

CALCULATION OF THE ZONE MOBILITIES OF ALIPHATIC AMMONIUM IONS IN PAPER ELECTROPHORESIS

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KUNKEL AND TISELIUS¹ suggested that the mobility u of an ion in a column of solution is reduced to u_p when the column is absorbed by a strip of filter paper of the same length, so that

$$u_p = \rho u \quad (1)$$

where ρ (< 1) is an "obstructive factor" characteristic of the paper strip. Methods of determining ρ have been described². The zone mobility u_z of the same ion may be defined as the velocity (corrected for electroendosmotic flow¹) of an observable zone of the ion in a paper strip moistened with a solution of other ions (the background electrolyte) when the apparent potential gradient³ along the strip is unity. It has been shown³ that $u_z \approx u_p$ when the amount spotted on the paper to form the zone is small, and when the conductance of the background solution is high. Then under these conditions

$$u_z/\rho \approx u \quad (2)$$

However, when the specific conductance of the background solution is low, u_p and u_z may be expected to diverge³. Because the conditions of concentration, etc., in the area of the zone are so ill-defined, it is impossible at the moment to delimit the conditions under which equation (2) is valid. It consequently becomes of interest to test it for ionic zones and background solutions of varying nature and concentration. Such a test is also implicitly a test of the hypothesis of KUNKEL AND TISELIUS¹, which has been generally^{4,5} but not universally⁶ accepted.

In the present work we have attempted to do this by observing the velocities of zones of monovalent and divalent ammonium ions in strips of Whatman No. 3 paper kept at 25°. These ions were chosen because they have widely differing mobilities⁷, but are all easily revealed by a ninhydrin spray. The distances travelled by the ionic zones were corrected for the electroendosmotic flow of the solution, as revealed by the movement of a zone of N- β -hydroxyethyl-2,4-dinitroaniline⁸ from the starting line. From a consideration of the pK_a values of related compounds⁹, the pK_a of this conveniently coloured water-soluble compound may be estimated as about -3. Hence it should not be appreciably ionized in the most strongly acidic background solution used in this work⁹. This was confirmed by experiments with tetramethyl glucose¹⁰ as neutral marker substance, which showed the same electroendosmotic flow. On the other hand, *s*-trinitrobenzene¹¹ was an unsatisfactory marker substance in aqueous solution, having an R_F of less than 1.00.

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In each experiment the value of ρ for the paper strip was determined by an empirical equation², and u_z/ρ (termed the "experimental mobility") compared with u .

EXPERIMENTAL

Materials

The amines, all commercial products, were used in 1% ethanolic solution. It was necessary to add a few drops of acetic acid to dissolve hexadecyl- and octadecylamine completely. A 1% solution of N- β -hydroxyethyl-2,4-dinitroaniline in 50% aqueous ethanol was used as neutral marker substance.

Electrophoretic procedure

A procedure essentially similar to that of KUNKEL¹² was employed for preparing the paper and applying 2- μ l samples of amine and marker solutions, using weighed strips, 57.1 \times 12.0 cm, of Whatman No. 3 paper and weighed glass plates, 50.8 \times 15.0 cm. The paper-glass plate "sandwich" was held tightly by screw clamps between brass plates (49.0 \times 15.2 cm) of FOSTER'S design¹⁰, kept at $25.0 \pm 0.5^\circ$ by circulating water. An attempt was made to tighten the clamps each time to the same extent. The protruding ends of the paper strips dipped into 600 ml of electrolyte solution in two plastic vessels, connected by agar-potassium chloride bridges with two beakers of saturated sodium chloride solution containing carbon electrodes. The levels in the two plastic vessels were equalized by a siphon connection.

The paper was allowed to equilibrate for 20 min. Separate experiments showed that during this time a flow of solution from each vessel towards the centre of the strip became complete^{11,12}, and the temperature of the paper became steady². The siphon was then removed. Two thin strips of aluminum foil were clamped across the paper strip immediately beside each end of the glass plates (*i.e.* 50.8 cm apart), and were connected to a Model 40 Universal Avometer. The D.C. potential applied to the carbon electrodes was adjusted to maintain a constant potential difference across the 50.8 cm strip of paper of 400 ± 5 V, and thus an apparent potential gradient of 7.88 ± 0.10 V/cm. The current varied with the concentration of background electrolyte, but was usually less than 10 mA. It was shown in separate experiments with a thermocouple in contact with the paper that such a current would lead to a temperature difference of less than 0.5° between the paper and the brass plates.

After 30-70 min, the current was stopped, the ends of the paper strip protruding beyond the glass plates were torn off, and the moist paper and glass plates were weighed. The absorbance α (in ml/g) of the paper strip was calculated in the manner described previously², and thence the obstructive factor ρ from the equation:

$$\rho = \frac{\alpha - 0.21}{\alpha + 0.67} \quad (3)$$

Under the conditions of our experiments the absorbance varied within narrow limits, being 1.55 ± 0.10 ml/g in 29 out of 31 experiments, corresponding to ρ values of 0.60 ± 0.02 .

The paper was removed from between the glass plates, dried, sprayed with a 1% ethanolic solution of ninhydrin containing a few drops of quinoline, and heated. The distances between the centres of the blue zones of the amines and a line drawn through the centres of zones of marker substance were measured, and the zone mobilities u_z (in $\text{cm}^2 \text{ volt}^{-1} \text{ sec}^{-1}$) and experimental mobilities u_z/ρ thence calculated. The latter are shown plotted as experimental points in Figs. 1, 3 and 4.

RESULTS AND DISCUSSION

Although most of the amines were applied to the paper in the free form and not as salts, their zone mobilities in dilute background solutions of hydrochloric acid and potassium chloride were almost the same (Fig. 1). This unexpected result indicated

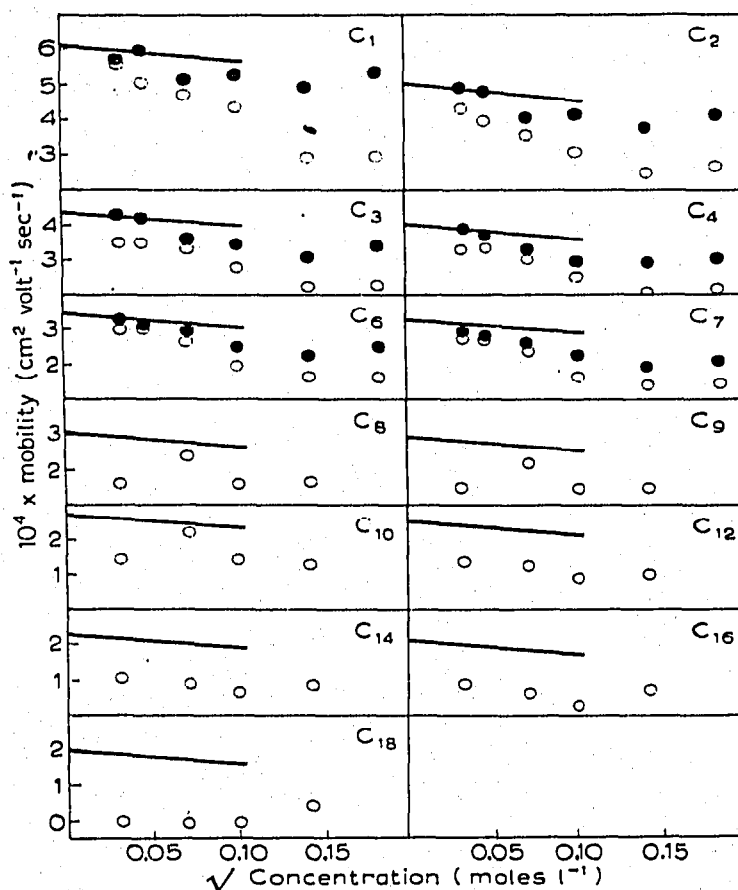
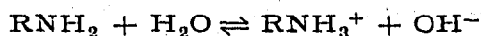


Fig. 1. Mobilities of alkylammonium ions, from methyl- (C_1) to octadecyl-ammonium (C_{18}), in various concentrations of aqueous hydrochloric acid (open circles) and potassium chloride (closed circles). Curves show mobilities calculated from equation (4).

that protonation of the amines in the latter solution rapidly became complete. A mechanism for this protonation is indicated in Fig. 2. The original amine solutions had concentrations of about 0.3–0.03 M , which in the spots on paper would become diluted twenty fold or more³. At this dilution the amines are 10–30% ionized according to the equation:



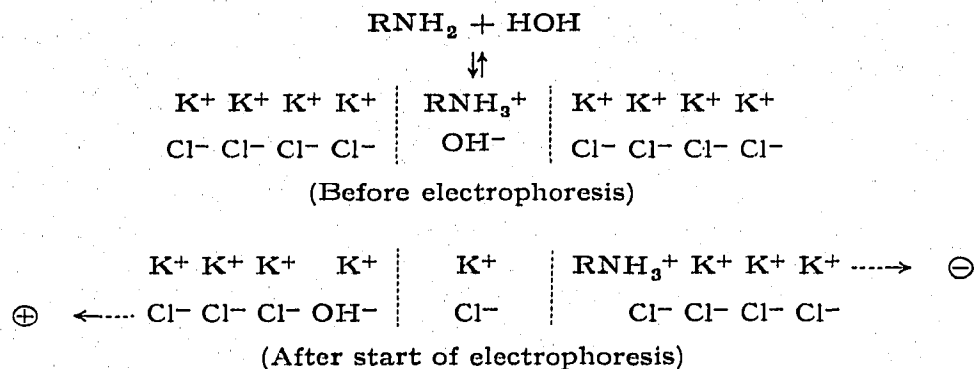


Fig. 2. Schematic representation of changes during electrophoresis of a spot of aliphatic amine in background potassium chloride solution.

The movement of RNH_3^+ and OH^- away from each other and from uncharged RNH_2 (Fig. 2) evidently enables ionization to go to completion.

In moderately concentrated (over 0.01 *N*) solutions of hydrochloric acid or potassium chloride, the zones of ammonium ions were round and well-defined; with more dilute solutions they became, as expected³, elongated in the direction of the current. With the most dilute solutions the zones were ill-defined, and these zone mobilities are likely to have the largest experimental errors. The theoretical mobilities u , given by the curves in Fig. 1, were calculated from the equation

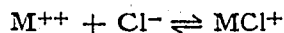
$$u = u_0 - (30.16 + 0.2605 u_0 F) \sqrt{I}/F \quad (4)$$

where u_0 is the mobility in infinitely dilute solution. This is a form of the Onsager equation¹³ giving the effect of the ionic strength I of a solution containing only univalent ions on the mobility of one ionic species in water at 25°, and holds to within about 1% for concentrations up to 0.01 *N*. We have assumed I equal to the concentrations of the background solutions, although the ionic strengths in the zones of ammonium ions were probably slightly greater³. Values of u_0 for the methylammonium¹⁴, ethylammonium¹⁵, propylammonium¹⁵ and dodecylammonium¹⁶ ions were calculated from equivalent conductances in the literature, and values for the other ions from an empirical equation⁷.

In view of the various possible sources of error, the experimental mobilities of the smaller monovalent ammonium ions in low concentrations of background electrolyte (Fig. 1) agreed reasonably well with the theoretical mobilities. However, they were lower than theoretical in the more concentrated background solutions of hydrochloric acid (Fig. 1). It is possible that for these solutions equation (3) yields erroneous values of ρ . The equation was derived from a study² of the conductances of paper strips soaked with solutions of neutral salts, in which the cellulose swelled so that its non-conducting volume increased by about 15%. Possibly with strong hydrochloric acid solutions the swelling is greater, and hence ρ smaller, than indicated in equation (3); a further investigation of the equation for these solutions is indicated.

Attempts to measure the zone mobilities of divalent ammonium ions (M^{++}) in various concentrations of background hydrochloric acid failed because of excessive streaking of the zones. This is probably explained by the fact that even in very dilute

solutions, divalent ions have a strong tendency to form ion-pairs with ions of opposite charge¹³:



Because of its reduced charge, the mobility of MCl^{+} will be about half that of M^{++} . Assuming the concentration of Cl^{-} within the zone of M^{++} to be about the same as in the rest of the paper, the concentration of M^{++} will be highest at the edges, and of MCl^{+} at the centre of the zone. Consequently, the amine at the front edge of the zone should migrate forward more rapidly than the amine at the centre. On the other hand, the rapidly moving M^{++} from the back edge of the zone will move into the more concentrated region of the centre and tend there to form MCl^{+} . This would explain the "streaking forward" appearance of the zone.

The sharp drop in experimental mobilities in 0.001 *N* hydrochloric acid for monovalent ions larger than heptylammonium, and in 0.005 *N* acid for monovalent ions larger than decylammonium (Fig. 1), is due to reversible adsorption of the ions

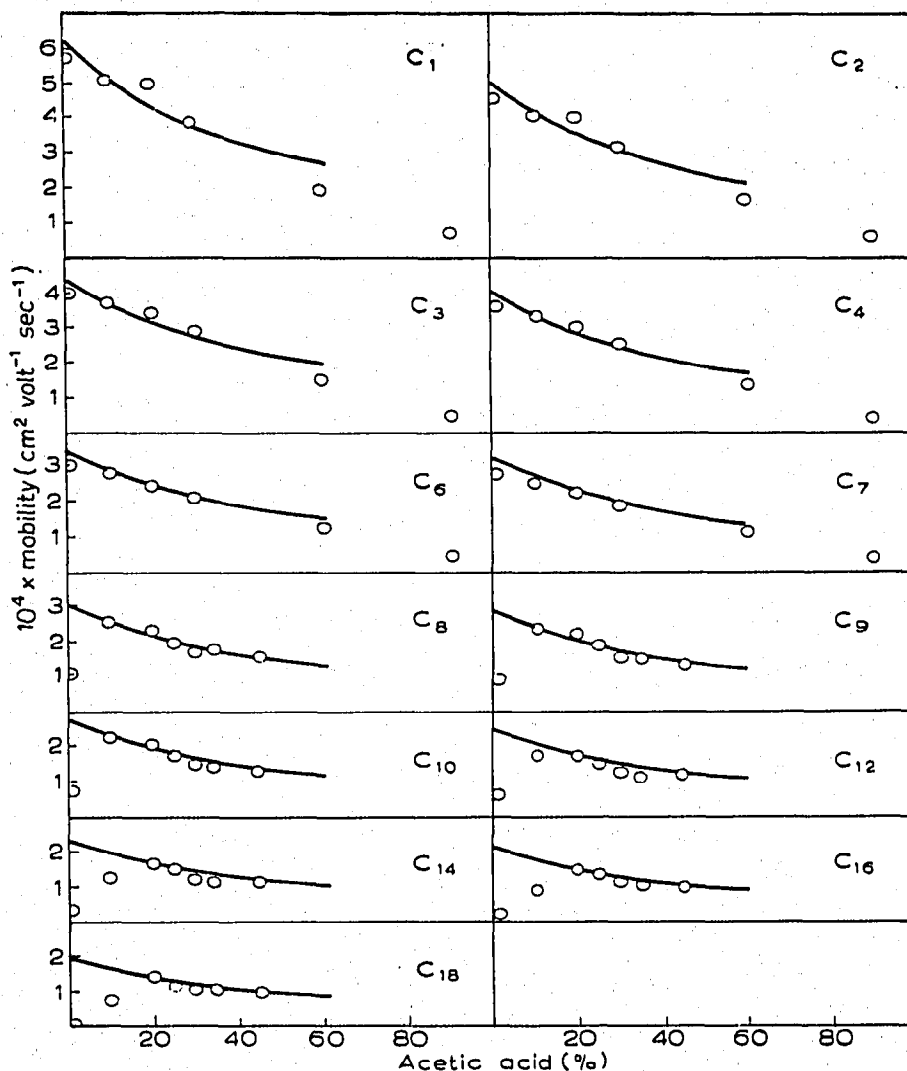


Fig. 3. Mobilities of alkylammonium ions in various concentrations of aqueous acetic acid. Curves calculated from equation (5).

by the paper^{17, 18}. This was shown by the use of strong aqueous acetic acid solutions, which are known to prevent adsorption of long chain ions¹⁸. Because of the changes in dielectric constant, etc., the calculation of ionic mobilities in these media by the Onsager equation is difficult. However, even fairly concentrated solutions are of low ionic strength, and so the curves giving the theoretical mobility u_a (Fig. 3) have been calculated from the equation

$$u_a \eta_a = u_o \eta_o \quad (5)$$

where η_o and η_a are the viscosities of water and of the aqueous acetic acid solutions at 25°¹⁹, the effect of ionic strength being ignored. The experimental mobilities are in rough agreement with this curve for concentrations of acetic acid up to about 50%; in stronger solutions interionic effects prevent equation (5) from being even approximately correct. Over the whole range of concentrations studied, the points to a good approximation lie on straight-line curves given by the empirical equation

$$u_a = u_o (1 - 0.01 a) \quad (6)$$

where a is the percentage of acetic acid in the solution.

In 1% acetic acid solution adsorptive effects become noticeable with the octylammonium ion; in 10% acetic acid, with dodecylammonium ion. In 20% acetic acid these effects are not perceptible with the octadecylammonium ion, the largest ion tested.

Divalent ammonium ions travelled as round, well-defined spots in aqueous acetic

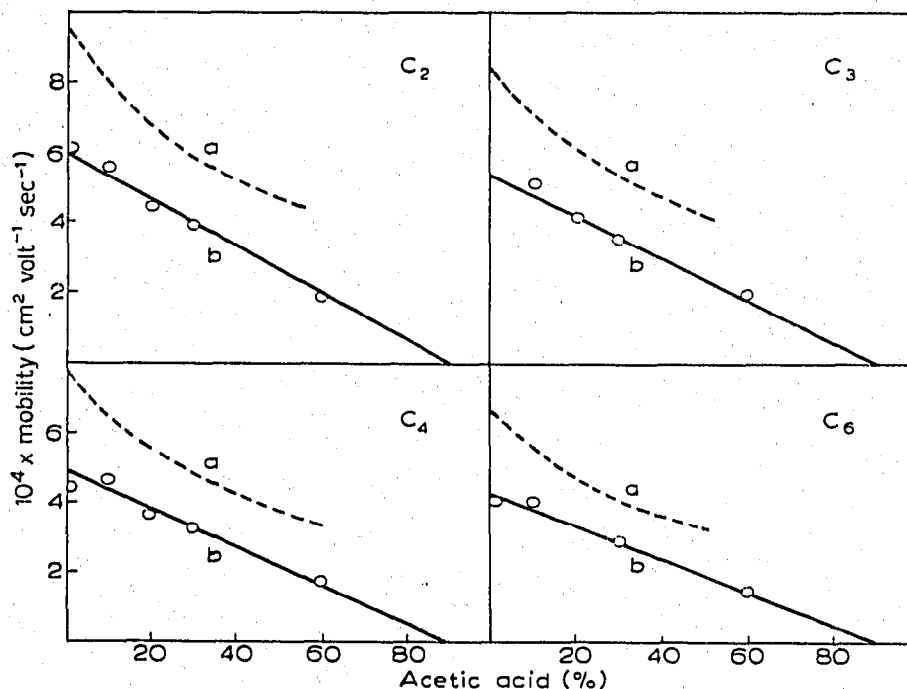
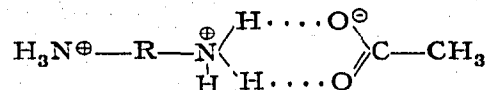


Fig. 4. Mobilities of ethylene- (C_2), trimethylene- (C_3), tetramethylene- (C_4), and hexamethylenediammonium (C_6) ions in various concentrations of aqueous acetic acid. Theoretical curves: a, equation (5); b, equation (7).

acid. As shown in Fig. 4, their experimental mobilities were considerably lower than mobilities calculated from equation (5), and in fact fitted reasonably well the empirical equation

$$u_a = 0.64 u_o (1 - 0.011 a) \quad (7)$$

Thus even in the most dilute background solution, the mobilities of the ions were less than two-thirds the theoretical mobility in infinitely dilute solution in water. This indicates that even in these solutions of low ionic strength, the divalent ammonium ions have a strong tendency to form ion-pairs with acetate ions; these ion-pairs of the general formula



may derive added stability from hydrogen-bonding¹⁸ as shown. It is possible that in solutions of weak electrolytes such as acetic acid the difference in degree of ion association for the inner and outer zones of polyvalent ions, postulated above for hydrochloric acid solutions, is not found, and hence the zones travel as round spots. It is difficult to envisage the exact mechanism of this "levelling effect", but further work on the mobilities of polyvalent ions in background solutions of weak electrolytes would be desirable. It is interesting to note that SLUYTERMAN²⁰ found that a background solution of 33% aqueous acetic acid (ionic strength about 0.02) gave much more compact spots of the polyvalent ion insulin than did a solution of glycine or citrate buffer.

In summary, the results with various background solutions indicate that if adsorptive effects are avoided, the zone mobilities of ions are proportional to their mobilities in free solution. Since the ions tested differed widely in their sizes, the results support the views of KUNKEL AND TISELIUS¹ as opposed to those of McDONALD⁶. The "barrier effects" postulated by the latter probably become of importance only when a fine-structured supporting medium such as a gel is used in place of filter paper²¹.

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SUMMARY

In a test of current theories of paper electrophoresis, the mobilities of zones of aliphatic ammonium ions were determined in moist paper between glass plates kept at $25.0^\circ \pm 0.5^\circ$. Using very dilute solutions of hydrochloric acid or potassium chloride as background electrolytes, the zone mobilities of small monovalent ions were approximately equal to the mobility calculated for the ion in free solution multiplied by an "obstructive factor" due to the paper. Large monovalent ions showed low zone mobilities because of adsorption by the paper. Zones of divalent ions moved in background hydrochloric acid solutions with excessive streaking. Mono- and divalent ions moved in well-defined zones in aqueous acetic acid of varying concentrations; their zone mobilities were given with fair precision by empirical equations taking account of the viscosities of the media.

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