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Measurement of Hydrodynamic Sizes of Ultralarge Water-Soluble Polymers by Nucleopore Filtration and Multicell Equilibrium Dialysis

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ABSTRACT

Membrane filtration and equilibrium dialysis using Nucleopore membranes have been carried out to determine the hydrodynamic sizes of large water-soluble polymers. The macromolecules were found to have been significantly deformed even under very low stress. Multicell equilibrium was somewhat long. The hydrodynamic radius obtained from this method was found to be in reasonable agreement with that found by ultracentrifugation and size exclusion chromatography.

INTRODUCTION

The hydrodynamic size of a water-soluble high polymer in aqueous solution has been shown to be directly related to the rheological properties of the polymer solution [1-3] and is an important parameter in the study of the mobility control behavior of the polymer solution. In order to understand the rheological behavior of a polymer in a particular aqueous solution, it is necessary to determine the hydrodynamic size of the polymer, which may be done by conventional

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methods such as light scattering, ultracentrifugation (UC), and size exclusion chromatography (SEC). When the polymer has a very large size, as in the case of polymers used in enhanced oil recovery and drag reduction, application of these methods in the size measurement has certain disadvantages. A light-scattering experiment will not give the whole size distribution and will be subject to interference by dust particles in the sample solution. The use of SEC is limited by the availability of packings with large enough pores. In addition, since mechanical degradation of high molecular weight polymers in high efficiency SEC columns (such as μ-Bondagel and Aquapore) has been observed by us and other research groups, packings of larger particle size have to be used. The fact that columns with large-pore packings normally have lower separation efficiency also makes the SEC analysis of ultralarge polymers difficult. The hydrodynamic size of a polymer can also be measured from its sedimentation velocity together with its intrinsic viscosity data [4]. However, the measurement of sedimentation velocity requires very accurate measurement of the specific volume and the use of an analytical ultracentrifuge, which is fairly expensive and not readily available to many of the research groups involved in enhanced oil recovery and drag reduction studies. Therefore, it was necessary to develop a method by which to measure the size and the size distribution of a high polymer under specified conditions. In this paper we describe a method designed to measure the hydrodynamic size of macromolecules in solution by membrane filtration and multicell equilibrium dialysis (MCED) experiments.

Membranes have been used to separate polymer molecules by their size [5], but quantitative size analysis by membrane filtration has not yet been achieved. Although many commercially available membranes have the range of selective permeability required to fractionate macromolecules in solution, they have to meet additional requirements if they are to be used in size determination. The desired membranes must 1) have well-defined pore geometry (the pore opening is a good measure of the pore size), 2) have their pore sizes well-characterized, 3) have a narrow pore size distribution, and 4) not cause significant loss of polymer sample in the substrate. Nucleopore membranes, prepared by a two-stage "track-etch" process [6], have straight-through cylindrical pores and appear to meet all the above requirements. In this work, Nucleopore membranes of nominal pore diameters of from 0.015 to 8 μm were used in the size determination. The Nucleopore filtration experiment involves the study of the retention characteristics of polymer samples which are filtered through Nucleopore membranes of various pore sizes. This experiment yields information on the equivalent hydrodynamic size of the macromolecules under certain shear stress. The effect of pressure on polymer retention has been studied on a polyacrylamide sample. In addition, the hydrodynamic size of this polymer sample at zero shear stress has been obtained from a MCED experiment. In this case the only driving force for the transport of the polymer across the membrane is the concentration gradient. Equilibrium dialysis has been extensively applied in the study of the

binding of low molecular weight ligands since it was first introduced by Davis [7] and Klotz [8]. In this experiment, molecules which are smaller than the pores of the membranes are able to diffuse back and forth across the membrane, and the concentration of such molecules on both sides of the membrane should be equal under equilibrium conditions. In an equilibrium dialysis of a polymer sample, this fact allows us to determine the size distribution of the sample from the concentration distribution at equilibrium.

The hydrodynamic size of polymer samples determined by membrane filtration and MCED are expressed in terms of the mean pore diameters of the Nucleopore membranes determined by electron microscopy. They are compared with hydrodynamic sizes determined by UC and SEC in order to examine the correlation between results obtained by this method and by conventional methods.

EXPERIMENTAL

Materials

One of the polyacrylamide samples tested was obtained from Polysciences, and the other was synthesized in our laboratory at 30°C with the use of $K_2S_2O_4$ as the initiator. Partially hydrolyzed polyacrylamide ($\overline{M}_w \approx 7 \times 10^6$) was obtained from Dow Chemical Co. and was used as received. The starch-g-acrylamide copolymer was synthesized by grafting acrylamide onto the water-soluble starch (Pfaltz & Bauer) at 30°C with the use of ceric nitrate as the initiator. The monomer was obtained from Matheson, Coleman & Bell and was recrystallized from benzene before use. Polymer solution samples were filtered through either a 12-μNucleopore membrane or a 5-μMillipore membrane prior to filtration or dialysis.

Membranes

Nucleopore polycarbonate membranes (47 mm in diameter) with nominal pore diameters of 0.015 to 8 μm were obtained from Nucleopore Co, and were soaked in fresh, deionized water for approximately 2 h before use. The pore size distribution was relatively narrow ($\leq \pm 10\%$) according to the specifications of the manufacturer (Table 1). Nominal thickness ranged from 5.4 to 12.0 μm, and nominal pore density ranged from 2×10^7 to 6×10^8 pores/cm^2. The two-stage "track-etch" process, developed by General Electric Co., made it possible to create straight-through cylindrical pores of which the openings were characterized by electron microscopy.

TABLE 1. Standard Nucleopore Membrane Specifications [6]

Specified pore size (μm)	Pore size range (μm)	Nominal thickness (μm)	Nominal pore density (pores/cm^2)	Fraction of[a] pore surface
8.0	6.9-8.0	8.0	1×10^5	.201
5.0	4.3-5.0	8.6	4×10^5	.314
3.0	2.5-3.0	11.0	2×10^6	.565
1.0	0.8-1.0	11.5	2×10^7	.627
0.8	0.64-0.80	11.6	3×10^7	.602
0.6	0.48-0.60	11.6	3×10^7	.339
0.4	0.32-0.40	11.6	1×10^8	.502
0.2	0.16-0.20	12.0	3×10^8	.376
0.1	0.08-0.10	5.3	3×10^8	.094
0.08	0.064-0.080	5.4	3×10^8	.060
0.05	0.040-0.050	5.4	6×10^8	.047
0.03	0.024-0.030	5.4	6×10^8	.017

[a]Calculated from pore size and density.

Methods of Membrane Filtration

The filtration was carried out under a constant pressure head with the use of membranes of various pore sizes. The reason for carrying out the filtration under constant pressure was to keep the change in shear stress and shear rate at a minimum when membranes of different pores are used. A constant flow-rate filtration would have caused drastic changes in shear rate when the pore size of the membrane is changed, because the shear rate at constant flow-rate is inversely proportional to the third power of the pore radius, while the number of pores per unit area does not show such a substantial change. For flow-through capillaries,

$$\dot{\gamma}_{wall} \propto \frac{4(Q/n)}{\pi R^3} \tag{1}$$

where $\dot{\gamma}_{wall}$ = shear rate at wall, Q = flow rate, n = total number of pores in the cross area of the membrane, and R = radius of the pore

opening. It was also found that a constant flow-rate filtration led to a substantial increase in pressure drop across the filter, even at a flow rate as low as 0.2 mL/min when membranes of small diameter were used without a prefilter.

In the study of the effect of shear stress on the retention of the polymer, two different apparatus were used to generate pressure heads ranging from 16 to 97,570 Pa (Fig. 1). The apparatus shown in Figure 1(A) was used in the pressure range of 7,500 to 97,570 Pa. The pressure of the nitrogen line was adjusted with valve 1 so that nitrogen gas bubbled slowly through the mercury column, and the pressure exerted on the polymer solution within the reservoir was controlled by the depth of immersion in mercury. The pressure forced the polymer solution to flow toward the stainless-steel filter assembly in which a Nucleopore membrane was supported by a stainless-steel screen. The whole filter assembly was dried under vacuum to remove the moisture before the filtration. The setting of valve 1 was kept constant in the series of experiments, and the line pressure was measured with a 15-psi pressure gauge. The filtrate was collected with a graduated cylinder which measured to 0.2 mL. The graduated cylinder was capped with a septum, and a 22-gauge needle was inserted to maintain atmospheric pressure inside the cylinder. This set-up allowed long-term filtration to be carried out without a significant change in the concentration. The volume of filtrate collected was 4 mL in the study of pressure effect, and was kept at 1 mL in the size-distribution measurements. The concentration of polymer in the filtrate was determined from its refractive index using a R-401 differential refractometer detector connected to a Waters GPC system. Concentration of the polymer sample solution varied from 0.004 to 0.76 g/dL, depending on the specific viscosity of the sample ($C < 0.7/[\eta]$). The concentrations used were considerably lower than the critical concentration estimated on the basis of Einstein's law for hard spheres ($C^* = 2.5/[\eta]$).

Method of Multicell Equilibrium Dialysis

Teflon cells (2 in. diameter, 1/4 in. thick) were used. Nucleopore membranes of pore sizes from 0.15 to 1.0 μm were placed in between two cells and were sealed with O-rings. The cell volume was 2 mL, and the dialyzing volume of the solution was 1.5 mL in all of the dialysis runs. The cell assembly (Fig. 2) was rotating at 20 rpm in a constant temperature bath (25°C). The cell volume, dialyzing volume, cell thickness, and rotating speed were designed to maintain a maximum concentration gradient across the membranes during the dialysis. The polymer solution was charged into the Nth cell, while all the other cells contained the same amount of solvent. A 0.2-M Na_2SO_4 aqueous solvent was used to eliminate the effect of charges on the polymer molecules (Donnan effect) [9, 10]. The concentrations of polymer in

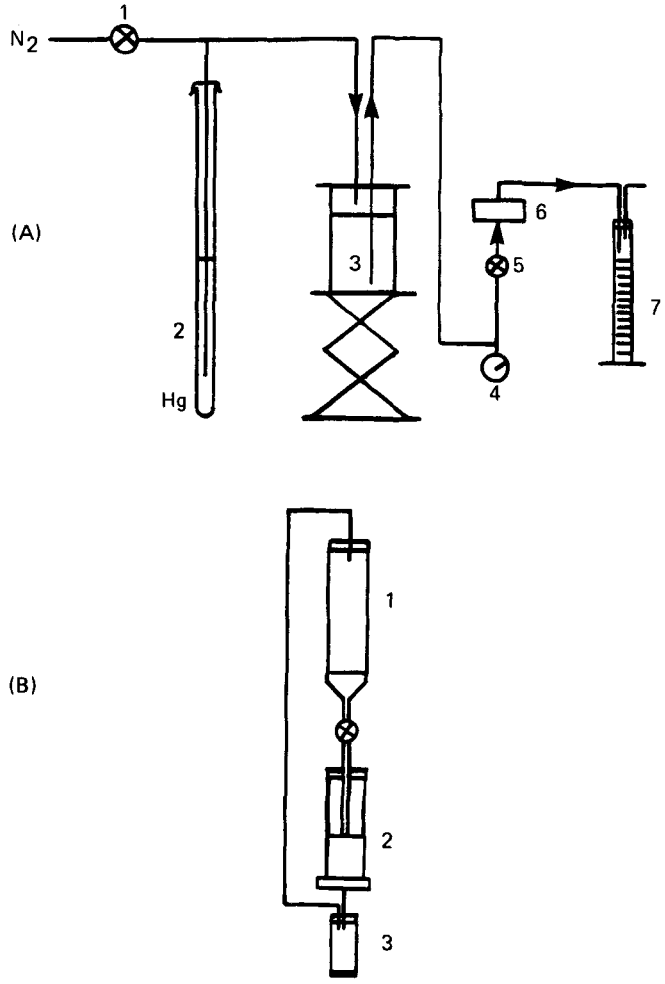

FIG. 1. Apparatus for constant-pressure filtration experiment. (A): 1, 5 = valves; 2 = mercury tube; 3 = polymer solution reservoir; 4 = pressure gauge; 6 = stainless steel filter assembly; 7 = filtrate collector. (B): 1 = polymer solution reservoir; 2 = sample solution holder; 3 = filtrate collector.

each cell after a certain dialyzing time were determined by monitoring the UV absorption at 214 nm where polyacrylamide absorption is fairly strong. The sample solution was passed through a Glyceryl CPG 75 Å column to separate any low molecular weight impurity from the polymer, so that the height of the polymer peak was directly proportional to the polymer concentration.

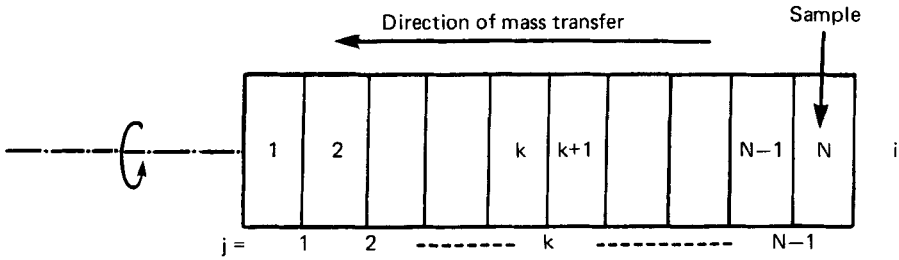

FIG. 2. A schematic picture of multicell equilibrium dialyzer.

When equilibrium was reached, the concentration or weight fraction of molecules of size smaller than the pore opening of the k-th membrane was determined by

$$W_k = [C_k(N - k + 1) + \sum_{i=1}^{k-1} C_i] / \sum_{i=1}^{N} C_i \qquad (2)$$

and the weight fraction of molecules of size R, where $R_{k-1} < R < R_k$, was simply

$$W_{k-1,k} = W_k - W_{k-1} \qquad (3)$$

where C_i = concentration of the i-th cell, W_k = weight fraction of molecules of size $< R_k$, $W_{k-1,k}$ = weight fraction of molecules of size $R_{k-1} < R < R_k$, and R_j = mean pore radius of the j-th membrane.

Equation (2) yields a cumulative size distribution curve, while Eq. (3) yields a size distribution curve of the polyacrylamide analyzed. The fraction in the k-th cell was considered to have an average hydrodynamic radius $R_\eta = (R_{k-1} + R_k)/2$. In this experiment the hydrodynamic radius, R_η, was so defined that it was equivalent to the radius of a hard sphere with the same retention characteristics.

Ultracentrifugation

The hydrodynamic sizes of the starch-g-acrylamide copolymer and the polyacrylamide were also determined by a sedimentation method using a Beckman Spinco Model-E ultracentrifuge. Sedimentation velocity of the polymer in 0.2 M KCl solution was determined at a speed of 44,770 rpm at 20°C. The molecular weight of the graft copolymer was determined from the sedimentation velocity (extrapo-

lated to infinite dilution), S_o, and $[\eta]$ at 20°C using the Mandelkern and Flory equation [11]:

$$M = \left[\frac{S_o[\eta]_{20}{}^{1/3}\eta_s N}{\phi(1 - \bar{\nu}_2\rho)}\right]^{3/2} \tag{4}$$

where η_s = solvent viscosity at 20°C, $\bar{\nu}_2$ = specific volume of the solute, ρ = density of the solution, $\phi = 2.5 \times 10^6$, a constant, and N = Avogadro number.

Intrinsic viscosities were measured with a Cannon-Fenske viscometer in deionized water and 0.2 M KCl at 20°C and in 0.2 M Na_2SO_4 aqueous solutions at 25°C.

The equivalent hydrodynamic radius of a high polymer (R_η) can be calculated from its $[\eta]$ and molecular weight by [4]

$$[\eta]M = \frac{10\pi N}{3}(R_\eta{}^3) \tag{5}$$

where R_η is the radius of a hard sphere which has the same intrinsic viscosity as the impermeable, flexible macromolecule, and is related to the radius of gyration, $\langle S^2\rangle^{\frac{1}{2}}$, by [2, 12]

$$R_\eta = 0.87\langle S^2\rangle^{\frac{1}{2}} \tag{6}$$

Size Exclusion Chromatography

The hydrodynamic radius of a starch-g-acrylamide copolymer sample (GS-28) was also measured by SEC. SEC is sufficient to yield a weight-average molecular size, even for a heterogeneous graft copolymer, if a calibration in terms of hydrodynamic volume is established.

A Waters Model 6000A GPC, equipped with a R401 refractive index detector and a Perkin-Elmer L75 variable wavelength UV-VIS detector, was used in this study. The packing material used was Glyceryl Controlled Porous Glass (GCPG) from Electro-Nucleonics, with an average particle size of ~50 μm, and a combination of four dry-packed, 90-cm columns (700/1400, 2000 + 2 × 3000 Å) was used. The mobile phase, 0.2 M sodium sulfate aqueous solution, was filtered through a 0.22-μMillipore membrane under vacuum before use. The absolute concentration of the two-component copolymer at varied retention vol-

ume was determined from the signals of the two detectors (refractive index and UV at 214 nm), with a correction for the dead volume between the detectors.

The calibration was done using polystyrene standards (Waters Associates and Pressure Chemicals, 2000 to 2×10^2 MW) in THF. The polystyrene standards had M_w/M_n ratios of <1.1, and the THF was HPLC grade. The use of these standards was necessary because reliable water-soluble standards which would cover the desired separation range were not readily available. The universal calibration curve thus obtained would be useful in aqueous SEC only if the pore size of the packing material would not alter in these two solvents. This had been demonstrated earlier for the GCPG columns where the total permeation and total exclusion volumes were found to be the same in THF and sodium sulfate aqueous solvent.

The band broadening and peak assymetry effects were corrected using the method described by Yau et al. [13], and all the calculations were done using a computer program (GPC S3). The band broadening and peak skew factors were determined from the elution peak of Schardinger-β-dextrin using the moment method.

RESULTS AND DISCUSSION

Hydrodynamic Size Determined by Membrane Filtration

Hydrodynamic sizes equivalent to the mean pore size of Nucleopore membranes for a nonionic starch-g-polyacrylamide copolymer and for the Dow Pusher 700 in deionized water were determined from the results of membrane filtrations carried out under varied pressure. The cumulative distribution curves are shown in Fig. 3. The filtration experiment was run under very low pressure, and the size of the filtrate collected was kept small to minimize the concentration polarization effect [6]. The concentration of the solution sample was kept as low as possible to minimize the interaction among macromolecules and also to minimize the formation of a solute cake. The hydrodynamic radius obtained from this method, together with values obtained from the UC experiment, are listed in Table 2. The hydrodynamic size of the graft copolymer (GS-28) determined from this method is lower than that obtained from sedimentation velocity and intrinsic viscosity data. This was explained as being due to the fact that the macromolecules were deformed by the stress exerted during the filtration, which was also apparent from the study of the pressure effect on retention.

The size of Dow Pusher 700, a polyelectrolyte in deionized water, was also estimated from its membrane filtration result and is reported here. Since a very dilute sample solution (0.004 g/dL) was used in this case, the concentration determination was subjected to larger error,

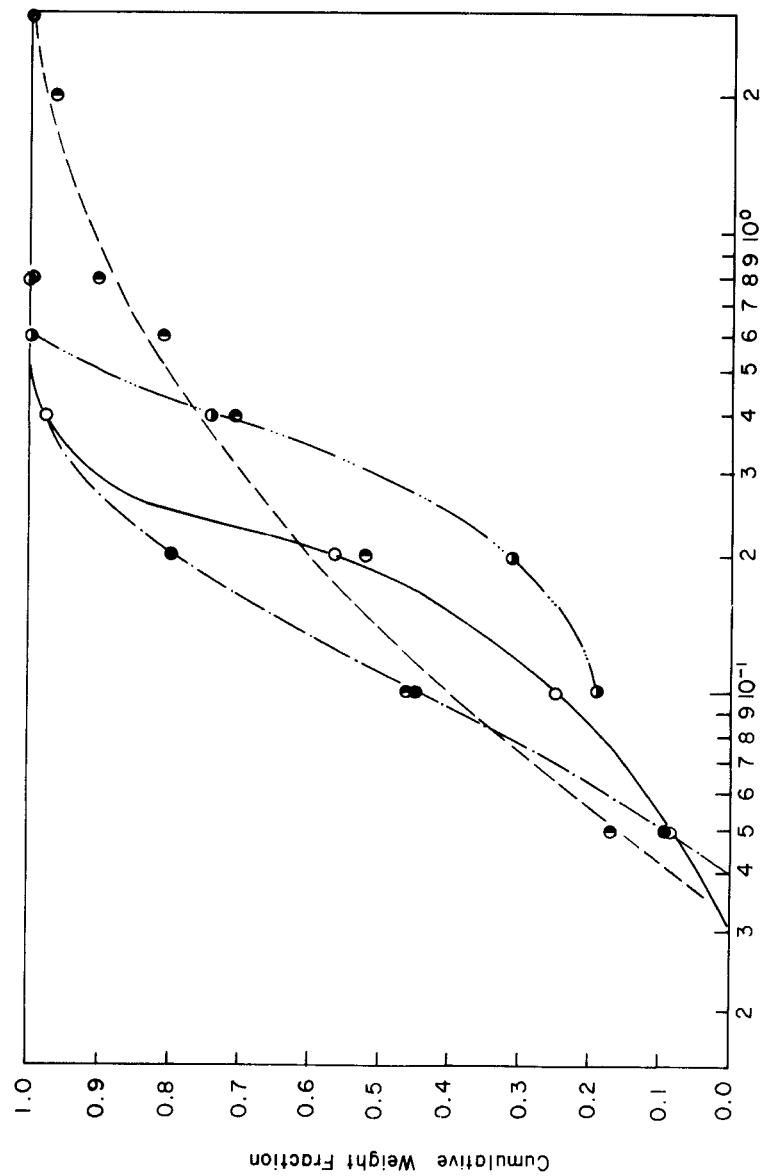

FIG. 3. Nucleopore membrane retentions of ST-g-PAM copolymer 28 [η] = 16.2 gL/g) and Dow Pusher 700 in water, varied pore sizes. Concentration: GS-28, 0.068 g/dL; Dow Pusher 700, 0.004 g/dL; filtrate size, 1 mL; (○) GS-28, 15.9 Pa pressure; (●) GS-28, 63.6 Pa; (◑) Pusher 700, 15.9 Pa; (◕) Pusher 700, 63.6 Pa.

TABLE 2
(A) Hydrodynamic Radius in Water by Nucleopore Filtration (R_{NF})

	Molecular weight $\times 10^6$	R_{NF}[a] (Å)	R_{SV}[b] (Å)
GS-28	6.92	956	1069
Dow Pusher 700	~7.0	1717	-

(B) Hydrodynamic Radius in 0.2 M Na_2SO_4 by Equilibrium Dialysis (R_{ED})

	Molecular weight $\times 10^6$	R_{ED} (Å)	R_{SV} (Å)	R_{SEC} (Å)
Polyacrylamide	6.88[e] (7.99)[f]	1232	1128	-
GS-28	6.92	1076	1069	1139

[a]Under a pressure head of 63.6 Pa.
[b]In 0.2 M Na_2SO_4 at 25°C.
[c]M_{SV} from S_o, $[\eta]$ at 20°C.
[d]Source: Dow Chemical Co.
[e]M_{SD} from $S_o = 8.17 \times 10^{-15}\ M^{0.31}$
[f]Calculated from R_{ED}, and k and a values from Ref. 14.

and the data were more scattered (Fig. 3). The value obtained is also suspected to be low because the highly extended macromolecules might have been easily deformed under the stress applied (63.6 Pa).

Effect of Pressure on Membrane Retention

Figure 4 demonstrates the effect of pressure applied on the fraction of polyacrylamide passed through a Nucleopore membrane of a 0.1-μm nominal pore diameter. The effect of pressure on the retention characteristics was found to be substantial even in the range of very low pressure. The curve did not level off in the lowest pressure range achieved, as was anticipated, and an extrapolation to zero pressure could not be accurately accomplished. This observation suggests that some other method is needed for the measurement of the size of an undeformed high polymer. The change of polymer retention was found to be moderate in the higher pressure range (1-13 psi). This might be an indication that the macromolecules had been deformed to such

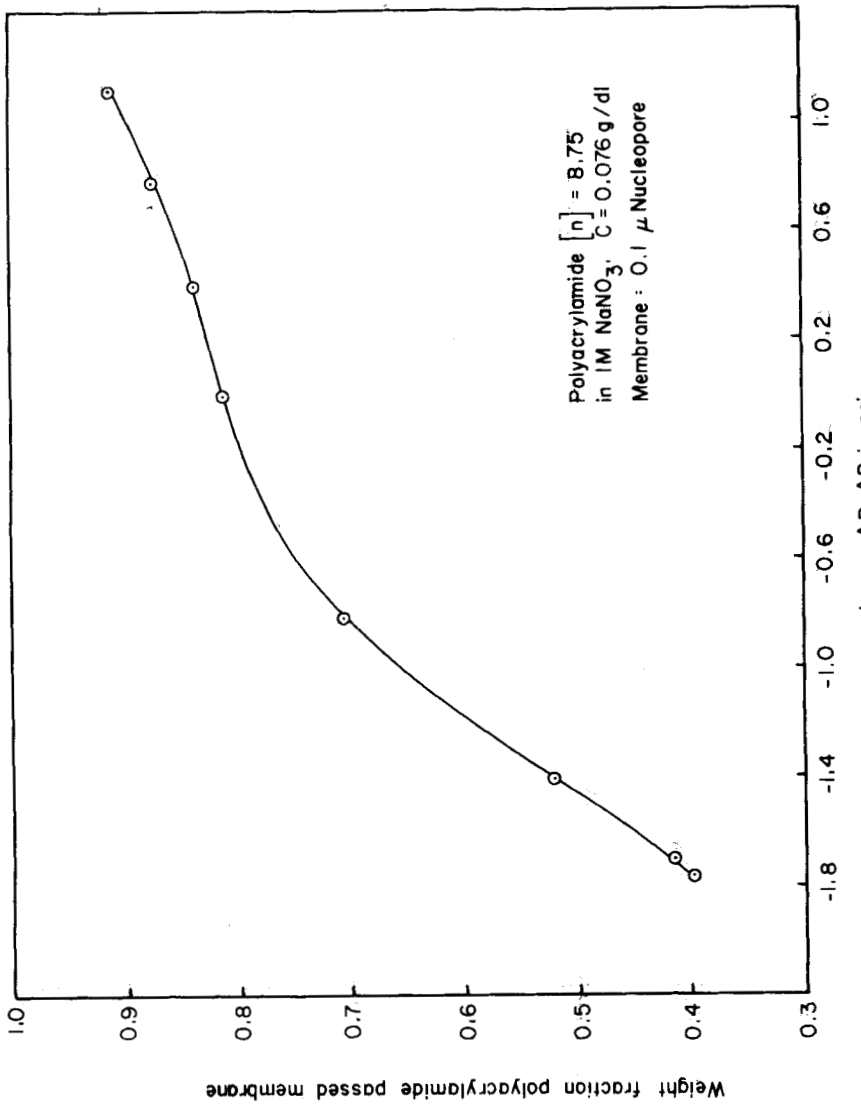

FIG. 4. Effect of pressure head on membrane retention.

an extent that additional pressure head would not deform the molecules much more, but instead would cause a portion of the molecules to become strained. However, this observation might also be a result of a concentration polarization effect which we tried to avoid during the experiment.

Although the membrane filtration experiment was not expected to give the correct hydrodynamic size of an undeformed high polymer in solution, it might still serve to yield information on the size of a high polymer under a certain amount of stress. This might eventually help in the prediction of mechanical entrapment of polymer molecules in a porous medium.

Multicell Equilibrium Dialysis

The equilibrium dialysis experiment was carried out on two high polymer samples, GS-28 ($M_{SV} = 6.92 \times 10^6$) and a nonionic polyacrylamide ($M_{SD} = 6.88 \times 10^6$). Size distribution curves of the polyacrylamide obtained at various times are shown in Fig. 5. Since the diffusion coefficient decreases with increasing molecular size, it required a fairly long period of time to establish the equilibrium for these large

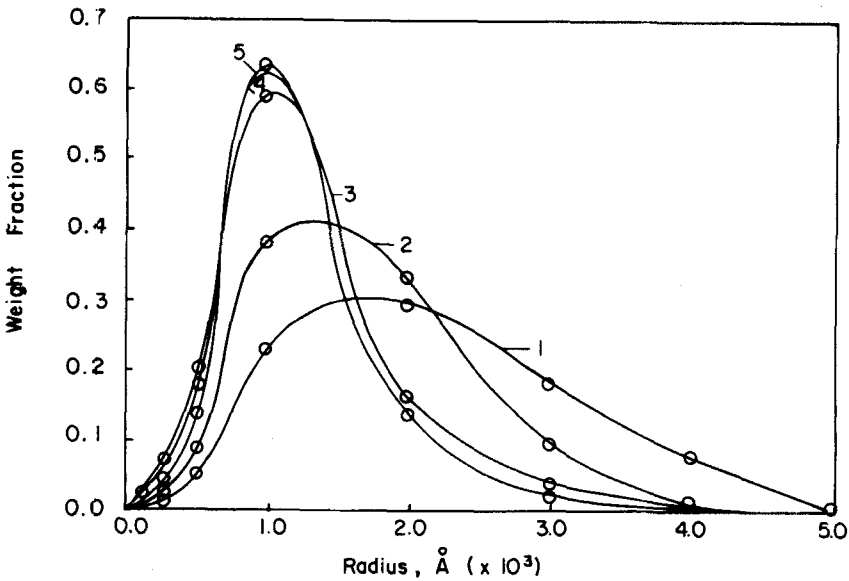

FIG. 5. Apparent size distribution curves of polyacrylamide in 0.2 M Na_2SO_4 aqueous solution at 25°C obtained at varied equilibrium times: (1) 24 h, (2) 48 h, (3) 72 h, (4) 96 h, (5) 120 h.

polymers. The average sizes were calculated in terms of the mean pore diameter listed in Table 1, and they are listed in Table 2. As was mentioned earlier, the R_η thus obtained was considered to be equivalent to the radius of a hard sphere having the same retention characteristics. This is somewhat different from the way the better-known equivalent hydrodynamic radius is defined. However, in order to compare the hydrodynamic radius obtained by membrane dialysis to that obtained by conventional methods, it was assumed that these hydrodynamic radii would relate to the radius of gyration of a flexible polymer in a similar way. The values of hydrodynamic radius obtained by this method were in good agreement with that obtained from sedimentation velocity and viscosity data and that obtained by SEC. This correlation is gratifying, even though the method itself does not require a calibration. It should be noted that the R_η of GS-28 determined by this method is larger ($\sim$11%) than that obtained from the membrane filtration experiment, probably indicating the deformation of GS-28 when filtered through the membrane under low pressure.

Good temperature control was found to be critical. A temporary temperature drop of 2° during the experiment was found to lead to a substantial change in the final concentration distribution in the cells. It was also important to use a SEC column to remove low molecular weight impurities from the polymer sample, so that the UV signal would be directly proportional to the concentration of the polymer. This is especially necessary when the establishment of equilibrium is not certain, e.g., in the study of the kinetics of the dialysis of an unknown sample. At equilibrium, the heights of total permeation peaks, which are due to either the presence of low molecular weight moiety in the cells or the difference in the salt concentration between the sample solution and the mobile phase or both, should be equal, because all these low molecular weight molecules are free to diffuse into any of the cells. Thus, the variation of the peak heights may be considered a measure of the experimental error associated with the procedure of concentration determination. Figure 6 shows a typical size exclusion chromatogram used in the concentration determination. For a sample size of 9, these peak heights were found to have an average value of 28.25 and a standard deviation of 0.26 or an estimated value of 28.25 ± 1.06% at 99% confidence interval.

Estimation of the Molecular Weight of Ultralarge Polymers

Although the resolution of this method is limited by the availability of membranes of desired pore sizes, this method may also be used to estimate the molecular weight of large polymers by relating the hydrodynamic radius (R_η) to the hydrodynamic volume, $[\eta]M$. For a polydisperse sample, Eq. (5) can be rewritten as

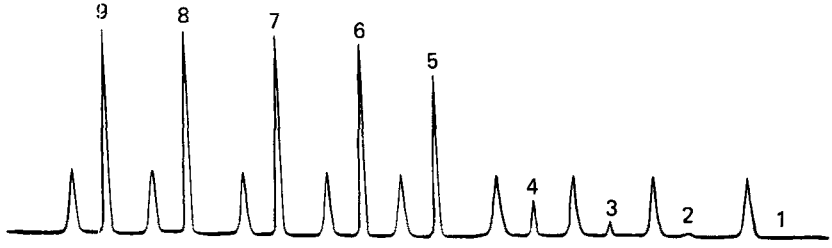

FIG. 6. A typical SEC chromatogram showing the concentration of polyacrylamide in the dialysis cells.

$$\overline{M}_w \cong \left(\frac{10N\pi}{3K}\right)^{1/1+a} \sum_i W_i (R_{\eta,i})^{3/1+a} \qquad (7)$$

where K and a are the Mark-Houwink constants, and $R_{\eta,i}$ is the hydrodynamic radius of the fraction in the i-th cell. If K and a are known for the polymer studied, molecular weight may be directly calculated from Eq. (7). If possible, Mark-Houwink constants obtained from the fractionated sample with a polydispersity close to that of the fraction in the dialysis cell should be used. With the use of Mark-Houwink constants reported by Klein and Conrad [14], the $\overline{M}_w$ of the polyacrylamide used in the dialysis study was estimated to be 7.99×10^6 (Table 2). It should be pointed out that the $\overline{M}_w$ thus obtained is only an approximation of the well-defined weight-average molecular weight, because the fractions in the cells are still polydisperse and the $R_{\eta,i}$ in Eq. (7) is an average itself. Before this method is further refined, molecular weights obtained in this way should only be considered as estimates. Mark-Houwink constants covering this molecular weight range generally will not be available in this case, and the $[\eta]$ measurement of each polymer fraction in the cell will have to be attempted in order to obtain the molecular weight distribution.

A Few Notes on the Equilibrium Dialysis Method

It should be pointed out that the results of the MCED method outlined are preliminary. Further work is clearly needed in order to establish the scope and limitations of this method. The time needed to reach equilibrium is long, but can probably be substantially re-

duced by suitable changes in cell design (larger membranes, thinner cells, etc.). The resolution may be improved, at least in principle, by a larger number of membranes of different pore size. The effect of concentration on the apparent size distribution is another factor that needs to be addressed in greater detail. Our experiments were run with an initial PAM concentration of 0.16 g/dL in the first cell, and the highest concentration at equilibrium (in the first cell) was only ~0.03 g/dL. As a result, the effect of concentration on the apparent size distribution is probably small, but this should be experimentally confirmed. An important remaining question is the size distribution in the cell enclosed by the smallest pore size membrane. The extent to which molecules with an equivalent hydrodynamic radius (R_η) larger than this pore radius are able to diffuse into this cell should be readily detectable by its size distribution.

The absence of temperature fluctuations is especially crucial in our experience. Even temporary temperature fluctuations (caused by a power blackout of short duration) rendered the experiment useless for reasons that are at present not well understood but could possibly be associated with temperature-induced expansion/contraction of the PAM molecule.

In conclusion, it appears that the MCED method shows promise for the determination of size distribution of polymers of large size. Future work designed to evaluate the method further will be attempted.

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REFERENCES

[1] P. E. Rouse Jr., J. Chem. Phys., 21, 1272 (1953).
[2] B. H. Zimm, Ibid., 24, 269 (1956).
[3] W. W. Graessley, Ibid., 43, 2696 (1965).
[4] H. Morawetz, Macromolecules in Solution, 2nd ed., Wiley-Interscience, New York, 1975.
[5] B. W. Baker, J. Appl. Polym. Sci., 13, 369 (1969).
[6] M. C. Porter, in Handbook of Separation Techniques for Chemical Engineers (P. A. Schweitzer, Ed.), McGraw-Hill, New York, 1979.
[7] B. D. Davis, J. Clin. Invest., 22, 753 (1943).
[8] I. M. Klotz, J. Am. Chem. Soc., 68, 2299 (1946).

[9] F. Karush and M. Sonenberg, Ibid., 71, 1369 (1949).
[10] H. B. Bull, An Introduction to Physical Biochemistry, 2nd ed., Davis, 1971.
[11] L. Mandelkern and P. J. Flory, J. Chem. Phys., 20, 212 (1952).
[12] R. L. Auer and C. S. Gardner, Ibid., 23, 1546 (1955).
[13] W. W. Yau, H. J. Stoklosa, C. R. Ginnard, and D. D. Bly, 12th Middle Atlantic Regional Meeting, ACS, April 5-7, 1978, Paper P013.
[14] J. Klein and K.-D. Conrad, Makromol. Chem., 181, 227 (1980).
[15] W. Scholtan, Ibid., 14, 169 (1954).

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