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Note

Isotachophoresis on paper

Part I. Investigation of general conditions and separation of some inorganic anions

Isotachophoresis is a relatively new term for an electromigration method in which the analogue of displacement chromatography is used in electromigration separations. Experiments based on this principle date from the 1920s, but the renewed interest in it is due mainly to the work of MARTIN AND EVERAERTS[1] and EVERAERTS AND VERHEGGEN[2], who included the use of thin capillaries for electromigration and coupled the method with modern instrumentation and recording methods.

In recent years there have also been published a number of papers dealing with column, thin-layer and membrane isotachophoresis[3,4], but to our knowledge nobody has examined the possibilities of an isotachophoretic arrangement on filter-paper in one of the usual paper electrophoretic arrangements. This paper reports our findings on this aspect as well as some separations of mixtures of inorganic anions.

Experimental and results

A simple low-voltage apparatus was constructed with Whatman No. 1 filter-paper strips (57 × 6 cm) sandwiched between 3 mm thick glass plates (50 × 7 cm) held together with clamps. The electrophoretic runs were performed at room temperature, which was 20 ± 2° during all experiments. A potential of 400 V applied for 3 h was used in the majority of experiments, and this caused no noticeable warming up of the glass plates.

Our experiments were planned by selecting leading and terminating electrolytes from tables of ionic mobilities or equivalent conductances at infinite dilution[5].

The separations of CNS^- and BrO_3^- using Cl^- as the leading and acetate as the terminating electrolyte. We chose this system of monovalent anions on the basis of the data for equivalent conductances at 25° ($Cl^- = 76$, $CNS^- = 66$, $BrO_3^- = 55.7$ and acetate = 40.9) as well as of the ease of detection of CNS^- with Fe^{3+} ions and BrO_3^- with KI–HCl without interference from each other. Solutions of their sodium salts of 0.1 *N* concentration were prepared and paper strips were impregnated as uniformly as possible with zones of the leading electrolyte, the terminating electrolyte and mixtures of CNS^- and BrO_3^-. The results of electrophoretic runs with various ratios and concentrations of CNS^- and BrO_3^- and various zone lengths are shown in Fig. 1. In all instances sharp fronts were formed at the front and rear of the CNS^- and BrO_3^- zones and by inspection it seemed evident that "equilibrium conditions" had been reached.

However, only a semi-quantitative correlation between zone length and con-

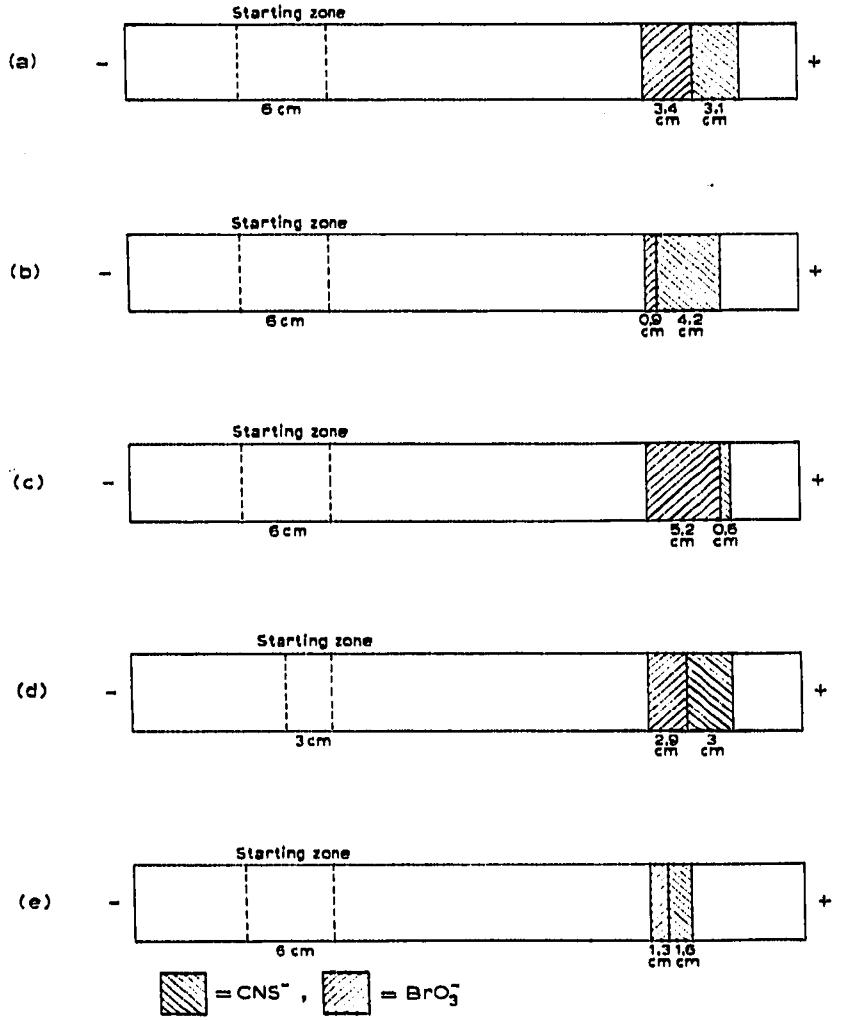

Fig. 1. Isotachophoretic separation of thiocyanate and bromate. Leading electrolyte Cl^-, 0.1 *N*; terminating electrolyte CH_3COO^-, 0.1 *N*; time 3 h; potential 400 V; run 21–27 cm. Sample composition: mixture of CNS^- and BrO_3^- in various ratios and concentrations: (a) CNS^- 0.1 *N* and BrO_3^- 0.1 *N* (1:1); (b) CNS^- 0.1 *N* and BrO_3^- 0.1 *N* (9:1); (c) CNS^- 0.1 *N* and BrO_3^- 0.1 *N* (1:9); (d) CNS^- 0.2 *N* and BrO_3^- 0.2 *N* (1:1); (e) CNS^- 0.05 *N* and BrO_3^- 0.05 *N* (1:1). Detection: Fe^{3+} for CNS^- and HCl–KI for BrO_3^-.

centration was obtained and we feel that this is due mainly to the rather crude method of applying the various electrolytes to the paper.

The separation of chromate and molybdate using thiosulphate as the leading and oxalate as the terminating electrolyte. Good separations with sharp boundaries were again obtained with various ratios and concentrations of ions, as shown in Fig. 2. Fig. 3 shows attempts to use sulphate as a "spacer" between CrO_4^{2-} and MoO_4^{2-}. The length of the sulphate zone is shorter than would be expected from its concen-

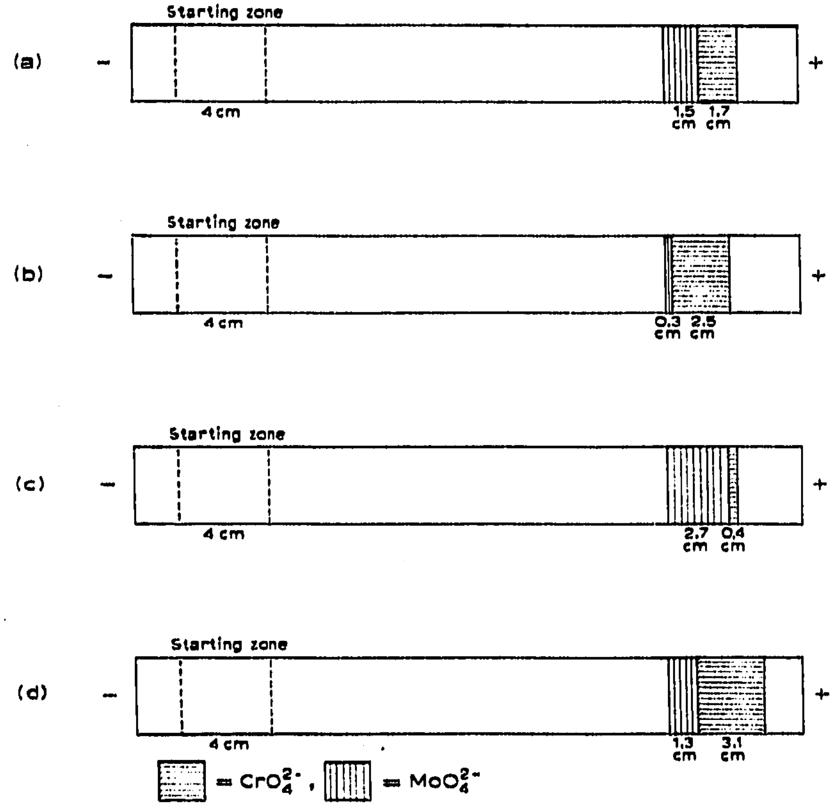

Fig. 2. Isotachophoretic separation of chromate and molybdate. Leading electrolyte $S_2O_3^{2-}$, 0.1 N; terminating electrolyte oxalate, 0.1 N; time 3 h; potential 400 V; run 23–27 cm. Sample composition: mixture of CrO_4^{2-} and MoO_4^{2-} in various ratios and concentrations: (a) CrO_4^{2-} 0.1 N and MoO_4^{2-} 0.1 N (1:1); (b) CrO_4^{2-} 0.1 N and MoO_4^{2-} 0.1 N (9:1); (c) CrO_4^{2-} 0.1 N and MoO_4^{2-} 0.1 N (1:9); (d) CrO_4^{2-} 0.2 N and MoO_4^{2-} 0.1 N (1:1). Detection: $AgNO_3$ for CrO_4^{2-} and $[Fe(CN)_6]^{4-}$ + HCl for MoO_4^{2-}.

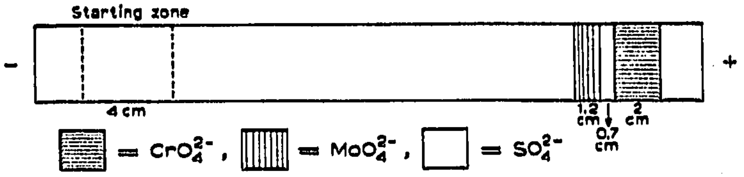

Fig. 3. Isotachophoretic separation of CrO_4^{2-}–SO_4^{2-}–MoO_4^{2-}. Conditions of run as in Fig. 2. Sample composition: mixture of CrO_4^{2-} 0.1 N, MoO_4^{2-} 0.1 N, and SO_4^{2-} 0.1 N (spacer) (1:1:1).

tration. We think we are just on the limit of "equilibrium conditions" in this instance, which may be overcome by using longer paper strips.

The concentration of CrO_4^{2-} from very dilute solutions. During the above separations, the separation of the yellow chromate zone could be followed visually during the isotachophoretic run and it was particularly striking how a dilute band of chromate became rapidly concentrated into a thin compact band. We thought that we could use this effect to demonstrate the possibilities of isotachophoresis in increasing the sensitivity of spot tests. The reaction of chromate with silver nitrate gives a red precipitate of silver chromate, but the sensitivity with dilute silver nitrate solution (0.1%) is rather low (10^{-3} *M*). When a solution which is 1000 times more dilute is subjected to isotachophoresis, the resulting band at "equilibrium conditions" should be about 0.1 *N*. A thin concentrated line of chromate actually forms and is readily detected with silver nitrate.

We feel that this use of isotachophoresis on paper has many potentialities, especially with solutions of rather high concentrations of other ions that would interfere in such techniques as the "Ring-Ofen" method. Furthermore, it may enable specific but not too sensitive reagents to be used.

Separations of some mixtures of three and four anions. A good separation of mixtures of CrO_4^{2-}, MoO_4^{2-} and WO_4^{2-} is obtained, as shown in Fig. 4. The equivalent conductances of these ions are $\frac{1}{2}$ CrO_4^{2-} = 83, $\frac{1}{2}$ MoO_4^{2-} = 74.5 and $\frac{1}{2}$ WO_4^{2-} = 69.4.

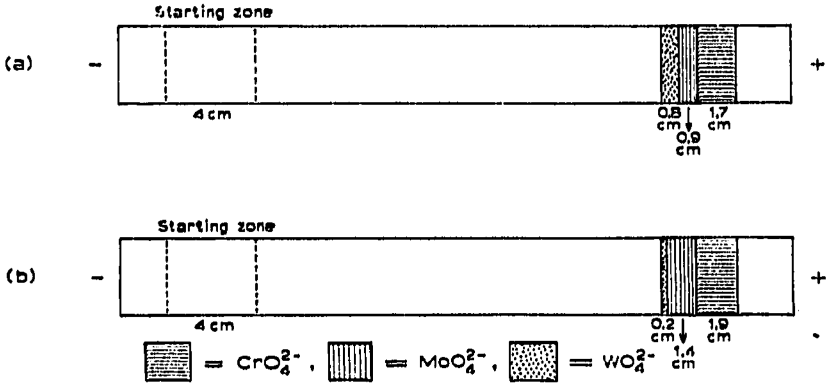

Fig. 4. Isotachophoretic separation of CrO_4^{2-}–MoO_4^{2-}–WO_4^{2-}. Conditions of run as in Fig. 2. Sample composition: mixture of CrO_4^{2-} 0.1 *N*, MoO_4^{2-} 0.1 *N*, and WO_4^{2-} 0.1 *N* in various ratios: (a) CrO_4^{2-} 0.1 *N*, MoO_4^{2-} 0.1 *N* and WO_4^{2-} 0.1 *N* (1:1:1); (b) CrO_4^{2-} 0.1 *N*, MoO_4^{2-} 0.1 *N* and WO_4^{2-} 0.1 *N* (5:5:1). Detection of WO_4^{2-} with $SnCl_2$–HCl.

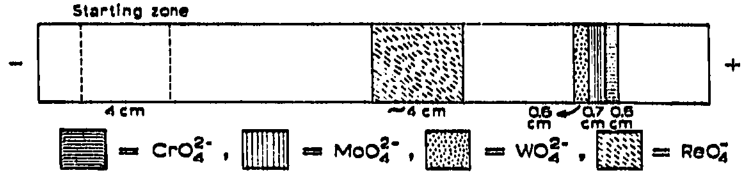

Fig. 5. Isotachophoretic separation of CrO_4^{2-}–MoO_4^{2-}–WO_4^{2-}–ReO_4^-. Conditions of run as in Fig. 2. Sample composition: mixture of CrO_4^{2-} 0.1 *N*, MoO_4^{2-} 0.1 *N*, WO_4^{2-} 0.1 *N*, and ReO_4^- 0.1 *N* (1:1:1). Detection of ReO_4^- with $SnCl_2$ + KCNS in HCl.

We then tried to separate a mixture of the above three ions with perrhenate. Theoretically, perrhenate should move faster than the terminating electrolyte, oxalate, but as shown in Fig. 5, perrhenate is left behind the oxalate and thus moves as a well separated zone but without isotachophoretic concentration, *i.e.*, as a zone that is slightly longer than the original zone and with a diffuse front and rear. This effect may be due to the fact that perrhenate is adsorbed rather strongly on filter-paper[6] and hence an adsorption effect is added to the isotachophoretic effect.

In conclusion, we found isotachophoresis to be a feasible method with a simple paper electrophoretic arrangement and with low voltages. We feel that the technique of isotachophoresis on paper will have applications in numerous problems, firstly as an elegant demonstration of the principle of isotachophoresis for teaching purposes, secondly as a very effective method for concentrating minor components from a mixture either for their detection or for preparative purposes, and finally as a separation method in which the zone length depends on the concentration of the constituent. Unlike zone electrophoresis on paper, it cannot furnish data for the identification of the separated zones, such as distances migrated (or mobilities), and it is also necessary to have an idea of the mobilities of the ions to be separated in order to be able to choose suitable leading and terminating electrolytes, whereas this is not necessary in zone electrophoresis. With the simple apparatus, we have separated only ions with large differences in mobilities and worked at relatively low electrolytic strength, but the use of longer paper strips and cooling devices could improve the range of paper isotachophoresis.

Work in this direction and also with cation mixtures is in progress.

Laboratorio di Cromatografia del C.N.R., V. TAGLIA
Via Romagnosi 18/A, Rome (Italy) M. LEDERER

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