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ISOTACHOPHORETIC DETERMINATION OF MOBILITY AND pK_a BY MEANS OF COMPUTER SIMULATION

V. EVALUATION OF m_0 AND pK_a OF TWENTY-EIGHT DIPEPTIDES AND ASSESSMENT OF SEPARABILITY

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SUMMARY

Isotachophoretic qualitative indices, R_E, for twenty-eight dipeptides were measured in the range pH 7.4–9.6. The absolute mobility, m_0, and pK_a values were evaluated by the use of the least-squares method, utilizing a simulation of the isotachophoretic steady state. The m_0 values were newly evaluated and the pK_a values were in good agreement with literature values. By comparison of the evaluated m_0 and pK_a values of the dipeptides with those of the constituent amino acids, simple relationships were found which may be used to estimate the m_0 and pK_a values of other dipeptides. The separability of the dipeptides was also evaluated by considering the differences between their simulated effective mobilities. It is concluded that isotachophoresis is very convenient for the separation of dipeptides and their constituent amino acids.

INTRODUCTION

In isotachophoresis (IP) no packings are used and the separability is determined simply by the differences in the effective mobilities of samples under the selected electrolyte conditions. On the contrary, in high-performance liquid chromatography (HPLC) the complex adsorption–desorption and ion-exchange phenomena occurring between samples and packings combine to increase the separability. Therefore the separability of IP is not as high as that in HPLC. However, the convenient sampling in isotachophoresis is worthy of special mention. In IP the pretreatment of samples is not necessary in many cases and only small amounts are required. Moreover, as has been emphasized in this series of papers, the separation equilibria can

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be simulated exactly, since the separation field of IP can be considered as a free solution contrary to that in LC. Therefore, isotachophoresis can be a powerful method to investigate the ionic characteristics in solution, such as mobilities, acid dissociation constants and complex stability constants.

Similarly to the previous paper which considered twenty-five amino acids[1], to increase the utility of the SIPS program for determination of the optimum separation conditions in isotachophoresis[2] and to obtain knowledge about how the mobility and pK_a values of dipeptides differ from those of the constituent amino acids, in this paper the absolute mobilities, m_0, and pK_a values of twenty-eight dipeptides were evaluated by our isotachophoretic methods[3,4].

Since the conventional conductivity method cannot be applied for amphoteric electrolytes such as dipeptides, their m_0 values have scarcely been reported. Together with the pK_a values these values are necessary for the simulation of the separability. The necessary thermodynamic pK_a values are not always available.

Using the evaluated constants, the effective mobilities of the twenty-eight dipeptides and the constituent amino acids under several typical electrolyte conditions were simulated to determine the limits of separability, taking into account results obtained for the amino acids[1]. Several practical examples are given of the analysis of the partly decomposed dipeptides forming the constituent amino acids. Due to the difference in the pK_a values between the amino acids and the dipeptides, an high separability is expected.

EXPERIMENTAL

The twenty-eight dipeptides were Ala-Ala, Ala-Amin (Amin = α-amino-*n*-butyric acid), Ala-Asn, Ala-Gly, Ala-Leu, Ala-Met, Ala-Phe, Ala-Ser, Ala-Val, β-Ala-His, Gly-Ala, Gly-Amin, Gly-Asn, Gly-Gly, Gly-Ile, Gly-L-Leu, Gly-Phe, Gly-L-Pro, Gly-Ser, Gly-D-Thr, Gly-Trp, Gly-Tyr, Gly-Val, Leu-Gly, Leu-Leu, Leu-Phe, L-Leu-L-Tyr and Leu-Val, which were obtained from Tokyo Kasei Co. and Sigma Chemical Co. in high purity. Except for β-Ala-His, the dipeptides are alanyl, glycyl and leucyl derivatives of amino acids. Monomer components for which the optical activity is not specified are mixtures, of the D- and L-isomers. Sample solutions (3–10 m*M*) were prepared by dissolving these dipeptides in distilled water. When the solubility was low, a small amount of 0.1 *M* sodium hydroxide solution was added.

In the literature, the pK_a values can be found for fifteen of the dipeptides studied. They are in the range of 1.5–3.6 for cations and 8–10 for anions. The pK_a values of the cations are larger than those of the constituent amino acids which are in turn greater than the values for the anions. Similarly to the amino acids[1], the dimer cations are not sufficiently mobile for isotachophoretic analysis; qualitative indices, R_E, were therefore measured for the anions.

The leading electrolyte systems 1–8 in Table II comprised 10 m*M* hydrochloric acid solutions and the pH_L was adjusted by adding imidazole (1), 2-amino-2-methyl-1,3-propanediol (amediol) (2–5) or, ethanolamine (6–8), respectively. The terminating electrolyte was 10 m*M* Tau (for 1) or 10 m*M* β-Ala (2–8), the pH being adjusted to *ca.* 10 by adding barium hydroxide to suppress the disturbance caused by HCO_3^-. The pH measurements were carried out by using a Horiba expanded pH meter, Model F7ss. All of the leading electrolytes contained 0.02% hydroxypropyl-

TABLE I

PHYSICO-CHEMICAL CONSTANTS USED IN SIMULATION (25°C)

m_0 = Absolute mobility ($cm^2\ V^{-1}\ s^{-1}$) · 10^5; pK_a = thermodynamic acid dissociation constant, assumed values being used for Cl^-; Tris = tris(hydroxymethyl)aminomethane; amediol = 2-amino-2-methyl-1,3-propanediol.

Cation	m_0	pK_a	*Anion*	m_0	pK_a
Imidazole	52.0*	7.15	Cl^-	79.08	−3
Tris	29.5*	8.076	Taurine	37.9*	4.756
Amediol	32.0*	8.79	β-Alanine	30.8*	10237
Ethanolamine	44.3*	9.498			

* Obtained isotachophoretically; other constants were taken from the literature[5–8].

cellulose to suppress electrode reactions and electroendosmosis. Table I shows the m_0 and pK_a values of the electrolyte constituents used in simulations of the isotachophoretic steady state. The pK_a values and some of the m_0 values were taken from the literature[5–8]. Most m_0 values were evaluated by our isotachophoretic method. Table II summarizes the leading electrolyte conditions together with the calculated concentrations and effective mobilities of the constituents.

The isotachopherograms were obtained using a Shimadzu isotachophoretic analyzer, IP-1B, equipped with potential-gradient detection (PGD). The temperature was thermostatted at 25°C. The separating tube used was *ca.* 40 cm × 0.5 mm I.D. The driving current was 50 μA and a single experiment took *ca.* 35 min. Fig. 1 shows three typical isotachopherograms obtained by the use of electrolyte systems 1 and 2 in Table II.

For the correction of the asymmetric potential of the PGD to obtain precise R_E values, the terminators, Tau and β-Ala, were used as the internal standard. Their

TABLE II

EXPERIMENTAL CONDITIONS FOR THE EVALUATION OF ABSOLUTE MOBILITIES AND pK_a OF DIPEPTIDES, CALCULATED CONCENTRATIONS AND EFFECTIVE MOBILITIES OF BUFFERS

Buffers used for pH adjustment: IM = imidazole; AM = amediol; EA = ethanolamine. pH_L = pH of leading electrolyte; $C^*_{B,L}$ = Total concentration (m*M*) of buffer ion; $\bar{m}_{B,L}$ = effective mobility ($cm^2\ V^{-1}\ s^{-1}$) of buffer ion · 10^5; Std (R_E) = internal standard used for correction of the asymmetric potential, with the R_E value in parentheses. The leading ion was 10.02 m*M* chloride and the effective mobility was 74.69 · 10^{-5} $cm^2\ V^{-1}\ s^{-1}$.

System	*Buffer*	pH_L	$C^*_{B,L}$	$\bar{m}_{B,L}$	*Std* (R_E)
1	IM	7.41	26.46	17.65	Tau (12.40)
2	AM	8.30	13.01	20.12	β-Ala (12.12)
3	AM	8.37	13.54	19.34	β-Ala (11.84)
4	AM	8.59	15.86	16.51	β-Ala (10.81)
5	AM	8.77	18.86	13.89	β-Ala (9.84)
6	EA	9.06	13.33	30.57	β-Ala (6.19)
7	EA	9.33	16.19	25.19	β-Ala (5.58)
8	EA	9.52	19.59	20.85	β-Ala (5.10)

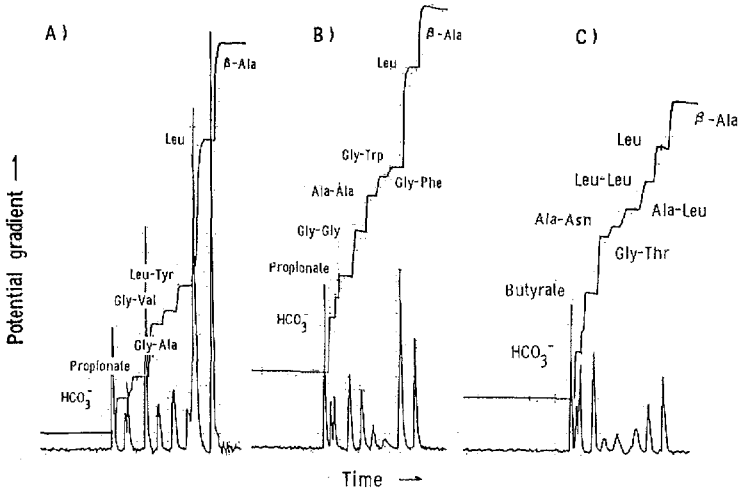

Fig. 1. The observed isotachopherograms of Gly-Ala, Gly-Val, Leu-Tyr (A), Gly-Gly, Ala-Ala, Gly-Phe, Gly-Trp (B) and Gly-Thr, Ala-Asn, Ala-Leu, Leu-Leu (C). The leading electrolytes used were 10.02 m*M* hydrochloric acid buffered by amediol at pH_L = 8.77 (A) and by ethanolamine at pH_L = 9.33 (B) and 9.52 (C). The terminator was 10 m*M* β-Ala, pH *ca.* 10 by addition of barium hydroxide. The sample amounts were 10–20 nmol and migration current was 50 μA.

R_E values are shown in Table II. The R_E values of the dipeptides were measured for several completely separable pairs. The experimental errors were less than 0.05 R_E units. The measurements were repeated three times and the averages were used for the m_0 and pK_a evaluation. Table III summarizes the observed R_E values of the dipeptides and Fig. 2 shows their pH_L dependence in the range pH 6–10.5 (buffers: imidazole, Tris, amediol and ethanolamine).

For the data processing and the simulation, SIPS programs on an NEC PC9801E microcomputer were used[2]. For the least-squares method, the SIPS-LSQ program on an NEC MS120 minicomputer was used. For plotting the figures, a Watanabe X-Y plotter WX4671 and a Roland DXY-980 were used.

RESULTS AND DISCUSSION

Using the observed R_E values listed in Table III, the m_0 and pK_a values were evaluated by the least-squares method, and were employed to plot the pH_L *vs.* R_E curves in Fig. 2. The buffers used were imidazole, Tris, amediol and ethanolamine. Although the R_E values were not measured in the pH range buffered by Tris, the simulated pH_L *vs.* R_E curves are shown in connection with a later section. Table IV shows the observed and the best-fitted R_E values, the effective mobilities and concentrations of the zone constituents of Ala-Ala, Ala-Gly, Ala-Leu, Gly-Ala, Gly-Gly, Gly-Leu, Leu-Gly and Leu-Leu. There is good agreement between the observed and the best-fitted R_E values. The mean errors were in the range of 0.31 (Ala-Amin)–1.81%(Ala-Phe). The evaluated m_0 and pK_a values are listed in Table V together with literature pK_a values obtained by conventional methods. Although the number of pK_a values reported is half that of those considered, most were smaller than the

TABLE III

OBSERVED R_E VALUES OF TWENTY-EIGHT DIPEPTIDES

Electrolyte systems as in Table II. R_E = Ratio of potential gradients, E_S/E_L.

Sample	*Electrolyte system and* pH_L						
	1 *7.41*	*2* *8.30*	*4* *8.59*	*5* *8.77*	*6* *9.06*	*7* *9.33*	*8* *9.52*
Ala-Ala	8.48	4.26	3.87	3.71	3.41	3.22	3.19
Ala-Amin	8.84	4.37*	–	–	3.50	3.42	3.34
Ala-Asn	8.73	4.44*	–	–	3.55	3.43	3.34
Ala-Gly	7.38	3.91	3.49	3.45	3.10	3.01	2.92
Ala-Leu	9.49	4.76*	–	–	3.83	3.68	3.58
Ala-Met	9.08	4.63*	–	–	3.78	3.64	3.50
Ala-Phe	9.47	4.95*	–	–	3.87	3.64	3.56
Ala-Ser	7.38	4.04*	–	–	3.35	3.28	3.23
Ala-Val	9.06	4.54*	–	–	3.66	3.50	3.36
β-Ala-His	–	9.23*	–	–	5.53	4.93	4.60
Gly-Ala	7.66	3.85*	3.59	3.47	3.12	2.97	2.98
Gly-α-Amin	7.92	4.12*	–	–	3.33	3.18	3.05
Gly-Asn	7.66	4.03	3.69	3.60	3.24	3.15	3.09
Gly-Gly	6.85	3.51	3.22	3.12	2.90	2.75	2.72
Gly-Ile	8.41	4.41	4.02	3.93	3.58	3.46	3.40
Gly-Leu	8.56	4.53	4.13	3.99	3.61	3.39	3.39
Gly-Phe	7.85	4.39	4.01	3.94	3.60	3.45	3.44
Gly-Pro	10.40	4.72	4.24	3.99	3.40	3.22	3.20
Gly-Ser	7.27	3.92	3.56	3.44	3.19	3.07	2.97
Gly-Thr	7.60	4.14*	–	–	3.38	3.27	3.20
Gly-Trp	8.44	4.74	4.31	4.19	3.71	3.57	3.56
Gly-Tyr	8.62	4.65*	–	–	3.58	3.27	3.00
Gly-Val	7.96	4.29*	3.92	3.79	3.42	3.27	3.28
Leu-Gly	7.51	4.28	3.85	3.89	3.49	3.40	3.40
Leu-Leu	9.43	5.05*	–	–	4.16	4.04	3.97
Leu-Phe	9.49	5.05*	–	–	4.16	4.02	3.91
Leu-Tyr	7.19	4.78*	4.51	4.36	3.87	3.61	3.42
Leu-Val	8.91	4.88*	–	–	3.98	3.90	3.78

* Electrolyte system 3, pH_L = 8.37.

evaluated values. This can be understood since most of the previous values were not corrected to the thermodynamic values and the ionic strengths, I, were in the range of 0.01–0.15. At I = 0.01 for example, according to the Debye–Hückel equation, the thermodynamic pK_a of a monovalent anion is 0.135 larger than the observed value. Taking into account this correction, the evaluated pK_a values are in good agreement with the previously reported values.

For the monovalent ions of the dipeptides, the evaluated m_0 values are in the range of $21.6 \cdot 10^{-5}$ (Leu-Leu)–$31.5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (Gly-Gly). Except for β-Ala-His (9.664), the pK_a values are in the narrow range of 8.269 (Leu-Gly)–8.746 (Gly-Pro). The m_0 and pK_a values of the constituent neutral amino acids are in the range of $26.4 \cdot 10^{-5}$ (Leu)–$37.4 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (Gly) and 9.030 (Asn)–9.857 (Ala). Therefore the separability of the dipeptides may be lower than that of the amino acids.

Of interest is how the mobilities and pK_a values of the dipeptides correlate with

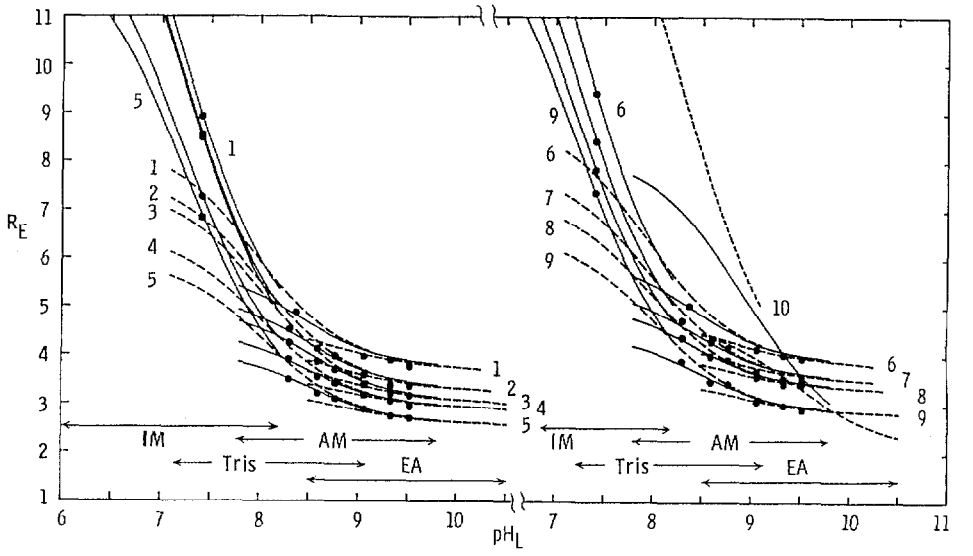

Fig. 2. The observed R_E values of Leu-Val (1), Gly-Leu (2), Ala-Ala (3), Gly-Ser (4), Gly-Gly (5), Leu-Leu (6), Gly-Trp (7), Gly-Phe (8) and Ala-Gly (9). The leading ion was 10 mM chloride. The curves were plotted using the best-fitted mobility and pK_a. The simulated curve for Gly (10) is also shown.

the corresponding values of the constituent amino acids. Fig. 3 shows the pH dependence of the effective mobility of Ala-Gly, Gly-Ala, Gly-Gly, Ala-Ala, Ala and Gly at $I = 0$. The mobilities of the dipeptides were smaller than those of Ala and Gly because of the increase in the ionic radii, and those of Ala-Gly and Gly-Ala were equal. The pK_a values of Gly-Gly and Ala-Ala were smaller than those of Gly (9.7796) and Ala (9.857). The shifts are very similar, 1.38 and 1.37 respectively. Such pK_a shifts for alanyl, glycyl and leucyl derivatives from the pK_a values of Ala, Gly and Leu (9.728) are summarized in Table VI, and the averages of the shifts are 1.400, 1.380 and 1.474 respectively. For twenty-seven dipeptides (β-Ala-His not included), the average of the shifts was 1.404 and the largest deviation from the average was found for Leu-Tyr (1.900). Thus pK_a values of monovalent anions of other dipeptides may be estimated conveniently as a first approximation. It should be noted that the dipeptides considered are limited to alanyl, glycyl and leucyl derivatives, and the pK_a values of the constituent amino acids are similar, in the range of 9.030 (Asn)–9.857 (Ala), except for Pro (10.64) and β-Ala (10.237). For Gly-Pro and β-Ala-His, the pK_a shifts from Gly and β-Ala were 0.86 and 0.573, deviating significantly from the above trend.

For many ions with relatively large molecular weights, MW, correlations between the mobilities and the molecular weights have been confirmed[9,10]. For the dipeptides considered a good correlation was also found, except for Gly-Tyr and Leu-Tyr

$$m_0 = (306.9/\sqrt{\mathrm{MW}} + 3.4) \cdot 10^{-5}\ \mathrm{cm^2\ V^{-1}\ s^{-1}} \quad (1)$$

TABLE IV

OBSERVED AND SIMULATED R_E VALUES OF EIGHT DIPEPTIDES, EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)

$\bar{m}_S$ = Effective mobility ($cm^2\ V^{-1}\ s^{-1}$) of sample ion · 10^5; pH_S = pH of sample zone; C_S^t = total concentration (mM) of sample; $C_{B,S}^t$ = total concentration (mM) of buffer ion; $\bar{m}_{B,S}$ = effective mobility ($cm^2\ V^{-1}\ s^{-1}$) of buffer ion · 10^5; I = ionic strength · 10^3.

System	R_E		$\bar{m}_S$	pH_S	C_S^t	$C_{B,S}^t$	$\bar{m}_{B,S}$	I
	Obs.	*Calc.*						
Ala-Ala								
1	8.48	8.48	8.81	8.19	5.53	22.0	4.37	1.91
2	4.26	4.25	17.6	8.85	6.26	9.33	14.2	4.46
4	3.87	3.92	19.1	8.99	6.25	12.2	11.8	4.85
5	3.71	3.72	20.1	9.11	6.24	15.2	9.92	5.12
6	3.41	3.36	22.2	9.43	5.65	9.17	23.3	5.13
7	3.22	3.24	23.0	9.64	5.60	12.1	18.2	5.30
8	3.19	3.18	23.5	9.81	5.54	15.6	14.3	5.37
	Mean error = 0.58%							
Ala-Gly								
1	7.38	7.39	10.1	8.14	5.77	22.2	4.85	2.15
2	3.91	3.84	19.4	8.81	6.50	9.55	14.9	4.80
4	3.49	3.56	21.0	8.96	6.49	12.4	12.3	5.19
5	3.45	3.39	22.0	9.08	6.48	15.4	10.4	5.45
6	3.10	3.11	24.0	9.39	5.90	9.39	24.0	5.44
7	3.01	3.01	24.8	9.61	5.86	12.3	18.9	5.60
8	2.91	2.95	25.3	9.78	5.82	15.8	14.9	5.67
	Mean error = 1.05%							
Ala-Leu								
1	9.49	9.50	7.86	8.22	5.07	21.6	4.15	1.78
3	4.76	4.73	15.8	8.90	5.81	9.42	13.3	4.23
6	3.83	3.81	19.6	9.46	5.17	8.75	19.6	4.72
7	3.68	3.68	20.3	9.68	5.11	11.7	17.3	4.86
8	3.58	3.61	20.7	9.85	5.03	15.2	13.4	4.90
	Mean error = 0.38%							
Gly-Ala								
1	7.66	7.67	9.74	8.16	5.78	22.3	4.67	2.07
3	3.85	3.83	19.5	8.85	6.50	10.1	14.1	4.81
4	3.59	3.61	20.7	8.97	6.50	12.4	12.1	5.12
5	3.47	3.43	21.8	9.08	6.49	15.4	10.3	5.38
6	3.12	3.13	23.9	9.40	5.91	9.40	23.8	5.40
7	2.97	3.02	24.7	9.62	5.87	12.3	18.8	5.57
8	2.98	2.96	25.2	9.79	5.81	15.8	14.8	5.64
	Mean error = 0.65%							
Gly-Gly								
1	6.85	6.91	10.8	8.14	6.18	22.6	4.91	2.22
2	3.51	3.53	21.2	8.80	6.83	9.88	14.9	5.00
4	3.22	3.27	22.9	8.95	6.83	12.7	12.5	5.41
5	3.12	3.11	24.0	9.06	6.82	15.7	10.6	5.69
6	2.90	2.85	26.3	9.38	6.27	9.73	24.4	5.74
7	2.75	2.75	27.2	9.59	6.23	12.6	19.4	5.92
8	2.72	2.70	27.7	9.76	6.18	16.1	15.5	6.00
	Mean error = 0.73%							

TABLE IV *(continued)*

System	R_E		$\bar{m}_S$	pH_S	C_S^t	$C_{B,S}^t$	$\bar{m}_{B,S}$	I
	Obs.	*Calc.*						
Gly-Leu								
1	8.56	8.58	8.70	8.18	5.25	21.7	4.49	1.94
2	4.53	4.47	16.7	8.84	5.99	9.06	14.4	4.39
4	4.13	4.13	18.1	8.99	5.97	11.9	11.8	4.76
5	3.99	3.93	19.0	9.11	5.96	14.9	9.89	5.00
6	3.61	3.59	20.8	9.44	5.36	8.90	23.1	4.93
7	3.39	3.48	21.5	9.66	5.30	11.8	17.8	5.07
8	3.39	3.42	21.8	9.83	5.23	15.3	13.9	5.11
	Mean error = 0.98%							
Leu-Gly								
1	7.51	7.52	9.93	8.11	5.24	21.7	5.14	2.23
2	4.28	4.22	17.7	8.78	5.98	9.04	15.3	4.67
4	3.85	3.94	19.0	8.95	5.97	11.9	12.4	5.01
5	3.89	3.78	19.8	9.08	5.96	14.9	10.3	5.23
6	3.49	3.52	21.2	9.41	5.36	8.88	23.6	5.05
7	3.40	3.43	21.7	9.64	5.30	11.8	18.1	5.14
8	3.40	3.39	22.0	9.82	5.23	15.3	14.0	5.16
	Mean error = 1.26%							
Leu-Leu								
1	9.43	9.43	7.92	8.19	4.70	21.2	4.39	1.85
3	5.05	5.03	14.9	8.88	5.43	9.05	13.7	4.16
6	4.16	4.16	17.9	9.47	4.78	8.39	22.2	4.47
7	4.04	4.04	18.5	9.70	4.71	11.4	16.7	4.55
8	3.97	3.98	18.8	9.89	4.62	14.9	12.8	4.62
	Mean error = 0.16%							

where m_0 are the absolute mobilities of the monovalent ions. The correlation coefficient was 0.94 and the standard deviation of m_0 was $0.79 \cdot 10^{-5}$. The mean deviation between the estimated and the observed m_0 was 2.5%. For the other dipeptides, this equation can give m_0 to a good approximation. On the contrary, for the amino acids the correlation was not so good. For twenty-two anionic amino acids, DL-Ala, β-Ala, DL-α-Amin, L-Asn, Asp, L-Cys, L-Glu, L-Gln, Gly, L-His, L-Hyp, DL-Ile, L-Leu, DL-Met, L-Phe, L-Pro, DL-Ser, Tau, DL-Thr, DL-Trp, L-Tyr and DL-Val, the correlation coefficient was 0.69 and the standard deviation was $2.7 \cdot 10^{-5}$. When Cys, Tau and Tyr were rejected, the correlation coefficient was 0.82, the standard deviation was $1.5 \cdot 10^{-5}$ and the mean deviation was 4.6%.

We also found a simple relationship to express the mobilities of the dipeptides in terms of those of the constituent amino acids. Since the m_0 values of the amino acids had already been evaluated[1], the corresponding Stokes radii at 25°C for monovalent anions can be calculated as

$$r = Ze/6\pi\eta m_0 = (95.104/m_0 \cdot 10^{-5}\ \text{Å} \qquad (2)$$

TABLE V

ABSOLUTE MOBILITIES AND DISSOCIATION CONSTANTS OF TWENTY-EIGHT DIPEPTIDES* (25°C)

Dipeptide	*Present method*		*Other methods pK_a*
	m_0	pK_a	
Ala-Ala	27.0	8.490	8.420 (6), 8.30 (7), 8.337 (5), 8.14 (7)
Ala-α-Amin	25.8	8.495	–
Ala-Asn	25.5	8.470	–
Ala-Gly	28.8	8.390	8.254 (7), 8.18 (6)
Ala-Leu	23.9	8.505	–
Ala-Met	24.2	8.463	–
Ala-Phe	23.9	8.502	–
Ala-Ser	26.2	8.297	–
Ala-Val	25.2	8.500	–
β-Ala-His	24.4	9.664	–
Gly-Ala	28.8	8.435	8.252 (5), 8.23 (6), 8.22 (7)
Gly-α-Amin	27.2	8.421	–
Gly-Asn	27.5	8.388	8.299 (5), 8.44 (8)
Gly-Gly	31.5	8.400	8.253 (6), 8.23 (7), 8.252 (5)
Gly-Ile	25.2	8.412	8.044 (7)
Gly-Leu	25.1	8.432	8.380 (7), 8.292 (5), 8.29 (6)
Gly-Phe	24.8	8.235	8.364 (7)
Gly-Pro	27.8	8.746	8.771(7), 8.66 (6) 8.622 (5)
Gly-Ser	28.1	8.350	8.380 (6), 8.34 (7)
Gly-Thr	26.3	8.334	–
Gly-Trp	23.6	8.359	8.124 (7)
Gly-Tyr	19.7**	8.211	8.25 (6)
	39.4**	9.981	10.03 (6)
Gly-Val	26.0	8.385	8.301 (5), 8.252 (5), 8.25 (6) 8.22 (7)
Leu-Gly	25.0	8.269	8.250 (6), 7.96 (7), 7.824 (5)
Leu-Leu	21.6	8.397	–
Leu-Phe	21.8	8.413	–
L-Leu-L-Tyr	18.2**	7.828	7.84 (7)
	36.4**	10.065	10.59 (7)
Leu-Val	22.3	8.364	–

* Unless otherwise noted, the dipeptides are DL isomers.
** The value was fixed in the least-squares method.

where Z is the charge of the ion (-1) and η is the viscosity of water. For Ala, Gly and Leu, the calculated Stokes radii were 2.95, 2.54 and 3.60 Å, and the absolute mobilities were $32.2 \cdot 10^{-5}$, $37.4 \cdot 10^{-5}$ and $26.4 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} respectively. On the assumption that the ions are spherical and the ionic volumes of the dipeptides are equal to the sum of the volumes of the constituent amino acids, V_A, V_B, the radii of the dipeptides and the mobilities can be expressed as

$$r_{AB} = [3(V_A + V_B)/4\pi[^{1-3} = (r_A{}^3 + r_B{}^3)^{1-3} \quad (3)$$

$$m_{AB} = (m_A{}^{-3} + m_B{}^{-3})^{1-3} \quad (4)$$

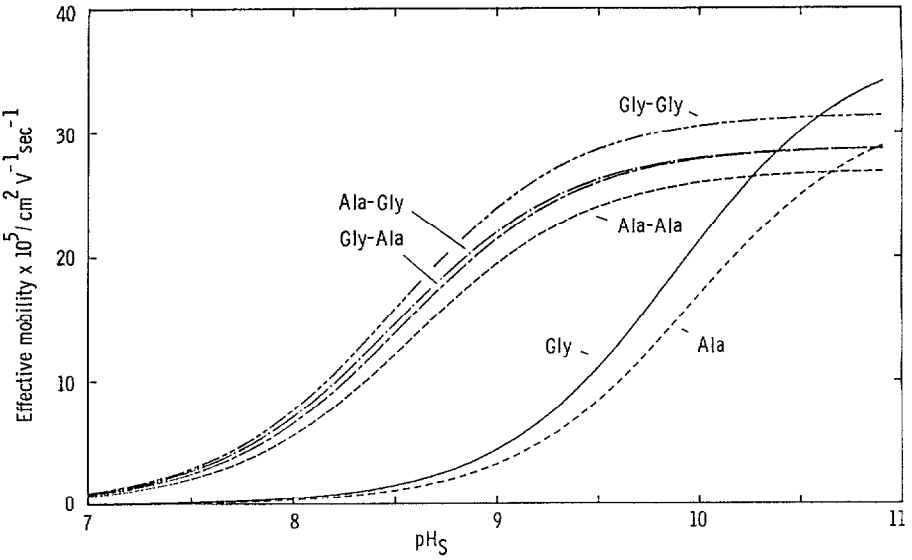

Fig. 3. The pH dependence of the effective mobility of Ala-Gly, Gly-Ala, Gly-Gly, Ala-Ala, Ala and Gly. The ionic strength is zero and the curves are not for the isotachophoretic steady state. pH_S = pH of sample zone.

where r_{AB} and m_{AB} are the Stokes radii and the absolute mobilities of the dipeptides. The estimated Stokes radii for Ala-Ala, Gly-Gly and Leu-Leu, for example, were 3.72, 3.20 and 3.60 Å and m_{AB} were $25.6 \cdot 10^{-5}$, $29.7 \cdot 10^{-5}$ and $21.0 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} respectively. By the least-squares method, for twenty-six dipeptides except for Gly-Tyr and Leu-Tyr, the following equation was obtained:

TABLE VI

DIFFERENCES IN pK_a VALUES AMONG Ala, Gly, Leu AND THE RELATED DIPEPTIDES (25°C)

ΔpK_a = pK_a (Ala, Gly, Leu) − pK_a (derivatives).

Dipeptide	*ΔpK_a*	*Dipeptide*	*ΔpK_a*	*Dipeptide*	*ΔpK_a*
Ala-Ala	1.367	Gly-Ala	1.345		
Ala-Amin	1.362	Gly-Amin	1.359		
Ala-Asn	1.387	Gly-Asn	1.392		
Ala-Gly	1.467	Gly-Gly	1.380	Leu-Gly	1.459
		Gly-Ile	1.368		
Ala-Leu	1.352	Gly-Leu	1.348	Leu-Leu	1.331
Ala-Met	1.394				
Ala-Phe	1.355	Gly-Phe	1.455	Leu-Phe	1.315
		Gly-Pro	1.034		
Ala-Ser	1.560	Gly-Ser	1.430		
		Gly-Thr	1.446		
		Gly-Trp	1.421		
		Gly-Tyr	1.569	Leu-Tyr	1.900
Ala-Val	1.357	Gly-Val	1.395	Leu-Val	1.364
Average	1.400		1.380		1.474

$$m_0 = 1.047 m_{AB} - 3 \cdot 10^{-6} \quad (5)$$

The coefficient of m_{AB} and the intercept of eqn. 5 should be 1 and 0 respectively when the estimated mobilities, m_{AB}, just fit the observed values, m_0. Eqn. 5 suggests that the estimated m_0 values were slightly underestimated in comparison with the observed values, due to the assumption that the ions are spherical. However the estimated values correlate well with the observed values. The correlation coefficient between the observed and the estimated m_0 values obtained using eqns. 2–5 was 0.97 and the standard deviation of the estimated m_0 was $0.59 \cdot 10^{-5}$. The mean deviation between the estimated and the observed m_0 values was 1.8%.

Table VII summarizes the observed and the estimated mobilities. The deviations are sufficiently small. By the use of this correlation equation, the m_0 values of the other dipeptides may be evaluated to a good approximation. The m_0 estimation from Stokes radii is meaningful in the sense that a good correlation exists between the Stokes radii of dipeptides and of the constituent amino acids.

The m_0 and pK_a values of dipeptides were evaluated independently except for Gly-Tyr and Leu-Tyr. For the latter, two pK_a values and two m_0 values for the monovalent and divalent anions should be evaluated; however, because of the pH_L conditions used, they could not be obtained independently, *i.e.*, reasonable convergence was not obtained in the least-squares method. Therefore the number of the unknown constants were decreased using the following assumptions: the monovalent mobilities, m_1, were estimated using eqn. 5 and the relationship $m_2 = 2m_1$ was adopted. In the least-squares method to obtain eqns. 1 and 5, the mobilities of these ions were not included.

TABLE VII

OBSERVED AND ESTIMATED ABSOLUTE MOBILITIES OF TWENTY-EIGHT DIPEPTIDES (25°C)

Dipeptide	m_0			*Dipeptide*	m_0		
	Obs.	*Est-1*	*Est-2*		*Obs.*	*Est-1*	*Est-2*
Ala-Ala	27.0	27.7	26.5	Gly-Ile	25.2	25.8	24.9
Ala-Amin	25.8	26.7	25.6	Gly-Leu	25.1	25.8	24.7
Ala-Asn	25.5	24.9	26.1	Gly-Phe	24.8	24.0	25.0
Ala-Gly	28.8	28.8	28.2	Gly-Pro	27.8	26.8	26.4
Ala-Leu	23.9	25.0	23.5	Gly-Ser	28.1	27.5	29.0
Ala-Met	24.2	24.1	25.1	Gly-Thr	26.3	26.5	27.5
Ala-Phe	23.9	23.4	23.8	Gly-Trp	23.6	22.4	24.0
Ala-Ser	26.2	26.5	27.0	Gly-Tyr	–	–	19.7
Ala-Val	25.2	25.8	24.7	Gly-Val	26.0	26.7	26.0
β-Ala-His	24.4	23.8	24.8	Leu-Gly	25.0	25.8	24.7
Gly-Ala	28.8	28.8	28.2	Leu-Leu	21.6	21.7	21.6
Gly-Amin	27.2	27.7	27.3	Leu-Phe	21.8	21.8	21.8
Gly-Asn	27.5	25.7	27.9	Leu-Tyr	–	–	18.2
Gly-Gly	31.5	30.1	30.8	Leu-Val	22.3	23.6	22.4

Separability assessment

Using the evaluated constants, the separability was assessed for Asp, Glu, Gly-Gly, Gly-Ser, Gly-Amin, Gly-Leu, Leu-Val, Tau, Thr, Gln and His. Fig. 4 shows the pH_L dependence of their simulated effective mobilities in the range pH_L 6–10. Fig. 5 shows the pH_L dependence of the simulated R_E values. The buffers used in the simulation were histidine, imidazole, Tris, amediol and ethanolamine. In Fig. 5, HCO_3^- is included, which originated from carbon dioxide dissolved in the solvent which is usually inevitable at thigh pH such that $pH_L \geqslant 8$. From Figs. 4 and 5, a

TABLE VIII

SIMULATED EFFECTIVE MOBILITIES OF TWENTY-EIGHT DIPEPTIDES AND THE FIFTEEN CONSTITUENT AMINO ACIDS

The leading ion is 10 m*M* chloride.

	G-G	*L-T*	*G-S*	*A-G*	*A-S*	*L-G*	*G-T*	*G-A*	*G-A*	*G-P*	*G-A*	*G-V*	*G-I*	*G-T*	*A-A*
pH_L 7.2, imidazole buffer															
1 Gly-Gly	9.4	0.2	0.5	0.6	0.6	0.8	0.9	0.9	1.0	1.1	1.3	1.3	1.7	1.8	1.8
2 Leu-Tyr		9.3	0.3	0.5	0.5	0.6	0.7	0.8	0.8	1.0	1.1	1.2	1.6	1.6	1.6
3 Gly-Ser			8.9	0.1	0.1	0.3	0.4	0.4	0.5	0.6	0.8	0.8	1.2	1.3	1.3
4 Ala-Gly				8.8	0.0	0.2	0.3	0.3	0.3	0.5	0.6	0.7	1.1	1.2	1.2
5 Ala-Ser					8.8	0.1	0.2	0.3	0.3	0.5	0.6	0.7	1.1	1.2	1.2
6 Leu-Gly						8.6	0.1	0.2	0.2	0.4	0.5	0.5	0.9	1.0	1.0
7 Gly-Thr							8.5	0.1	0.1	0.3	0.4	0.4	0.8	0.9	0.9
8 Gly-Asn								8.5	0.0	0.2	0.3	0.4	0.8	0.8	0.8
9 Gly-Ala									8.5	0.2	0.3	0.4	0.8	0.8	0.8
10 Gly-Phe										8.3	0.1	0.2	0.6	0.6	0.6
11 Gly-Amin											8.2	0.1	0.4	0.5	0.5
12 Gly-Val												8.1	0.4	0.5	0.5
13 Gly-Ile													7.7	0.1	0.1
14 Gly-Trp														7.6	0.0
15 Ala-Ala															7.6
16 Gly-Leu															
17 Gly-Tyr															
18 Ala-Asn															
19 Ala-Amin															
20 Leu-Val															
21 Ala-Val															
22 Ala-Met															
23 Leu-Leu															
24 Leu-Phe															
25 Ala-Phe															
26 Ala-Leu															
27 Gly-Pro															
28 Asn															
29 Thr															
30 Ser															
31 Phe															
32 Met															
33 Tyr															
34 Gly															

good separability is expected for these samples, using Tris as the pH buffer. The optimum pH_L range may be *ca.* 7.5–8.2.

Fig. 6 shows the simulated and the observed isotachopherograms at pH 8 using Tris as buffer. The terminator was Gly. At pH_L 8, the order of elution agreed with the decreasing order of the simulated effective mobilities, namely HCO_3^- ($41.8 \cdot 10^{-5}$), Asp ($27.3 \cdot 10^{-5}$), Glu ($24.5 \cdot 10^{-5}$), Gly-Gly ($17.1 \cdot 10^{-5}$), Gly-Ser ($15.8 \cdot 10^{-5}$), Gly-Amin ($14.7 \cdot 10^{-5}$), Gly-Leu ($13.5 \cdot 10^{-5}$), Leu-Val ($12.6 \cdot 10^{-5}$), Tau ($11.4 \cdot 10^{-5}$), Thr ($9.3 \cdot 10^{-5}$), Gln ($8.6 \cdot 10^{-5}$), His ($7.7 \cdot 10^{-5}$) and Gly ($6.6 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}). The

G-L	*G-T*	*A-A*	*A-A*	*L-V*	*A-V*	*A-M*	*L-L*	*L-P*	*A-P*	*A-L*	*G-P*	*Asn*	*Thr*	*Ser*	*Phe*	*Met*	*Tyr*	*Gly*
1.9	1.9	2.0	2.1	2.2	2.3	2.3	2.6	2.6	2.6	2.6	3.3	4.2	5.1	5.3	5.8	5.9	6.0	6.7
1.7	1.7	1.9	2.0	2.0	2.1	2.1	2.4	2.4	2.5	2.5	3.1	4.1	5.0	5.1	5.7	5.7	5.8	6.5
1.4	1.4	1.5	1.6	1.7	1.8	1.8	2.1	2.1	2.1	2.1	2.8	3.7	4.6	4.8	5.3	5.4	5.5	6.2
1.3	1.3	1.4	1.5	1.5	1.7	1.7	1.9	2.0	2.0	2.0	2.6	3.6	4.5	4.7	5.2	5.3	5.4	6.1
1.2	1.3	1.4	1.5	1.5	1.7	1.7	1.9	2.0	2.0	2.0	2.6	3.6	4.5	4.6	5.2	5.2	5.4	6.0
1.1	1.1	1.3	1.3	1.4	1.5	1.5	1.8	1.8	1.8	1.9	2.5	3.5	4.4	4.5	5.1	5.1	5.2	5.9
1.0	1.0	1.2	1.2	1.3	1.4	1.4	1.7	1.7	1.7	1.8	2.4	3.3	4.3	4.4	5.0	5.0	5.1	5.8
0.9	0.9	1.1	1.2	1.2	1.3	1.4	1.6	1.7	1.7	1.7	2.3	3.3	4.2	4.3	4.9	4.9	5.0	5.7
0.9	0.9	1.1	1.2	1.2	1.3	1.3	1.6	1.6	1.6	1.7	2.3	3.3	4.2	4.3	4.9	4.9	5.0	5.7
0.7	0.7	0.9	1.0	1.0	1.1	1.1	1.4	1.4	1.5	1.5	2.1	3.1	4.0	4.1	4.7	4.7	4.8	5.5
0.6	0.6	0.8	0.9	0.9	1.0	1.0	1.3	1.3	1.3	1.4	2.0	3.0	3.9	4.0	4.6	4.6	4.7	5.4
0.6	0.6	0.7	0.8	0.9	1.0	1.0	1.2	1.3	1.3	1.3	1.9	2.9	3.8	4.0	4.5	4.6	4.7	5.4
0.2	0.2	0.3	0.4	0.5	0.6	0.6	0.8	0.9	0.9	0.9	1.5	2.5	3.4	3.6	4.1	4.2	4.3	5.0
0.1	0.1	0.2	0.3	0.4	0.5	0.5	0.8	0.8	0.8	0.8	1.5	2.4	3.3	3.5	4.0	4.1	4.2	4.9
0.1	0.1	0.2	0.3	0.4	0.5	0.5	0.8	0.8	0.8	0.8	1.5	2.4	3.3	3.5	4.0	4.1	4.2	4.9
7.5	0.0	0.2	0.2	0.3	0.4	0.4	0.7	0.7	0.7	0.8	1.4	2.4	3.3	3.4	4.0	4.0	4.1	4.8
	7.5	0.1	0.2	0.3	0.4	0.4	0.7	0.7	0.7	0.7	1.4	2.3	3.2	3.4	3.9	4.0	4.1	4.8
		7.4	0.1	0.1	0.3	0.3	0.5	0.6	0.6	0.6	1.2	2.2	3.1	3.3	3.8	3.9	4.0	4.7
			7.3	0.1	0.2	0.2	0.4	0.5	0.5	0.5	1.1	2.1	3.0	3.2	3.7	3.8	3.9	4.6
				7.3	0.1	0.1	0.4	0.4	0.4	0.5	1.1	2.1	3.0	3.1	3.7	3.7	3.8	4.5
					7.1	0.0	0.3	0.3	0.3	0.3	1.0	1.9	2.8	3.0	3.5	3.6	3.7	4.4
						7.1	0.3	0.3	0.3	0.3	1.0	1.9	2.8	3.0	3.5	3.6	3.7	4.4
							6.9	0.0	0.1	0.1	0.7	1.7	2.6	2.7	3.3	3.3	3.4	4.1
								6.8	0.0	0.0	0.7	1.6	2.5	2.7	3.2	3.3	3.4	4.1
									6.8	0.0	0.6	1.6	2.5	2.7	3.2	3.3	3.4	4.1
										6.8	0.6	1.6	2.5	2.6	3.2	3.2	3.4	4.0
											6.2	1.0	1.9	2.0	2.6	2.6	2.7	3.4
												5.2	0.9	1.1	1.6	1.7	1.8	2.5
													4.3	0.1	0.7	0.7	0.9	1.6
														4.1	0.5	0.6	0.7	1.4
															3.6	0.1	0.2	0.9
																3.5	0.1	0.8
																	3.4	0.7
																		2.7

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TABLE VIII *(continued)*

	G-G	*A-G*	*G-S*	*G-A*	*A-S*	*G-A*	*G-T*	*L-G*	*G-A*	*G-V*	*G-P*	*A-A*	*G-I*	*G-L*	*L-T*	*A-A*	*A-A*	*G-T*	*A-V*	*A-M*
pH_L 8.0, Tris buffer																				
1 Gly-Gly	17.1	1.3	1.3	1.7	1.9	2.0	2.1	2.3	2.4	2.7	2.8	3.1	3.4	3.6	3.6	3.7	3.7	3.8	4.1	4.3
2 Ala-Gly		15.8	0.0	0.4	0.6	0.7	0.8	1.0	1.1	1.4	1.5	1.8	2.1	2.3	2.3	2.4	2.4	2.5	2.8	3.0
3 Gly-Ser			15.8	0.4	0.6	0.7	0.8	1.0	1.1	1.4	1.5	1.8	2.1	2.3	2.3	2.4	2.4	2.5	2.8	3.0
4 Gly-Ala				15.4	0.2	0.2	0.4	0.6	0.7	1.0	1.1	1.4	1.6	1.9	1.9	2.0	2.0	2.1	2.3	2.5
5 Ala-Ser					15.2	0.1	0.3	0.4	0.5	0.8	0.9	1.2	1.5	1.7	1.7	1.8	1.8	1.9	2.2	2.4
6 Gly-Asn						15.1	0.2	0.4	0.4	0.8	0.9	1.1	1.4	1.6	1.6	1.7	1.8	1.8	2.1	2.3
7 Gly-Thr							14.9	0.2	0.3	0.6	0.7	1.0	1.2	1.4	1.5	1.5	1.6	1.6	1.9	2.1
8 Leu-Gly								14.7	0.1	0.4	0.5	0.8	1.0	1.2	1.3	1.3	1.4	1.5	1.7	1.9
9 Gly-Amin									14.7	0.3	0.4	0.7	1.0	1.2	1.2	1.3	1.3	1.4	1.7	1.9
10 Gly-Val										14.3	0.1	0.4	0.6	0.8	0.9	1.0	1.0	1.1	1.3	1.5
11 Gly-Phe											14.2	0.3	0.5	0.7	0.8	0.9	0.9	1.0	1.2	1.4
12 Ala-Ala												14.0	0.3	0.5	0.5	0.6	0.6	0.7	1.0	1.2
13 Gly-Ile													13.7	0.2	0.2	0.3	0.4	0.4	0.7	0.9
14 Gly-Leu														13.5	0.0	0.1	0.2	0.2	0.5	0.7
15 Leu-Tyr															13.5	0.1	0.1	0.2	0.5	0.7
16 Ala-Asn																13.4	0.1	0.1	0.4	0.6
17 Ala-Amin																	13.3	0.1	0.3	0.5
18 Gly-Trp																		13.3	0.3	0.5
19 Ala-Val																			13.0	0.2
20 Ala-Met																				12.8
21 Gly-Tyr																				
22 Leu-Val																				
23 Ala-Phe																				
24 Ala-Leu																				
25 Gly-Pro																				
26 Leu-Leu																				
27 Leu-Phe																				
28 Asn																				
29 Thr																				
30 Ser																				
31 Met																				
32 Phe																				
33 HIs																				
34 Tyr																				
35 Gly																				
36 Trp																				
37 Val																				
38 Ala																				
39 Amin																				
40 Leu																				
41 β-Ala-His																				
42 Ile																				
43 β-Ala																				

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G-T	*L-V*	*A-P*	*A-L*	*G-P*	*L-L*	*L-P*	*Asn*	*Thr*	*Ser*	*Met*	*Phe*	*His*	*Tyr*	*Gly*	*Trp*	*Val*	*Ala*	*Amin*	*Leu*	*A-H*	*Ile*	*β-Ala*
4.4	4.5	4.7	4.7	5.0	5.1	5.1	6.1	7.7	7.8	9.2	9.3	9.4	9.7	10.5	11.5	11.6	11.7	11.8	12.0	12.1	12.1	13.5
3.1	3.2	3.4	3.4	3.7	3.8	3.8	4.8	6.4	6.5	7.9	8.0	8.1	8.4	9.2	10.2	10.3	10.4	10.5	10.7	10.8	10.8	12.2
3.1	3.2	3.4	3.4	3.7	3.8	3.8	4.8	6.4	6.5	7.9	7.9	8.1	8.4	9.2	10.2	10.3	10.4	10.5	10.7	10.7	10.8	12.2
2.7	2.8	3.0	3.0	3.3	3.4	3.4	4.4	6.0	6.1	7.5	7.5	7.6	8.0	8.8	9.8	9.9	10.0	10.1	10.3	10.3	10.4	11.8
2.5	2.6	2.8	2.8	3.1	3.2	3.2	4.2	5.8	5.9	7.3	7.4	7.5	7.8	8.6	9.6	9.7	9.9	10.0	10.1	10.2	10.2	11.6
2.4	2.6	2.7	2.8	3.0	3.1	3.2	4.1	5.8	5.9	7.2	7.3	7.4	7.8	8.6	9.6	9.6	9.8	9.9	10.0	10.1	10.2	11.5
2.3	2.4	2.6	2.6	2.8	3.0	3.0	4.0	5.6	5.7	7.1	7.1	7.2	7.6	8.4	9.4	9.4	9.6	9.7	9.9	9.9	10.0	11.4
2.1	2.2	2.4	2.4	2.6	2.8	2.8	3.8	5.4	5.5	6.9	6.9	7.0	7.4	8.2	9.2	9.3	9.4	9.5	9.7	9.7	9.8	11.2
2.0	2.1	2.3	2.3	2.6	2.7	2.7	3.7	5.3	5.4	6.8	6.9	7.0	7.3	8.1	9.1	9.2	9.3	9.4	9.6	9.6	9.7	11.1
1.7	1.8	2.0	2.0	2.2	2.4	2.4	3.4	5.0	5.1	6.5	6.5	6.6	7.0	7.8	8.8	8.9	9.0	9.1	9.3	9.3	9.4	10.8
1.6	1.7	1.9	1.9	2.1	2.3	2.3	3.3	4.9	5.0	6.4	6.4	6.5	6.9	7.7	8.7	8.8	8.9	9.0	9.2	9.2	9.3	10.7
1.3	1.4	1.6	1.6	1.9	2.0	2.0	3.0	4.6	4.7	6.1	6.2	6.3	6.6	7.4	8.4	8.5	8.6	8.7	8.9	8.9	9.0	10.4
1.0	1.2	1.4	1.4	1.6	1.8	1.8	2.7	4.4	4.5	5.9	5.9	6.0	6.4	7.2	8.2	8.2	8.4	8.5	8.6	8.7	8.8	10.1
0.8	0.9	1.1	1.2	1.4	1.5	1.5	2.5	4.2	4.3	5.6	5.7	5.8	6.2	6.9	8.0	8.0	8.2	8.3	8.4	8.5	8.6	9.9
0.8	0.9	1.1	1.1	1.4	1.5	1.5	2.5	4.1	4.2	5.6	5.6	5.8	6.1	6.9	7.9	8.0	8.1	8.2	8.4	8.4	8.5	9.9
0.7	0.8	1.0	1.1	1.3	1.4	1.4	2.4	4.1	4.2	5.5	5.6	5.7	6.1	6.8	7.9	7.9	8.1	8.2	8.3	8.4	8.5	9.8
0.7	0.8	1.0	1.0	1.2	1.4	1.4	2.4	4.0	4.1	5.5	5.5	5.6	6.0	6.8	7.8	7.9	8.0	8.1	8.3	8.3	8.4	9.8
0.6	0.7	0.9	0.9	1.2	1.3	1.3	2.3	3.9	4.0	5.4	5.5	5.6	5.9	6.7	7.7	7.8	8.0	8.1	8.2	8.3	8.3	9.7
0.3	0.5	0.6	0.7	0.9	1.0	1.1	2.0	3.7	3.8	5.1	5.2	5.3	5.7	6.5	7.5	7.5	7.7	7.8	7.9	8.0	8.1	9.4
0.1	0.2	0.4	0.5	0.7	0.8	0.9	1.8	3.5	3.6	4.9	5.0	5.1	5.5	6.3	7.3	7.3	7.5	7.6	7.7	7.8	7.9	9.2
12.7	0.1	0.3	0.3	0.6	0.7	0.7	1.7	3.3	3.4	4.8	4.9	5.0	5.3	6.1	7.1	7.2	7.3	7.4	7.6	7.7	7.7	9.1
	12.6	0.2	0.2	0.5	0.6	0.6	1.6	3.2	3.3	4.7	4.7	4.8	5.2	6.0	7.0	7.1	7.2	7.3	7.5	7.5	7.6	9.0
		12.4	0.0	0.3	0.4	0.4	1.4	3.0	3.1	4.5	4.5	4.6	5.0	5.8	6.8	6.9	7.0	7.1	7.3	7.3	7.4	8.8
			12.3	0.2	0.4	0.4	1.4	3.0	3.1	4.5	4.5	4.6	5.0	5.8	6.8	6.9	7.0	7.1	7.3	7.3	7.4	8.8
				12.1	0.1	0.1	1.1	2.8	2.9	4.2	4.3	4.4	4.8	5.5	6.6	6.6	6.8	6.9	7.0	7.1	7.2	8.5
					12.0	0.0	1.0	2.6	2.7	4.1	4.1	4.2	4.6	5.4	6.4	6.5	6.6	6.7	6.9	6.9	7.0	8.4
						12.0	1.0	2.6	2.7	4.1	4.1	4.2	4.6	5.4	6.4	6.5	6.6	6.7	6.9	6.9	7.0	8.4
							11.0	1.6	1.7	3.1	3.2	3.3	3.6	4.4	5.4	5.5	5.6	5.7	5.9	6.0	6.0	7.4
								9.3	0.1	1.5	1.5	1.6	2.0	2.8	3.8	3.9	4.0	4.1	4.3	4.3	4.4	5.8
									9.2	1.4	1.4	1.5	1.9	2.7	3.7	3.8	3.9	4.0	4.2	4.2	4.3	5.7
										7.9	0.0	0.1	0.5	1.3	2.3	2.4	2.5	2.6	2.8	2.8	2.9	4.3
											7.8	0.1	0.5	1.3	2.3	2.3	2.5	2.6	2.8	2.8	2.9	4.2
												7.7	0.4	1.2	2.2	2.2	2.4	2.5	2.6	2.7	2.8	4.1
													7.3	0.8	1.8	1.9	2.0	2.1	2.3	2.3	2.4	3.8
														6.6	1.0	1.1	1.2	1.3	1.5	1.5	1.6	3.0
															5.5	0.1	0.2	0.3	0.5	0.5	0.6	2.0
																5.5	0.2	0.3	0.4	0.5	0.5	1.9
																	5.3	0.1	0.3	0.3	0.4	1.8
																		5.2	0.2	0.2	0.3	1.7
																			5.1	0.0	0.1	1.5
																				5.0	0.1	1.4
																					4.9	1.4
																						3.6

TABLE VIII *(continued)*

	G-G	*A-G*	*G-S*	*G-A*	*G-A*	*A-S*	*G-A*	*G-T*	*A-A*	*G-V*	*L-G*	*G-P*	*G-I*	*A-A*	*A-A*	*G-L*	*A-V*	*G-P*	*G-T*	*A-M*
pH_L 8.6, amediol buffer																				
1 Gly-Gly	22.9	1.9	2.1	2.2	2.8	3.2	3.2	3.3	3.8	3.9	4.0	4.4	4.6	4.7	4.8	4.8	5.2	5.3	5.5	5.6
2 Ala-Gly		21.1	0.2	0.3	0.9	1.3	1.4	1.5	2.0	2.0	2.1	2.5	2.7	2.8	2.9	2.9	3.3	3.4	3.6	3.8
3 Gly-Ser			20.8	0.1	0.7	1.1	1.1	1.2	1.7	1.8	1.8	2.3	2.5	2.6	2.6	2.7	3.0	3.1	3.4	3.5
4 Gly-Ala				20.8	0.6	1.0	1.0	1.2	1.7	1.7	1.8	2.2	2.4	2.5	2.6	2.6	3.0	3.1	3.3	3.4
5 Gly-Asn					20.1	0.4	0.4	0.5	1.0	1.1	1.2	1.6	1.8	1.9	2.0	2.0	2.4	2.4	2.7	2.8
6 Ala-Ser						19.7	0.0	0.1	0.6	0.7	0.8	1.2	1.4	1.5	1.6	1.6	2.0	2.0	2.3	2.4
7 Gly-Amin							19.7	0.1	0.6	0.6	0.7	1.2	1.4	1.5	1.5	1.6	1.9	2.0	2.2	2.4
8 Gly-Thr								19.6	0.5	0.5	0.6	1.1	1.3	1.4	1.4	1.5	1.8	1.9	2.1	2.3
9 Ala-Ala									19.1	0.0	0.1	0.6	0.8	0.9	0.9	1.0	1.3	1.4	1.6	1.8
10 Gly-Val										19.1	0.1	0.5	0.7	0.8	0.9	0.9	1.3	1.4	1.6	1.8
11 Leu-Gly											19.0	0.4	0.7	0.8	0.8	0.8	1.2	1.3	1.5	1.7
12 Gly-Phe												18.5	0.2	0.3	0.4	0.4	0.8	0.8	1.1	1.2
13 Gly-Ile													18.3	0.1	0.1	0.2	0.5	0.6	0.9	1.0
14 Ala-Amin														18.2	0.0	0.1	0.4	0.5	0.8	0.9
15 Ala-Asn															18.2	0.0	0.4	0.5	0.7	0.9
16 Gly-Leu																18.1	0.4	0.4	0.7	0.8
17 Ala-Val																	17.8	0.1	0.3	0.5
18 Gly-Pro																		17.7	0.2	0.4
19 Gly-Trp																			17.5	0.1
20 Ala-Met																				17.3
21 Asn																				
22 Gly-Tyr																				
23 Ala-Phe																				
24 Ala-Leu																				
25 Leu-Val																				
26 Leu-Tyr																				
27 Leu-Phe																				
28 Leu-Leu																				
29 Ser																				
30 Thr																				
31 Met																				
32 His																				
33 Phe																				
34 Tyr																				
35 Gly																				
36 Val																				
37 Ala																				
38 Trp																				
39 Amin																				
40 Leu																				
41 Ile																				
42 β-Ala-His																				
43 β-Ala																				

Asn	*G-T*	*A-P*	*A-L*	*L-V*	*L-T*	*L-P*	*L-L*	*Ser*	*Thr*	*Met*	*His*	*Phe*	*Tyr*	*Gly*	*Val*	*Ala*	*Trp*	*Amin*	*Leu*	*Ile*	*A-H*	*β-Ala*
5.7	5.9	6.1	6.1	6.5	6.6	7.1	7.1	7.5	7.7	9.7	10.0	10.1	10.5	11.0	13.1	13.1	13.2	13.3	13.8	14.0	14.0	16.0
3.8	4.1	4.2	4.2	4.6	4.8	5.2	5.3	5.8	5.8	7.8	8.1	8.2	8.6	9.1	11.2	11.2	11.3	11.5	11.9	12.1	12.1	14.1
3.6	3.8	4.0	4.0	4.3	4.5	4.9	5.0	5.4	5.6	7.6	7.9	7.9	8.4	8.8	11.0	11.0	11.1	11.2	11.7	11.9	11.9	13.9
3.5	3.7	3.9	3.9	4.3	4.5	4.9	4.9	5.3	5.5	7.5	7.8	7.9	8.3	8.8	10.9	10.9	11.0	11.1	11.6	11.8	11.8	13.8
2.9	3.1	3.3	3.3	3.7	3.8	4.3	4.3	4.7	4.9	6.9	7.2	7.2	7.7	8.2	10.3	10.3	10.4	10.5	11.0	1.2	11.2	13.2
2.5	2.7	2.9	2.9	3.3	3.4	3.9	3.9	4.3	4.5	6.5	6.8	6.8	7.3	7.8	9.9	9.9	10.0	10.1	10.6	10.8	10.8	12.8
2.4	2.7	2.8	2.9	3.2	3.4	3.8	3.9	4.3	4.4	6.5	6.8	6.8	7.3	7.7	9.8	9.9	10.0	10.1	10.6	10.7	10.8	12.8
2.3	2.6	2.7	2.7	3.1	3.3	3.7	3.8	4.1	4.3	6.4	6.7	6.7	7.2	7.6	9.7	9.8	9.9	10.0	10.5	10.6	10.7	12.7
1.8	2.1	2.2	2.2	2.6	2.8	3.2	3.3	3.6	3.8	5.9	6.2	6.2	6.7	7.1	9.2	9.3	9.4	9.5	10.0	10.1	10.2	12.2
1.8	2.1	2.2	2.2	2.6	2.8	3.2	3.3	3.6	3.8	5.8	6.1	6.2	6.6	7.1	9.2	9.2	9.3	9.5	9.9	10.1	10.1	12.1
1.7	2.0	2.1	2.1	2.5	2.7	3.1	3.2	3.5	3.7	5.8	6.1	6.1	6.6	7.0	9.1	9.1	9.2	9.4	9.9	10.0	10.1	12.0
1.3	1.5	1.7	1.7	2.1	2.2	2.7	2.7	3.1	3.3	5.3	5.6	5.6	6.1	6.6	8.7	8.7	8.8	8.9	9.4	9.6	9.6	11.6
1.1	1.3	1.5	1.5	1.8	2.0	2.4	2.5	2.9	3.1	5.1	5.4	5.4	5.9	6.3	8.5	8.5	8.6	8.7	9.2	9.4	9.4	11.4
1.0	1.2	1.4	1.4	1.7	1.9	2.4	2.4	2.8	3.0	5.0	5.3	5.3	5.8	6.3	8.4	8.4	8.5	8.6	9.1	9.3	9.3	11.3
0.9	1.2	1.3	1.3	1.7	1.9	2.3	2.4	2.7	2.9	5.0	5.3	5.3	5.8	6.2	8.3	8.3	8.4	8.6	9.1	9.2	9.3	11.2
0.9	1.1	1.3	1.3	1.7	1.8	2.3	2.3	2.7	2.9	4.9	5.2	5.2	5.7	6.2	8.3	8.3	8.4	8.5	9.0	9.2	9.2	11.2
0.5	0.8	0.9	0.9	1.3	1.5	1.9	2.0	2.3	2.5	4.6	4.9	4.9	5.4	5.8	7.9	7.9	8.0	8.2	8.7	8.8	8.9	10.8
0.4	0.7	0.8	0.8	1.2	1.4	1.8	1.9	2.2	2.4	4.5	4.8	4.8	5.3	5.7	7.8	7.9	8.0	8.1	8.6	8.7	8.8	10.8
0.2	0.4	0.6	0.6	1.0	1.2	1.6	1.7	2.0	2.2	4.2	4.5	4.6	5.0	5.5	7.6	7.6	7.7	7.9	8.3	8.5	8.5	10.5
0.0	0.3	0.4	0.5	0.8	1.0	1.4	1.5	1.9	2.0	4.1	4.4	4.4	4.9	5.3	7.4	7.5	7.6	7.7	8.2	8.4	8.4	10.4
17.3	0.3	0.4	0.4	0.8	1.0	1.4	1.5	1.8	2.0	4.0	4.3	4.4	4.8	5.3	7.4	7.4	7.5	7.7	8.1	8.3	8.3	10.3
	17.0	0.1	0.2	0.5	0.7	1.1	1.2	1.6	1.7	3.8	4.1	4.1	4.6	5.0	7.1	7.2	7.3	7.4	7.9	8.1	8.1	10.1
		16.9	0.0	0.4	0.6	1.0	1.1	1.4	1.6	3.6	3.9	4.0	4.4	4.9	7.0	7.0	7.1	7.3	7.7	7.9	7.9	9.9
			16.9	0.4	0.6	1.0	1.0	1.4	1.6	3.6	3.9	4.0	4.4	4.9	7.0	7.0	7.1	7.3	7.7	7.9	7.9	9.9
				16.5	0.2	0.6	0.7	1.0	1.2	3.3	3.6	3.6	4.1	4.5	6.6	6.6	6.7	6.9	7.4	7.5	8.7	9.5
					16.3	0.4	0.5	0.8	1.0	3.1	3.4	3.4	3.9	4.3	6.4	6.5	6.6	6.7	7.2	7.3	7.4	9.4
						15.9	0.1	0.4	0.6	2.7	3.0	3.0	3.4	3.9	6.0	6.0	6.1	6.3	6.8	6.9	6.9	8.9
							15.8	0.4	0.5	2.6	2.9	2.9	3.4	3.8	5.9	6.0	6.1	6.2	6.7	6.9	6.9	8.9
								15.5	0.2	2.2	2.5	2.6	3.0	3.5	5.6	5.6	5.7	5.9	6.3	6.5	6.5	8.5
									15.3	2.0	2.3	2.4	2.8	3.3	5.4	5.4	5.5	5.7	6.1	6.3	6.3	8.3
										13.2	0.3	0.3	0.8	1.2	3.4	3.4	3.5	3.6	4.1	4.3	4.3	6.3
											12.9	0.0	0.5	0.9	3.1	3.1	3.2	3.3	3.8	4.0	4.0	6.0
												12.9	0.5	0.9	3.0	3.1	3.2	3.3	3.8	3.9	4.0	6.0
													12.4	0.5	2.6	2.6	2.7	2.8	3.3	3.5	3.5	5.5
														12.0	2.1	2.1	2.2	2.4	2.9	3.0	3.0	5.0
															9.9	0.0	0.1	0.3	0.7	0.9	0.9	2.9
																9.8	0.1	0.2	0.7	0.9	0.9	2.9
																	9.7	0.1	0.6	0.8	0.8	2.8
																		9.6	0.5	0.6	0.7	2.7
																			9.1	0.2	0.2	2.2
																				9.0	0.0	2.0
																					8.9	2.0
																						6.9

(Continued on p. 218/219)

TABLE VIII *(continued)*

	G-G	*A-G*	*G-A*	*Asn*	*G-S*	*Ser*	*G-A*	*G-T*	*G-A*	*A-A*	*G-P*	*G-T*	*Thr*	*A-S*	*G-V*	*A-A*	*A-A*	*L-G*	*G-I*	*G-L*
pH_L 9.4, ethanolamine buffer																				
1 Gly-Gly	27.4	2.4	2.5	2.8	2.9	3.4	3.5	3.7	3.9	4.2	4.3	4.5	4.5	4.5	4.8	5.3	5.5	5.5	5.6	5.7
2 Ala-Gly		25.0	0.1	0.4	0.5	1.1	1.2	1.4	1.5	1.8	2.0	2.1	2.1	2.1	2.5	2.9	3.1	3.2	3.2	3.4
3 Gly-Ala			24.9	0.3	0.4	1.0	1.0	1.3	1.4	1.7	1.8	2.0	2.0	2.0	2.4	2.8	3.0	3.1	3.1	3.3
4 Asn				24.6	0.1	0.7	0.7	1.0	1.1	1.4	1.5	1.7	1.7	1.7	2.1	2.5	2.7	2.8	2.8	3.0
5 Gly-Ser					24.5	0.5	0.6	0.8	0.9	1.3	1.4	1.6	1.6	1.6	1.9	2.3	2.6	2.6	2.7	2.8
6 Ser						23.9	0.1	0.3	0.4	0.8	0.9	1.0	1.0	1.1	1.4	1.8	2.0	2.1	2.2	2.3
7 Gly-Asn							23.9	0.2	0.3	0.7	0.8	1.0	1.0	1.0	1.3	1.7	1.9	2.0	2.1	2.2
8 Gly-Tyr								23.6	0.1	0.5	0.6	0.7	0.7	0.8	1.1	1.5	1.7	1.8	1.9	2.0
9 Gly-Amin									23.5	0.3	0.5	0.6	0.6	0.7	1.0	1.4	1.6	1.7	1.8	1.9
10 Ala-Ala										23.2	0.1	0.3	0.3	0.3	0.7	1.1	1.3	1.3	1.4	1.5
11 Gly-Pro											23.1	0.2	0.2	0.2	0.5	0.9	1.1	1.2	1.3	1.4
12 Gly-Thr												22.9	0.0	0.0	0.4	0.8	1.0	1.1	1.1	1.3
13 Thr													22.9	0.0	0.4	0.8	1.0	1.1	1.1	1.2
14 Ala-Ser														22.9	0.3	0.7	1.0	1.0	1.1	1.2
15 Gly-Val															22.5	0.4	0.6	0.7	0.8	0.9
16 Ala-Amin																22.1	0.2	0.3	0.4	0.5
17 Ala-Asn																	21.9	0.1	0.1	0.3
18 Leu-Gly																		21.8	0.1	0.2
19 Gly-Ile																			21.8	0.1
20 Gly-Leu																				21.6
21 Ala-Val																				
22 Gly-Phe																				
23 Leu-Tyr																				
24 Gly																				
25 Tyr																				
26 Ala-Met																				
27 Met																				
28 Ala-Phe																				
29 Ala-Leu																				
30 Gly-Trp																				
31 His																				
32 Phe																				
33 Leu-Val																				
34 Leu-Phe																				
35 Leu-Leu																				
36 Ala																				
37 Amin																				
38 Val																				
39 Trp																				
40 Leu																				
41 Ile																				
42 β-Ala-His																				
43 β-Ala																				

A-V	*G-P*	*L-T*	*Gly*	*Tyr*	*A-M*	*Met*	*A-P*	*A-L*	*G-T*	*His*	*Phe*	*L-V*	*L-P*	*L-L*	*Ala*	*Amin*	*Val*	*Trp*	*Leu*	*Ile*	*A-H*	*β-Ala*
5.8	5.9	6.0	6.1	6.4	6.6	6.7	6.9	6.9	6.9	7.2	7.7	8.1	8.6	8.8	9.5	10.1	10.2	10.9	11.4	11.5	12.0	13.6
3.4	3.5	3.6	3.8	4.0	4.2	4.3	4.6	4.6	4.6	4.9	5.3	5.7	6.2	6.4	7.2	7.7	7.8	8.5	9.0	9.1	9.6	11.2
3.3	3.4	3.5	3.7	3.9	4.1	4.2	4.4	4.5	4.5	4.7	5.2	5.6	6.1	6.3	7.1	7.6	7.7	8.4	8.9	9.0	9.5	11.1
3.0	3.1	3.2	3.4	3.6	3.8	3.9	4.1	4.1	4.1	4.4	4.9	5.3	5.8	6.0	6.8	7.3	7.4	8.1	8.6	8.7	9.2	10.8
2.9	3.0	3.1	3.2	3.5	3.7	3.8	4.0	4.0	4.0	4.3	4.8	5.2	5.7	5.9	6.6	7.2	7.2	8.0	8.4	8.6	9.1	10.7
2.3	2.4	2.5	2.7	2.9	3.1	3.2	3.5	3.5	3.5	3.8	4.3	4.7	5.2	5.3	6.1	6.6	6.7	7.5	7.9	8.0	8.5	10.1
2.3	2.3	2.4	2.6	2.8	3.1	3.1	3.4	3.4	3.4	3.7	4.2	4.6	5.1	5.2	6.0	6.6	6.6	7.4	7.8	8.0	8.4	10.0
2.0	2.1	2.2	2.4	2.6	2.8	2.9	3.2	3.2	3.2	3.5	4.0	4.4	4.9	5.0	5.8	6.3	6.4	7.2	7.6	7.7	8.2	9.8
1.9	2.0	2.1	2.3	2.5	2.7	2.8	3.1	3.1	3.1	3.4	3.8	4.2	4.8	4.9	5.7	6.2	6.3	7.1	7.5	7.6	8.1	9.7
1.6	1.7	1.8	2.0	2.2	2.4	2.5	2.7	2.7	2.7	3.0	3.5	3.9	4.4	4.6	5.3	5.9	6.0	6.7	7.2	7.3	7.8	9.4
1.5	1.5	1.6	1.8	2.0	2.3	2.3	2.6	2.6	2.6	2.9	3.4	3.8	4.3	4.4	5.2	5.8	5.8	6.6	7.0	7.2	7.6	9.2
1.3	1.4	1.5	1.7	1.9	2.1	2.2	2.4	2.5	2.5	2.7	3.2	3.6	4.1	4.3	5.1	5.6	5.7	6.4	6.9	7.0	7.5	9.1
1.3	1.4	1.5	1.7	1.9	2.1	2.2	2.4	2.4	2.4	2.7	3.2	3.6	4.1	4.3	5.1	5.6	5.7	6.4	6.9	7.0	7.5	9.1
1.3	1.4	1.4	1.6	1.8	2.1	2.2	2.4	2.4	2.4	2.7	3.2	3.6	4.1	4.3	5.0	5.6	5.6	6.4	6.8	7.0	7.5	9.1
0.9	1.0	1.1	1.3	1.5	1.7	1.8	2.1	2.1	2.1	2.4	2.9	3.3	3.8	3.9	4.7	5.2	5.3	6.1	6.5	6.6	7.1	8.7
0.5	0.6	0.7	0.9	1.1	1.3	1.4	1.7	1.7	1.7	2.0	2.4	2.8	3.4	3.5	4.3	4.8	4.9	5.7	6.1	6.2	6.7	8.3
0.3	0.4	0.5	0.7	0.9	1.1	1.2	1.5	1.5	1.5	1.8	2.2	2.6	3.2	3.3	4.1	4.6	4.7	5.5	5.9	6.0	6.5	8.1
0.2	0.3	0.4	0.6	0.8	1.0	1.1	1.4	1.4	1.4	1.7	2.2	2.6	3.1	3.2	4.0	4.5	4.6	5.4	5.8	5.9	6.4	8.0
0.2	0.3	0.3	0.5	0.7	1.0	1.1	1.3	1.3	1.3	1.6	2.1	2.5	3.0	3.2	3.9	4.5	4.5	5.3	5.7	5.9	6.4	8.0
0.1	0.1	0.2	0.4	0.6	0.8	0.9	1.2	1.2	1.2	1.5	2.0	2.4	2.9	3.0	3.8	4.4	4.4	5.2	5.6	5.7	6.2	7.8
21.6	0.1	0.2	0.4	0.6	0.8	0.9	1.1	1.1	1.1	1.4	1.9	2.3	2.8	3.0	3.8	4.3	4.4	5.1	5.6	5.7	6.2	7.8
	21.5	0.1	0.3	0.5	0.7	0.8	1.1	1.1	1.1	1.4	1.8	2.2	2.7	2.9	3.7	4.2	4.3	5.0	5.5	5.6	6.1	7.7
		21.4	0.2	0.4	0.6	0.7	1.0	1.0	1.0	1.3	1.7	2.1	2.7	2.8	3.6	4.1	4.2	5.0	5.4	5.5	6.0	7.6
			21.2	0.2	0.4	0.5	0.8	0.8	0.8	1.1	1.6	2.0	2.5	2.6	3.4	3.9	4.0	4.8	5.2	5.3	5.8	7.4
				21.0	0.2	0.3	0.6	0.6	0.6	0.9	1.3	1.7	2.3	2.4	3.2	3.7	3.8	4.6	5.0	5.1	5.6	7.2
					20.8	0.1	0.3	0.3	0.3	0.6	1.1	1.5	2.0	2.2	3.0	3.5	3.6	4.3	4.8	4.9	5.4	7.0
						20.7	0.3	0.3	0.3	0.6	1.0	1.4	2.0	2.1	2.9	3.4	3.5	4.3	4.7	4.8	5.3	6.9
							20.5	0.0	0.0	0.3	0.8	1.2	1.7	1.8	2.6	3.2	3.2	4.0	4.4	4.6	5.0	6.6
								20.4	0.0	0.3	0.8	1.2	1.7	1.8	2.6	3.2	3.2	4.0	4.4	4.5	5.0	6.6
									20.4	0.3	0.8	1.2	1.7	1.8	2.6	3.2	3.2	4.0	4.4	4.5	5.0	6.6
										20.2	0.5	0.9	1.4	1.5	2.3	2.9	2.9	3.7	4.1	4.3	4.7	6.3
											19.7	0.4	0.9	1.1	1.8	2.4	2.4	3.2	3.6	3.8	4.3	5.9
												19.3	0.5	0.7	1.4	2.0	2.1	2.8	3.3	3.4	3.9	5.5
													18.8	0.2	0.9	1.5	1.5	2.3	2.7	2.9	3.4	5.0
														18.6	0.8	1.3	1.4	2.2	2.6	2.7	3.2	4.8
															17.8	0.5	0.6	1.4	1.8	1.9	2.4	4.0
																17.3	0.1	0.8	1.3	1.4	1.9	3.5
																	17.2	0.8	1.2	1.3	1.8	3.4
																		16.5	0.4	0.6	1.0	2.6
																			16.0	0.1	0.6	2.2
																				15.9	0.5	2.1
																					15.4	1.6
																						3.8

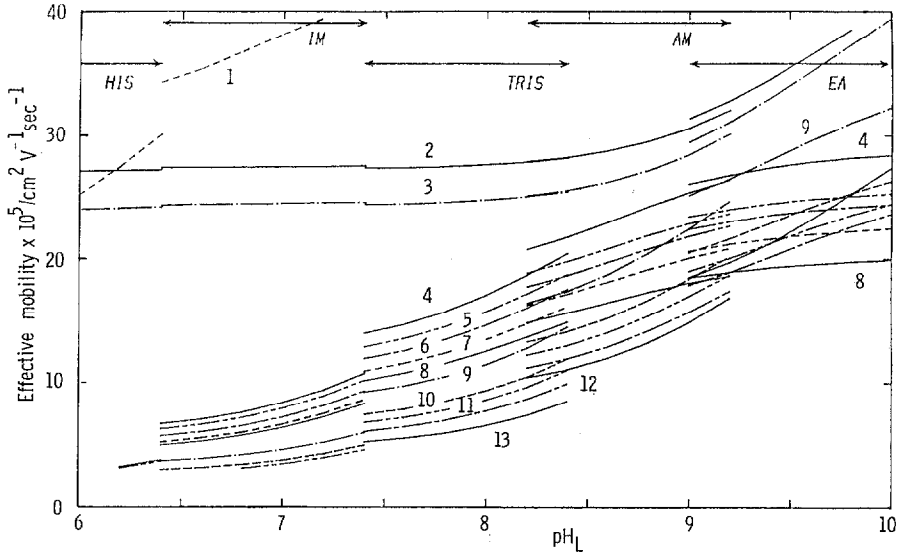

Fig. 4. The pH_L dependence of the effective mobility of HCO_3^- (1), Asp (2), Glu (3), Gly-Gly (4), Gly-Ser (5), Gly-Amin (6), Gly-Leu (7), Leu-Val (8), Tau (9), Thr (10), Gln (11), His (12) and Gly (13) at the isotachophoretic steady state.

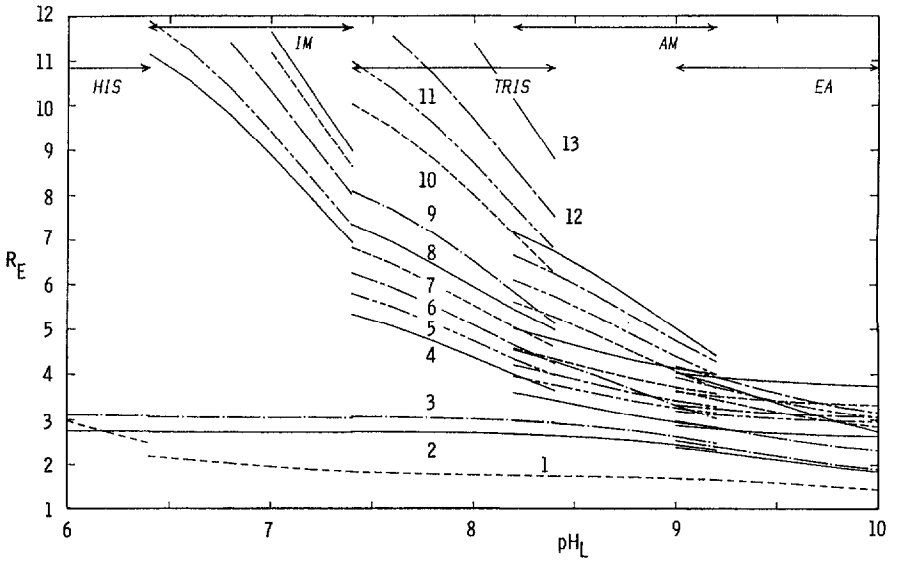

Fig. 5. The pH_L dependence of the R_E of HCO_3^- (1), Asp (2), Glu (3), Gly-Gly (4), Gly-Ser (5), Gly-Amin (6), Gly-Leu (7), Leu-Val (8), Tau (9), Thr (10), Gln (11), His (12) and Gly (13) at the isotachophoretic steady state.

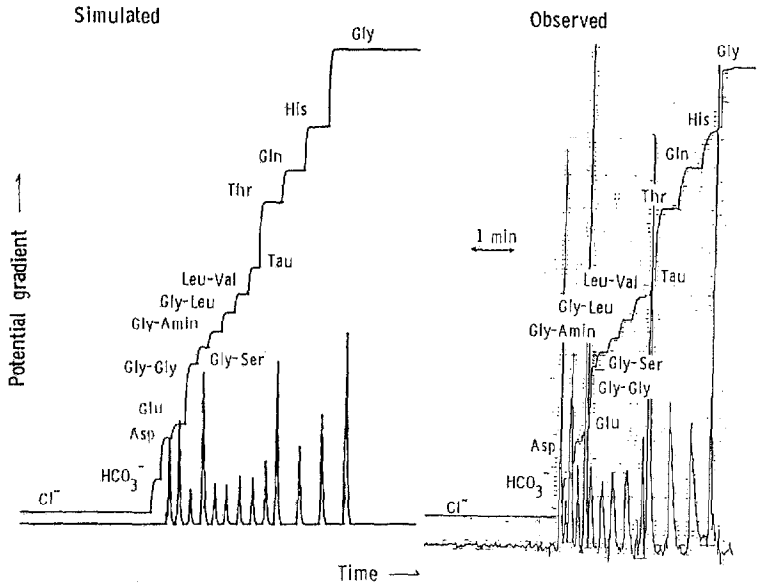

Fig. 6. The simulated and the observed isotachopherograms of HCO_3^-, Asp, Glu, Gly-Gly, Gly-Ser, Gly-Amin, Gly-Leu, Leu-Val, Tau, Thr, Gln, His and Gly at pH_L 8.00 buffered by Tris. The leading ion was 10.02 mM chloride. The sample amounts were 10–20 nmol and the migration current was 50 μA.

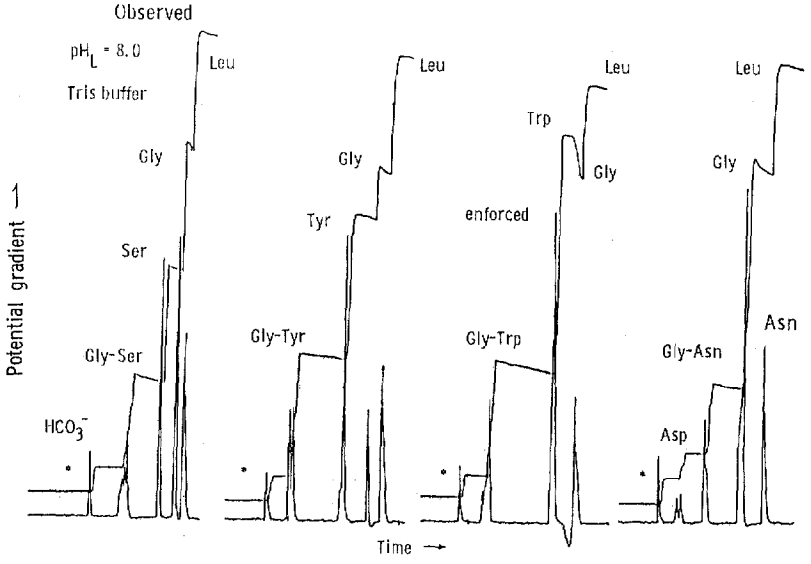

Fig. 7. The observed isotachopherograms of Gly-Ser, Gly-Tyr, Gly-Trp and Gly-Asn partly decomposed to the constituent amino acids at pH_L 8.00 buffered by Tris. Other details as in Fig. 6.

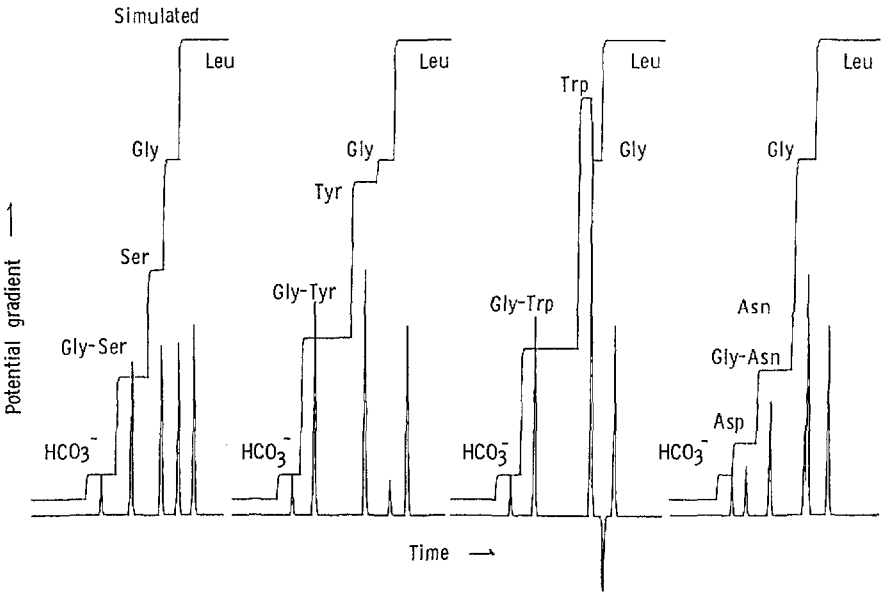

Fig. 8. The simulated isotachopherograms of Gly-Ser, Gly-Tyr, Gly-Trp and Gly-Asn partly decomposed to the constituent amino acids at pH_L 8.00 buffered by Tris. For other conditions, see Fig. 7.

differences in the effective mobilities of the neighbouring samples were $14.5 \cdot 10^{-5}$, $2.8 \cdot 10^{-5}$, $7.5 \cdot 10^{-5}$, $1.3 \cdot 10^{-5}$, $1.1 \cdot 10^{-5}$, $1.2 \cdot 10^{-5}$, $0.9 \cdot 10^{-5}$, $1.2 \cdot 10^{-5}$, $2.1 \cdot 10^{-5}$, $0.7 \cdot 10^{-5}$, $0.9 \cdot 10^{-5}$ and $1.1 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ respectively. As expected from the simulation, the separation was complete and the simulated and the observed electropherograms were in good agreement.

The differences between the effective mobilities of the neighbouring samples from HCO_3^- to terminating β-Ala in Fig. 1A, B, C were $9.4 \cdot 10^{-5}$, $12.2 \cdot 10^{-5}$, $1.9 \cdot 10^{-5}$, $2.7 \cdot 10^{-5}$, $7.2 \cdot 10^{-5}$ and $2.4 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ for samples A, $11.6 \cdot 10^{-5}$, $6.6 \cdot 10^{-5}$, $4.2 \cdot 10^{-5}$, $1.6 \cdot 10^{-5}$, $1.1 \cdot 10^{-5}$, $4.7 \cdot 10^{-5}$ and $2.2 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ for samples B and $16.1 \cdot 10^{-5}$, $7.5 \cdot 10^{-5}$, $0.9 \cdot 10^{-5}$, $1.5 \cdot 10^{-5}$, $1.9 \cdot 10^{-5}$, $2.0 \cdot 10^{-5}$ and $2.1 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ for samples C respectively.

It has been concluded that the differences in the effective mobilities among samples is a good measure of separability to a first approximation[1]. By comparing the observed separation behaviour of amino acids with the difference in the simulated mobilities at the isotachophoretic steady state, it has become apparent that the critical threshold of the difference is *ca.* $1 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$, although this value changes with the sample amount and the length of the separating tube. For the dipeptides, such a separability assessment also seems valid.

Table VIII summarizes the simulated effective mobilities (diagonals) and the differences between them (off-diagonals) for the twenty-eight dipeptides considered and the fifteen constituent amino acids at the steady state. Two of the pH_L values employed 8.6 and 9.4) were the same as those used for the similar simulation for amino acid[1]. At pH_L 7.2, thirty-four dipeptides and amino acids are listed in Table VIII, since the R_E values of the rest exceeded 30. Under such conditions the isotacho-

phoretic separation will be difficult. Apparently, the differences in the effective mobilities of adjacent dipeptides were very small and sometimes zero, suggesting that all of them cannot be separated at once. Considering the threshold of the difference of *ca.* $1 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, the number of practically separable samples may be five to six. This is less than that for the amino acids, eight to ten practically, at most fourteen at pH_L 8.64 buffered by amediol[1]. On the other hand, as seen in Table VIII, except at pH_L 9.4, the R_E values of the listed amino acids were larger than those of the dipeptides, because the pK_a values of the amino acids and dipeptides are sufficiently different. Therefore a good separability may be expected for a given mixture of them.

In Fig. 7 the observed electropherograms are shown for some partly decomposed dipeptides forming monomers. The sample solutions were stored in a refrigerator for 6 months. The decomposition products of Gly-Asn were Gly, Asn and Asp. The effective mobilities of Asp are not shown in Table VIII. The values were $27.5 \cdot 10^{-5}$, $27.6 \cdot 10^{-5}$, $28.8 \cdot 10^{-5}$ and $34.6 \cdot 10^{-5}$ for pH_L 7.2, 8.0, 8.6 and 9.6 respectively. Apparently Asp can be separated from all the other compounds in Table VIII. In Fig. 8, the simulated electropherograms are shown. A good agreement with Fig. 7 was obtained including the enforced phenomena found for Trp and Gly, which were simulated by analyzing the transient mixed zone. The zone lengths used in Fig. 8 were taken from the observed electropherograms. As shown in Figs. 7 and 8, the IP method is very convenient for analysis of the purity of dipeptides and/or their decomposition rate. Similar utility may be expected for other oligopeptides. Whether or not the proposed method for mobility estimation using the Stokes radii of the constituents can be adopted for other oligopeptides is now under investigation.

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