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Transport Phenomena in Zonal Centrifuge Rotors. XI. Gradient Properties of Sorbitol and Sucrose in Sodium Phosphate Buffer Solutions

V. S. Shah[a]; H. W. Hsu[a]
[a] DEPARTMENT OF CHEMICAL AND METALLURGICAL ENGINEERING, THE UNIVERSITY OF TENNESSEE, KNOXVILLE, TENNESSEE

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NOTE

Transport Phenomena in Zonal Centrifuge Rotors. XI. Gradient Properties of Sorbitol and Sucrose in Sodium Phosphate Buffer Solutions

V. S. SHAH and H. W. HSU*

DEPARTMENT OF CHEMICAL AND METALLURGICAL ENGINEERING
THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE 37916

Abstract

The gradient properties of sucrose and sorbitol in sodium phosphate buffer solutions were measured. Concentration-dependent diffusivities were measured by a microinterferometric method. Empirical formulas for the diffusivity and an activity coefficient of each solute in various concentrations of buffer are presented as a function of solute concentrations.

In the application of zonal centrifugation of biological materials, sucrose, cesium, chloride, Ficoll, and methyl cellulose are often used to produce the liquid density gradient solution. For virus separation or isolation, the use of potassium citrate or potassium tartrate has been suggested (*1*) because of their favorable ionization constants for virus stability. In this study we are reporting the effect of sodium phosphate buffer on the gradient solutions of sucrose and sorbitol. Sorbitol is generally used as a sweetening agent for diabetics and has properties to increase absorption of vitamins and other nutrients in pharmaceutical preparations. Therefore, it is ideal for use in the zonal centrifugation for purification of cellular materials, from *in vivo* or in *vitro*, without altering their metabolic state.

*To whom correspondence should be addressed.

We have used the same microinterferometric method and equipment as reported previously (*1*, *2*) to determine the gradient properties of sucrose and sorbitol in sodium phosphate buffer solutions.

RESULTS AND DISCUSSION

The refractive index–concentration relationships were first measured. The results are presented in Figs. 1a–1c, which show that the refractive index is a linear function of concentration. Therefore, the theory used in the previous measurements (*1*, *2*) can be used without modification. Viscosities, densities, refractive indices, and partial specific volumes of both solutes at various concentrations are listed in Table 1. Measured diffusivities of sucrose in 0.025 *M* sodium phosphate buffer and sorbitol in distilled water at 25°C are tabulated with their respective activity coefficients in Table 2. Experimental literature data points of diffusivities of sucrose-distilled water (*4*–*6*) are shown along with the correlation curves in Fig. 2. Measured diffusivities of sorbitol in four different sodium phosphate buffer solutions along with activity coefficients are presented in Table 3. The experimental data points together with estimation by the correlated formula are shown in Fig. 3.

Empirical formulas for the diffusion coefficient obtained from the measurements and the activity coefficient calculated from Eq. (1) of Ref. *2*

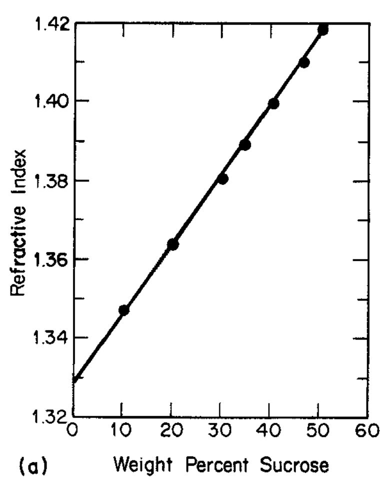

FIG. 1a. Refractive index for various sucrose concentrations.

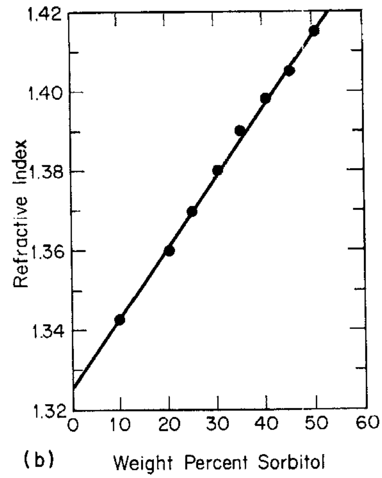

FIG. 1b. Refractive index for various sorbitol concentrations.

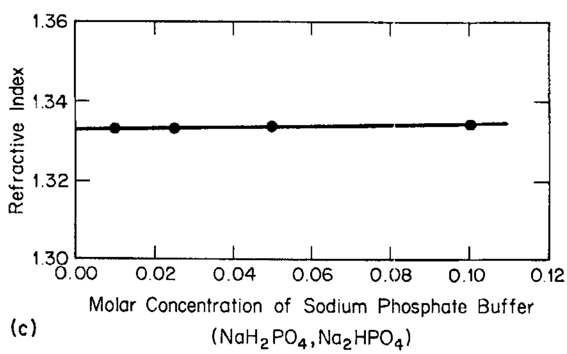

FIG. 1c. Refractive index for various sodium phosphate buffer concentrations.

TABLE 1

Physical Properties of Various Concentrations of Sucrose and Sorbitol at 25 °C

Wt of solute / Wt of solution	Density of solution (g/ml)	Solute concentration (g-solute/ml)	Partial specific volume of solvent (ml/g solution)	Viscosity (cP)	Refractive index
		Sucrose			
30.00	1.1250	0.33750	1.0000	2.7384	1.3807
34.40	1.1464	0.39435	0.9987	3.5283	1.3888
40.20	1.1755	0.47257	0.9965	5.2199	1.3996
45.40	1.2055	0.54730	0.9943	7.9508	1.4100
50.30	1.2322	0.61980	0.9919	12.7500	1.4197
		Sorbitol			
10.00	1.0250	0.1025	1.0028	1.2290	1.3471
20.00	1.0600	0.2120	1.0028	1.8363	1.3627
30.00	1.1015	0.3305	1.0028	2.9397	1.3795
40.00	1.1360	0.4544	1.0028	4.6915	1.3965
50.00	1.1705	0.5853	1.0028	9.4778	1.4160

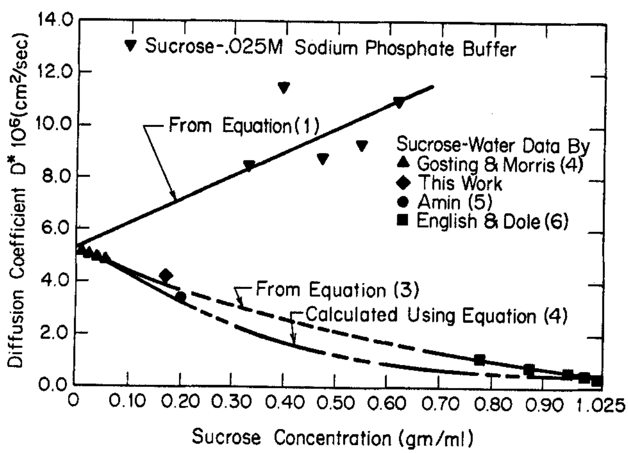

FIG. 2. Concentration-dependent diffusivities of sucrose in distilled water and in 0.25 *M* phosphate buffer solution.

TABLE 2

Diffusivities and Activity Coefficients of Sucrose and Sorbitol at 25 °C

Solute concentration (g/ml)	$\beta = \frac{\eta}{\eta_0 v_0 \rho}$	Diffusivity $D \times 10^6$ (cm²/sec)	Activity coefficient $\ln \gamma^{(c)}$
Sucrose—0.025 *M* Phosphate Buffer			
0.33750	2.72364	8.4510	0.13089
0.39435	3.44851	11.4870[a]	0.79460
0.47257	4.98604	8.7900	2.12319
0.54730	7.42225	9.3000	3.8938
0.61980	11.80776	10.9000	6.1377
Sorbitol—Distilled Water			
0.10250	1.3380	5.5240	1.3421
0.21200	1.9331	7.6250	3.8064
0.33045	2.9781	13.4760	8.2943
0.45440	4.6085	16.5270	16.1080
0.58525	9.0359	18.8460	28.9975

[a]Rejected data point using Chauvenet's criterion (*3*) when the formula was obtained. The quantities η, v, and ρ are viscosity, specific volume, and density, respectively. The subscript zero is the quantity of solvent.

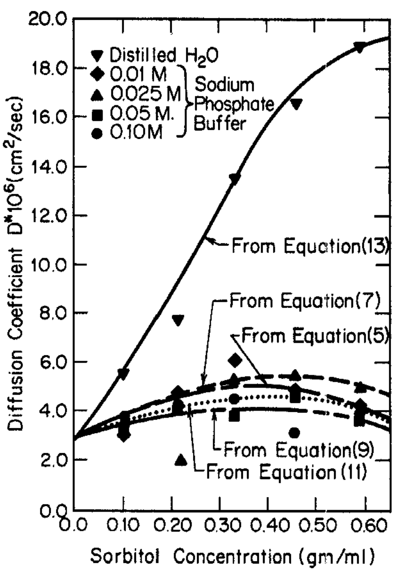

FIG. 3. Concentration-dependent diffusivities of sorbitol in distilled water and in four different concentrations of phosphate buffer solutions.

TABLE 3

Diffusion and Activity Coefficients of Sorbitol–Sodium Phosphate Buffer Systems at 25 °C

Sorbitol concentration (g/ml)	Diffusivity $D \times 10^6$ (cm²/sec)	Activity coefficient $\ln \gamma^{(c)}$	Standard deviation	Diffusivity $D \times 10^6$ (cm²/sec)	Activity coefficient $\ln \gamma^{(c)}$	Standard deviation
	Phosphate Buffer Concentration 0.010 *M*			Phosphate Buffer Concentration 0.025 *M*		
0.10250	2.9935[a]	0.7160	1.0227	3.4937	0.6490	0.2335
0.21200	4.7849	1.6115	0.0826	2.0776[a]	1.4906	1.6752
0.33045	6.1026[a]	2.9006	0.8355	5.3158	2.7624	1.0287
0.45440	4.8847	4.7551	0.2208	5.4413	4.7225	0.5936
0.58525	4.2006	7.3174	0.0908	4.9659	7.6456	0.4752
	Phosphate Buffer Concentration 0.50 *M*			Phosphate Buffer Concentration 0.10 *M*		
0.10250	3.2034	0.4609	0.2598	3.7102	0.4902	0.2454
0.21200	4.1099	1.0635	0.1618	4.1315	1.1333	0.1975
0.33045	3.8559	1.9849	0.3564	4.5025	2.1004	0.3412
0.45440	4.7031[a]	3.3993	0.5037	3.1449	3.5674	0.9423
0.58525	3.6613	5.4718	0.2030	4.0863	5.6793	0.4201

[a]Rejected data points using Chauvenet's criterion (*3*) when the correlation formula was obtained.

were correlated as a function of solute concentration at 25 °C (D in unit of cm^2/sec).

Sucrose—0.025 M *Sodium Phosphate Buffer* ($c = 0.3375$ to 0.6198 g/ml)

$$D \times 10^6 = 5.2659 + 8.2675c, \qquad \text{standard deviation} = 0.4008 \tag{1}$$

$$\ln \gamma^{(c)} = -6.2787 + 12.1543c^2 + 22.5125c^3 \tag{2}$$

Sucrose–Distilled Water ($c = 0.010$ to 1.025 g/ml)

$$D \times 10^6 = 5.2659 - 9.0681c + 6.6518c^2 - 2.4550c^3, \quad \text{standard deviation} = 0.1351 \tag{3}$$

$$\ln \gamma^{(c)} = -9.7571c + 26.7647c^2 - 25.5977c^3 + 13.0724c^4 - 3.5839c^5 \tag{4}$$

Sorbitol–0.010 M *Sodium Phosphate Buffer* ($c = 0.1025$ to 0.5853 g/ml)

$$D \times 10^6 = 2.8000 + 13.5397c - 19.7006c^2, \quad \text{standard deviation} = 0.6013 \tag{5}$$

$$\ln \gamma^{(c)} = 6.7665c + 1.1449c^2 + 6.6876c^3 + 40.6578c^4 - 47.3264c^5 \tag{6}$$

Sorbitol–0.025 M *Sodium Phosphate Buffer* ($c = 0.1025$ to 0.5853 g/ml)

$$D \times 10^6 = 2.8000 + 11.5405c - 13.1166c^2, \quad \text{standard deviation} = 0.9484 \tag{7}$$

$$\ln \gamma^{(c)} = 6.0502c + 1.6321c^2 + 8.1993c^3 + 34.6545c^4 - 31.5098c^5 \tag{8}$$

Sorbitol–0.050 M *Sodium Phosphate Buffer* ($c = 0.1025$ to 0.5853 g/ml)

$$D \times 10^6 = 2.8000 + 6.6653c - 8.9353c^2, \quad \text{standard deviation} = 0.4349 \tag{9}$$

$$\ln \gamma^{(c)} = 4.3090c + 0.6998c^2 + 9.1592c^3 + 20.0149c^4 - 21.4651c^5 \tag{10}$$

Sorbitol–0.100 M *Sodium Phosphate Buffer* ($c = 1.025$ to 0.5853 g/ml)

$$D \times 10^6 = 2.8000 + 7.5500c - 10.3814c^2, \quad \text{standard deviation} = 0.5065 \tag{11}$$

$$\ln \gamma^{(c)} = 4.6250c + 0.7463c^2 + 8.8272c^3 + 22.6718c^4 - 24.9319c^5 \tag{12}$$

Sorbitol–Distilled Water (c = 1.025 to 0.5853 g/ml)

$$D \times 10^6 = 2.8000 + 20.8788c + 64.9780c^2 - 91.9200c^3,$$
$$\text{standard deviation} = 0.7764 \qquad (13)$$

$$\ln \gamma^{(c)} = 9.3853c + 33.9806c^2 + 15.1881c^3 + 46.8682c^4 + 156.0958c^5 - 184.0151c^6 \qquad (14)$$

The experiments show that the diffusivities of sucrose in distilled water decrease with an increase in sucrose concentration and increase with an increase in sucrose concentration in a sodium phosphate buffer solution. The diffusivities of sorbitol in distilled water increase rather significantly with an increase in sorbitol concentration; however, less variation in diffusivities was observed as sorbitol concentration varies in a sodium phosphate buffer. The information may play some role in the membrane transport in the biological metabolism.

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