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THE THEORETICAL STUDY OF THE PAPER ELECTROPHORETIC SEPARATION OF LOW MOLECULAR WEIGHT SUBSTANCES

I. APPLICATION TO AMINO ACIDS AND PEPTIDES

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(First received November 17th, 1970; revised manuscript received February 8th, 1971)

SUMMARY

The relative zone mobility of substances with dissociable protons is expressed in terms of the molecular weight, the charge, the dissociation constant of the proton, and the pH of the background solution. Using the derived equation the separability of given mixtures is discussed.

Relative mobility against pH curves of amino acids and peptides were obtained using this equation. Many previously published results were compared with those calculated and were found to be in good agreement with the exception of a few amino acids. The differences between the observed and the calculated results is discussed based on the charge, the molecular weight, the chemical structure, and the degree of hydration of the substances under consideration.

Thus, if pK values and molecular weights of given substances are known, the optimum conditions for separation can be read off from calculated mobility–pH curves rather than obtained by laborious experimental tests.

INTRODUCTION

Paper electrophoresis is used mainly for the separation of various substances. As electrophoretic experiments are simple and usually fast this method soon became very popular in chemistry, biochemistry, and medicine[1,2]. Recently it has also been applied in the chemistry[3–6] of complexes and for the study of ion pair formation[7,8].

In spite of its frequent usage and the advances in techniques and apparatus, it is probable that no physico-chemical method has ever been used so extensively with so little knowledge of its fundamentals[9].

Because of the various parameters influencing the process the prospects of deriving a generally applicable theory are rather poor. The first approach to a theory was the application of the STOKES' law[10] and the adaptation of this law for the calculation of the mobilities of smaller size particles by changing the numerical factors of this law[11–13]. JOKL derived an equation for electrophoretic mobilities by studying the dependence of these mobilities on the mass of the species[14]. The mobilities in free solution and the decrease of the mobility on a paper strip due to an obstructive

factor[15–20] and an adsorptive factor[17–20] were investigated. CONSDEN *et al.* studied the mobilities of dissociating substances depending on their pK values[21]. Their equation could be applied to substances with only one dissociable proton.

Recently an equation for the mobility of substances with more than one dissociable proton, as a function of their molecular weight, the charge of the migrating species, their proton dissociation constant and the pH of the background solution, was derived[22].

In this paper we report detailed studies on this theory, its theoretical applicability in different cases, and experimental and calculated results are compared. We try to predict optimum conditions for separations with special emphasis on the study of amino acids and peptides.

THEORETICAL CONSIDERATIONS

The effective charge of molecules with intrinsic ionized groups such as organic acids, organic bases or amino acids depends on the pH of the medium and the pK(s) of the ionized group(s). Amphoteric molecules like amino acids (H_nA) form cations in an acidic solution by binding proton(s), while in a basic solution they form anions by dissociating proton(s). The degree of association or dissociation of the protons is a function of the pH of the background solution and the pK(s) of the ionized substance(s). The equilibrium relation between the proton dissociation of amino acids and the pH of the medium can be expressed as follows:

$$H_{m+n}A^{m} \overset{-H^+}{\rightleftharpoons} H_{m+n-1}A^{m-1} \ldots H_{m+n-(i-1)}A^{m-(i-1)} \overset{-H^+}{\rightleftharpoons} H_{m+n-i}A^{m-i}$$

$$\ldots HA^{-(n+1)} \overset{-H^+}{\rightleftharpoons} A^{-n} \qquad (1)$$

where m represents the maximum number of protons which can be bound by the imino or amino groups, n is the maximum number of dissociable protons which are due to –COOH, –OH, or –SH groups.

In this proton equilibrium system, eqn. 1, the proton-exchange reaction is reversible and the reaction rate is usually very high. Consequently the mean charge of all the ionic species in the equilibrium system can be expressed as follows:

$$Z = \frac{\sum_{i=0}^{m+n} (m - i)\,[H_{m+n-i}A^{m-i}]}{\sum_{i=0}^{m+n} [H_{m+n-i}A^{m-i}]} \qquad (2)$$

Eqn. 3. is the definition of the consecutive proton dissociation constant.

$$K_i = \frac{[H_{m+n-i}A^{m-i}]\,[H^+]}{[H_{m+n-(i-1)}A^{m-(i-1)}]} \qquad i = 1 \sim m + n \qquad (3)$$

By substitution of eqn. 3 into eqn. 2, we get

$$Z = \frac{m + (m-1)K_1/[H^+] + (m-2)K_1K_2/[H^+]^2 + \ldots + (m-i)K_1K_2 \ldots K_i/[H^+]^i + \ldots + (-n)K_1K_2 \ldots K_{m+n}/[H^+]^{m+n}}{1 + K_1/[H^+] + K_1K_2/[H^+]^2 + \ldots + K_1K_2 \ldots K_i/[H^+]^i + \ldots + K_1K_2 \ldots K_{m+n}/[H^+]^{m+n}} \quad (4)$$

As the calculation of the mean charge using eqn. 4 is complicated, we tried to simplify it.

If we choose only two successive ion species, such as $H_{m+n-(i-1)}A^{m-(i-1)}$ and $H_{m+n-i}A^{m-i}$ from all the species in the equilibrium system (1), the mean charge is given by

$$Z = \frac{(m-(i-1))\,[H_{m+n-(i-1)}A^{m-(i-1)}] + (m-i)\,[H_{m+n-i}A^{m-i}]}{[H_{m+n-(i-1)}A^{m-(i-1)}] + [H_{m+n-i}A^{m-i}]}$$

$$= (m-(i-1)) \cdot \frac{1 + \alpha_i K_i/[H^+]}{1 + K_i/[H^+]}$$

$$= (m-(i-1)) \cdot \left[\frac{1+\alpha_i}{2} + \frac{1-\alpha_i}{2} \cdot \frac{1 - K_i/[H^+]}{1 + K_i/[H^+]}\right]$$

$$= \frac{1}{2} + (m-i) + \frac{1}{2} \cdot \frac{1 - K_i/[H^+]}{1 + K_i/[H^+]} \quad (5)$$

where $\alpha_i = (m-i)/(m-(i-1))$
Now, if we define

$$K_i/[H^+] = e^{-2x} \quad (6)$$

we obtain

$$x = \frac{2.303}{2}(pK_i - pH) \quad (7)$$

Further, combining the well known relation

$$\frac{e^x - e^{-x}}{e^x + e^{-x}} = \frac{1 - e^{-2x}}{1 + e^{-2x}} = \tanh x \quad (8)$$

with eqns. 6 and 7, eqn. 5 is altered to eqn. 9:

$$Z_i = (m-(i-1))\left[\frac{\alpha_i + 1}{2} + \frac{1-\alpha_i}{2} \tanh\left\{\frac{2.303}{2}(pK_i - pH)\right\}\right]$$

$$= \left[\left(m - i + \frac{1}{2}\right) + \frac{1}{2} \tanh\left\{\frac{2.303}{2}(pK_i - pH)\right\}\right] \quad (9)$$

If $i < m$, Z_i in eqn. 9 is positive. On the other hand, if $i > m$, Z_i is negative. The mean charge is represented schematically as a function of the pH in Fig. 1. The first term, $m - i + \frac{1}{2}$ (eqn. 9), corresponds to the distance between the inflection point of the sigmoidal curve and the pH-axis, the $\frac{1}{2}$ in the second term represents the amplitude of the curves, and pK_i the distance between the inflection point of the

curves and the Z axis, respectively. The mean charge Z in Fig. 1 only refers to two successive ionic species. The overall charge achieved by the presence of various ionic species as a function of the pH is shown in Fig. 2, the calculation being based on eqn. 4. An equation representing this continuous Z–pH curve is not the simple summation

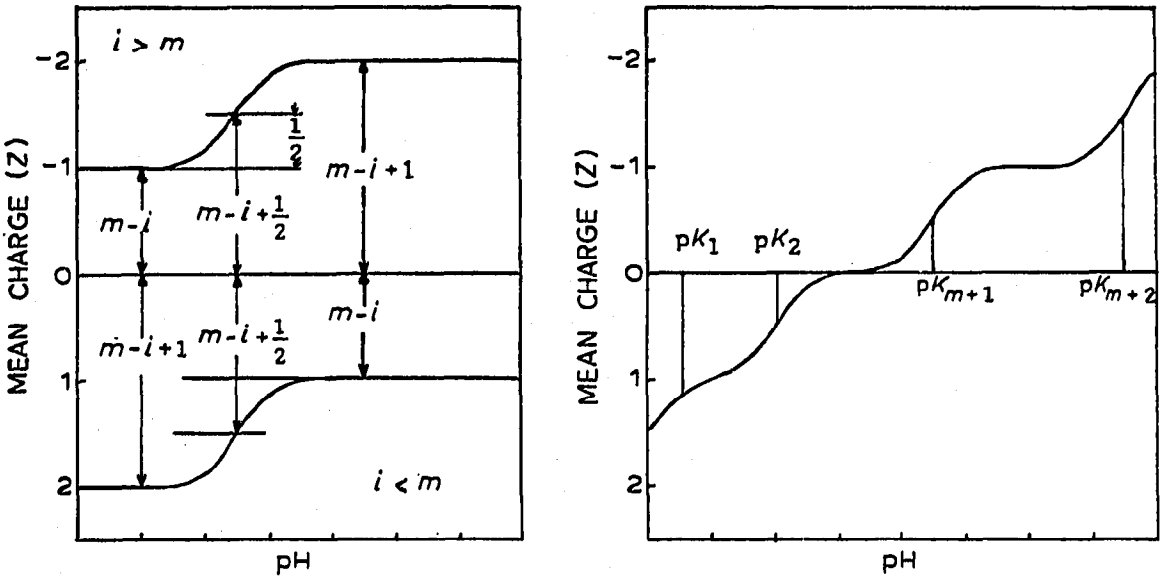


Fig. 1. pH dependence of the mean charge for proton dissociation and protonation equilibrium, respectively.

Fig. 2. pH dependence of the mean charge for more than one consecutive protonation or dissociation equilibrium.

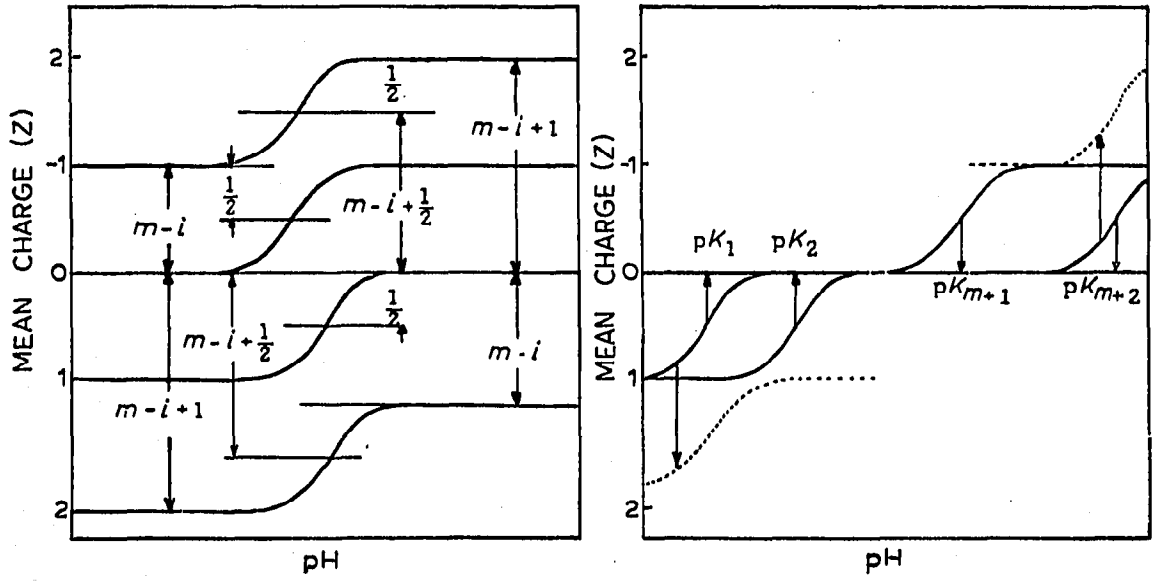


Fig. 3. pH dependence of the mean charge showing the modification of eqn. 9 to eqn. 10 graphically.

Fig. 4. pH dependence of the mean charge for various consecutive protonation or dissociation equilibria showing the modification of eqn. 9 to eqn. 10 schematically.

of eqn. 9 from $i = 1$ to $i = m + n$. In order to get a component function of the desired equation, the first term of eqn. 9 should be modified as follows:

$$Z_i = \frac{1}{2}\left[1 + \tanh\left\{\frac{2.303}{2}(pK_i - pH)\right\}\right] \tag{10}$$

The modification in eqn. 10 is due to the shift of the Z axis from $m - i$ to zero as shown in Fig. 3.

In general, the relationship between $pK_i = 1 \sim m + n$ is as follows:

$$pK_1 < pK_2 < \ldots pK_{i-1} < pK_i < \ldots < pK_{m+n} \tag{11}$$

The Z_i–pH curves obtained with eqns. 10 and 11 are drawn as solid lines in Fig. 4.

$$\begin{aligned} Z &= \sum_{i=1}^{m} Z_i + \sum_{m+1}^{m+n} Z_i \\ &= \frac{1}{2}\sum_{i=1}^{m}\left[1 + \tanh\left\{\frac{2.303}{2}(pK_i - pH)\right\}\right] \\ &\quad - \frac{1}{2}\sum_{i=m+1}^{m+n}\left[1 + \tanh\left\{\frac{2.303}{2}(pH - pK_i)\right\}\right] \end{aligned} \tag{12}$$

The modification of eqn. 9 to eqn. 10 may be understood from the schematic diagram in Fig. 4. As the charge of the ionic species in the proton equilibrium system (eqn. 1) is always positive in the range $i < m$, Z can be expressed by eqn. 13 which is derived from the first or the second term of eqn. 12 by adding or subtracting the shift of the coordinate Z, respectively.

$$\begin{aligned} Z &= \frac{1}{2}\sum_{i=1}^{m+n}\left(1 + \tanh\left\{\frac{2.303}{2}(pK_i - pH)\right\}\right) - n \\ Z &= m - \frac{1}{2}\sum_{i=1}^{m+n}\left[1 + \tanh\left\{\frac{2.303}{2}(pH - pK_i)\right\}\right] \end{aligned} \tag{13}$$

m in eqn. 13 is zero for organic acids whereas for substances like amines, purine and pyrimidine bases, n is zero.

THE RELATIONSHIP BETWEEN THE ZONE MOBILITY AND THE MASS OF MIGRATING SUBSTANCES

Recently a relationship between the zone mobility of the migrating substances and their molecular weight has been derived. It proved to be in good agreement with many experimental results[14, 22–24]

$$U = a\frac{Z}{\sqrt{M}} - b \tag{14}$$

where U is the relative zone mobility of the migrating species, M is the mass, *i.e.* molecular weight, a and b are constants related to a standard mobility.

Dissociation of a few protons from a molecule or their addition to a molecule (protonation) does not change the mass or the size of the migrating substances a great deal, whereas in aqueous solution the degree of hydration of an ionic species generally increases with increasing charge.

Consequently the mass M in eqn. 14 should be expressed as a sum of the mass of the molecule itself and the mass of the water of hydration. Moreover, the degree of hydration depends on the intrinsic molecular weight and/or the chemical structure. The degree of hydration decreases with increasing molecular weight[22] and is lower in aromatic than in aliphatic compounds. If a discrepancy between the observed and the calculated mobilities is due to a mass increment as a result of hydration, we can get eqn. 15 using eqn. 14

$$\frac{U_{\text{cal.}}}{U_{\text{obs.}}} = \frac{\sqrt{M + \Delta M}}{\sqrt{M}} \cdot \frac{a + b\sqrt{M}}{a + b\sqrt{M + \Delta M}} \tag{15}$$

where $U_{\text{cal.}}$ is the calculated relative mobility, $U_{\text{obs.}}$ is the observed relative mobility, and ΔM is the mass increment due to hydration. Eqn. 15 can be altered to eqn. 16:

$$\frac{\Delta M}{M} = \left(\frac{aRu}{a + b\sqrt{M}(1 - Ru)}\right)^2 - 1 \tag{16}$$

$$Ru = \frac{U_{\text{cal.}}}{U_{\text{obs.}}} \tag{17}$$

Z in eqn. 14 is a function of the pK(s) and the pH. Now we can derive eqn. 18 combining eqn. 14 with eqn. 13:

$$U_{\text{cal.}} = \frac{1}{2}\left\{\left(\frac{a}{\sqrt{M}} + b\right)\sum_{i=1}^{m+n}\left(1 + \tanh\left[\frac{2.303}{2}(\text{p}K_i - \text{pH})\right]\right\} - n$$

$$U_{\text{cal.}} = -\frac{1}{2}\left\{\left(\frac{a}{\sqrt{M}} + b\right)\sum_{i=1}^{m+n}\left(1 + \tanh\left(\frac{2.303}{2}(\text{pH} - \text{p}K_i)\right)\right\} + m \tag{18}$$

The exact mass M in eqn. 18 should rather be represented as the mass of the hydrated ion as mentioned above.

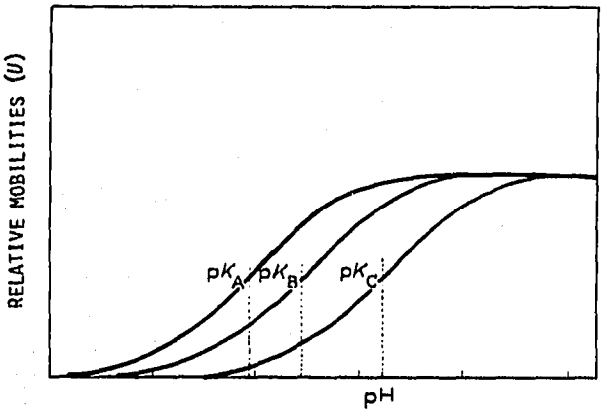
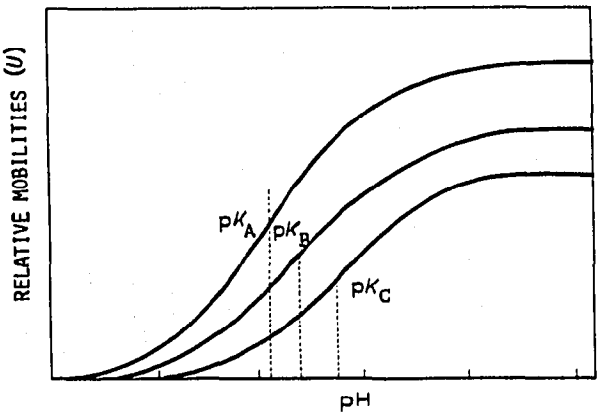


Fig. 5. pH dependence of the electrophoretic mobility. A, B, C: substances with equal molecular weight but different pK values.



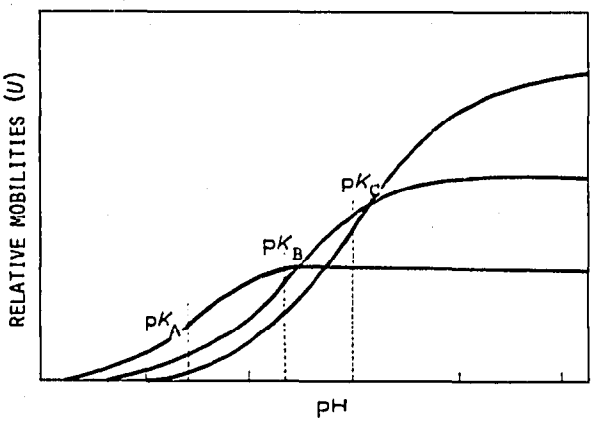


Fig. 6. pH dependence of the electrophoretic mobility. A, B, C: substances with increasing molecular weight and also increasing pK values.

Fig. 7. pH dependence of the electrophoretic mobility. A, B, C: substances with decreasing molecular weight but increasing pK values.

TABLE I

CALCULATED RELATIVE MOBILITIES OF AMINO ACIDS AND THEIR pK VALUES[25,26]

Amino acid	*Mol. wt.*	U_1	pK_1	pK_2	pK_3
Alanine (Ala)	89.1	0.85	2.43	9.69	
β-Alanine (β-Ala)	89.1	0.85	3.60	10.19	
α-Aminobutyric acid (α-Amin)	103.1	0.77	2.55	9.60	
Arginine (Arg)	174.2	0.55	2.17	9.64	12.48
Asparagine (Asn)	132.2	0.66	2.02	8.8	
Aspartic acid (Asp)	133.1	0.66	2.1	3.86	9.82
L-Cysteine (Cys)	121.2	0.70	1.71	8.33	10.78
Cystine (Cysti)	240.3	0.44	<1	2.1	8.71
Glutamic acid (Glu)	147.1	0.62	2.19	4.25	9.67
L-Glutamine (Glu NH_2)	146.2	0.62	2.17	9.13	
Glycine (Gly)	75.1	0.94	2.34	9.60	
Histidine (His)	155.2	0.59	1.78	5.97	8.97
L-Hydroxyproline (Hyp)	131.1	0.66	1.82	9.65	
Isoleucine (Ile)	131.2	0.66	2.26	9.62	
Leucine (Leu)	131.2	0.66	2.36	9.60	
Lysine (Lys)	146.2	0.62	2.20	8.90	10.28
Hydroxylysine	162.2	0.58	2.13	8.62	9.67
Methionine (Met)	149.2	0.61	2.28	9.21	
Ornithine (Orn)	132.2	0.66	1.94	8.65	10.76
Phenylalanine (Phe)	165.2	0.57	1.83	9.13	
Proline (Pro)	115.1	0.72	1.99	10.60	
Serine (Ser)	105.1	0.77	2.21	9.15	
Taurine (Tau)	125.2	0.68	1.5	8.74	
Threonine (Thr)	119.1	0.71	2.15	9.12	
Tryptophan (Try)	204.2	0.49	2.38	9.36	
Tyrosine (Tyr)	181.2	0.53	2.20	9.11	10.07
Valine (Val)	117.2	0.71	2.32	9.62	
$(Gly)_2$	130.2	0.67	3.12	8.17	
Ala–Gly	146.2	0.617	3.11	8.18	
Gly–Tyr	238.3	0.438	2.98	8.40	10.40
Leu–Gly	188.3	0.519	3.18	8.29	
Gly–GluNH_2	203.3	0.491	2.88	8.29	
$(Gly)_3$	186.3	0.522	3.26	7.91	

ESTIMATION OF THE SEPARABILITY OF A MIXTURE

The separability of a mixture A, B, C, ... with different pK(s) and masses is discussed for various cases below.

(1) If the masses of the different ionic species A, B, C, ... are equal and the number of dissociable protons in every species is one:

$$M_A = M_B = M_C = \ldots$$

$$pK_A < pK_B < pK_C > \ldots \qquad (19)$$

$$pK_{max} - pK_{min} < 4$$

pK_{max} and pK_{min} are the maximum and the minimum pK values among all the pK values of the substances to be separated. In this case the optimum pH value for the separation is:

$$pH = \frac{pK_A + pK_B + pK_C + \ldots}{n} \qquad (20)$$

where n is the number of substances to be separated (see Fig. 5).

(2) If the relation between the masses and the pK values of the substances is:

$$M_A < M_B < M_C < \ldots$$

$$pK_A < pK_B < pK_C < \ldots \qquad (21)$$

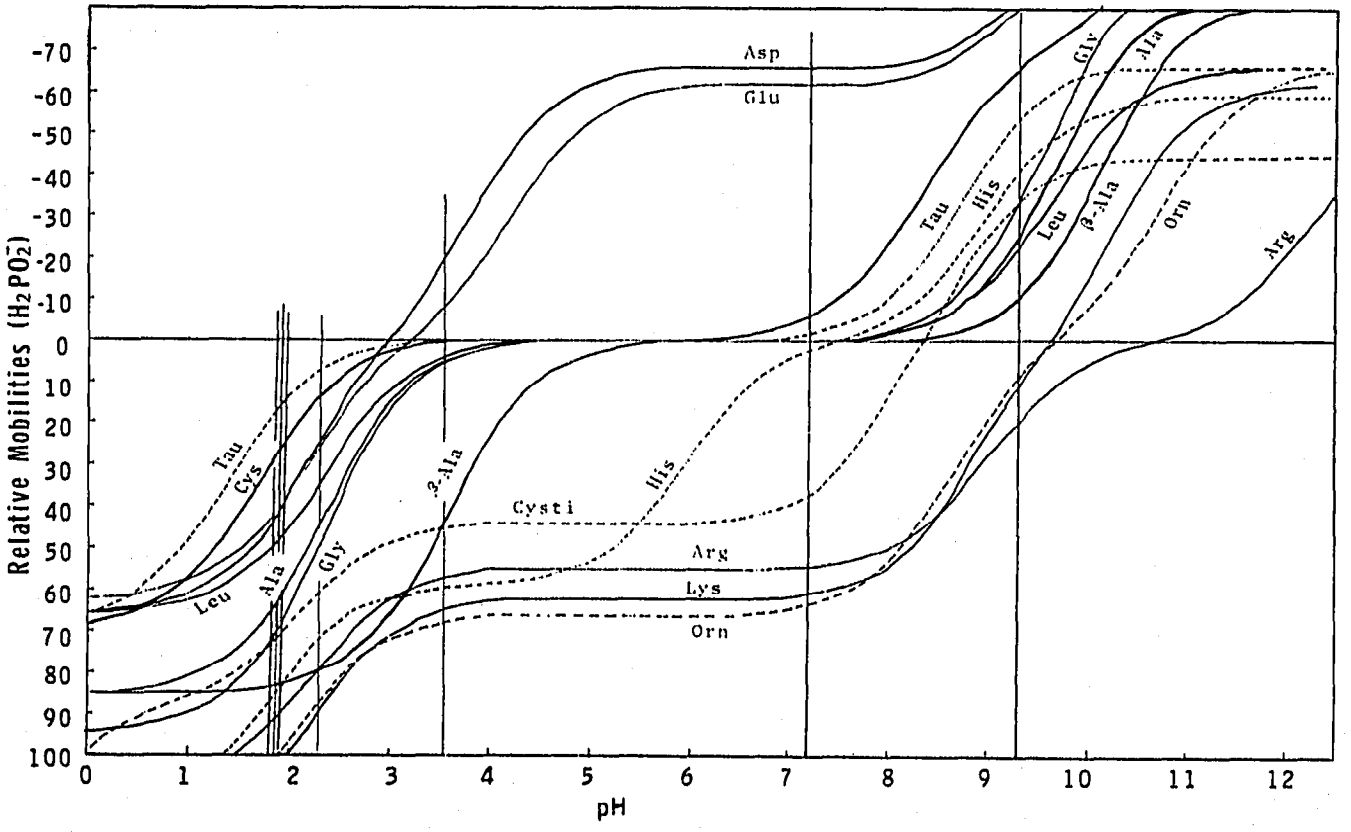


Fig. 8. pH dependence of the electrophoretic mobilities of amino acids.

In this case the separation is easy in the range pH $>$ pK_{max} $-$ 2 (see Fig. 6).

(3) If the relation between the masses and pK values is:

$$M_A > M_B > M_C > \ldots$$
$$pK_A < pK_B < pK_C < \ldots \quad (22)$$

In this case the optimum pH for a separation cannot be determined without consulting the zone mobility–pH curves for those substances (see Fig. 7).

CALCULATION OF ZONE MOBILITY–pH CURVES OF AMINO ACIDS

To obtain the zone mobility–pH curves of polyanionic substances the calculation of zone mobilities of monoanionic substances first is essential. Eqn. 23 was derived from experimental data[22]:

$$U_1 = 10 \frac{1}{\sqrt{M}} - 0.21 \quad (23)$$

TABLE II

COMPARISON BETWEEN OBSERVED AND CALCULATED RELATIVE MOBILITIES OF AMINO ACIDS

Compounds	*pH 1.81*					*pH 1.85*				
	Cal.	*Cal.*[c]	*Obs.*[a]	*Obs.*[c]	*Δ %*	*Cal.*	*Cal.*[d]	*Obs.*[b]	*Obs.*[d]	*Δ %*
Ala	0.65	119	100	123	—4	0.64	77	100	69	— 8
β-Ala						0.834	100	145	100	0
α-Amin						0.646	77	90	62	—16
Arg						0.919	110	131	90	—20
Asn						0.393	47	71	49	2
Asp	0.354	65	59	73	8	0.339	41	61	42	1
Cys						0.293	35	60	41	6
Cysti						0.313	38	59	41	3
Glu	0.433	79	66	82	3	0.421	50	67	48	— 2
$GluNH_2$						0.417	50	69	47	— 3
Gly						0.712	85	114	79	— 6
His						0.860	107	131	90	—17
Hyp						0.32	38	54	37	— 1
Ile	0.517	95	80	99	4	0.477	57	77	53	— 4
Leu	0.517	95	78	96	1	0.507	61	77	53	— 8
Lys						1.043	125	147	102	—23
Met						0.442	53	71	49	— 4
Orn						1.012	121	152	105	—16
Phe	0.290	53	61	75	12	0.291	35	61	42	7
Pro	0.435	80	69	85	5	0.417	50	69	48	— 2
Ser	0.547	100	81	100	0	0.533	64	83	57	— 7
Tau						0.212	25	30	20	— 5
Thr	0.484	89	73	90	1	0.470	56	75	52	— 4
Try						0.377	45	46	32	—13
Tyr	0.378	69	53	65	—4	0.368	44	53	37	— 7
Val	0.545	100	81	100	0	0.532	64	81	56	— 1

[a] 2.0% (w/v) formic acid–20% (w/v) acetic acid–0.4 mM cadmium acetate; 100 V/cm, mobility relative to alanine[27].

[b] 2.5% (w/v) formic acid–7.8% (w/v) acetic acid; 100 V/cm; mobility relative to alanine[27].

[c] Standard: valine.

[d] Standard: β-alanine.

TABLE III

COMPARISON BETWEEN OBSERVED AND CALCULATED RELATIVE MOBILITIES OF AMINO ACIDS

Compounds	pH 1.9									
	Cal.	Cal.[c]	Obs.[a]	Obs.[c]	Δ %	Cal.	Cal.[c]	Obs.[b]	Obs.[c]	Δ %
Ala	0.622	90	24.2	90	0	0.622	90	100	87	— 3
β-Amin	0.632	92	21.7	82	—10					
Aspn						0.375	55	73	63	— 8
Asp	0.403	59	8.0	70	11					
Cys	0.342	50								
Cysti	0.302	43	14.3	53	10	0.302	50	67	58	— 8
Glu	0.405	59	15.5	58	— 1					
Gly	0.688	100	26.9	100	0	0.688	100	115	100	0
Hyp	0.302	44	130	48	— 4	0.302	44	60	52	8
Leu	0.490	71	20.9	78	6					
Lys						1.03	149	141	127	—22
Met	0.429	62	17.6	67	5					
Orn						1.01	146	141	123	—23
Phe	0.261	38	16.6	63	25	0.261	38	62	54	16
Pro	0.398	58	15.4	56	— 2	0.398	58	75	65	7
Ser	0.513	75	19.7	74	— 1					
Tau	0.106	15	6.4	22	7					
Thr	0.595	87	18.2	67	—10	0.595	87	78	68	—29
Try	0.367	53	15.5	59	6	0.367	53	48	42	—11
Tyr	0.354	52	16.2	59	7					
Val	0.515	75	21.2	78	3					

[a] 2 *N* acetic acid–0.6 *N* formic acid (1 : 1), pH 1.9; Whatman No. 1 paper; 70 V/cm; 200 min; unit is cm[38].

[b] Acetic acid–formic acid–water (150:50:800, v/v/v), pH 1.9; Whatman No. 3 filter paper; 85 V/cm; cooling fluid temperature, —5°; mobility relative to that of alanine[20].

[c] Standard: glycine.

U_1 was the zone mobility of monoanionic substances relative to that of monoanionic hypophosphorous acid as migration standard. The calculated relative zone mobilities of monoanions of amino acids, which were calculated using eqn. 23 and their pK values, are given in Table I. Fig. 8 shows the U–pH curves of amino acids derived from eqn. 18 and Table I. The relative zone mobilities of di-cations and di-anions generated by proton association and dissociation were just twice as large as those of the monoanions shown in Table I. In Tables II–V, observed and calculated mobilities at various pHs of the background solutions are recorded. The calculated values are obtained with eqn. 18. The comparison of the calculated with the experimental values is simplified giving ratios of the relative zone mobilities and showing them in a clear graph (see Figs. 9 and 10). From these figures it can be seen that the calculated and the experimental values are usually in good agreement. The crossing of a few connecting lines in this graph means that the migration order found experimentally is different from the expected one. Those amino acids deviating rather often from the theory are phenylalanine, tryptophan, tyrosine, lysine, arginine, ornithine, and histidine. Hydrophobic phenyl groups are present in the phenylalanine, tryptophan, and tyrosine molecules. In lysine, ornithine, arginine, and histidine the mean charge in the low pH range is greater than unity whereas in the other amino acids the mean charge in this pH range is usually less than unity. The greater charge gives rise to

TABLE IV

COMPARISON BETWEEN OBSERVED AND CALCULATED RELATIVE MOBILITIES OF AMINO ACIDS

Compounds	*pH 2.3*					*pH 3.3*				
	Cal.	*Cal.*[c]	*Obs.*[a]	*Obs.*	*Δ %*	*Cal.*	*Cal.*[a]	*Obs.*[b]	*Obs.*[a]	*Δ %*
Ala	0.444	125	15	117	— 8	0.084	14	5	7	—7
β-Ala						0.565	93	61	90	—3
α-Amin	0.495	139	13.1	102	—37					
Arg						0.585	96	68	100	4
Asp	—0.153	—43	—6.8	—53	—10	—0.167	—27	—21	—31	4
Cysti	0.166	47	8.5	66	19					
Glu	0.268	76	3.8	30	—46	—0.027	— 4	— 7	—10	7
Gly	0.493	139	16.4	128	—11	0.094	15	7	10	—5
His						0.609	100	68	100	0
Hyp	0.165	47	8.0	63	16					
Leu	0.354	100	12.8	100	0					
Met	0.297	84	10.9	85	1					
Phe	0.143	40	10.1	79	39					
Pro	0.239	68	9.6	75	7	0.033	5	0	0	—5
Ser	0.309	86	11.9	93	7					
Tau	0.116	33	5.8	45	12					
Thr	0.293	83	11.2	88	5					
Tyr	0.236	67	9.3	73	6					
Val	0.348	98	12.8	100	2					

[a] 1 *N* acetic acid–0.6 *N* formic acid (1:1), pH 2.3; Whatman No. 1 paper; 70 V/cm; 180 min[28].
[b] Pyridine–formic acid buffer, pH 3.3; Whatman No. 4 paper; 10 V/cm; 1–2 h; mobility relative to amaranth[30].
[c] Standard: leucine.
[d] Standard: histidine.

TABLE V

COMPARISON BETWEEN OBSERVED AND CALCULATED RELATIVE MOBILITIES OF AMINO ACIDS

Compound	*pH 7.2*					*pH 9.3*				
	Cal.	*Cal.*[c]	*Obs.*[a]	*Obs.*[c]	*Δ %*	*Cal.*	*Cal.*[c]	*Obs.*[b]	*Obs.*[c]	*Δ %*
Ala						—0.25	—28.5	—10	— 12.3	17
β-Ala						—0.098	— 11.2	— 3	— 4	7
Arg	0.55	83	62	81	—2	0.338	39	45	56	17
Asp	—0.66	—100	—76	—100	0	—0.876	—100	—81	—100	0
Glu	—0.61	— 94	—72	— 95	1	—0.824	— 94.0	—77	— 95	1
Gly	0.0	0.0	0	0	0	—0.317	— 36.2	—12	— 15	16
His	0.08	12	8	11	—1	—0.189	— 22	—10	— 12	10
Pro	0.0	0.0	0	0	0	—0.003	— 3	0	0	— 3
Ser						—0.447	— 51	—28	— 35	16

[a] Dimethylaminopropionitrile–acetic acid buffer; Whatman No. 4 paper; 10 V/cm; 1–2 h; mobility relative to amaranth[30].
[b] 2-Dimethylaminoethanol–acetic acid buffer[30].
[c] Standard: aspartic acid.

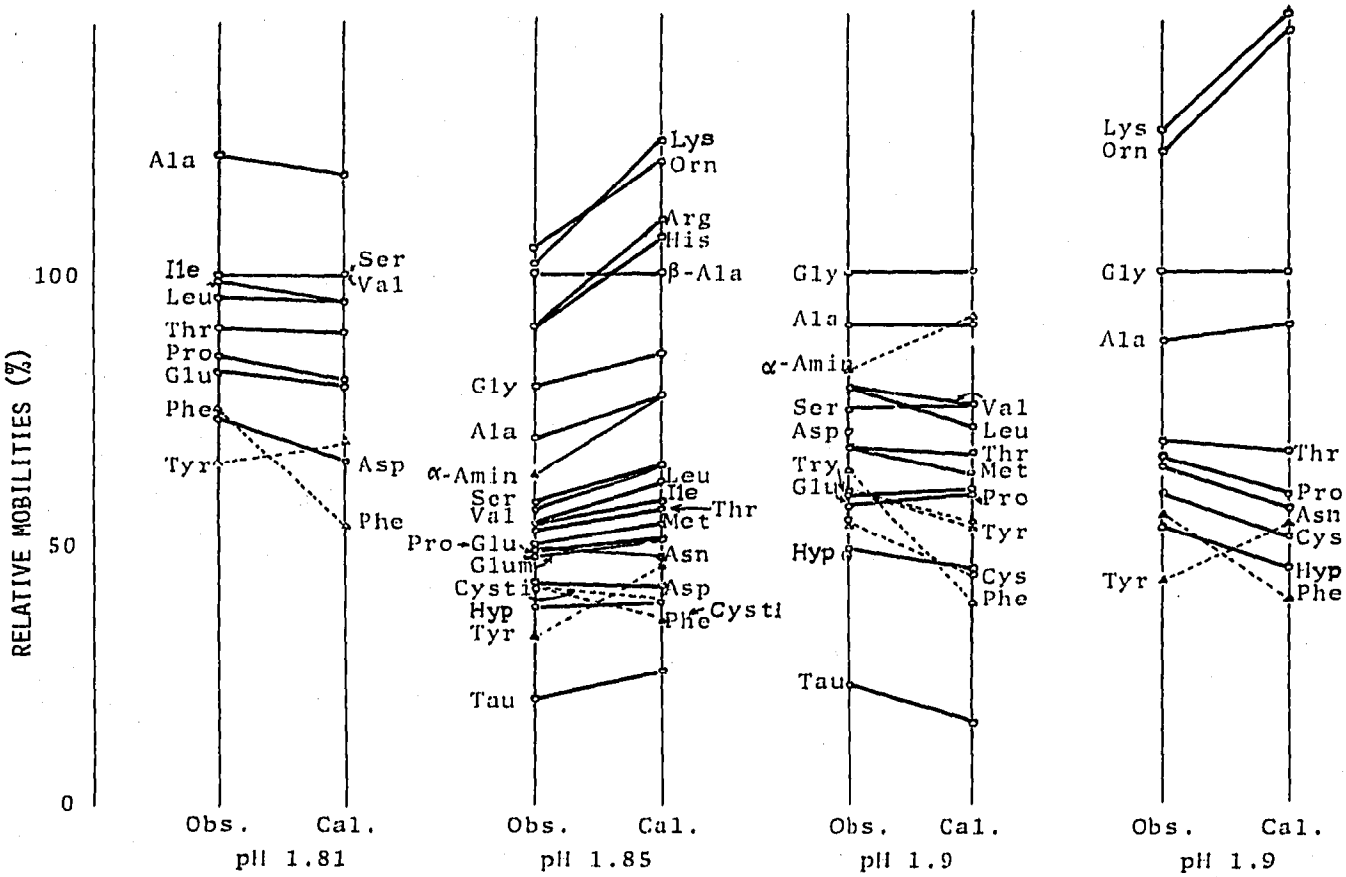


Fig. 9. Schematic comparison between observed and calculated relative mobilities of various amino acids. Observed data published by different authors.

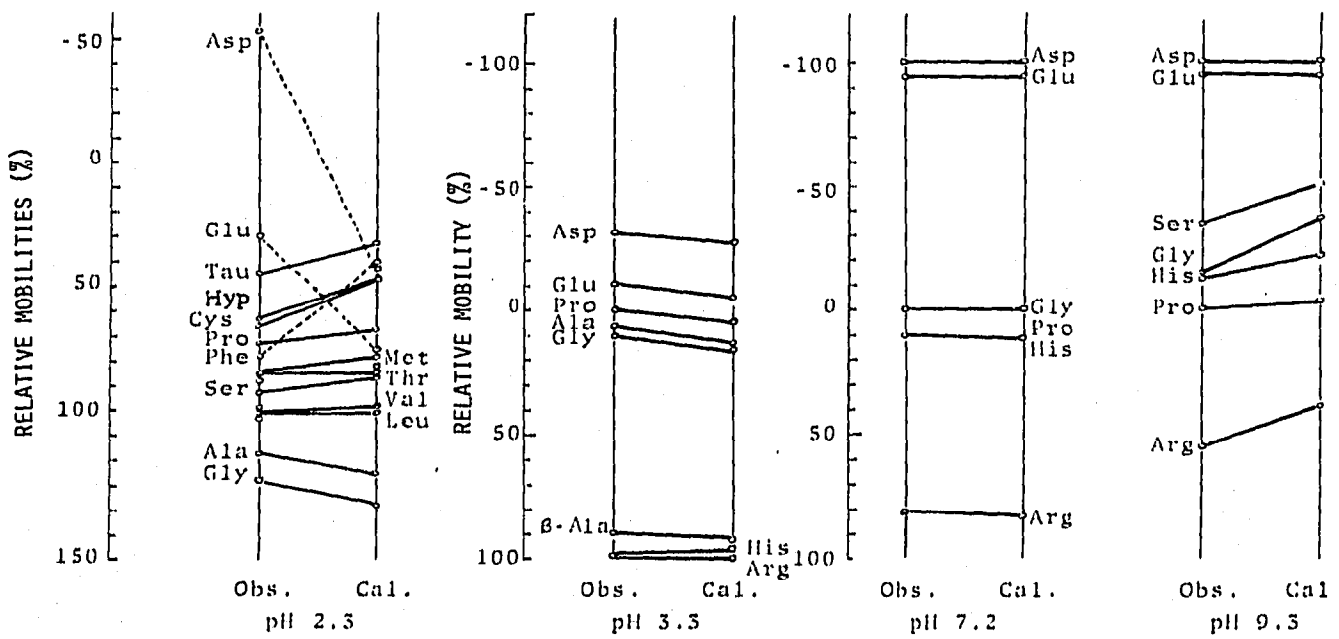


Fig. 10. Schematic comparison between observed and calculated relative mobilities of various amino acids at different pHs.

a higher degree of hydration which is neglected in the calculations hence leading to calculated mobility values which consequently are too high. At pH 7.2 and 9.3 (Table V) the charges of all amino acids are about equal and thus the calculated and the experimental mobilities are in good agreement here.

TABLE VI

COMPARISON BETWEEN THE OBSERVED AND THE CALCULATED RELATIVE MOBILITIES OF SOME PEPTIDES AT pH 1.9

Compound	*Cal.*	*Obs.*[a]	*Cal.*[c]	*Obs.*[a,c]	*Δ %*	*Cal.*[d]	*Obs.*[b,d]	*Δ %*
Gly	0.688	16.4	109.7	68.6	−41.1			
Ala	0.622					100	100	0
$(Gly)_2$	0.627	23.9	100	100	0			
Ala–Gly	0.554	22.8	88.4	95.4	7.0			
Gly–Tyr	0.404	16.5	64.4	69.0	4.6	64.9	64	0.9
Leu–Gly	0.492	19.4	78.5	81.2	2.7	79.1	81	0.9
Gly–GluNH$_2$	0.465					74.7	82	7.3
$(Gly)_3$	0.499	21.4	79.6	89.5	9.9			

[a] Buffer (2 *N* acetic acid–0.6 *N* formic acid, 1:1, v/v); Whatman No. 1 paper; 70 V/cm; 60 min; unit is cm[28].

[b] Buffer (glacial acetic acid–formic acid–water, 150:50:800, v/v/v); Whatman No. 3 paper; 85 V/cm; mobility relative to alanine[29].

[c] Standard: $(Gly)_2$.

[d] Standard: alanine.

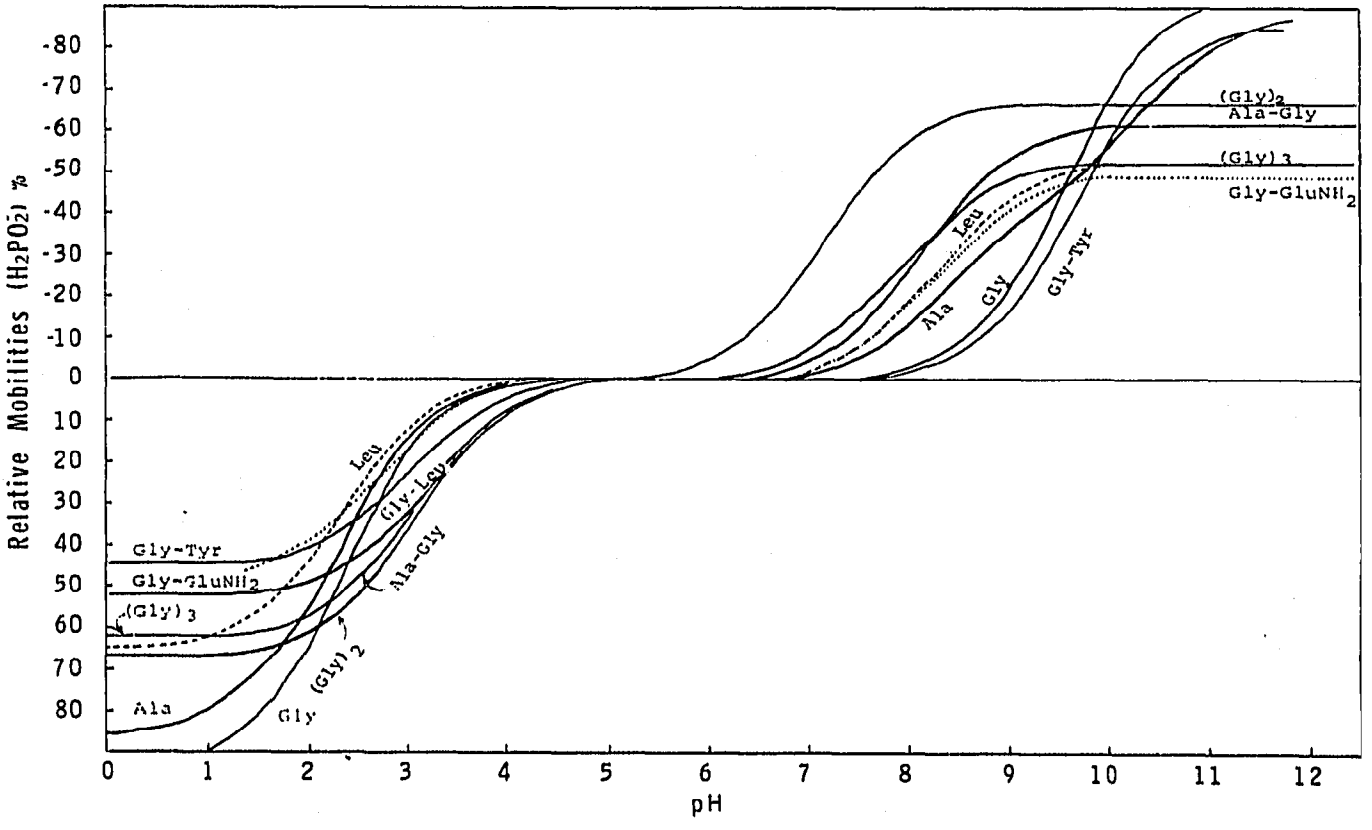


Fig. 11. pH dependence of the relative mobilities of selected oligopeptides.

Table VI shows a comparison between the observed and the calculated mobilities of a few peptides at pH 1.9. The negative percentage deviation of glycine in Table VI is very large. This is due to a high degree of hydration relative to that of the other dimer or trimer peptides. On the other hand, the positive percentage deviation of $(Gly)_3$ shows a lower degree of hydration in comparison with that of the other dimer and trimer amino acids. The observed mobilities of the other oligopeptides agree fairly well with the calculated ones. A few mobility–pH curves of amino acids and oligopeptides are given in Fig. 11. The pH value of the background electrolyte suitable for the separation of a certain mixture can be read easily from this graph. Below pH = 1 and above pH = 11 the mobility depends only on the difference in the weights of the species and not on the pH of the background solution. Between pH 4 and 6 the peptides have no or only a negligibly small charge and hence do not migrate at all. The optimum pH for a separation will vary according to which species are to be separated.

The simplicity and rapidity of this method compared with finding the optimum pH from trial and error tests is obvious in such complicated cases.

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