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CAPILLARY ISOTACHOPHORESIS WITH ELECTROLYTE COUNTER-FLOW TEMPERATURE AND CONCENTRATION PROFILES OF THE ZONE BOUNDARY

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SUMMARY

A conductivity sensor was constructed which can be incorporated into instruments for capillary isotachopheresis. The appropriate electronic equipment was developed using the perspective inland component base. A new method of proportional counter-flow regulation was designed which can be applied to any apparatus, irrespective of the detection method. It was shown that the temperature profile of the zone boundary can be sharpened by decreasing the boundary velocity by means of an appropriate counter-flow. The temperature and conductivity profiles of the zone boundary were characterized by means of second central moments. By this comparison it was shown unambiguously that conductivity detection is more advantageous. Comparison of thermometric and conductivity detections showed that the latter is more sensitive by more than two orders.

INTRODUCTION

Applications of capillary isotachopheresis as an analytical method in the diverse fields of chemistry, biology and medicine require appropriate instruments with a reliable and sensitive detection of individual components in the capillary tube. In nearly all the papers which have appeared since this method was developed¹⁻¹⁸, thermometric detection was generally involved. However, at the present time more and more papers are appearing that deal with other methods of detection. The aim of this paper is to compare conductometric and thermometric detection by means of a study of temperature and concentration profiles of zone boundaries in capillary isotachopheresis with electrolyte counter-flow.

EXPERIMENTAL

Apparatus

An additional conductivity detector was attached to the instrument for capillary isotachopheresis with electrolyte counter-flow, as described in previous papers¹⁷⁻¹⁹. This instrument was equipped with a PTFE capillary with an outside diameter of 0.4 mm and a length of 30 cm and a thermometric detector. The counter-flow of elec-

trolyte, which was due to the difference in level between the electrolyte in the electrode compartment and that in the plunger compartment, was proportionally controlled by means of the signal derived from a regulation thermocouple. The thermometric detector consisted of a thermocouple or thermistor connected with an appropriate amplifier; this allowed measurement of the Joule heat evolved, which—provided the electric current passed is constant—is proportional to the conductivity and, consequently, also to the effective mobility in a given zone. To provide for a proportional counter-flow control in the case of conductivity detection, the apparatus was equipped with an additional device by means of which the control signal for the proportional counter-flow controller is derived from the instantaneous voltage over the capillary.

At the first stage in developing an apparatus for a complex automatic evaluation of results, a device was constructed which (being equipped with a digital chronometer and a printer) could record the time needed for individual zones to pass the detector. This makes quantitative analysis of the mixtures under separation more accurate. A detailed description of this device will be presented in a separate paper. An additional device for qualitative analysis is being tested.

Conductivity detector. For measuring conductivity in the capillary tube several methods have been tested²⁰⁻²³. In testing a conductometric contact detector the main purpose was to develop a reproducible technology for manufacturing a reliable and sensitive conductivity sensor which could be mounted on the capillary system, and to design convenient evaluating electronics, making use preferentially of the perspective Czechoslovak component base. The best results were achieved with the detector shown in Fig. 1. Here, the sensor itself consists of a PMMA cylinder with two platinum contact

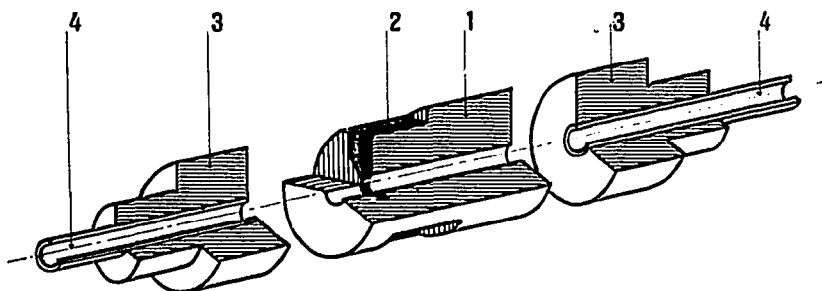


Fig. 1. Schematic diagram of the conductivity sensor. 1 = Body of the sensor, 2 = platinum contacts, 3 = centering cylinders fixing the capillary, 4 = capillary tube of PTFE.

foils (of a thickness of 0.05 mm) fixed in it by cement. In this cylinder there is a hole drilled along the axis, the diameter of which is equal to the inside diameter of the capillary tube. The capillary is fixed in two stepped centering rings. All this is covered by a casing with lead wires from the contact electrodes passing through it. The electrodes are platinum black coated.

Evaluation of conductivity G is based on the measurement of changes in resistance of the sensor S (Fig. 2) that are transformed in the primary winding of the separating transformer, Tr . The quantity measured is the voltage generated by oscillation in the primary winding of the transformer, which is fed from an a.c. generator. This

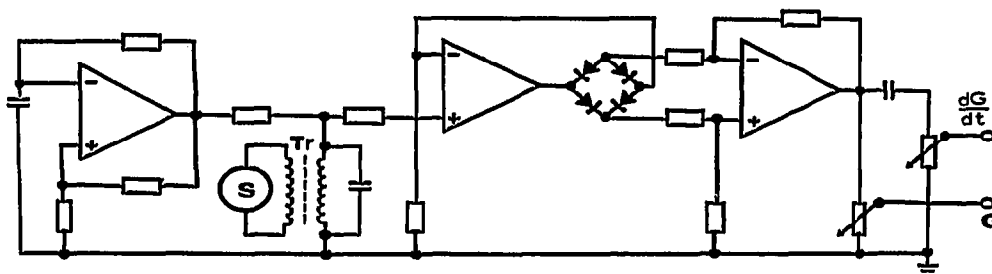


Fig. 2. Schematic diagram of the electronics for conductivity detection.

voltage is proportional to the resonance resistance of the primary winding and, consequently, also to the resistance of the sensor which is connected to the secondary winding. The over-all circuit diagram of the evaluation electronics* is shown in Fig. 2.

Proportional counter-flow controller. In designing an instrument for conductivity detection some problems may arise concerning the method of counter-flow regulation. In the method described previously the signal for the proportional counter-flow controller was derived from another (regulation) thermocouple located in about the middle of the capillary. By this location of the thermocouple the position is given at which the zone boundary is to stop due to counter-flow control. However, it is impractical to use a regulation thermocouple or a regulation conductivity sensor in an instrument with conductivity detection (particularly in those with an immediate cooling of the capillary tube by means of kerosene), as this would make such an instrument rather complicated from both the electronic and mechanical respects. Therefore, a new method of counter-flow control was developed which is based on the instantaneous value of the applied voltage. It can be used with any possible detection method and allows the zone boundary to be stationary at any point in the capillary. The control signal for the proportional counter-flow controller is obtained by comparing a part of the instantaneous voltage, V_N , across the capillary tube with an adjustable reference voltage, U_{ref} , on the input of an operational amplifier. If the voltage over the capillary surpasses the preselected value, a signal will appear on the output of the amplifier which will put the process of automatic counter-flow regulation into operation (there will be a change in the voltage across the electromagnet, M , which determines the position of the plunger and, consequently, the level of electrolyte in the plunger compartment). An overall schematic diagram of this device is shown in Fig. 3.

The same principle of regulation was applied and tested with a two-position counter-flow control using a special micropower valve pump.

Experimental conditions

A model eight-component mixture of anions was prepared for separation, containing sulphate, perchlorate, chromate, malonate, pyrazole-3,5-dicarbonate, adipate, acetate and β -chloropropionate ions, each of $10^{-2} N$ concentration. It was used at this concentration only for the experiment described in Fig. 6; for all other experiments it was used diluted. Unless otherwise stated, $0.01 M$ histidine plus $0.01 M$

* The electronics described here were developed in close cooperation with the research group of Dr. Everaerts, University of Technology, Eindhoven, The Netherlands.

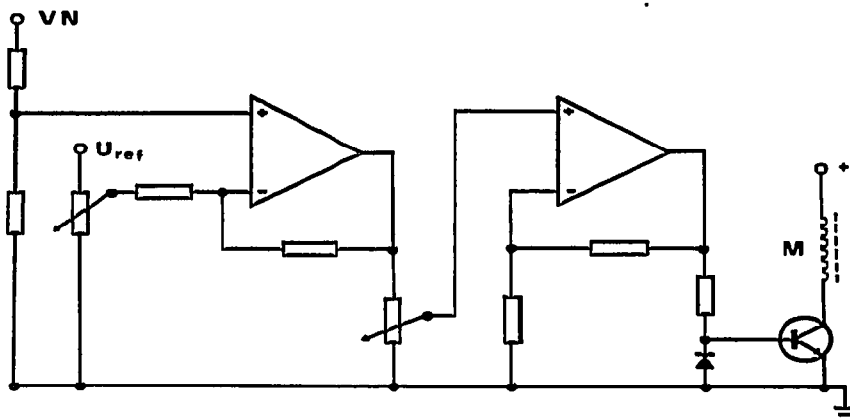


Fig. 3. Schematic diagram of the electronics of the proportional counter-flow controller.

histidine monochloride was used as leading electrolyte and 0.01 *M* phenylacetic acid as terminating electrolyte. All the experiments with this leading electrolyte were carried out at a constant current of 60 μA , which makes the zone boundary move at a constant velocity of 2.5×10^{-4} m/sec. In the experiments with counter-flow of electrolyte aimed at an improved separation, a proportionally regulated counter-flow was involved (the single exception being the experiment the results of which are shown in Fig. 6, for which a two-position regulation was used). For measuring the temperature profiles of the zone boundary as a function of the velocity at which the boundary moves, this velocity was adjusted to a given value each time by means of an appropriate constant counter-flow. As the profiles of the zone boundaries obtained for different boundary velocities cannot be compared with each other, the temperature distribution along the capillary at a given time, $T(x)$, was calculated from the changes of temperature with time, $T(t)$, measured at one point of the capillary (due to the constant velocity of the boundary this could easily be done).

Temperature (T) curves and conductivity (G) curves were recorded with a convenient speed of the chart drive of 5–180 mm/min. All the other conditions of the experiments are given in the legends to the figures showing the results.

RESULTS AND DISCUSSION

Experiments with counter-flow

Enlargement of the effective capillary length. By using an appropriate counter-flow an apparent extension of the separating capillary can be achieved. Thus, even in a relatively short capillary a multicomponent mixture can be separated which would otherwise require an instrument with a long capillary. The advantages of applying a proportionally regulated counter-flow have been described previously^{8,9,17}. In addition to this, further evidence is given here by comparing the separation of a larger amount of mixture (8 μl of mixture with component concentrations of 2×10^{-3} *N*) without and with counter-flow (Figs. 4 and 5). The separation results for an even still larger amount of mixture (5 μl of the initial mixture) obtained with two-position counter-flow regulation are shown in Fig. 6.

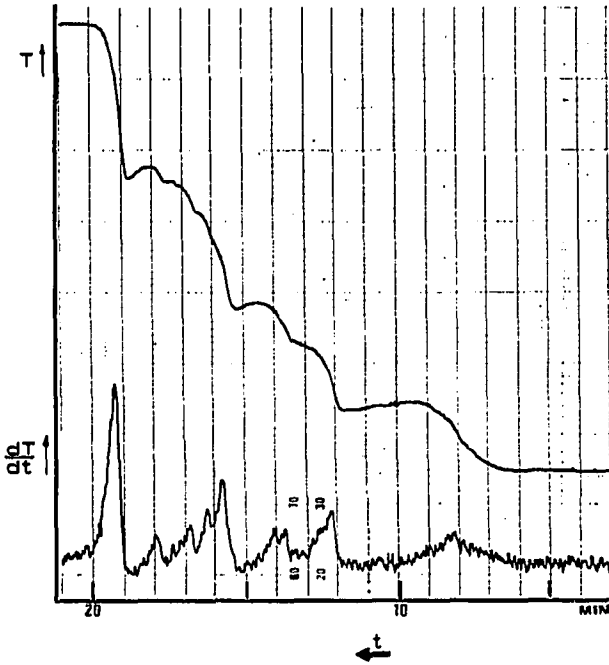


Fig. 4. Electropherogram of 8 μ l of the model mixture (with component concentrations of $2 \times 10^{-3} N$) separated without counter-flow.

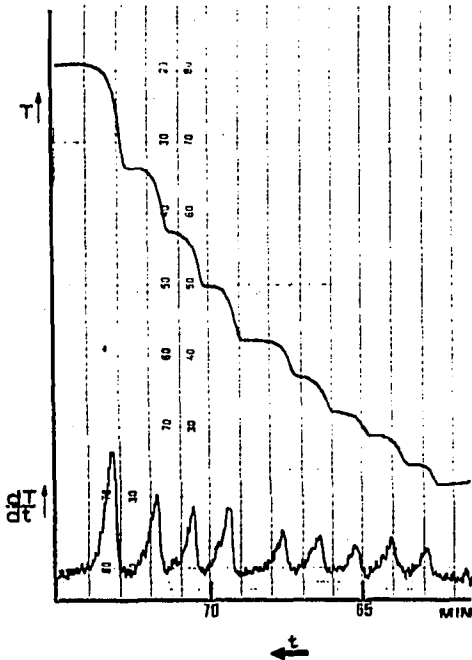


Fig. 5. Electropherogram of 8 μ l of mixture (with component concentrations of $2 \times 10^{-3} N$) after separation with counter-flow (50 min).

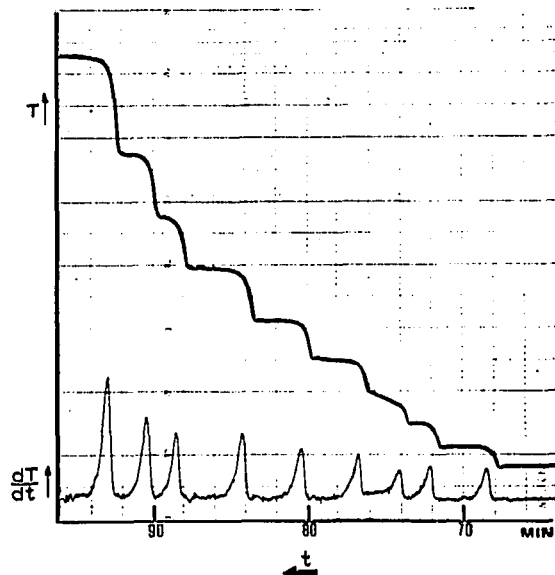


Fig. 6. Electropherogram of 5 μ l of the initial model mixture after separation with counter-flow (applied for 1 h by means of a valve pump, using a two-position regulation).

Enhancement of the sensitivity of thermometric detection. In using thermometric detection the temperature profiles of the zone boundary are recorded instead of the concentration profiles. Due to the self-sharpening effect of differing potential gradients on both sides of the zone boundary, which is typical of isotachopheresis, the concentration profiles are very sharp. Unfortunately, this is not true for the temperature profiles, which are rather diffuse (Fig. 9).

The shape of a temperature profile is given by the mechanism of heat transfer from the electrolyte moving inside the PTFE capillary towards the thermocouple and it depends, amongst other factors, on the velocity at which the zone boundary moves along the capillary. Therefore, a change in the velocity at which the boundary passes the point where the detection thermocouple is attached must affect the shape of the temperature profile measured. To characterize the temperature profile of a zone boundary, the second central moment, μ , was used, defined as

$$\mu = \frac{\int x^2 f(x) dx}{\int f(x) dx}$$

where $f(x)$ is a function representing the shape of the boundary profile (the argument x being the position coordinate along the capillary). This moment, which is also called the dispersion variance, corresponds to the mean quadratic deviation. (A detailed treatment of the influence of counter-flow on the temperature profiles of various zone boundaries in terms of appropriate moments, including the methods of calculation of these moments, is the subject of a separate paper to be published.)

The boundary between the leading and the terminating electrolytes was used for characterization. The temperature profiles measured (Fig. 7), as well as the second

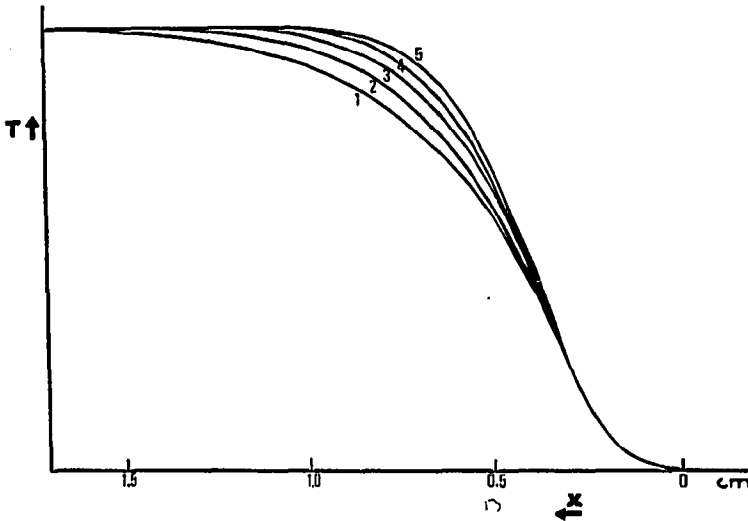


Fig. 7. Temperature profiles of the zone boundary in a capillary for various boundary velocities: (1) 2.5×10^{-4} (without counter-flow); (2) 2×10^{-4} ; (3) 1.4×10^{-4} ; (4) 1.1×10^{-4} ; (5) 0.7×10^{-4} m/sec.

central moments of these (Fig. 8), indicate their dependence on the boundary velocity; with lower boundary velocities (achieved by an appropriate constant counter-flow) a somewhat sharper temperature profile of the zone boundary can be obtained, which leads to a higher sensitivity of thermometric detection. However, if the boundary velocity was lowered by decreasing the electric current, the sharpening observed was not the same; this is due to a smaller Joule's heat resulting in a lower sensitivity of the thermocouple.

Comparison of thermometric and conductivity detections

An apparatus equipped with both thermometric and conductivity detectors

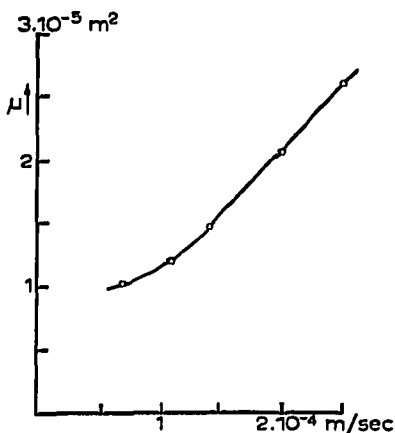


Fig. 8. Second central moment, μ , as a function of the velocity of the zone boundary passage.

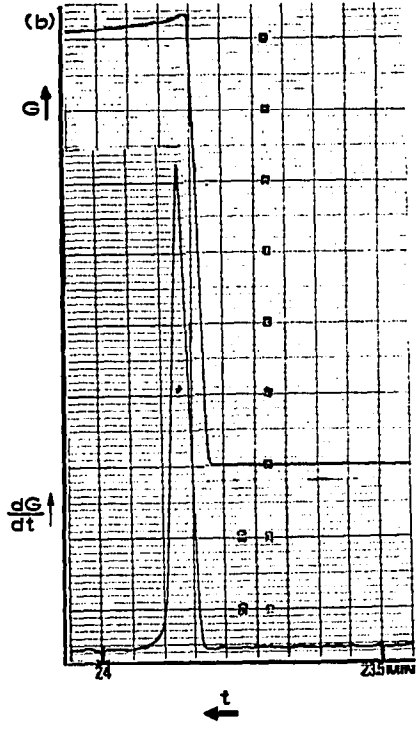
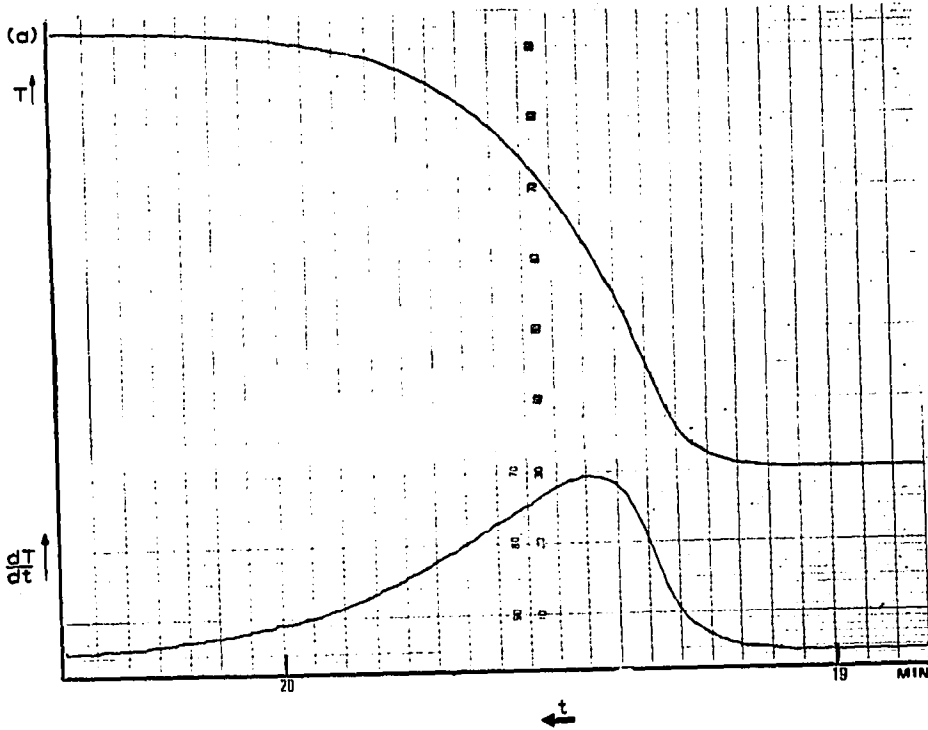


Fig. 9. Temperature (a) and conductivity (b) profiles of the zone boundary.

allows a comparison of the records derived from each during the same experiment. Fig. 9 shows the temperature and concentration profiles of the leading and terminating electrolytes. From these curves the second central moments were found to be $2.6 \times 10^{-5} \text{ m}^2$ and $4 \times 10^{-9} \text{ m}^2$ for the temperature and the concentration profiles, respectively. Figs. 10–15 show the results obtained in separation experiments with various

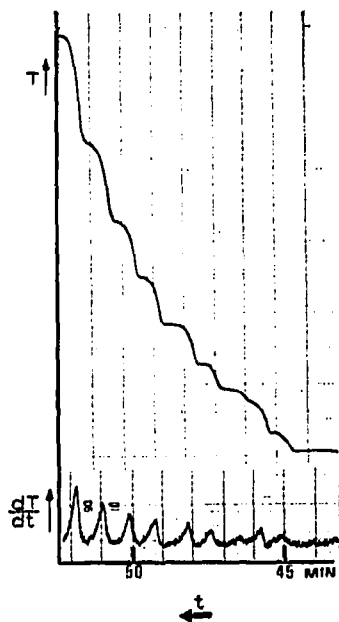


Fig. 10. Electropherogram of $3 \mu\text{l}$ of mixture (with component concentrations of $2 \times 10^{-3} \text{ N}$) with counter-flow (30 min).

amounts of the model mixture, including those with lower concentrations of the leading electrolyte. These results can be summarized as follows:

(1) The temperature profiles are much less sharp than the concentration profiles (the second central moment of the latter being smaller by nearly four orders), but they may become somewhat sharper if the velocity of the zone boundary is depressed by using an appropriate counter-flow (Figs. 7 and 8).

(2) With the same concentration of the leading electrolyte (0.01 M) a conductivity detector allows reliable determination of an amount of sample ten times less than that for a thermometric detector. With such a small amount of sample a thermometric detector would only show a smooth temperature profile, indicating no separation (Fig. 12).

(3) A leading electrolyte concentration of about 0.01 M was found to be optimal for thermometric detection (with concentrations lower than this, the value of Joule's heat is so much decreased that the effective signal becomes comparable with the electronic background noise). For conductivity detection the concentration of the leading electrolyte may be lower by one or two orders.

(4) The plots of the effective mobility of components against the leading

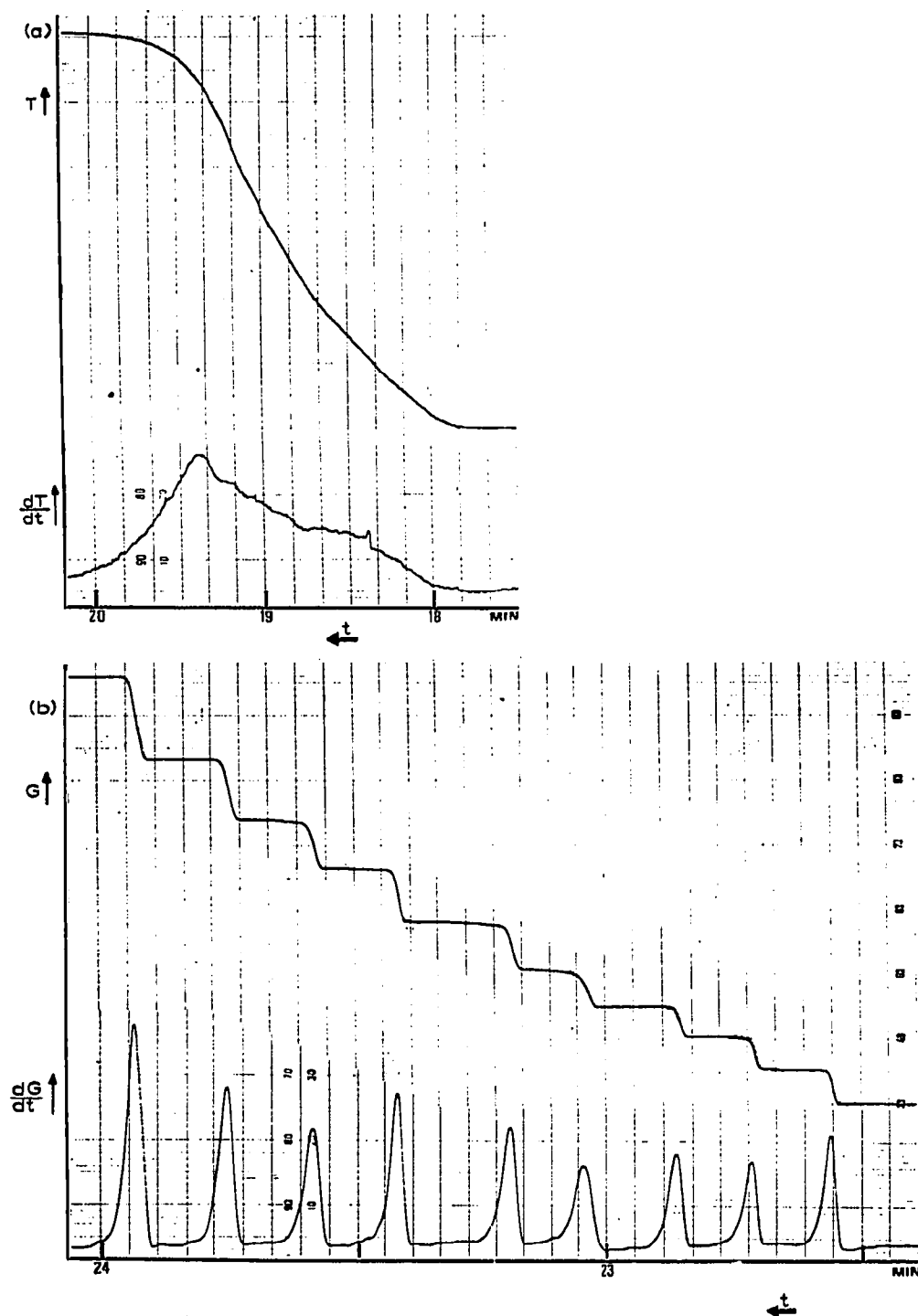


Fig. 11. Electropherogram of $1 \mu\text{l}$ of mixture (with component concentrations of $2 \times 10^{-3} N$). (a) Temperature record; (b) conductivity record.

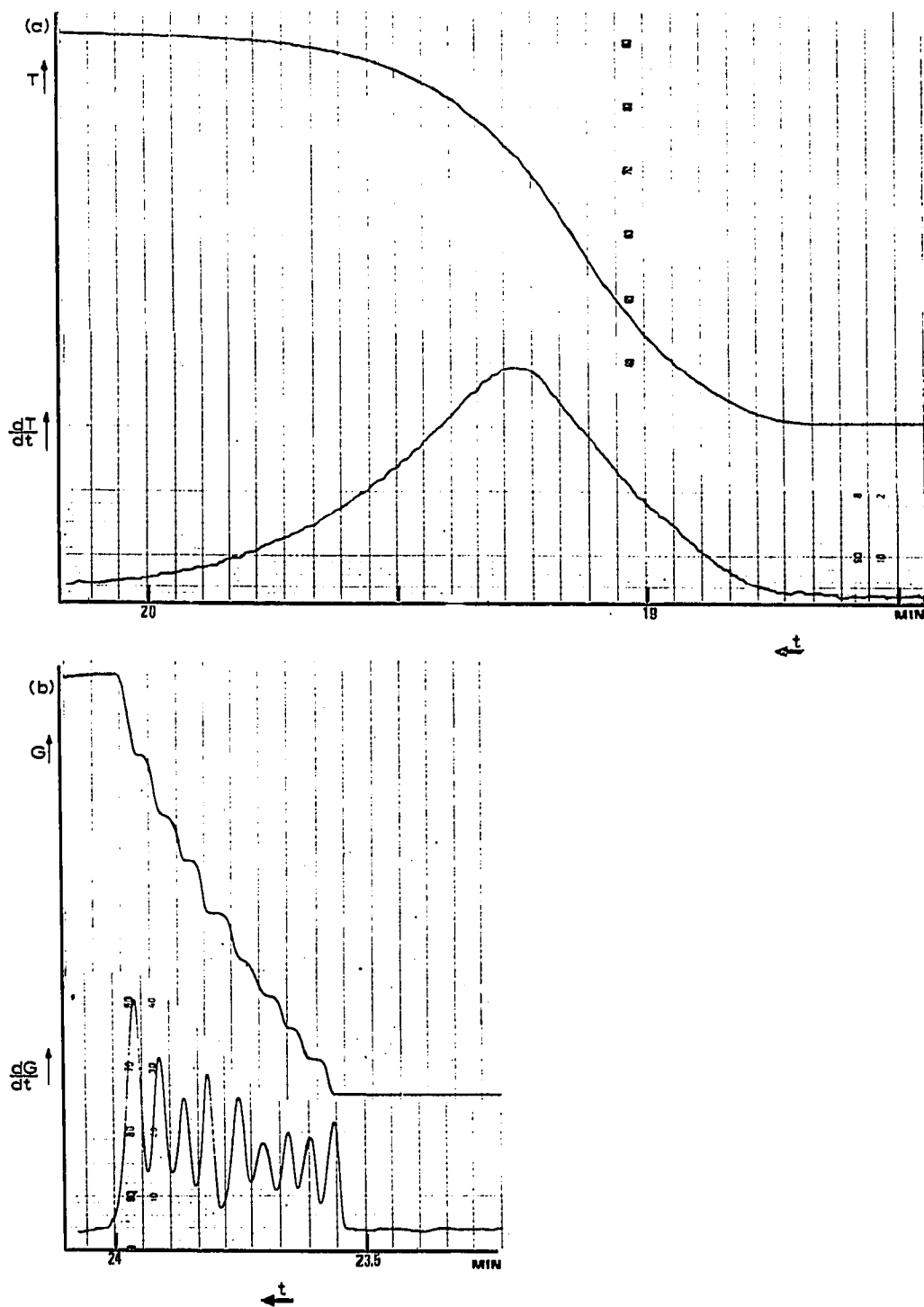


Fig. 12. Electropherogram of 0.3 μ l of mixture (with component concentrations of $2 \times 10^{-3} N$). (a) Temperature record; (b) conductivity record.

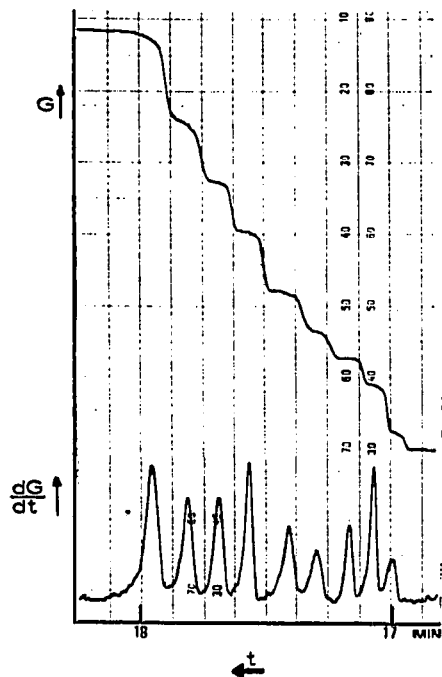


Fig. 13. Electropherogram of $0.4 \mu\text{l}$ of mixture (with component concentrations of $10^{-3} N$). Concentration of the leading electrolyte, $5 \times 10^{-3} M$; current, $40 \mu\text{A}$.

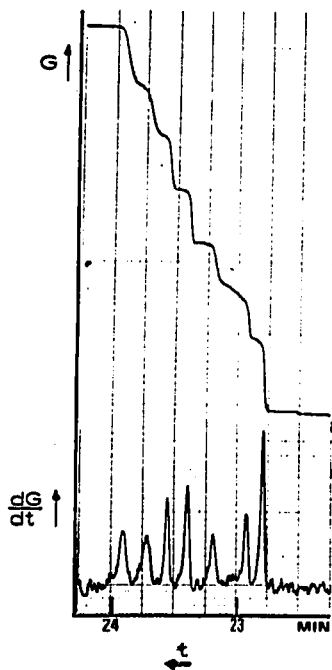


Fig. 14. Electropherogram of $0.3 \mu\text{l}$ of mixture (with component concentrations of $5 \times 10^{-4} N$). Concentration of the leading electrolyte, $10^{-3} M$; current, $7 \mu\text{A}$.

electrolyte concentration are not the same for all individual components of the mixture, thus allowing the concentration of the leading electrolyte for which the differences in the effective mobilities of individual components are the greatest to be found. Thus, in addition to pH value, solvent quality (aqueous or non-aqueous),

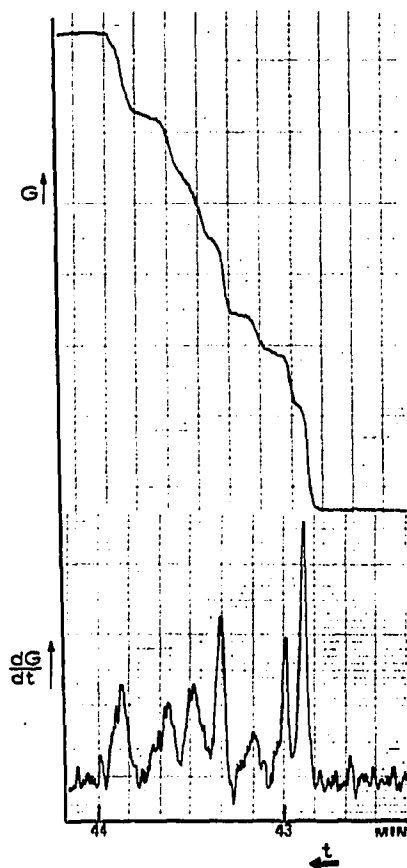


Fig. 15. Electropherogram of $0.3 \mu\text{l}$ of mixture (with component concentrations of $2 \times 10^{-4} N$). Concentration of the leading electrolyte, $5 \times 10^{-4} M$; current, $5 \mu\text{A}$. Separated with counter-flow (10 min).

etc., we have here a further parameter, which, properly chosen, may contribute to the optimization of the conditions for the separation of a given mixture (*e.g.*, in the case of our model mixture, a concentration of the leading chloride ions of $10^{-3} M$ or less was found inconvenient for the separation of sulphates, as under these conditions the mobility of sulphate ion is higher than that of chloride).

(5) In the separation of the model mixture an amount of 4×10^{-11} equiv. of each component was sufficient for conductivity detection (*i.e.*, 2.4×10^{-9} g of acetates), whereas for thermometric detection an amount of 6×10^{-9} equiv. (*i.e.*, 3.6×10^{-7} g of acetates) was needed. Thus, the conductivity detection is more sensitive by more than two orders.

(6) The very sharp concentration profiles of the zone boundary, as detected by the conductivity sensor, are much more convenient for further automatic treatment of results (*i.e.*, a digital chronometer in combination with a printer, or a punching machine and a computer).

REFERENCES

- 1 A. J. P. Martin and F. M. Everaerts, *Anal. Chim. Acta*, 38 (1967) 233.
- 2 F. M. Everaerts, *Thesis*, University of Technology, Eindhoven, 1968.
- 3 A. J. P. Martin and F. M. Everaerts, *Proc. Roy. Soc. London, Ser. A*, 316 (1970) 493.
- 4 F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, 53 (1970) 315.
- 5 F. M. Everaerts and T. P. E. M. Verheggen, *Sci. Tools*, 17 (1970) 17.
- 6 F. M. Everaerts and W. M. L. Hoving-Keulemans, *Sci. Tools*, 17 (1970) 25.
- 7 L. Arlinger and R. J. Routs, *Sci. Tools*, 17 (1970) 21.
- 8 F. M. Everaerts, J. Vacik, T. P. E. M. Verheggen and J. Zuska, *J. Chromatogr.*, 49 (1970) 262.
- 9 F. M. Everaerts, J. Vacik, T. P. E. M. Verheggen and J. Zuska, *J. Chromatogr.*, 60 (1971) 397.
- 10 R. J. Routs, *Thesis*, University of Technology, Eindhoven, 1971.
- 11 F. M. Everaerts and R. J. Routs, *J. Chromatogr.*, 58 (1971) 415.
- 12 F. M. Everaerts, *J. Chromatogr.*, 65 (1972) 3.
- 13 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 65 (1972) 287.
- 14 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 68 (1972) 207.
- 15 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 69 (1972) 165.
- 16 J. L. Beckers, *Thesis*, University of Technology, Eindhoven, 1973.
- 17 J. Vacik and J. Zuska, *Chem. Listy*, 66 (1972) 416.
- 18 J. Vacik, J. Zuska, F. M. Everaerts and T. P. E. M. Verheggen, *Chem. Listy*, 66 (1972) 545.
- 19 J. Vacik, J. Zuska, F. M. Everaerts and T. P. E. M. Verheggen, *Chem. Listy*, 66 (1972) 647.
- 20 J. Božičević, *Electron. Lett.*, 7 (1971) 688.
- 21 J. Božičević, *Elektrotehnika*, 5-6 (1972) 432.
- 22 T. P. E. M. Verheggen, E. C. van Ballegooijen, C. H. Massen and F. M. Everaerts, *J. Chromatogr.*, 64 (1972) 185.
- 23 F. M. Everaerts and T. P. E. M. Verheggen, *J. Chromatogr.*, 73 (1972) 193.