Column-coupling in electrophoresis

COLUMN-COUPLING IN ELECTROPHORESIS

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ABSTRACT

A system is discussed that makes use of two teflon tubes with different internal diameters. In the pre-separation tube, which has the larger internal diameter, a high pre-separation current is permitted. At a well defined distance from a "tell-tale" detector (conductivity type), a special construction permits the branching-off of the final separation tube, which has the smaller internal diameter. The zones of interest can easily be separated from the sample zones, migrating all at equal speed in the pre-separation tube, via the "tell-tale" detector and are separated and detected in the separation tube by both an u.v.-absorption and a conductivity detector.
Examples are shown of the determination of metabolites (e.g. urate and valproate) at serum level. The serum has been ultra-filtered. Moreover an example is given of the combination of zone-electrophoresis and isotachophoresis.

INTRODUCTION

As in other analytical separation techniques, also in isotachophoresis various methods (Everaerts, 1979) can be applied to obtain reliable qualitative and quantitative information about sample constituents present in low concentrations, especially if the concentration of the other compounds is an order of magnitude higher. In almost all cases it is a rule, that if more specific information of trace compounds in complex mixtures is asked for, the time needed to collect this information increases drastically with the decreasing concentration of these compounds in such complex mixtures.
The method presented, i.e. the column-coupling system (Everaerts, 1979), is a rather unique solution for the problems, as discussed above, without a necessary increase in analysis time. We have to keep in mind that in isotachophoretic analyses the sample zones, migrating consecutively in order of their effective mobilities with equal speed, are not diluted as a function of time. The self-sharpening effect (Everaerts, 1976) keeps the zone-boundary sharp. The concentration of the compound in its zone is adjusted to the concentration of the leading electrolyte and not by other operational conditions (Table 1).

INSTRUMENTATION

Basically the equipment consists of two teflon tubes with inside diameters of 0.8 mm (pre-separation tube) and 0.2 mm (separation tube). In the pre-separation tube a high electric current is permitted. At a well defined distance from a "tell-tale" detector (conductivity type), mounted in the pre-separation compartment, a special construction (bifurcation) allows the separation tube to branch off the pre-separation tube. The zones of interest can be easily selected via the "tell-tale" detector and separated from the "sample-train", migrating isotachophoretically in the pre-separation tube. In fact the very efficient separation characteristic of isotachophoresis is applied for both sample pre-separation and final separation. A high sample load is permitted, because the analysis time is increased negligibly; high ratios of concentrations between sample constituents are permitted; moreover different operational systems can be applied in one analysis or even different electrophoretic principles (e.g. isotachophoresis and zone-electrophoresis) can be applied (Everaerts, 1976).

MATERIALS

The chemicals used were all of the highest purity commercially available. The hydroxyethyl cellulose (Polyscience, Inc., Warrington, PA 18976, Cat 5568) was purified by shaking the solution (½%) with a mixed-bed ion-exchanger (Merck V, Darmstadt, GFR).

TABLE 1. OPERATIONAL SYSTEMS

	System no			
	1	2	3	4
pH of (leading) electrolyte	5	5	6	6
Anionic constituent	Cl	Cl	Cl	HEPES
concentration (M)	0.01	0.005	0.01	0.1
Cationic constituent	EACA	EACA	HIST	HIST
Terminating anion	MES	MES	MES	---
Concentration (ca)	0.005	0.005	0.005	---
Additive to electrolyte (HEC) %	0.2	0.2	0.2	0.2
Temperature	Ambient	Ambient	Ambient	Ambient
Electric driving current:				
d_i = 0.8 mm: 0.07 A cm^{-2}				
d_i = 0.2 mm: 0.08 A cm^{-2}				

MES = 2-(N-morpholino)ethanesulfonic acid; HEPES = N-2-Hydroxyethylpiperazine-N'-2-etanesulfonic acid; HIST = histidine; EACA = epsilon-aminocaproic acid; HEC = hydroxyethyl cellulose.

EXPERIMENTAL

Figure 1 shows the analysis of urate and valproate at serum level. Injected was 0.8 µl ultra-filtered, undiluted human serum under operational conditions listed in Table 1 (no 1). In Fig. 1.A the separation in a conventional equipment (Everaerts, 1976) is shown. The total analysis time was 12 minutes. During 10 minutes the electric current has been doubled to speed up the analysis ("chloride-trans-

port"). In Fig. 1.B the separation of 5 µl of this serum is shown in the equipment with coupled columns. In the pre-separation tube the electrolyte listed in Table 1 (no 1) has been applied, while in the separation tube the electrolyte listed in Table 1 (no 2) has been chosen. The indicated zones (Fig. 1.A: u.v.-trace) have been trapped and are finally separated and detected. The analysis time again was ca 12 minutes.

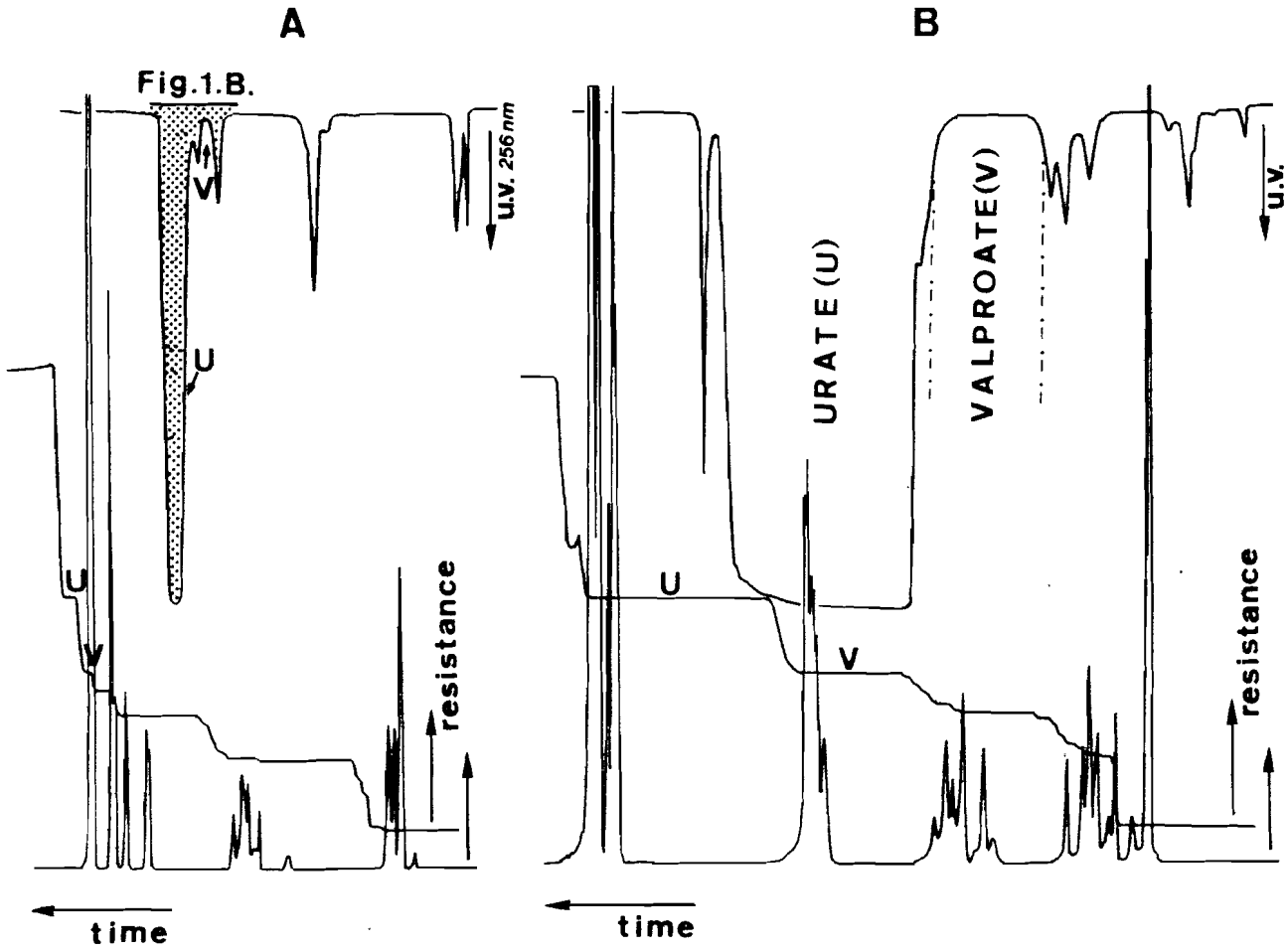

Fig. 1. Isotachophoretic analysis of urate and valproate in human serum.
A: 0.8 µl of serum has been analysed in a conventional equipment. Analysis time was 12 minutes.
B: 5 µl of serum has been analysed with the device with coupled columns. Analysis time was 12 minutes.
The indicated zones (Fig. 1.A: u.v.-trace) have been trapped.
The operational conditions are listed in Table 1 (no 1 and no 2).

Easily this analysis time can be decreased (ca 6 minutes), by choosing the lengths of pre-separation and separation tubes correctly. The amplification of both u.v.-adsorption and conductivity detector have been changed compared to the results shown in the Fig. 1.A. From various metabolites e.g. urate and valproate, easily protein-bound and free concentrations could be determined (Oerlemans, 1979). In the Fig. 2 a zone-electrophoretic separation of various dyes is

shown. The pre-separation tube was filled with the electrolyte listed in the Table 1 (no 3). The separation tube was filled with the electrolyte listed in the Table 1 (no 4). Calibration curves shown that these dyes reproducibly could be analysed at picomol level and even lower. (Mikkers, 1979).

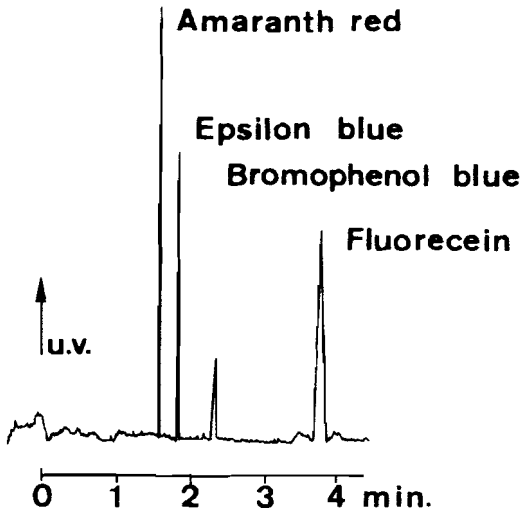

Fig. 2. Zone-electrophoretic separation of amaranth red, epsilon blue, bromo-phenol blue and fluorocein.

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