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Note

High-performance paper electrophoresis

III. Influence of various parameters on electrophoretic separations

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A very simple apparatus for electrophoretic separations was recently developed in our Institute[1–4]. This method, which has been called "high-performance paper electrophoresis" (HPPE), yields good separations within a few minutes. The extreme rapidity of the separations makes HPPE particularly suitable for the products of syntheses, the identification of reaction intermediates, etc. We felt that it would be interesting to extend this investigation further and therefore investigated the different factors that influence the electrophoretic behaviour, especially the distance between the electrodes.

EXPERIMENTAL AND RESULTS

A simple arrangement was employed, consisting of an LKB Model 337 IE power supply, a plastic electrophoretic cell without a cooling system and glass plates 5 cm wide and varying in length from 6 to 20 cm and in thickness from 2 to 4 mm. Whatman No. 1 and 3MM paper strips were used. The length of the experiments did not exceed 10 min. Sometimes the glass plates were held together with two sturdy office clamps. The separations described below were chosen in order to establish the influence of the various parameters (distance between the electrodes, type of paper, electric field, etc.) on the separations.

All the substances utilized for this investigations had already been separated by high-voltage paper electrophoresis. We used the following substances: (a) cobalt(III) complexes with mixed ligands; (b) inorganic anions; (c) inorganic cations; and (d) amino acids.

The parameters that were varied in the electrophoretic experiments were the distance between the electrode (from 6 to 20 cm), voltage and electric field, type of paper (Whatman No. 1 or 3MM), thickness of the glass plates (from 2 to 4 mm) and the length of the experiments (from 5 to 10 min).

Distance between electrodes, voltage and electric field

Figs. 1–5 show the results obtained in all the systems studied when the distance between the electrodes was varied at a constant total voltage. It is possible to see that

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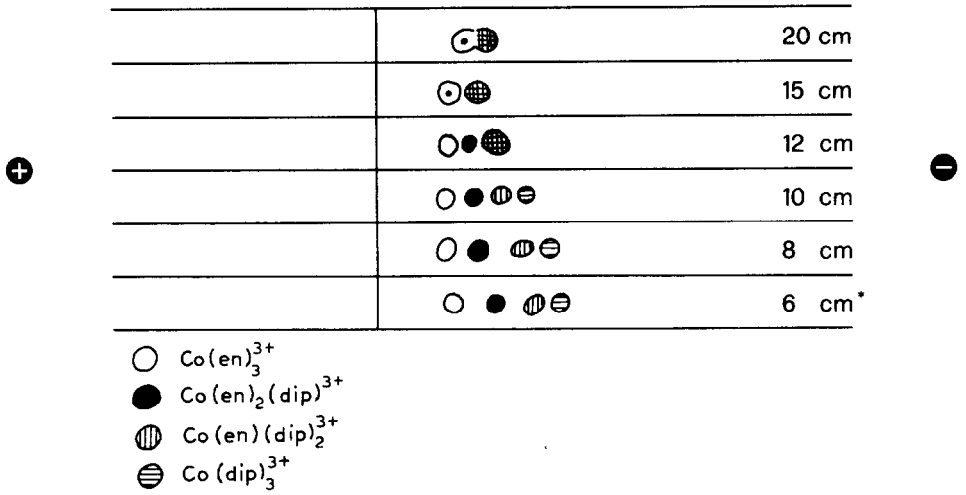

Fig. 1. Electropherograms of cobalt(III) complexes with mixed ligands on Whatman No. 1 paper at 500 V for 5 min and electrode distances varying from 20 to 6 cm. Glass thickness, 4 mm; electrolyte, 0.3 *N* sodium sulphate solution. The asterisk denotes that a field of 400 V over these 6 cm has been applied.

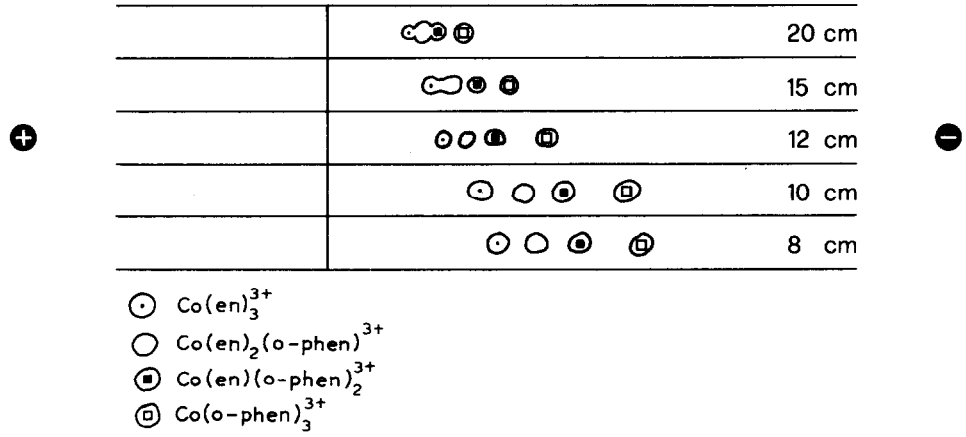

Fig. 2. Electropherograms of cobalt(III) complexes with mixed ligands on Whatman No. 1 paper at 600 V for 5 min and electrode distances varying from 20 to 8 cm. Glass thickness, 4 mm without clamps; electrolyte, 0.3 *N* sodium sulphate solution.

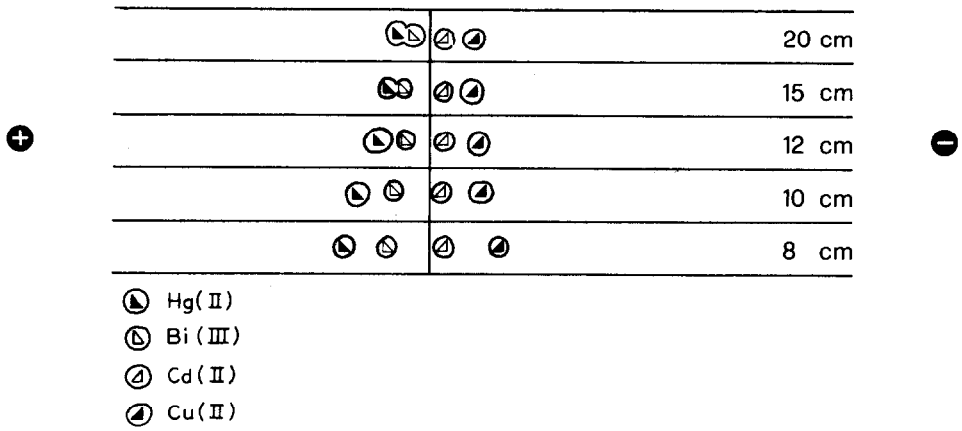

Fig. 3. Separation of some inorganic ions by HPPE at 150 V for 5 min with Whatman No. 3MM paper. Glass thickness, 2 mm with clamps; electrolyte, 0.5 *N* hydrochloric acid.

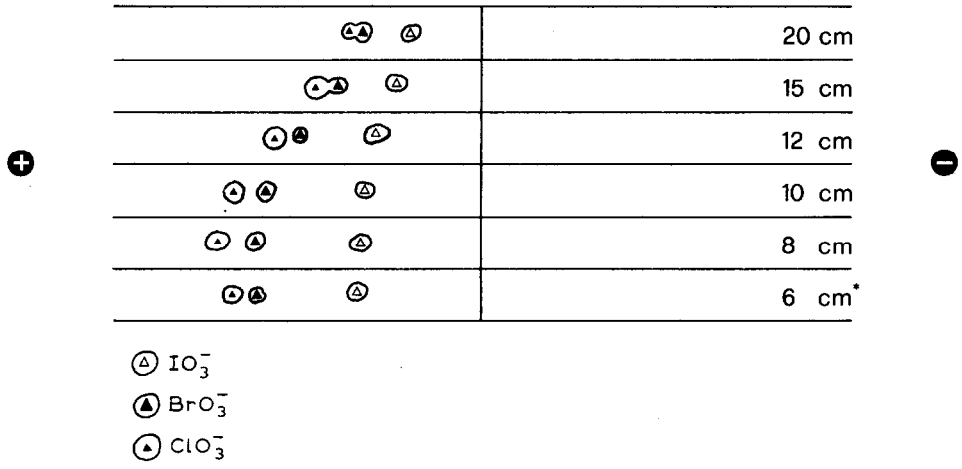

Fig. 4. Separation of some inorganic anions by HPPE at 600 V for 3 min with Whatman No. 3MM paper. Glass thickness, 4 mm with clamps; electrolyte, 0.1 *N* hydrochloric acid. The asterisk denotes that a field of 500 V has been applied over these 6 cm.

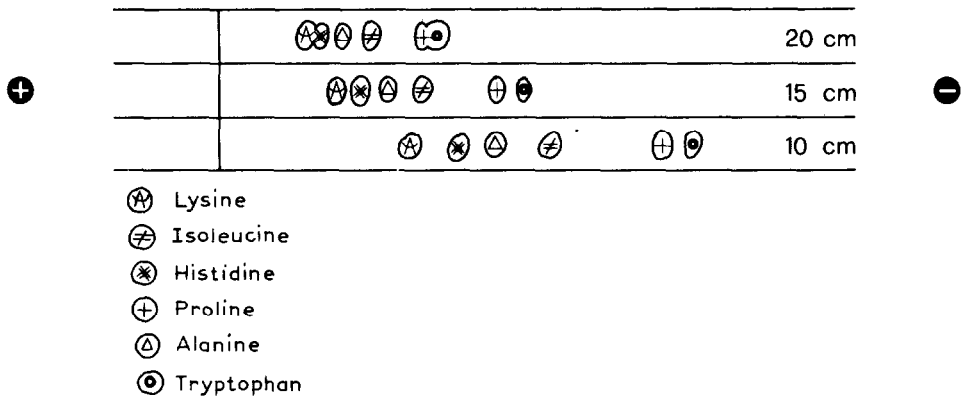

Fig. 5. Electropherograms of some amino acids on Whatman No. 3MM paper at 1000 V for 6 min and electrode distances varying from 20 to 10 cm. Glass thickness, 4 mm with clamps; electrolyte, 1 *N* formic acid.

the separations improve when the distances become smaller. The best results were obtained with distances of 8–10 cm. It is not convenient to reduce the distances below 8 cm; at 6 cm, for example, for the increase in temperature due to the Joule effect (it is important to remember that the apparatus has no cooling system) there is considerable evaporation of the solutions on the strips and consequently a current interruption in the course of the experiments might occur. Figs. 6 and 7 show the results obtained when the electric field was kept constant. It can be seen that the separations are very similar; it is better, however, to use small distances as the voltage used is then smaller. With a distance of 8–10 cm for the same electric field there is a good reproducibility of the results. Probably under those conditions the effects of the other parameters are negligible or have similar effects.

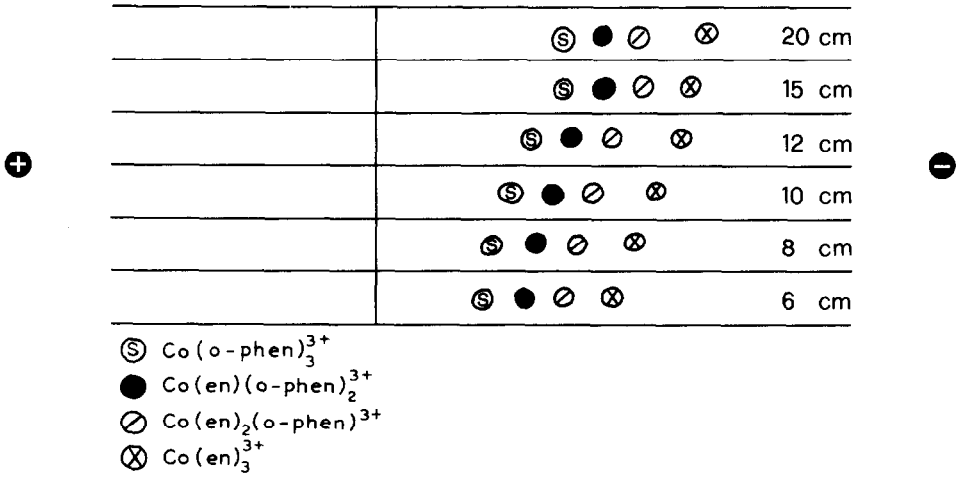

Fig. 6. Electropherograms of cobalt(III) complexes with mixed ligands on Whatman No. 1 paper at 60 V/cm for 5 min and electrode distances varying from 20 to 6 cm. Glass thickness, 4 mm without clamps; electrolyte, 0.3 *N* sodium perchlorate.

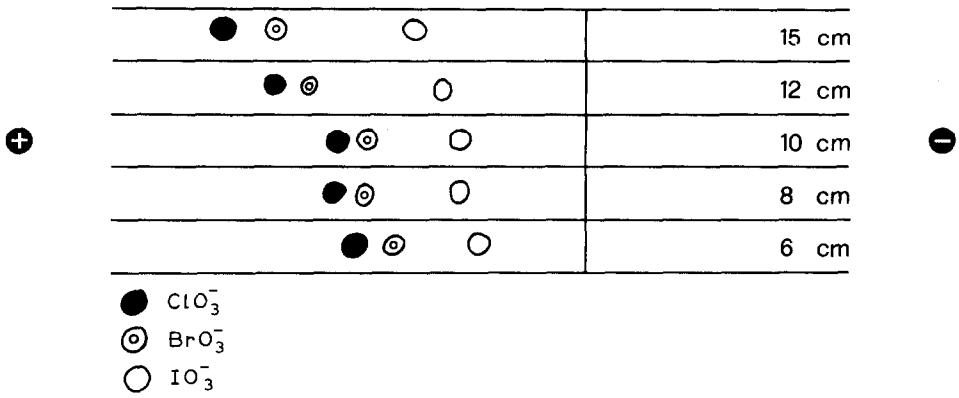

Fig. 7. Separation of some inorganic anions by HPPE on Whatman No. 1 paper at 50 V/cm for 5 min and electrode distances varying from 15 to 6 cm. Glass thickness, 4 mm with clamps; electrolyte, 0.1 *N* hydrochloric acid.

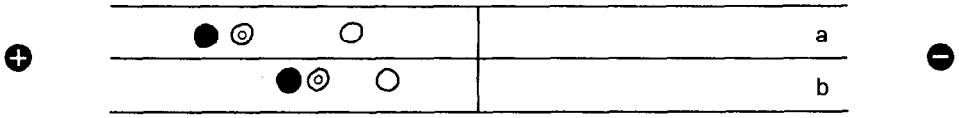

Fig. 8. Electrophoretic separation of some inorganic anions at 600 V for 3 min and an electrode distance of 10 cm. Glass thickness, 4 mm with clamps; electrolyte, 0.1 *N* hydrochloric acid. (a) Whatman No. 1 paper; (b) Whatman No. 3MM paper. Symbols as in Fig. 7.

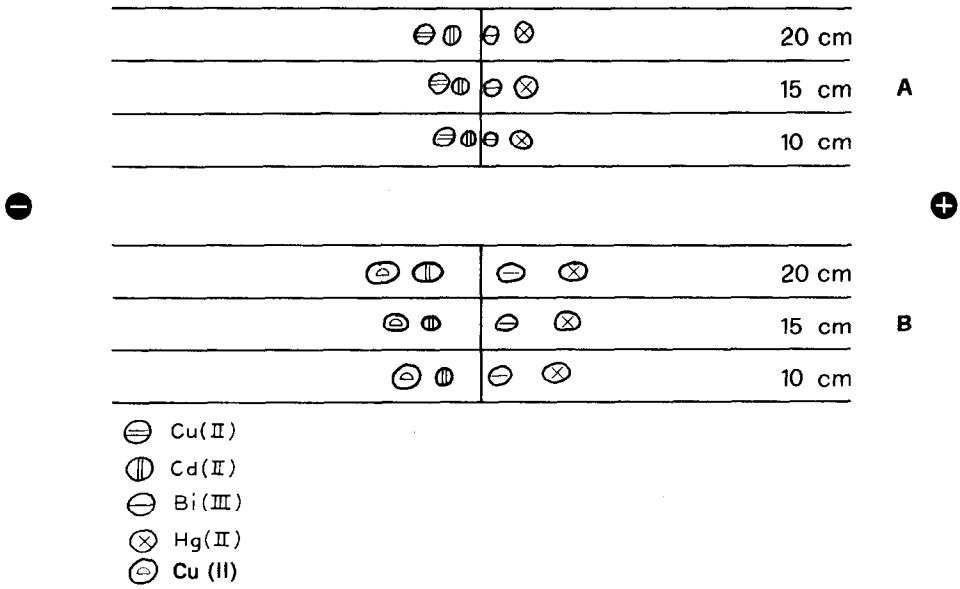

Fig. 9. Electrophoretic separation of some inorganic ions at 10 V/cm on Whatman No. 3MM paper and electrode distances varying from 20 to 10 cm. Glass thickness, 4 mm with clamps; electrolyte, 0.5 *N* hydrochloric acid. (A) 5 min; (B) 10 min.

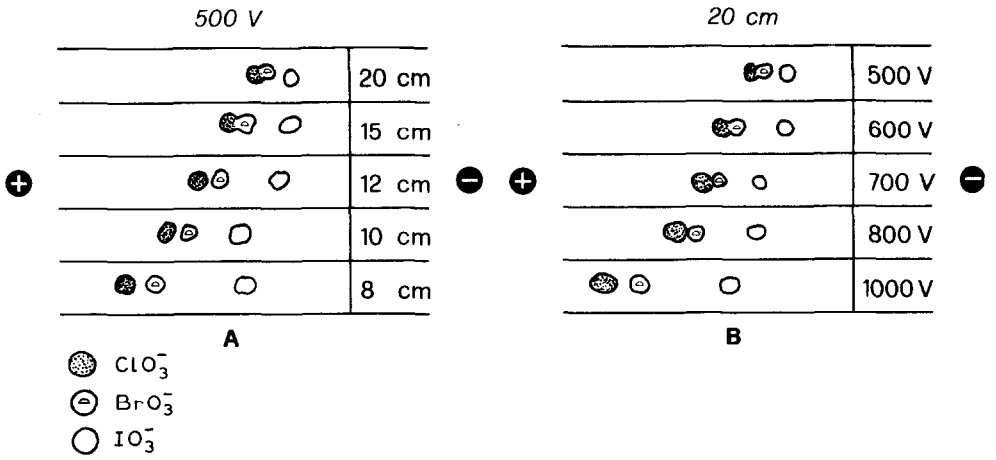

Fig. 10. Behaviour of some inorganic anions on Whatman No. 1 paper. Glass thickness, 4 mm with clamps; electrolyte, 0.1 *N* hydrochloric acid. (A) Constant voltage of 500 V and electrode distance varying from 20 to 8 cm; (B) electrode distance constant at 20 cm and voltage varying from 500 to 1000 V.

Type of paper, thickness of the glass plates, length of the experiments

Fig. 8 shows electrophoretic separations with two different types of paper (Whatman No. 1 and 3MM). The electrophoretic mobilities are higher with the 3MM paper because thick paper causes more heating owing to the Joule effect, which favours the separation. It is not convenient to use this type of paper with thermolabile substances.

Experiments were carried out with glass plates varying in thickness from 2 to 4 mm; the latter are more suitable because they permit the dissipation of more Joule heat originating during the passage of the electric current. Sometimes it is better to use office clamps to hold together the glass plates. This is useful when 3MM paper is used. In fact, under these conditions the Joule heat is lower, the diffusion is negligible and the separations are good.

Fig. 9 shows separations obtained at 5 or 10 min. In both instances there are good separations; the electrophoretic mobilities are greater in the latter instance.

Fig. 10 shows two series of electropherograms obtained at constant voltage (Fig. 10A) and constant electrode distance (Fig. 10B). The results are very similar and therefore it is more convenient to use a lower voltage and an electrode distance of 8–10 cm.

CONCLUSION

For all the experiments reported we found that the best operating conditions are a small distance between the electrodes (8–10 cm), a run time of 5 min and an electric field between 40 and 80 V/cm. With these conditions there is good reproducibility of the results and it is possible to achieve separations that either are impossible to obtain with other systems or require a very long time and/or a high voltage. The several parameters that can be changed and the great influence that they have on the separation make this method very versatile.

ACKNOWLEDGEMENT

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