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# POST-COLUMN AMPEROMETRIC DETECTION IN CAPILLARY ISOTACHOPHORESIS

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## SUMMARY

The basic requirements for the coupling of electrochemical (amperometric) detection to isotachophoretic separation were investigated. To minimize disturbances due to the driving current, a post-column detector configuration was employed. The separated constituents were hydrodynamically transported from the separation compartment, provided with a "T"-piece, into the detection cell. When leaks of the driving current through the detector were comparable to its background current, satisfactory experimental conditions for the detection of oxidizable anionic constituents were achieved. The transport of the separated components to the detection cell led to dispersions of the zone boundaries. Means of improving the performance characteristics of the detector are proposed.

## INTRODUCTION

Capillary isotachophoresis (ITP) is able to separate trace amounts of ionic constituents present in complex ionic mixtures. However, less favourable detection limits, decreased certainty of identification and/or quantitative analysis biased by a systematic error, *e.g.*, due to co-migrating constituents, are problems which can be encountered when current universal (conductivity, potential gradient) and selective (fixed-wavelength photometric) detectors are employed for the evaluation of such separations. Although the recently developed dual-wavelength photometric[1,2], fluorometric[2,3] and radiometric[4,5] detectors are examples of the efforts made to increase the selectivity and/or sensitivity of the detection in ITP, further developments exploiting other physico-chemical principles seem necessary.

Considering the physico-chemical properties of potential analytes (see, *e.g.*, refs. 6–8), the availability of an electrochemical detector suitable for ITP would appear desirable. However, the development of any detector belonging to this category, *e.g.*, amperometric, must overcome some inherent incompatibilities of the electrophoretic separation process with the electrochemical detection (ED). The main prob-

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lems can be expected from the disturbances associated with the driving current. Of these, the bipolar behaviour of electrically conducting materials inserted into the driving electric field (see ref. 6, p. 176) probably represents the most serious hindrance to design of an on-column configuration for an electrochemical detector. Elimination of the disturbances due to leaks of the driving current through the detection electrodes is an additional but less serious problem since it can be solved in a similar way to that used for the conductivity or potential gradient detectors (ref. 6, p. 150). Further, ITP separations are carried out in a quiescent solution, *i.e.*, under hydrodynamic conditions less favourable from the point of view of the sensitivity of ED (see, *e.g.*, refs. 9 and 10).

These facts probably provide an explanation of why no attempts to couple ED to ITP or to capillary zone electrophoresis have been reported. The aim of this work was to investigate possibilities of solving the above problems by post-column coupling of ED to the ITP separation. Such detector seems the most practical one in many respects:

(i) it provides a way to perform the detection without (or with only minimum) disturbance due to the driving current as the detection takes place out of its path in the separation unit;

(ii) the detector is operating at a low potential (relative to the electric ground, *i.e.* potential of the counter electrode, of the ITP instrument) and, therefore, its galvanic insulation can be achieved by simple means;

(iii) transport of the separated constituents into the detector by a stream of the solution improves the hydrodynamic conditions for the detection.

A low dispersion of the zone boundaries is a typical feature of ITP. Therefore, an increase in the dispersion due to the hydrodynamic transport is unavoidable for any post-column detector in ITP. Experiments with a counter flow of the leading electrolyte carried out by Everaerts *et al.* (ref. 6, p. 379) are very instructive in this respect. Thus, the resolving power of post-column ED should be *a priori* lower in comparison, *e.g.*, with the conductivity detector. However, this disadvantage can be outweighed by its inherent selectivity and/or sensitivity.

The results presented here summarize our introductory investigation of a post-column amperometric detector for ITP.

## EXPERIMENTAL

### *Instrumentation*

A block diagram of the ITP separation unit used is shown in Fig. 1. It was assembled from modules similar to those employed by Everaerts *et al.*[6] and from ones developed in this laboratory[5]. All modules were manufactured from acrylic.

A laboratory-made syringe pump, connected to the refilling block (P, in Fig. 1), delivered the solution necessary for transport of the separated constituents into the electrochemical detector. The pumping rate was 3.4 μl/min, unless stated otherwise.

A conductivity detector[5,11] served for the visualization of the separations in a 0.3 mm I.D. capillary tube made from a copolymer of fluorinated ethylene and propylene (FEP) and for the evaluation of the dispersion due to the post-column ED arrangement.

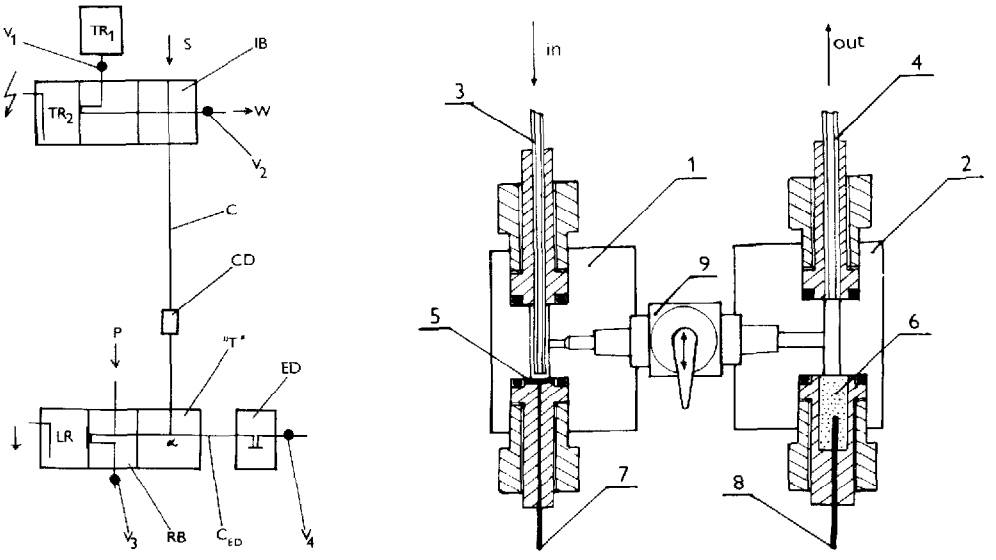



Fig. 1. Block diagram of the separation unit provided with a post-column amperometric detector. C = Capillary tube; IB = injection block; $TR_1$ = terminating electrolyte reservoir; $TR_2$ = terminating electrolyte compartment with the driving electrode (the compartment is separated by a cellophane membrane from the separation compartment); "T" = elution "T"-piece; α = bifurcation point; RB = refilling block; LR = counter-electrode compartment; CD = conductivity detection cell; $C_{ED}$ = capillary tube connecting the elution "T"-piece with the amperometric detection cell (ED); S = position for the injection of the sample solution; W = waste; $V_1$–$V_4$ = valves; P = position for the connection of the syringe pump.

Fig. 2. Amperometric detection cell. 1,2 = Acrylic body of the detector; 3 = inlet capillary tube ($C_{ED}$ in Fig. 1); 4 = outlet tube; 5 = working electrode; 6 = reference electrode; 7,8 = connecting cables to the measuring unit; 9 = 1MM1 Hamilton microvalve.

The driving current was delivered by a high-voltage power supply suitable for ITP (VVZ PJT, Spišská Nová Ves, Czechoslovakia).

A PA-3 polarographic analyser (Laboratorní přístroje, Prague, Czechoslovakia) provided with a detection cell (Fig. 2) served for ED in a two-electrode amperometric mode. The isotachopherograms from both the conductivity and amperometric detectors were registered by a TZ 4200 line recorder (Laboratorní přístroje).

*Chemicals*

Commercially available chemicals (after purification by conventional methods[12]) were used for preparation of the solutions of the leading and terminating electrolytes. Triply distilled water was used throughout. Hydroxyethylcellulose (HEC) obtained from Hercules (Natrosol 250 HR, Rijswijk, The Netherlands) was added (to suppress electroosmosis) to the leading electrolyte solutions employed for refilling the separation compartment. The solutions used for the elution of the separated constituents from the "T"-piece (Fig. 1) did not contain this additive.

Electrochemically oxidizable anionic constituents were investigated. As some of them are easily oxidized by oxygen, their stock solutions (2–10 m*M*) in water were

thoroughly deoxygenated by nitrogen. The solutions used for the analyses were prepared daily by appropriate dilution of the stock solutions in deoxygenated water and the final solutions were kept under a nitrogen atmosphere.

To prevent losses of analytes caused by their interactions with metallic parts of the syringe used for the sample injections, ethylenediaminetetraacetic acid (EDTA) was added to the sample solutions[13].

## RESULTS AND DISCUSSION

### *Separation unit*

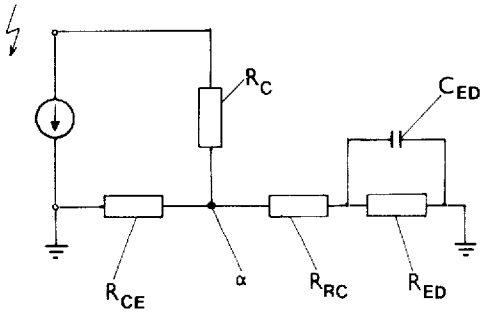
For comparison with current single-column separation units (see, *e.g.*, ref. 6), we used one which was hydrodynamically closed by cellophane membranes from the side of the counter-electrode compartment as well as from the side of the terminating electrolyte compartment (LR and $TR_2$ in Fig. 1, respectively). Similarly, as in continuous preparative ITP[14], undesirable movement of the solution in the separation compartment was prevented.

A "T"-connecting piece attached to the column downstream of the conductivity detection cell served to connect the separation compartment with the refilling block (counter-electrode compartment) and the electrochemical detector. It had a 0.30 mm I.D. channel for the connection to the column, and a 0.45 mm I.D. channel for the connection to the counter-electrode. A 0.25 mm I.D. FEP tube (80–100 mm) served for the transport of the separated components from the bifurcation point ($\alpha$ in Fig. 1) to the amperometric detector.

Experiments carried out with anionic dyes spaced by colourless constituents revealed that the dispersion of the train of separated components in the "T"-piece was negligible relative to that due to the connecting tube. A further decrease in the I.D. and/or the length of the tube is an obvious way to minimize the overall dispersion of the hydrodynamically transported zones[15,16]. This aspect, important for optimization of post-column ED in ITP, was not investigated in detail in this work as measuring electronics designed for the use in electrophoretic separation methods (leak currents through the electrodes of the detector well below its background current) would have been necessary.

The amperometric detection cell shown in Fig. 2 was used. It consisted of two compartments interconnected by a 1MM1 valve (Hamilton, Bonaduz, Switzerland). Either platinum or glassy carbon disks, 3 mm in diameter, served as the working electrodes and the cell was employed in a two-electrode mode for the detection of oxidizable anions. A low dead volume of the detection part was achieved by placing the end of the connecting tube (O.D. of the tube in the cell was 2.5 mm) 0.03–0.04 mm from the surface of the working electrode. In this way a reasonable conversion of the electrochemically active constituents was achieved. Judging from the evaluation of a geometrically similar arrangement of the sensing part of an amperometric detector suitable for liquid chromatography in open-tubular columns[17], our cell behaves as a thin-layer type rather than a wall-jet arrangement with which it resembles.

Some of the problems associated with the use of post-column ED in ITP can best be explained by using a simplified electrical equivalent of the separation unit provided with such a detector (Fig. 3). From this electrical equivalent it is apparent that the total electrical resistance of the branch of the "T"-piece in the direction of



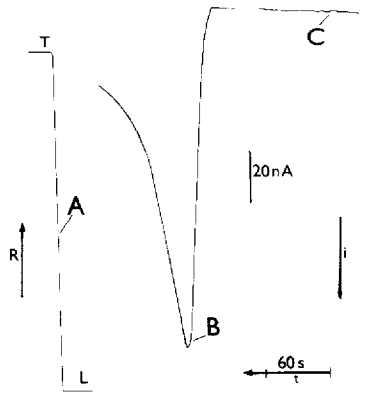




Fig. 3. Electrical equivalent of the separation unit provided with a post-column electrochemical detector. $R_C$ = Separating capillary tube; $R_{CE}$ = resistance of the channel to the counter-electrode; $R_{RC}$ = resistance of the channel to the detector; $C_{ED}$, $R_{ED}$ = electrochemical detector; α = bifurcation point.

Fig. 4. Responses of the amperometric detector upon entry of the terminating anion into the bifurcation point: (C) with a continuous elution of the material from the bifurcation point by a solution of the leading electrolyte at a rate of 3.4 μl/min; (B) without pumping; (A) isotachopherogram from the conductivity detector. System 1 (Table I) was used. The driving current was 45 μA. T = Terminating electrolyte; L = leading electrolyte; R = response; i = current; t = time.

the counter-electrode should be minimized, while the resistance to the ground potential through the detector should be maximized. Thus, simultaneously, the bifurcation point (α in Figs. 1 and 3) will be at a low potential and the driving current will be forced to flow in the direction of the counter-electrode. Therefore, our design of the "T"-piece as well as the dimensions of the connecting channels in it fulfil this requirement (see above). In addition, the I.D. of the channel in the refilling block (3.0 mm) also favoured the desired branching of the driving current. The resistance in the direction of the detector was maximized by using a connecting tube of the above dimensions ($R_{RC}$ in Fig. 3) and by disconnecting the mechanical earthing of the measuring electronics of the detector and recorder. Insulating the resistance of the power transformers of these devices ($R_{ED}$, Fig. 3) contributed to a final decrease in the leak currents to 0.05–0.1% of the driving currents applied to the separation (30–50 μA).

The total background current of the detector immediately after the driving current had been applied was higher than *ca.* 300 nA. Within 3–4 min it decayed to 30–35 nA [$E_W$ (potential working electrode) = +400 mV *vs.* Ag/AgCl (1 *M* potassium chloride) reference electrode]. The latter value of the background current was approximately twice that which was inherent to the detector before the driving current was applied or when it was disconnected. This difference in the background currents must be ascribed to imperfections of the galvanic insulation of the detector employed.

*Evaluation of the detector in conjunction with ITP*

The disturbance caused by the leak currents through the working electrode as observed in analytical experiments is illustrated by the isotachopherograms in Fig. 4. These experiments were also evaluated by the amperometric detector. In one (C,

TABLE I

OPERATING PARAMETERS

BALA = β-Alanine; HIS = histidine; HAc = acetic acid; HEC = hydroxyethylcellulose (the elution buffer did not contain this additive); MES = 2-(N-morpholino)ethanesulphonate.

| *Parameter* | *System No.* | |
|---|---|---|
| | *1* | *2* |
| Leading constituent | $Cl^-$ | $SO_4^{2-}$ |
| Concentration (m*M*) | 10 | 5 |
| Counter constituent | HIS | BALA |
| pH of the leading electrolyte | 6.0 | 3.5 |
| Additive to the leading electrolyte | HEC | HEC |
| Concentration (%, w/v) | 0.1 | 0.1 |
| Terminating constituent | MES | HAc |
| Concentration (m*M*) | 5 | 5 |

Fig. 4) the solution of the leading electrolyte was continuously pumped through the amperometric cell and as neither the sample was applied nor the amount of oxidizable impurities present within the leading-terminating mobility interval (*i.e.* the values of the effective mobilities higher than that of the terminating ion and, at the same time, lower than that of the loading ion) was higher than the detection limit, no detector response was registered. On the other hand, in an identical experiment with the pump switched off (B, Fig. 4), the detector response indicated the presence of oxidizable constituents. This analytically false signal was associated with an increase in potential at the bifurcation point upon entry of the zone of the terminating anions. This was primarily due to the imperfections in the galvanic insulation of the detector and we eliminated this disturbance in experiments described below by eluting the separated components at such a rate (see Experimental) as to prevent the false response.

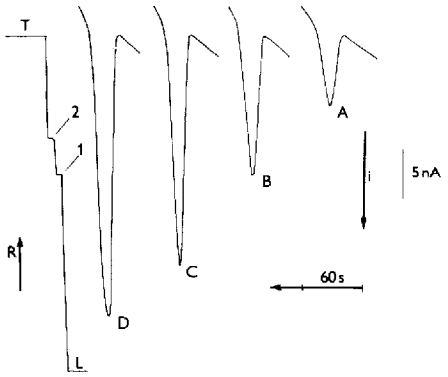



Fig. 5. Responses of the amperometric detector to various amounts of gallic acid: (A) 25 pmol; (B) 50 pmol; (C) 75 pmol; (D) 100 pmol. Glutamate (1) and 2,4,6-trimethoxybenzoate (2) served as spacers (see the isotachopherogram from the conductivity detector). The separations were performed with system 1 (Table I) and a driving current of 45 μA. A platinum working electrode (+400 mV *vs* Ag/AgCl reference electrode) was used.

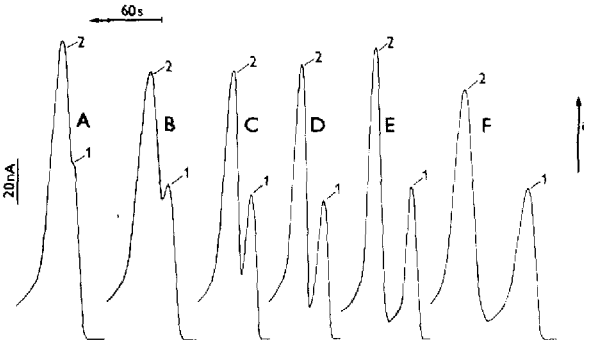



Fig. 6. Resolution of two separated oxidizable anions by a post-column amperometric detector. 1 = 2,6-Dihydroxybenzoate (400 pmol); 2 = *p*-aminosalicylate (200 pmol). Different amounts of lactate were used for spacing the oxidizable separands. The zonelengths of lactate in the separation compartment were: (A) 6 mm; (B) 7 mm; (C) 8.5 mm; (D) 11.5 mm; (E) 17 mm; (F) 34 mm. The separations were carried out with system 2 (Table I) and a driving current of 35 μA. A platinum working electrode (+600 mV *vs.* Ag/AgCl reference electrode) was used.

When the precautions concerning the sample handling were taken (for details, see Experimental) and the disturbances due to the driving current reduced, reproducible results were achieved. For example, in a series of isotachopherograms of *p*-aminosalicylate both the peak heights and peak areas were reproduced within *ca.* 4% relative standard deviations.

Isotachopherograms obtained for 25–100 pmol of gallic acid (Fig. 5) show that the peak heights as well as the peak areas were proportional to the injected amounts. However, in analyses designed to evaluate the day-to-day validity of the calibration lines we sometimes obtained considerable deviations in slopes. This long-term irreproducibility was caused by a fouling of the working electrode. It is typical of ED with solid electrodes and we eliminated it in several ways:

(1) by repeating the switching on and off of the driving current; (2) for the platinum electrode a cleaning effect could be achieved when the cell was thoroughly washed with an efficient detergent (Decon 90; Decon Labs., Brighton, U.K.) and with twice distilled water; (3) when the first two procedures were not effective, the original sensitivity was restored by mechanical polishing of the surface of the working electrode.

In spite of the fact that we did not observe electrode fouling caused by the constituents of the leading electrolyte, *e.g.*, histidine, the selection of the latter for ED must take account of this possibility. This is especially true for separations of anions where amines are used as counter ions.

The dispersion of the zone boundaries due to the transport of the separated components from the separation compartment into the detector was evaluated in experiments in which the same amounts of two oxidizable anions were analyzed with various amounts of an electrochemically inactive spacer. The minimum zonelength of the spacer, as registered by the conductivity detector, sufficient for a baseline resolution of the oxidizable components was taken as a measure of the dispersive effects of the post-column detection system. Isotachopherograms from the amperometric detector shown in Fig. 6 reveal that the post-column detector used in this work required a 23-s zone of lactate to achieve a baseline resolution of *p*-aminosalicylate and 2,6-dihydroxybenzoate. It is obvious that its resolving power was slightly lower than that of the thermometric detector (for comparative data see ref. 6, p. 122).

## CONCLUSIONS

This work clearly shows that post-column coupling of amperometric detection to ITP is a suitable way to overcome inherent incompatibilities of the electrophoretic separation process with ED methods.

Further improvements in the performance of the detector, mainly its resolving power and sensitivity, are strictly associated with a further minimization of the post-column dispersion of the zone boundaries. This would necessitate a design of the measuring electronics of the detector which suppresses the leak currents through the electrodes to a low pA level.

Also the problems concerning the electrode fouling require special attention. Recent progress in the field of flow-through electrochemical sensors, however, provides some alternatives such as composite electrodes[8,19], fibre electrodes[20,21] and coating of the electrodes by polymeric films[22,23].

The applicability of the described approach to ED is not restricted only to the oxidative or reductive amperometric modes, but it provides a way to detect some constituents by exploiting their complex-forming properties by using copper[24] or silver[25] working electrodes or through the use of potentiomeric sensors (see, *e.g.*, ref. 26).

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