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NOTE

Transport Phenomena in Zonal Centrifuge Rotors. XIII. Gradient Properties of Metrizamide*

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Abstract

The gradient properties of Metrizamide in three buffer solutions (0.005 *M* phosphate buffer at pH 6.2, 0.5 *M* acetate buffer at pH 4.6, and 1.0 *M* borate buffer at pH 8.2) were measured. Concentration-dependent diffusivities were measured by a microinterferometric method over a solute concentration range from 0.1054 to 0.4862 g/m*L*. Empirical formulas for the diffusivity and activity coefficient of Metrizamide in three buffer solutions are presented as a function of solute concentration. A generalization factor was obtained for the use of Metrizamide in different buffers at different pH values.

Separation of intact cells by means of density gradient centrifugation has become one of the powerful techniques in recent years. However, application has been somewhat limited by the lack of suitable gradient materials. At the densities required for cell separation (1.05 to 1.16 g/cm^3) of most available substances, sucrose, albumin, methyl cellulose, Ficoll, etc. form solutions of undesirably high osmolality or high viscosity. In liquid density centrifugation the use of potassium citrate or potassium tartrate has been suggested (*1*) for virus isolation or purification because of their favorable ionization constants for virus stability. Sorbitol has also been suggested (*2*) as a gradient material for purification of cellular materials because of its property to increase absorption of vitamins and

*Metrizamide is a product of Nyegaard & Co., Norway, and is distributed by Gallard-Schlesinger Chemical Mfg. Corp., 584 Mineola Ave., Carle Place, New York 11514.
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other nutrients in pharmaceutical preparations. Therefore it is ideal for use in density gradient centrifugation for purification of cellular materials without altering their metabolic state either *in vivo* or *in vitro*.

Recently a new gradient material, Metrizamide [a tri-iodinated benzamido derivative of glucose with the systematic name 2-(3-acetamido-5-*N*-methyl-acetamido-2,4,6-tri-iodobenzamido)-2-deoxy-D-glucose] has been successfully utilized in the separation of nuclei (*3*) mitochondria and lysosomes (*4*) and various subcellular particles and macromolecules (*5–7*). Metrizamide gives dense solutions of low viscosity and osmolality, and would appear particularly well suited for the separation of intact cells.

We have used the same microinterferometric method and equipment as reported previously (*1, 2*) to determine the gradient properties of Metrizamide in three different pH buffer solutions: 0.005 *M* phosphate buffer at pH 6.2, 0.5 *M* acetate buffer at pH 4.6, and 1.0 *M* borate buffer at pH 8.2.

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. Therefore the theory used in the previous measurements (*1, 2*) can be used without modification.

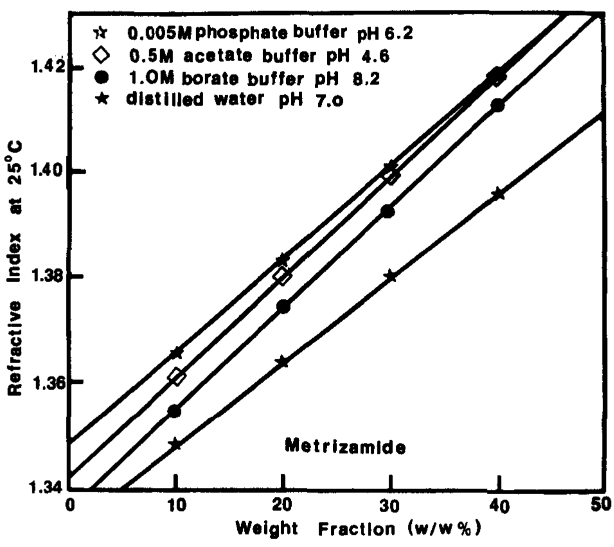

FIG. 1. The refractive index of Metrizamide in various buffer solutions as a function of concentration at 25°C.

The measured kinematic viscosity ratio [$\beta = \mu/(\mu_0 \nu_0 \rho)$], diffusivity, and their respective activity coefficients at various concentrations of Metrizamide in aqueous solution and in three different buffer solutions are listed in Table 1. Empirical formulas for the diffusivities obtained from the microinterferometric measurements and the activity coefficients calculated from the measured diffusivities with Eq. (14) of Ref. *1* were correlated as a function of Metrizamide concentration ranging from 0.1054 to 0.4862 g solute/mL solution. The van Laar forms of the activity coefficient in terms of weight percent concentration were also correlated. The equivalent concentrations for weight percent are from 10 to 40%. (D in unit of cm^2/sec).

TABLE 1

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

$\frac{\text{Wt of solute}}{\text{Wt of solution}}$	Solute concentration $\left(\frac{\text{g solute}}{\text{mL}}\right)$	$\beta = \frac{\mu_0}{\mu_0 \nu_0 \rho}$	$D \times 10^5$ (cm^2/sec)	Activity coefficient, $\ln \gamma^{(c)}$
Metrizamide–Distilled Water				
10	0.1054	1.1837	0.4180	1.8000
20	0.2213	1.4966	0.6416	4.0000
30	0.3486	2.0223	0.8512	6.9000
40	0.4862	2.7619	0.8821	10.6100
Metrizamide–0.005 M Phosphate Buffer at pH 6.2				
10	0.1054	1.1837	0.6337	3.1120
20	0.2213	1.4966	1.1427	7.3000
30	0.3486	2.0223	1.7552	13.3260
40	0.4862	2.7619	2.3069	22.2000
Metrizamide–0.5 M Acetate Buffer at pH 4.6				
10	0.1054	1.1837	0.5004	2.1000
20	0.2213	1.4966	0.7799	5.0230
31	0.3486	3.0223	1.2007	9.1480
40	0.4862	2.7619	1.3658	14.6700
Metrizamide–1.0 M Borate Buffer at pH 8.2				
10	0.1054	1.1837	0.3558	1.6990
20	0.2213	1.4966	0.6128	3.8720
30	0.3486	2.0223	1.0810	7.0490
40	0.4862	2.7619	1.0146	11.4860

Metrizamide–Distilled Water

$$D \times 10^5 = 0.1725 + 2.6412C - 4.2837C^2 + 12.3238C^3 - 17.3343C^4, \quad (SD = 0.037)$$

$$\ln \gamma^{(w)} = 16.8269C - 0.4324C^2 + 19.9460C^3 + 50.9723C^4 - 142.3057C^5 + 230.8316C^6 - 374.2156C^7 + 195.7153C^8$$

$$\ln \gamma^{(w)} = 0.1302\left(1.000 + \frac{108.931w}{1.000 - w}\right)^{1.027}$$

Metrizamide–0.005 *M* Phosphate Buffer at pH 6.2

$$D \times 10^5 = 0.1725 + 4.4325C, \quad (SD = 0.035)$$

$$\ln \gamma^{(c)} = 27.2092C + 19.8622C^2 + 11.2099C^3 + 92.4010C^4 - 80.0733C^5$$

$$\ln \gamma^{(w)} = 0.3580\left(1.000 + \frac{45.920w}{1.000 - w}\right)^{1.1945}$$

Metrizamide–0.5 *M* Acetate Buffer at pH 4.6

$$D \times 10^5 = 0.1725 + 2.7920C + 2.7850C^2 - 9.5860C^3 + 9.3180C^4 - 8.9406C^5, \quad (SD = 0.046)$$

$$\ln \gamma^{(c)} = 17.7007C + 20.7162C^2 - 1.4420C^3 + 52.1049C^4 - 4.0631C^5 - 187.3444C^6 + 234.0011C^7 - 202.3248C^8 + 89.7292C^9$$

$$\ln \gamma^{(w)} = 0.141\left(1.000 + \frac{84.330w}{1.000 - w}\right)^{1.150}$$

Metrizamide–1.0 *M* Borate Buffer at pH 8.2

$$D \times 10^5 = 0.1725 + 2.5186C - 5.6888C^2 + 27.1757C^3 - 37.6827C^4, \quad (SD = 0.085)$$

$$\ln \gamma^{(c)} = 16.1163C - 5.0435C^2 + 44.3479C^3 + 50.0149C^4 - 187.8279C^5 + 452.8310C^6 - 818.4722C^7 + 425.4621C^8$$

$$\ln \gamma^{(w)} = 0.3218\left(1.000 + \frac{24.362w}{1.000 - w}\right)^{1.256}$$

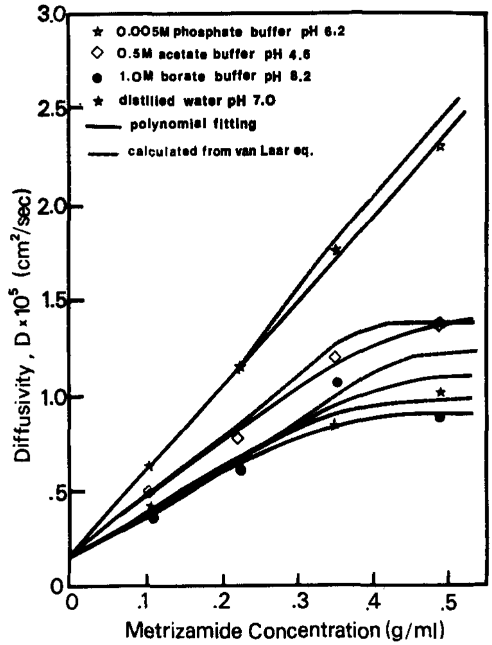

FIG. 2. Diffusion coefficients of Metrizamide in various buffer solutions as a function of concentration at 25°C.

A comparison of the concentration-dependent diffusivities between experimental data points and calculated values using the polynomial and the empirical van Laar forms of the activity coefficient is presented in Fig. 2.

The experiments show that the diffusivities of Metrizamide in various solutions increase with an increase in concentration. Diffusivity of Metrizamide is the highest in 0.005 *M* phosphate buffer at pH 6.2, in 0.5 *M* acetate buffer at pH 4.6 is the next, and in 1.0 *M* borate buffer at pH 8.2 is the lowest among the three buffer solutions. Diffusivity of Metrizamide in distilled water above 0.3 *M* is lower than that in buffer solutions. At a lower concentration range, diffusivities of Metrizamide in distilled water are slightly higher than those in 1.0 *M* borate buffer at pH 8.2; however, this may be due to experimental errors. The experiments also show that the diffusivity of Metrizamide depends highly on the concentration and pH value of the buffers in which it dissolved. This is probably due to the molecular weight, ion charge, ionization constant, etc. of the buffers.

A trial-and-error procedure was used to obtain a generalization factor,

ϕ, which could be used to estimate the diffusivities of Metrazamide in buffer solutions other than those experimentally measured; the diffusivities obtained in various buffer solutions multiplied by the generalization factor will yield the diffusivity of an equivalent concentration in distilled water. It was found that if the ratio of a quantity obtained from the formula weight of ion per valence multiplied by its pK value, between water and buffer solution, is used as a generalization factor, diffusivities obtained in the experiments can be reduced to that in distilled water at the equivalent concentration within $\pm 12\%$. The generalization factor for three buffer solutions and their respective properties used are tabulated in Table 2. Recalculated diffusivities using the generalization factor for Metrizamide in three buffer solutions are presented in Fig. 3 for comparison.

Thus, if one knows the properties of a new buffer solution, formula weight per valence, and its pK value, diffusivities of Metrizamide in this buffer solution can be estimated from the value of diffusivity of Metrizamide in distilled water and the generalization factor calculated from those buffer properties.

TABLE 2

Properties of Buffer Solutions and Values of Their Generalization Factor

	Phosphate buffer	Acetate buffer	Borate buffer	Distilled water
Formula weight of ion	96.0	59.0	42.81	17.0
Valence of ion	2	1	1	1
pK value	7.198	4.77	4.81	14.00
Generalization factor	0.689	0.846	1.156	1.000

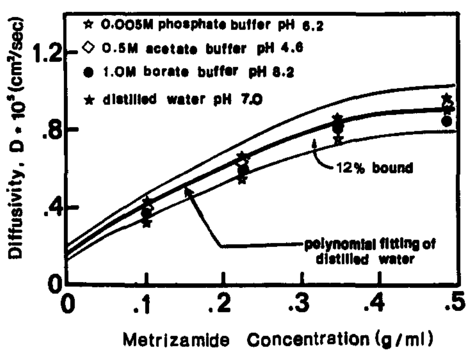

FIG. 3. Comparison of diffusion coefficients of Metrizamide in distilled water and in various buffer solutions corrected by a generalization factor at 25°C.

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