ELSEVIER

Journal of Chromatography A, 663 (1994) 264–266

JOURNAL OF CHROMATOGRAPHY A

Short Communication

Chiral separations on cellulose in electrophoresis

T.K.X. Huynh, L. Ossicini*, C. Polcaro

Istituto di Cromatografia del CNR, Casella Postale 10, 00016 Monterotondo Scalo (Rome), Italy

(First received September 14th, 1993; revised manuscript received November 24th, 1993)

Abstract

Chiral separations are possible in zone electrophoresis if a chiral support such as microcrystalline cellulose is used as the support. This was shown by separating DL-methyltryptophans by electrophoresis on microcrystalline cellulose with 0.05 *M* Na_2HPO_4 or 0.05 *M* $(NH_4)_2SO_4$ (pH range 2–12) as electrolyte. The separations were best at intermediate pH and with electrolyte concentrations not higher than 0.05 *M*.

1. Introduction

In adsorption chromatography on cellulose it became evident that cellulose can produce separations of enantiomers under suitable conditions [1–3]. Such chiral behaviour can vary considerably depending on whether the cellulose is "native" or "microcrystalline" [4]. There is an extensive literature on paper electrophoresis, which in the "high-voltage" mode can produce separations comparable to those now obtained in capillary zone electrophoresis [5]. Although numerous asymmetric compounds have been examined electrophoretically, there has been no mention, to our knowledge, of chiral separations due to the cellulose support.

Chiral separations in paper electrophoresis have been reported [6] using optically active ion-pairing counter ions in the electrolyte. In this paper, some experiments are described to show that under suitable conditions chiral separations can be achieved by paper electrophoresis that are due to the chiral properties of the cellulose used as the support.

* Corresponding author.

2. Experimental

A Camag (Muttenz, Switzerland) high-voltage paper electrophoresis apparatus was used. From work on adsorption chromatography on cellulose it was evident that only microcrystalline cellulose was likely to give separation effects with short migration distances. The only microcrystalline cellulose available was in the form of Merck 5577 20-cm long thin layers (Merck, Darmstadt, Germany). As the electrophoresis chamber is designed for 40-cm long paper strips, the thin layers were connected to the electrolyte vessels with Whatman 3MM paper (Whatman, Maidstone, UK) holding the same electrolyte. The spots were detected with 1% ninhydrin in acetone.

3. Results and discussion

Preliminary results showed that separations occurred and these improved if the electrolyte was not more concentrated than 0.05 *M* and when run for 60 min at 2500 V.

Figs. 1 and 2 show electropherograms of DL-

0021-9673/94/$07.00
SSDI 0021-9673(93)E0044-U

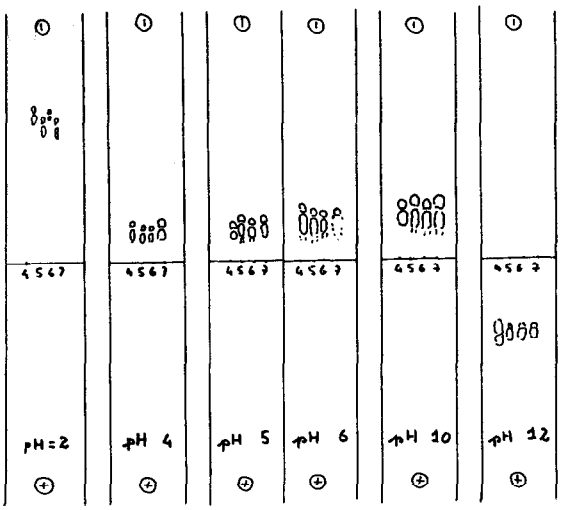

Fig. 1. Thin-layer electrophoresis of DL-methyltryptophans on Merck 5577 microcrystalline cellulose in a Camag electrophoresis apparatus operated at 2500 V for 60 min. Electrolyte, 0.05 *M* Na_2HPO_4 at pH 2, 4, 5, 6, 10 and 12 (from left to right). The samples (from left to right) are DL-4-methyltryptophan, DL-5-methyltryptophan, DL-6-methyltryptophan and DL-7-methyltryptophan. Note that "baseline" separations occur for DL-4-methyltryptophan from pH 4 to 10.

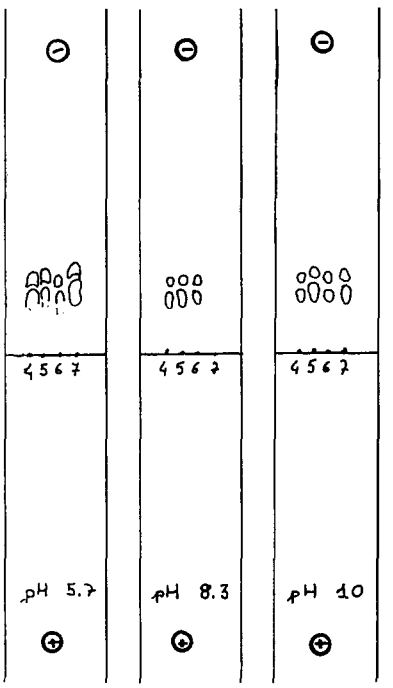

Fig. 2. As Fig. 1, except that 0.05 *M* $(NH_4)_2SO_4$ was used as the electrolyte at pH 5.7, 8.3 and 10 (from left to right). Note that all four methyltryptophans yield baseline separations of their enantiomers.

methyltryptophans at different pH values in 0.05 *M* Na_2HPO_4 and in 0.05 *M* $(NH_4)_2SO_4$. The separations are better in the intermediate pH range when the electrophoretic movement is small, *i.e.*, when much of the tryptophan is in a non-ionized form. This agrees with the results found in TLC on cellulose, where the best separations are obtained in the neutral range. Various cations were tried in the electrolyte and Li^+, Na^+, K^+ and Mg^{2+} yielded essentially the same results (Fig. 3). The anion of the electrolyte also has little effect on the separation; sulphate, chloride and acetate yielded the same results as phosphate buffers (Figs. 4 and 5). The separations could be due to a displacement by electroosmotic flow and differential adsorption on cellulose or to electrophoretic movement and differential adsorption, or both.

It was shown, however, that complete separations are possible in favourable circumstances and they must be considered as a possibility in electrophoretic work with cellulose as a stabilizing medium.

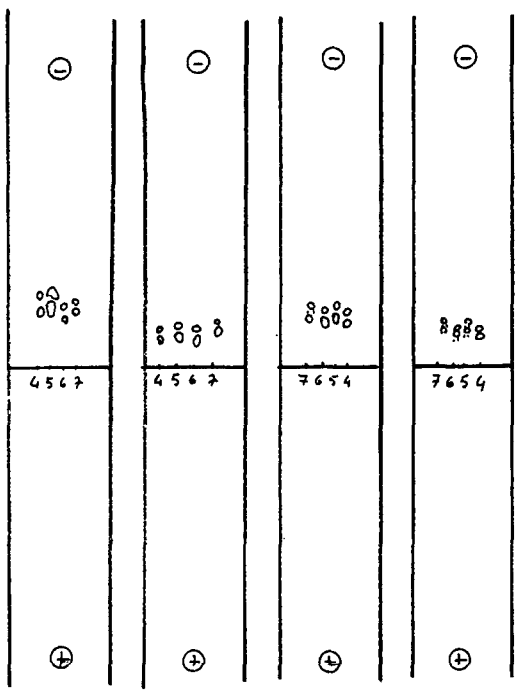

Fig. 3. As Fig. 1, using unbuffered 0.05 *M* LiCl, NaCl, KCl and $MgCl_2$ (from left to right) as electrolytes. All four methyltryptophans examined yield baseline separations.

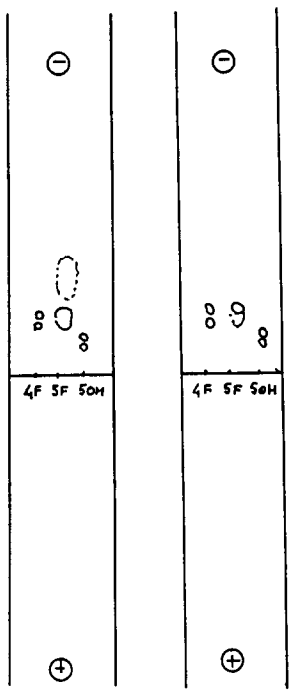

Fig. 4. As Fig. 1 with (left) 0.05 *M* $(NH_4)_2SO_4$ and (right) 0.05 *M* NaCl as electrolytes. The compounds examined were DL-4-fluorotryptophan, DL-5-fluorotryptophan and DL-5-hydroxytryptophan; only DL-4-fluorotryptophan yields a complete separation.

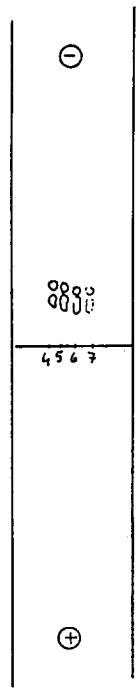

Fig. 5. As Fig. 1, with 0.05 *M* sodium acetate at pH 5 as electrolyte. Samples (from left to right): DL-4-methyltryptophan, DL-5-methyltryptophan, DL-6-methyltryptophan and DL-7-methyltryptophan.

4. References

[1] C.E. Dalgliesh, *Biochem. J.*, 64 (1956) 483.
[2] R. Weichert, *Acta Chem. Scand.*, 8 (1954) 1542.
[3] A.O. Kuhn, M. Lederer and M. Sinibaldi, *J. Chromatogr.*, 469 (1989) 253.
[4] M. Lederer, *J. Chromatogr.*, 604 (1992) 55.
[5] R. Clotten and A. Clotten, *Hochspannungselektrophorese*, Georg. Thieme, Stuttgart, 1962.
[6] L. Ossicini and C. Celli, *J. Chromatogr.*, 115 (1975) 655.