BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 3093—3099 (1969)

A Study of the Properties of Dilute Polymer Solutions by the Archibald Ultracentrifugal Method[*1]

Akira KOTERA, Hideomi MATSUDA[*2] and Yuji MIYAZAWA[*3]
Department of Chemistry, Faculty of Science, Tokyo Kyoiku University, Otsuka, Bunkyo-ku, Tokyo
and Etsuo JOKO
The Tokyo Metropolitan Technical College, Higashioi, Shinagawa-ku, Tokyo

(Received May 1, 1969)

A modification of the Archibald method for determining the molecular weight of a polydisperse sample in ideal and non-ideal solutions is developed in this paper. A reliable value of the weight-average molecular weight is obtained from the plot of an apparent weight-average molecular weight against the centrifugal time rather than against the amount of the solute sedimented from the meniscus. This fact is explained on the assumption of a fractionation effect of the solute caused by the centrifugal potential.

The sedimentation equilibrium method, one of the most reliable procedures for the molecular-weight determination of a polymer, is based on the strict thermodynamical treatment, but the time required for the attainment of the equilibrium is, in general, very long.

Archibald[1)] has suggested that the molecular weight of a polymer can be determined from the data obtained during the approach to the sedimentation equilibrium for a monodisperse sample in an ideal solution.

For the system of a monodisperse sample in a non-ideal solution, Fujita and Inagaki[2,3)] have derived a relationship according to which the apparent weight-average molecular weight of the solute at the meniscus at a given time is corrected to the value of that at zero time.

For a polydisperse sample in ideal and non-ideal solutions a weight-average molecular weight can be, in principle, determined in the same way, but the apparent weight-average molecular weight calculated at a given time must be extrapolated to zero time for the determination of the weight-average molecular weight of the sample.[1)] It was shown in the experiments for the polydisperse sample in non-ideal solutions that the value of the apparent weight-average molecular weight of the sample at each centrifugal time at the meniscus, $M_{(app)}(t)$, does not change linearly with time, but that the extrapolated value at the meniscus to zero time is usually larger than that at the bottom of the cell.[4–7)] According to the other reports, the values of $M_{(app)}(t)$ at the meniscus and the bottom of the cell vary approximately linearly with time for some cases of polydisperse samples in non-ideal solutions, and these values give the same intercept at zero time.[2,8,9)] The purpose of this paper is to find out, experimentally, a suitable procedure for the extrapolation of the $M_{(app)}(t)$ of a polydisperse sample to the starting condition, and to obtain a reliable value for the molecular weight.

Experimental

Materials. Poly-α-methylstyrene 17[*4] (PαMST-17, $\bar{M}_w=37\times10^4$), poly-α-methylstyrene 18[*4] (PαMST-18,

---

[*1] Parts of this work were presented at the 15th Annual Meeting of the Society of Polymer Science, Japan (Nagoya University, May 22, 1966), and at the 16th Polymer Symposium in Japan (Kyushu University, October 21, 1967).

[*2] Present address: Department of Chemical Engineering, Faculty of Textile Science, Shinshu University, Ueda, Nagano.

[*3] Present address: Department of Chemistry, Faculty of Science, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo.

1) W. J. Archibald, *J. Phys. Chem.*, **51**, 1204 (1947).

2) H. Fujita, H. Inagaki, T. Kotaka and H. Utiyama, *J. Phys. Chem.*, **66**, 4 (1962).

3) A. Nakazawa and H. Inagaki, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **87**, 1049 (1966).

4) A. Ginsburg, P. Appel and H. K. Schachman, *Arch. Biochem. Biophys.*, **65**, 545 (1956).

5) H.-G. Elias, *Angew. Chem.*, **73**, 209 (1961).

6) H. A. Swenson, A. J. Morak and S. Kurath, *J. Polymer Sci.*, **51**, 231 (1961).

7) A. Kotera, N. Iso, K. Umezawa and Y. Miyazawa, This Bulletin, **35**, 699 (1962).

8) H. Inagaki, S. Kawai and A. Nakazawa, *J. Polymer Sci., Part A*, **1**, 3303 (1963).

9) H. Inagaki, A. Nakazawa and T. Kotaka, *Bull. Inst. Chem. Res., Kyoto Univ.*, **43**, 135 (1965).

[*4] Polymerized anionically in the laboratory of Professor M. Nagasawa, Nagoya University.

$\bar{M}=59\times10^4$), and polystyrene NBS705[*5](PST-NBS705, $\bar{M}=19\times10^4$) were used as samples with a narrow molecular-weight distribution. PαMST-mixt, which is a 1 : 1 mixture in weight of PαMST-17 and PαMST-18, and polystyrene SM1[*6] (PST-SM1, $\bar{M}_w=30\times10^4$) were used as the polydisperse samples.

Solvents. Cyclohexane was repeatedly washed with a sodium hydroxide solution and with water after being treated with a mixture of sulfuric acid and nitric acid; then it was distilled twice and fractionally distilled after being dried over calcium chloride and metallic sodium. The physical constants were as follows: bp 80.8°C, n_D^{20} 1.4264, d^{38} 0.7619 g/m*l*.

Toluene was repeatedly washed with sodium hydroxide and water after being treated with sulfuric acid. It was dried over calcium chloride and then distilled fractionally. The physical constants were as follows: bp 110.8°C, d^{30} 0.8579 g/m*l*.

Butanone was distilled after being treated with potassium permanganate; then it was distilled fractionally after being dried over sodium sulfate. The physical constants were as follows: bp 79.4°C, d^{25} 0.7986 g/m*l*.

Ultracentrifugation. The measurements were made with a Hitachi Analytical Ultracentrifuge, UCA-1, equipped with a schlieren optical system. A double-sector cell was used. The experimental conditions in this study are summarized in Table 1. The values of the partial specific volume, $\bar{v}$, the density of the solvent, ρ, and the refractive index increment, d*n*/d*C*, used in this experiment are also listed in Table 1. The concentration in the cell was determined in accordance with the Klainer and Kegeles method.[10]

Results and Discussion

Determination of *M*(*t*). *M*(*t*), a weight-average molecular weight at each centrifugal time, was calculated by the following equation:[1]

$$M(t) = \{\boldsymbol{R}T/(1-\bar{v}\rho)\omega^2\}(1/C_m x_m)(\mathrm{d}C/\mathrm{d}x)_m \qquad (1)$$

where $\boldsymbol{R}$ is the gas constant, T is the absolute temperature, $\bar{v}$ is the partial specific volume of the solute, ρ is the density of the solvent, ω is the angular velocity of the rotation, x_m is the distance from the axis of rotation to the meniscus or to the bottom of the cell, C_m is the concentration of the solute at the meniscus or the bottom, and $(\mathrm{d}C/\mathrm{d}x)_m$ is the concentration gradient at the meniscus or the bottom.

The schlieren pattern at the bottom of the cell was so indistinct that it was impossible to determine the concentration gradient. Therefore, only the results obtained at the meniscus will be discussed in this report.

In the calculation of $M(t)$ at the meniscus by Eq. (1), the value of $(1/C_m x_m)(\mathrm{d}C/\mathrm{d}x)_m$ was obtained by extrapolating a plot of log $(1/Cx)(\mathrm{d}C/\mathrm{d}x)$ against a given point in the cell, x, to the meniscus.[2]

The values of $M(t)$ obtained were plotted against time, t; the value extrapolated to $t=0$ gives the weight-average molecular weight of the solute at zero time, $M(t=0)$. The centrifugal time was calculated as follows; one-third of the time before the rotor attained its final speed was added to the time which elapsed after the operational speed was reached.[19]

The Results for Ideal Systems. The results of experiments for ideal systems are summarized in Table 2, where the value of $\Delta M(t)/\Delta t$ shows the

TABLE 1. THE EXPERIMENTAL CONDITIONS AND THE VALUES OF THE PHYSICAL CONSTANT USED IN THE SEDIMENTATION MEASUREMENTS

Sample	Solvent	Temp. (°C)	$\bar{v}$ (m*l*/g)	ρ (g/m*l*)	d*n*/d*C* (m*l*/g) (546 mμ)
PαMST-17	Cyclohexane	38.0 (θ)	0.8658[11]	0.7619[11]	0.201[11]
PαMST-18	Cyclohexane				
PαMST-mixt	Cyclohexane				
PαMST-18	Toluene	30.0	0.726[12]	0.8579[12]	0.1287[12]
PST-SM 1	Cyclohexane	35.0 (θ)	0.940[13]	0.7650[14]	0.173[15]
	Butanone	25.0	0.925[12]	0.7986[12]	0.221[16]
	Toluene	30.0	0.913[17]	0.8579[12]	0.109[18]
PST-NBS705	Toluene				

(θ) : θ-temperature for the polymer solution

[*5] A standard sample distributed by the National Bureau of Standards.

[*6] A sample distributed by the Committee on Molecular Weight and Molecular Weight Distribution of the Society of Polymer Science, Japan.

10) S. M. Klainer and G. Kegeles, *J. Phys. Chem.*, **59**, 952 (1955).

11) C. Hosoi, Thesis, Tokyo Kyoiku University, 1964.

12) C. Hosoi, unpublished data, 1965.

13) B. Rosen, *J. Polymer Sci.*, **17**, 559 (1955).

14) International Critical Tables, Vol. 3, 1st Ed., pp. 27, 29 (1928).

15) W. R. Krigbaum and D. K. Carpenter, *J. Phys. Chem.*, **59**, 1166 (1955).

16) I.U.P.A.C., *J. Polymer Sci.*, **10**, 129 (1953).

17) W. Heller and A. C. Thompson, *J. Colloid Sci.*, **6**, 57 (1951).

18) H. P. Frank and H. F. Mark, *J. Polymer Sci.*, **17**, 1 (1955).

19) E. G. Pickels, "Ultracentrifugation," in "Methods in Medical Research," Vol. 5, ed. by A. C. Corcoran, Year Book Publishers, Chicago (1953), p. 123.

TABLE 2. THE VALUES OF $\Delta M(t)/\Delta t$, AND M FOR IDEAL SYSTEM

Sample	Solvent and Temp.	Initial concn. (g/dl)	Rotor speed (rpm)	$\Delta M(t)/\Delta t$ $\times 10^{-4}$ (min^{-1})	M $\times 10^{-14}$
PαMST-17 (monodisperse)	Cyclohexane 38.0°C (θ)	0.211	7640	-0.17_5	$37._0$
		0.325	7640	-0.18_7	$39._8$
		0.459	8270	-0.17_7	$36._1$
		0.541	8950	-0.21_1	$41._1$
		0.661	8270	-0.18_5	$38._3$
PαMST-18 (monodisperse)	Cyclohexane 38.0°C (θ)	0.203	7060	-0.24_4	$58._0$
		0.286	7060	-0.24_2	$57._0$
		0.406	7060	-0.26_8	$56._3$
		0.557	7060	-0.31_9	$55._8$
		0.667	7060	-0.31_2	$55._6$
PαMST-mixt (polydisperse)	Cyclohexane 38.0°C (θ)	0.224	7060	-0.28_9	$48._7$
		0.411	7060	-0.35_7	$50._3$
		0.601	7060	-0.35_3	$49._0$
		0.795	7060	-0.35_5	$50._3$
		0.979	7640	-0.38_0	$48._3$
PST-SM 1 (polydisperse)	Cyclohexane 35.0°C (θ)	0.136	8270	-0.16_0	$29._0$
		0.209	8950	-0.14_8	$31._3$
		0.568	9690	-0.18_2	$31._8$
		0.699	8270	-0.13_1	$31._6$

$\Delta M(t)/\Delta t$: the magnitude of the slope of the plot $M(t)$ against t
M: the molecular weight of sample

TABLE 3. THE VALUES OF $\Delta M_{(app)}(t)/\Delta t$, AND $M_{(app)}$ FOR NON-IDEAL SYSTEM

Sample	Solvent and Temp.	Initial concn. (g/dl)	Rotor speed (rpm)	$\Delta M_{(app)}(t)/\Delta t$ $\times 10^{-4}$ (min^{-1})	$M_{(app)}$ $\times 10^{-4}$
PαMST-18 (monodisperse)	Toluene 30.0°C	0.202	7060	$+0.12_9$	$33._0$
		0.311	8270	$+0.02_6$	$26._8$
		0.408	10490	-0.05_3	$21._3$
		0.515	10490	-0.04_8	$19._1$
		0.614	10490	-0.02_7	$17._0$
PST-NBS705 (monodisperse)	Toluene 30.0°C	0.480	10490	$+0.04_4$	$12._9$
		0.648	11350	$+0.02_8$	$11._4$
		0.821	13310	$+0.01_3$	$10._3$
PST-SM1 (polydisperse)	Butanone 25.0°C	0.101	10490	-0.15_0	$26._2$
		0.338	10490	-0.11_9	$22._2$
		0.515	10490	-0.13_0	$17._8$
		0.649	10490	-0.09_4	$14._4$
		1.084	10490	-0.06_2	$12._2$
	Toluene 30.0°C	0.346	10490	-0.02_4	$23._5$
		0.446	11350	-0.02_7	$21._8$
		0.487	11350	-0.02_5	$19._9$
		0.711	13310	-0.03_8	$15._8$

$\Delta M_{(app)}(t)/\Delta t$: the magnitude of the slope of the plot $M_{(app)}(t)$ against t
$M_{(app)}$: the apparent molecular weight of the solute depending on the initial concentration

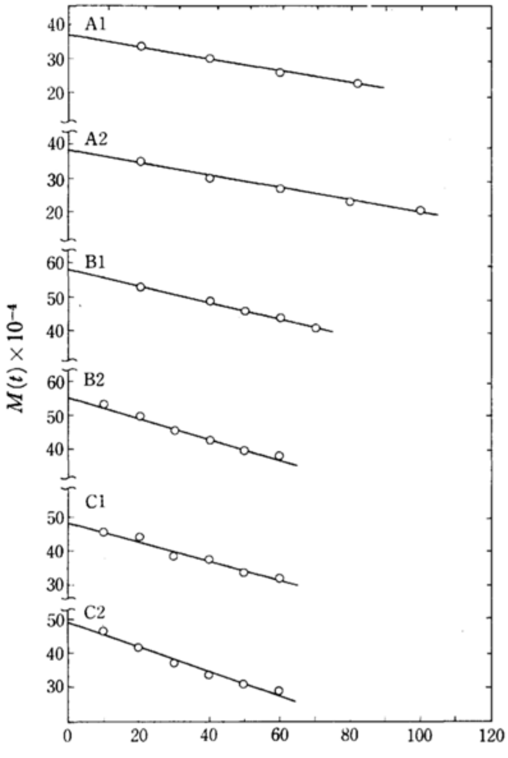

Fig. 1. Plots of $M(t)$ against t for PαMST in cyclohexane at 38°C.

PαMST-17: (A1) $C_0=0.211$ g/dl
rotor speed=7640 rpm
(A2) $C_0=0.661$ g/dl, 8270 rpm
PαMST-18: (B1) $C_0=0.203$ g/dl, 7060 rpm
(B2) $C_0=0.667$ g/dl, 7060 rpm
PαMST-mixt: (C1) $C_0=0.224$ g/dl, 7060 rpm
(C2) $C_0=0.601$ g/dl, 7060 rpm
$M(t)$: Molecular weight of the solute at each centrifugal time t
C_0: Initial concentration of the solute

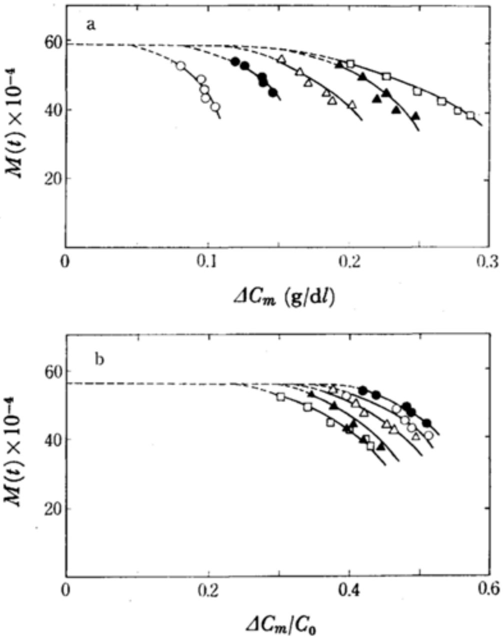

Fig. 2. (a) Plots of $M(t)$ against ΔC_m for PαMST-18 in cyclohexane at 38°C.
(b) Plots of $M(t)$ against $\Delta C_m/C_0$ for PαMST-18 in cyclohexane at 38°C.

(○) $C_0=0.203$ g/dl (●) $C_0=0.286$ g/dl
(△) $C_0=0.406$ g/dl (▲) $C_0=0.557$ g/dl
(□) $C_0=0.667$ g/dl
The measurements were carried out at 7060 rpm.
$M(t)$: Molecular weight of the solute at each centrifugal time t
ΔC_m: Amount of the solute sedimented from the meniscus
C_0: Initial concentration of the solute

magnitude of the slope in the plot of $M(t)$ against t. A typical plot of $M(t)$ against t is shown in Fig. 1 for three samples of PαMST in the theta solvent.

The data for ideal systems in Table 2 and Fig. 1 show that: (1) the values of $M(t)$ vary linearly with the time (though it has no theoretical basis), and (2) the plots have a negative slope.

The measured value of the molecular weight, at the meniscus, of a monodisperse solute in an ideal solution should be independent of the centrifugal time. When the time dependence of $M(t)$ observed in the sample of an ideal system is supposed to be caused by the sedimentational fractionation of the solute molecules at the meniscus, the more heterogeneous the solute is, the more negative the time dependence to be expected for $M(t)$. In the present study, it is consistent with the above expectation that $M(t)$ for PαMST-mixt in the theta solvent shows a negative time dependence, and that the magnitude of its time dependence is larger than that of either PαMST-17 or PαMST-18 in the theta solvent. A distinct negative time dependence of PαMST-17 and PαMST-18 in an ideal solution, whose molecular-weight distribution is fairly narrow, suggests the strong sensitivity of this method to sample heterogeneity. The larger gradient of the plot of $M(t)$ against t for PαMST-18 in cyclohexane than that for PαMST-17 in cyclohexane is concluded on the basis of a comparison of the sedimentation patterns for the two samples. Both samples show the schlieren pattern with a leading minor peak of the second component; the area under the minor peak in PαMST-18 in cyclohexane is a little larger than that in PαMST-17 in cyclohexane.[12)]

It seems appropriate to use amounts of the solute sedimented from the meniscus, ΔC_m and $\Delta C_m/C_0$, as variables in the analysis of $M(t)$ to obtain $M(t=0)$. A typical plot of $M(t)$ against ΔC_m is shown in Fig. 2-a for PαMST-18 in the theta solvent. The plot shows a downward-curving line for a solution of a given initial concentration. When the fractionation of the solute by the sedimentation is independent of C_0, the plots of $M(t)$ against $\Delta C_m/C_0$ for the different values of C_0 will fall on a master curve because the molecular-weight distributions of the solute at the meniscus for the different concentrations may be expected to be identical for the same value of $\Delta C_m/C_0$. Figure 2-b shows a typical plot of $M(t)$ against $\Delta C_m/C_0$ for PαMST-18 in the theta solvent. This plot also gives curved lines for each C_0 value, though the lines are close to each other. It might be reasonable to assume that the fractionation of the solute at the meniscus does not always proceed regularly in the order of the molecular weight, but that the hydrodynamic interactions between the solute molecules are influenced by the initial concentration of the solute under the conditions used in this experiment.

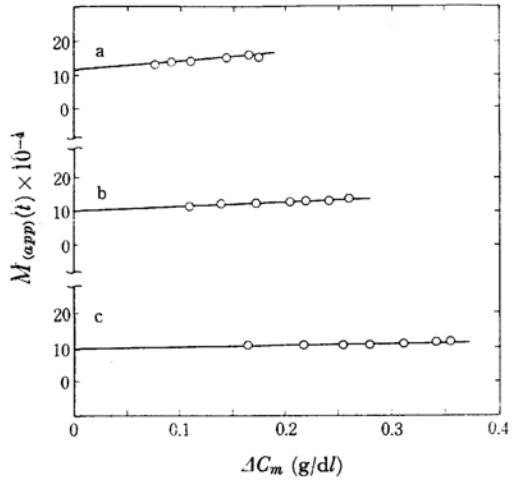

Fig. 3. Plots of $M_{(app)}(t)$ against ΔC_m for PST-NBS705 in toluene at 30°C.

(a) $C_0=0.480$ g/dl, rotor speed$=10490$ rpm
(b) $C_0=0.648$ g/dl, 11350 rpm
(c) $C_0=0.821$ g/dl, 13310 rpm
$M_{(app)}(t)$: Molecular weight of the solute at each centrifugal time t
ΔC_m: Amount of the solute sedimented from the meniscus
C_0: Initial concentration of the solute

The Results for Non-ideal Systems. For a non-ideal system the value of $M(t)$ calculated from Eq. (1) is an apparent weight-average molecular weight, $M_{(app)}(t)$, because the value depends on C_0. The results for non-ideal systems are summarized in Table 3. The gradient of the plot of $M_{(app)}(t)$ against t decreases slightly with an increase in C_0 at the same rotor speed, as is shown in the systems of PαMST-18 in toluene, PST-SM1 in butanone, and PST-SM1 in toluene. All of the plots for PST-SM1 show negative slopes; moreover, the slopes become small in magnitude in the order of the cyclohexane, the butanone, and the toluene solutions. The plot of $M_{(app)}(t)$ against t for PST-NBS705 in toluene shows a positive slope, while that for PαMST-18 in toluene shows either a positive slope or a negative one. A positive time dependence in a non-ideal system, such as PST-NBS705 in toluene and PαMST-18 in toluene, is expected from the theory of Fujita and Inagaki[2] for the sample of a sufficiently narrow molecular-weight distribution. It may be concluded from the results in this study that the time dependence of $M_{(app)}(t)$ in a non-ideal system becomes smaller with an increase in C_0. This result shows that the fractionation effect of the solute during sedimentation is superposed on the thermodynamic non-ideality of the solution.

For a monodisperse sample in a non-ideal solution, the following equation was given by Fujita and Inagaki:[2]

$$M_{(app)}(t) = M_{(app)} + M^2\{B_{11}+(\bar{v}/M)\}\Delta C_m + \text{(higher terms in } \Delta C_m) \tag{2}$$

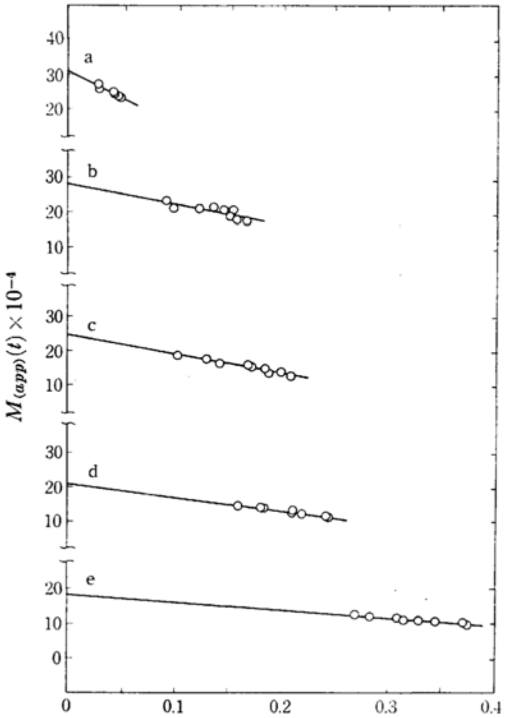

Fig. 4. Plots of $M_{(app)}(t)$against ΔC_m for PST-SM1 in butanone at 25°C.

(a) $C_0=0.101$ g/dl (b) $C_0=0.338$ g/dl
(c) $C_0=0.515$ g/dl (d) $C_0=0.649$ g/dl
(e) $C_0=1.084$ g/dl
The measurements were carried out at 10490 rpm.
$M_{(app)}(t)$: Molecular weight of the solute at each centrifugal time t
ΔC_m: Amount of the solute sedimented from the meniscus
C_0: Initial concentration of the solute

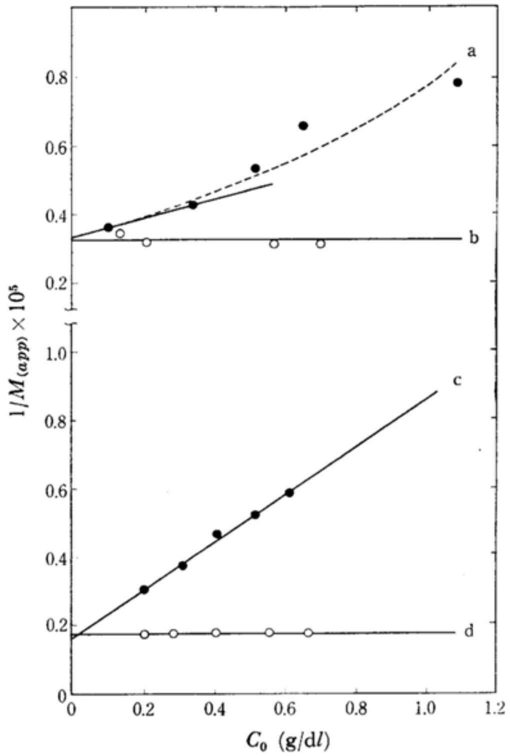

Fig. 5. Plots of $1/M_{(app)}$ against C_0.

(a): (●) PST-SM1 in butanone at 25°C
(b): (○) PST-SM1 in cyclohexane at 35°C (θ)
(c): (●) PαMST-18 in toluene at 30°C
(d): (○) PαMST-18 in cyclohexane at 38°C(θ)
The solid line in (a) is the initial tangent of the plot.
$M_{(app)}$: Apparent molecular weight of the solute
C_0: Initial concentration of the solute

TABLE 4. WEIGHT-AVERAGE MOLECULAR WEIGHT $\overline{M}_w$

Sample	Solvent Temp. (°C)	$\overline{M}_w \times 10^{-4}$ This method	$\overline{M}_w \times 10^{-4}$ Literature values
PαMST-17	Cyclohexane 38.0 (θ)	$38._5$	$40._7$(SE)[12] $40._0$(LS)[21]
PαMST-18	Cyclohexane 38.0 (θ)	$56._5$	$60._6$(LS)[21]
	Toluene 30.0	$60._6$	
PαMST-mixt	Cyclohexane 38.0 (θ)	$49._3$ $(48._6)$*	
PST-SM 1	Cyclohexane 35.0 (θ)	$30._9$	28.9 (LS)[20] 28.2 (A)[20]
	Butanone 25.0	$28._4$	27.9 (LS)[20] 28.2 (A)[20]
	Toluene 30.0	$31._7$	
PST-NBS705	Toluene 30.0	$20._7$	17.9 (LS)[22] 19.0 (SE)[22]

Asterisk (*): Calculated from $\overline{M}_w$ of PαMST-17 and PαMST-18
SE: Sedimentation equilibrium method
LS: Light scattering method
A: Archibald method

where $M_{(app)}$ is the apparent molecular weight of the solute (it is independent of time), M is the molecular weight of the solute, B_{11} is the parameter for thermodynamic interaction between the solutes, and $\bar{v}$ is the partial specific volume of the solute. Generally speaking, the value of $\varDelta C_m$ depends on time and takes a positive value. Equation (2) suggests a linear relation between $M_{(app)}(t)$ and $\varDelta C_m$ for a monodisperse solute by neglecting the higher terms. Nakazawa and Inagaki[3] reported that the plot of $M_{(app)}(t)$ against $\varDelta C_m$ has a linear relation for a monodisperse solute in a non-ideal solution, as is to be expected from Eq. (2), and concluded that the effect of the rotor speed on the plot of $M_{(app)}(t)$ against t can be eliminated by the plot of $M_{(app)}(t)$ against $\varDelta C_m$. Consequently, $M_{(app)}$ is obtained more satisfactorily when $M_{(app)}(t)$ is extrapolated to $\varDelta C_m=0$ than when it is extrapolated to $t=0$. The plot of $M_{(app)}(t)$ against $\varDelta C_m$ for PST-NBS705 in toluene is shown in Fig. 3, where it shows a positive slope and a straight line, as may be expected from Eq. (2). However, the value of B_{11} obtained from the slope depends on the initial concentration and the rotor speed; this finding suggests that the effect of the fractionation of the solute is fairly large, even on this sample with a narrow molecular-weight distribution.

The plot of $M_{(app)}(t)$ against $\varDelta C_m$ for PST-SM1 in butanone is shown as a typical example for a non-ideal polydisperse system in Fig. 4. The plot is approximately a straight line showing a negative slope. However, the observed values of $M_{(app)}(t)$ were localized in a limited range of $\varDelta C_m$, so the values of $M_{(app)}$ obtained by the extrapolation might be considered to be more ambiguous than that shown on the plot of $M_{(app)}(t)$ against t.

The weight-average molecular weight of the sample is determined from the intercept on the plot of $1/M_{(app)}$ against C_0, where $M_{(app)}$ is the value obtained by the plot of $M_{(app)}(t)$ against t. Figure 5 shows typical plots of $1/M_{(app)}$ against C_0. For an ideal system the reciprocals of M obtained by the extrapolation of the plot of $M(t)$ against t are independent of C_0 as was expected. The reciprocals of $M_{(app)}$ for a polydisperse solute in a non-ideal solution, such as PST-SM1 in butanone and PST-SM1 in toluene, show a dependence on C_0 with an upward curvature; this finding agrees with that reported by Toyoshima and Fujita.[20] On the

---

[20] Y. Toyoshima and H. Fujita, *J. Phys. Chem.*, **68**, 1378 (1964).

[21] A. Wada, Thesis, Tokyo Kyoiku University, 1964.

[22] National Bureau of Standards, Certificate.

other hand, the plot of $M_{(app)}$ against C_0 for the sample of a narrow molecular-weight distribution in a non-ideal solution shows that the contribution of a third term to the virial expansion of Eq. (2) can be neglected. The values of M_w obtained by this method are summarized in Table 4; the values agree with those in literature.

The authors wish to express their hearty thanks to Professor Mitsuru Nagasawa of Nagoya University for his kind help in supplying the samples. They also wish to express their hearty thanks to Dr. Takahide Saito of Tokyo Kyoiku University for his many helpful discussions, and to Miss Yasuko Nishibe and Miss Makiko Muraki for their assistance in the experimental work.