Estimation of the Molecular Weight of Ions by Isotachophoresis. IV.\textsuperscript{1)}
Comparison of the Correlations of Mobility with Molecular Volume and with Molecular Weight\textsuperscript{2,3)}

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(Received March 7, 1988)

The determination of the molecular volumes of organic substances for which values of specific gravity (d) are not available was investigated. The values of van der Waals volume (VA: Å\textsuperscript{3}/molecule) for aliphatic saturated monocarboxylic acids are closer to those of molecular weight per specific gravity (M/d: cm\textsuperscript{3}/mol) than those of Vw (cm\textsuperscript{3}/mol). The slope of the correlative equation between VA and M/d for organic anionic substances is 1.03, but that between Vw and M/d is 0.62.

The values of VA for organic anionic substances show a good correlation with the absolute ionic mobility (m\textsubscript{A}) as well as M/d. The values of VA for amino acids and dipeptides also showed a better correlation with m\textsubscript{A} than the molecular weight (M). Thus, the values of mobility could be better estimated from the molecular volume than the molecular weight.

Keywords——isotachophoresis; molecular weight estimation; molecular volume; ionic mobility; amino acid isotachophoresis; peptide isotachophoresis; β-lactam antibiotic isotachophoresis

In the previous study,\textsuperscript{1)} we reported the correlative equations between the molecular volume, that is, the molecular weight (M) per specific gravity (d), and R\textsubscript{E} for organic anionic substances, i.e., R\textsubscript{E} = a + b(M/d)\textsuperscript{2/3} / |Z|, where R\textsubscript{E} is the ratio of the potential gradient of the sample zone to that of the leading zone,\textsuperscript{4)} and Z, a and b are the electric charge of ions, and two constants, respectively. According to these equations, the molecular volume or the molecular weight of ions may be estimated from the value of R\textsubscript{E}, when the electric charge is known. The values of R\textsubscript{E} were more significantly correlated with molecular volume (M/d) than molecular weight (M).\textsuperscript{1)}

However, the values of specific gravity (d) of many organic substances are not available in the literature.\textsuperscript{5)} Thus, we wished to calculate the values of molecular volume without knowledge of the values of specific gravity (d). This paper describes our studies\textsuperscript{2,3)} on the relationship of van der Waals volume (VA) with the molecular weight per specific gravity (M/d) for organic substances,\textsuperscript{2)} on the relationship of the molecular volume (VA) with the molecular weight (M) for amino acids\textsuperscript{2,3)} and dipeptides,\textsuperscript{3)} and on the application of these results to β-lactam antibiotics.\textsuperscript{3)}

Calculation

The significance of the correlation coefficient (r) was tested by means of the t-test in each case after transformation of r to z. The significance of the difference of r\textsubscript{1} and r\textsubscript{2} was tested by means of the following equation: \[ z_0 = \frac{|z_1 - z_2|}{\sqrt{1/(N_1 - 3) + 1/(N_2 - 3)}} \]
TABLE I. The Values of van der Waals Volume (Å³/molecule)\(^a\) for Some Atoms or Groups

<table>
<thead>
<tr>
<th>Atom or group</th>
<th>(V_A) (^a)</th>
<th>Atom or group</th>
<th>(V_A)</th>
<th>Atom or group</th>
<th>(V_A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OH</td>
<td>13.35</td>
<td>-H(^b)</td>
<td>5.73</td>
<td>-O-(c.e.)</td>
<td>8.63</td>
</tr>
<tr>
<td>&gt;C = O</td>
<td>19.43</td>
<td>&gt;C &lt;</td>
<td>5.53</td>
<td>-O-(a.e.)</td>
<td>6.14</td>
</tr>
<tr>
<td>-COOH(^c)</td>
<td>32.78</td>
<td>&gt;C &lt; H</td>
<td>11.26</td>
<td>-O-(ph.e.)</td>
<td>5.31</td>
</tr>
<tr>
<td>-NH(_2)</td>
<td>17.50</td>
<td>&gt;CH(_2)</td>
<td>16.99</td>
<td>-S</td>
<td>17.93</td>
</tr>
<tr>
<td>&gt;N&lt;</td>
<td>13.42</td>
<td>-CH(_3)</td>
<td>22.70</td>
<td>-SH</td>
<td>24.58</td>
</tr>
<tr>
<td>-NO(_2)</td>
<td>7.19</td>
<td>=C</td>
<td>11.56</td>
<td>=O</td>
<td>11.13</td>
</tr>
<tr>
<td>&gt;C = O</td>
<td>27.90</td>
<td>&gt;C = H</td>
<td>16.64</td>
<td>=C = N</td>
<td>24.41</td>
</tr>
<tr>
<td>&gt;C-(alkyl)(^d)</td>
<td>7.87</td>
<td>=C &lt; H</td>
<td>14.06</td>
<td>&gt;C = CH(_2)</td>
<td>28.15</td>
</tr>
<tr>
<td>&gt;C-H(^e)</td>
<td>13.38</td>
<td>-C</td>
<td>13.37</td>
<td>=C-H</td>
<td>19.18</td>
</tr>
<tr>
<td>Phenyl</td>
<td>76.12</td>
<td>Naphthyl</td>
<td>118.64</td>
<td>-F(ph)</td>
<td>9.63</td>
</tr>
<tr>
<td>-F(pr)</td>
<td>9.50</td>
<td>-F(s, t)</td>
<td>10.30</td>
<td>-F(p)</td>
<td>9.96</td>
</tr>
<tr>
<td>-Cl(pr)</td>
<td>19.30</td>
<td>-Cl(s, t, p)</td>
<td>20.32</td>
<td>-Cl(ph)</td>
<td>19.90</td>
</tr>
<tr>
<td>-Br(pr)</td>
<td>23.91</td>
<td>-Br(s, t, p)</td>
<td>24.24</td>
<td>Br(ph)</td>
<td>25.11</td>
</tr>
<tr>
<td>-I(pr)</td>
<td>31.85</td>
<td>-I(s, t, p)</td>
<td>33.79</td>
<td>-I(ph)</td>
<td>32.61</td>
</tr>
</tbody>
</table>

\(^a\) The values (cm\(^3\)/mol) reported by Bondi\(^6\) were transformed into (Å³/molecule): 1 cm\(^3\)/mol = 1.66 Å³/molecule.

Correlation of van der Waals Volume (\(V_A\)) with the Molecular Weight per Specific Gravity (\(M/d\)) for Organic Anionic Substances

The values of van der Waals volume (\(V_A\)) for organic anionic substances were calculated after transformation of the values (\(V_w\)) as cm\(^3\)/mol given by Bondi\(^6\) to the values as Å³/molecule for some atoms or groups, that is, 1 cm\(^3\)/mol = 1.66 Å³/molecule. The values of van der Waals volume (Å³/molecule) for some atoms or groups are listed in Table I. The values of \(M/d\) were calculated from those of molecular weight (\(M\)) and specific gravity (\(d\)) taken from the literature.\(^5\)

Derivation of the Correlative Equations between Molecular Volume (\(V_A\)) and the Absolute Mobility (\(m_0\)), the Molecular Weight per Specific Gravity (\(M/d\)) and \(m_0\), and \(V_A\) and \(R_E\) for Organic Anionic Substances

The values of \(V_A\) were calculated from those in Table I\(^6\) and \(M/d\) values were calculated as described previously. Substances of known specific gravity were selected (\(N=115\)),\(^5\) as described in the previous paper.\(^1\) The values of \(m_0\), \(R_E\) and electric charge (\(Z\)) at pH\(_L\) 10.0 given by Hirokawa et al.\(^4\) were used. pH\(_L\) is the pH of the leading electrolyte.

Comparison of the Correlative Equation between Molecular Volume (\(V_A\)) and the Absolute Mobility (\(m_0\)), with That between Molecular Weight (\(M\)) and \(m_0\) for Amino Acids

The values of \(m_0\) of amino acids\(^7\) given by Hirokawa et al. were used. The values of \(V_A\) were calculated from the data in Table I.\(^6\) The values of \(M\) were taken from the literature.\(^5\) The mean deviation (MD: %) was calculated by means of the following equation: MD (\(\%\)) = \(1/N \cdot \sum |(a + bx - y)/y|\), where \(a\) and \(b\) are the constants of the correlative equations.

Comparison of the Correlative Equation between Molecular Volume (\(V_A\)) and the Absolute Mobility (\(m_0\)), with That between Molecular Weight (\(M\)) and \(m_0\) for Dipeptides

The values of \(m_0\) of dipeptides\(^8\) given by Hirokawa et al. were used. The values of \(V_A\) were calculated from the data in Table I.\(^6\) The values of \(M\) were taken from the literature.\(^5\) The mean deviation (MD: %) was calculated as described previously.
Derivation of the Correlative Equations between Molecular Volume \( (V_A) \) and \( R_E \), and \( V_A \) and the Absolute Mobility \( (m_0) \) for Some \( \beta \)-Lactam Antibiotics

The values of \( V_A \) of \( \beta \)-lactam antibiotics were calculated from the data in Table I.\(^6\) The values of \( R_E \) were obtained experimentally, as follows. The approximate values of \( m_0 \) were calculated from \( R_E \), that is, \( R_{E,\text{min}} \) obtained using only one \( \text{pH}_L \) in the case of maximum electric charge.\(^1\)

**Experimental**

**Values of \( R_E \) for Some \( \beta \)-Lactam Antibiotics at \( \text{pH}_L 8.6 \)** — Samples and Reagents: The sodium salts of cefalotin \((\text{CET}, \text{M: molecular weight}=418.41, \text{pK}_a=3.6 \pm 0.13)\),\(^9\) cefazolin \((\text{CEZ, M}=476.49, \text{pK}_a=2.1)\),\(^9,10\) cefapirin \((\text{CEPR}, \text{M}=445.44, \text{pK}_a=\text{ca. 5.3})\),\(^9,10\) cefmetazole \((\text{CMZ}, \text{M}=493.51, \text{pK}_a=2.34)\),\(^9,10\) and cefotaxime \((\text{CTX, M}=477.44, \text{pK}_a=\text{ca. 3.4})\)\(^9\) were purchased from Shionogi & Co., Ltd., Fujisawa Pharmaceutical Co., Ltd., Bristol-Myers Co., Sankyo Co., Ltd., and Hoechst Japan Ltd., respectively. Other reagents were purchased from Nakarai Chemicals, Ltd. Hydroxypropyl cellulose (HPC, 1000–4000 cps) was purchased from Tokyo Kasei Kogyo Co., Ltd.

Electrolyte System for Isotachophoresis and Instruments: The leading ion was 0.01 M chloride. The pH of the leading electrolyte (pH\(_L\)) was adjusted to 8.6 by using Amediol (2-amino-2-methyl-1,3-propanediol),\(^4,11\) and 0.02% HPC was added as a surfactant in order to suppress electrophoresis and to increase the sharpness of the zone boundaries.\(^7,8\) The terminating ion was 0.01 M \( \beta \)-alanine. The pH of the terminating electrolyte (pH 11.4) was adjusted by using Ba(OH)\(_2\); Ba\(^{2+}\) precipitates with CO\(_2^{-}\). The migration current was 100 \( \mu \)A. An IP-1B isotachophoretic analyzer with the separating tube of 0.5 mm i.d. and 15 cm length, and a PGD-1 potential gradient detector (Shimadzu Seisakusho Ltd., Kyoto, Japan) were used.\(^12\)

**Results and Discussion**

Correlation of van der Waals Volume \( (V_A) \) with the Molecular Weight per Specific Gravity \( (M/d) \) for Organic Anionic Substances

First, the correlation of van der Waals volume \( (V_A) \) with the molecular weight per specific gravity \( (M/d) \) for aliphatic saturated monocarboxylic acids was investigated. The values of \( M/d \) reported by Dorinson et al.\(^{13}\) and those of \( V_A \) calculated from van der Waals volume \( (V_w) \) by Bondi\(^6\) are listed in Table II. The unit of \( M/d \) is \( \text{cm}^3/\text{mol} \), because the unit of \( M \) is \( \text{g/mol} \),\(^{14} \) and the unit of \( d \) is \( \text{g/cm}^3. \)\(^{14} \) The unit of \( V_w \) reported by Bondi\(^6\) is also \( \text{cm}^3/\text{mol} \). However, as shown in Table II, the values of \( M/d \) (\( \text{cm}^3/\text{mol} \)) are different from those of \( V_w \) (\( \text{cm}^3/\text{mol} \)), and the values of \( M/d \) are fairly close to \( V_A \) (\( \text{\AA}^3/\text{molecule} \)). The mean deviation \( \text{MD} (\%) = \frac{1}{N} \sum |100 (V_A - (M/d)) / (M/d)| = 1.75 (\%) \).

In order to confirm that the values calculated from van der Waals volume \( (V_A) \) can

**Table II. Molecular Volume for Aliphatic Saturated Monocarboxylic Acids**

<table>
<thead>
<tr>
<th>Acid</th>
<th>( M/d^{a} ) (cm(^3/)mol)</th>
<th>( V_w^{b} ) (cm(^3/)mol)</th>
<th>( V_A^{c} ) (\text{\AA}^3/\text{molecule})</th>
<th>( D^{d} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic</td>
<td>37.71</td>
<td>23.19</td>
<td>38.51</td>
<td>2.12</td>
</tr>
<tr>
<td>Acetic</td>
<td>57.21</td>
<td>33.41</td>
<td>55.48</td>
<td>3.02</td>
</tr>
<tr>
<td>Propionic</td>
<td>74.55</td>
<td>43.64</td>
<td>72.47</td>
<td>2.79</td>
</tr>
<tr>
<td>Butyric</td>
<td>91.93</td>
<td>53.87</td>
<td>89.46</td>
<td>2.69</td>
</tr>
<tr>
<td>Valeric</td>
<td>108.69</td>
<td>64.11</td>
<td>106.45</td>
<td>2.06</td>
</tr>
<tr>
<td>Caproic</td>
<td>125.04</td>
<td>74.34</td>
<td>123.44</td>
<td>1.28</td>
</tr>
<tr>
<td>Enantoic</td>
<td>141.89</td>
<td>84.57</td>
<td>140.43</td>
<td>1.03</td>
</tr>
<tr>
<td>Caprylic</td>
<td>158.57</td>
<td>94.80</td>
<td>157.42</td>
<td>0.73</td>
</tr>
<tr>
<td>Pelargonic</td>
<td>174.53</td>
<td>105.03</td>
<td>174.41</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\( a \) The values reported by Dorinson et al.\(^{13}\)  \( b \) The values calculated from van der Waals volumes \( (V_w) \) by Bondi\(^6\)  \( c \) The values calculated from the values \( (V_A) \) in Table I.  \( d \) The deviation between \( V_A \) (\( \text{\AA}^3/\text{molecule} \)) and \( M/d \) \( \text{MD} (\%) = |100 (V_A - (M/d)) / (M/d)| \). The mean deviation \( \text{MD} = 1.75 \%(\).
substitute for the molecular weight per specific gravity \((M/d)\), the following correlative equation between \(V_A\) (\(\text{Å}^3/\text{molecule}\)) and \(M/d\) (\(\text{cm}^3/\text{mol}\)) for organic anionic substances \((N = 111)\) was derived.

\[
V_A = 9.69 + 1.03M/d \quad (r = 0.910, p < 0.001) \quad (1)
\]

The significance level \((p)\) shows that the values calculated from van der Waals volume may be used as the molecular volume of organic substances. When the unit of van der Waals volume \((V_w)\) is shown as \(\text{cm}^3/\text{mol}\), Eq. 1 is as follows \((N = 111)\).

\[
V_w = 5.84 + 0.62M/d \quad (r = 0.910, p < 0.001) \quad (1')
\]

Of course, the correlation coefficients \((r)\) and the significance level \((p)\) of Eq. 1 and Eq. 1' are the same, and the constants of Eq. 1' are \(1/1.66\) times of those of Eq. 1, because \(1 \text{cm}^3/\text{mol} = 1.66 \text{Å}^3/\text{molecule}\), as mentioned previously. The slope of Eq. 1 is 1.03 and that of Eq. 1' is 0.62, so Eq. 1 \((V_A: \text{Å}^3/\text{molecule})\) is clearly better than Eq. 1' \((V_w: \text{cm}^3/\text{mol})\).

Further, since we are investigating the relationship between the mobility of an ion and the molecular volume of an ion \((\text{Å}^3/\text{molecule})\), the unit of \(\text{Å}^3/\text{molecule}\) is suitable.

**Derivation of the Correlative Equations between Molecular Volume \((V_A)\) and the Absolute Mobility \((m_0)\), the Molecular Weight per Specific Gravity \((M/d)\) and \(m_0\), and \(V_A\) and \(R_E\) for Organic Anionic Substances**

In the previous study,\(^{1,1}\) we investigated the relationships between the value of \(R_E\), which is defined as the ratio of the potential gradient of the sample zone to that of the leading zone, and the molecular volume \((M/d)\). We used \(R_E\), the ratio of the potential gradients,\(^{4,1}\) the value of which is corrected for the relative step height \((h_R)\) in an isotachopherogram.\(^{15,16}\) That is, the molecular volume or molecular weight may be estimated from an isotachopherogram, when the electric charge is known. For example, in the case of \(pH_1 = 10\) we may employ the maximum electric charge of the sample ion, but in other cases, we should calculate the electric charge \((Z)\) and \(pH\) of the sample zone,\(^{1,16}\) by using the absolute mobilities \((m_0)\).\(^{4,1}\) The following correlative equations between \(V_A\) and \(m_0\) \((10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1})\) and \(M/d\) and \(m_0\) for organic anionic substances were derived.

\[
m_0 = 8.44 + 572|Z|/V_A^{2/3} \quad (r = 0.976, N = 111, p < 0.001) \quad (2)
\]

\[
m_0 = 12.8 + 452|Z|(M/d)^{2/3} \quad (r = 0.962, N = 115, p < 0.001) \quad (3)
\]

The values of \(m_0\) of divalent and trivalent anionic substances were used in the cases of maximum electric charge, that is, \(Z = 2\) and \(Z = 3\), respectively.\(^{1,4,1}\)

The exponent of \(V_A\) or \(M/d\) was 2/3, because the drag ratio is obtained as a function of \(V^{2/3}\), that is, 2/3 represents the cross section per volume of the ion.\(^{1,1}\)

The correlation coefficient \((r)\) in the case of \(V_A\) (Eq. 2) is slightly better than that in the case of \(M/d\) (Eq. 3). However, the difference was not significant. Thus, the values of the absolute mobility \((m_0)\) may be estimated from either the molecular volume \((V_A; \text{Eq. 2})\) or the molecular weight per specific gravity \((M/d; \text{Eq. 3})\), when the electric charge \((Z)\) is known.

The values of \(V_A\), \(M/d\) and \(m_0\) for amino acids for which specific gravity \((d)\) values are available in the literature\(^5\) are listed in Table III. The values of \(m_0\) calculated from Eq. 2 \((V_A)\) are closer to those reported by Hirokawa et al.\(^7\) than those calculated from Eq. 3 \((M/d)\).

The following correlative equation between \(V_A\) and \(R_E\) for organic anionic substances was derived.

\[
R_E = 0.544 + 0.083V_A^{2/3}/|Z| \quad (r = 0.953, N = 111, p < 0.001) \quad (4)
\]

When the electric charge is known, the molecular volume for compounds whose specific gravity is not available in the literature\(^5\) may be estimated from the value of \(R_E\) calculated...
table of the Molecular Volume and the Mobility for Some Amino Acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$V_A$</th>
<th>$M/d$</th>
<th>$m_o$</th>
<th>$m_0^i$</th>
<th>$m_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>84.24</td>
<td>62.22</td>
<td>38.2</td>
<td>41.6</td>
<td>32.2</td>
</tr>
<tr>
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<td>115.46</td>
<td>85.63</td>
<td>32.6</td>
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<td>31.6</td>
</tr>
<tr>
<td>Asp</td>
<td>111.31</td>
<td>80.12</td>
<td>33.2</td>
<td>37.1</td>
<td>30.1</td>
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<tr>
<td>Glu</td>
<td>128.30</td>
<td>95.66</td>
<td>30.9</td>
<td>34.4</td>
<td>27.0</td>
</tr>
<tr>
<td>Gly</td>
<td>67.27</td>
<td>64.66</td>
<td>43.0</td>
<td>40.9</td>
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</tr>
<tr>
<td>Leu</td>
<td>135.19</td>
<td>101.44</td>
<td>30.2</td>
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<tr>
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<td>30.9</td>
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</tr>
<tr>
<td>Val</td>
<td>118.20</td>
<td>95.24</td>
<td>32.2</td>
<td>34.5</td>
<td>28.4</td>
</tr>
</tbody>
</table>

$V_A$ a) The molecular volumes ($Å^3$/molecule) calculated from the values in Table I. $M/d$ b) The molecular volumes (cm$^3$/mol) calculated from literature values. $m_o$ c) The mobilities calculated from Eq. 2: $m_o = 8.44 + 572/Z/VA^{2/3}$. $m_0^i$ d) The mobilities calculated from Eq. 3: $m_0^i = 12.8 + 452/Z/(M/d)^{2/3}$. $m_o$ e) The absolute mobilities reported by Hirokawa et al. The units of $m_o$ (c, d and e) are $10^{-5}$ cm$^2$ V$^-1$s$^-1$.

Comparison of the Correlative Equation between Molecular Volume ($V_A$) and the Absolute Mobility ($m_0$), with That between Molecular Weight ($M$) and $m_0$ for Amino Acids

We reported that molecular volume ($M/d$) is more significantly correlated with $R_E$ than molecular weight ($M$) for organic acids. In order to confirm that the molecular volume ($V_A$) also correlates well with the absolute mobility ($m_0$) for amino acids, the following correlative equations between $V_A$ and $m_0$ for amino acids were derived. The exponent of $V_A$ was 2/3, as discussed previously.

$$m_0 = 7.4 + 530.4/Z/VA^{2/3} \quad (r = 0.955, \text{MD} = 6.2\%, N = 22)$$ (5)

$$m_0 = 6.2 + 546.7/Z/VA^{2/3} \quad (r = 0.950, \text{MD} = 7.0\%, N = 26)$$ (6)

Equation 5 was derived using the values of $m_0$ of divalent amino acids ($N=4$, Asp, Cys, Glu, Tyr) in the case of $Z=2$, and Eq. 6 was derived using the values of $m_0$ of those in the case of $Z=1$ and $Z=2$. The difference between these equations was not regarded as significant. However, Hirokawa et al. reported that the correlation coefficient ($r$) between the mobilities and $M^{1/2}$ for amino acids ($N=22$, $Z=1$) was only 0.69, significantly different from that of Eq. 5 (or Eq. 6, $p < 0.01$). Hirokawa et al. obtained $r = 0.82$ except for Cys, Tau and Tyr ($N = 19$), but the difference from Eq. 5 (or Eq. 6) is still significant (Eq. 5, $p < 0.05$; Eq. 6, $p < 0.06$).

Figure 1 shows the relationship between the exponent ($n$) of molecular weight ($M$) or molecular volume ($V_A$) for amino acids, and (A) the values of the correlation coefficient ($r$), and (B) the mean deviation (MD: %). The best exponent of $V_A$ was not 2/3 in Fig. 1, but we used 2/3 as the exponent of $V_A$ in any case, because it represents the cross section per the volume of the ion, as discussed previously.

On the whole, Fig. 1 shows that the correlation coefficient ($r$) between the absolute mobility ($m_0$) and $V_A$ is better than that between $m_0$ and $M$, and shows that MD ($\%$) in the case of $V_A$ is smaller than in the case of $M$.

Comparison of the Correlative Equation between Molecular Volume ($V_A$) and the Absolute Mobility ($m_0$), with That between Molecular Weight ($M$) and $m_0$ for Dipeptides

The following correlative equations between $V_A$ and $m_0$ for dipeptides were derived. The
Fig. 1. Relationships between the Exponent (n) of Molecular Weight or Molecular Volume for Amino Acids and (A) the Correlation Coefficient (r), and (B) Mean Deviation (MD: %)

\[ m_0 = 7.6 + 570.5 \frac{Z}{V_A^{2/3}} \]  \( r = 0.985, MD = 1.9\%, N = 28 \)  \( \text{(7)} \)

\[ m_0 = 6.4 + 602.9 \frac{Z}{V_A^{2/3}} \]  \( r = 0.968, MD = 3.0\%, N = 30 \)  \( \text{(8)} \)

Equation 7 was derived using the values of \( m_0 \) of divalent dipeptides \((N=2, \text{Gly–Tyr, Ley–Tyr})\) in the case of \( Z=2 \), and Eq. 8 was derived using the values of the \( m_0 \) in the cases of \( Z=1 \) and \( Z=2 \). The difference between these equations was not regarded as significant. Hirokawa et al. reported that the correlation coefficient \( r \) between the mobilities and \( M^{1/2} \) for monovalent dipeptides except for Gly–Tyr and Ley–Tyr \((N=26)\) was 0.94. The difference between the correlation coefficient \( r \) reported by Hirokawa et al. and that of Eq. 8 was not significant, though in the case of Eq. 7 there was a significant difference \((p<0.03)\).

Figure 2 shows the relationship between the exponent (n) of molecular weight \((M)\) or molecular volume \((V_A)\) for dipeptides, and (A) the values of the correlation coefficient \( r \), and (B) the mean deviation \( (MD: \%) \). The best exponent of \( V_A \) was near 2/3 in Fig. 2, probably because the influence of the drag coefficients \((C_D)^1\) may be small. The correlation coefficient \( r \) between the molecular volume \((V_A^{2/3})\) of the dipeptides of Ala and the absolute mobilities \((m_0)\), is better \( (r=0.982) \) than for other constituent amino acids \( (r=0.930, N=9) \), and \( r \) between \( V_A^{2/3} \) of the dipeptides of Gly and \( m_0 \) is better \( (r=0.963) \) than that of the other amino acids \( (r=0.911, N=13) \). Further, \( r \) between \( V_A^{2/3} \) of the dipeptides of Leu and \( m_0 \) is better \( (r=0.986) \) than that of the other constituent amino acids \( (r=0.962, N=5) \).

On the whole, Fig. 2 also shows that the correlation coefficient \( r \) between the absolute mobility \((m_0)\) and molecular volume \((V_A)\) is better than that between \( m_0 \) and molecular weight \((M)\), and shows that MD \( (\%) \) in the case of \( V_A \) is smaller than in the case of \( M \).

When the values of the \( m_0 \) of amino acids or dipeptides are taken for \( Z=1 \), \( r \) and MD \( (\%) \) are almost independent of the exponent \( (n) \) of \( M \) \((0<n<1)\). The values of MD \( (\%) \) of amino acids are smaller than 7\% (Fig. 1), and those of dipeptides are smaller than 4\% (Fig. 2).

The absolute mobilities \((m_0)\) for dipeptides\(^8\)) are invariably smaller than those of the
constituent amino acids,7) and the values of molecular volume ($V_A$) or the molecular weight ($M$) of dipeptides are naturally larger than those of the constituent amino acids. When the electric charge is constant, $M$ or $V_A$ is inversely proportional to $m_0$ and proportional to $R_E$.

### Derivation of the Correlative Equation between Molecular Volume ($V_A$) and $R_E$, and $V_A$ and the Absolute Mobility ($m_0$) for Some β-Lactam Antibiotics

Figure 3 shows the observed isotachopherogram of a β-lactam antibiotic, cefotaxime (CTX). The value of $R_E$ of CTX was calculated from the isotachopherogram, and those of other β-lactam antibiotics were obtained similarly. The values of $R_E$, $V_A$ and $m_0$ for some β-lactam antibiotics are listed in Table IV. The values of $m_0$ calculated from $R_E$ are close to those calculated from Eq. 5.

The following correlative equation between $V_A$ and $R_E$ for some β-lactam antibiotics was derived.

$$R_E = -0.08 + 0.092 \frac{V_A^{2/3}}{|Z|} \quad (r = 0.939, N = 5) \quad (9)$$

The optimum pH of electrolytes for the estimation of the molecular volume or for the separation of β-lactam antibiotics may be determined from the $R_E$-pH$_L$ curves obtained on the basis of this equation.$^{11}$

The following correlative equation between $V_A$ and $m_0$ for some β-lactam antibiotics was derived.

$$m_0 = 0.1 + 867.6 \frac{|Z|}{V_A^{2/3}} \quad (r = 0.932, N = 5) \quad (10)$$

β-Lactam antibiotics consist of cysteine and valine.$^{17}$ Then, the values of $m_0$ of these amino acids were added to those of the β-lactam antibiotics, and the following correlative equations were derived.

$$m_0 = 7.6 + 508.6 \frac{|Z|}{V_A^{2/3}} \quad (r = 0.999, N = 7) \quad (11)$$

$$m_0 = 7.4 + 499.8 \frac{|Z|}{V_A^{2/3}} \quad (r = 0.994, N = 8) \quad (12)$$
The value of $R_E$ of CTX was calculated according to the following equations:

$$ RE = \frac{Es}{EL} = \frac{h_s + \Delta h}{h_L + \Delta h}, \quad \Delta h = \frac{h_{std} - h_L}{RE_{std} - 1}, \quad RE_{std} = \frac{E_{std}}{EL} = \frac{m_L}{m_{std}} = 79.08/42.4 = 1.865.$$

$R_E$ is the ratio of the potential gradient of the sample zone ($Es$) to that of the leading zone ($EL$), $h_s$ is the observed step height of the sample zone (CTX), $h_L$ and $h_{std}$ are the apparent step height of the leading zone (Cl⁻) and that of the zone of the internal standard (acetic acid), and $m$ is the absolute mobility. The relative step height ($h_R$) was defined as $h_s/h_L$.15,16)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>$R_E \pm S.D.$</th>
<th>$V_a$</th>
<th>$m_0$</th>
<th>$m_0^{d)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CET</td>
<td>4.09 ± 0.14 (N=4)</td>
<td>308.64</td>
<td>19.3</td>
<td>19.0</td>
</tr>
<tr>
<td>CEZ</td>
<td>4.37 ± 0.21 (N=3)</td>
<td>326.58</td>
<td>18.1</td>
<td>18.6</td>
</tr>
<tr>
<td>CEPR</td>
<td>4.34 ± 0.40 (N=4)</td>
<td>329.21</td>
<td>18.2</td>
<td>18.5</td>
</tr>
<tr>
<td>CTX</td>
<td>4.38 ± 0.05 (N=4)</td>
<td>344.01</td>
<td>18.1</td>
<td>18.2</td>
</tr>
<tr>
<td>CMZ</td>
<td>4.55 ± 0.10 (N=5)</td>
<td>354.13</td>
<td>17.4</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Equation 11 was derived using the value of $m_0$ of Cys in the case of $Z=2$, and Eq. 12 was derived using the values of $m_0$ in the cases of $Z=1$ and $Z=2$.7) The difference between these equations was not regarded as significant, but there were significant differences between the correlation coefficients of Eq. 10 and Eq. 11 (or Eq. 12, $p < 0.01$).

As described previously, the values of $m_0$ of dipeptides are invariably smaller than those of the constituent amino acids, that is, the zones of dipeptides in the isotachopherogram invariably follow those of the constituent amino acids, when the electric charge is constant. Further studies are in progress.

Acknowledgement The authors are grateful to Dr. Takeshi Hirokawa and Professor Yoshiyuki Kiso, Faculty of Engineering, Hiroshima University, for valuable advice.

References and Notes

3) M. Hirakawa, O. Fujishita, S. Higuchi, K. Otsubo, T. Aoyama and H. Karasawa, Abstracts of Papers, 7th