

MOBILITIES OF AMINO ACIDS AND PEPTIDES IN PAPER ELECTROPHORESIS

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INTRODUCTION

The relative zone mobility u_r of an ion X is defined as the ratio of its zone mobility to the zone mobility of a standard ion (we have used butylammonium as the standard cation and picrate as the standard anion¹). In a previous communication¹ we showed that u_r is given by the equation:

$$u_r = u\rho/u_{(\text{std})}\rho_{(\text{std})} \quad (1)$$

where u and $u_{(\text{std})}$ are the mobilities of X and of the standard ion in free solution, and ρ and $\rho_{(\text{std})}$ are the adsorptive factors of X and of the standard ion. The adsorptive factor measures the retardation of an ion by reversible adsorption on to the cellulose fibres of the paper and for most small ions can be ignored (*i.e.* $\rho = 1$). However, for ions having large flat hydrophobic faces, adsorptive effects become appreciable and $\rho < 1$.

The possibility of calculating u_r depends on the ability to calculate $u/u_{(\text{std})}$ and $\rho/\rho_{(\text{std})}$.

Our previous communication¹ dealt with the calculation of the first term, when the shape of the ion under consideration was approximately spherical or ellipsoidal. In the present paper we extend the calculations to several amino acids and peptides, some of them of irregular shape, measured against butylammonium as the standard cation. The second term $\rho/\rho_{(\text{std})}$ has been evaluated experimentally; the instances where it may safely be assumed equal to unity are discussed below.

CALCULATION OF $u/u_{(\text{std})}$

The amino acids and peptides listed in Table I were run against butylammonium as standard ion in hydrochloric acid at pH 1.51 ± 0.04 (measured with a glass electrode). The experimental procedure has already been described¹. Since all the ions under investigation are monovalent, they will suffer about the same proportional retardation from interionic effects in a solution of given ionic strength, so that approximately:

$$u^+/u_{(\text{std})}^+ = u_0^+/u_0^+_{(\text{std})} \quad (2)$$

where u_0^+ and $u_0^+_{(\text{std})}$ are cation mobilities in infinitely dilute solution.

TABLE I
RELATIVE ZONE MOBILITIES (u_r^+) OF AMINO ACIDS AND PEPTIDES AT pH 1.5

No.	Compound	z	r_w^\pm/r_w^+	u_r^+ (calc.)	u_r^+ (obs.)
1	Leucine	0.890	1.01	0.65	0.67
2	Norleucine	0.890	1.01	0.65	0.68
3	Valine	0.880	1.01	0.69	0.68
4	Norvaline	0.898	1.01	0.70	0.65
5	Threonine	0.823	1.01	0.66	0.67
6	Serine	0.853	1.01	0.76	0.70
7	Proline	0.772	1.01	0.69	0.66
8	Alanine	0.895	1.02	0.82	0.83
9	Asparagine	0.810	1.01	0.68	0.68
10	Phenylalanine	0.720	1.01	0.51	0.53
11	Tryptophan	0.904	1.01	0.48	0.49
12	Glycine	0.895	1.02	0.96	0.97
13	β -Alanine	0.994	1.10	1.02	1.02
14	<i>o</i> -Aminobenzoic acid	0.831	1.01	0.61	0.19
15	<i>m</i> -Aminobenzoic acid	0.982	1.10	0.68	0.65
16	<i>p</i> -Aminobenzoic acid	0.910	1.10	0.69	0.66
17	Glycylglycine	0.892	1.10	0.86	0.82
18	Diglycylglycine	0.985	1.07	0.70	0.72

acid zwitterions (II). It has been shown that a modified STOKÉ's equation applies to the diffusion in water of these and other neutral organic molecules⁷:

$$D_0^\pm = kT/5\pi r_w^\pm \eta (f/f_0) \quad (6)$$

$$(f/f_0) = kT/5\pi D_0^\pm r_w^\pm \eta \quad (6a)$$

where k is Boltzmann's constant, T the absolute temperature, and r_w^\pm the Van der Waals radius of the zwitterion. Equation (6) has been found to hold fairly well for glycine and alanine (which are sufficiently ellipsoidal for (f/f_0) to be obtainable from PERRIN's equations)⁸, and hence may be assumed to apply to the more irregular amino acids which deviate widely from ellipsoidal shapes. Values of D_0^\pm for the amino acids and peptides of Table I are available from the careful work of LONGSWORTH⁹. LONGSWORTH's experimental values are for low solute concentrations, rather than for infinite dilution, but will differ by only 1-2 % from values for the latter condition.

(c) Calculation of the ionic radius r_w^+

The Van der Waals volume V_w of organic ions may be obtained by the addition of the volumes of the constituent atoms or groups, which have already been listed^{1, 2, 10, 11}. The Van der Waals radius r_w is thus given by the relation: $V_w = 4\pi r_w^3/3$. However, in aqueous solution it was sometimes found necessary to assume an increase in volume because of hydration, in order to compute correct ionic mobilities by the use of Eqn. (3). Thus it was found that the volume of the ionized carboxylate group (29.5 Å³) had to be increased by about 40 Å³ to account satisfactorily for the mobilities of ions containing this group². On the other hand, it was not found necessary to increase the Van der Waals volume (11.5 Å³) of the —NH₃⁺ group to account satisfactorily for the mobilities of substituted ammonium ions in water^{2, 3}, in

spite of the fact that other types of evidence¹² indicate this group also to be hydrated. It would seem that with substituted ammonium ions the effect of hydration is counterbalanced by other effects, such as loosening of the surrounding water structure¹³, insofar as mobilities are concerned.

The Van der Waals volumes calculated in this way prove satisfactory in explaining the mobilities of monofunctional and some simple polyfunctional compounds^{2,3}. However, there is some evidence that hydration increases when several polar groups are near each other in an ion², perhaps through cooperative effects in binding water molecules. Thus the cations of glycine (I; R = H) and alanine (I; R = CH₃) were found to have mobilities considerably less than calculated from Eqn. (3)¹. The experimental mobilities may be brought into line with calculated values if it is assumed that the Van der Waals volumes of these cations are increased by about 30 Å³.

The work described in the present paper with the more extended series of amino acids of Table I corroborates this finding. The relative mobilities of these cations were obtained from Eqn. (7), obtained by combining Eqns. (3) and (6):

$$u_0^+ = 1.602 \times 10^{-12} z r_w^{\pm} D_0^{\pm} / k r_w^+ T \quad (7)$$

For water solutions at 25° this becomes:

$$u_0^+ = 38.9 z D_0^{\pm} r_w^{\pm} / r_w^+ \quad (8)$$

D being measured in cm²·sec⁻¹. When butylammonium ($u_0 = 3.88 \times 10^{-4}$ cm²·sec⁻¹·V⁻¹ at 25°) is used as the standard ion:

$$u_0^+ / u_{0(\text{std})}^+ = 1.00 \times 10^5 z D_0^{\pm} r_w^{\pm} / r_w^+ \quad (9)$$

The mobility ratio calculated from Eqn. (9) is for aqueous solutions at 25°. However, the relative mobilities of organic ions having radii larger than about 3 Å are not affected by temperature². In the present work temperatures were about 30°, but were not controlled closely.

(d) Determination of $\rho/\rho_{(\text{std})}$

The adsorptive factor ($\rho_{(\text{std})}$) of the butylammonium ion is known to be 1.00¹. The same adsorptive factors would be anticipated for the cations of all the amino acids and peptides listed in Table I, except perhaps phenylalanine, tryptophan and the aminobenzoic acids, which contain aromatic rings. The adsorptive factors were determined by the method described earlier¹, and were found to be approximately 1.00 for all the amino acids (including phenylalanine) in aqueous hydrochloric acid (pH 1.5) on Whatman No. 1 paper, with the exception only of *o*-aminobenzoic acid ($\rho = 0.86$), *m*-aminobenzoic acid ($\rho = 0.82$), *p*-aminobenzoic acid ($\rho = 0.82$), and tryptophan ($\rho = 0.80$). The different values obtained for phenylalanine and the aminobenzoic acids illustrate the fact that while the adsorptive factor is in general governed by the area of flat hydrophobic surface in the molecule, other more specific factors are also involved².

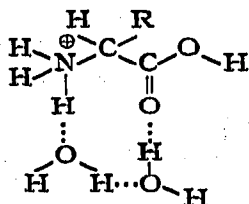
COMPARISON OF CALCULATED AND OBSERVED RELATIVE ZONE MOBILITIES

Combining Eqns. (1), (2) and (9) gives:

$$u_{r^+} = 1.00 \times 10^5 \rho z D_0^\pm r_w^\pm / r_w^+ \quad (10)$$

when butylammonium is the standard ion. If no hydration of the amino acid cation (I) is assumed, r_w^\pm / r_w^+ varies from 1.18 for the smallest ion (glycine) to 1.07 for the largest (tryptophan), because of the increase in volume on ionization of unhydrated $-\text{CO}_2\text{H}$ to hydrated CO_2^- . The use of such values led to systematically high values of u_{r^+} , the error being greater for the small ions than for the large ions. However, when it was assumed that V_w^+ of the cations was increased by 33 \AA^3 by hydration, and the consequent values of r_w^\pm / r_w^+ given in Table I were obtained, the calculated values of u_{r^+} were found to be in reasonably good agreement with the observed values*. Better agreement could not be expected, considering the various approximations in the calculations, and the precision of only about 3-4% in the observed values.

The unexpected hydration of the cations (I) may be a consequence of a cooperative effect of the ammonium and carboxyl groups in binding water molecules:



In that case the effect should drop off with separation of the groups. Some support for this idea comes from the cations after No. 12 in Table I. An increase of 10 \AA^3 in the V_w^+ for the β -amino acids (Nos. 13 and 14), and of zero for the remaining acids and peptides (Nos. 15-18) has been assumed in arriving at the calculated values of u_{r^+} in the table. It is evident that these values are in rough agreement with observed values, except in the case of *o*-aminobenzoic acid. We cannot explain the anomalously low mobility of this cation, which merits further investigation.

It is realized that in this treatment a naive picture of the conduction process is used: the decrease in mobility is attributed solely to an increased volume of migrating ion because of hydration. In fact the retardation of ions in aqueous solutions is due to a number of causes¹⁶, of which this is only one. However, this treatment is justified by its operational usefulness. It suggests that the mobilities of ammonium ions having aldehyde, keto or nitro groups near the $-\text{NH}_3^+$ group may be less than those having these groups more distant from the $-\text{NH}_3^+$ group.

Attempts were made to analyse the relative zone mobilities of amino acid

* The question of the relative sizes of amino acid cations, anions and zwitterions seems first to have been considered by SVENSSON¹⁴, who, however, lacked any experimental evidence on the subject.

** In a previous treatment¹⁶ relating u^+ and D_0^\pm , it was assumed that $r_w^\pm \sim r_w^+$. It is apparent, from inspection of r_w^\pm / r_w^+ values in Table I, that this assumption is in rough agreement with the results in the present paper.

anions (III) in aqueous piperidine at pH 11.4, using picrate as the standard anion. Qualitatively, the results appeared to indicate hydration of the anions (III) considerably in excess of the hydration assumed for simple carboxylate ions. However, the mobilities of α -amino acids in this and in other alkaline media varied in an erratic manner, for reasons not yet understood. The attempt to clarify this behaviour is being continued.

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SUMMARY

The relative zone mobilities of several protonated amino acids and two peptides have been calculated from the diffusion coefficients of the zwitterionic forms of the compounds. Good agreement with observed values was obtained if it was assumed that the Van der Waals volume of protonated α -amino acids is increased by 33 \AA^3 by hydration.

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