter detector can be poor for constituents having smaller molar absorptivities at a given wavelength[11,17]. Similar conclusions can be drawn for the simultaneous use of conductivity and radiometric detectors[18].

In this laboratory, ITP separations in solvents other than water were studied in order to optimize the analyses of some compounds that cannot be separated in water (solubilities, formation of micelles, insufficient differences in mobilities)[19]. In the study cited[19], a conductivity detector was used as a universal means of detection of the zones. Detection cells made of acrylic (giving a very reliable performance in aqueous solutions) could not be used in methanolic and ethanolic solutions[11]. Therefore, a new type of the detection cell suitable for capillary ITP in both aqueous and non-aqueous solutions was developed[13]. This paper describes the construction of this very simple detection cell and shows some typical isotachopherograms obtained with it.

EXPERIMENTAL

An instrument for capillary ITP similar to that described by Everaerts *et al.*[11] was used. In experiments with methanolic solutions parts of the equipment which are in contact with the solutions were made of poly(tetrafluoroethylene) (PTFE). Capillary tubes of 0.15 and 0.30 mm I.D. made of a fluorinated ethylene–propylene copolymer (FEP) were used throughout (for the description of the detection cells, see below).

Leak currents through the measuring transformer[11] were lower than 10 pA when the cell was at a potential of *ca.* 1 kV (measured against the ground potential of the conductimeter). The leak current increased to 50–90 pA when the cell worked at a potential of *ca.*10 kV.

The driving current was supplied by a unit developed by Havaši[20].

RESULTS AND DISCUSSION

Design of the conductivity cell

Capillary tubes made of fluoropolymers (PTFE, FEP) are mostly used in instruments for capillary ITP. We found empirically that a wire of small diameter (*e.g.*, made of a Pt–Ir alloy) when heated to a temperature close to the melting temperature of FEP or PTFE can be welded into the wall of the capillary tube (made of one of these materials) into such a position that part of the surface of the wire becomes part of the inner wall of the capillary tube. When the diameter of the wire is sufficiently small (0.03–0.05 mm), the wall remains liquid-tight in the position where the wire was welded. The cells described in this work utilized these empirical findings.

A procedure for the manufacture of the detection cells (suitable for capillary tubes as narrow as 0.1 mm I.D.) currently used in our laboratory and giving a high

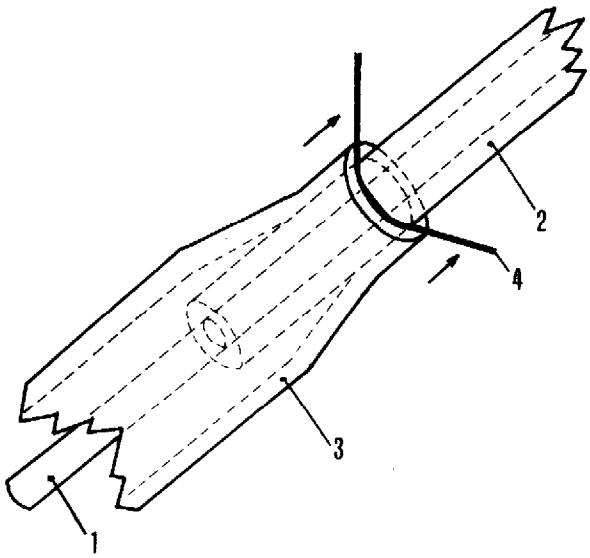

Fig. 1. Procedure for the welding of Pt–Ir measuring electrodes into the wall of the capillary tube used for ITP separation. 1 = Wire (glass capillary) defining the depth of the welding of the electrodes; 2 = FEP capillary tube; 3 = conical glass tube fixing the capillary tube (2) during the welding; 4 = Pt–Ir wire.

yield of cells with good performance is as follows (see also Fig. 1):

(1) A wire (or a glass tube) of diameter *ca.* 0.01 mm smaller than I.D. of the capillary tube is inserted into the hole of the capillary tube.

(2) The capillary tube is then fixed in a glass tube having at the end an inner diameter of the hole identical with the outer diameter of the capillary tube.

(3) An approximately 3.5 cm long wire (diameter 0.04 mm) of which the measuring electrode is made connected to a d.c. power supply. Current flowing through the wire (*ca.* 500 mA) heats it to a temperature sufficient for rapid and tight welding into the wall of the capillary tube. The second electrode is welded in the same way into a position opposite the first electrode. The wire (or glass capillary) inserted into the hole of the capillary tube serves as a heat sink when the wire to be welded is in a suitable position and simultaneously prevents the wire electrodes being welded very deeply.

(4) The tightness of the capillary tube in the position of the measuring electrodes is checked by water pressure after the inner wire (glass tube) has been pulled out.

(5) The measuring electrodes are fixed on the outer surface of the capillary tube with an epoxy glue.

(6) A solution of electrolyte (*e.g.*, 10^{-2} *M* potassium chloride) is allowed to flow through the capillary tube and the rest of the combustion products from the surface of the measuring electrodes are removed by two 1000 V pulses from a d.c. power supply (the polarity of the electrodes is reversed after the first pulse).

(7) When the resistance of the solution (used for the electrochemical cleaning in the preceding step) in the cell has the expected value, the hole in the capillary tube is thoroughly rinsed with an aqueous solution of surfactant and with doubtly distilled water.

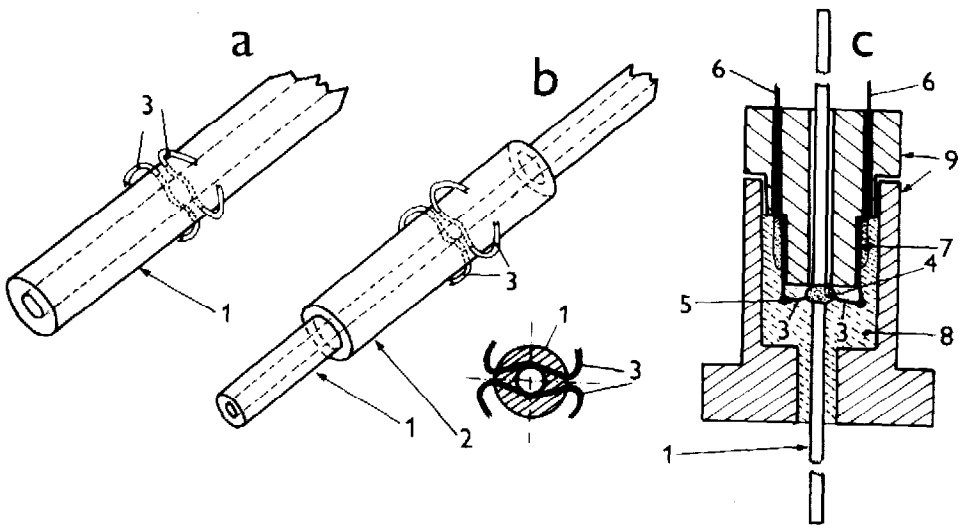

Fig. 2. Capillary tubes with welded conductivity detection cells. (a) Arrangement for capillary tubes having a wall thickness greater than *ca.* 0.15 mm; (b) arrangement for thin wall capillary tubes; (c) encapsulated detection cell. 1 = FEP capillary tube; 2 = piece of FEP capillary tube tightly welded on to the tube (1) in the position where the conductivity detection cell is mounted; 3 = measuring electrodes; 4 = epoxy glue fixing the measuring electrodes; 5 = soldering of the measuring electrodes to the cables (6) for the connection to the conductivity meter; 7 = fixing of the cables (6) by glue to the wall of the housing (9); 8 = paraffin.

(8) The cell is encapsulated in the way shown in Fig. 2c.

The above procedure was found to be very effective for capillary tubes made of FEP (PTFE gave poorer results). A wall thickness of at least 0.15 mm is required to achieve good welding of the wire into the wall. For capillary tubes having thinner walls a modified procedure is used (see Fig. 2b).

Maintenance of the cell

To achieve a reliable performance of the conductivity detection cell in capillary ITP, it is necessary to maintain the surface of the measuring electrodes clean (assuming the conductivity detector was designed properly[11]). To fulfill this requirement recommendations given by Everaerts *et al.*[11] should be followed. For the cell described above they can be summarized by the following practical rules: after a series of experiments during a day the whole equipment (and also the cell) is thoroughly rinsed with a solution of detergent and with doubly distilled water; when the performance of the cell indicates adsorption of solutes or the signal of the conductivity detector gives evidence of the electrode reactions[11], the measuring electrodes are cleaned by two 100 V pulses from a d.c. power supply connected to the electrodes (the polarity is reversed after the first pulse); when for any reason the above cleaning procedures are not effective the cell is replaced by a new one (the typical life of the cell is 1 year or more) as the cost of manufacturing a cell of this type is low.

Examples of the use of the conductivity detection cell

The simplicity of the construction of the cell and the possibility of making

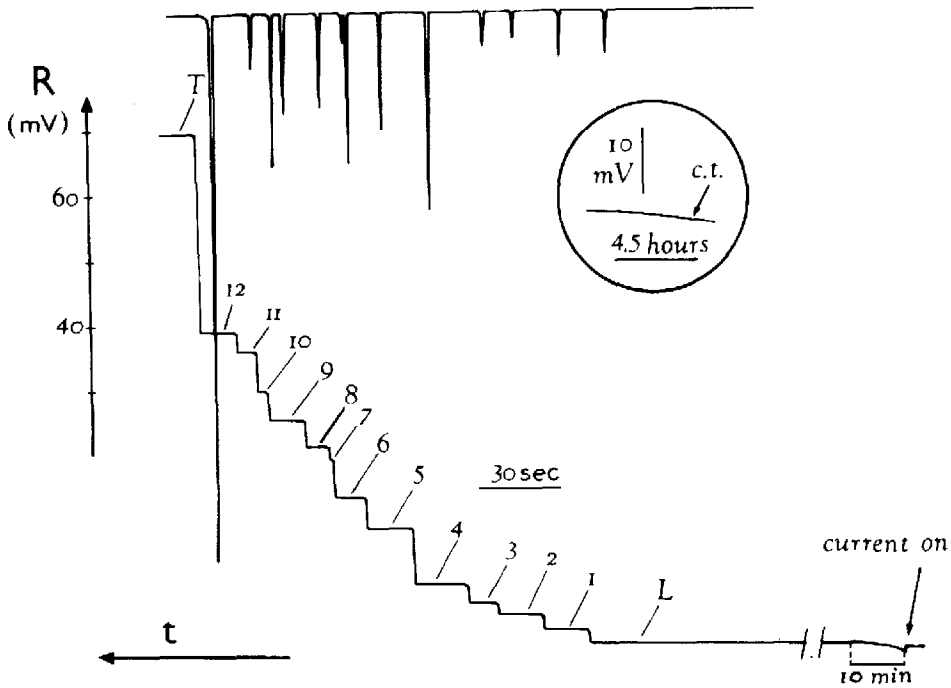

Fig. 3. Isotachopherogram of the separation of a mixture of anions in an FEP capillary tube of 0.15 mm I.D. The detection cell shown in Fig. 2b was used for the detection of the zones. Leading electrolyte, 10 m*M* hydrochloric acid titrated to pH 6.0 with histidine; 0.2% hydroxyethylcellulose was used as additive. Terminating electrolyte: 5 m*M* morpholinoethanesulphonic acid (MES). Driving current, 10 μA. R = signal of the conductivity meter (increasing resistance); t = increasing time. L = Chloride; 1 = sulphate; 2 = chlorate; 3 = chromate; 4 = malonate; 5 = adipate; 6 = benzoate; 7 = impurity; 8 = acetate; 9 = β-bromopropionate; 10 = naphthalene-2-sulphonate; 11 = glutamate; 12 = enantate; T = MES. The signal from the conductivity detector for the first 10 min of the analysis was recorded at a chart speed of 0.2 cm/min; the zones were registered at a chart speed of 6 cm/min. An evaluation of the drift of the signal from the conductivity cell filled with the leading electrolyte is given in the circle above the isotachopherogram (c.t. = trace of the signal from the conductivity meter; no driving current was applied during this experiment).

conductivity detectors suitable for capillary tubes as narrow as 0.1 mm I.D. are very advantageous. The isotachopherogram shown in Fig. 3 was chosen to illustrate the separation of a model mixture of anions in a capillary tube of 0.15 mm I.D. provided with a detection cell of the described construction. The reasons for using tubes of such a small inner diameter are clear from the investigation carried out by Verheggen *et al.*[21]. Excellent sharpness of the registered zones and high stability of the conductivity signal during the detection are clear from this isotachopherogram.

A reliable performance of the cell was also achieved in methanolic solutions in long-term testing[19]. An isotachopherogram for the separation of anionic constituents in methanol is given in Fig. 4 to illustrate the use of the detection cell in non-aqueous solutions. In this instance an FEP capillary tube of 0.30 mm I.D. was used.

In analytical ITP, additives suppressing convective disturbances (electroosmosis, thermal convection) are used in the leading electrolytes[11,12]. A search for additives suitable for methanolic solutions carried out in our laboratory did not give

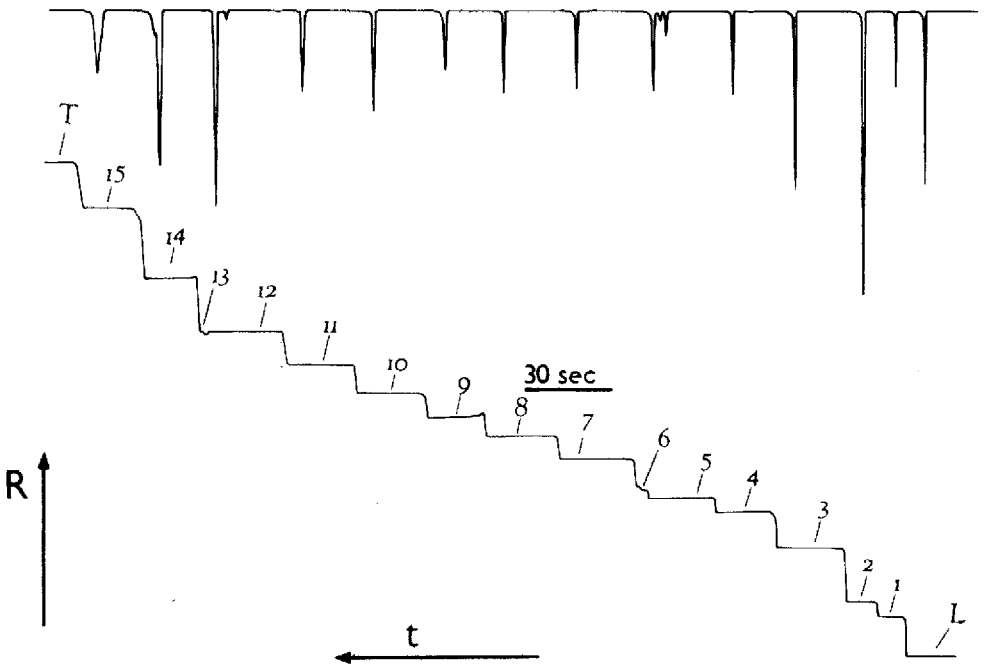

Fig. 4. Isotachopherogram of the separation of a mixture of anions in methanol. The cell shown in Fig. 2a was used for detection. Leading electrolyte: 1 m*M* perchloric acid in 98% methanol was titrated with N-ethylmorpholine to pH* 6.7 (see ref. 11). Terminating electrolyte: 1 m*M* caproic acid in 99% methanol. The sample was introduced in 99% methanol. The driving current was 10 μA during the separation and 6 μA during the detection. L = Perchlorate; 1 = bromide; 2 = chloride; 3 = monochloroacetate; 4 = fluoride; 5 = *m*-nitrobenzoate; 6 = impurities; 7 = mesaconate; 8 = hippurate; 9 = gluconate; 10 = *p*-aminohippurate; 11 = benzoate; 12 = α-naphthylacetate; 13 = impurities; 14 = levulate; 15 = propionate; T = capronate.

successful results[19]. Therefore, a substitute solution giving satisfactory results was used when separations in methanol and ethanol solutions were performed. Before a series of experiments the capillary tube was rinsed with a 0.3% aqueous solution of methylhydroxyethylcellulose 30,000 (Serva, Heidelberg, G.F.R.). Water–methanol was applied in the following step and finally the capillary tube was filled with the methanol (or ethanol) leading electrolyte. The sharpness of the zones was unaffected even in a long series of experiments.

The simplicity of the design of the cell is also advantageous in the investigation of the dynamics of ITP separation[22]. Another field of application is a multi-detection system for the evaluation of the analysis in a way similar to that developed by Schumacher *et al.*[23].

CONCLUSIONS

A simple conductivity detection cell suitable for capillary ITP in both aqueous and non-aqueous solutions with good analytical performance has been developed. In general, its advantages can be summarized as follows: simple construction and low cost; applicability of the design to capillary tubes as narrow as 0.1 mm I.D.; Uni-

versality from the point of view of the solvent used; the procedure described gives the possibility of building several detectors along the capillary tube to monitor the separation or to perform the analytical evaluation from several channels; mechanically undisturbed surface of the measuring electrodes is achieved; connections close to the detection point are eliminated.

Disadvantages of the design of the detection cell can also be simply summarized: a small contact area of the electrodes with the solution to be measured (as a consequence, higher sensitivity to the adsorption of solutes can be expected); higher dispersion of the cell constant compared with some other cell designs can be expected[1–15]; hardly any possibility of mechanical cleaning being applied as recommended for some disturbances[11].

In general, this type of the conductivity detection cell can be very advantageous for cheap ITP equipment having the capability of high-resolution universal detection. Obviously, when an excellent long-term analytical performance is required, all recommendations concerning conductivity detection in ITP must be followed[11].

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