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Note

"High-performance" paper electrophoresis

MICHAEL LEDERER

Laboratorio di Cromatografia del C.N.R., Via Romagnosi 18A, Rome (Italy)

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In recent years much effort has been expended in speeding up separation processes. The main aim has been to improve separation efficiencies, but even when such improvements were small or not very evident, higher speeds seemed to be attractive to many workers.

Actually, the first of these "high-performance" methods dates from 1951, when Michl¹ showed how paper electrophoresis could be speeded up by the use of high potentials and pointed out that the diffusion term of the separation process could be decreased considerably. His separations were carried out in 20 min when previously 1-12 h were necessary for paper electrophoresis. Since then there has been no lack of fast electrophoretic separations. Hjertén² separated metal ions in free capillary zone electrophoresis in as little as 2-3 min and more complicated mixtures in 10-20 min. Similar speeds were obtained in capillary isotachopheresis³ and isoelectric focusing^{4,5}. Hence there indeed seems to be no valid reason why zone electrophoretic separations should usually be carried out in 30-120 min in most high-voltage arrangements.

Prompted by these considerations, we decided to establish which separations could be obtained within 5 min with the simplest apparatus available. We therefore set up the usual glass plate apparatus and noted that much higher potentials (up to 20 V/cm) could be applied if short paper strips were employed for not more than 5 min. In such short times, the Joule heat generated is still taken up by the cold glass plates and the electrolyte flow from the electrolyte vessels towards the centre of the paper strip is not as extensive as in 1-h experiments, simply because the flow-rate of the electrolyte inside the paper is not high enough. It is hence possible to operate at such high potentials, but of course without maintaining a constant temperature. There is even a considerable temperature increase during the run, which, however, is beneficial to the separation effect, the increase in electrophoretic mobility due to the temperature increase giving the system a speed gradient.

EXPERIMENTAL AND RESULTS

A simple glass plate arrangement was employed, consisting of an LKB power supply, 3371E (1200Y), attached to two platinum wire electrodes, two microscope slide staining jars, which served as electrode vessels, and two pieces of glass (5 × 10 cm) held together with two sturdy office clamps. The whole arrangement is shown in fig. 1.

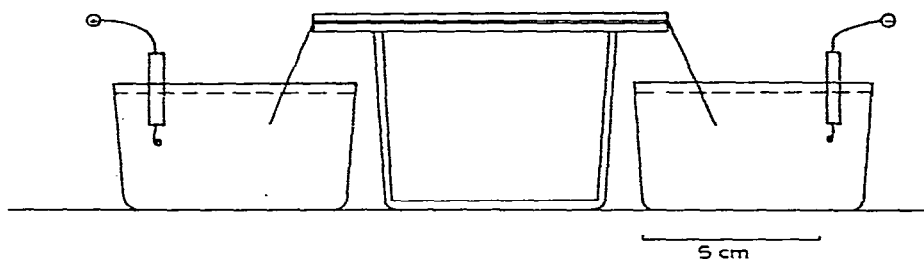


Fig. 1. Glass plate apparatus used (side view).

We used Whatman 3MM paper throughout the experiments because thin papers are more likely to permit evaporation of the electrolyte and subsequent drying of the exposed parts of the paper. The width of the paper strips is important. More current passes through wide than through narrow strips and our aim was to pass the maximal current that the apparatus would permit without charring of the paper. Paper strips 5–7 mm wide were found to give the best performance. The spots were

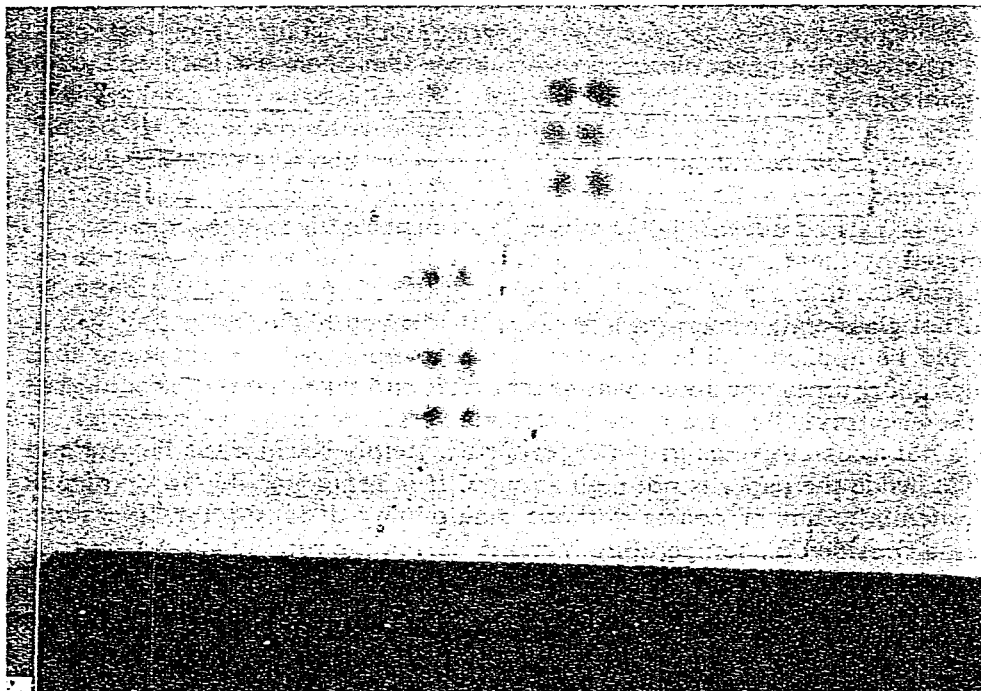


Fig. 2. Some electrophoretic separations. Top: three consecutive separations of a mixture of (from left to right) Cu(II)–Cd(II) (hardly visible at the point of application)–Bi(III)–Hg(II) in 0.5 *N* hydrochloric acid at 180 V and about 45 mA. The paper strips were 6 mm wide. Centre: three separations of [Co(III) trisorthophenanthroline]³⁺–[Co(III) diorthophenanthroline monoethylenediamine]³⁺–[Co(III) monoorthophenanthroline diethylenediamine]³⁺ in 0.5 *N* ammonium sulphate at 400 V and 20 mA. Bottom: one separation of the same mixture of Co(III) complexes as in the centre in 5% perchloric acid at 220 V and 45 mA. In perchloric acid the sequence of the complexes is reversed

placed with a glass capillary on the centre of the paper strip and the current was allowed to pass for exactly 5 min, using a stop-watch. The potential applied varied for each electrolyte and was adjusted to give a current of 45–50 mA for the system.

Typical results are shown in Fig. 2, repeating separations carried out previously in either a simple plate apparatus in 30 min or in a Camag high-voltage apparatus in 30 or 60 min. The results seem to be as good or almost as good as with other apparatus. Two of the separations shown were repeated three times each and the reproducibility, in spite of the completely uncontrolled temperature gradient, seemed very satisfactory.

Fig. 3 shows the effect of the thickness of the glass plates on the separation. As pointed out by Kunkel and Tiseius⁶, the thermal conductivity of glass is about the same as that of water, and hence the thickness of the glass plates will alter the cooling effect of the "sandwich". With thicker glass plates a slower movement (especially of the Cu^{2+} spot) can be seen, but this effect does not seem to be critical and equally good separations are obtained with thin and thick glass.

DISCUSSION

The main purpose of this paper is to show that a simple electrophoresis apparatus is capable of producing separations in as little as 5 min. We can, of course, imagine various ways of improving such fast separations, such as the application of

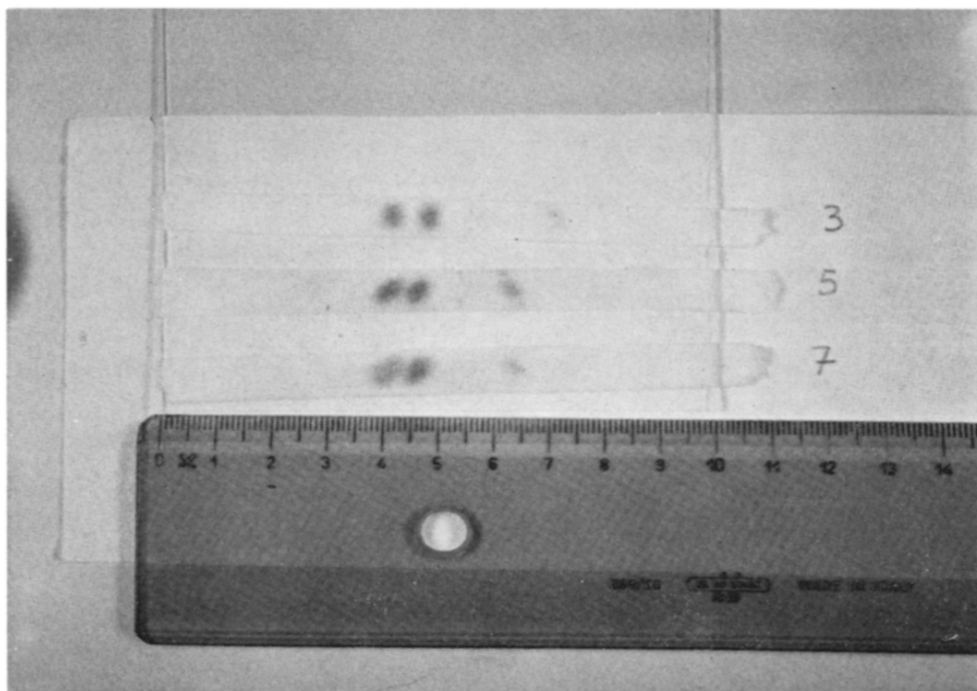


Fig. 3. Separation of (from left to right) Hg(II) – Bi(III) – Cd(II) – Cu(II) in 0.5 N hydrochloric acid as in Fig. 2, but with three different thicknesses of glass plates; top, 3 mm; centre, 5 mm; bottom, 7 mm. The yellow CdS spot situated halfway between the Cu^{2+} and Bi^{3+} spots was still visible on the photograph but not any more on the block.

smaller sample spots and the use of water-cooled glass plates. However, without any further refinements, the technique can give reproducible results and we intend to investigate its possibilities further. It is evident that the high final temperature would make it unsuitable for application to thermolabile substances, e.g., proteins and nucleic acids.

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