

The Archibald Ultracentrifugation Method in Binary Solvent Systems

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The generalized theory of the Archibald ultracentrifugation method has been extended for describing polymer-binary solvent systems. The Archibald functions are derived in terms of the osmotic virial expansion, based on two types of possible definitions of three component systems.

To demonstrate the feasibility of the Archibald method in binary solvent systems, the method has been applied to a system of polystyrene in methyl ethyl ketone and methyl chloroacetate mixture. The molecular weight obtained by this method is in good agreement with those determined by other methods.

INTRODUCTION

The Archibald method^{1,2)} is one of the most common methods for determining molecular weights with the analytical ultracentrifuges. Recent developments of the theory to polydisperse, nonideal systems have shown that the method allows one to determine the weight average molecular weights and the second virial coefficients equivalent to those obtained by the light scattering method³⁻⁵⁾. In this study an attempt has been made to extend the method for describing binary solvent systems and to find out what kind of information we could expect to obtain by the binary solvent experiments.

The use of binary solvent systems is essentially required in experiments with polyelectrolytes, *i.e.*, the measurements are usually made with polyelectrolytes in properly buffered solutions. For these cases, we have examined the applicability of the Archibald method by taking bovine serum albumin in NaCl solutions as an example⁶⁾.

Another possible merit of the use of binary solvent systems is that one could expect to extend the applicable limit of the method to a higher molecular weight region. As is well known, the Archibald method is based on the boundary condition in the ultracentrifuge cell¹⁾. The advantage of this method, which permits relatively rapid measurements, stems from the fact that the boundary condition stands valid at any time even in the early stage of centrifugation, as is opposed to the sedimentation equilibrium method²⁾. At the same time, however, the use of the boundary condition causes a disadvantage of the method, *i.e.*, an ambiguity is often involved in determining concentration gradients at the ends of the solution column.^{5,7)} This is particularly true for solutes with high sedimentation coefficients, in which the concentration gradients at the ends are usually too steep to be determined with high precision. By using a binary solvent consisting of a light

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and a heavy solvent with respect to the given polymer, the sedimentation rate should be considerably reduced (while the diffusion rate should not be much influenced) so that one would obtain workable Archibald patterns even with high molecular weight solutes.

In this paper the generalized theory of the Archibald method⁴⁾ is extended to binary solvent systems and the results are compared with those of the osmotic pressure and the light scattering methods. Also this paper demonstrates the feasibility of the Archibald method with a binary solvent system by taking a system of polystyrene in a mixture consisting of methyl ethyl ketone and methyl chloroacetate as an example.

THEORY

We consider a three component system consisting of a principal solvent (designated as component 1), a monodisperse polymer solute (component 2) and a second solvent (component 3). We also consider a reference system consisting of the complete solution less the polymer solute. In the Archibald experiment the complete solution is introduced into one side of a double sector cell and an equal amount of the reference solution into the other side of the cell. The cell is centrifuged at a constant angular velocity ω and at a constant temperature T . In the complete solution, we obtain for the change in the total chemical potential per mole of component i ($i=2, 3$), $\bar{\mu}_i = \mu_i - M_i \omega^2 r^2 / 2$, at radial distance r in the centrifugal field: ^{8,9)}

$$-d\bar{\mu}_i = M_i \omega^2 r dr - \left(\sum_{j=2}^3 \mu_{ij} dm_j + \bar{V}_i dP \right) \quad (1)$$

$$\mu_{ij} = (\partial \mu_i / \partial m_j)_{T, P, m} = \mu_{ji} \quad (2)$$

$$\bar{V}_i = (\partial V_m / \partial m_i)_{T, P, m} \quad (3)$$

where m_i is the molality (number of moles in the solution containing 1 kg of component 1); M_i and $\bar{V}_i$ are the molecular weight and the partial molal volume, respectively; V_m is the volume of the solution containing 1 kg of component 1; P is the pressure; the suffix i denotes the quantities referred to component i . The subscript m in equations (2) and (3) denotes constancy of all the m_i not specifically indicated as variable in the derivative. The flow J_i relative to the cell is given by: ¹⁰⁾

$$J_i = \sum_{j=2}^3 L_{ij} d(\bar{\mu}_j / M_j) / dr, \quad (i=2, 3) \quad (4)$$

where L_{ij} is the Onsager phenomenological coefficient as defined by Hooyman.¹⁰⁾ The use of the condition for mechanical equilibrium in the centrifuge cell, *i.e.*, $dP = \rho \omega^2 r dr$, where ρ is the local density of the solution, and the use of the boundary condition that the flow of each component is always zero at the ends of the solution column lead to: ¹¹⁾

$$\omega^2 r M_i' = \sum_{j=2}^3 \mu_{ij} (dm_j / dr) \quad (5)$$

($i=2, 3$; $r=r_a$ or r_b ; for any time t)

$$M_i' = V_m(\partial\rho/\partial m_i)_{T,P,m} = M_i(1 - \bar{v}_i\rho), \quad (6)$$

where r_a and r_b are the radial distance to the meniscus and to the bottom of the solution column, respectively; and $\bar{v}_i = \bar{V}_i/M_i$ is the partial specific volume. On the other hand, for the reference solution which contains only m_3 moles of component 3 per 1 kg of component 1, we obtain:

$$\omega^2 r M_3'^0 = \mu_{33}^0 (dm_3/dr) \quad (7)$$

($r = r_a$ or r_b ; for any time t)

Here the superscript "0" denotes the quantities referred to the reference solution. Reminding that in the ultracentrifuges in common use the concentration gradient is measured in terms of refractive index gradient:

$$d\tilde{n} = \Psi_2 dm_2 + \Psi_3 dm_3; \Psi_i = (\partial\tilde{n}/\partial m_i)_{T,P,m} \quad (8)$$

we obtain from equations (5), (6) and (8):

$$\frac{1}{\omega^2 r} \frac{d\tilde{n}}{dr} = \frac{(M_2' + M_3' \Gamma_m)(\Psi_2 + \Psi_3 \Gamma_m)}{(\mu_{22} - \mu_{33} \Gamma_m^2)} + \frac{\Psi_3 M_3'}{\mu_{33}} \quad (9)$$

for the complete solution, and also from equations (7) and (8):

$$\frac{1}{\omega^2 r} \frac{d\tilde{n}^0}{dr} = \Psi_3^0 M_3'^0 / \mu_{33}^0 \quad (10)$$

($r = r_a$ or r_b ; for any time t)

for the reference solution. Here the quantity Γ_m is the "adsorption" coefficient defined by:

$$\Gamma_m = -\mu_{23}/\mu_{33} = (\partial m_3/\partial m_2)_{T,P,\mu_3} \quad (11)$$

Now we define a quantity, which is termed as the apparent molecular weight at time t , $M_{app}(t)$, and has the dimension of molecular weight by using experimentally measurable quantities as:

$$M_{app}(t) = \frac{RT}{\omega^2 r} \frac{(d\tilde{n}/dr) - (d\tilde{n}^0/dr)}{(1 - \phi_2 \rho^0)(\tilde{n} - \tilde{n}^0)} \quad (12)$$

($r = r_a$ or r_b)

Here ϕ_2 is the apparent specific volume of component 2; and $(\tilde{n} - \tilde{n}^0)$ is the concentration of component 2 (appropriately expressed in terms of refractive index increment) at either the meniscus or the bottom of the solution column at time t . They may be calculated from a sedimentation pattern by using:

$$(\tilde{n} - \tilde{n}^0)_{r_a} = (\tilde{n} - \tilde{n}^0)_{t=0} - r_a^{-2} \int_{r_a}^{r_b} r^2 \left(\frac{d\tilde{n}}{dr} - \frac{d\tilde{n}^0}{dr} \right) dr \quad (13)$$

(for the bottom, replace r_a by r_b) r_b being an arbitrary point where the integrand is zero (see Fig. 1). And $(\tilde{n} - \tilde{n}^0)_{t=0}$ is the initial concentration of component 2 (again expressed in terms of refractive index increment) and can be calculated by multiplying the initial polymer concentration by an appropriate optical factor. The optical factor is, in turn, determined by some other independent measurements such as boundary forming experiments.^{2,5)} Then the apparent molecular weight, M_{app} , is obtained from $M_{app}(t)$ by taking the limit as $t \rightarrow 0$ (or perhaps better by $t^{1/2} \rightarrow 0$ as suggested by Yphantis¹¹⁾). Thus the quantity M_{app} should be a function of the initial concentrations of the solution. Another possible alternative for determining M_{app} is first to extrapolate the quantity $[(d\tilde{n}/dr) - (d\tilde{n}^0/dr)]$ at the meniscus or the bottom back to zero time and then multiply it by $(RT/\omega^2 r)/(1 - \phi_2 \rho^0)(\tilde{n} - \tilde{n}^0)_{t=0}$ with $r = r_a$ or r_b , respectively, thus avoiding the graphical integration involved in equation (13).

At this point, we introduce the condition of osmotic equilibrium for a three

component system. We consider a three component system (at pressure $P = \pi + P'$) at the osmotic equilibrium against a binary solvent (at pressure P' and concentration m_3') through a membrane impermeable only to the component 2. In the three component system, the equilibrium condition is:⁹⁾

$$V_m \left(\frac{\partial P}{\partial m_2} \right)_{\mu_1, \mu_3} = V_m \left(\frac{\partial \pi}{\partial m_2} \right)_{\mu_3} = m_2 \left(\frac{\partial \mu_2}{\partial m_2} \right)_{\mu_1, \mu_3} \quad (14)$$

Reminding that:

$$d\mu_i = \sum_{j=2}^3 \mu_{ij} dm_j + \bar{V}_i dP \quad (i=2, 3) \quad (15)$$

we obtain:

$$[(V_m/m_2) - (\bar{V}_2 + \bar{V}_3 \Gamma_m)] \left(\frac{\partial \pi}{\partial m_2} \right)_{\mu_3} = \mu_{22} - \mu_{33} \Gamma_m^2 \quad (16)$$

Finally from equations (9)-(16), we obtain:

$$\begin{aligned} M_{app} &= \lim_{t \rightarrow 0} M_{app}(t) \\ &= \frac{RT}{(1 - \phi_2 \rho^\circ)(n - \bar{n}^\circ)_{t=0}} \left(\frac{\partial \pi}{\partial m_2} \right)_{\mu_3}^{-1} \frac{(M_2' + M_3' \Gamma_m)(\psi_2 + \psi_3 \Gamma_m)}{(V_m/m_2) - (\bar{V}_2 + \bar{V}_3 \Gamma_m)} \end{aligned} \quad (17)$$

It should be noted that all the quantities involved in the above equation are all referred to the initial state of the solution. Upon deriving the above equation (17), we assumed that the last term in equation (9) and equation (10) cancels with each other under the condition $m_2 \ll m_3$ and $t \rightarrow 0$.

Equation (17) can be transformed from the molality to the more conventional c -scale (polymer concentration in gram per milliliter of the solution) by using the relations:

$$m_2/V_m = c_2/M_2 \quad (18)$$

$$V_m = (10^3 + m_2 M_2 + m_3 M_3)/\rho \quad (19)$$

and also the osmotic virial expansion:

$$\pi/RTc_2 = 1/M_2 + A_2 c_2 + \dots \quad (20)$$

We further expand the initial concentration of component 2 as:

$$(n - n^\circ)_{t=0} = (\partial n / \partial c_2)^\circ_{m_3} c_2 \quad (21)$$

and we notice that the bouyancy factor is expressed as:

$$(1 - \phi_2 \rho^\circ) = (\partial \rho / \partial c_2)^\circ_{m_3} \quad (22)$$

From equations (17)-(22) and after some calculations, we finally obtain:

$$\frac{1}{M_{app}} = \frac{1}{(1 + \alpha^\circ \Gamma_w)(1 + \beta^\circ \Gamma_w)} \left[\frac{1}{M_2} + \left(2A_2 + \frac{\bar{V}_3^2 RT}{\mu_{33} V_m^\circ M_2^2} + \frac{2\bar{V}_3 \mu_{23}}{M_2^2 \mu_{33}} \right) c_2 + \dots \right] \quad (23)$$

$$\alpha^\circ = \frac{(\partial n / \partial c_3)^\circ_{m_2}}{(\partial n / \partial c_2)^\circ_{m_3}}; \quad \beta^\circ = \frac{(\partial \rho / \partial c_3)^\circ_{m_3}}{(\partial \rho / \partial c_2)^\circ_{m_3}} \quad (24)$$

$$\Gamma_w = (\partial c_3 / \partial c_2)^\circ_{\mu_3} \quad (25)$$

In the above equations the quantities with the superscript "0" denote the values in the limit $c_2 = 0$. The correction term to A_2 is negligible except when M_2 is very small or A_2 is made to vanish (osmotic θ -condition). The plot of M_{app} (or $1/M_{app}$) versus c_2 yields as the limit $c_2 = 0$:

$$\lim_{c_2 \rightarrow 0} M_{app} = M_2 (1 + \alpha^\circ \Gamma_w) (1 + \beta^\circ \Gamma_w) \quad (26)$$

The result is equivalent to that of the light scattering method^{9,12)} except the

correction factor, *i.e.*, in the latter, the factor is $(1 + \alpha^\circ \Gamma_w)^2$. To determine the correct value of M_2 , we need to know the adsorption coefficient by some other measurements. However, as pointed out by Eisenberg,^{9,13)} by employing a different definition of components we can avoid this difficulty.

Such a choice in the definition of components in three component systems is to take a three component system equilibrated through exhaustive dialysis against a given binary solvent.¹³⁾ The procedure is quite commonly employed for protein solutions. In such cases, the density and refractive index differences are measured between the equilibrated three component solution and the binary solvent. They lead $(\partial\rho/\partial c_2)^\circ_{\mu_3}$ and $(\partial\tilde{n}/\partial c_2)^\circ_{\mu_3}$ instead of $(\partial\rho/\partial c_2)^\circ_{m_3}$ and $(\partial\tilde{n}/\partial c_2)^\circ_{m_3}$, respectively. When these conventions are employed, the factor $(1 - \phi_2\rho^\circ)(\tilde{n} - \tilde{n}^\circ)_{t=0}$ in equations (12) and (17) can be replaced by :

$$(\partial\rho/\partial c_2)^\circ_{\mu_3}(\partial\tilde{n}/\partial c_2)^\circ_{\mu_3}c_2$$

The new definition of the apparent molecular weight, which is denoted by M_{app}^* , takes a simpler form :

$$\frac{1}{M_{app}^*} = \frac{1}{M_2} + (2A_2 + \frac{\bar{V}_3^2 RT}{\mu_{33}^\circ V_m^\circ M_2^2})c_2 + \dots \quad (27)$$

In this case, the molecular weight M_2 can be directly obtained without any knowledge of the adsorption coefficient Γ_w , as suggested by Eisenberg.^{9,13)}

It should be noted that in the above derivations, the polymer solute was assumed as monodisperse. For a homologous but polydisperse (in molecular weights) solute with $(\partial\tilde{n}/\partial c)$ and $(\partial\rho/\partial c)$ being independent of molecular weights, it can be shown that M_{app} versus c , c being the total polymer concentration, yields the weight average molecular weight, M_w , *i.e.*, the M_2 in equations (23), (26) and (27) is M_w . These equations would be useful in describing synthetic polyelectrolytes in buffered solutions.

EXPERIMENTAL

Materials

The system investigated was polystyrene in a methyl ethyl ketone and methyl chloroacetate mixture (0.456 : 0.544 by weight, respectively) at 30°C. For the sake of comparison, solutions of the same polymer in methyl ethyl ketone (MEK) and in methyl chloroacetate (MCA) both at 30°C were also examined. The solvents were fractionally distilled for two or three time, whenever necessary, just before use. The purity of MCA was examined by gas chromatographic analysis.

The polystyrene sample was one of the fractions prepared previously in our laboratory¹⁴⁾ by thermal bulk polymerization at a conversion less than 10 % and subsequently fractionated with benzene and *n*-butanol at 30°C. The weight average molecular weight was 6.58×10^5 as determined by the light scattering measurement in MEK at 20°C.

Density and bouyancy factor determination

Density measurements of the solvent mixtures with various compositions were made with several Welde type pycnometers (each has the volume about 20 ml in a thermostated bath at $30 \pm 0.01^\circ\text{C}$). Table 1 lists the density as a function of solvent composition. Densities of polymer solutions were measured in the similar way.

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Table 1. Density of methyl ethyl ketone and methyl chloroacetate mixture at 30 deg. C.

MCA CONTENT (wt%)	DENSITY (g/ml)
0.000	0.7942
6.241	0.8138
11.44	0.8311
15.99	0.8425
22.82	0.8668
32.33	0.8983
38.69	0.9216
42.93	0.9381
52.79	0.9781
54.40	0.9860
58.55	1.0025
63.38	1.0252
72.78	1.0700
77.97	1.0958
84.57	1.1319
87.27	1.1466
92.67	1.1778
100.0	1.2191

The bouyancy factor for the second solvent, $(1-\bar{v}_3\rho^\circ)$, was calculated from the density data by :

$$(1-\bar{v}_3\rho^\circ) = \frac{(\rho^\circ - c_3)(\partial\rho/\partial c_3)}{\rho^\circ - c_3(\partial\rho/\partial c_3)},$$

while for the bouyancy factor for the polymer, equation (22) was used.

The values of the bouyancy factor of polystyrene were 0.079_s for the mixed solvent, -0.168 for MCA and 0.274 for MEK (all at 30°C). The bouyancy factor of the second solvent was 0.203. Thus the factor in equation (24) was evaluated as $\beta^\circ=2.55$ in the binary solvent.

Refractive index increment

Refractive indices of the solvent mixtures were determined with a Shimadzu-Abbe type Refractometer Model II using sodium D line at $30\pm0.5^\circ\text{C}$. Table 2 lists the refractive index as a function of solvent composition.

Table 2. Refractive index $\tilde{n}_D^{30}$ of methyl ethyl ketone and methyl chloroacetate mixture.

MCA CONTENT (wt%)	REFRACTIVE INDEX ($\tilde{n}_D^{30}$)
0.000	1.374
44.06	1.395
54.40	1.398
61.06	1.402
74.91	1.408
100.00	1.417

The refractive increments of polystyrene solutions in three solvents were determined directly with the ultracentrifuge by synthetic boundary cell experiments.²⁾ The specific increments, $(\partial\tilde{n}/\partial c_2)^\circ$, of polystyrene (in unit ml/g) were 0.235 in MEK, 0.157 in MCA and 0.189 in the mixed solvent. The factor in equation (24) was evaluated as $\alpha^\circ=0.185$ by using the specific increment in the mixed solvent and the data in Table 2.

Ultracentrifugation

A "Spinco" Model E analytical ultracentrifuge equipped with a RTIC temperature controlling unit was used. Schlieren optics was employed using a phase plate and a phase angle 80° . The centrifuge cells used were a single sector (Kel-F) cell and a double sector (Epon-filled) cell for Archibald runs and a double sector synthetic boundary cell for synthetic boundary runs. The Archibald runs were performed at a speed 15,220 rpm in MCA and in the mixed solvent and at speeds less than 10,000 rpm in MEK. The synthetic boundary runs were made at a speed 31,410 rpm. All the measurements were made at $30\pm0.1^\circ\text{C}$.

Schlieren photographs were taken at times from start up to about 60 min. The patterns were read on a Nikon Profile Projector Model V-16 (Nihon Kogaku). From the patterns, values of $(d\tilde{n}/dr)$ were evaluated by multiplying and optical factor of the ultracentrifuge.^{2,5)}

RESULTS AND DISCUSSION

Fig. 1 shows typical examples of Archibald patterns obtained with polystyrene in the mixed solvent (above) and in MCA (below). In the mixed solvent polysty-

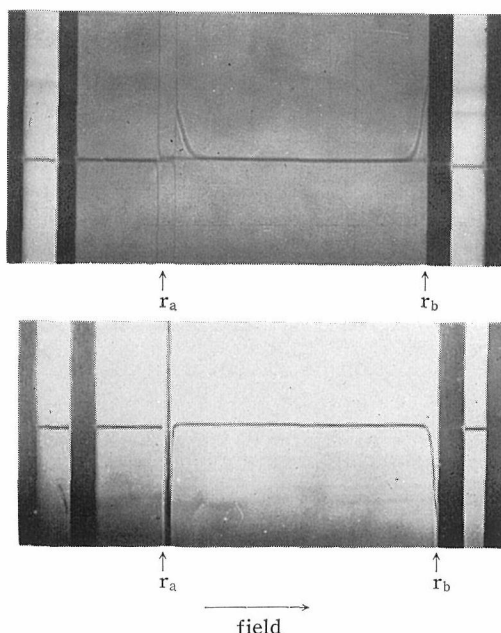


Fig. 1 Schlieren photographs of typical Archibald runs.

- Above : polystyrene in MEK-MCA (0.456 : 0.544) mixture at 30°C .
 polymer conc.=0.593g/dl ; speed=15,220 rpm ; time=55min.
 Below : polystyrene in MCA at 30° .
 polymer conc.=0.488g/dl ; speed=15,220 rpm ; time=50min.

rene slowly sediments toward the bottom of the cell. In MCA the polymer floats toward the meniscus rather than sediments toward the bottom, so that the accumulation of solute at the meniscus leads to forming a thick line and this makes it difficult to evaluate the correct value of $(d\tilde{n}/dr)$. On the other hand, the pattern at the bottom appears to be fairly good, and we use the bottom data for later analysis. However, since the shadow of the cell bottom obscures the detail of the pattern, it is not certain that the pattern gives the correct reading of $(d\tilde{n}/dr)$ value at the bottom. In the two experiments shown in Fig. 1, which were carried out under almost same conditions (concentration, speed, centrifugation time etc.), the pattern for the mixed solvent system is seen to be less steeper and easier to be analyzed than that for MCA system. This suggests that in the mixed solvent system the solutes with much higher molecular weights can be examined by the Archibald analysis.

As is well known, an error involved in calculating $M_{app}(t)$ is resulted mostly from an ambiguity in evaluating $(d\tilde{n}/dr)$ at either ends of the solution column. Direct reading of the values from a schlieren picture can be safely done perhaps as close as 0.02 cm apart from the ends. The values at the ends, therefore, must be obtained by some sort of extrapolation procedure.^{1,4,5)} Among the various procedures proposed so far,⁵⁾ the linear extrapolation of $(d\tilde{n}/dr)$ versus r plot near the ends seems to be most promising.^{7,15,16)} For monodisperse, ideal solutes, it has been shown both theoretically and experimentally that the extrapolation should be essentially linear for appropriate combinations of centrifugation time, speed and molecular weights. LaBar has worked out a criterion for the linear extrapolation.⁷⁾ While for polydisperse, nonideal solutes, there is no theoretical justification. However, it has been demonstrated by Weston and Billmeyer¹⁶⁾ that even in such cases the linear extrapolation leads to molecular weights in good agreement with those obtained by light scattering. For these reasons we also have adopted the linear extrapolation procedure for evaluating $M_{app}(t)$, and any data which did not have a linear extrapolation region near the ends have been discarded. The values of $M_{app}(t)$ thus obtained are plotted against $t^{1/2}$ and extrapolated back to zero time and M_{app} is determined.

The values of M_{app}^{-1} are plotted as a function of polymer concentration for three systems in Fig. 2. In all these cases, we find rather high upward concavity

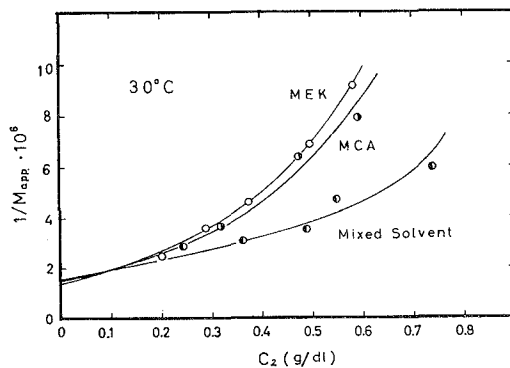


Fig. 2 Plots of $1/M_{app}$ versus c_2 in MEK, in MCA and in the mixed solvent.

in the plots. This makes it difficult to obtain high precision in the extrapolation to zero concentration. To avoid this difficulty, the data are replotted in Fig. 3 according to a semi-empirical procedure proposed by Inagaki,¹⁷⁾ which consists of plotting $-\log M_{app}$ versus c_2 .

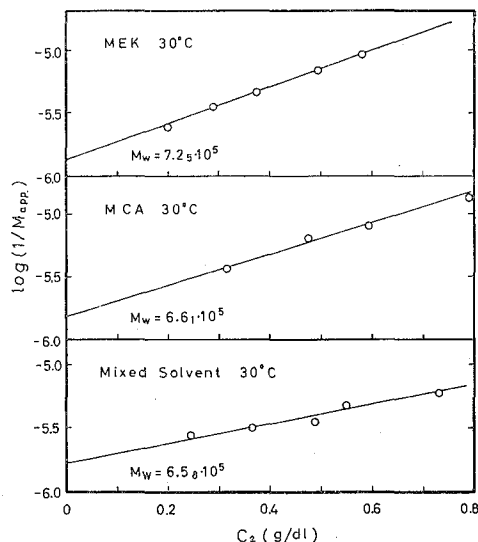


Fig. 3. Plots of $\log(1/M_{app})$ versus c_2 in MEK, in MCA and in the mixed solvent.

In Fig. 3, the plots respectively extrapolate to the values of molecular weights of 7.25×10^5 in MEK, 6.61×10^5 in MCA and 6.58×10^5 in the mixed solvent, which are in good agreement with the value from light scattering, 6.58×10^5 . The discrepancies in the values obtained in three systems seem to be due to an experimental error. The factor Γ_w in the MEK-MCA mixture seems to be negligible. We anticipate that further refinement of the experimental procedures will lead to more detailed information on the factor Γ_w .

From the plots in Fig. 3, the second virial coefficients (in cgs) are determined as 2.24×10^{-4} in MEK, 1.71×10^{-4} in MCA and 1.52×10^{-4} in the mixed solvent, which are compared with a value of 1.52×10^{-4} obtained by light scattering in MEK at 20°C. The values obtained by the Archibald method seem to be somewhat higher than they should be. Particularly, in view of the fact that the light scattering A_2 has been known to vanish at 27.5°C in MCA,¹⁸⁾ the present result of high A_2 value must be an artifact in the Archibald analysis and the agreement in molecular weight in this system seems to be fortuitous. This must be due to an ambiguity in evaluating $(d\tilde{n}/dr)$ at the bottom of the cell, which are apparently underestimated. The error may be larger in systems with higher concentrations. The slightly higher values in A_2 and the appearance of gross curvature in the plots of $1/M_{app}$ versus c_2 in the other two systems might also be due to the same reason. Further refinement of the ultracentrifuge techniques, for example, the reduction in the centrifugation speed, will lead to better values of A_2 .

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