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Transport Phenomena in Zonal Centrifuge Rotors. IX. Gradient Properties of Ficoll and Methyl Cellulose (M-278)

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Abstract

Concentration-dependent diffusivities of Ficoll and methyl cellulose (M-278) in aqueous solution were measured by a microinterferometric method for solute concentrations ranging from 0.050 to 0.700 g solute/ml of solution for Ficoll and from 0.050 to 0.200 g solute/ml of solution for methyl cellulose. Empirical formulas for the diffusivity and an activity coefficient of each solute are presented as a function of solute concentrations.

In the application of zonal centrifugation for separation of macromolecules, viruses, bacteria, cell organelles, yeast, and mammalian cells, sucrose or cesium chloride solutions are often used as gradient solutions. However, these solutions are often not desirable for use with bioparticles that behave as osmometers. Recently there have been reported studies using Ficoll (the commercial name of a synthetic high polymer made by the copolymerization of sucrose and epichlorohydrin by Pharmacia Fine Chemical AB, Uppsala, Sweden) and methyl cellulose as gradient materials in zonal centrifugations to isolate red blood cells (*1–3*), mitochondria (*4*, *5*), peroxisomes (*6*), and lysosomes (*7*) in a more "native" state. These

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gradient materials were used because of their low osmotic characteristics, high molecular weight, and low content of dialyzable material. Because of lesser shrinkage, the cells can be collected at lower densities in gradient solutions made with these materials than in those made with sucrose or cesium chloride.

In order to use gradient solutions of Ficoll or methyl cellulose for biophysical separation or for isolations in a more native state, it is necessary to know the physical properties of the solutions, such as density, viscosity, and diffusivity, at various concentrations. For the determination of a sedimentation coefficient and a molecular weight by ultracentrifugation, it is also necessary to have information on diffusivity at the banding concentration and the activity coefficient for the thermodynamic term (*8*). In improving the separation resolution, diffusivity at various concentrations is the prime information required for evaluating the maximum loading capacity (*9*, *10*).

We have used the same microinterferometric method as reported previously (*11*) to determine the properties of two gradient materials, Ficoll and methyl cellulose (M-278).

MATERIALS

Ficoll and methyl cellulose aqueous solutions were prepared using distilled water. Methyl cellulose was supplied by Fisher Scientific Co., Fair Lawn, New Jersey in USP 15 cP grade for laboratory use.

RESULTS AND DISCUSSION

The refractive index-concentration relationships were first measured. The results are presented in Fig. 1, which shows that the refractive index is a linear function of concentration. The theory discussed in the previous paper (*11*) can therefore be used without modification.

Viscosities at various concentrations for both solutions were measured in a Hewlett-Packard Auto-Viscometer, Model 5901-B with an Ubbelohde viscometer tube made by Cannon Instrument Co. Densities at various concentrations were determined by a specific gravity digital recorder made by the Oak Ridge Gaseous Diffusion Plant. The data points obtained from both measurements are presented in Table 1. The partial specific volumes $\bar{v}_0$ of water were determined graphically by the method of intercepts from the measured densities, which are also presented in Table 1.

Binary diffusion coefficients were measured by the previously described

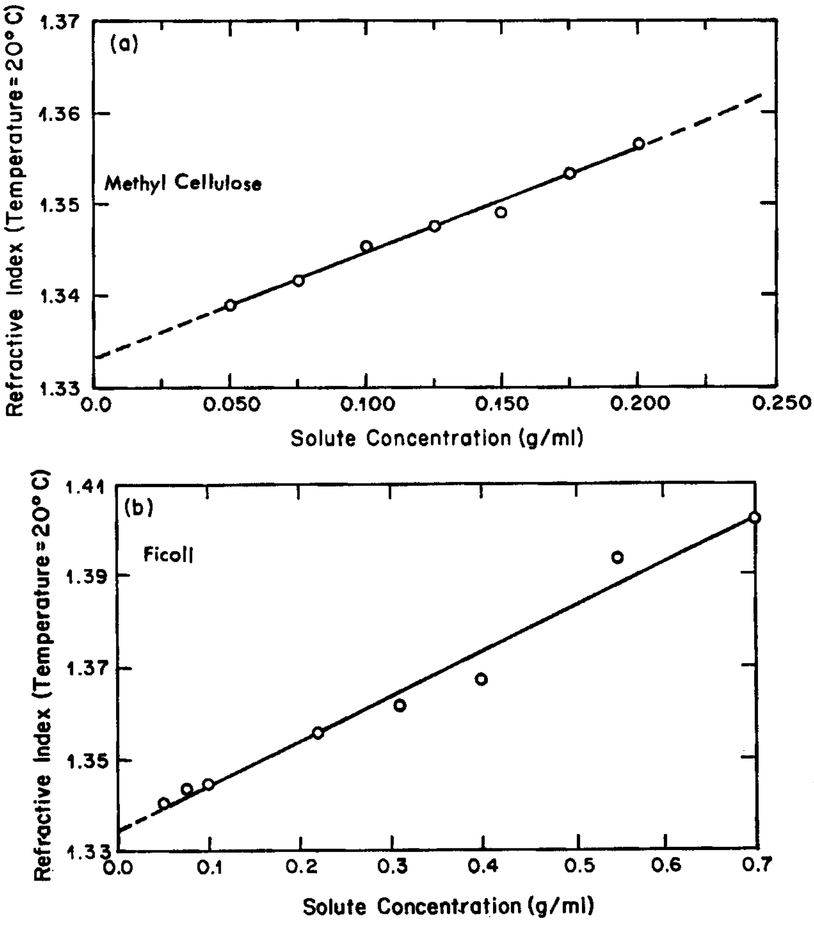

FIG. 1. Refractive index at various concentrations.

TABLE 1

Density, Viscosity, and Partial Specific Volume at 25 °C at Various Concentrations

C (g-solute/ml)	η (centipoise)	ρ (g-solution/ml)	$\bar{v}_0$ (ml/g-solution)
		Ficoll	
0.050	11.8	1.013	1.010
0.075	12.6	1.021	1.010
0.100	13.2	1.028	1.010
0.220	18.0	1.059	1.010
0.310	23.0	1.077	1.010
0.400	28.0	1.096	1.010
0.550	41.0	1.119	1.010
0.700	60.0	1.139	1.010
		Methyl Cellulose	
0.050	1.78	1.012	1.001
0.075	6.15	1.017	1.001
0.100	17.89	1.022	1.001
0.125	56.38	1.028	1.001
0.150	158.45	1.034	1.001
0.175	500.00	—	—
0.200	1,690.00	2.136	1.001

microinterferometric method by counting the total number of fringes. Solutions of five different concentrations of each material were allowed to diffuse into distilled water. Then, using the total number of fringes, the diffusivity at that concentration was obtained (*11*). They are given in Table 2. In order to evaluate the thermodynamic term, the quantities $[\eta/\eta_0\bar{v}_0\rho]$ at various concentrations were also computed from the measurements and the results are listed in Table 2. The quantity D_0 is obtained by extrapolation to an infinite dilution from a diffusivity vs concentration plot. It is found that D_0 is 1.161×10^{-6} and 0.110×10^{-6} cm²/sec for Ficoll and methyl cellulose (M-278), respectively.

The activity coefficient was obtained by integrating

$$\ln \gamma^{(c)} = \int_0^c \left[\frac{D\eta}{D_0\eta_0\bar{v}_0\rho} - 1\right] d\ln c \tag{1}$$

The quantities ρ, $\bar{v}_0$, and η are measured quantities at various concentrations, and the diffusivity is a sequence of measured values at various concentrations. Hence all the quantities are concentration dependent except D_0 and η_0. Thus, by substituting the value determined experimentally at

TABLE 2

Diffusion Coefficient and the Calculated Physical Parameters at 25°C at Various Concentrations

C (g-solute/ml)	$D \times 10^6$ (cm^2/sec)	$\eta/\eta_0\bar{v}_0\rho$	$\ln \gamma^{(c)}$
	Ficoll ($D_0 \times 10^6 = 1.161$)		
0.100	1.586	0.0702	14.44
0.220	2.415	0.0524	36.59
0.310	2.660	0.0416	51.56
0.400	2.750	0.0349	66.13
0.550	4.390	0.0242	98.54
0.700	5.620	0.0169	150.93
	Methyl Cellulose ($D_0 \times 10^6 = 0.110$)		
0.075	0.1659	0.147	0.25×10^3
0.100	0.1769	0.115	1.00×10^3
0.125	0.1858	0.162×10^{-1}	2.50×10^3
0.150	0.2140	0.058×10^{-1}	6.00×10^3
0.175	0.2886	—	—
0.200	0.3630	0.112×10^{-2}	43.75×10^3

each concentration into the integrand and performing a numerical integration to that concentration, the activity coefficient at the concentration was obtained. The results of numerical integration by an IBM 360 Model 65 are presented in Table 2 and in Fig. 2.

Empirical formulas for the diffusion coefficient obtained from measurements and the activity coefficient calculated from Eq. (1) were correlated as a function of the solute concentration:

Ficoll (at 25°C)

$$D \times 10^6 = 1.161 + 5.488C - 5.594C^2 + 9.949C^3 \tag{2}$$

(std dev = 0.195)

$$\ln \gamma^{(c)} = 81.25C + 938.00C^2 - 3581.20C^3 + 5485.94C^4 - 2703.55C^5 \tag{3}$$

(std dev = 0.680)

in weight percent of solute (W)

$$\ln \gamma^{(W)} = 17.00\left[1.00 + \frac{0.50W}{1.00 - W}\right]^{7.36} \tag{4}$$

(std dev = 1.67)

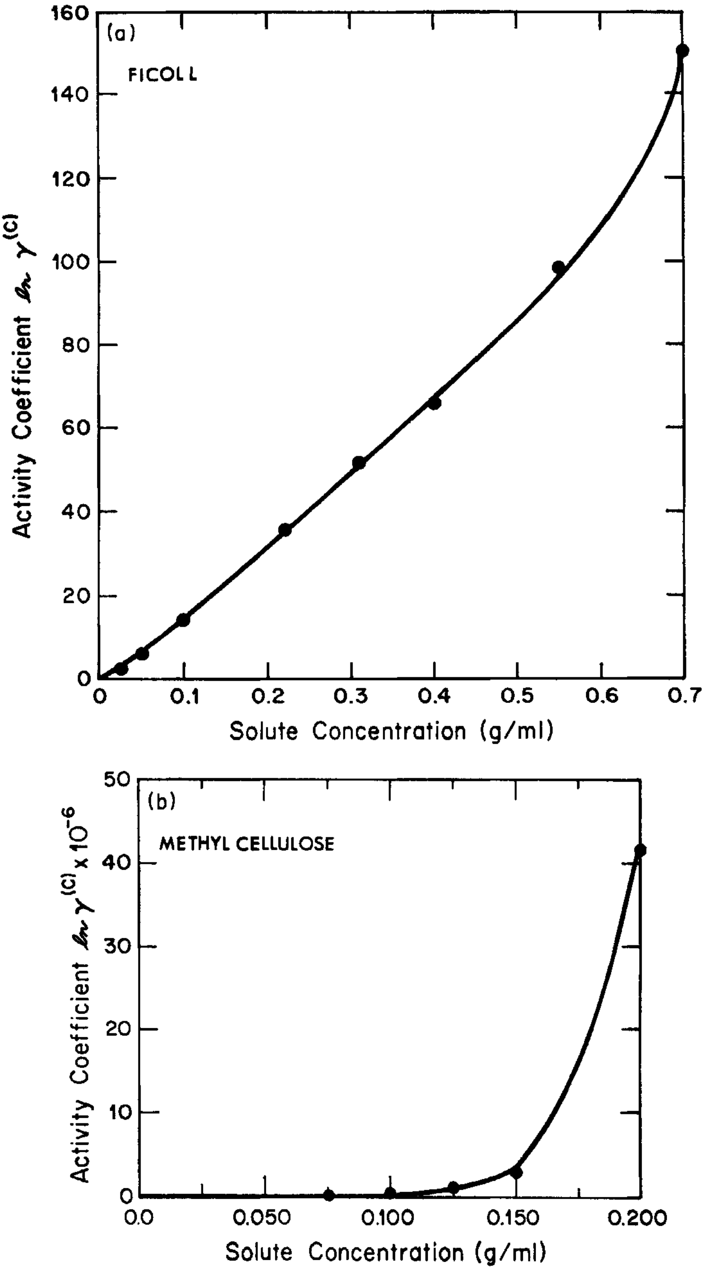

FIG. 2. A plot of activity coefficients vs solute concentrations.

Methyl cellulose (at 25°C)

$$D \times 10^6 = 0.110 + 1.715C - 19.527C^2 + 86.851C^3 \quad (5)$$
$$(\text{std dev} = 0.0124)$$

$$\ln \gamma^{(c)} = 10.75C + 168.97C^2 - 1153.33C^3 + 3015.750C^4 \quad (6)$$
$$(\text{std dev} = 0.046)$$

in weight percent of solute (W)

$$\ln \gamma^{(W)} = 0.10 + 640\left[1.00 + \frac{10.00W}{1.00 - W}\right]^{10.1} \quad (7)$$
$$(\text{std dev} = 0.597)$$

A comparison of the concentration-dependent diffusivities between experimental data points and calculated values using the polynomial and the empirical formulas of the activity coefficient is presented in Fig. 3. A good agreement exists between the polynomials and the experimental data points. The diffusivities calculated from the van Laar-type activity coefficient formula are slightly lower than the experimental data points. The thermodynamic term involves a differentiation of the activity coefficient with respect to the concentration. The van Laar-type activity coefficients may have a good fit, but they give a poorer result for a thermodynamic term than that of a polynomical form.

Band broadening is greater in a Ficoll gradient solution than it is in a methyl cellulose gradient, reflecting the fact that the diffusivity of Ficoll in water is about 10 times higher than that of methyl cellulose; however, the viscosity is less. Since the viscosity of a gradient solution determines the sedimentation rate (*12*), the $D\eta$ were computed at various concentrations for the evaluation of both solutes as a gradient material. The results are plotted in Fig. 4. The unit of $D\eta$ is the dyne, the unit for force. The quantity $D\eta$ is the force required to move a particle in the solution at that concentration. Naturally, one would prefer a lower energy for a given separation task; therefore, one would choose a lower force requirement to move a particle, and in this respect the Ficoll gradient solution is the better one.

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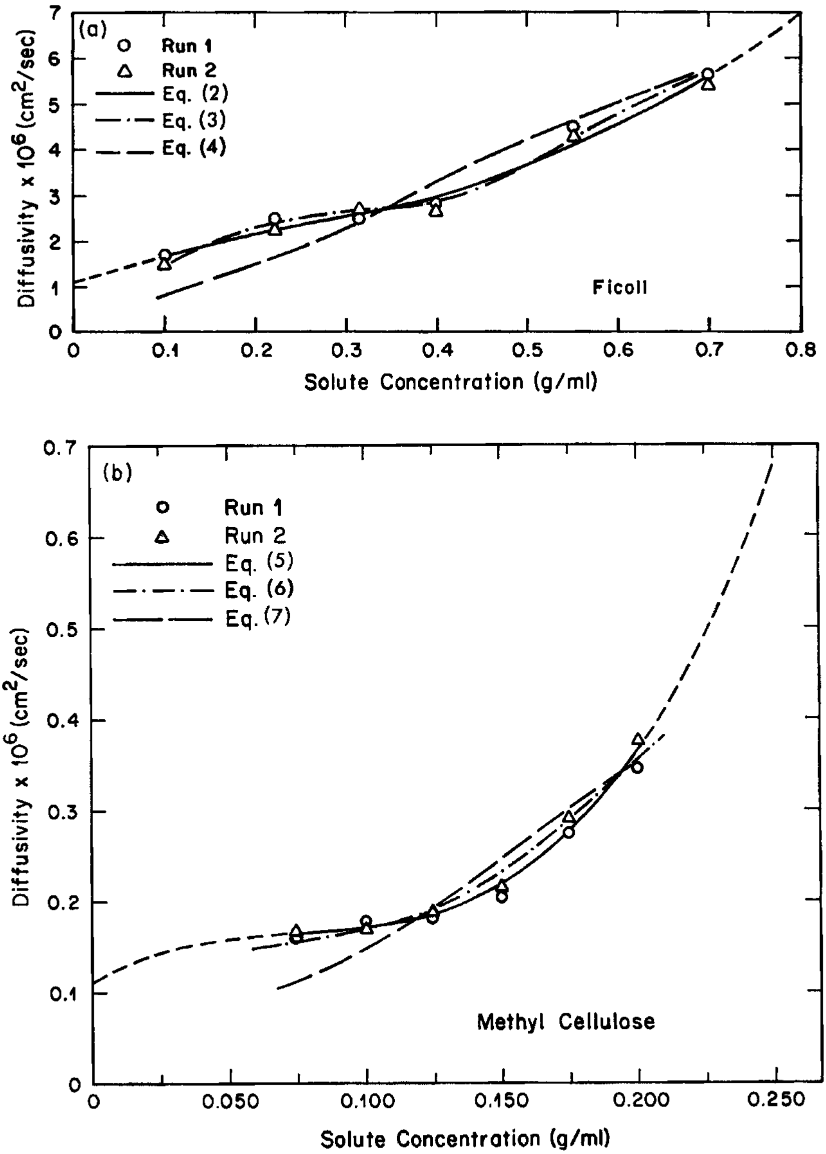

FIG. 3. Comparison of diffusivities between experimentally measured values and estimated values from empirical formulas.

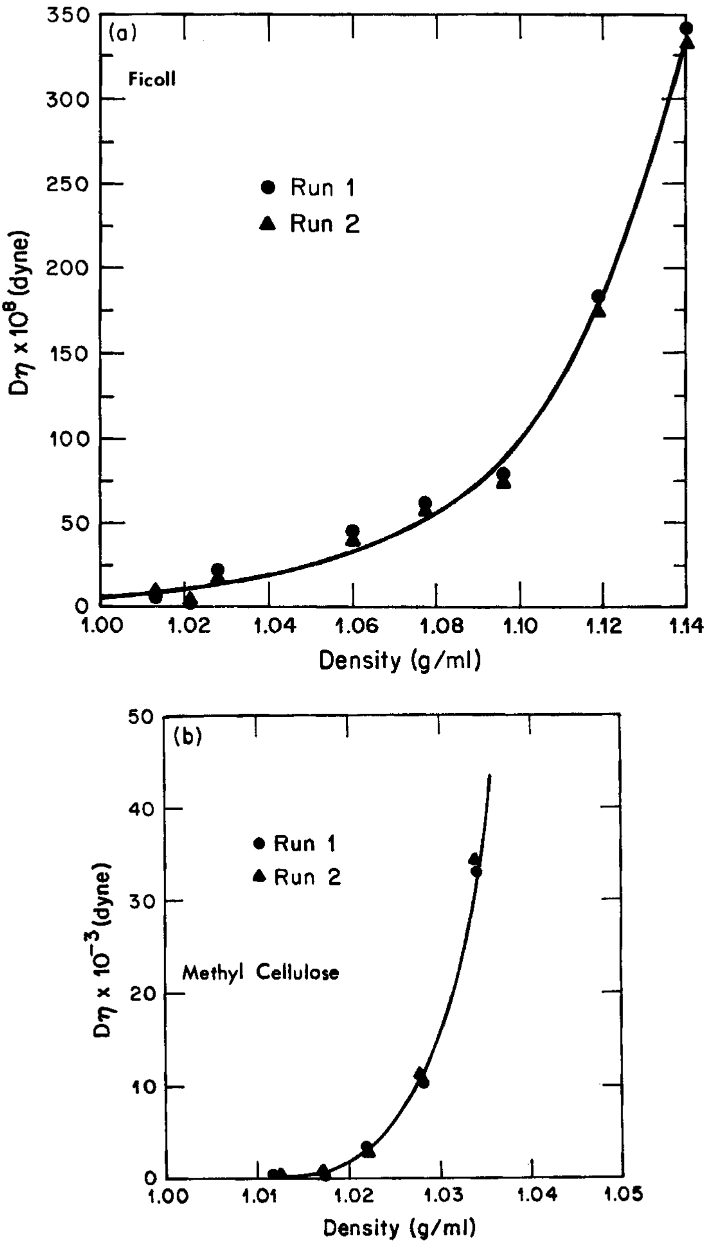

FIG. 4. The $D\eta$ vs various densities of Ficoll and methyl cellulose (M-278).

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