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## Note

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### Electrophoretic behaviour of congo red dyes in aqueous dimethylformamide containing an electrolyte

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Electrophoresis using a filter-paper is a very convenient method for the separation of ionizable compounds<sup>1</sup>, but adsorption of migrating substances on the filter-paper is unavoidable. Congo red dyes, used for dyeing cotton materials, are strongly adsorbed on filter-paper and would not move in the usual buffer solutions under the influence of an electric field. It is therefore necessary to find a solution that will weaken the interactions between the dyes and the filter-paper. Using an aqueous dimethylformamide (DMF) solution containing sodium chloride, we were able to produce good migration of the dyes. The electrophoretic behaviour of the dyes using such a solution is described in this paper.

#### EXPERIMENTAL

Congo red dyes were synthesized by coupling benzidine and naphthionic acids<sup>2</sup>. Chemicals other than the dyes were purchased from Wako (Osaka, Japan).

The apparatus was described in a previous paper<sup>3</sup>. Dyes were dissolved in aqueous or pure DMF depending on their solubilities at room temperature (*ca.* 20°). A 5- $\mu$ l volume of sample solution ( $\leq 10^{-2}$  M) was spotted at a position marked with pencil on a filter-paper (Toyoroshi No. 50, 1  $\times$  40 cm) wetted with a supporting solution and migration was carried out in a usual manner. Supporting solutions were prepared by mixing an aqueous sodium chloride solution with DMF.

#### RESULTS AND DISCUSSION

Although we tried to electrophorize congo red dyes in supporting solutions such as 0.1 M sodium chloride, 0.1 M hydrochloric acid, 30% acetic acid, 28% aqueous ammonia and Miller-Golder solution<sup>4</sup>, the dyes did not migrate. We therefore tried buffer solutions different from the above, and found that a water-DMF-electrolyte system separated the dyes successfully. In order to prevent evaporation of supporting solution from the surface of the filter-paper, the spotted filter-paper is dipped in *n*-hexane during electrophoretic migration. Hence the supporting solution

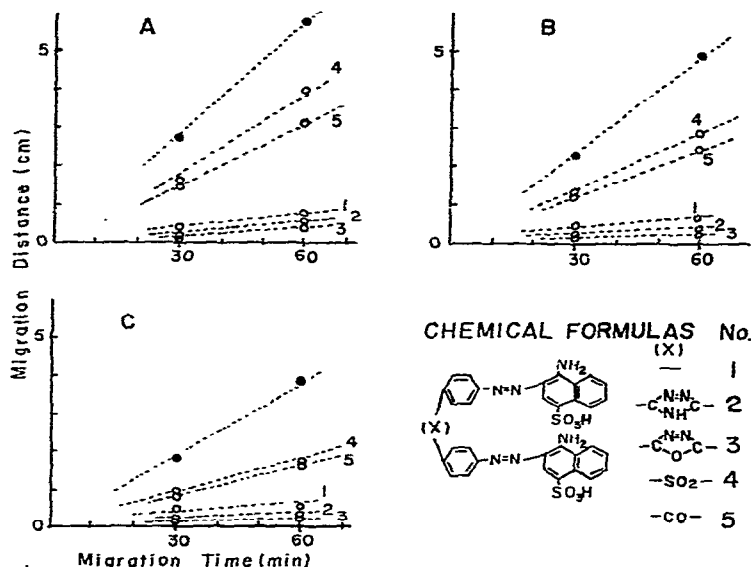


Fig. 1. Observed mobilities of congo red dyes at different spotting positions. Conditions: voltage gradient, 1000 V per 30 cm; supporting solution, DMF-0.1 M NaCl (1:1), migration temperature, ca. 17°. Spotting positions: A, 5 cm to the cathodic side from the centre of the filter-paper; B, centre of the filter-paper; C, 5 cm to the anodic side of the centre. Positive movement is towards the anode, negative movement towards the cathode. ●, Picric acid. The numbers on the graphs indicate the dyes represented by the formula.

should not be miscible with *n*-hexane; DMF is immiscible with *n*-hexane. An appropriate concentration of electrolyte (sodium chloride in this work) must be used because the electrolyte controls the electric current and the width of the migration zones. The results at different spotting positions are shown in Fig. 1.

The order of distances migrated by compounds 1-5 did not change with the different spotting positions A-C, while the overall distances increased in the order C < B < A. The migration distances increased in the order 2  $\approx$  3 < 1  $\ll$  5 < 4. The differences among the spotting positions are due to capillary action<sup>5,6</sup>, as shown in Fig. 2.

Although evaporation of supporting solution from the surface of the filter-paper is suppressed by dipping the spotted filter-paper into *n*-hexane, the flow of supporting solution due to a capillary action was not stopped completely. The capillary action should ideally be symmetrical at the centre of the filter-paper and depends on the nature of the filter-paper and the supporting solution. The capillary action in this DMF solution did not disappear even 1 h after introducing the filter-paper into the migration chamber. This result is different from that in electrolyte-dissolved water solutions<sup>6-9</sup>, in which the capillary action disappears almost completely after 1 h.

A result illustrating the relationship between the chemical structures of the dyes and the migration distance is that compounds 4 and 5, the aromatic rings of which are bent at the SO<sub>2</sub> or CO group at a definite angle, migrated further than 1, 2 and 3, in which the aromatic rings rotate freely. The separation of compounds 1, 2 and 3 will be achieved by using a higher voltage gradient and a longer migration time.

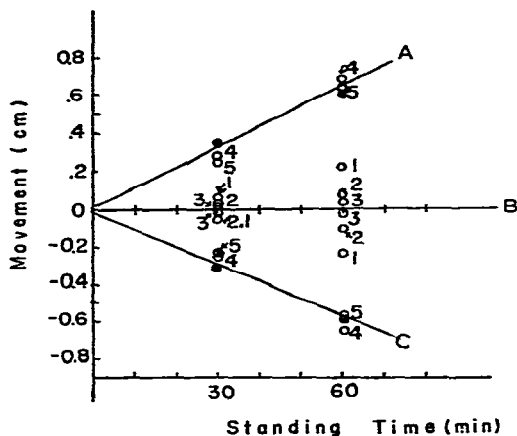


Fig. 2. Movement of dyes on a filter-paper as a function of the standing time. Conditions: sample solution,  $5 \mu\text{l}$ ; supporting solution, DMF- $0.1 M$  NaCl (1:1); standing temperature, *ca.*  $15^\circ$ . Spotting positions: A, B and C (see Fig. 1). All compounds spotted at position B (the centre) did not move. The upper line shows the movement of the compounds spotted at A and the lower line that of the compounds spotted at C.

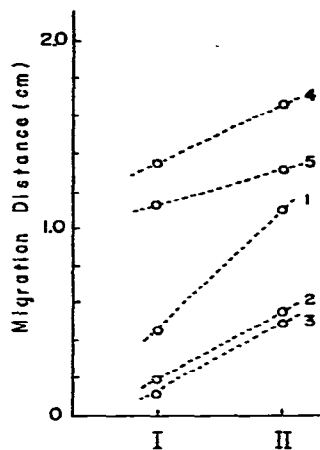


Fig. 3. Relationship between the migration distance and the concentration of DMF in the supporting solution. Conditions: voltage gradient,  $1000 V$  per  $30 cm$ ; migration time,  $30 min$ ; spotting position, A (see Fig. 1); migration temperature, *ca.*  $15^\circ$ . Supporting solution: I, DMF- $0.1 M$  NaCl (1:1); II, DMF- $0.1 M$  NaCl (5:1).

An increase in the concentration of DMF in the supporting solution increased the mobilities of all of the dyes, as shown in Fig. 3. The characteristics of this solution are that the chemical interactions between the dyes and the filter-paper are weakened and that the capillary action still remains after 1 h.

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