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ISOTACHOPHORETIC DETERMINATION OF MOBILITY AND pK_a BY MEANS OF COMPUTER SIMULATION

II. EVALUATION OF m_0 AND pK_a OF 65 ANIONS

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SUMMARY

A computational method has been applied for the evaluation of m_0 and pK_a of 65 organic and inorganic anions from the qualitative indexes R_E. For nine dicarboxylic acids, $HOOC(CH_2)_nCOOH$ (n = 0–8), the R_E values have been obtained, and by the use of the least squares method the absolute mobilities have been evaluated. In order to confirm the validity of the proposed method, step heights observed previously by Everaerts *et al.* have been converted into R_E values, and the m_0 and pK_a values evaluated for 58 anions. The agreement between the evaluated and the previously reported values is satisfactory, confirming the utility and the general applicability of the proposed method for the determination of m_0 and pK_a of weak acids and bases.

INTRODUCTION

The utility of isotachophoresis as a technique for the measurement of physicochemical constants of ionic samples, such as absolute mobility, acid dissocation constants and stability constants, has been reported previously[1–6]. The method of evaluation of the constants is based on an extension of Everaerts' theory for an isotachophoretic equilibrium in a steady state[4] and on a computational least squares technique for the analysis of the observed R_E values under different electrolyte conditions[5].

The proposed method has an unique feature in comparison with conventional techniques: the sample mixtures can be treated at once, only small amounts being necessary (a few tenths of nmol) and so-called conductivity water is not required for the determination of mobility. All of these advantages result from the high separability of isotchophoresis. Moreover, the electrolyte constituents migrate in a nar-

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row-bore tube without any packing, thus avoiding adsorption problems, and the equilibria can be treated theoretically like free solutions. As discussed in the preceding paper[6], the expected accuracy of the evaluated constants is sufficiently high, at present to three significant figures, if a R_E value can be measured with high reproducibility (± 0.02).

In order to confirm the general applicability of the proposed method, the absolute mobilities of nine different divalent organic acids have now been obtained by analysing R_E data in six different electrolyte systems. Further, the step heights observed using a conductometric detector by Everaerts *et al.*[7] have been converted into R_E values for about 60 different inorganic and organic anions, applying the R_E index of acetic acid as an internal standard. From the resulting R_E values, m_0 and pK_a values were evaluated and compared with the values obtained by conventional methods.

EXPERIMENTAL

The absolute mobilities of the following divalent organic acids, $HOOC(CH_2)_nCOOH$, were determined: oxalic ($n = 0$); malonic (1); succinic (2); glutaric (3), adipic (4); pimelic (5); suberic (6); azelaic (7) and sebacic (8) acids. Six kinds of leading electrolytes were used, differing in the pH (pH_L). The electrolytes were buffered in the pH range 3.92–7.95. Values of pH_L lower than 3.92 were avoided so as the suppress excessive increases in R_E. Although the precise evaluation of the mobilities of monovalent components from high R_E may be possible in principle[6], the reproducibility and reliability may decrease unless a low driving current is applied. To apply such a condition is sometimes time-consuming. In the preliminary calculations and measurements of R_E, the R_E values of the treated acids were in the range 1–ca. 5. The leading electrolyte was 5 m*M* hydrochloric acid, the pH of which was adjusted by ε-aminocaproic acid (ε-AMC: 3.92 and 4.12), creatinine (Crea: 4.40 and 4.80), histidine (His: 5.84) and tris(hydroxymethyl)aminomethane (Tris: 7.95). The terminating electrolyte was 10 m*M* caproic acid, the pH of which was adjusted to the value of the leading electrolyte using the same buffers. The measurements of pH were carried out using a HORIBA expanded scale pH meter, Model F7ss.

The isotachopherograms were obtained using a Shimadzu isotachophoretic analyzer, IP-1B, equipped with a home-made potential gradient detector (PGD). The temperature was thermostatted at 25°C and the separating tube used was 40 cm × 0.5 mm I.D. The driving currents applied were in the range 25–50 μA. The nine samples (10 m*M*) were divided into two groups, although in the low pH_L range they were separable at once.

For the correction of the asymmetric potential of a PGD, as discussed in the preceding paper, the R_E values of the terminating ion, caproate, were used. The simulated R_E values at 25°C were 5.816 ($pH_L = 3.92$), 5.395 (4.12), 4.362 (4.40), 3.769 (4.80), 2.852 (5.84) and 2.696 (7.95), obtained by the use of the absolute mobilities and dissociation constants shown in Table I. The method of correction was as follows. First, the step height caused by an assymmetric potential, Δh, was estimated by

$$\Delta h = [h(\text{std}) - h_L \cdot R_E(\text{std})]/[R_E(\text{std}) - 1] \quad (1)$$

TABLE I

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

m_0 = Absolute mobility ($cm^2\ V^{-1}\ sec^{-1}$) × 10^5. pK_a = Thermodynamic acidity constant, assumed values being used for Cl^-. The absolute mobilities marked with an asterisk were obtained isotachophoretically; the other constants were taken from refs. 8–10. Cap^- = Caproate; Ac^- = acetate.

Cation	m_0	pK_a	*Anion*	m_0	pK_a
β-Ala^+	31.0*	3.55	Cl^-	79.08	−3
ε-AMC^+	28.8*	4.43	Cap^-	30.2*	4.852
$Crea^+$	37.2*	4.828	Ac^-	42.4	4.756
His^+	29.6*	6.04			
$Tris^+$	29.5*	8.08			

where R_E(std) is the simulated R_E value of a standard sample, h(std) the observed (apparent) step height of it and h_L that of the leading zone. On the assumption that h_L and h(std) had the same Δh, by subtracting Δh from the apparent step height of the leading and sample zones, h_S, the apparent R_E value, h_S/h_L, can be corrected as follows:

$$R_E = (h_S - \Delta h)/(h_L - \Delta h) \quad (2)$$

The above correction was made for each determination independently. The averages of the corrected R_E values obtained from four or five determinations were used in the evaluation of the absolute mobility. For example, one of the results of the data processing is shown in Table II. The samples were oxalic, malonic, glutaric, pimelic, azelaic and caproic acids and the pH_L was 4.12. The average of the apparent step height was 35.06 mm and the average of the corrected step height from the asymmetric potential was 33.52 mm, suggesting the existence of a positive asymmetric potential of 4.6%. Although errors in R_E values caused by the potential were small when R_E was small, the influence on large R_E was marked. If the correction was not applied, the reproducibility of the R_E values exceeded ±0.06 for azelaic acid. On the other hand, the reproducibility was less than ±0.02 for all samples, if the internal standard was used. The corrected R_E values are summarized in Table III.

In a similar manner, the step heights of organic and inorganic anions obtained by Everaerts *et al.*[7] were converted into R_E values. A conductometric detector had been used and the step heights of the samples were relative to that of chlorate ion, assumed as 100. The pK_a of chloric acid is out of the pH range (−1.37), *i.e.*, its effective mobility is not influenced by pH_L, therefore chloric acid is a good internal standard. However, the errors, in reading the step height of the chlorate zone may sometimes cause an error for samples with high relative step heights due to the fact that the step height of chlorate ion is smaller than almost all those of the other samples. Thus, we used the different internal standard, acetic acid, for which the R_E values are sufficiently high in the low pH_L range. The use of the standard enables one to check the general applicability of the above method of R_E correction (in this case, not due to the asymmetric potential) and the linearity of the detector used. If the method is reliable, the errors in readings are small and the linearity is high, the R_E

TABLE II

CORRECTION OF ASYMMETRIC POTENTIAL FOR THE OBSERVED R_E VALUES OF OXALIC (OX), MALONIC (MAL), GLUTARIC (GLU), PIMELIC (PIM), AZELAIC (AZE) AND CAPROIC (CAP) ACIDS

pH_L = 4.12 (ε-AMC buffer).

Expt. No.	*L*	*OX*	*MAL*	*GLU*	*PIM*	*AZE*	*CAP*
Apparent step heights (mm)							
1	36.0	48.5	70.8	119.8	141.8	157.3	183.5
2	35.5	47.4	70.0	119.0	141.0	157.0	184.0
3	35.0	46.5	69.3	117.3	138.6	154.3	181.8
4	34.3	45.8	68.5	117.5	138.9	154.3	181.2
5	34.5	46.0	69.3	117.7	139.4	154.8	181.3
av.	35.06	46.84	69.58	118.26	139.94	155.54	182.36
Apparent R_E values							
1		1.347	1.967	3.328	3.939	4.369	5.097
2		1.335	1.972	3.352	3.972	4.423	5.183
3		1.329	1.980	3.351	3.960	4.409	5.194
4		1.335	1.997	3.426	4.050	4.499	5.283
5		1.333	2.009	3.412	4.041	4.487	5.255
av.		1.336	1.985	3.374	3.992	4.437	5.203
Corrected step heights (mm), $R_E(Cap)$ = 5.395							
1	33.56	46.06	68.36	117.36	139.36	154.86	181.06
2	33.79	45.69	68.29	117.29	139.29	155.29	182.29
3	33.40	44.90	67.70	115.70	137.00	152.70	180.20
4	33.42	44.92	67.62	116.62	138.02	153.42	180.32
5	33.40	44.90	68.20	116.60	138.30	153.70	180.20
av.	33.52	45.30	68.04	116.72	138.40	154.00	180.82
Corrected R_E values							
1		1.372	2.037	3.497	4.152	4.614	5.395
2		1.352	2.021	3.471	4.122	4.596	5.395
3		1.344	2.027	3.464	4.102	4.572	5.395
4		1.344	2.023	3.489	4.129	4.590	5.395
5		1.344	2.042	3.491	4.141	4.602	5.395
av.		1.351	2.030	3.482	4.129	4.595	5.395

value of chlorate ion is expected to be constant within the reproducibility of the earlier experiments.

Four different electrolyte systems were used: the leading ion was 10 mM Cl^- and the buffers were β-Ala (pH_L = 3), ε-AMC (4.5), histidine (His: 6) and Tris (7.5). The relative step heights of acetic acid were reported[7] as 3880 (pH_L = 3), 1090 (4.5), 484 (6) and 466 (7.5). The simulated R_E values are 8.409, 3.094, 1.983 and 1.913, respectively. Since the relative step heights of the leading zone, h_L, were not reported, they were estimated by

$$h_L = h(\mathrm{Ac})/[R_E(\mathrm{Ac}) - 1] \tag{3}$$

where h(Ac) and R_E(Ac) are the relative step height and R_E value of acetic acid. The

TABLE III

CORRECTED AND BEST-FITTED R_E VALUES OF NINE DICARBOXYLIC ACIDS AND EFFECTIVE MOBILITIES AND CONCENTRATIONS OF ZONE CONSTITUENTS (25°C)

Electrolyte systems: 1, pH_L = 3.92 (ε-AMC); 2, 4.12 (ε-AMC); 3, 4.40 (Crea); 4, 4.80 (Crea); 5, 5.84 (His); 6, 7.95 (Tris). R_E = Ratio of potential gradients, E_S/E_L. $\bar{m}_S$ = Effective mobility (cm^2 V^{-1} sec^{-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} sec^{-1}) of buffer ion × 10^5. I = Ionic strength × 10^3.

Electrolyte system	R_E		$\bar{m}_S$	pH_S	C_S^t	$C_{B,S}^t$	$\bar{m}_{B,S}$	I
	*Corr.**	*Fit.***						
Oxalic acid								
1	1.42	1.417	53.51	4.085	2.932	5.926	−18.48	5.60
2	1.35	1.348	56.24	4.244	2.813	6.875	−16.33	5.89
3	1.25	1.250	60.66	4.514	2.641	6.427	−23.73	6.31
4	1.16	1.171	64.69	4.852	2.540	9.137	−17.39	6.77
5	1.11	1.100	68.84	5.860	2.464	7.823	−16.62	7.30
6	1.09	1.092	69.37	7.965	2.453	8.344	−15.61	7.36
Malonic acid								
1	2.06	2.045	37.07	4.084	3.899	5.298	−18.72	3.92
2	2.03	1.994	38.01	4.261	3.828	6.211	−16.28	4.02
3	1.90	1.915	39.60	4.564	3.539	5.647	−23.10	4.06
4	1.77	1.800	42.09	4.940	3.278	8.433	−15.77	4.42
5	1.39	1.419	53.40	5.936	2.582	7.518	−15.58	6.14
6	1.29	1.264	59.95	7.983	2.352	8.137	−15.35	7.04
Succinic acid								
1	3.42	3.359	22.57	4.480	3.381	5.047	−13.09	2.66
2	3.18	3.123	24.26	4.565	3.330	5.974	−11.77	2.86
3	2.59	2.628	28.85	4.801	3.040	5.433	−18.63	3.28
4	2.21	2.270	33.38	5.049	2.873	8.226	−13.66	3.85
5	1.56	1.585	47.81	5.958	2.429	7.361	−15.27	5.93
6	1.43	1.397	54.22	7.997	2.276	7.987	−15.17	6.81
Glutaric acid								
1	3.83	3.808	19.91	4.545	2.835	4.960	−12.09	2.60
2	3.48	3.484	21.75	4.622	2.783	5.909	−10.90	2.84
3	2.78	2.784	27.23	4.845	2.528	5.416	−17.73	3.41
4	2.32	2.334	32.47	5.066	2.421	8.218	−13.33	4.10
5	1.65	1.657	45.72	5.946	2.252	7.259	−15.45	6.09
6	1.55	1.538	49.26	8.011	2.200	7.838	−14.98	6.59
Adipic acid								
1	4.22	4.207	18.02	4.581	2.718	4.860	−11.54	2.44
2	3.85	3.836	19.76	4.656	2.671	5.809	−10.41	2.68
3	3.02	3.026	25.06	4.874	2.426	5.307	−17.16	3.24
4	2.50	2.519	30.08	5.087	2.333	8.106	−12.94	3.94
5	1.76	1.767	42.89	5.956	2.197	7.158	−15.30	5.95
6	1.65	1.638	46.27	8.020	2.150	7.738	−14.84	6.44

(Continued on p. 54)

TABLE III *(continued)*

Electrolyte system	R_E		$\bar{m}_S$	pH_S	C^t_S	$C_{B,S}$	$\bar{m}_{B,S}$	I
	*Corr.**	*Fit.***						
Pimelic acid								
1	4.53	4.535	16.72	4.612	2.687	4.793	−11.09	2.30
2	4.13	4.131	18.35	4.684	2.641	5.739	−10.01	2.53
3	3.25	3.238	23.42	4.898	2.391	5.222	−16.68	3.09
4	2.68	2.687	28.20	5.106	2.299	8.013	−12.60	3.77
5	1.86	1.866	40.61	5.966	2.159	7.071	−15.16	5.81
6	1.73	1.725	43.93	8.029	2.108	7.654	−14.73	6.32
Suberic acid								
1	4.79	4.798	15.80	4.620	2.632	4.706	−10.97	2.23
2	4.38	4.368	17.35	4.693	2.588	5.651	−9.89	2.46
3	3.43	3.422	22.16	4.908	2.336	5.125	−16.50	2.99
4	2.83	2.837	26.71	5.116	2.245	7.914	−12.42	3.66
5	1.95	1.972	38.43	5.975	2.114	6.980	−15.04	5.68
6	1.84	1.822	41.58	8.038	2.063	7.565	−14.60	6.18
Azelaic acid								
1	5.03	5.049	15.02	4.633	2.571	4.641	−10.78	2.16
2	4.60	4.589	16.51	4.705	2.529	5.587	−9.71	2.38
3	3.60	3.579	21.18	4.919	2.281	5.058	−16.26	2.91
4	2.96	2.958	25.61	5.126	2.196	7.847	−12.22	3.57
5	2.03	2.045	37.05	5.982	2.082	6.920	−14.94	5.59
6	1.89	1.887	40.13	8.044	2.033	7.506	−14.52	6.09
Sebacic acid								
1	5.34	5.343	14.19	4.634	2.515	4.533	−10.77	2.09
2	4.88	4.850	15.63	4.709	2.476	5.482	−9.66	2.30
3	3.76	3.780	20.06	4.927	2.231	4.957	−16.12	2.80
4	3.11	3.114	24.33	5.138	2.155	7.759	−12.02	3.44
5	2.11	2.115	35.82	5.991	2.060	6.871	−14.81	5.48
6	1.94	1.934	39.18	8.048	2.013	7.466	−14.46	6.03

* Corrected values; the internal standard was caproic acid.
** Best-fitted values by the least squares method.

h_L values so obtained were 523.7 (pH_L = 3), 520.5 (4.5), 492.4 (6) and 510.4 (7.5). The relative step heights of the samples from the baseline at which electric conductivity is infinitely large or resistance is zero were expressed by the sum of the obtained h_L and the original relative step heights, giving the objective R_E values:

$$R_E(\text{converted}) = (h_S + h_L)/h_L \qquad (4)$$

The converted R_E values are given in a later section.

For the data processing and the simulations, a SORD microcomputer M223 Mk III was used.

RESULTS AND DISCUSSION

For the nine dicarboxylic acids, the absolute mobilities of the mono- and divalent components, m_1 and m_2, were obtained simultaneously by the least squares method using the literature pK_a values[8–10] to reproduce the observed R_E values. The pK_a values of sebacic acid could not be found in literature so we used approximate values. Table III shows the observed and the best-fitted R_E values, the concentrations and the effective mobilities of the sample zone constituents. The mean errors between the observed and the best-fitted R_E values were 0.40% for oxalic acid, 1.51% for malonic acid, 1.93% for succinic acid, 0.44% for glutaric acid, 0.46% for adipic acid, 0.23% for pimelic acid, 0.50% for suberic acid, 0.35% for azelaic acid and 0.32% for sebacic acid. The agreement between these two sets of R_E values is thus satisfactory.

The evaluated m_1 and m_2 are listed in Table IV, together with the dispersions and the literature values of the mobility and pK_a. The dispersion of m_2 for malonic and succinic acid is relatively large, suggesting that in these cases m_2 is less accurate, although the values for the other acids seem to be reliable on the assumption that the pK_a values used are valid. For pimelic, suberic and azelaic acids, the evaluation of the absolute mobility was carried out using two different sets of pK_a values. The obtained constants are shown in Table IV as sets I and II. The mean errors using m_0 and pK_a (set II) were 0.88% for pimelic and suberic acids and 0.50% for azelaic acid. Since the agreement was relatively good, perhaps the reliability of the different m_0 should be judged from a different viewpoint.

The absolute mobility of the acids $HOOC(CH_2)_nCOOH$ decreases regularly with increasing number of methylene groups, n, when the pK_a values of set I is used. This decrease is probably due to the expected increase in ionic size, therefore, we judged the values of set I to be more exact. The slight difference between the two sets of pK_a values causes the apparent difference in m_0. The pK_a values of set I, may therefore be more exact than the others. Since the agreement between the observed and the best-fitted R_E values was good, a simultaneous evaluation of m_0 and pK_a was not carried out except as follows. According to tentative evaluations for malonic and succinic acids for which the mean errors were relatively large, m_1, m_2 and pK_2 of malonic acid are 39.7 (σ = 0.33), 65.9 (0.44) $\times$ 10^{-5} cm^2 V^{-1} sec^{-1} and 5.489 (0.045), and m_1, m_2, pK_1 and pK_2 of succinic acid are 31.7 (6.72), 60.0 (0.47) $\cdot$ 10^{-5} cm^2 V^{-1} sec^{-1}, 4.257 (0.31) and 5.461 (0.17). The mean errors decreased to 0.45 and 0.52% respectively. However, the number of the different R_E values in the present work is not satisfactory for the precise simultaneous determintion of m_1, m_2, pK_1 and pK_2 as is apparent from the above example.

Fig. 1 shows the observed R_E values. The curves were plotted using the best-fitted m_0 for the isotachophoretically steady state, therefore the R_E values at the different pH$_L$ can be estimated from Fig. 1. It is to be noted that the nine curves do not cross each other at any pH$_L$.

The agreement between the present and the previous absolute mobilities obtained by conductometric measurements of sodium and disodium salts was good for oxalic acid (m_1 and m_2), malonic acid (m_2) and succinic acid (m_1 and m_2), taking into account the reproducibility of the previous measurements. On the other hand, there were a distinct differences for the other samples. In order to check the reliability of the present results, computer simulations of R_E values were carried out using the litera-

TABLE IV

EVALUATED ABSOLUTE MOBILITIES OF NINE DICARBOXYLIC ACIDS (25°C)

m_i = Absolute mobility ($cm^2\ V^{-1}\ sec^{-1}$) × 10^5 · pK_a = Thermodynamic acid dissociation constant. The values were fixed in the least squares method. σ = Dispersion. ITP = Evaluated values. Lit. = Literature[8–10].

Sample	*pK$_a$*	*(Lit.)*	*m_1*			*m_2*			
			ITP	*σ*	*Lit.*	*ITP*	*σ*	*Lit.*	
Oxalic acid	1.271	4.266	42.4	0.74	41.7	77.0	0.30	76.7	75.3
Malonic acid	2.847	5.696	40.7	0.47	36.5	67.0	1.00	66.7	62.4
Succinic acid	4.207	5.638	33.0	0.61	32.3	60.9	1.10	63.1	57.1
Glutaric acid	4.343	5.272	26.6	0.21	30.8	55.6	0.24	58.6	
Adipic acid	4.430	5.277	24.6	0.20	29.3	52.4	0.22	54.5	
Pimelic acid I	4.509	5.312	24.0	0.10	27.3	49.9	0.11	51.6	
II	4.484	5.424	25.6	0.33		50.4	0.43		
Suberic acid I	4.524	5.327	23.0	0.23	25.7	47.4	0.26	48.8	
II	4.517	5.403	24.2	0.34		47.7	0.42		
Azelaic acid I	4.550	5.333	22.0	0.14	–	45.9	0.26	–	
II	4.523	5.395	22.6	0.19		46.2	0.24		
Sebacic acid	4.53	5.38	20.7	0.12	–	44.9	0.14	–	

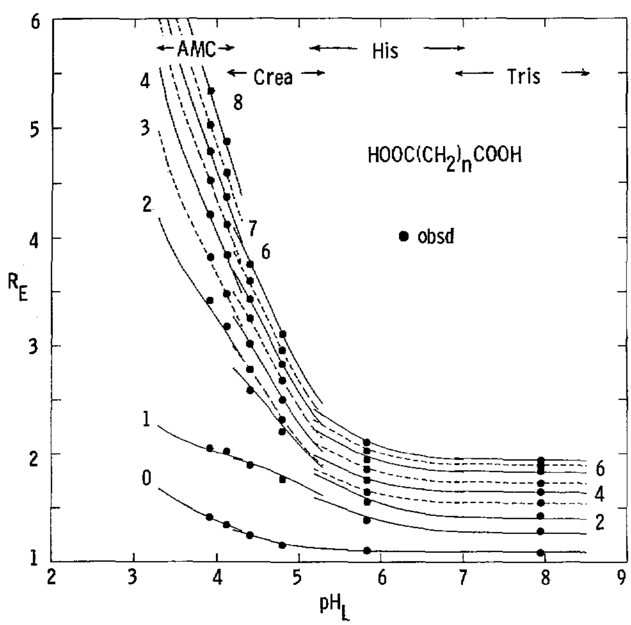

Fig. 1. Observed R_E values of oxalic (0), malonic (1), succinic (2), glutaric (3), adipic (4), pimelic (5), suberic (6), azelaic (7) and sebacic (8) acids. The concentration of the leading ion, Cl^-, was 5 mM. The buffers used were ε-aminocaproic acid (AMC), creatinine (Crea), histidine (His) and tris(hydroxymethyl)-aminomethane (Tris). The best-fitted curves are also shown, plotted using the determined absolute mobilities. For pimelic, suberic and azelaic acids, set I in Table IV was used for simulation.

ture values. Fig. 2 shows the dependency of pH_L and buffer on the simulated R_E values, together with the observed R_E values. Apparently, the curves are different from those in Fig. 1. For example, those for succinic and glutaric acids cross each other. The simulated R_E values of succinic acid are 3.35 (pH_L = 3.92), 3.11 (4.12), 2.61 (4.40), 2.25 (4.80), 1.54 (5.84) and 1.35 (7.95) and those of glutaric are 3.41, 3.14, 2.54, 2.15, 1.56 and 1.45, respectively. These values suggest that the separation of these acids is difficult, even in the low pH_L range. Moreover, at pH_L = 4.40 and 4.80, the R_E values of glutaric acid are smaller than those of succinic acid. These observations are inconsistent with the present experimental results. To illustrate this, an example of the obtained isotachopherograms including these acids is shown in Fig. 3A, together with the simulated isotachopherograms using both the present (B) and the previous (C) absolute mobilities. The pH_L was 3.92 (ε-AMC buffer). The agreement between the observed and the simulated isotachopherogram using the present m_0 is striking; on the other hand, there is serious disagreement between that simulated using the previous values. The discrepancy in the different pH_L ranges could be estimated from Fig. 2. Especially in the pH_L range adjacent to pK_a, the curves of R_E did not coincide with the experimental results in part.

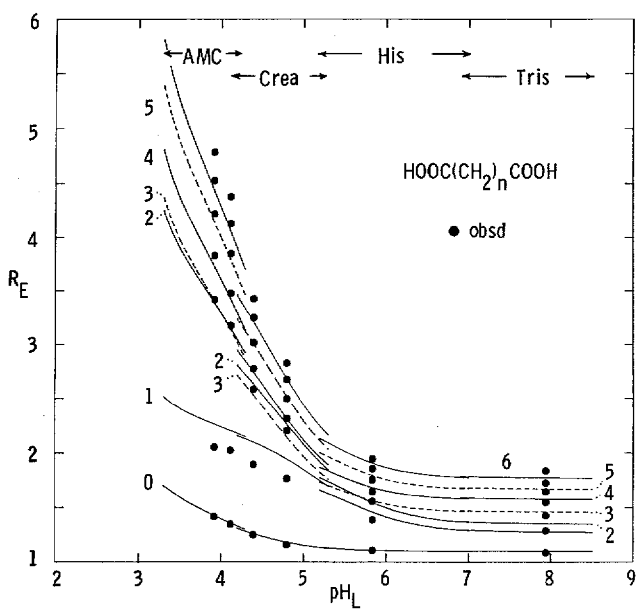

Fig. 2. Dependency of pH_L on the simulated R_E values using the estimated absolute mobilities from the literature conductivities of the sodium salts. Samples 1–6 as in Fig. 1. The observed R_E values are also shown. The values of m_2 used are those in the first m_2 column in Table IV. The electrolyte conditions were as in Fig. 1.

The converted R_E values using eqns. 3 and 4 for the anions considered by Everaerts *et al.*, are listed in Table V. The conversion from the relative step heights into the R_E values was successful, since the converted R_E values of chloric acid, 1.19 (pH_L = 3), 1.19 (4.5), 1.20 (6) and 1.20 (7.5), are consistent with each other within the experimental accuracy. The simulated R_E value was 1.19 at any pH_L (m_0 =

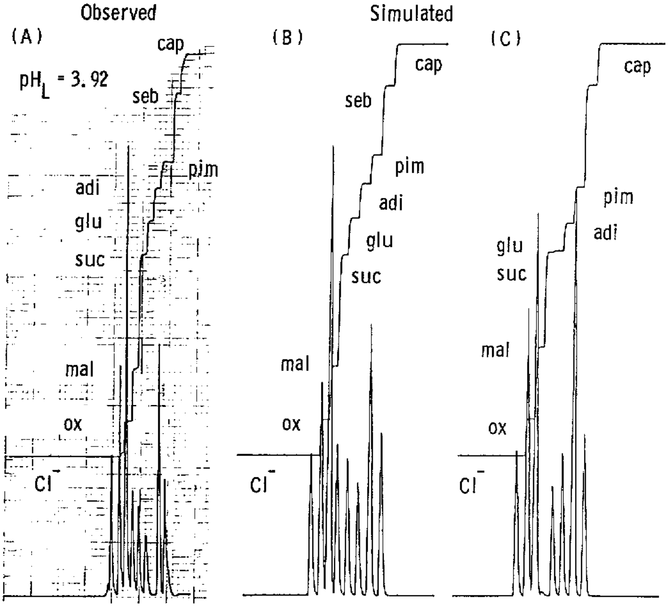

Fig. 3. Observed isotachopherogram at pH_L = 3.92 (A), and simulated isotachopherograms using the evaluated absolute mobilities (B) and the literature mobilities (C). Samples: oxalic (ox), malonic (mal), succinic (suc), glutaric (glu), adipic (adi), pimelic (pim) and sebacic acids (seb). The terminating ion was caproate (cap).

TABLE V

CONVERTED R_E, BEST-FITTED R_E AND MEAN ERRORS FOR 62 ANIONS

pH_L = pH of leading electrolyte. m.e. = Mean error (%). Total concentration of leading ion was 0.01 *M* for all electrolyte systems. Buffers used were β-alanine (pH_L = 3), ε-aminocaproic acid (4.5), histidine (6) and tris(hydroxymethyl)aminomethane (7.5). Std. = Standard.

Sample	*pH_L = 3*		*pH_L = 4.5*		*pH_L = 6*		*pH_L = 7.5*		*m.e.*
	*Conv.**	*Fit.***	*Conv.*	*Fit.*	*Conv.*	*Fit.*	*Conv.*	*Fit.*	
DL-Benzyl aspartic acid	–		–		3.34	3.34	3.23	3.23	0.02
Acetic acid	(8.409)		(3.094)		(1.983)		(1.912)		Std.
Chloroacetic acid	2.42	2.49	1.99	1.96	1.99	1.93	1.91	1.93	2.10
Dichloroacetic acid	2.10	2.08	2.03	2.06	2.10	2.06	2.03	2.06	1.44
Trichloroacetic acid	2.25	2.26	2.34	2.25	2.26	2.25	2.17	2.25	2.09
Benzoic acid	6.37	6.57	3.31	3.02	2.47	2.50	2.42	2.48	3.98
p-Aminobenzoic acid	13.01	12.92	4.42	4.48	2.70	2.66	2.50	2.54	1.30
2,4-Dihydroxybenzoic acid	3.79	3.81	2.79	2.66	2.63	2.57	2.44	2.57	3.27
p-Nitrobenzoic acid	4.06	4.07	2.71	2.66	2.57	2.55	2.49	2.55	1.33
Butyric acid	11.08	11.08	3.96	3.96	2.53	2.52	2.42	2.43	0.25
Cacodylic acid	–		13.70	13.65	4.06	4.09	2.84	2.83	0.53
Capric acid	–		13.03	–	4.02	3.97	3.76	3.81	1.23
Caproic acid	13.45	13.56	4.71	4.66	2.84	2.82	2.68	2.70	0.89
Caprylic acid	14.90	15.30	5.46	5.20	3.16	3.16	2.98	3.03	2.35
Chloric acid	1.19	1.20	1.19	1.20	1.20	1.20	1.20	1.20	0.41

TABLE V *(continued)*

Sample	*pH_L = 3*		*pH_L = 4.5*		*pH_L = 6*		*pH_L = 7.5*		*m.e.*
	*Conv.**	*Fit.***	*Conv.*	*Fit.*	*Conv.*	*Fit.*	*Conv.*	*Fit.*	
Chromic acid	1.35	1.36	1.33	1.34	1.27	1.23	1.06	1.07	1.49
Bichromic acid	1.40	1.37	1.32	1.35	1.27	1.27	1.07	1.07	1.26
Citric acid	3.62	3.60	2.00	2.03	1.53	1.51	1.35	1.36	1.07
Enanthic acid	13.89	14.04	4.92	4.84	3.01	2.99	2.86	2.88	0.96
Formic acid	2.93	2.91	1.55	1.58	1.42	1.41	1.41	1.40	0.98
Fumaric acid	2.87	2.87	1.65	1.64	1.44	1.45	1.45	1.45	0.50
Glutaric acid	–		–		1.72		–		
Glucuronic acid	5.20	5.26	3.52	3.28	3.10	3.12	3.00	3.12	3.11
Glutamic acid	8.16	8.40	3.90	3.63	2.88	2.90	2.74	2.81	3.31
Glycerinic acid	4.19	4.20	2.46	2.43	2.28	2.25	2.20	2.25	1.19
Glycolic acid	4.09	4.07	2.13	2.16	1.93	1.92	1.92	1.91	0.71
Gluconic acid	–		–		3.08		–		
Hippuric acid	3.62	3.70	3.27	3.21	2.95	2.93	1.81	1.81	1.21
Iodic acid	1.95	1.94	1.96	1.93	1.94	1.93	1.90	1.94	0.96
α-Ketoglutaric acid	2.75	2.75	1.97	1.97	1.53	1.53	1.49	1.49	0.08
Lactic acid	4.65	4.84	3.04	2.56	2.24	2.29	2.16	2.28	6.90
Levulinic acid	9.06	9.22	3.67	3.55	2.51	2.50	2.41	2.45	1.70
Maleic acid	1.97	2.03	1.94	1.88	1.62	1.61	1.41	1.42	1.84
Malic acid	3.79	3.79	2.01	2.00	1.53	1.53	1.50	1.50	0.10
Malonic acid	2.43	2.48	1.90	1.85	1.42	1.42	1.33	1.33	1.33
Methacrylic acid	7.42	7.49	3.11	3.05	2.26	2.26	2.22	2.23	0.82
Naphthalene-2-sulphonic acid	2.58	2.61	2.75	2.61	2.62	2.61	2.53	2.62	2.49
Nicotinic acid	10.62	10.84	4.02	3.89	2.48	2.45	2.31	2.36	2.16
Nitric acid	1.04	1.05	1.04	1.05	1.07	1.05	1.06	1.05	1.18
Nitrous acid***	1.06	1.05	1.06	1.05	1.05	1.05	1.04	1.05	0.71
Orotic acid	2.85	2.85	2.56	2.50	2.50	2.48	2.41	2.48	1.59
Oxalic acid	1.76	1.75	1.23	1.27	1.19	1.16	1.16	1.16	1.72
Pelargonic acid	12.40	12.45	4.61	4.61	3.34	3.18	3.00	3.11	2.20
Perchloric acid	1.13	1.13	1.12	1.13	1.15	1.13	1.14	1.13	0.88
Phenylacetic acid	8.03	7.97	3.32	3.38	2.67	2.63	2.59	2.60	1.09
Phosphoric acid	2.60	2.62	2.44	2.40	2.25	2.26	1.64	1.64	0.74
Phthalic acid	3.44	3.08	1.96	2.18	1.78	1.73	1.68	1.67	6.19
Picric acid	2.57	2.58	2.71	2.57	2.56	2.57	2.48	2.58	2.46
Pimelic acid	9.27	9.62	3.44	3.19	1.91	1.92	1.81	1.85	3.45
Pivalic acid	14.54	14.30	4.67	4.78	2.74	2.74	2.61	2.60	1.10
Propionic acid	9.55	9.88	3.80	3.58	2.29	2.29	2.18	2.21	2.66
3-Chloropropionic acid	4.48	4.54	2.58	2.46	2.28	2.22	2.11	2.21	3.39
Pyrazine-2,3-dicarboxylic acid	2.18	2.18	1.71	1.71	1.60	1.59	1.57	1.58	0.44
Pyrazole-3,5-dicarboxylic acid	2.67	2.63	1.58	1.65	1.60	1.55	1.56	1.54	2.53
Salicylic acid	3.02	3.02	2.32	2.34	2.31	2.30	2.31	2.30	0.41
Succinic acid	6.19	6.24	2.62	2.58	1.61	1.64	1.55	1.53	1.30
Sulphamic acid	1.59	1.59	1.60	1.59	1.60	1.59	1.59	1.60	0.33
Sulphanilic acid	3.41	3.42	2.51	2.48	2.40	2.42	2.41	2.42	0.61
Sulphuric acid	1.06	1.10	1.09	1.08	1.12	1.08	1.09	1.08	2.19
Sulphurous acid	1.70	1.72	1.64	1.63	1.58	1.57	1.34	1.34	0.53
Tartaric acid	2.91	2.90	1.62	1.64	1.47	1.45	1.44	1.45	0.73
Tartronic acid	2.33	2.30	1.53	1.57	1.32	1.29	1.28	1.28	1.50

* Converted from the relative step heights observed by Everaerts *et al.*[7].
** Best-fitted by the least squares method.
*** The sample may be oxidized to nitric acid, see text.

$67.0 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}). The detector used had a high linearity and the error in readings was negligibly small, at least in the R_E range 1–*ca.* 8.5. Evaluation of m_0 and pK_a was carried out by the least-squares method using the R_E values listed in Table V. The best-fitted R_E values and the mean errors (%) are also shown in Table V.

Since the electrolyte conditions used for measurements of the step height were four for a sample, m_0 and pK_a could not always be evaluated simultaneously. For monovalent anions, the simultaneous evaluation may be possible, if the pK_a of the samples are in the pH range. On the other hand, for samples having small pK_a values only the absolute mobility was determined. These samples were dichloroacetic, trichloroacetic, chloric, iodic, naphthalene-2-sulphonic, nitric, perchloric, picric and sulphamic acids. Appropriate pK_a values were used in the least squares method. For nitrous acid, the pK_a has been reported[9] as 3.22 (30°C), suggesting an increase in R_E values in the low pH_L range. However, no such increase has been observed. This suggests that the nitrous acid might be oxidized to nitric acid. Therefore, the difference between the converted R_E values of nitric and "nitrous" acid can be regarded as the reproducibility of the previous experiments, ± 0.01, which coincides with the reproducibility of the R_E values of chlorate ion. Therefore, the m_0 evaluation of nitric acid was carried out using two sets of R_E values. For glutaric and glucuronic acids, m_0 or pK_a could not be evaluated, since only one R_E value was available.

For divalent anions, the simultaneous evaluation of m_0 and pK_a by the least squares method is not statistically significant since the number of available R_E values is four. Therefore, the evaluation was limited to the absolute mobility. However, for several samples without m_0 and pK_a data, the simultaneous evaluation was unavoidable. These samples were bichromic, α-ketoglutaric, pyrazine-2,3-dicarboxylic, and pyrazole-3,5-dicarboxylic acids. For the last two acids, the pK_1 was fixed at 1 according to preliminary calculations.

Table VI shows the evaluated pK_a and m_0 and their dispersions, together with the previous values obtained by different methods. Although all of the present constants could not be compared with the previous ones due to lack of data, the agreement was satisfactory, especially for the absolute mobility. For many samples the difference was less than $1 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. However, for some samples the difference exceeds $3 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. Nevertheless, we believe that the present results are more reliable. For example, the m_0 of levulinic acid obtained by the present method was 33.4 and by the previous was $29.2 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. If the previous value were correct, the R_E value at pH_L 7.5 should be *ca.* 0.3 higher than the present value. The detector used could easily resolve such a difference. The two sets of R_E values for nitric acid gave slightly different m_0 values of $75.4 \cdot 10^{-5}$ and $75.3 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}.

For pK_a, the agreement between the present and the previous values was also satisfactory for many samples, taking into account the dispersions. It should be noted that for all samples the four observed R_E values could be employed for m_0 evaluation, on the other hand only the two R_E values at low pH_L (3.0 and 4.5) were effective for pK_a evaluation of many of the samples. In spite of this disadvantage, a significant discrepancy between the evaluated and the literature pK_a values was observed only for peralgonic and pivalic acids. The differences were negative and positive with respect to the previous values, therefore it may not be due to the temperature increase. The applied current for the measurements was 90, 80, 70 and 80 μA at pH_L 3, 4.5, 6 and 7.5, respectively[7].

TABLE VI

EVALUATED ABSOLUTE MOBILITIES AND THERMODYNAMIC ACID DISSOCIATION CONSTANTS OF 58 ANIONS (25°C)

For m_i and pK_a, values in parentheses were assumed.

Sample	*pK_a*			*m_0*		
	ITP	*σ*	*Lit.*	*ITP*	*σ*	*Lit.*
DL-Benzylaspartic acid	–		4.855	25.7	0.02	–
Chloroacetic acid	–		2.865	41.9	0.53	41.3
Dichloroacetic acid	–		1.257	39.4	0.32	39.7
Trichloroacetic acid	–		0.635	36.2	0.54	37.9
Benzoic acid	4.166	0.106	4.203	32.9	1.44	33.6
			4.200			34.4
p-Aminobenzoic acid	–		2.38	(32.3)	–	–
	4.939	0.024	4.89	32.3	0.43	33.4
2,4-Dihydroxybenzoic acid	3.239	0.133	3.297	31.8	0.99	34.4
p-Nitrobenzoic acid	3.391	0.049	3.425	32.1	0.41	–
Butyric acid	4.807	0.006	4.820	33.7	0.11	33.8
			4.824			31.9
Cacodylic acid	6.182	0.013	6.194	29.9	0.26	32.3
			6.387			
Capric acid	–		(5)	22.1	0.26	–
Caproic acid	4.913	0.016	4.857	30.5	0.27	30.3, 30.2*
			4.854			29.9
Caprylic acid	4.930	0.050	4.894	27.4	0.78	28.2
			4.851			
Chloric acid	–		–2.7	66.6	0.15	67.0
Chromic acid	–		0.745	59.3	0.94	–
	–		6.49	81.1	1.82	–
Bichromic acid	–		(0.745)	58.9	1.42	–
	6.723	0.386	–	82.1	3.50	–
Citric acid	–		3.128	31.0	0.85	28.7*
	–		4.761	53.4	1.68	54.7*
	–		6.396	70.8	1.36	72.8, 74.4*
Enanthic acid	4.887	0.018	4.893	28.7	0.29	28.2
Formic acid	3.796	0.030	3.752	57.1	0.58	56.6
Fumaric acid	–		3.02	35.1	0.38	31.0*
	–		4.384	60.5	0.29	61.2*
Glucuronic acid	3.516	0.115	–	26.6	0.87	–
Glutamic acid	–		2.162	(28.7)	–	28.9*
	4.302	0.086	4.288	28.7	1.09	29.2, 28.9*
	–		9.387	(54.9)	–	(54.9)
Glycerinic acid	3.656	0.038	3.517**	36.3	0.42	–
Glycolic acid	3.850	0.020	3.831	42.3	0.30	41.7
			3.827			
Hippuric acid	(2.5)		–	25.9	0.53	–
	7.346	0.249	–	55.3	3.71	–
Iodic acid	–		0.772	41.9	0.27	42.0
α-Ketoglutaric acid	2.800	–	–	37.5	–	–
	5.272	–	–	59.0	–	–
Lactic acid	3.854	0.212	3.858	35.8	2.69	36.5
			3.866, 3.860			
Levulinic acid	4.594	0.037	4.602	33.4	0.63	29.2
			4.648			

(Continued on p. 62)

TABLE VI *(continued)*

Sample	pK_a			m_0		
	ITP	σ	*Lit.*	*ITP*	σ	*Lit.*
Maleic acid	–		1.943	42.5	0.89	41.3*
	–		6.225	62.0	1.54	62.7*
Malic acid	–		3.46	34.9	0.05	–
	–		5.05	58.5	0.06	–
Malonic acid	–		2.847	42.4	0.80	36.5, 39.5*
	–		5.696	65.4	1.17	66.7, 66.6*
Methacrylic acid	4.458	0.021	–	36.6	0.36	–
Naphthalene-2-sulphonic acid	–		(−2)	31.3	0.53	–
Nicotinic acid	–		2.07	(34.6)	–	–
	4.819	0.040	–	34.6	0.77	–
Nitric acid	–		−1.37	75.4	0.52	74.1
Orotic acid	2.519	0.136	–	32.9	0.52	–
Oxalic acid	–		1.271	44.9	1.53	41.7
	–		4.266	74.6	1.33	76.7
Pelargonic acid	4.678	0.055	4.955	26.7	0.77	–
Perchloric acid	–		(−2)	70.0	0.38	69.8
Phenylacetic acid	4.351	0.024	4.311	31.5	0.34	31.7
			4.264			32.3
Phosphoric acid	–		2.148	34.1	0.26	34.2, 34.3*
	–		7.22	58.3	0.79	59.1, 59.5*
	–		11.75	–		71.5
Phthalic acid	–		2.95	35.3	3.23	–
	–		5.408	52.7	4.00	–
Picric acid	–		0.708	31.7	0.56	31.1
						31.5
Pimelic acid	–		4.509	27.6	1.73	27.3
	–		5.312	48.4	1.92	51.6
Pivalic acid	5.007	0.025	4.860	31.6	0.46	–
			4.842			
Propionic acid	4.779	0.061	4.874	36.9	1.24	37.1
3-Chloropropionic acid	3.804	0.095	–	36.8	1.18	–
Pyrazine-2,3-dicarboxylic acid	–		(1)	36.6	0.62	–
	4.308	0.120		55.7	0.45	–
Pyrazole-3,5-dicarboxylic acid	–		(1)	25.4	13.4	–
	3.894	0.755	–	56.9	2.25	–
Salicylic acid	2.937	0.022	3.079	35.3	0.14	–
Succinic acid	–		4.207	35.2	0.56	32.3
	–		5.638	57.5	0.77	63.1
Sulphamic acid	–		(−2)	50.3	0.09	–
Sulphanilic acid	3.127	0.028	3.227	33.7	0.20	–
Sulphuric acid	–		1.78	49.3	0.26	–
	–		6.991	67.1	0.61	–
Sulphurous acid	–		(−2)	(45)	–	–
	–		1.921	79.5	1.19	82.9
Tartaric acid	–		3.036	34.6	0.55	32.4*
	–		4.366	60.5	0.41	66.3, 60.6*
Tartronic acid	–		2.366	38.9	1.04	–
	–		4.735	67.8	1.09	68.0*

* Unpublished data obtained in our laboratory.
** At 20°C.

Among many anions, the absolute mobilities of oxalic and pimelic acids are suitable for comparison with those based on the data of Everaerts *et al.* For oxalic acid, the two sets of values were $42.4 \cdot 10^{-5}$–$77.0 \cdot 10^{-5}$ and $44.9 \cdot 10^{-5}$–$74.6 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}; for pimelic acid, they were $24.0 \cdot 10^{-5}$–$49.9 \cdot 10^{-5}$ and $27.6 \cdot 10^{-5}$–$48.4 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}, respectively. As the reproducibility of the R_E values was lower when the data of Everaerts *et al.* were used, the dispersions of the evaluated m_0 were large. If these dispersions are taken into account, it can be said that the values are in agreement. For several samples, we had determined m_0 previously, as shown by an asterisk in Table IV. There is good agreement between our previous mobility and the present results.

Thus, the absolute mobility of *ca.* 30 samples and the pK_a values of 8 samples were newly determined using the converted R_E values. Figs. 4–7 show the dependencies on pH$_L$ of the simulated R_E values of 55 anions. The curves are for the isotachophoretically steady state and were plotted using the evaluated m_0 and pK_a. The pH$_L$ ranges buffered by β-Ala, ε-AMC, His and Tris were 3–4, 3.9–5, 5.9–7 and 6.9–8.5, respectively. By the use of Figs. 4–7, the R_E values of the samples can be estimated at different pH$_L$.

It can be concluded that the proposed method is generally applicable for the measurement of m_0 and pK_a. The use of an internal standard for the correction of an asymmetric potential could be helpful for the estimation of exact R_E values. The qualitative index, R_E, may be useful even for the usual qualitative analysis in isotachophoresis, as well as the IRM proposed by Deml *et al.*[11], since it well reflects the pH$_L$ dependency of effective mobility, characteristic of a sample. For the estimation of R_E values, exact values of m_0 and pK_a are necessary. Now, the m_0 and pK_a values of *ca.* 300 different organic and inorganic anions are stored in a data bank in our laboratory, and using a computer system the R_E value under any aqueous elec-

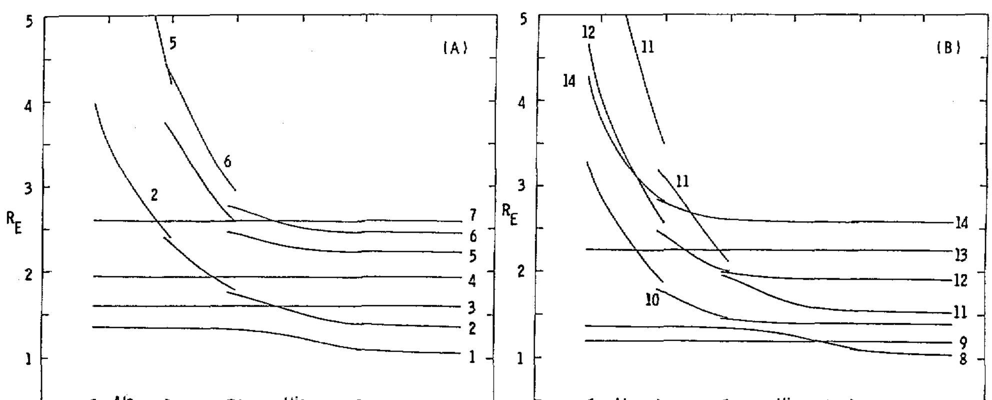

Fig. 4. Dependency on pH$_L$ and buffer of the simulated R_E values using m_0 and pK_a evaluated by analysing the relative step heights obtained by Everaerts *et al.* Acids: A, chromic (1), citric (2), sulphamic (3), iodic (4), methacrylic (5), levulinic (6) and picric (7); B, bichromic (8), chloric (9), formic (10), succinic (11), glycolic (12), trichloroacetic (13) and 2,4-dihydroxybenzoic (14).

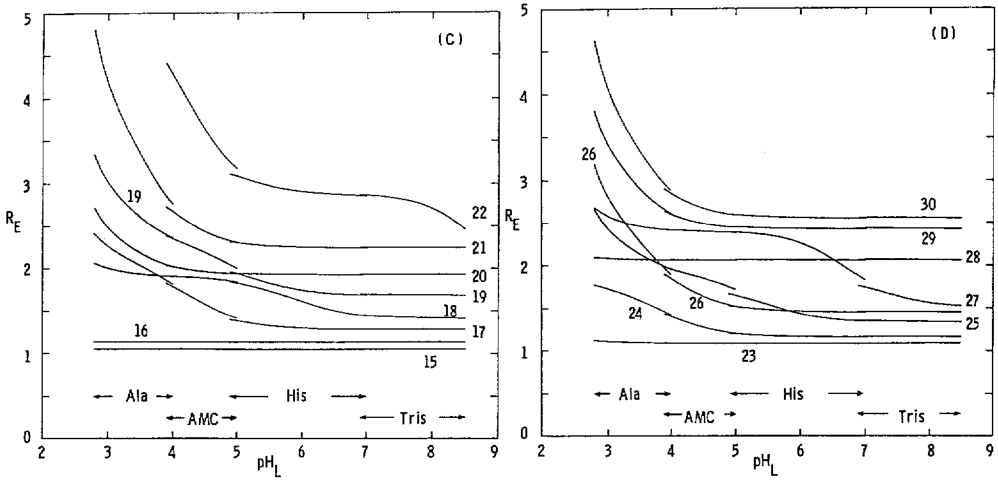

Fig. 5. Dependency on pH_L and buffer of the simulated R_E values as in Fig. 4. Acids: C, nitric (15), perchloric (16), tartronic (17), maleic (18), phthalic (19), chloroacetic (20), glycerinic (21) and glutamic (22); D, sulphuric (23), oxalic (24), malonic (25), fumaric (26), phosphoric (27), dichloroacetic (28), sulphanilic (29) and *p*-nitrobenzoic (30).

trolyte conditions can quickly be simulated. The separability of a given sample mixture can also be judged by plotting of simulated isotachopherograms as in Fig. 3. The details of the method of plotting the simulated isotachopherograms will be reported elsewhere.

The R_E values can easily be converted into different indexes. A table of simulated R_E values under different leading electrolyte conditions will be published else-

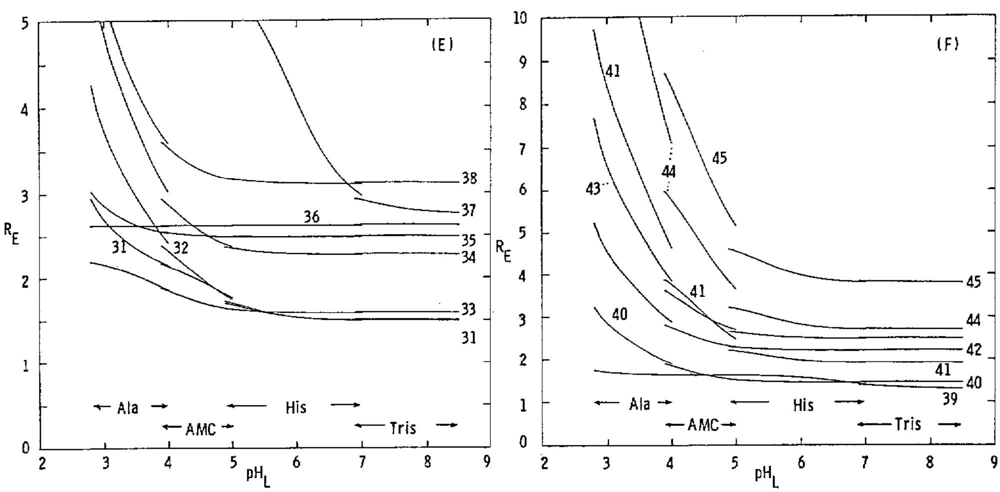

Fig. 6. Dependency on pH_L and buffer of the simulated R_E values as in Fig. 4. Acids: E, α-ketoglutaric (31), malic (32), pyrazole-2,3-dicarboxylic (33), lactic (34), orotic (35), naphthalene-2-sulphonic (36), cacodylic (37) and glucuronic (38); F, sulphurous (39), tartaric (40), acetic (41), 3-chloropropionic (42), benzoic (43), caproic (44) and capric (45).

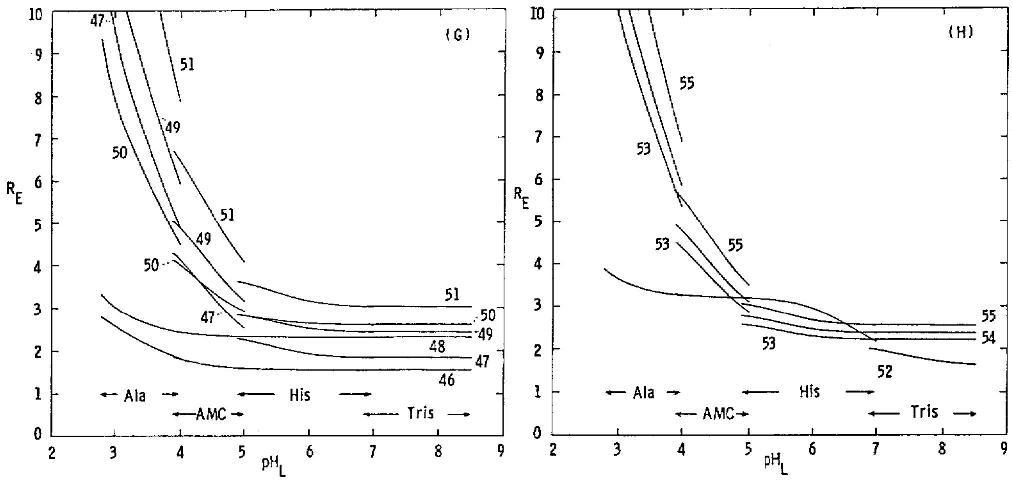

Fig. 7. Dependency on pH_L and buffer of the simulated R_E values as in Fig. 4. Acids: G, pyrazine-3,5-dicarboxylic (46), pimelic (47), salicylic (48), butyric (49), phenylacetic (50) and caprylic (51); H, hippuric (52), propionic (53), nicotinic (54) and *p*-aminobenzoic (55).

where. The table may be useful for the estimation and/or the assessment of observed isotachopherograms when a computational simulation of the isotachophoretic equilibrium is not available.

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