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Transport Phenomena in Zonal Centrifuge Rotors. VI. Concentration-Dependent Diffusivities of Potassium Citrate and Potassium Tartrate in Aqueous Solutions

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Abstract

Concentration-dependent diffusivities of potassium citrate and potassium tartrate in aqueous solution were measured by a microinterferometric method for solute concentrations ranging from 0.103 to 0.670 grams solute per ml. Empirical formulas for the diffusivity and an activity coefficient of both solutes are presented as a function of solute concentrations.

INTRODUCTION

Sucrose or cesium chloride solutions are often used as density gradient solutions in zonal centrifugation for the separation of biomaterials.

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However, for virus separation or isolation, the use of potassium citrate or potassium tartrate as gradient materials has some advantages over sucrose or cesium chloride solutions. A density higher than that of sucrose solutions is obtainable by the use of cesium chloride solutions, but the use of cesium chloride solutions at higher density produces deleterious effects. The structure of a virus protein may be destroyed due to the high ionic strength of cesium chloride solution.

Potassium citrate solutions are chemically more complex than sucrose solutions because potassium citrate is a uni-trivalent electrolyte of a strong base and a weak tribasic acid that has relatively small differences in the ionization constants for the three hydrogen ions. Consequently, the hydrolysis of the pure salt in water produces solutions that are too basic for virus stability, the pH approaching 9.4 at saturation (*1*). The addition of citric acid will lower the pH into the range considered satisfactory for virus isolations, but the amount of citric acid required to obtain a given pH changes with the potassium citrate concentration. The use of potassium tartrate as gradient solutions reduces those deficiencies to an acceptable range of virus stability.

In order to use potassium citrate or potassium tartrate as a gradient solution for the isolation or separation of viruses, it is necessary to know their physical properties, such as density, viscosity, and diffusivity, at various concentrations. For the determination of a sedimentation coefficient and a molecular weight, it is necessary to have information on diffusivity at the banding concentration and also the activity coefficient for the thermodynamic term (*2*). In an improving of the separation resolution, diffusivity at various concentrations is the primal information required for evaluating the maximum load capacity (*3*) or the band broadening effect (*4*).

EXPERIMENTAL METHOD AND THEORY

The accurate measurement of a diffusion coefficient is not easily accomplished. Small changes in temperature or slight mechanical vibrations can cause a convective flow and the disruption of the pure molecular diffusion processes. A large number of methods of measuring diffusion coefficients are available. With two reasons discussed later, the microinterferometric method was used for the present study. The microinterferometer most used today was developed by Nishijima and Oster (*5*) in 1956. The instrument consisted of a light source, a diffusion cell, and a microscope-camera set up. The diffusion cell was made by placing two half-metallized slides together, separated only by a cover slip. This interferometric wedge, first described by Searle (*6*), employs light interference methods suitable for measurement of the refractive index and thus of the concentration of a medium. Introducing a continuous low power (1 mW) helium gas laser (6328 Å) as the monochromatic light source, the usual heat effects present in a conventional light source can be eliminated (*7*).

The time requirement in the microinterferometric method is very short. According to the theory of Brownian movement, the average of the square of the distance over which a particle is randomly wandering is proportional to the time during which it was traveling. Therefore, if the diffusion is observed over a small distance, the time required for the observation can be reduced by the square of the magnification factor. Thus, if the diffusion measurement is carried out under a microscope with a magnification factor of 50, the time scale is reduced by 2500 so that hours in the conventional diffusion apparatus become seconds in the microdiffusion apparatus. Another feature of the microdiffusion apparatus is that only microgram amounts of solutions (drop-size sample) are required. Other various advantages of the microinterferometric method over several types of optical interference methods in measuring diffusion coefficients were presented by Duda et al. (*8*).

Theory

Molecular diffusion is a process that leads to an equalization of the chemical potential within a single phase at constant temperature and pressure. If the assumption is made that the system is ideal, the activity coefficient is equal to 1.0 and concentration and activity are equivalent. Therefore, the equation describing molecular diffusion without convection fluxes can be given by Fick's second law:

$$\partial c/\partial t = \nabla(D\nabla c) \tag{1}$$

For the microinterferometric diffusion, the differential equation describing two droplet liquids diffusing into one another in the optical wedge can be adequately given by the one-dimensional diffusion equation in rectilinear coordinates:

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial c}{\partial x}\right) \tag{2}$$

Since the diffusion time and path are both very short, we can assume that the instantaneous diffusivity of the system is constant without a significant error, then Eq. (2) becomes

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{3}$$

In a one-dimensional model of the diffusion process, a medium of concentration c_1 and a medium of concentration c_2 are diffusing into one another, the initial and boundary conditions are:

$$\begin{array}{lll} \text{at } t \leq 0 & c = c_1{}^0 & \text{for all } x > 0 \\ \text{at } t \leq 0 & c = c_2{}^0 & \text{for all } x < 0 \\ \text{at } x = \infty & c = c_1{}^0 & \text{for all } t \\ \text{at } x = -\infty & c = c_2{}^0 & \text{for all } t \end{array} \tag{4}$$

The solution of Eq. (3) with the conditions given in Eq. (4) yields

$$c(x, t) = c_1{}^0 - \frac{c_1{}^0 - c_2{}^0}{2}\left[1 - \operatorname{erf}\left(\frac{x}{2(Dt)^{1/2}}\right)\right] \tag{5}$$

Differentiation of Eq. (5) with respect to x yields

$$\frac{dc}{dx} = \frac{(c_1{}^0 - c_2{}^0)}{2(\pi Dt)^{1/2}} \exp\frac{-x^2}{4Dt} \tag{6}$$

Direct measurement of this concentration gradient is difficult, time consuming, and generally inaccurate. A rapid indirect method which relates the concentration gradient to a refractive index gradient is generally used. A brief introduction to the theory of this correlation is discussed below.

A beam of monochromatic light directed through an optical wedge interferes with the light which is partially reflected. If the refractive index of the medium is constant in the wedge, then equally spaced interference fringes result.

In the case of two diffusing substances in the wedge, the refractive index along their interface varies continuously so that a curved interference pattern is observed. If a reference line is drawn perpendicular to the original interface, the refractive index gradient curve is obtained by a plot of the density of fringes along this line. If the refractive index is assumed to be a linear function of concentration, then dn/dc is constant. Hence,

$$c_1{}^0 - c_2{}^0 = K(n_2 - n_1) \tag{7}$$

where n_2 and n_1 are the refractive indices corresponding to the solute concentration c_1^0 and c_2^0, respectively. The refractive index gradient can be written as

$$\frac{dn}{dx} = \frac{dn}{dc}\frac{dc}{dx} = \frac{1}{K}\frac{dc}{dx} \tag{8}$$

Substitution of Eq. (7) into Eq. (6) and then substitution of the resulting expression in Eq. (8) yields

$$\frac{dn}{dx} = \frac{(n_2 - n_1)}{2(\pi Dt)^{1/2}} \exp\left[\frac{-x^2}{4Dt}\right] \tag{9}$$

Equation (9) is the relationship between the refractive index gradient dn and the distance x from the original boundary. This is true, provided the refractive index is a linear function of the solute concentration. In order to compare Eq. (9) with a normal distribution the following substitution is made

$$\sigma = (2D't')^{1/2} \tag{10}$$

Then Eq. (9) becomes

$$\frac{dn}{dx} = \frac{(n_2 - n_1)}{\sigma(2\pi)^{1/2}} \exp\left[\frac{-x^2}{2\sigma^2}\right] \tag{11}$$

Equation (11) is the normal distribution of Gauss, where σ is the standard deviation and the term $(n_2 - n_1)$ is the cumulative frequency. The primed notation is used because of the necessity of a zero-time correction in the computation of the experimental diffusion coefficient. D' is the observed diffusion coefficient and t' is the observation time.

Method

The method of measuring the diffusivities of a system is outlined below. It is assumed that, during the measurement, the ambient temperature will be unchanging and the mechanical vibrations will be held to a minimum.

One drop (0.02 ml) of each medium (not touching) is placed on the microscope slide coated by silver; the nonmetallized portion of the slide is resting on the microscope stage. The other slide is positioned very gently in place to form the wedge and to start the diffusion process. The stopwatch is started when the wedge is formed.

The microscope is adjusted until the ground-glass viewer depicts a region where the interface is perpendicular to the interference fringes. After the initial turbulence of the contacting has died out (about 60 sec), photographs are taken at successive intervals until the diffusing interface spreads out of the photographic field. The refractive index gradient curve is obtained by a plot of the density of fringes along the reference line versus the distance along the line. Then, by Eq. (11), the diffusion coefficient of the substance under investigation can be calculated.

Thermodynamic Term

From irreversible thermodynamic considerations, Gosting (*9*) has introduced a term called the thermodynamic term for nonideal mixtures. Then, an expression for an isothermal, isotropic diffusion coefficient in nonreacting mixtures of the absence of external forces can be written as

$$D = D_0\left[1 + \frac{d\ln\gamma^{(c)}}{d\ln c}\right] \tag{12}$$

where D_0 is the diffusivity at infinite dilution by extrapolating experimental data points, in which the activity coefficient $\gamma^{(c)}$ becomes 1.0. The expression in the bracket is the thermodynamic term.

The method we used in the measurement of diffusivity is essentially a transient technique. In order to compensate the frictional coefficient with concentration, following Gosting's approach (*9*), Eq. (12) was modified to

$$D = D_0\left[\frac{\eta_0\bar{v}_0\rho}{\eta}\right]\left[1 + \frac{d\ln\gamma^{(c)}}{d\ln c}\right] \tag{13}$$

in which η, $\bar{v}_0$, and ρ are viscosity, specific volume of solvent, and density at a given concentration, respectively, and η_0 is viscosity of pure solvent.

MATERIALS

Potassium citrate and potassium tartrate solutions were prepared from distilled water, and both solutes were supplied by the J. T. Baker Chemical Co., Phillipsburg, New Jersey, in crystal reagent grade. The assay of potassium citrate [$HOC(COOK)(CH_2COOK)_2 \cdot H_2O$] is 100.5% with insoluble matter 0.0005%, and the potassium tartrate [$K_2C_4H_4O_6 \cdot \frac{1}{2}H_2O$] is 99.7% with insoluble matter 0.002%.

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 Experimental section can be used without modification.

Viscosities at various concentrations for both solutions were measured by a Hewlett-Packard Auto-Viscometer, Model 5901-B, with an Ubbelohde viscometer tube made by Cannon Instrument Company. Densities at various concentrations were determined by 1 ml serological

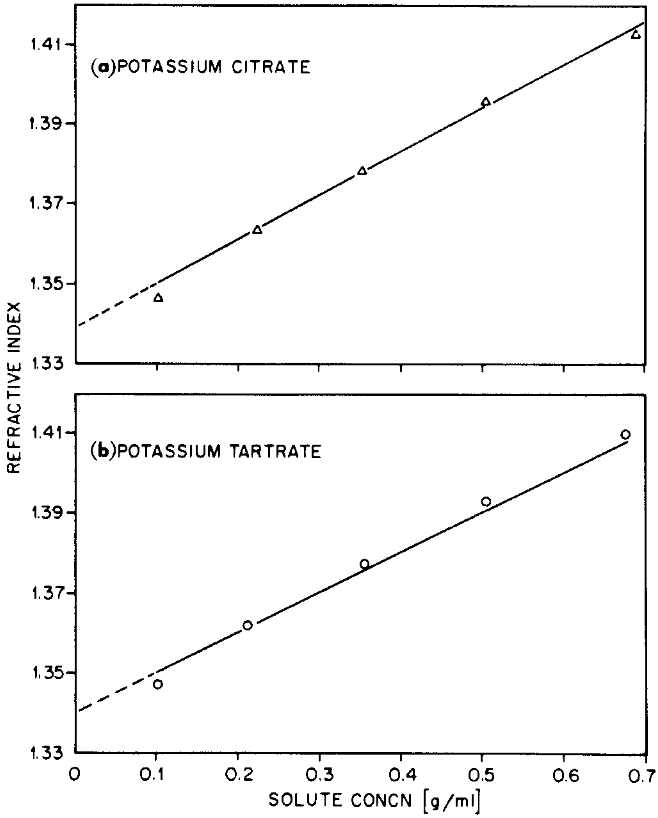

FIG. 1. Refractive index of potassium citrate (a) and potassium tartrate (b) at various concentrations at 20°C.

pipet (sterile) supplied by Falcon Plastics Division of BioQuest. The data points obtained from both measurements are presented in Table 1. The partial specific volumes $\bar{v}_0$ of water were determined by the method of intercept by a graphical method from the measured densities, which are also presented in Table 1.

Binary diffusion coefficients were measured by the previously described microinterferometric method by means of counting the total number of fringes. Five different concentrations of the solutions were diffused into distilled water for both materials. Then, by using Eqs. (10) and (11) together with the total number of fringes, the diffusivity at that concentration was obtained. They are given in Table 2. In order to evaluate the thermodynamic term, the quantity $[\eta/\eta_0\bar{v}_0\rho]$ at various concentrations was also computed and the results are also listed in Table 2. The quantity D_0 was obtained by extrapolation to an infinite dilution from a diffusivity vs. concentration plot. It was found that $D_0 = 2.305 \times 10^{-6}$ and 1.000×10^{-6} for potassium citrate and potassium tartrate, respectively.

The activity coefficient was obtained by rearranging Eq. (13):

$$\ln \gamma^{(c)} = \int_0^c \left[\frac{D\eta}{D_0\eta_0\nu_0\rho} - 1\right] d\ln c \qquad (14)$$

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)
	Potassium Citrate		
0.1031	1.065	1.031	1.000
0.2207	1.383	1.104	1.000
0.3530	1.970	1.176	1.000
0.5045	3.496	1.260	0.955
0.6740	5.350	1.345	0.955
	Potassium Tartrate		
0.1038	1.022	1.039	1.000
0.2199	1.258	1.100	1.000
0.3546	1.695	1.182	0.969
0.5021	2.500	1.256	0.959
0.6701	4.280	1.340	0.959

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)	$\eta/\eta_0\bar{v}_0\rho$	$\ln \gamma^{(c)}$	$D\eta \times 10^8$ (dyne)
		Potassium Citrate ($D_0 \times 10^6 = 2.305$)		
0.1031	3.149	1.156	0.4774	3.354
0.2207	3.539	1.402	1.0536	4.894
0.3530	3.712	1.874	1.8022	7.313
0.5045	3.620	3.251	2.8449	12.656
0.6740	4.420	4.660	4.4540	23.647
		Potassium Tartrate ($D_0 \times 10^6 = 1.000$)		
0.1038	2.191	1.100	1.4029	2.239
0.2199	3.387	1.280	3.0175	4.261
0.3546	4.207	1.656	5.1823	7.131
0.5021	5.116	2.322	8.2074	12.915
0.6701	6.629	3.724	15.2857	28.372

The quantities, D, ρ, $\bar{v}_0$, and η are all functions of concentration. Hence, substituting experimentally determined values of each quantity at various concentrations into the integrand and performing a numerical integration to that concentration, the activity coefficient at that concentration was obtained. The results of numerical integration by an IBM 360 Model 67 series are presented in Table 2 and in Fig. 3.

Empirical formulas for the diffusion coefficient and the activity coefficient as a function of the solute concentration were also obtained. They are listed below:

Potassium citrate:

$$D \times 10^6 = 2.305 + 10.986c - 20.080c^2 + 27.289c^3 \text{ (cm}^2\text{/sec)}; \ (0.0556) \tag{15}$$

$$\ln \gamma^{(c)} = 4.727c - 2.435c^2 + 16.942c^3 - 26.687c^4 + 19.326c^5 \tag{16a}$$

or

$$\ln \gamma^{(c)} = -0.31 + 1.91\left[0.37 + \frac{2.90c}{1.75 - 0.79c}\right]^{1.35}; \ (0.0370) \tag{16b}$$

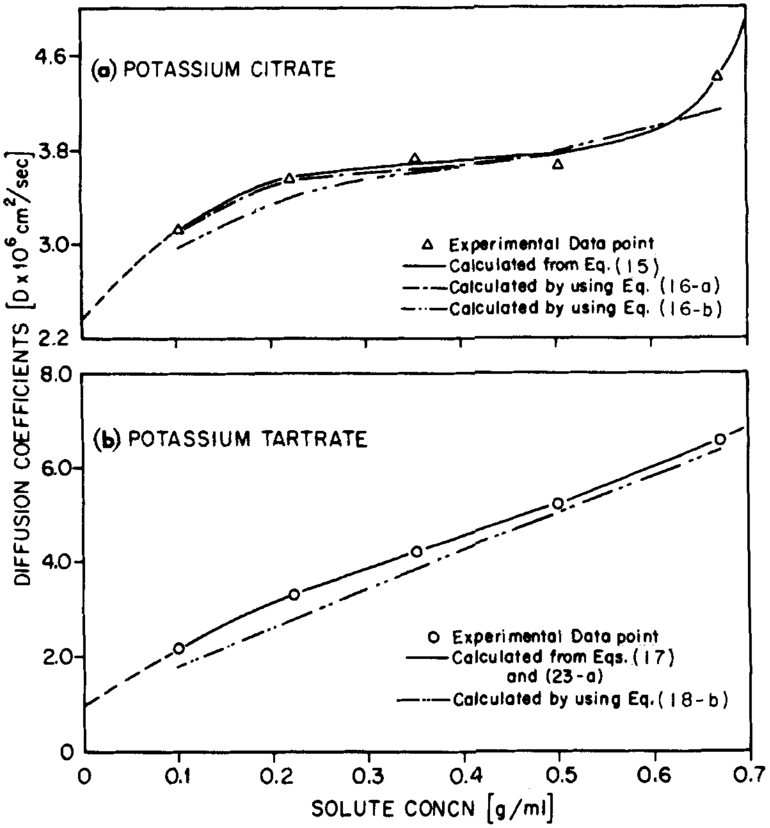

FIG. 2. Comparison of diffusivities between experimentally measured values and estimated values from empirical formulas. (a) Potassium citrate. (b) Potassium tartrate.

in wt% of solute (w)

$$\ln \gamma^{(w)} = -0.31 + 1.91 \left[0.31 + \frac{1.62w}{1 - w}\right]^{1.35} \tag{16c}$$

Potassium tartrate:

$$D \times 10^6 = 1.000 + 13.903c - 19.484c^2 + 16.785c^3 \text{ (cm}^2\text{/sec)}; \ (0.0588) \tag{17}$$

$$\ln \gamma^{(c)} = 13.903c - 6.827c^2 + 32.613c^3 - 28.398c^4 + 19.571c^5 \tag{18a}$$

or

$$\ln \gamma^{(c)} = 1.58 \left[0.91 + \frac{4.82c}{1.75 - 0.79c}\right]^{1.64}; \ (0.1077) \tag{18b}$$

in wt% of solute (w)

$$\ln \gamma^{(w)} = 1.58 \left[0.91 + \frac{2.69w}{1 - w}\right]^{1.64} \tag{18c}$$

The numbers in the parentheses after the equations are the standard deviation.

The comparison of the concentration-dependent diffusivities between experimental data points and calculated values using the polynomial and the empirical formulas of the activity coefficient are presented in Fig. 2 for both systems. A good agreement exists between the polynomials and experimental data points. The diffusivities computed from the polynomial-activity-coefficient give 0.051 and 0.0593 as the standard deviations for potassium citrate and potassium tartrate, respectively. The diffusivities calculated from the van Laar-type activity coefficient formula are slightly lower than the experimental data points and give 0.234 and 0.482 as the standard deviations for potassium citrate and potassium tartrate, respectively. 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,

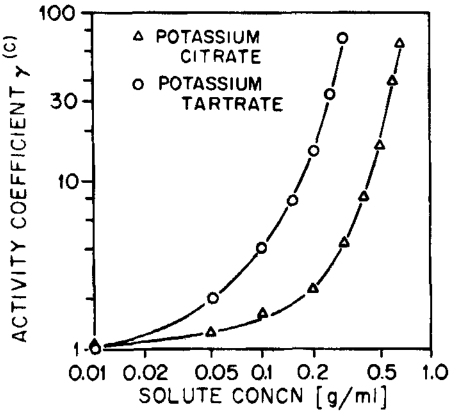

FIG. 3. A plot of activity coefficients vs. solute concentrations of potassium citrate and potassium tartrate.

but they give a poorer result for a thermodynamic term than that of a polynomial form.

As gradient solutes it is interesting to note that a potassium tartrate gradient will yield a higher band-broadening effect at higher concentrations than that of a potassium citrate, whereas the diffusivity of a potassium tartrate in water is about 50% higher, while the viscosity of a potassium tartrate is lower than that of a potassium citrate. Since the viscosity of a gradient solution determines the sedimentation rate (*10*), values of $D\eta$ 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 dyne, the unit for force. The quantity $D\eta$ means 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. In this respect it is concluded that a potassium citrate solution would be a better gradient solution if a gradient density higher than 1.25 g/cc is

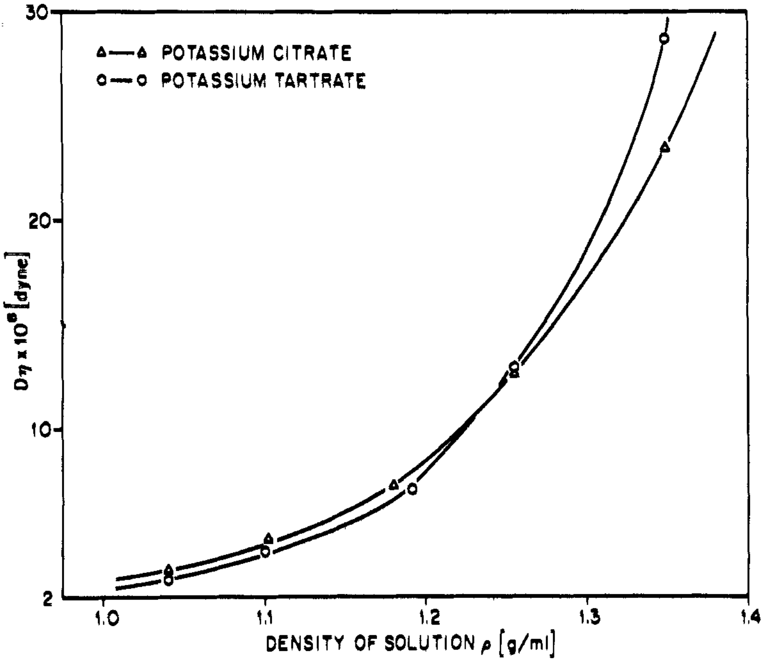

FIG. 4. The $D\eta$ vs. various densities of potassium citrate and potassium tartrate.

required, and a potassium tartrate would be better if a gradient density lower than 1.25 g/cc is required.

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