CHROM. 4669

DISPLACEMENT ELECTROPHORESIS

EXPERIMENTS WITH COUNTERFLOW OF ELECTROLYTE

F. M. EVERAERTS, J. VACÍK*, T. P. E. M. VERHEGGEN AND J. ZUSKA*
Department of Instrumental Analysis, Eindhoven, University of Technology, Eindhoven (The Netherlands)

(Received February 11th, 1970)

SUMMARY

Where ions with small differences in mobility need to be separated, the counterflow technique makes it possible to obtain a complete separation, even in short tubes. If an ion is present in a mixture in too great an amount it normally causes "mixed zones". These mixed zones can be resolved by use of counterflow. The voltages required are relatively low.

INTRODUCTION

Displacement electrophoresis has been shown to be of analytical value in many cases[1–10]. Only small amounts of sample are needed and the self-sharpening characteristic of displacement techniques, is of value wherever ions are present with small differences in mobility.

Because the inside diameter of the capillary tubes is very small and the concentration of the leading-electrolyte is small, high voltages are needed for the separation of ions with small mobilities. If the sample consists of ions with only small differences in mobility, long capillaries are used and hence still higher voltages are needed. A counterflow of electrolyte can be used to overcome these problems. Instead of voltages up to 30 kV, voltages of 2 kV can be used, because a capillary tube with a length of 30 cm can be taken and used irrespective of the mixture to be separated.

EXPERIMENTAL

The apparatus consists of a teflon capillary with an O.D. of 0.6 mm and an I.D. of 0.4 mm. This capillary is connected between two electrodes, the anode and cathode compartment, respectively. These electrodes are described in ref. 5.

If we consider a sample of acids injected into the capillary, these acids will move in the electric field and because of the difference in mobility, zones of the separate acids will be formed. These zones will all move at the same speed if the steady-

* Present address: Department of Physical Chemistry, Charles University, Prague, Czechoslovakia.

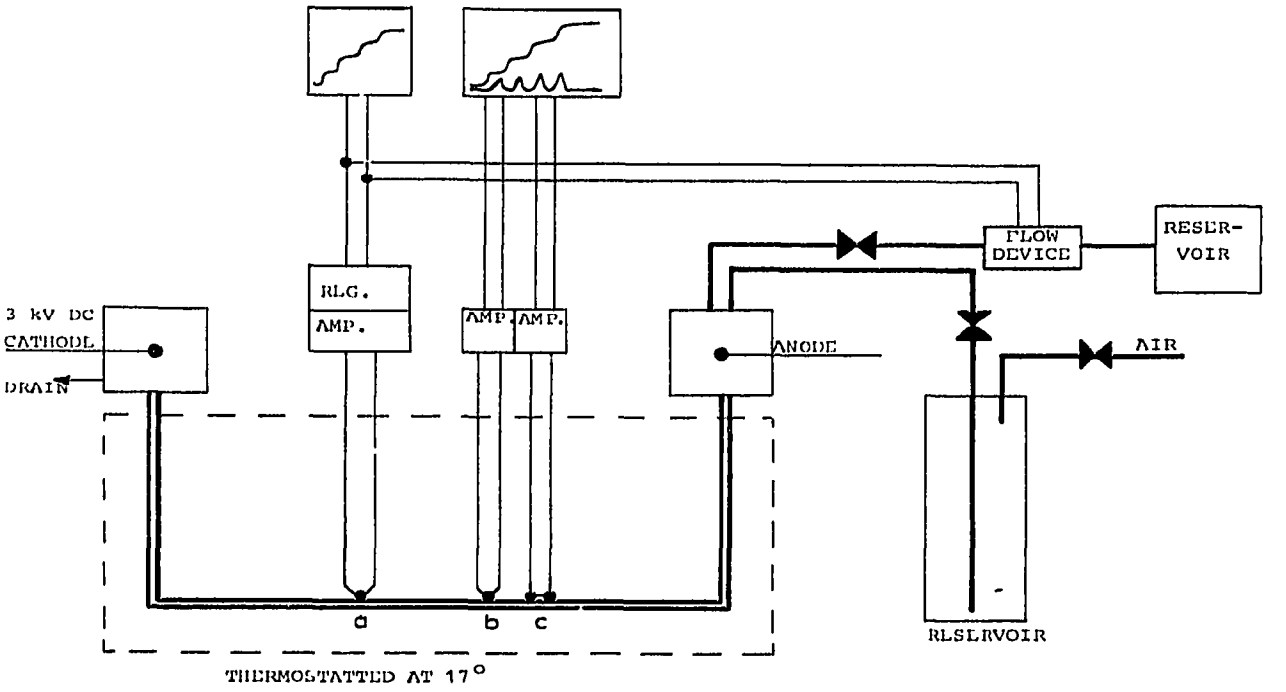


Fig. 1. Schematic diagram of the apparatus used for the analysis with counterflow. The teflon capillary, mounted between two electrode compartments, is thermostated at 17°. The capillary can be filled with electrolyte by a pressure system. The flow device and the reservoir is filled with the leading electrolyte.

state is reached. The ion with the highest mobility is at the front, while that with the least will be at the rear. As a result of this the electric resistivity increases in a stepwise fashion from the front zone to the rear zone. As the current is maintained constant, the rate of heat production correspondingly increases from the front to the rear. This makes it possible to detect the zones by means of three thermocouples, which are mounted around the capillary tube (Fig. 1).

The thermocouples a and b measure the temperature of the capillary tube. The reference junction is thermostated at 17°. Thermocouple c measures the difference in temperature between two points on the capillary tube. If a zone reaches *e.g.* thermocouple a, the signal from it is amplified and registered by a recorder. In addition the signal is, after amplification, led to a regulator which controls the counterflow device in such a way that the zone stands still at this thermocouple.

However, due to the potential drop which is still present, the separation ultimately reaches a steady state where the diffusion, the selfsharpening of the zones, the electroendosmosis and the disturbance of the zones by the counterflow of electrolyte come into equilibrium. The counterflow is then stopped. The zones start moving again and they are detected by a second set of thermocouples (b and c). Thermocouple b measures the temperature of the capillary, which is determined by the ion-species present in the zone just passing. Thermocouple c measures the temperature-drop along the capillary. The recorded curve allows the length of a zone in the capillary to be determined from the distance between two successive peaks and gives information about the amount of the given ion-species (ref. 1). The counterflow technique was tested with different systems.

A peristaltic pump giving a counterflow of 100 μl/h disturbed the zones too much; instead of a better separation mixed zones were formed where under normal conditions, without a counterflow of electrolyte, a full separation was obtained. The separation was better if the viscosity of the leading electrolyte, also used as the electrolyte for the counterflow, was increased up to 100 c.p.s. by dissolving a suitable polymer in it.

A syringe-pump gives much better results, the disturbance of the zones being less. In this case, addition of a polymer to the leading electrolyte did not give better separations.

The best results, however, were obtained where the counterflow of electrolyte was created by a higher level of electrolyte in the electrode compartment[9,10], towards which the sample is moving (Fig. 2). The rate of counterflow can be controlled by the signal from the leading electrolyte as in ref. 10. A change in this reference due to a hot zone means that this zone cannot pass the "control-thermocouple", because the disturbance of the reference-signal is eliminated by increasing the level in the anode compartment.

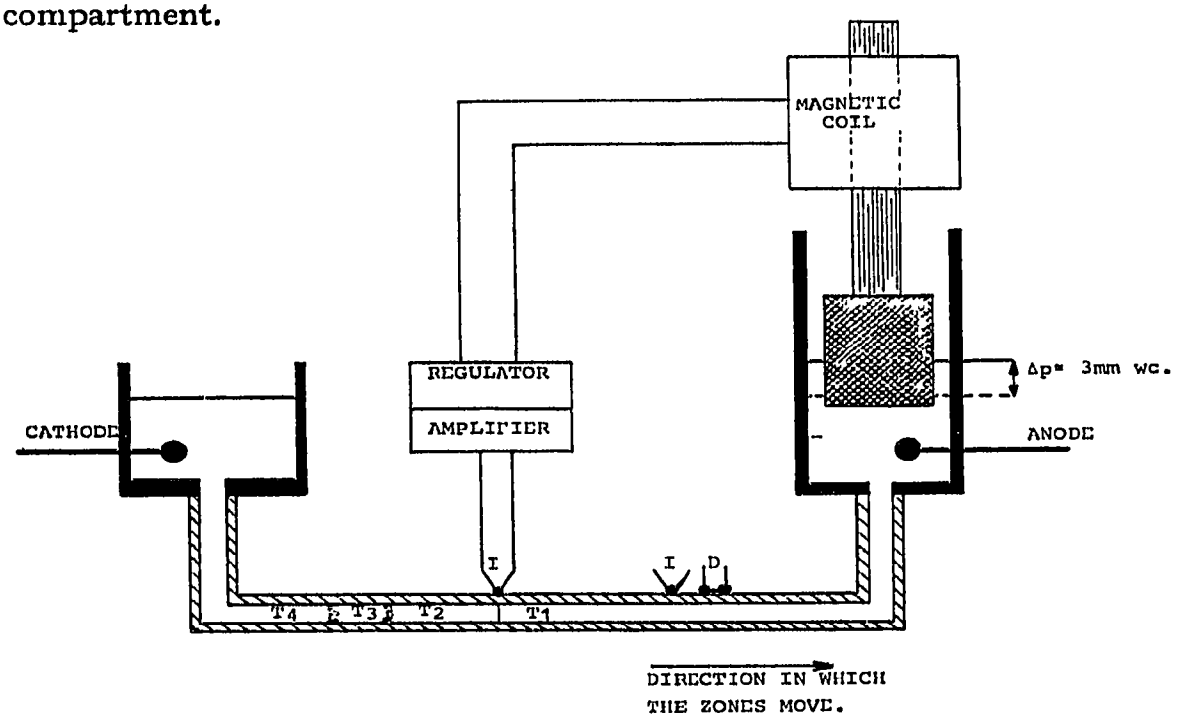


Fig. 2. Schematic diagram of the flow device, which gives the best results for experimental work with counterflow. A temperature change in the capillary tube is detected by the thermocouple mounted around the capillary. This signal is, after amplification and comparison with a reference signal, led to a regulator which increases the current in a magnetic coil, which in turn operates a plunger. This movement creates a higher level in the electrode compartment and gives an adequate counterflow.

RESULTS AND DISCUSSION

The experiments show that a counterflow technique can be used with success. Counterflow of electrolyte in a capillary with the dimensions described and a length of 30 cm gives, if a voltage of 2 kV is applied, the same separation as that in a capillary tube with a length of 100 cm, and the same inside and outside diameter and an applied

voltage of 15 kV, but with no counterflow. The analysis time will, however, be increased by 30%. The detection system, based on temperature measurements on the outside of the capillary, could perhaps be further developed. A minimum value for the difference in mobility, where it is possible to separate the ions in two different zones, cannot be given yet. At the present time, a difference in mobility of 0.5% is needed to separate ions into two successive zones. The step heights, characteristic for the ion-species, measured with and without counterflow were not the same. The distance between two successive peaks was changed if a counterflow technique was used. To elucidate this a series of experiments was performed. Fig. 3 shows the result. The —●— values were measured in a glass capillary with an I.D. of 0.6 mm and an O.D. of 0.8 mm (Fig. 3, A).

The values in section B of Fig. 3 are measured in a teflon capillary, 30 cm long and with the same diameter as before. As leading electrolyte a solution of histidine (0.01 *M*) and histidine HCl (0.01 *M*) in water was used in both cases. From Fig. 3 it is clear that use of a counterflow of electrolyte causes the step heights to be changed. The reason for this is as follows.

In our apparatus the anode compartment has a membrane in it, so that the capillary can be rinsed and filled again with electrolyte without disturbing the electrolyte in the anode compartment[5]. This membrane is made of cellulose acetate. The

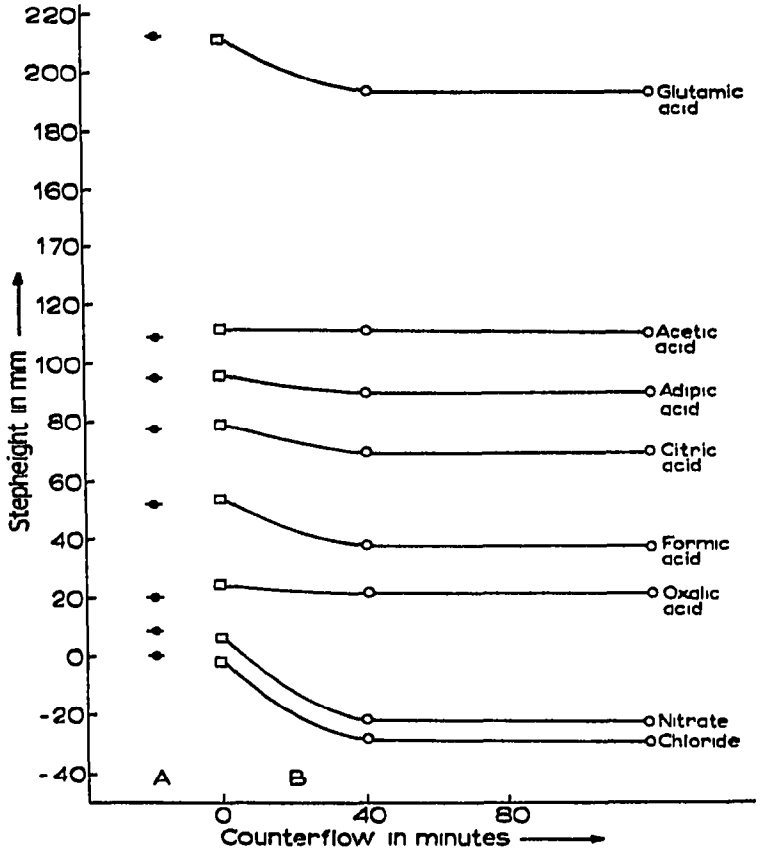


Fig. 3. This shows step heights of different materials. The —●— values were measured in a glass capillary without counterflow. The accuracy was about 2%. The values of section B are measured in a teflon capillary and counterflow was also used. For further explanations see text.

cellulose acetate is more permeable for H^+ than it is for the histH^+ used as counterion. During the analysis a pH change occurs as a result. Because the H^+ ion is very mobile, this pH shift influences the separation during the experiment. The more that is injected, the longer the analysis will be. The step height will be constant when twice the amount is injected. In the case of other acids being analysed, the analysis time is different and so is the step height. The bad reproducibility (2%) found in our early experiments in measuring step heights has the same cause.

In counterflow experiments the counterflow enters the system in the same way as water for rinsing or the electrolyte for filling the capillary. The counterflow will carry with it a pH disturbance and a new steady-state will be formed dependent on the current used. In our experiments this steady state was reached after 40 min. Due to the pH shift the "chloride-line" or base-line drops after a while (Fig. 3). The nitrate ion is also related to a strong acid and therefore the nitrate drops in a similar way to the "chloride-line". The reason for this is that the H^+ ions increase the conductivity of the electrolyte in the capillary in the presence of a strong acid. The current is maintained constant and so the heat generated is less.

The other acids, however, are weak. The velocity of the zones must be constant in spite of a lower pH. Since less of the weak acid is ionised a higher voltage is needed at this lower pH to give the zone the same velocity as before, hence the heat generated

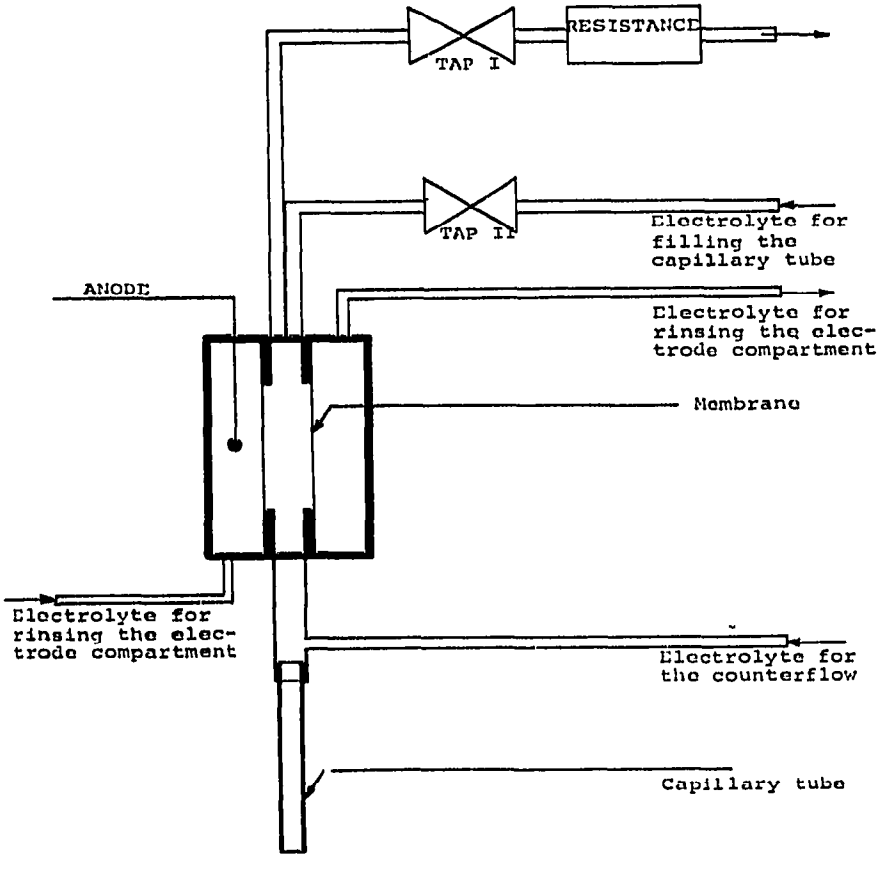
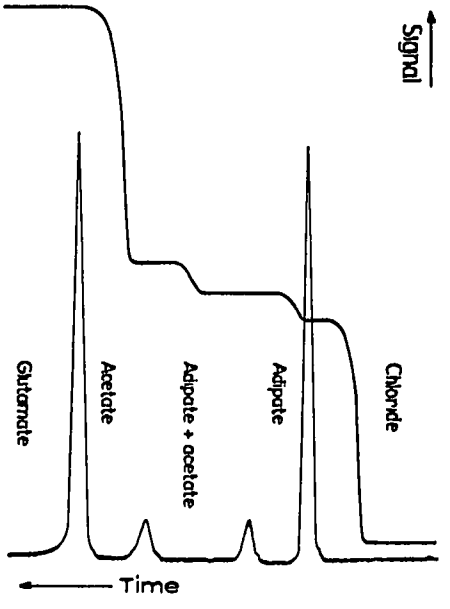


Fig. 4. The anode compartment, and the arrangement for counterflow are illustrated. Thus constructed, a counterflow can be given in the capillary tube as well as a flow in the anode compartment. This prevents disturbances to occur in the capillary tube, due to the use of a membrane.

is greater and the step height is enlarged with respect to the "chloride-line". To overcome these problems an electrode compartment was constructed as shown in Fig. 4. Experiments without counterflow now gave the same results as experiments with counterflow. Using counterflow, and tap I (Fig. 4) open, the right resistance of flow can be chosen so that a part of the electrolyte flows into the capillary tube and a part through tap I. Thus a pH-shift cannot enter into the capillary. Figs. 5 and 6 show a mixture of acids separated without and with counterflow. Both electropherograms were reproducible. For simplicity only two acids were separated. In Fig. 5 the sepa-



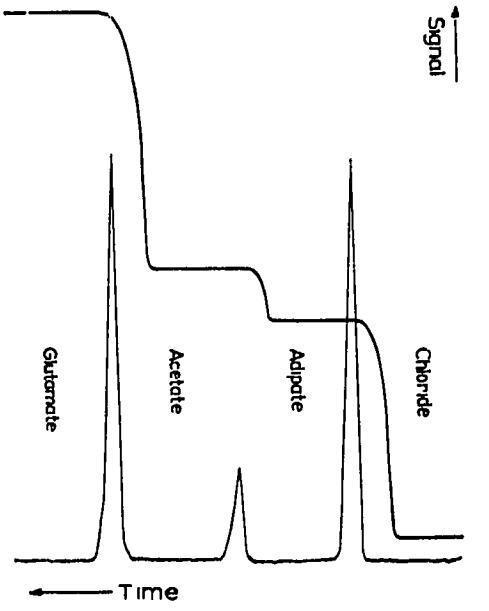


Fig. 5. Electropherogram of adipate and acetate. Injection of too much material results in a zone of unseparated material persisting. The capillary was too short for a complete separation.

Fig. 6. Electropherogram of the same mixture of adipate and acetate as shown in Fig. 5. By using counterflow the length of the capillary is effectively enlarged. The separation is complete.

ration was poor, too much was injected and the capillary was too short for a complete separation. Fig. 6 shows an electropherogram without the mixed zone consisting of the ions not yet separated. The analysis time was increased by 40% by using the counterflow technique. The conditions for these analyses were:

The current was 60 μA.

The system was thermostated at 17°.

The capillary tube was filled with a solution of histidine (0.01 *M*) and histidine HCl (0.01 *M*) in water.

The displacer was glutamic acid (0.01 *M*).

The capillary had an effective length of 80 cm.

In both cases 4 μl of a solution containing adipic acid (0.02 *M*) and acetic acid (0.04 *M*) were injected.

ACKNOWLEDGEMENT

Thanks are due to Prof. Dr. A. I. M. KEULEMANS for giving Dr. J. VACÍK and Dr. J. ZUSKA the opportunity of working on this method for a period of some months at Eindhoven University of Technology.

REFERENCES

1 A. J. P. MARTIN AND F. M. EVERAERTS, *Anal. Chim. Acta*, 38 (1967) 233.
2 F. M. EVERAERTS, *Thesis*, Eindhoven University of Technology, 1968.
3 A. VESTERMARK, Personal communication, *Dept. of Biochem. of the University of Stockholm*, 1966.
4 A. J. P. MARTIN AND F. M. EVERAERTS, *Proc. Roy. Soc. (London)*, *Ser. A*, (1970), in press.
5 F. M. EVERAERTS AND T. P. E. M. VERHEGGEN, *Sci. Tools*, 17 (1970), in press.
6 J. DVORÁK, O. GRUBNER AND Z. SPURNÝ, *Chem. Listy*, 47 (1953) 899.
7 O. GRUBNER AND J. DVORÁK, *Chem. Listy*, 49 (1955) 1767.
8 W. PREETZ, *Talanta*, 13 (1966) 1649.
9 W. PREETZ AND H. L. PFEIFER, *Talanta*, 13 (1967) 143.
10 J. VACÍK AND J. ZUSKA, *Chem. Listy*, (1970), in press.