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# The Electric Mobilities of Glycine, Alanine, and Glycyl-glycine

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Mobility measurements on weak electrolytes are very scarce in the literature. For amino acids, there are only a few investigations on transference numbers and conductivities, which data have been brought together and discussed by Schmidt [1] in his monograph on proteins and amino acids. Recently, ionophoresis has been used for the separation of amino acids from each other and from peptides (Consden, Gordon, and Martin [2]; Butler and Stephen [3]). An extensive use of this method, however, necessitates a thorough knowledge of the mobilities of different amino acids and peptides under different conditions, especially pH.

## EXPERIMENTAL

The moving boundary systems used for the mobility studies are shown schematically in Fig. 1. The system 1 a gives a sharp descending boundary in the acid range, 1 b a similar boundary in the alkaline range. The amino acid or peptide radical is denoted by $A^-$, its zwitterionic form by $^+HA^-$, and its positive ion by $H_2A^+$. In some experiments, the formic acid was replaced by acetic acid.

This type of moving boundary systems for amino acids was proposed to one of us (H. S.) by Professor Tiselius in the year 1937. It was used by him and Eriksson-Quensel[4] in an electrophoretic study of the peptic digestion products of egg albumin. The sharpening of the amino acid boundary is due to a considerable pH change across the boundary and not, as is generally the case, to a great change in conductivity. The pH in phase (2), Fig. 1 a, is much lower than that in phase (3). Since the degree of dissociation of an

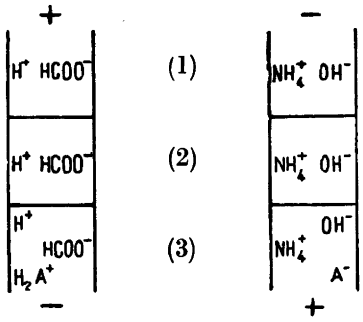



*Fig. 1. The moving boundary system.*

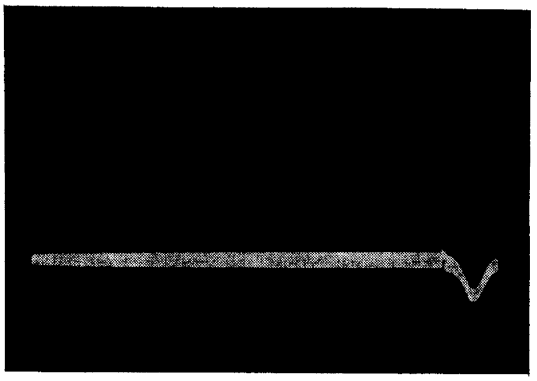


*Fig. 2. Optical pattern of glycine in ammonia. Plus is to the left.*

amino acid, and thus even its mobility, rises with an increasing acidity in the medium, amino acid radicals lagging behind the moving boundary by diffusion will find themselves in a medium where they possess a higher mobility, with the result that they will speed up and take over the boundary again very soon. In Fig. 1 b the situation is the same. Moving boundaries at which the sharpening effect is contributable to different states of dissociation of weak electrolytes were predicted by Miller [5]. In the system sodium acetate-acetic acid proposed by him, the sharpening of the $Na^+/H^+$ boundary is also a pH effect, the constituent mobility of the hydrogen radical being a function of the acetate ion concentration.

The concentration of the amino acid or peptide was in most cases 0.1 per cent. The formic acid and the ammonia concentrations in the bottom solutions (3) were varied to give different pH:es. Although rather low, the ionic strength was therefore not constant throughout the investigation. Estimated values of this function are included in the Tables 1—6. In the top solution (1), the concentration was chosen to secure gravitational stability at the stationary boundary between (1) and (2). Thus the formic acid concentration in (1) was constantly appreciably lower than that in (3), whereas the ammonia concentration in (1) was always higher than that in (3), due to the negative density increment of ammonia. The refractive index increment of the ammonical solutions seemed to parallel the concentration of ammonia, as is evident from Fig. 2.

The electrophoresis apparatus according to Tiselius was used, essentially in the form described by Svensson [6]. The electrodes were silver-silver chloride surrounded by 1 *M* sodium chloride solution. The positive limb was kept

closed during the runs, and no volume correction was carried out. This correction is well below the experimental errors since the current densities were very low in these experiments.

The conductivities of the amino acid solutions were measured in an ice bath, and the experiments were also run at a water-bath temperature of 0.0° C., ethyl alcohol being added to the water in order to make this possible.

The pH measurements were carried out at room temperature with a hydrogen electrode. Potassium biphthalate was used as a standard (pH 4.00); in order to avoid drifting readings due to catalytic reduction of the phthalate, the adjustment of the potentiometer was made while the hydrogen gas was bubbling through. The pH:es of the ammoniacal solutions were measured using hydrogen gas coming from a washing flask containing ammonia of the same concentration as in the sample in the pH cell. In this way systematic errors due to evaporation of ammonia were avoided; the readings were both constant and reproducible.

## RESULTS

The experimental data have been collected in the Tables 1—6. The first column gives the computed ionic strength, the second gives the concentration of formic acid (acetic acid; ammonia) used in the bottom solutions, the third the concentration of amino acid or peptide. In the fourth to sixth columns, we have the observed pH:es, conductivities, and mobilities. In Figure 3, the experimental mobilities have been plotted against pH. Since the spreading of the points is fairly great, the following method of finding the most probable mobility curve has been adopted.

The dissociation of a monovalent amino acid in the acid range is governed by the equation:

$$(H^+)\ (^+HA^-) = K\ (H_2A^+) \tag{1}$$

where $K$ is the thermodynamic dissociation constant and ( ) denotes activities. Passing from activities to concentrations except for the hydrogen ion, we have:

$$(H^+)\ [^+HA^-] = K\ f\ [H_2A^+] \tag{2}$$

where [ ] denotes concentrations and $f$ is the activity coefficient for a monovalent ion. From this equation, we can derive the degree of dissociation

$$\alpha = \frac{(H^+)}{(H^+) + Kf} \tag{3}$$

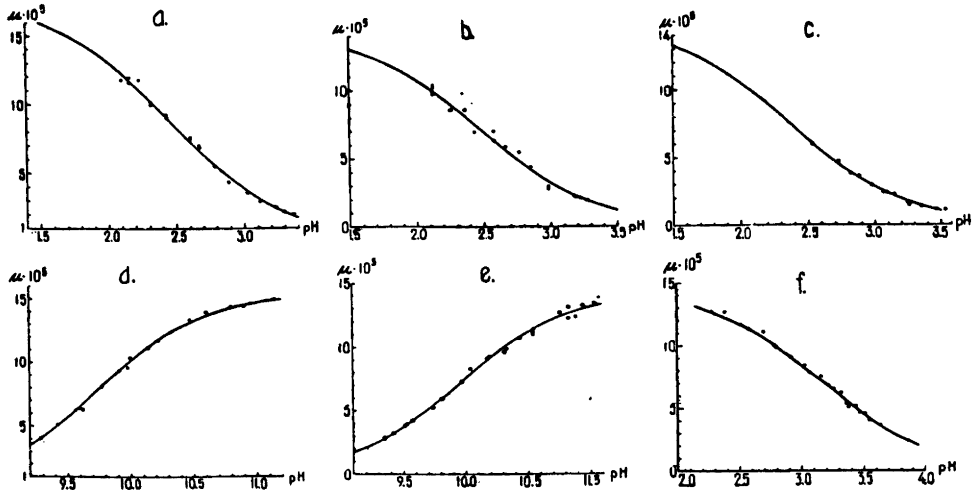



*Fig. 3. The Mobilities of glycine, alanine, and glycyl-glycine at 0° C as functions of pH.*

*a. The mobility of glycine in formic acid solutions.*
*b. The mobility of alanine in formic acid solutions.*
*c. The mobility of alanine in acetic acid solutions.*
*d. The mobility of glycine in solutions of ammonia.*
*e. The mobility of alanine in solutions of ammonia.*
*f. The mobility of glycyl-glycine in formic acid solutions.*

If the observed mobility is $u$ and the mobility of the free ion $H_2A^+$ is $u_o$ (in the same medium), we have the relation:

$$u = \alpha u_o \tag{4}$$

which gives with the aid of (3):

$$(H^+) = u_o \frac{(H^+)}{u} - Kf \tag{5}$$

Thus, if $f$ and $u_0$ are constant throughout the series of experiments, it can be concluded that the hydrogen ion activity should be a linear function of the ratio $(H^+)/u$. This is of course not exactly true since both the ionic strength and the concentration of formic acid (acetic acid) varies. Thus the experimental material cannot be interpreted in this simple way if the plot of $(H^+)$ versus $(H^+)/u$ shows a definite deviation from a straight line. If it does not,

*Table 1. The mobility of glycine at 0° C in formic acid solutions.*

| $\mu \cdot 10^3$ | mC HCOOH | per cent glycine | pH | $\varkappa \cdot 10^3$ | $u \cdot 10^5$ |
|---|---|---|---|---|---|
| 2 | 6 | 0.1 | 3.36 | 0.200 | 1.98 |
| | 8 | 0.1 | 3.29 | 0.230 | 2.19 |
| | 10 | 0.1 | 3.23 | 0.239 | 2.53 |
| | 15 | 0.1 | 3.11 | 0.296 | 2.47 |
| 3.5 | 20 | 0.1 | 3.02 | 0.353 | 3.57 |
| | 33 | 0.1 | 2.88 | 0.461 | 4.35 |
| 5.5 | 50 | 0.1 | 2.78 | 0.578 | 5.53 |
| | 75 | 0.1 | 2.67 | 0.729 | 6.90 |
| | 75 | 0.1 | 2.67 | 0.729 | 6.93 |
| 7 | 100 | 0.1 | 2.60 | 0.849 | 7.53 |
| | 100 | 0.1 | 2.60 | 0.845 | 7.45 |
| 9 | 200 | 0.1 | 2.43 | 1.283 | 9.02 |
| | 200 | 0.1 | 2.43 | 1.273 | 9.17 |
| 10 | 300 | 0.1 | 2.315 | | 10.01 |
| | 300 | 0.1 | 2.315 | 1.520 | 10.18 |
| 11.5 | 400 | 0.1 | 2.23 | 1.852 | 11.81 |
| | 500 | 0.1 | 2.16 | 2.092 | 11.60 |
| | 500 | 0.1 | 2.16 | 2.086 | 11.98 |
| 14.5 | 600 | 0.1 | 2.10 | 2.329 | 11.78 |

*Table 2. The mobility of alanine at 0° C in formic acid solutions.*

| $\mu \cdot 10^3$ | mC HCOOH | per cent alanine | pH | $\varkappa \cdot 10^3$ | $u \cdot 10^5$ |
|---|---|---|---|---|---|
| 2 | 10 | 0.1 | 3.18 | 0.233 | 2.22 |
| 3 | 20 | 0.1 | 2.98 | 0.342 | 2.84 |
| | 20 | 0.1 | 2.98 | 0.351 | 2.88 |
| | 35 | 0.1 | 2.85 | 0.478 | 4.37 |
| 4.5 | 50 | 0.1 | 2.76 | 0.590 | 5.46 |
| | 75 | 0.1 | 2.66 | 0.748 | 5.85 |
| 6 | 100 | 0.1 | 2.575 | 0.862 | 6.29 |
| | 100 | 0.1 | 2.575 | 0.948 | 6.99 |
| | 160 | 0.1 | 2.43 | 1.111 | 6.94 |
| 8 | 200 | 0.1 | 2.36 | 1.283 | 8.57 |
| 8.5 | 285 | 0.1 | 2.245 | 1.535 | 8.53 |
| | 500 | 0.1 | 2.12 | 2.100 | 10.20 |
| 11.5 | 500 | 0.1 | 2.12 | 2.060 | 9.77 |
| | 500 | 0.1 | 2.12 | 2.082 | 9.83 |
| | 500 | 0.1 | 2.12 | | 10.37 |

*Table 3. The mobility of alanine at 0° C in acetic acid solutions.*

| mC HAc | per cent alanine | pH | $u \cdot 10^5$ |
|---|---|---|---|
| 20 | 0.25 | 3.35 | 1.40 |
| 100 | 0.25 | 3.26 | 1.57 |
| 130 | 0.25 | 3.25 | 1.66 |
| 150 | 0.25 | 3.14 | 2.24 |
| 200 | 0.25 | 3.08 | 2.37 |
| 300 | 38.7 mC | 3.06 | 2.39 |
| 300 | 100 mC | 3.26 | 1.62 |
| 500 | 100 mC | 3.46 | 1.16 |
| 500 | 50 mC | 2.975 | 2.94 |
| 500 | 0.25 | 2.88 | 3.60 |
| 500 | 25 mC | 2.82 | 3.80 |
| 500 | 0.1 | 2.73 | 4.72 |
| 1000 | 0.1 | 2.54 | 6.00 |

*Table 4. The mobility of glycine at 0° C in solutions of ammonia.*

| $\mu \cdot 10^3$ | mC $NH_3$ | per cent glycine | pH | $\varkappa \cdot 10^3$ | $u \cdot 10^5$ |
|---|---|---|---|---|---|
| 3 | 6.35 | 0.1 | 9.28 | 0.207 | — 4.05 |
| | 10.0 | 0.1 | 9.42 | 0.262 | — 5.13 |
| 5 | 15.0 | 0.1 | 9.56 | | — 6.23 |
| | 16.5 | 0.1 | 9.59 | 0.330 | — 6.39 |
| | 25.34 | 0.1 | 9.77 | 0.423 | — 8.04 |
| | 36.2 | 0.1 | 9.92 | 0.489 | — 9.28 |
| | 43.1 | 0.1 | 9.98 | 0.514 | — 9.51 |
| 7 | 45.0 | 0.1 | 10.00 | 0.517 | — 10.27 |
| | 70.0 | 0.1 | 10.14 | 0.573 | — 11.05 |
| | 90.5 | 0.1 | 10.24 | 0.595 | — 11.66 |
| 8.5 | 165.2 | 0.1 | 10.47 | 0.686 | — 13.32 |
| | 226 | 0.1 | 10.60 | 0.751 | — 13.93 |
| 9 | 362 | 0.1 | 10.80 | 0.775 | — 14.32 |
| 10.5 | 452 | 0.1 | 10.91 | 0.819 | — 14.39 |
| 12 | 905 | 0.1 | 11.14 | 0.896 | — 14.94 |

*Table 5. The mobility of alanine at 0° C in solutions of ammonia.*

| $\mu \cdot 10^3$ | mC $NH_3$ | per cent alanine | pH | $\varkappa \cdot 10^3$ | $u \cdot 10^5$ |
|---|---|---|---|---|---|
| 2 | 5 | 0.1 | 9.35 | 0.1460 | — 2.86 |
| | 5 | 0.1 | 9.415 | 0.1460 | — 3.24 |
| 3.5 | 9 | 0.1 | 9.53 | 0.1936 | — 3.81 |
| 3.5 | 10 | 0.1 | 9.52 | 0.220 | — 3.87 |
| | 10.9 | 0.1 | 9.57 | 0.2091 | — 4.20 |
| | 15 | 0.1 | 9.74 | 0.2830 | — 5.24 |
| 4.5 | 20 | 0.1 | 9.81 | 0.3148 | — 5.92 |
| | 30 | 0.1 | 9.97 | 0.3703 | — 7.25 |
| 5.5 | 37.5 | 0.1 | 10.04 | 0.3804 | — 8.31 |
| | 50 | 0.1 | 10.18 | 0.4438 | — 9.04 |
| | 52 | 0.1 | 10.19 | 0.4459 | — 9.15 |
| | 75 | 0.1 | 10.31 | 0.4831 | — 9.56 |
| 6.5 | 80 | 0.1 | 10.33 | 0.4840 | — 9.80 |
| | 105 | 0.1 | 10.43 | 0.514 | — 10.69 |
| | 144.8 | 0.1 | 10.545 | 0.547 | — 11.00 |
| | 144.8 | 0.1 | 10.545 | | — 11.15 |
| 7.5 | 150 | 0.1 | 10.555 | 0.561 | — 11.40 |
| | 250 | 0.1 | 10.755 | 0.621 | — 12.74 |
| | 300 | 0.1 | 10.83 | 0.638 | — 12.27 |
| 8.0 | 300 | 0.1 | 10.83 | 0.641 | — 13.18 |
| | 350 | 0.1 | 10.89 | 0.652 | — 12.39 |
| | 400 | 0.1 | 10.95 | 0.667 | — 13.29 |
| | 500 | 0.1 | 11.04 | 0.681 | — 13.49 |
| 8.0 | 600 | 0.1 | 11.075 | 0.714 | — 13.91 |

however, the interpretation outlined may be accepted as a means of levelling the experimental data.

In ammoniacal solutions, the same treatment leads to a somewhat different equation:

$$(H^+) = \frac{Ku_0}{f}\frac{1}{u} - \frac{K}{f} \tag{6}$$

In this case, therefore, $(H^+)$ should be a linear function of $1/u$.

The plots of the hydrogen ion activity, taken as the negative antilogarithm of pH, against $(H^+)/u$ in acid and against $1/u$ in alkaline solutions are shown in Figure 4. It is evident that a definite curvature cannot be seen, which means that the assumption of a constant $f$ and of a constant $u_0$ does not violate the

*Table 6. The mobility of glycyl-glycine at 0° C in formic acid solutions.*

| $\mu \cdot 10^3$ | mC HCOOH | per cent peptide | pH | $\varkappa \cdot 10^3$ | $u \cdot 10^5$ |
|---|---|---|---|---|---|
| 4 | 10 | 0.2 | 3.545 | 0.260 | 4.05 |
| | 12 | 0.2 | 3.51 | 0.261 | 4.61 |
| 5.5 | 15 | 0.2 | 3.44 | 0.314 | 5.16 |
| | 18 | 0.2 | 3.38 | | 5.12 |
| | 19 | 0.2 | 3.355 | 0.345 | 5.34 |
| 6 | 21 | 0.2 | 3.33 | 0.360 | 6.24 |
| | 24.7 | 0.2 | 3.26 | 0.401 | 6.50 |
| | 35 | 0.2 | 3.16 | 0.493 | 7.51 |
| | 98.9 | 0.5 | 3.08 | | 7.81 |
| 8 | 49.5 | 0.2 | 3.03 | 0.575 | 8.41 |
| | 70 | 0.2 | 2.915 | 0.687 | 9.06 |
| 10 | 98.9 | 0.2 | 2.79 | 0.815 | 10.00 |
| | 98.9 | 0.2 | 2.79 | 0.816 | 9.94 |
| 11.5 | 180 | 0.2 | 2.58 | 1.098 | 11.30 |
| | 297 | 0.2 | 2.39 | 1.433 | 12.63 |
| 13 | 400 | 0.2 | 2.28 | 1.681 | 12.70 |

experimental results. The straight lines in these figures have been constructed with the aid of the method of least squares.

The two parameters of the straight lines give the limiting mobilities for complete dissociation and approximate figures for the p$K$:s. These data have been collected in Table 7. The p$K'$ values given there are the negative logarithms of $Kf$ in acid and of $K/f$ in alkaline solutions. Their deviations from the thermodynamic p$K$:s, also given in the table (taken from Cohn and Edsall [7]), are due in part to the factor $f$, in part to slight variations in $f$ and $u_0$ from experiment to experiment. Also, the thermodynamic p$K$ values refer to room temperature, whereas this investigation was carried out at 0° C. The limiting mobilities given in Table 7 cannot be regarded as very accurate. Mobility values from experiments performed at the most extreme pH:es exert a great influence on the values of $u_0$. These experiments are the least reliable ones since the sharpening of the boundaries was not very pronounced in high concentrations of formic acid or ammonia.

The curves in Figure 3 are the theoretical curves derived from the $u_0$ and p$K'$ values in Table 7. Numerical values taken from these curves for every tenth of a pH unit are presented in Table 8.

It was tried to extend the investigation to glycyl-glycine in ammonia, but certain difficulties arose in these experiments. The cause of the disturbances is not quite understood for the present.

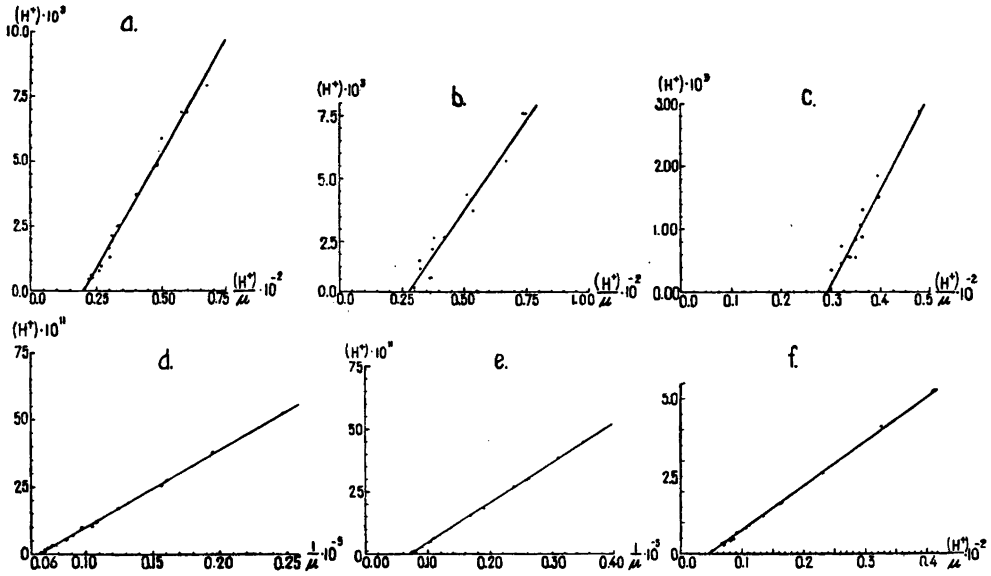



*Fig. 4.*

*a. Plot of $(H^+)$ versus $(H^+)/u$ for glycine in formic acid solutions.*
*b. Plot of $(H^+)$ versus $(H^+)/u$ for alanine in formic acid solutions.*
*c. Plot of $(H^+)$ versus $(H^+)/u$ for alanine in acetic acid solutions.*
*d. Plot of $(H^+)$ versus $1/u$ for alanine in ammoniacal solutions.*
*e. Plot of $(H^+)$ versus $1/u$ for alanine in ammoniacal solutions.*
*f. Plot of $(H^+)$ versus $(H^+)/u$ for glycyl-glycine in formic acid solutions.*

*Table 7. The mobilities at 0° C of the completely ionized forms, and the apparent pK values, of glycine, alanine, and glycyl-glycine in media of aqueous formic acid, acetic acid, and ammonia.*

| | Glycine in | | Alanine in | | | Glycyl-glycine in |
|---|---|---|---|---|---|---|
| | HCOOH | $NH_3$ | HCOOH | $CH_3COOH$ | $NH_3$ | HCOOH |
| $u_0 \cdot 10^5$ | 17.8 | − 15.4 | 14.5 | 15.1 | − 14.3 | 14.3 |
| p$K'$ | 2.44 | 9.73 | 2.46 | 2.36 | 9.95 | 3.18 |
| p$K$ | 2.35 | 9.78 | 2.35 | 2.35 | 9.87 | 3.08 |

*Table 8. The mobilities of glycine, alanine, and glycyl-glycine.*

| pH | Glycine in | Alanine in | | Glycyl-glycine in |
|---|---|---|---|---|
| | HCOOH | HCOOH | $CH_3COOH$ | HCOOH |
| 2.2 | 11.3 | 9.35 | | 12.9 |
| 2.3 | 10.35 | 8.6 | | 12.6 |
| 2.4 | 9.3 | 7.7 | | 12.2 |
| 2.5 | 8.3 | 6.9 | 6.3 | 11.8 |
| 2.6 | 7.3 | 6.1 | 5.5 | 11.25 |
| 2.7 | 6.35 | 5.3 | 4.7 | 10.7 |
| 2.8 | 5.4 | 4.5 | 4.0 | 10.0 |
| 2.9 | 4.6 | 3.85 | 3.35 | 9.3 |
| 3.0 | 3.9 | 3.2 | 2.8 | 8.6 |
| 3.1 | 3.2 | 2.7 | 2.3 | 7.8 |
| 3.2 | 2.65 | 2.2 | 1.9 | 6.95 |
| 3.3 | 2.2 | 1.8 | 1.55 | 6.1 |
| 3.4 | 1.75 | 1.5 | 1.25 | 5.3 |
| 3.5 | | | | 4.55 |
| 3.6 | | | | 3.9 |
| 3.7 | | | | 3.3 |
| 3.8 | | | | 2.8 |
| 3.9 | | | | 2.3 |
| 4.0 | | | | 1.9 |
| pH | $NH_3$ | $NH_2$ | | |
| 9.2 | − 3.5 | − 2.1 | | |
| 9.3 | − 4.2 | − 2.6 | | |
| 9.4 | − 4.9 | − 3.1 | | |
| 9.5 | − 5.7 | − 3.7 | | |
| 9.6 | − 6.6 | − 4.4 | | |
| 9.7 | − 7.45 | − 5.1 | | |
| 9.8 | − 8.3 | − 5.9 | | |
| 9.9 | − 9.2 | − 6.7 | | |
| 10.0 | − 10.0 | − 7.5 | | |
| 10.1 | − 10.8 | − 8.35 | | |
| 10.2 | − 11.5 | − 9.1 | | |
| 10.3 | − 12.15 | − 9.85 | | |
| 10.4 | − 12.7 | − 10.5 | | |
| 10.5 | − 13.2 | − 11.1 | | |
| 10.6 | − 13.6 | − 11.65 | | |
| 10.7 | − 13.9 | − 12.1 | | |
| 10.8 | − 14.2 | − 12.5 | | |
| 10.9 | − 14.45 | − 12.85 | | |
| 11.0 | − 14.6 | − 13.1 | | |

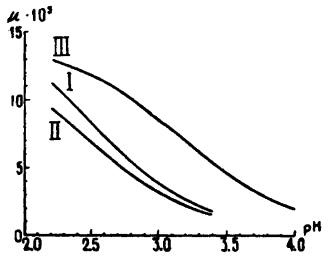



*Fig. 5. Comparison between the mobilities of glycine (I), alanine (II), and glycyl-glycine (III) in formic acid solutions.*

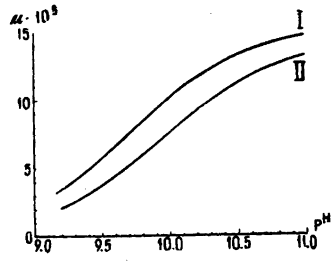
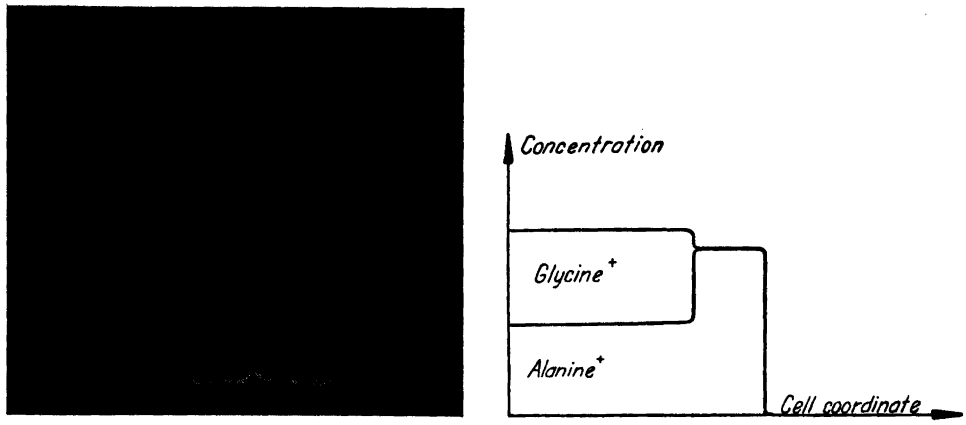



*Fig. 6. Comparison between the mobilities of glycine (I) and alanine (II) in ammoniacal solutions.*

## DISCUSSION

In the Figures 5 and 6, the pH-mobility curves of the two amino acids and the peptide have been put together for comparison. It is realized that an electrophoretic separation of them should be possible. In the case of glycine and alanine, this is due to a difference in limiting mobility since their pK:s are practically the same. The separation of glycyl-glycine from anyone of the amino acids is still more favourable, but this depends upon the appreciable difference in pK.

In order to demonstrate the applicability of the experimental conditions prevailing in these experiments for analytical and for preparative purposes, two more experiments were performed. Fig. 7 a shows the electrophoretic pattern of a mixture of equal parts (0.5 %) of glycine and alanine in formic acid solution at a pH of 2.79. The big spike is the (slower) alanine boundary. The glycine boundary appears very small because it is superimposed by a considerable negative concentration increment of alanine, as illustrated in Fig. 7 b. The optical pattern, therefore, does not give direct information about the composition of the original solution. For separation purposes it is favourable that the concentration of alanine in the isolated fraction is higher than in the original solution, but it also implies the risk that, with other mixtures of amino acids and peptides, the density decrement given by the surrounding amino acids may be greater than the increment due to the leading one (the one that disappears in the boundary). Then a gravitationally unstable system would result, which constitutes one limitation in the method. The exposure in Fig. 7 a was taken after the passage of 114 coulombs; thus the separation speed was very low, and it was not possible to prolong the experiment until the adjusted solution between the moving boundaries could be removed in sufficient quantity for analysis. Integration of the pattern gave 8.74 as the ratio




a. Fig. 7. b.

*a. Optical pattern of the system 0.5 % glycine and 0.5 % alanine in 0.3—C formic acid. Plus is to the right.*

*b. Schematic representation of the concentration changes of glycine and alanine across the boundaries in the same system.*

between the areas, roughly corresponding to 0.90 % alanine in the adjusted solution.

With a mixture of glycine and glycyl-glycine in the same pH range, it is evident from Fig. 5 that the conditions for separation are much more favourable, and, in addition, their density increments (per equivalent) parallel their mobilities. Fig. 8 shows the pattern from an electrophoresis experiment where the initial bottom solution contained 0.5 % glycine and 0.5 % glycyl-glycine in a formic acid medium of pH 2.76. The exposure was taken after

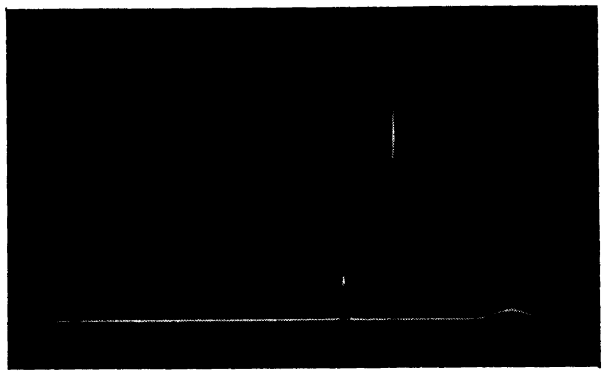


*Fig. 8.*

*Optical pattern of the system 0.5 % glycine and 0.5 % glycyl-glycine in 0.3—C formic acid. Plus is to the right.*

passage of 54 coulombs; thus the speed of separation was much greater than in the first experiment. The picture shows that the enclosed areas correspond more closely to the true composition of the original mixture than in the glycine-alanine experiment. Analysis of the adjusted solution containing glycine showed that pH was lowered to 2.69. At this pH glycine has a higher mobility than in the original solution of pH 2.76. The separation velocity between the boundaries is therefore somewhat smaller than what corresponds to the difference in mobility in the original solution. The glycine concentration in the isolated fraction was 0.77 %, as determined by Kjeldahl analysis. Integration on the plate gave an apparent concentration of 0.74 %.

In the moving boundary systems used in this investigation, we are very far from the »ideal» electrophoresis, where the migrations in the two limbs are mirror image processes. Deviations from this behaviour were called boundary anomalies by Tiselius [8]. The cause of this »anomalous» behaviour is twofold. First, the buffering action of amino acids and peptides, per unit weight, is much greater than in the case of proteins. Second, the concentrations of other ions are low. As a matter of fact, the amino acids themselves contribute with a considerable part to the ionic strength, and their effects on the pH and the conductivity of the solution are pronounced. In the experiments with two amino acids reported here, it was found that very great changes in these properties took place at the slower boundary, whereas at the faster boundary a considerable decrease in the concentration of the slowest component tended to counteract these changes. In agreement with theoretical expectations, the deviation from the state of »ideal» electrophoresis are greater the smaller the difference in mobility between the components. This is strikingly illustrated by the disproportion between the two peaks in each of the Figures 7 a and 8.

As in the case of the classical two-salt boundary systems for the measurement of transference numbers, advantage is here taken of these »anomalous» effects. For mobility measurements the sharp descending boundaries are very useful, and in separation runs the increased concentration of the slowest component should be advantageous. The limitations of the moving boundary method can be overcome by using powders or gels as stabilizers against convection. In this way it seems possible to isolate each component in a mixture in one run and with a high yield (for references, see Svensson [9]).

If a complete separation is required, the experimental conditions used here with only a weak acid or base in the medium is not suitable because the sharpening of the descending boundaries is accompanied by a corresponding blurring of the ascending boundaries. A more symmetrical migration can of course be obtained by the use of buffered solutions, although certain difficulties due to the high buffering capacity of the amino acids themselves will most

probably remain. In the acid range, it can be expected that formate buffers will be useful. In cases where the ionic strength is increased, the mobilities given here are of course not applicable unless they are supplemented with information about the influence of the medium. They can be expected to retain a qualitative significance, however, at least indicating the sequence of the different amino acids as to their mobilities at a given pH.

## SUMMARY

The electric mobilities of glycine, alanine, and glycyl-glycine have been measured in solutions of formic acid, acetic acid, and ammonia as functions of pH. The results were used to discuss the possibilities for electrophoretic separation of amino acids and peptides. A couple of experiments with mixtures of these substances illustrated, in addition, pronounced and interesting »boundary anomalies» which are easily explained by the moving boundary equation.

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