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Estimation of the Molecular Weight of Ions by Isotachophoresis

Osamu Fujishita,* Shun Higuchi, Manabu Yoshikawa,
Toshinobu Aoyama and Masayoshi Horioka

Hospital Pharmacy, Medical Department, Kyushu University, 1-1, Maidashi 3-chome, Higashi-ku, Fukuoka 812, Japan

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A correlative equation relating the molecular weight of ions to the relative step height in an isotachopherogram was derived: $h_R=a+b\ M/|Z|$, in which h_R, M, $|Z|$, a and b are the relative step height with chloride ion as a standard, the molecular weight, the electric charge of ions and two constants, respectively. Therefore, the molecular weight of ions may be estimated from the relative step height in an isotachopherogram, when the electric charge is known. The equation for carboxylic acids was: $h_R=0.65+0.01\ M/|Z|$.

The optimum pH of electrolytes for the estimation of molecular weight or for the separation of ions may be determined from the relative step height-pH curves obtained on the basis of this equation.

Keywords——molecular weight correlative equation; electrophoretic mobility and molecular weight; isotachophoresis; ion separation isotachophoresis; carboxylic acid isotachophoresis

Correlative equations between the molecular weight and the electrophoretic mobility of ions were reported by Jokl[1] and Offord.[2] The former author found that the minus one-half power of the molecular weight ($M^{-1/2}$) was proportional to the mobility, and the latter author indicated that the minus one-third or two-thirds power ($M^{-1/3}$ or $M^{-2/3}$) was proportional to the mobility.

On the other hand, the relationship between the electrophoretic mobility and the potential gradient of ions was shown by MacInnes *et al.*[3] and Preetz[4] to be as follows: $U_1 \cdot E_1 = U_2 \cdot E_2 = v$=constant, in which U ($cm^2 \cdot V^{-1} \cdot s^{-1}$), E (V/cm) and v (cm/s) are the mobility, the potential gradient and the velocity, respectively. The name of isotachophoresis (Greek: *iso*=equal, *tacho*=speed) results from the principle that all ions move with equal velocity.[5] The relative potential gradient can be obtained from the step height in the isotachopherogram,[6,7] and a relationship between the electrophoretic mobility of ions and the relative step height in the isotachopherogram was reported by Deml *et al.*[7]: $U_1/U_2=h_2/h_1$, in which U and h are the mobility and the step height.

This paper describes our studies on the relationship between the molecular weight of ions and the relative step height in the isotachopherogram, the estimation of the molecular weight of ions from the relative step height, and how to choose a suitable pH of the electrolytes for the estimation of the molecular weight or for the separation of ions.

Calculation

Examination of the Relationship between the Order of the Molecular Weight and the Coefficient of Correlation

The coefficients of correlation between the molecualr weight and the mobility were calculated for various orders of the molecular weight. The values for the molecular weight and the mobility per electric charge given in Jokl's paper[1] were used (N=60).

Derivation of the Correlative Equation between the Molecular Weight of Ions and the Relative

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Step Height in the Isotachopherogram

Determination of the Electrophoretic Mobility and the Electric Charge of Ions——The mobility of ions was calculated from the equation given by MacInnes *et al.*[3,8]: $\kappa = |Z|CFU \times 10^{-3}$, in which κ, $|Z|$, C, F and U are the specific conductance, the electric charge, the concentration, the Faraday equivalent and the mobility, respectively. The values for the concentration and the specific conductance of carboxylic acids at pH 7.05 given by Beckers *et al.*[9] were used.[10] The electric charge of ions at pH 7.05 was calculated according to the equation of Kiso *et al.*[11]: $Z = -1/2 \sum[1+\tanh\{2.303/2(\mathrm{pH}-\mathrm{p}K)\}]$. The p$K$ values of ions were taken from the literature.[12]

Derivation of the Correlative Equation between the Electrophoretic Mobility and the Molecular Weight of Ions——The values for the mobility and the electric charge of ions given in Table I were used. The values for the molecular weight of ions were taken from the literature.[13]

Derivation of the Correlative Equation between the Electrophoretic Mobility and the Step Height in the Isotachopherogram——The values of mobility in Table I and the step height for carboxylic acids at pH 7.05 given by Beckers *et al.*[9] were used.

Derivation of the Correlative Equation between the Molecular Weight of Ions and the Step Height in the Isotachopherogram——The values of electric charge, molecular weight[13] and step height[9] in Table I were used.

Modification of the Correlative Equation between the Molecular Weight of Ions and the Step Height in the Isotachopherogram——The values of electric charge, molecular weight[31] and step height[9] in Table I were used.

Conversion of Step Height into Relative Step Height——The step height in equation (6) was converted into the relative step height[7] with chloride ion as a standard. The step height of chloride ion was determined from equation (6).

Relation between pH and the Relative Step Height in the Isotachopherogram

The relative step height was calculated by the use of equation (7). The electric charge was calculated according to the equation of Kiso *et al.*[11] The values of molecular weight[13] and pK[12] of ions in Table I were used.

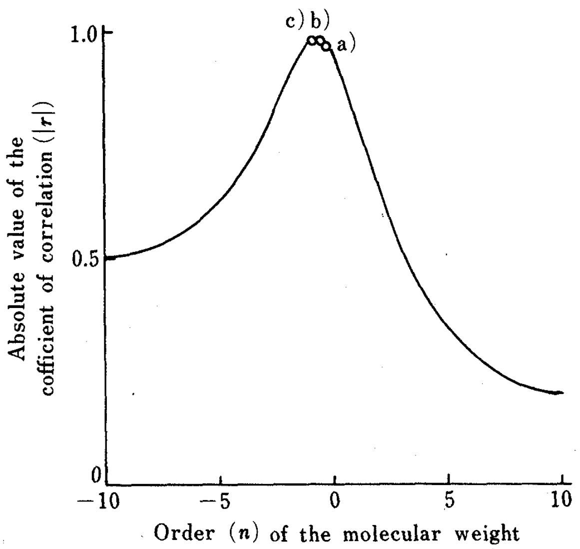

Fig. 1. Relationship between the Order (n) of the Molecular Weight and the Absolute Value of the Coefficient of Correlation ($|r|$)

a) $n=-1/3$: $U/Z=-0.94+9.97\,M^{-1/3}$ ($r_1=0.977$, $p<0.0001$).
b) $n=-1/2$: $U/Z=-0.29+14.8\,M^{-1/2}$ ($r_2=0.983$, $p<0.0001$).
c) $n=-2/3$: $U/Z=-0.03+24.3\,M^{-2/3}$ ($r_3=0.984$, $p<0.0001$).
Where U, Z and M are the electrophoretic mobility, the elevtric charge and the molecular weight of ions, respectively ($N=60$).

Results and Discussion

Examination of the Relationship between the Order of the Molecular Weight and the Coefficient of Correlation

Figure 1 shows the relationship between the order (n) of the molecular weight and the absolute value of the coefficient of correlation($|r|$). The coefficients of correlation between the n-th power of the molecular weight (M^n) and the mobility were calculated for various orders (n) of the molecular weight. The correlations were good, and the differences among the three orders, $-1/3$, $-1/2$ and $-2/3$, cannot be regarded as clear-cut, because $|z_1-z_2|/(2/N-3)^{1/2}=0.7398$, $|z_2-z_3|/(2/N-3)^{1/2}=0.2299$ and $|z_1-z_3|/(2/N-3)^{1/2}=0.9697$, in which z_1, z_2, z_3 and N are the transformations of r_1, r_2, r_3 in Fig. 1 and the number of samples, respectively. Therefore, we used the minus one-half power

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TABLE I. Some Values for Carboxylic Acids at pH 7.05

	Acid	$U^{a)}$	p$K^{b)}$	$\lvert Z \rvert^{c)}$	$M^{d)}$	$h^{e)}$
1[f]	Acetic	87.78	4.757	0.995	60.05	28.1
2	Benzoic	80.97	4.212	0.999	122.12	34.0
3	Caprylic	76.86	4.894	0.993	144.21	40.0
4	Crotonic	81.97	4.698	0.996	86.09	32.6
5	Formic	99.26	3.752	0.999	46.02	21.6
6	Pelargonic	76.65	4.955*	0.992	158.23	39.3
7	Isovaleric	80.08	4.78	0.995	102.13	36.0
8	Adipic	92.32	4.409 5.296	1.980	146.14	25.2
9	Maleic	108.1	1.921 6.225	1.870	116.07	21.6
10	Malonic	106.2	2.855 5.696	1.958	104.06	20.4
11	Oxalic	109.9	1.271 4.266	1.998	90.04	18.0
12	Pimelic	90.00	4.484* 5.424*	1.974	160.17	26.4
13	Succinic	100.5	4.207 5.635	1.962	118.09	22.4

a) Electrophoretic mobility $\times 10^5$ ($cm^2 \cdot V^{-1} \cdot s^{-1}$), according to the equation of MacInnes *et al.*[3,8]
b) Values for pK[12a] and pK,*[12b] are from the literature.
c) Absolute values of the electric charge at pH 7.05 are calculated from the equation of Kiso *et al.*[11]
d) Values of molecular weight were taken from the literature.[13]
e) Values for the step height (cm) at pH 7.05 according to Beckers *et al.*[9]
f) 1-7, Monocarboxylic acids; 8-13, bicarboxylic acids.

of the molecular weight to calculate the correlation between the molecular weight of ions and the step height in the isotachopherogram.

Derivation of the Correlative Equation between the Molecular Weight of Ions and the Relative Step Height in the Isotachopherogram

The significance of the correlation coefficient (r) was tested by means of the t-test in each case after transformation of r to z ($N=13$; Table I).

Determination of the Electrophoretic Mobility and the Electric Charge of Ions——The first column of Table I shows the calculated mobilities of carboxylic acids. A good correlation was found between the mobility and the relative mobility according to the equation of Kiso *et al.*[11] ($r=0.9634$, $P<0.0003$). Therefore, we used these electrophoretic mobility values of ions to determine the correlation between the molecular weight of ions and the step height in the isotachopherogram. The third column shows the electric charges of carboxylic acids at pH 7.05.

Derivation of the Correlative Equation between the Electrophoretic Mobility and the Molecular Weight of Ions——The following correlative equation between the mobility and the molecular weight was derived:

$$U = 56 + 258\,|Z|/M^{1/2} \qquad (r = 0.9508, P<0.0004) \qquad (1)$$

Derivation of the Correlative Equation between the Electrophoretic Mobility and the Step Height in the Isotachopherogram——The following correlative equation between the mobility and the step height was derived:

$$U = 46 + 1197\,1/h \qquad (r = 0.9812, P<0.0002) \qquad (2)$$

Derivation of the Correlative Equation between the Molecular Weight of Ions and the Step Height in the Isotachopherogram——The following correlative equation between the molecular weight and the step height was derived:

$$1/h = 0.0082 + 0.2134\,|Z|/M^{1/2} \qquad (r = 0.9616, P<0.0003) \qquad (3)$$

Chart 1 shows the relationships among these equations. Equation (3) can be derived

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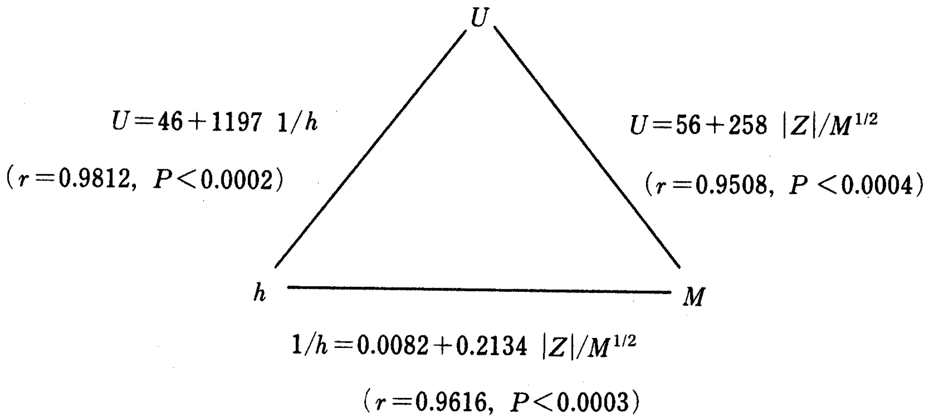

Chart 1. Relationships among Mobility(U), Step Height(h) and Molecular Weight(M) of Ions

This chart shows that the correlative equation between the molecular weight (M) and the step height (h) can be derived directly. These equations were derived using the values in Table I (N=13). $|Z|$: absolute value of the electric charge.

directly, and is in reasonably good accord with that derived from equations (1) and (2) : $1/h=0.0084+0.2155\,|Z|/M^{1/2}$. These results demonstrate the correlation between the molecular weight of ions and the step height in the isotachopherogram, and confirm the utility of the finding of Okuyama *et al.*[14]

Modification of the Correlative Equation between the Molecular Weight of Ions and the Step Height in the Isotachophelogram——Equation (3) shows that the reciprocal of the step height is proportional to the reciprocal of the molecular weight, and Fig. 1 shows that the use of the three different orders of molecular weight has little effect on the relationship between the molecular weight and the mobility. Therefore, ebuation(3) was modified:

$$h = 7.092+2.637\,M^{1/2}/|Z| \quad (r = 0.9711, P<0.0002) \quad (4)$$

$$h = 8.648+1.116\,M^{2/3}/|Z| \quad (r = 0.9720, P<0.0002) \quad (5)$$

$$h = 12.29+0.188\,M/|Z| \quad (r = 0.9323, P<0.00006) \quad (6)$$

The correlation between the one-half or two-thirds power of molecular weight and the step height (eq. 4 or 5) was good, as was the correlation between the minus one-half power of the molecular weight and the reciprocal of the step height (eq. 3) ($|z_4-z_7|/(2/N-3)^{1/2}=0.3232$ or $|z_5-z_7|/(2/N-3)^{1/2}=0.3590$). The correlation between the molecular weight and the step height (eq. 6) was good in the range of molecular weight of ions from about 50 to 150 daltons ($|z_6-z_7|/(2/N-3)^{1/2}=0.6508$). z_4, z_5, z_6 and z_7 are the transformations of r of equations (4), (5), (6) and (3), respectively.

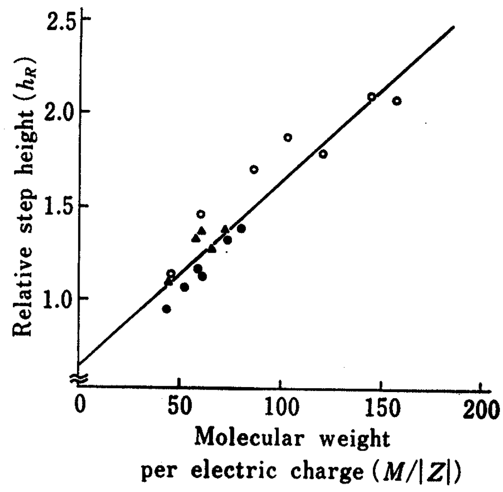

Fig. 2. Correlation between the Molecular Weight and the Relative Step Height

1) Calculated result, eq. (7): $hR=0.65+0.01\,M/|Z|$.
2) Experimental results,
○: Monocarboxylic acids by Beckers *et al.*[9]
●: Bicarboxylic acids by Beckers *et al.*[9]
▲: Bicarboxylic acids by Boček *et al.*[15,16]
The results of Beckers *et al.*[9] in Table I were converted by the use of equations (6) and (7). The results of Boček *et al.*[15,16] are those for oxalate, fumarate, succinate, oxaloacetate and α-ketoglutarate at pH 7.4.

Conversion of Step Height into Relative Step Height——Since the step height in the isotachopherogram depends upon the electrophoretic conditions, the step height in equation (6) was converted into the relative step height[7]

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with chloride ion as a standard:

$$h_R = 0.65+0.01\ M/|Z| \qquad (7)$$

Figure 2 shows the correlation between the molecular weight per electric charge and the relative step height according to equation (7), together with the results of Boček *et al.*[15,16] and the converted results of Beckers *et al.*[9] The relative step heights of bicarboxylic acids were roughly consistent with those of monocarboxylic acids of half the molecular weight. Therefore, this correlation may be expressed by the equation: $h_R=a+b\ M/|Z|$, in which a and b are constants, and the molecular weight of ions may thus be estimated from the relative step height, when the electric charge is known.

Relation between pH and the Relative Step Height in the Isotachopherogram

Figure 3 shows the relation between pH and the relative step height for some carboxylic acids based on equation (7), together with the results of Bocčk *et al.*[15,16] The optimum pH of electrolytes for the estimation of molecular weight may be: pH>pK+3, when the electric charge is maximum, and that for the separation of ions may be near the pK on the relative step height-pH curves.

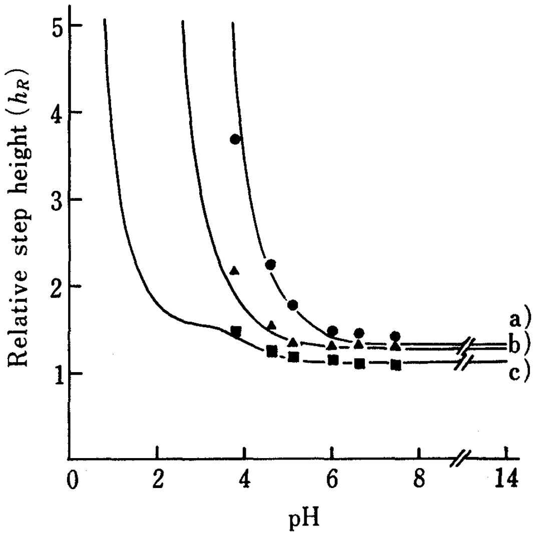

Fig. 3. Relative Step Height-pH Curves According to Equation (7): $h_R=0.65+0.01\ M/|Z|$

1) Calculated results:
a) succinic acid; b) fumaric acid; c) oxalic acid.
2) Experimental results of Boček *et al.*:[15,16]
●, succinate; ▲, fumarate; ■, oxalate.

References and Notes

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