CHROM. 4848

## Isotachophoresis*

## Experiments in methanol

Volatile liquids can be used for electrophoretic separations by means of capillaries and closed systems, however, not any liquid can be used as the solvent. The mean properties should be about the same as those of water. This means that all substances to be separated should reasonably dissolve and be ionised in it, and the solvent must solvate the ions. It is also important that the dielectric constant $E$ as well as the dipole moment $\mu$ have about the same values as those of water.

Methanol was chosen because it is similar to water in that the molecule is small, the boiling point is $64.7°$, the dielectric constant $E = 32.63$ $(25°)$, the dipole moment $\mu = 1.70$ (gas phase) and it has an amphoteric character.

Whereas the mobility $(cm^2/Vsec)$ of $K^+$ and $NH_4^+$ in water is about the same, in methanol their mobility differs considerably. The mobility of the hydroxyl ion in water has the highest value of all anions; in methanol however the hydroxyl ion can be a zone sandwiched between, for instance, nitrate and acetate.

TABLE I

THE MOBILITIES OF SOME ION SPECIES

The mobilities are calculated as average values and are taken from Ref. 5. The step-heights are measured from our electropherograms.

| Ion | Water | | Methanol | |
| --- | --- | --- | --- | --- |
| | $m \times 10^5$ $(cm^2/Vsec)$ | $h$ $(mm)$ | $m \times 10^5$ $(cm^2/Vsec)$ | $h$ $(mm)$ |
| $OH^-$ | 204.6 | — | 54.8 | 158 |
| $Br^-$ | 81.3 | 105 | 58.6 | 153 |
| $I^-$ | 79.8 | 106 | 66.1 | 138 |
| $Cl^-$ | 79.0 | 107 | 54.1 | 164 |
| $NO_3^-$ | 74.0 | 111 | 63.9 | 148 |
| $F^-$ | 56.5 | 134 | 42.2 | 196 |
| $HCOO^-$ | 56.6 | 136.5 | 51.7 | 176 |
| $CH_3COO^-$ | 42.6 | 162 | 40.8 | 191 |
| $H^+$ | 362.2 | 34 | 149.7 | 94 |
| $Cs^+$ | 81.3 | 132 | 62.5 | 163 |
| $Rb^+$ | 80.3 | 131 | 58.4 | 174 |
| $NH_4^+$ | 76.9 | 138 | 58.7 | 170 |
| $K^+$ | 76.7 | 138 | 54.4 | 181 |
| $Na^+$ | 52.8 | 182 | 46.8 | 206 |
| $Li^+$ | 40.2 | 220 | 40.4 | 236 |

Table I shows the mobility of some ion species in water and methanol and their corresponding step-heights measured in our electropherograms.

* In earlier papers the name Displacement Electrophoresis'' was used. Ref. 1 gives the reason why the name is changed to "Isotachophoresis".
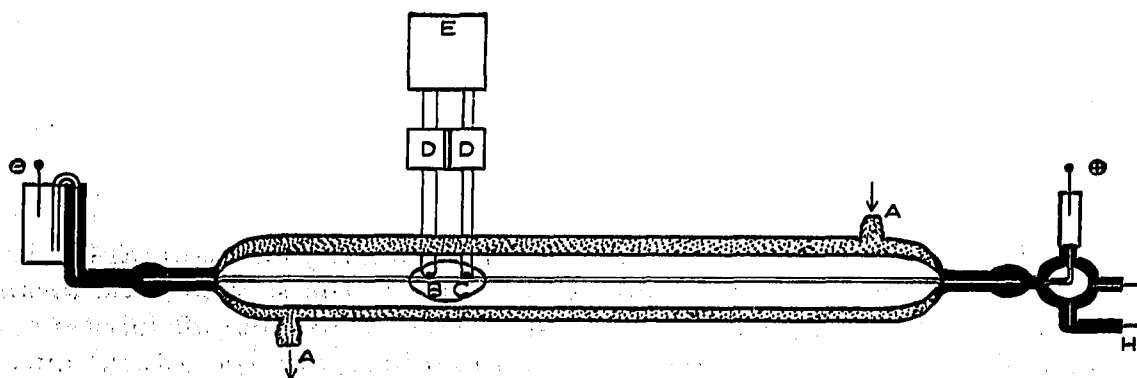
Fig. 1. Apparatus for isotachophoresis. A = thermostated water, B = integral thermocouple, C = differential thermocouple, D = preamplifier, E = two-pen recorder, H = flat four-way tap.

## Apparatus

For the experimental work an apparatus similar to that described in ref. 2 was used, because methanol will destroy our injection systems made of perspex[3]. Fig. 1 shows the apparatus schematically. A teflon tube mounted in a double walled glass vessel (like a Liebig-condenser), leads to a flat four-way tap H. The tap can be used
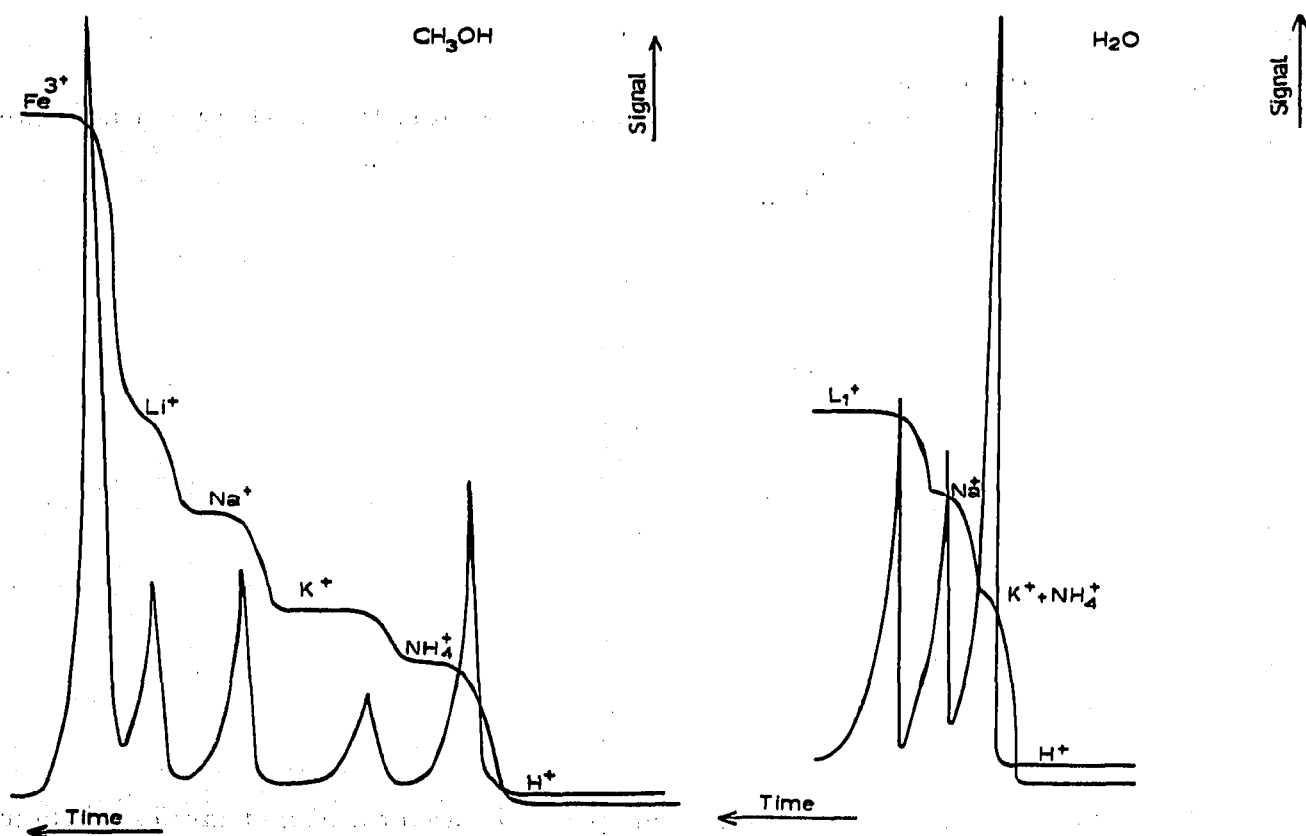


Fig. 2. Electropherogram of the separation of cations where methanol instead of water is used as a solvent.

Fig. 3. Electropherogram of the separation of cations with water as solvent. Under the conditions described no separation between $K^+$ and $NH_4^+$ was found.

as a sample tap and connects the capillary and a compartment filled with the terminal electrolyte.

Because it was difficult to make the flat tap fit tightly, the reproducibility of the analysis was the same as that in earlier experiments (2%). At the other end, the capillary was dipped into a reservoir containing the same electrolyte as that filling the capillary. Thermocouples were again mounted as the detectors[3,4].

The signals from these thermocouples are led to a preamplifier and from these to a two-pen recorder. It should be mentioned that the electropherogram must be interpreted as follows[2]: The step height of the integral curve is a measure for the ion species present in the sample. The length of the step or the distance between two successive peaks is a measure of the amount of an ion species injected.

*Separation of cations*

Fig. 2 shows an electropherogram of a separation of cations. The conditions for the analysis were as follows: The leading electrolyte was HCl ($1 \cdot 10^{-2} M$) in methanol. No polymer for stabilisation or buffer was added. As intermediate ions[1] 50 $\mu$l of a mixture of $NH_4Cl$ ($5 \cdot 10^{-4} M$), KCl ($7 \cdot 10^{-4} M$), NaCl ($5 \cdot 10^{-4} M$) and LiCl ($5 \cdot 10^{-4} M$) in methanol was used. The terminator[1] was $FeCl_3$ ($1 \cdot 10^{-2} M$) in methanol. The current was stabilised and was 70 $\mu$A. The starting-voltage was about 5 kV; the end-voltage was about 13 kV. The capillary tube (teflon) was 100 cm long, the outside diameter was 0.7 mm and the inside diameter was 0.45 mm. The analysis time was 45 min. The speed of the recorder paper was 10 mm/min.

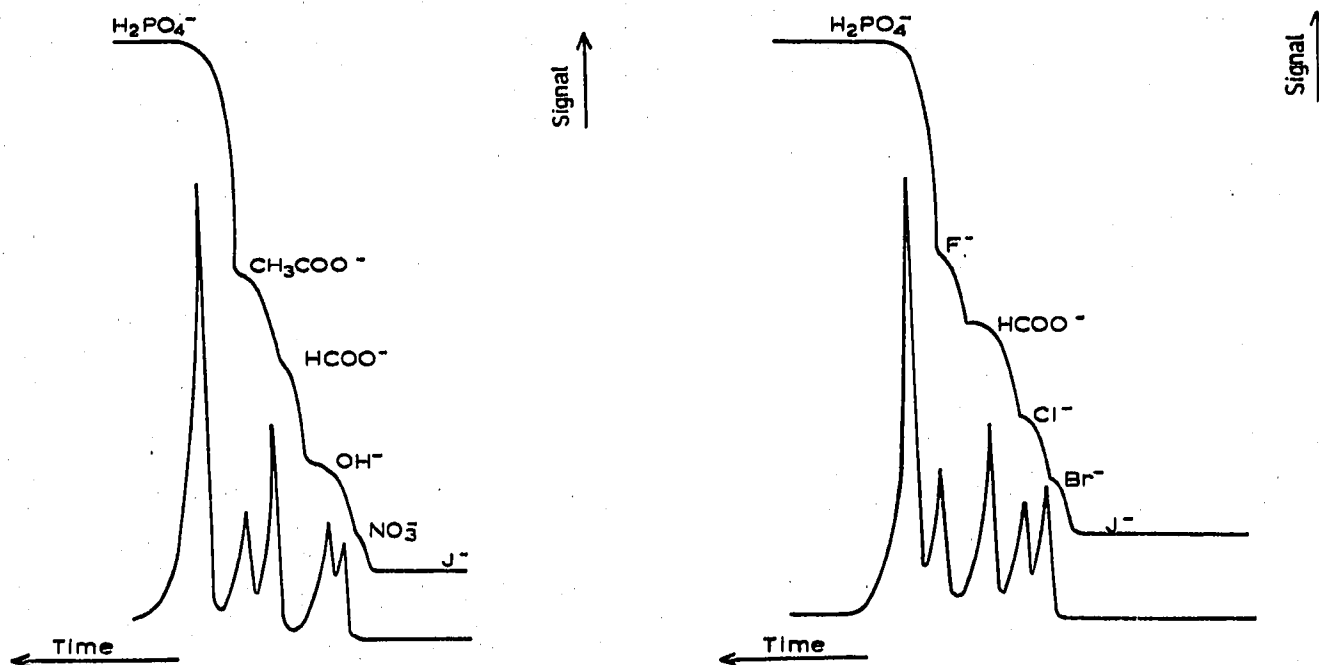Fig. 3 shows the same electropherogram if water is used as a solvent. Instead of



Fig. 4. Electropherogram of the separation of anions. The hydroxyl ion in methanol is not any longer the fastest moving anion.

Fig. 5. Electropherogram of the separation of the halides. Formic acid was added to make it possible to compare the electropherogram with the one in Fig. 4.

$Fe^{3+}$, $Li^+$ was used as the terminator. The analysis time was 30 min. The speed of the recorder paper was 5 mm/min.

It will be clear from Figs. 2 and 3 that $K^+$ and $NH_4^+$ are not difficult to separate if methanol is used as a solvent.

*Separation of anions*

Fig. 4 shows an electropherogram of a separation of anions. The conditions for this analysis were as follows: The leading electrolyte was NaI ($1 \cdot 10^{-2}$ $M$) in methanol. No polymer for stabilisation or buffer was added. As intermediate ions 50 $\mu$l of a mixture of $NaNO_3$ ($5 \cdot 10^{-4}$ $M$), NaOH ($2 \cdot 10^{-4}$ $M$), HCOONa ($5 \cdot 10^{-4}$ $M$) and $CH_3$-COONa ($5 \cdot 10^{-4}$ $M$) in methanol was used. The terminating electrolyte was $H_3PO_4$ ($1.10^{-2}$ $M$) in methanol. The analysis time was about 50 min. The speed of the recorder paper was 5 mm/min. The current was stabilised and was 70 $\mu$A. The starting-voltage was about 7 kV; the end-voltage was 13 kV. The hydroxyl ion is sandwiched between nitrate and formate.

Fig. 5 shows an electropherogram of the separation of the halides. The conditions of this analysis were about the same as those mentioned above. The terminator was $H_3PO_4$.

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