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A NEW METHOD FOR DETERMINATION OF DISSOCIATION CONSTANTS OF SIMPLE ORGANIC ACIDS BY ISOTACHOPHORESIS

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Dissociation constants of formic, acetic, butyric, caproic, succinic, and glutaric acids were obtained together with absolute mobilities by means of isotachophoresis. It was found that the obtained values agreed satisfactorily with those in literature and the present method was very useful for the mixed samples.

Dissociation constants have been obtained by the measurements of electrical conductivity, electromotive force, *etc*. However, in these methods, the values were determined only for each individual samples, not for the mixed samples.

In the previous work$^{1)}$, we have shown a method by isotachophoresis for the determination of absolute mobilities m_0 of the mixed carboxylic acids by the use of least-squares method for ratios of the observed potential gradients (E_V/E_L ; E_V and E_L are potential gradients of samples and leading electrolytes) at fully charged state. In principle, dissociation constants K may also be estimated if the ratio can be obtained at the other unsatisfactorily dissociated state: The ratio E_V/E_L can be expressed as a function of effective mobilities of leading ions ($\bar{m}_L$) and sample ions ($\bar{m}_V$), their acidity constants defined by Brönsted (k_a) and concentration of H^+ ions at sample zone (C_{H^+}), for mononocarboxylic acids, as follows;

$$(E_V/E_L) = (\bar{m}_L/\bar{m}_V) = (\bar{m}_L/m_V^0\,\alpha_V) = (\bar{m}_L/m_V^0)\,(k_a + C_{H^+})/k_a\ , \qquad (1)$$

where m_V^0 and α_V are the absolute mobility of sample ion and the degree of dissociation. Thermodynamic dissociation constant K_a is defined by Eq.(2);

$$K_a = (C_{H^+}\cdot C_{A^-})(f_{H^+}\cdot f_{A^-})/(C_{HA}\cdot f_{HA}) = k_a f_{\pm}^{2}\ , \qquad (2)$$

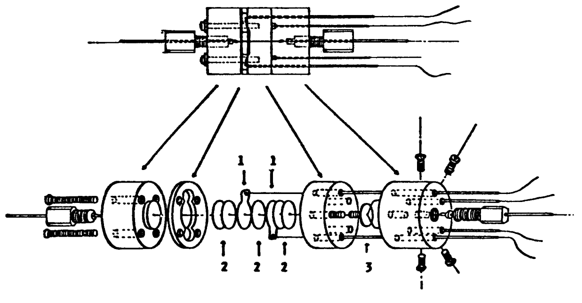
where C_{A^-} and C_{HA} are the concentration of A^- ion and acid HA, and f_{H^+}, f_{A^-}, f_{HA}, and $f_{\pm}$ are the activity coefficients of H^+, A^-, HA, and averaged acivity coefficient. Eq.(3) can be derived from Eqs (1) and (2);

$$pK_a = p\,\frac{(E_V/E_L)^0\ C_{H^+}}{(E_V/E_L)-(E_V/E_L)^0} - 2\log f_{\pm}\ , \qquad (3)$$

where $(E_V/E_L)^0$ is the potential ratio at fully charged state of samples. The value $f_{\pm}$ for monovalent pair ions with ionic strength I at 25°C was estimated using the following Eq.(4).

$$\log f_{\pm} = -0.5092\sqrt{I}\ /\ (1+0.3286\ a\sqrt{I}\ )\ , \qquad (4)$$

where 4 Å was assumed for a. The values of C_{H^+} and $f_{\pm}$ were estimated from the calculation of isotachophoretic equilibrium equation. Similar equations to (1)-



1. Platinum electrodes (20μ).
2. PTFE insulator (50μ).
3. Copper-Constantan thermocouple (50μ).

Fig.1.
A newly designed PGTD cell.

(4) can be written for dicarboxylic acids. In the practical calculation, pK_a was obtained by a least-squares method taking into account several values of E_V/E_L obtained by the use of different leading electrolytes.

The isotachopherogram was obtained using a capillary type isotachophoretic analyzer (Shimadzu Seisakusho Ltd., Model IP-1B) equipped with a newly designed potential gradient and thermometric detector (PGTD). Figure 1 shows a PGTD cell made of acryl resin (30mm ϕ, 40 mm in length). The platinum electrodes can be easily exchanged when they were contaminated with deposits formed by electrode reactions. The PGTD cell together with the capillary systems were placed in a thermostatted box to controll the temperature in the regions from *ca.* 0°C to 50°C within ± 0.1°C. A cooler and a heater were attached to the box and the heater was controlled by a regulator (Type ST-15A by Gasukulo Kogyo Ltd.). Temperature of solution and box were monitored by digital thermometers (Yokogawa Electric Co., Ltd. Type 2575). All experiments were carried out at 25°C. Particullar care was taken for the electric insulation among electrodes, thermocouples and ground.

The leading and terminating ions were chloride and caproate ions. Concentration of both ions was 0.005 mol/l (factor = 1.022 for chloride). The pH of leading solutions (pH_L) were adjusted to 3.64 by β-alanine, 4.17 and 4.61 by ε-aminocaproic acid, 5.19 and 6.02 by histidine, and 7.87 by Tris, for the analysis of monocarboxylic acids. Similar electrolytes were used for dicarboxylic acids. The pH of terminating solution was also adjusted approximately to that of leading solution using the same buffer. The length of the capillary tube for separation was 20 cm (0.5 mm i.d.) and the applied current was 40 μA for all experiments.

Figure 2 shows the relation between pH and mobilities of monocarboxylic acids. Circles in Fig. 2 show position of pKa. These curves were obtained by the calculation using known absolute mobilities of chloride ion and samples and their pK_a values. The dotted curves in Fig. 3 shows the relation between E_V/E_L values and pH. E_V/E_L values used in the following treatment were the mean values for five observed ones at each pH_L. The mean values are shown in Fig. 3. In the least-squares method, value of function S;

$$S = \sum_i W_i (E_V/E_L(\text{obsd}) - E_V/E_L(\text{calcd}))^2$$

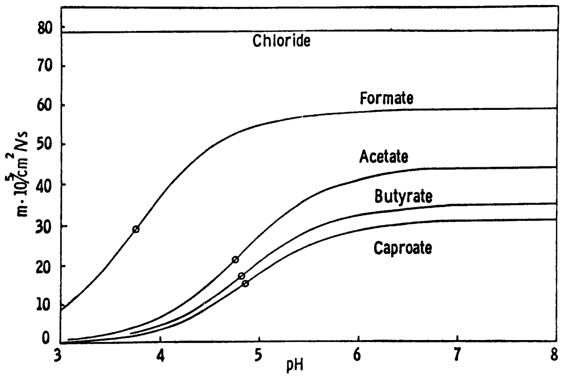


Fig. 2. Mobility *vs*. pH.

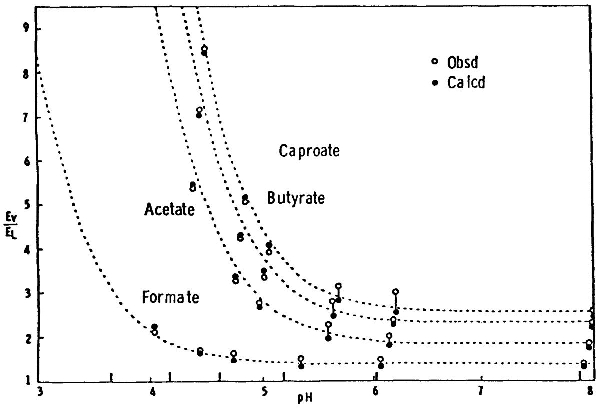


Fig. 3. Observed and calculated values of E_V/E_L for monocarboxylic acid.

was minimized varying m_0 and/or pK_a of the samples. W_i is the weights of the observed values; the inverses of the mean values were used. Concentration of ions, pH of zones *etc.* were calculated at each cycle of minimization of S, taking into account Onsager's correction for effective mobilities. Brute force method was employed, since damped least-squares method has failed to obtain m_0 and pK_a simultaneously due to close relation between them. The dispersions (σ) of the obtained m_0 and/or pK_a were calculated by the following equation;

$$\sigma = ((\tilde{J}WJ)^{-1}_{hh}\, S/(q-p))^{1/2},$$

where J, W, q and p are Jacobian matrix, weight matrix, number of observed values, and number of fixed constants. The mobilities of buffer ions used were 31.7, 29.7, 34.1 and $49.5 \times 10^{-5} cm^2/Vs$ at 25°C for Tris, histidine, ε-aminocaproic acid, and β-alanine, respectively, which have been obtained by isotachophoresis. All calculations were carried out using HITAC 8700/8800 of Hiroshima University.

Table 1 shows the calculated m_0 and pK_a and those in literatures[2,3] for mono and dicarboxylic acids. The values in column I were obtained simultaneously. Although the obtained m_0 agreed well with those by conductometric method, the dispersions were large. Therefore m_0 were obtained at first by the use of E_V/E_L values at fully charged state (pH=7.87), and then, pK_a were refined. The results were shown in Column II of Table 1. The best-fitted values are shown in Fig. 3.

Table 1. Calculated m_0 ($10^{-5} cm^2/Vs$) and pK_a at 25°C.

	I		II		Literature
Samples	m_0	σ	m_0	σ	m_0
Formate	54.8	2.09	58.2	0.37	56.6
Acetate	41.6	1.35	43.5	0.40	42.9, 42.4
Butyrate	33.7	1.69	35.4	0.28	33.8, 31.9
Caproate	30.2	3.21	31.8	0.29	30.3
Succinate	-	-	34.1 / 64.3	0.44 / 0.77	32.9 / 62.8
Glutarate	-	-	28.8 / 58.3	0.08 / 0.10	30.8 / 58.6
Samples	pK_a	σ	pK_a	σ	pK_a
Formate	3.70	0.10	3.84	0.10	3.752
Acetate	4.63	0.05	4.69	0.04	4.756
Butyrate	4.71	0.08	4.77	0.05	4.820
Caproate	4.82	0.05	4.89	0.06	4.857
Succinate	-	-	4.24 / 5.56	0.02 / 0.05	4.207 / 5.636
Glutarate	-	-	4.34 / 5.27	0.01 / 0.01	4.343 / 5.272

Table 2. Calculated pH_V, concentration, averaged activity coefficient, and effective mobility of monocarboxylic acid.$^{1)}$

	$pH_L = 3.64$				$pH_L = 7.87$			
Samples	pH_V	C_A^t	$f_{\pm}$	$\bar{m}$	pH_V	C_A^t	$f_{\pm}$	$\bar{m}$
Formate	4.005	4.474	0.9443	34.30	7.920	4.596	0.9296	55.14
Acetate	4.375	3.823	0.9605	14.08	7.976	4.076	0.9333	40.86
Butyrate	4.439	3.343	0.9635	10.98	8.022	3.691	0.9362	33.00
Caproate	4.505	3.105	0.9661	9.05	8.048	3.488	0.9378	29.51

1) For symbols, see text. Units of C_A^t and $\bar{m}$ are mmol/l and $10^{-5}cm^2/Vs$.

Table 3. Observed pH_L, E_V/E_L, calculated pH_V, E_V/E_L, concentration, averaged activity coefficient, and effective mobility of dicarboxylic acid.$^{1)}$

	Obsd		Calcd				
Samples	pH_L	E_V/E_L	pH_V	E_V/E_L	C_A^t	$f_{\pm}$	$\bar{m}$
Succinate	8.00	1.35	8.049	1.33	2.276	0.9163	56.63
	6.21	1.41	6.279	1.41	2.343	0.9183	53.54
	4.40	2.67	4.728	2.69	3.021	0.9403	28.10
	4.10	3.13	4.559	3.13	3.129	0.9441	24.43
	3.81	3.99	4.313	4.00	2.968	0.9525	18.89
Glutarate	8.00	1.48	8.067	1.48	2.189	0.9177	51.06
	6.21	1.53	6.285	1.53	2.226	0.9187	49.43
	4.40	2.86	4.755	2.87	2.605	0.9397	26.38
	4.10	3.39	4.599	3.38	2.701	0.9441	22.36
	3.81	4.58	4.362	4.58	2.564	0.9538	16.50

1) See footnote of Table 2.

for monocarboxylic acids. The m_0 and pK_a obtained agreed well with those reported and the dispersions were small enough. Table 2 listed the calculated pH of zones (pH_V), total concentration of samples (C_A^t), averaged activity coefficients ($f_{\pm}$), and effective mobilities of monocarboxylic acids, for pH_L = 3.64 and 7.87. Table 3 listed those of dicarboxylic acids together with observed and calculated E_V/E_L.

As shown above, the present method for determination of pKa of simple acids by isotachophoresis was revealed a powerful technique for mixed samples. This fact implies that a qualitative analysis could be made by isotachophoresis , which needs no internal standard.

References

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