

Note

Effect of aqueous organic supporting solutions on the electrophoretic mobilities of aliphatic amines

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The supporting solution plays an important role in the separability obtained in electrophoresis¹. Much attention has been paid to aqueous supporting solutions, but not to organic solutions^{2,3}. It is unusual to obtain circular and well defined migration zones in pure organic solvents. Werum *et al.*⁴ found that organic buffers in 30% formamide were useful for rapid paper electrophoresis in the range pH 3.3-9.3, and successfully applied the buffers to the separation of various biochemicals⁵. The addition of an electrolyte is recommended in some cases⁵ in order to improve the shape of the zones. The electrophoresis of substances that are insoluble or sparingly soluble in water has been achieved by the use of an organic solvent miscible with water and which contains an electrolyte⁶. The effects of aqueous organic supporting solutions, such as dimethylformamide (DMF), dimethyl sulphoxide (DMSO), methanol, ethanol, and propanol, on the electrophoretic behaviour of aliphatic amines are described in this paper.

EXPERIMENTAL

Paper electrophoresis

A migration apparatus equipped with multicompartment cells was used essentially as described previously⁷. Chemicals of guaranteed grade were used. The supporting solutions were prepared by mixing an aqueous solution of sodium chloride with an organic solvent. A 5- μ l portion of sample solution [*ca.* $5 \cdot 10^{-2}$ M *n*-propyl-(C₃)-, *n*-amyl(C₅)-, *n*-heptyl(C₇)-, *n*-nonyl(C₉)-, lauryl(C₁₂)- or stearylamine(C₁₈)] were spotted at a position 5 cm to the anodic side of the centre of a filter paper (Toyoroshi No. 51A, 1 \times 40 cm) wetted with a supporting solution. Picric acid (10^{-3} M) as a marker was also spotted in each run. The filter paper was then dipped in *n*-hexane in the migration chamber, and in the supporting solution in the electrode cells. The electrophoresis was carried out at a constant stabilized voltage. The position of the amines on migration was detected by 1% ethanolic ninhydrin solution^{8,9}.

Paper chromatography

Sample solutions similar to those used in the paper electrophoresis were developed for 5-8 h at 20° on Toyoroshi No. 51A filter paper (2 \times 40 cm) by an ascending method. The results are shown in Table I. Since the supporting solutions

used in the paper electrophoresis were not always good developing solvents for the paper chromatography, well defined spots were not obtained for higher alkylamines such as nonyl-, lauryl- and stearylamine.

TABLE I

 R_F -VALUES OF AMINES IN VARIOUS SOLVENTS

Solvents: A = 0.1 M NaCl; B = 0.1 M NaCl-DMF (1:1); C = 0.1 M NaCl-methanol (1:1); D = 0.1 M NaCl-ethanol (1:1); E = 0.1 M NaCl-propanol (1:1); F = 0.1 M NaCl-DMSO (1:1).

Sample	Solvent					
	A	B	C	D	E	F
Picric acid	0.53	0.93	0.68	0.86	0.90	0.84
C ₃	0.95	0.93	0.88	0.86	0.71	1.0
C ₅	0.94	0.95	0.91	0.90	0.82	1.0
C ₇	0.93	0.97	0.87	0.90	0.91	1.0
C ₉	0.97*	0.94	0.88	0.95	0.96	1.0
C ₁₂	0.98*	0.90	0.86	0.97	0.95	1.0
C ₁₈	*	0	*	*	0	0

* The spreading of the spot prevented the determination of the precise R_F -value.

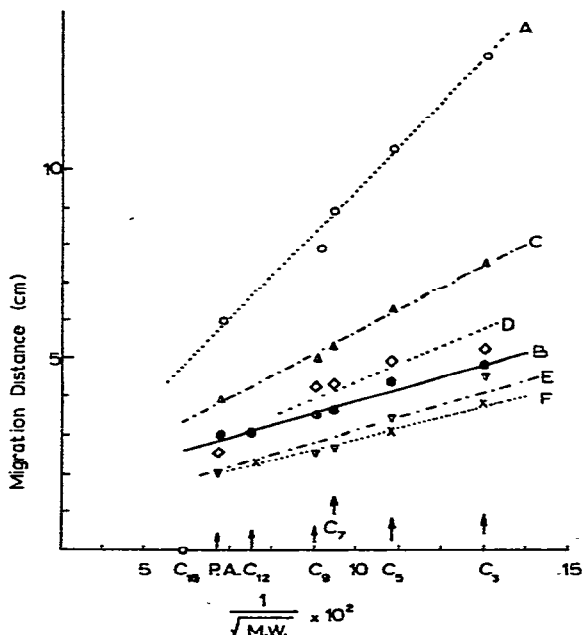


Fig. 1. Migration distances of aliphatic amines in various supporting solutions. Conditions: voltage gradient, 1000V per 30 cm; migration time, 30 min; migration temperature, *ca.* 17°; supporting solutions, 0.1 M NaCl (A), 0.1 M NaCl-DMF (1:1) (B), 0.1 M NaCl-methanol (1:1) (C), 0.1 M NaCl-ethanol (1:1) (D), 0.1 M NaCl-propanol (1:1) (E), 0.1 M NaCl-DMSO (1:1) (F); sample solution, C₃, C₅, C₇, C₉, C₁₂, C₁₈ and picric acid (P.A.).

RESULTS AND DISCUSSION

The mobilities of ions in electrophoresis on a filter paper depend on the natures of the ions, the supporting solution and the filter paper. It is difficult to estimate the amount of adsorption of amines on a filter paper¹⁰. One of the methods employed is to observe the R_F values in a developing solvent similar to the supporting solution. The R_F values obtained in the present study were not so low that the adsorption of amines other than stearylamine could not be assumed to be strong.

Since the pK_a values of the amines used are 10.5–10.7 (ref. 11), almost all of the amine molecules are considered to be dissociated in aqueous and mixed aqueous organic supporting solutions. Thus, the mobility of the amines will be mainly determined by the nature of the amine cations and by the components of the supporting solutions. Since the extent of the chemical interaction between the amine cations and the given solutions is not considered to differ greatly for the various amine homologues, the differences in the migration distances in a given solution are considered to represent the effect of the molecular weight on the mobility. Previously, Edward and Crawford⁸ found that the mobilities of amines decreased with increasing molecular weight.

The migration distances of the amines in the various solvents are shown in Fig. 1. The distances were inversely proportional to the square root of the molecular

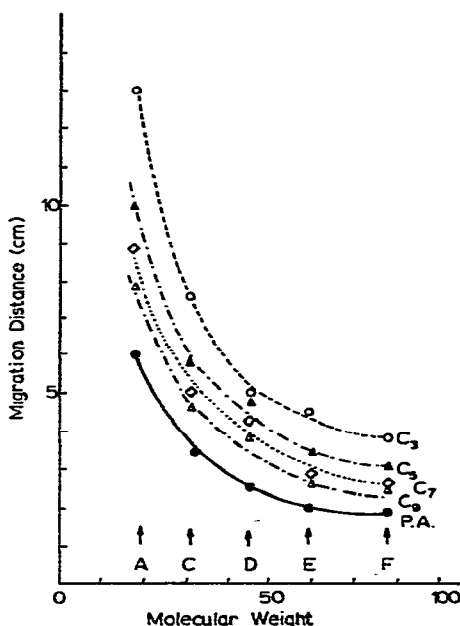
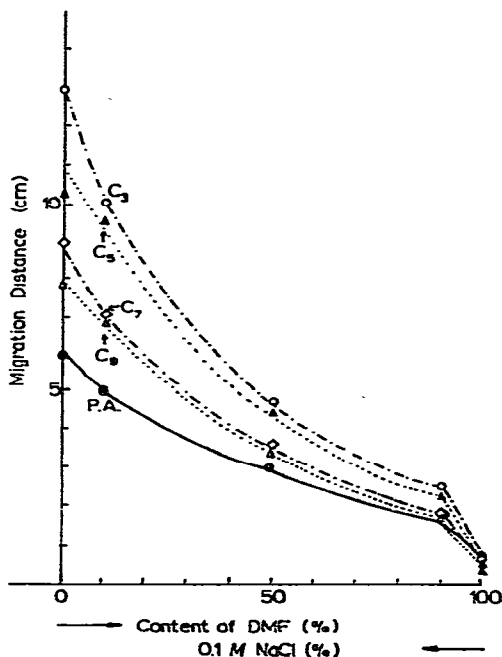


Fig. 2. Relation between migration distance and content of DMF. Conditions: voltage gradient, 1000 V per 30 cm; migration time, 30 min; migration temperature, *ca.* 20°; supporting solution, a mixture of 0.1 M NaCl and DMF; sample solution, C₃, C₅, C₇, C₉ and picric acid (P.A.).

Fig. 3. Relation between migration distance and molecular weight of the organic component in the supporting solution. Conditions: voltage gradient, 1000 V per 30 cm; migration time, 30 min; migration temperature, *ca.* 17°; sample solution, C₃, C₅, C₇, C₉ and picric acid (P.A.). Organic component: A = water; C = methanol; D = ethanol; E = propanol; F = DMSO.

weight. However, longer linear molecules such as stearylamine did not migrate in any of the supporting solutions, probably owing to adsorption on the filter paper and/or a molecular sieving effect. The maximum migration due to capillary action at a position 5 cm from the centre of the filter paper was 0.5 cm under these experimental conditions^{6,10}. The migration distances shown in Fig. 1 were not corrected for the capillary action.

The relation between the migration distance and the content of DMF in the supporting solution is shown in Fig. 2. The migration distance largely decreased with increasing content of DMF, in sharp contrast to the migration of anions⁶. Solvation of the amine cations by DMF probably causes the mobility of the amines to decrease with increasing content of DMF in the supporting solution. The electric current decreased with decreasing content of sodium chloride in the solution. This is not considered to be the main cause of the decrease in mobility because the mobilities of amines in 0.1 M NaCl-DMF (1:1) were identical to those in 0.5 M NaCl-DMF (1:1).

The migration distances of amines varied when the supporting solutions contained different organic constituents as shown in Fig. 3. All of the solutions consisted of 0.1 M aqueous sodium chloride solution-organic solvent (1:1). Thus, these results show that the organic components specifically affect the migration of the amines. Surprisingly, the migration distance decreased with increasing molecular weight of the organic solvent. According to Stoke's law, the mobility should be inversely proportional to the viscosity of the supporting solution and to the radius of the migrant¹. This is not borne out by the lower mobilities of the amines in methanol than in water which has a higher viscosity. The difference in mobilities also can not be explained by the difference in dielectric constants of the supporting solutions. Thus, we conclude that the decrease in the mobilities with increasing molecular weight of the organic component in the supporting solution is due mainly to a solvation effect.

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