SHORT COMMUNICATIONS

A demonstration of continuous "electrochromatography" and "electrophoresis"

During a study of different supporting media for continuous paper electrophoresis an anomalous migration of a cationic dye (Brilliant Blue) was observed. Whereas the cation migrated towards the negative pole when an electrical field was applied across a glass fiber curtain, the ion exhibited slight anionic movement on cellulose paper. An anionic dye (Amaranth) exhibited anionic movement in both media.

A Spinco-Beckman Model CP Continuous Flow Paper Electrophoresis apparatus was used. Barbital buffer, pH 8.6 at an ionic strength of 0.05 and a constant voltage of 300 V d.c. were employed. The flow of buffer on the vertical edges was regulated so that no migration occurred in the absence of an electrical field[1]. Two water-soluble dyes were selected for this study, Brilliant Blue, the disodium salt of 4-{[4-(N-ethyl-N-*p*-sulfobenzylamino)-phenyl]-(2-sulfoniumphenyl)-methylene}-1-(N-ethyl-N-*p*-sulfobenzyl)-$\Delta^{2,5}$-cyclohexadienimine, and Amaranth, the trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid. The dyes were made up as 1% solution in the barbital buffer.

With a cellulose filter paper curtain (Whatman 3 MM), it was observed that both Brilliant Blue and Amaranth (red) migrated towards the anode (Fig. 1) with Brilliant

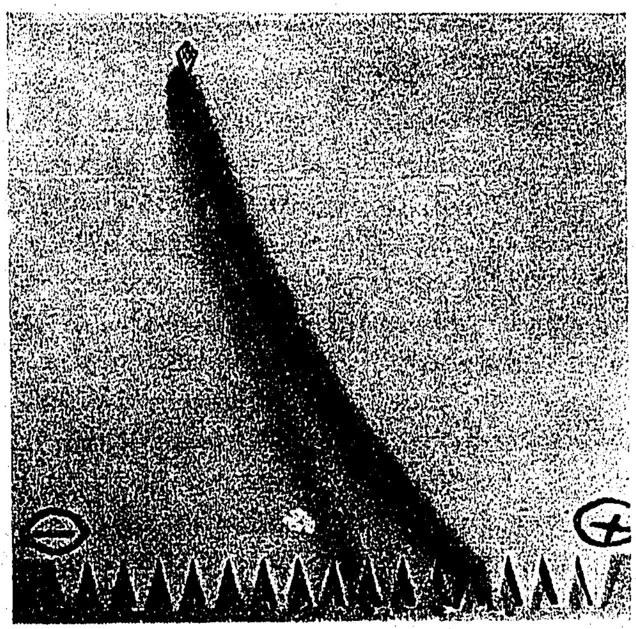

Fig. 1. Separation of dyes on cellulose. Left: Brilliant Blue; right: Amaranth.

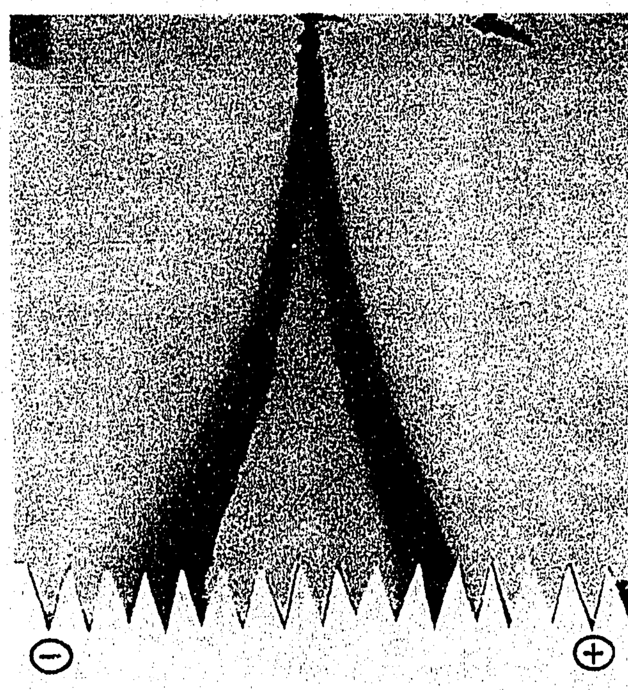

Fig. 2. Separation of dyes on glass. Left: Brilliant Blue; right: Amaranth.

Blue moving at a slower rate. When a glass paper curtain (Reeve Angel glass paper) was substituted for the cellulose, the red dye still moved towards the anode, while Brilliant Blue migrated towards the cathode (Fig. 2). In Table I are listed the numbers of the respective tabs at which the bulk of each dye was collected.

It was also observed that when both dyes were fed onto the cellulose curtain, without the application of an electrical field, Brilliant Blue moved vertically about twice as fast as Amaranth, while on glass fiber both dyes moved at the same speed. This was an indication that Amaranth was more strongly adsorbed on cellulose than Brilliant Blue.

On the basis of these results, it is strongly indicated that the vectors operative in the movement of the cation (Brilliant Blue—a quaternary amine) and the anion (Amaranth—a trisulfonic acid) on glass fiber are the vertical flow of buffer and the transverse electrical field. The resultant migration is "continuous electrophoresis".

TABLE I

	Glass	*Cellulose*
Brilliant Blue	+5	—3
Amaranth	+8	+3

On the other hand, the apparently "anionic" behavior of Brilliant Blue on cellulose paper indicates that other forces are operative, *e.g.* endosmosis. This view is supported by the larger anionic displacement of Amaranth on cellulose than on glass. Even if endosmosis made a small contribution in the migration of Amaranth, one would expect a larger displacement due the adsorption of this dye on cellulose. The use of cellulose filter paper as a supporting medium precludes the term "continuous electrophoresis", and the usage of the term "electrochromatography" becomes more applicable[2].

PUČAR[3], as quoted by STRAIN[4], concluded that migration of sorbed ions on cellulose paper, due to chromatographic and electrical forces, resulted in no greater separation than electrical migration alone in a nonsorptive medium. The anomalous behavior of the cationic dye (Brilliant Blue) on cellulose, as shown here, seems to indicate, however, that sorption in an electrical field and in solvent flow is not proportional, and that other forces (like endosmosis) must be considered.

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1 R. J. BLOCK, E. DURRUM AND G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, Inc., New York, 1958, p. 551.
2 H. H. STRAIN AND T. R. SATO, *Chem. Eng. News*, 32 (1954) 1190.
3 Z. PUČAR, *Croat. Chem. Acta*, 28 (1956) 195; *C. A.*, 51 (1957) 11809f.
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