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Partial Molar Isentropic and Isothermal Compressions of the Nucleosides Adenosine, Cytidine, and Uridine in Aqueous Solution at 298.15 K

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ABSTRACT: Sound speeds have been measured for aqueous solutions of the nucleosides adenosine, cytidine, and uridine at T = 298.15 K and at ambient pressure. For adenosine and uridine, the partial molar isentropic compressions at infinite dilution, $K^{\circ}_{S,2}$, were derived from the sound speed data using conventional methods. These $K^{\circ}_{S,2}$ results were combined with the partial molar heat capacities and partial molar isobaric expansions at infinite dilution available from the literature to evaluate, for the first time, the partial molar isothermal compressions at infinite dilution, $K^{\circ}_{T,2}$ $\{K^{\circ}_{T,2} = -(\partial V^{\circ}_2/\partial p)_T$, where V°_2 is the partial molar volume of the solute at infinite dilution$\}$. An alternative method is described for the calculation of the $K^{\circ}_{S,2}$ and $K^{\circ}_{T,2}$ values for cytidine from the sound speed data. The $K^{\circ}_{T,2}$ results for the nucleosides have been rationalized in terms of the likely solute—water interactions. For cytidine, an analysis of the molality dependence of the partial molar isothermal compression in terms of self-association has also been explored.

INTRODUCTION

As a consequence of the significant impact of solvent water on the structure and function of the nucleic acids RNA and DNA in aqueous solution,[1−3] various experimental methods have been used to investigate nucleic acid hydration. The determination of volumetric properties is one method that has provided valuable insights into aspects of nucleic acid—solvent interactions.[4,5] Since nucleic acids have a diverse array of constituent functional groups, the volumetric properties of the basic building blocks, such as the bases and nucleosides, have proved useful in the interpretation of the results for RNA and DNA polymers.[4,6] Although volumetric properties for these constituent molecules are available for ambient conditions,[4,7−10] and also as a function of temperature at ambient pressure,[10−12] no data are available for high pressures. There is growing interest in understanding the behavior of biopolymers at high pressures[13,14] as a consequence, for example, of the discovery of deep-sea organisms that are well adapted to life at high pressures.[15]

As part of a research program to determine the volumetric properties of important biological molecules at high pressures, we reported recently[16] a method to derive, from sound speed data as a function of pressure, the partial molar volumes at infinite dilution, V°_2, the partial molar isentropic compressions at infinite dilution, $K^{\circ}_{S,2}$, and the partial molar isothermal compressions at infinite dilution, $K^{\circ}_{T,2}$ $\{K^{\circ}_{T,2} = -(\partial V^{\circ}_2/\partial p)_T\}$, for solutes in aqueous solution over a wide pressure range. A necessary requirement of the method is the availability of a range of reliable thermodynamic properties at a pressure of 1 bar for the solutes of interest. As a prerequisite for the determination of the volumetric properties of the constituent nucleosides of RNA at high pressures, we have reported[10] the partial molar heat capacities at infinite dilution, $C^{\circ}_{p,2}$, V°_2 data, and the partial molar isobaric expansions at infinite dilution, E°_2 $\{E^{\circ}_2 = (\partial V^{\circ}_2/\partial T)_p\}$, for the nucleosides adenosine, cytidine, and uridine in aqueous solution at p = 1 bar and T = 298.15 K. As an extension to this work, we report herein $K^{\circ}_{S,2}$ results for the three nucleosides in aqueous solution at p = 1 bar and T = 298.15 K. Although $K^{\circ}_{S,2}$ values have been reported for all three nucleosides in two previous studies,[8,12] the large estimated uncertainties negate use of these results in our high-pressure studies. Also described in this paper are the first determinations of the $K^{\circ}_{T,2}$ values for adenosine, cytidine, and uridine in aqueous solution at p = 1 bar and T = 298.15 K.

EXPERIMENTAL SECTION

The nucleosides cytidine and uridine were samples recovered from aqueous solutions used in previous experimental work.[10] The solids were recrystallized (cytidine from {water + ethanol} and uridine from {methanol + diethylether}) using the procedures described in detail elsewhere.[10] The white crystalline products were dried under vacuum at room temperature for 24 h. The sample of adenosine used was material remaining from previous studies.[10]

The water used to prepare solutions and as the reference solvent was glass-distilled and thoroughly degassed immediately prior to use. All solutions (volumes typically (5 to 6) cm^3) were prepared by mass using a Mettler Toledo AX205 analytical balance (readability 0.01 mg), and corrections were made for the effect of air buoyancy. To avoid possible water adsorption by the solids through prolonged exposure to the atmosphere, solutions were prepared in batches, typically four at a time, after which the solids were dried further under vacuum.

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Table 1. Sound Speeds and Apparent Molar Isentropic Compressions for Aqueous Solutions of Nucleosides at T = 298.15 K

m	u^a	$10^{15}\,K_{S,\phi}$	m	u^a	$10^{15}\,K_{S,\phi}$
(mol·kg^{-1})	(m·s^{-1})	(m^3·mol^{-1}·Pa^{-1})	(mol·kg^{-1})	(m·s^{-1})	(m^3·mol^{-1}·Pa^{-1})
		Adenosine			
0.02054	1497.996	$-4.7_4 \pm 0.1_7$	0.01851	1497.868	$-4.7_7 \pm 0.1_9$
0.02001	1497.965	$-4.8_2 \pm 0.1_8$	0.01799	1497.835	$-4.7_6 \pm 0.2_0$
0.01950	1497.934	$-4.8_6 \pm 0.1_8$	0.01736	1497.796	$-4.8_1 \pm 0.2_0$
0.01911	1497.903	$-4.6_7 \pm 0.1_9$	0.01701	1497.775	$-4.8_3 \pm 0.2_1$
		Cytidine			
0.12736	1505.981	-14.69 ± 0.03	0.03696	1499.449	$-16.08 \pm 0.1_0$
0.11547	1505.132	-14.83 ± 0.03	0.03088	1498.997	$-16.1_5 \pm 0.1_1$
0.10221	1504.191	-15.05 ± 0.03	0.02709	1498.717	$-16.2_4 \pm 0.1_3$
0.08896	1503.232	-15.19 ± 0.04	0.02424	1498.508	$-16.3_6 \pm 0.1_5$
0.07638	1502.327	-15.40 ± 0.05	0.01993	1498.185	$-16.3_9 \pm 0.1_8$
0.06353	1501.390	-15.57 ± 0.06	0.01484	1497.807	$-16.5_9 \pm 0.2_4$
0.04979	1500.385	-15.76 ± 0.07	0.01300	1497.667	$-16.5_5 \pm 0.2_7$
0.04298	1499.888	-15.91 ± 0.08			
		Uridine			
0.11086	1503.822	-10.92 ± 0.03	0.03520	1498.988	$-11.90 \pm 0.1_0$
0.09859	1503.048	-11.08 ± 0.04	0.02798	1498.520	$-12.01 \pm 0.1_3$
0.08550	1502.214	-11.22 ± 0.04	0.02455	1498.293	$-11.98 \pm 0.1_4$
0.07233	1501.380	-11.43 ± 0.05	0.02081	1498.057	$-12.24 \pm 0.1_7$
0.05897	1500.520	-11.56 ± 0.06	0.01946	1497.961	$-12.02 \pm 0.1_8$
0.04644	1499.715	-11.75 ± 0.08	0.01599	1497.734	$-12.05 \pm 0.2_2$
0.04005	1499.300	-11.80 ± 0.09	0.01510	1497.676	$-12.06 \pm 0.2_4$

[a] The repeatability of u is ± 0.005 m·s^{-1}.

Table 2. Coefficients of Equation 2[a]

solute	a_1/(kg·m^{-3})	a_2/(kg·m^{-3})
adenosine	95.81 ± 0.06	[b]
cytidine	89.50 ± 0.02	$-13.4_9 \pm 0.1_2$
uridine	91.62 ± 0.02	$-15.1_3 \pm 0.1_2$

[a] From ref 10. [b] Value of a_2 was not statistically significant.

Sound speed measurements were carried out using a rubidium clock sound velocity meter, the details of which have been described elsewhere.[17] The temperature of the thermostat bath was maintained at ± 0.001 K using methods described in earlier work from the Bergen laboratory.[18] The reproducibility of a speed of sound measurement was to better than ± 0.005 m·s^{-1}.

RESULTS AND DISCUSSION

The measured sound speeds, u, for aqueous solutions of the nucleosides are given in Table 1. The isentropic compressibility, κ_S $\{\kappa_S = -(1/V)(\partial V/\partial p)_S\}$, for each solution was obtained using the Newton−Laplace equation[19]

$$\kappa_S = 1/(u^2\rho) \qquad (1)$$

where ρ is the solution density. For each solution, the value for ρ was calculated using a power series in the solution molality, m, of the form

$$\rho - \rho_1^* = a_1(m/m^\circ) + a_2(m/m^\circ)^2 \qquad (2)$$

where ρ_1^* is the density of the pure solvent (997.047 kg·m^{-3} at T = 298.15 K);[20] a_1 and a_2 are adjustable parameters; and m° = 1.0 mol·kg^{-1}. For each nucleoside, the parameters a_1 and a_2 and their estimated uncertainties, which are the results taken from previous work,[10] are summarized in Table 2.

The values of κ_S were used to calculate the apparent molar isentropic compression, $K_{S,\phi}$, which is defined by the relation[21,22]

$$K_{S,\phi} = (M_2\kappa_S/\rho) - (\kappa_{S,1}^*\rho - \kappa_S\rho_1^*)/(m\rho\rho_1^*) \qquad (3)$$

where $\kappa_{S,1}^*$ is the isentropic compressibility for solvent water ($\kappa_{S,1}^*$ = 4.47736·10^{-10} Pa^{-1} at T = 298.15 K);[23] M_2 is the solute molar mass; and the remaining symbols are as defined for eq 2. The $K_{S,\phi}$ results along with their uncertainties, which were estimated using propagation of errors methods[24] applied to eqs 1 and 3, are given in Table 1.

For dilute solutions of nonelectrolytes, the molality dependence of $K_{S,\phi}$ can usually be represented by the simple linear equation[25−28]

$$K_{S,\phi} = K_{S,2}^\circ + S_k m \qquad (4)$$

where $K_{S,2}^\circ$ is the partial molar isentropic compression of the solute at infinite dilution and S_k is the experimental slope. An analysis of the $K_{S,\phi}$ data for adenosine by weighted least-squares using eq 4 gave a value for S_k that was not statistically significant because the accessible molality range is too narrow. The $K_{S,2}^\circ$ result given in Table 3 is actually the mean of the $K_{S,\phi}$ values, and the uncertainty given is the standard deviation. Included in Table 3 are the $K_{S,2}^\circ$ values for adenosine reported in two previous studies.[8,12] Although our result is significantly less negative than

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Table 3. Partial Molar Isentropic Compressions at Infinite Dilution and the S_k Values for the Nucleosides in Aqueous Solution at T = 298.15 K

	$10^{15}\,K^{o}_{S,2}$	$10^{15}\,S_k$
solute	$(m^3\cdot mol^{-1}\cdot Pa^{-1})$	$(m^3\cdot kg\cdot mol^{-2}\cdot Pa^{-1})$
adenosine	-4.78 ± 0.06[a]	[b]
	-5.8 ± 0.9[c]	
	-5.6 ± 1.0[d]	
cytidine	-16.77 ± 0.06[a,e]	
	-17.0 ± 0.8[c]	
	-15.0 ± 1.0[d]	
	-18.2 ± 0.4[f]	
uridine	-12.33 ± 0.02[a]	$12.7_4 \pm 0.2_6$
	-13.8 ± 0.7[c]	
	-11.4 ± 1.0[d]	
	-17.0 ± 0.4[f]	

[a] This work. [b] See text. [c] From ref 8. [d] From ref 12. [e] Calculated using eq 6. See text. [f] From ref 7.

those reported previously, the very large estimated uncertainties given by both Buckin et al.[8] and Lee and Chalikian[12] are such that all three results are almost concordant, within the combined estimated uncertainties.

The molality dependences of $K_{S,\phi}$ for uridine and cytidine at T = 298.15 K are shown in Figure 1. Since the plot of $K_{S,\phi}$ for uridine against molality is linear, the data were analyzed by weighted least-squares using eq 4, with weighting factors taken as the inverse squares of the uncertainties of the apparent molar compressions. The solid line drawn in Figure 1(a) is that calculated by the least-squares analysis. The $K^{o}_{S,2}$ and S_k values obtained are given in Table 3. The $K^{o}_{S,2}$ values for uridine that are reported in the literature[7,8,12] are also included in Table 3. The large variation among the $K^{o}_{S,2}$ results is, at first sight, rather discouraging. Our result lies between those reported by Buckin et al.[8] and by Lee and Chalikian[12] but is, in fact, in agreement with the latter result within the admittedly large combined uncertainties. In the first determination of $K^{o}_{S,2}$ for uridine at T = 298.15 K by Høiland and co-workers,[7] the curvature observed in their plot of the apparent molar compression against molality is irrefutable. The more negative value of $K^{o}_{S,2}$ reported by Høiland et al.[7] can be accounted for, at least in part, by this curvature. However, it is clear from Figure 1(a) that for the sample of uridine used in this study there is no curvature in the plot of $K_{S,\phi}$ versus molality. It is worth stressing that of the four determinations of $K^{o}_{S,2}$ for uridine only in our work was the commercial sample subjected to further careful purification and analysis.[10]

In contrast, for cytidine the plot of $K_{S,\phi}$ against molality shown in Figure 1(b) has a distinct curvature. The dashed line, which actually represents the weighted least-squares linear fit for the data that span the molality range m = (0.128 to 0.048) mol·kg^{-1}, was added to Figure 1(b) as a visual aid to emphasize the deviation from linearity as the molality decreases. Weighted least-squares analyses of the $K_{S,\phi}$ data were carried out using a polynomial in molality of the form

$$K_{S,\phi} = b_0 + b_1(m/m^o) + b_2(m/m^o)^2 + b_3(m/m^o)^3 \qquad (5)$$

where b_i, i = 0 to 3, are adjustable parameters and the remaining symbols are as defined vide supra. Values of the b_i coefficients,

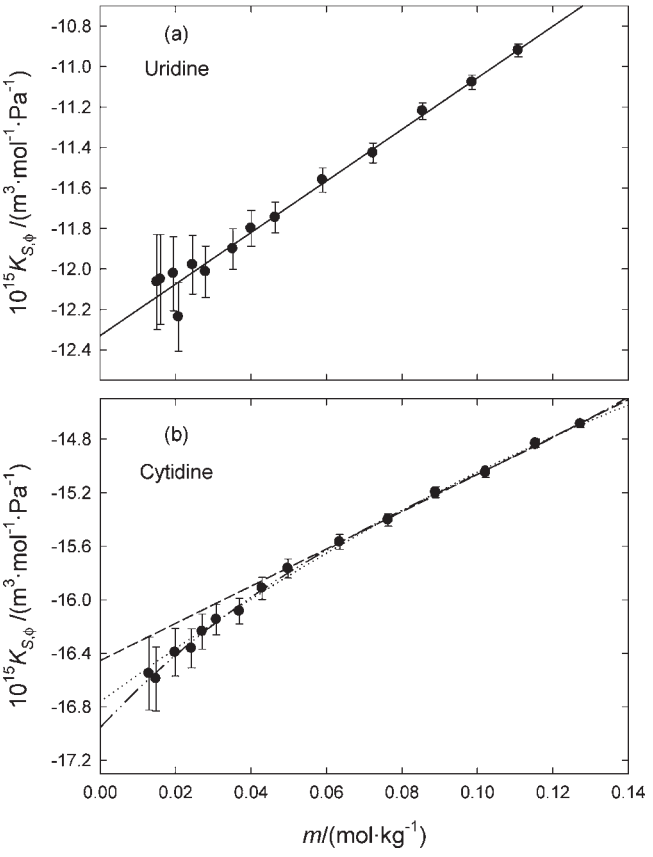

Figure 1. Molality dependences of the apparent molar isentropic compressions for the nucleosides in aqueous solution: (a) uridine; (b) cytidine; ·····, curve obtained using eq 5 order 2; —··—··, curve obtained using eq 5 order 3; — — — —, see text.

along with their estimated uncertainties, obtained using both second-order and third-order polynomials are given in Table 4. The key feature of these results is that the value obtained for the b_0 coefficient, which is equivalent to $K^{o}_{S,2}$, depends upon the equation chosen for the data analysis. The calculated $(K_{S,\phi}, m)$ curves based on the coefficients given in Table 4 are also displayed in Figure 1(b). Clearly, it is desirable to find a more satisfactory method to evaluate $K^{o}_{S,2}$ for cytidine from the sound speed data.

The thermodynamic quantities E^{o}_{2} and $C^{o}_{p,2}$ determined in previous work,[10] along with various properties of the solvent, were used to convert the $K^{o}_{S,2}$ values for adenosine and uridine into the more useful partial molar isothermal compressions at infinite dilution, $K^{o}_{T,2}$ $\{K^{o}_{T,2} = -(\partial V^{o}_{2}/\partial p)_T\}$. The expression used for this conversion is[21,29]

$$K^{o}_{T,2} = K^{o}_{S,2} + \delta^{*}_{1}(2E_2{}^{o}/\alpha^{*}_{1} - C^{o}_{p,2}/\sigma^{*}_{1}) \qquad (6)$$

where the quantities δ^{*}_{1}, α^{*}_{1}, and σ^{*}_{1} are all properties of the pure solvent: σ^{*}_{1} is the heat capacity per unit volume, σ^{*}_{1} = 4.1670 J·K^{-1}·cm^{-3} at T = 298.15 K;[30] α^{*}_{1} is the isobaric expansibility $\{\alpha^{*}_{1} = (\partial V^{*}_{1}/\partial T)_p/V^{*}_{1}\}$, $10^6\alpha^{*}_{1}$ = 257.21 K^{-1} at T = 298.15;[31] δ^{*}_{1} is the difference between the isothermal compressibility $\kappa^{*}_{T,1}$ $\{\kappa^{*}_{T,1} = -(\partial V^{*}_{1}/\partial p)_T/V^{*}_{1}\}$ and the isentropic compressibility $\kappa^{*}_{S,1}$ $\{\kappa^{*}_{S,1} = -(\partial V^{*}_{1}/\partial p)_S/V^{*}_{1}\}$, $\delta^{*}_{1} = \kappa^{*}_{T,1} - \kappa^{*}_{S,1} = 0.4736\cdot10^{-11}$ Pa^{-1} at T = 298.15 K.[23,31] The values of $K^{o}_{T,2}$ for adenosine and uridine at T = 298.15 K, and their uncertainties estimated by the application of propagation of error methods to eq 6, are given in Table 5.

Table 4. Coefficients of Equation 5

	$10^{15}\ b_0$	$10^{15}\ b_1$	$10^{15}\ b_2$	$10^{15}\ b_3$
polynomial	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$
order 2	-16.76 ± 0.05	$20._5 \pm 1$	$-33._6 \pm 8$	
order 3	-16.96 ± 0.08	$30._3 \pm 4$	-171 ± 52	574 ± 215

Table 5. Partial Molar Expansions, Heat Capacities, Isentropic Compressions, and Isothermal Compressions at Infinite Dilution for the Nucleosides in Aqueous Solution at T = 298.15 K

	$E_2^{o\,a}$	$C_{p,2}^{o\,a}$	$10^{15}\ K_{S,2}^{o}$	$10^{15} K_{T,2}^{o}$
solute	$(cm^3 \cdot mol^{-1} \cdot K^{-1})$	$(J \cdot K^{-1} \cdot mol^{-1})$	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$	$(m^3 \cdot mol^{-1} \cdot Pa^{-1})$
adenosine	0.2074 ± 0.0008	488.1 ± 2.4	-4.78 ± 0.06	2.30 ± 0.07^b
uridine	$0.18_7 \pm 0.01$	387.7 ± 1.8	-12.33 ± 0.02	$-5.8_8 \pm 0.3_7{}^b$
cytidine	0.1865 ± 0.0007	397.3 ± 2.1	-16.77 ± 0.06^d	-10.35 ± 0.06^c

[a] From ref 10. [b] Derived using eq 6. [c] Derived using eq 19. [d] Derived using eq 6. See text.

The value of $K_{T,2}^{o}$ for cytidine can be obtained directly from the partial molar compressions, $K_{T,2}$ $\{K_{T,2} = -(\partial V_2/\partial p)_T\}$, which were evaluated using the following procedure. Starting with the definition for the partial molar volume of a solute, $V_2 = (\partial V/\partial n_2)_{T,p,n1}$, where n_2 and n_1 are, respectively, the number of moles of solute and solvent, the volume of any solution V of molality m at constant T and p, when expressed in terms of its mass and density, leads to the equation[32]

$$V_2 = (M_2/\rho) - (1 + M_2 m)(\partial\rho/\partial m)/\rho^2 \qquad (7)$$

Differentiation of eq 7 with respect to pressure at constant temperature gives, after some manipulation, an expression for the partial molar isothermal compression of the form

$$K_{T,2} = -(\partial V_2/\partial p)_T = (1 + M_2 m)(\partial\kappa_T/\partial m)/\rho + V_2\kappa_T \qquad (8)$$

where κ_T is the isothermal compressibility, $\kappa_T = -(\partial V/\partial p)_T/V$, and the other symbols are as defined for eqs 3 and 7. The isothermal compressibility was evaluated using the expression[16,21]

$$\kappa_T = \kappa_S + T\alpha^2/\sigma \qquad (9)$$

where σ is the heat capacity per unit volume, and α is the isobaric expansibility, which is defined by the equation[16,21]

$$\alpha = (\partial V/\partial T)_p/V = -(\partial\rho/\partial T)_p/\rho \qquad (10)$$

Density data are available for aqueous solutions of cytidine at T = (288.15, 298.15, 303.15, and 313.15) K.[10] Each (ρ, m) data set was analyzed using eq 2 to give the fitted coefficients a_1 and a_2, from which the solution densities for the molalities used in this work were then obtained. For each solution, the equation

$$\rho - \rho_1^* = c_0 + c_1(T - T_m) + c_2(T - T_m)^2 \qquad (11)$$

was fitted to the density data, where T_m is the midpoint temperature of the range used (T_m = 300.65 K) and c_i, i = 0 to 2, are the fitted coefficients. Differentiation of eq 11 with respect to temperature at constant pressure gives the equation

$$(\partial\rho/\partial T)_p = (\partial\rho_1^*/\partial T)_p + c_1 + 2c_2(T - T_m) \qquad (12)$$

The derivative $(\partial\rho/\partial T)_p$ was obtained using eq 12 and was used to calculate the isobaric expansibility for each solution at T = 298.15 K. The relevant quantities for pure water used in the calculations were those reported by Kell.[31] These α values and their uncertainties, which were assessed by the application of propagation of errors to eqs 10 and 12, are given in Table 6.

The heat capacity per unit volume, σ, for each solution was obtained by multiplying the specific heat capacity, c_p, by the solution density, which was calculated using eq 2 and the coefficients given in Table 2. The specific heat capacities were calculated from the apparent molar heat capacities, $C_{p,\phi}$, reported previously[10] using the following rearranged form of the standard expression for $C_{p,\phi}$

$$c_p = (C_{p,\phi} + c_{p,1}^*/m)/(M_2 + 1/m) \qquad (13)$$

where $c_{p,1}^*$ is the specific heat capacity of pure water ($c_{p,1}^*$ = 4.1793 $J \cdot K^{-1} \cdot g^{-1}$ at T = 298.15 K[30]) and the remaining symbols are as defined vide supra. These c_p values are given in Table 6.

The isothermal compressibilities calculated using eq 9, along with their estimated uncertainties obtained by the application of propagation of errors, are shown in Table 6. The κ_T data were analyzed by weighted least-squares using the equation

$$\kappa_T = \kappa_{T,1}^* + d_1(m/m^o) + d_2(m/m^o)^2 + d_3(m/m^o)^3 \qquad (14)$$

where d_i, i = 1 to 3, are the fitted coefficients, and the isothermal compressibility of water is $\kappa_{T,1}^*$ = $4.52472 \cdot 10^{-10}$ Pa^{-1} at T = 298.15 K.[31] A third-order polynomial was chosen because the fit to the data was slightly better than that obtained using a quadratic equation. The values of the coefficients, and their uncertainties obtained from the least-squares analysis, are as follows: $d_1 = -(7.975_6 \pm 0.005_8) \cdot 10^{-11}$ Pa^{-1}; $d_2 = (3.2_3 \pm 0.1_5) \cdot 10^{-11}$ Pa^{-1}; $d_3 = -(5.0_6 \pm 0.8_8) \cdot 10^{-11}$ Pa^{-1}. The term $(\partial\kappa_T/\partial m)$ in eq 8 was evaluated using the expression obtained by differentiation of eq 14 with respect to molality, viz.

$$\partial\kappa_T/\partial m = d_1 + 2d_2(m/m^o) + 3d_3(m/m^o)^2 \qquad (15)$$

The V_2 values required for the calculation of partial molar isothermal compressions were obtained using eq 7, with the

Table 6. Calculated Isobaric Expansibilities, Specific Heat Capacities, Isothermal Compressibilities, Partial Molar Volumes, and Partial Molar Compressions for Aqueous Solutions of Cytidine at T = 298.15 K

m	$10^6\alpha$	$c_p{}^a$	$10^{10}\kappa_T{}^b$	V_2	$10^{15}K_{T,2}$
(mol·kg^{-1})	K^{-1}	(J·mol^{-1}·g^{-1})	Pa^{-1}	(cm^3·mol^{-1})	(m^3·mol^{-1}·Pa^{-1})
0.12736	$273.61_9 \pm 0.01_8$	4.09212	4.42734	153.95 ± 0.05	$-7.5_1 \pm 0.8_7$
0.11547	272.282 ± 0.008	4.09988	4.43616	153.94 ± 0.05	$-7.5_8 \pm 0.7_6$
0.10221	270.744 ± 0.001	4.10864	4.44599	153.93 ± 0.05	$-7.7_1 \pm 0.6_4$
0.08896	269.158 ± 0.007	4.11748	4.45597	153.92 ± 0.04	$-7.8_9 \pm 0.5_3$
0.07638	$267.60_6 \pm 0.01_2$	4.12596	4.46545	153.91 ± 0.04	$-8.1_0 \pm 0.4_3$
0.06353	$265.97_4 \pm 0.01_5$	4.13472	4.47525	153.91 ± 0.03	$-8.3_7 \pm 0.3_4$
0.04979	$264.17_7 \pm 0.01_6$	4.14417	4.48578	153.90 ± 0.03	$-8.7_0 \pm 0.2_6$
0.04298	$263.26_6 \pm 0.01_5$	4.14890	4.49102	153.90 ± 0.03	$-8.8_9 \pm 0.2_2$
0.03696	$262.45_0 \pm 0.01_4$	4.15310	4.49565	153.90 ± 0.03	$-9.0_6 \pm 0.1_9$
0.03088	$261.61_6 \pm 0.01_3$	4.15735	4.50039	153.90 ± 0.03	$-9.2_5 \pm 0.1_6$
0.02709	$261.09_0 \pm 0.01_2$	4.16002	4.50334	153.90 ± 0.03	$-9.3_7 \pm 0.1_4$
0.02424	$260.69_2 \pm 0.01_1$	4.16203	4.50555	153.90 ± 0.02	$-9.4_6 \pm 0.1_3$
0.01993	$260.08_5 \pm 0.01_0$	4.16508	4.50894	153.91 ± 0.02	$-9.6_1 \pm 0.1_2$
0.01484	$259.36_2 \pm 0.00_8$	4.16869	4.51293	153.91 ± 0.02	$-9.7_9 \pm 0.1_0$
0.01300	$259.09_8 \pm 0.00_7$	4.17000	4.51439	153.91 ± 0.02	$-9.8_6 \pm 0.09$

[a] The estimated uncertainties for c_p are typically $2 \cdot 10^{-4}$ J·mol^{-1}·g^{-1}. [b] The estimated uncertainties for κ_T are typically (3.22 to 3.31)·10^{-15} Pa·s^{-1}.

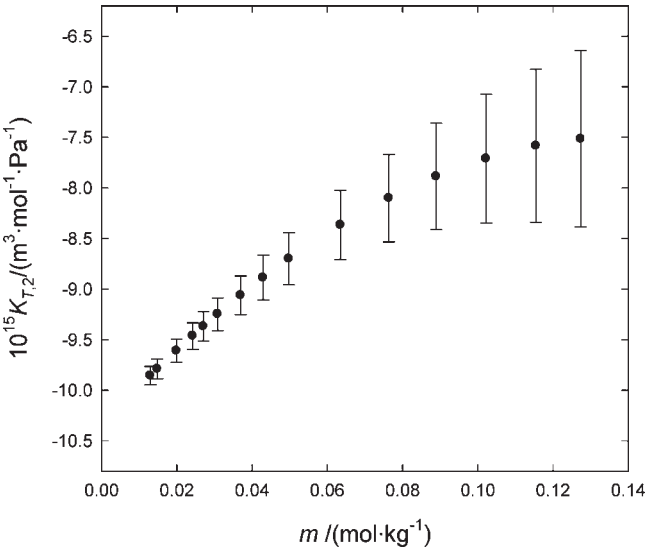

Figure 2. Molality dependence of the partial molar isothermal compression of cytidine in aqueous solution at T = 298.15 K.

term $(\partial\rho/\partial m)$ evaluated using the expression

$$\partial\rho/\partial m = a_1 + 2a_2(m/m^\circ) \quad (16)$$

obtained by differentiating eq 2. The partial molar volumes and partial molar isothermal compressions for the aqueous solutions of cytidine are given in Table 6. In the calculation of the uncertainties for V_2 and $K_{T,2}$ using propagation of error methods, extra cross terms were included in the standard formula to allow for the fact that the input quantities a_1 and a_2 of eq 16 and d_1, d_2, and d_3 of eq 15 are correlated.[33] The molality dependence of $K_{T,2}$ is displayed in Figure 2. The counterintuitive increase in the uncertainty for $K_{T,2}$ with increasing molality arises because of the significant contributions in the propagation of errors analysis from the second and third terms on the right-hand side of eq 15.

At first sight, it appears that as a consequence of the curvature displayed in Figure 2 the determination of the infinite dilute isothermal property is fraught with the same difficulties as described above for the apparent molar isentropic compression. However, the partial molar isothermal compression of a solute at infinite dilution, $K^\circ_{T,2}$, can be evaluated directly from eq 8. In the limit as $m \rightarrow 0$, eq 8 can be recast in the form

$$K^\circ_{T,2} = d_1/\rho_1^* + V_2^\circ\kappa^*_{T,1} \quad (17)$$

Similarly, the limiting condition $m \rightarrow 0$ when applied to eq 7 leads to the expression[32]

$$V_2^\circ = M_2/\rho_1^* - a_1/(\rho_1^*)^2 \quad (18)$$

Combining eqs 17 and 18 gives the equation

$$K^\circ_{T,2} = d_1/\rho_1^* + \{M_2/\rho_1^* - a_1/(\rho_1^*)^2\}\kappa^*_{T,1} \quad (19)$$

The value of $K^\circ_{T,2}$ calculated using eq 19 and its estimated uncertainty obtained by the application of error propagation methods to eq 19 are given in Table 5.

This $K^\circ_{T,2}$ result, along with the E_2° and $C^\circ_{p,2}$ values for cytidine reported in earlier work,[10] can be used to calculate a value for $K^\circ_{S,2}$ using the appropriately rearranged form of eq 6. The result obtained and its uncertainty estimated by propagation of errors methods are given, for completeness, in both Tables 3 and 5. It is worth noting that this $K^\circ_{S,2}$ result is in exceedingly good agreement with the b_0 coefficient obtained from an analysis of the apparent molar isentropic compressions with eq 5 as a second-order polynomial (see Table 4). The values of $K^\circ_{S,2}$ for cytidine in aqueous solution at T = 298.15 K that are available from the literature are shown in Table 3. Our result is in good agreement with that reported by Buckin et al.,[8] but it differs significantly from the other two results.[7,12]

The partial molar compressions presented in Table 5 show that for each nucleoside the value for $K^\circ_{T,2}$ is more positive than the corresponding $K^\circ_{S,2}$ result. For adenosine, the difference between the two partial molar compressions is such that the

values of $K^{o}_{T,2}$ and $K^{o}_{S,2}$ have opposite signs. Such a sign change, which was also noted previously in a study of some neutral *N*-acetyl amino acid amides,[28] is worthy of further comment. In studies of the partial molar compressions of solutes in aqueous solution, it is desirable to determine both the isentropic and isothermal quantities because, from a theoretical viewpoint, $K^{o}_{S,2}$ is more difficult to interpret than $K^{o}_{T,2}$.[21,34] However, as the E^{o}_{2} and $C^{o}_{p,2}$ results that are required to convert the $K^{o}_{S,2}$ into $K^{o}_{T,2}$ using eq 6 are not always available, one is often restricted to using $K^{o}_{S,2}$ results alone. If the $K^{o}_{S,2}$ values for the solutes of interest happen to be reasonably large, e.g., for most electrolytes[35] and for the zwitterionic amino acids and peptides,[36] then the relatively small corrections to convert $K^{o}_{S,2}$ into $K^{o}_{T,2}$ will not substantially alter the interpretation of the results. On the other hand, if the $K^{o}_{S,2}$ values are small, and consequently the conversions of $K^{o}_{S,2}$ into $K^{o}_{T,2}$ can lead to sign changes, as is the case for adenosine, then interpretations based on $K^{o}_{S,2}$ results alone will lead to incorrect conclusions about the hydration of the solutes.

For any solute of low molar mass, the intrinsic volume of the solute molecule is, to a first approximation, independent of pressure.[6,37] Consequently, the partial molar isothermal compression at infinite dilution for a solute in aqueous solution is a property that reflects primarily solute−water interactions. If it is assumed that there are minimal interactions between the base unit and the ribose moiety in nucleosides, as has been suggested by the results of previous studies,[8,12] then the differences among the $K^{o}_{T,2}$ values for the nucleosides can be rationalized in terms of the hydration of the base units. The positive $K^{o}_{T,2}$ value for adenosine indicates that, on average, the water molecules in the hydration shell of the molecule are more compressible than those in the bulk solvent. The value of $K^{o}_{T,2}$ for D-ribose at $T = 298.15$ K, calculated using eq 6 and data taken from the literature ($K^{o}_{S,2} = -(12.4 \pm 0.2)\cdot 10^{-15}$ m$^3\cdot$mol$^{-1}\cdot$Pa^{-1} at $T = 298.15$ K;[38] $E^{o}_{2} = (0.14_4 \pm 0.01)$ cm$^3\cdot$mol$^{-1}\cdot$K^{-1} at $T = 298.15$ K, calculated using V^{o}_{2} data reported by Chalikian;[39] $C^{o}_{p,2} = (283.5 \pm 0.9)$ J$\cdot$K$^{-1}\cdot$mol^{-1} at $T = 298.15$ K[40]), is $K^{o}_{T,2} = -(7.42 \pm 0.4)\cdot 10^{-15}$ m$^3\cdot$mol$^{-1}\cdot$Pa^{-1}. On the basis of this result, it is reasonable to assume that the contribution from the ribose moiety to $K^{o}_{T,2}$ of adenosine will also be negative, and hence the contribution from the base unit to $K^{o}_{T,2}$ must be positive. Presumably this positive contribution arises because the "structure-breaking" nature of the planar purine ring is not sufficiently compensated by hydrogen bonding between water molecules and the polar functional groups of the purine ring. By contrast, the $K^{o}_{T,2}$ values for uridine and cytidine, which have smaller pyrimidine base units, are both negative. The close proximity of polar functional groups on the pyrimidine rings of both cytidine and uridine allows for cooperative hydrogen bonding with water molecules, a feature which is expected to make a significant negative contribution to the $K^{o}_{T,2}$ value.[41]

It is well established that nucleosides self-associate to various degrees in aqueous solution.[42,43] Since the underlying interactions responsible for the association involve parallel alignment of the base moieties, the term base stacking is usually used to describe the aggregates formed.[42] In previous volumetric studies of nucleosides,[7,10] the observed curvature in some of the plots of the apparent molar volumes and apparent molar isentropic compressions as a function of molality was taken as evidence for base stacking. The apparent molar quantities were analyzed accordingly using suitable self-association models.[7,10] The results for cytidine obtained in this work can be analyzed in a similar manner. Using an association model in which each of the successive steps to form a dimer, trimer, tetramer, etc. is assumed to have the same association constant, the stoichiometric molality can be expressed by[44]

$$m = m_1/(1 - Km_1)^2 \qquad (20)$$

where m_1 is the molality of the cytidine monomer and K is the association constant. The value of K determined from an analysis of osmotic coefficient data is 0.87 at $T = 298.15$ K.[45] It is possible to express the partial molar isothermal compression of cytidine in terms of the contributions from the monomer and the molecules in the aggregate as

$$K_{T,2} = x_1 K_{T,2}(\text{mono}) + x_2 K_{T,2}(\text{stack}) \qquad (21)$$

where $K_{T,2}$(mono) is the partial molar isothermal compression of the cytidine monomer; $K_{T,2}$(stack) is the average partial molar isothermal compression of cytidine in the various associated species; and x_1 and x_2 are, respectively, the corresponding mole fractions. Values of x_1 and x_2 were obtained from the m_1 values calculated using eq 20. The molality dependences of $K_{T,2}$(mono) and $K_{T,2}$(stack) can be taken into account using the expression

$$K_{T,2} = x_1\{K^{o}_{T,2}(\text{mono}) + S_k(\text{mono})m_1\} + x_2\{K^{o}_{T,2}(\text{stack}) + S_k(\text{stack})m_2\} \qquad (22)$$

where S_k represents the slope factor and m_2 is the molality of the stacked species. The value of $K^{o}_{T,2}$ calculated using eq 19 corresponds to $K^{o}_{T,2}$(mono) because at infinite dilution only free monomers should exist in solution. Consequently, eq 22 can be rearranged to give

$$K_{T,2} - x_1 K^{o}_{T,2} = S_k(\text{mono})x_1 m_1 + K^{o}_{T,2}(\text{stack})x_2 + S_k(\text{stack})x_2 m_2 \qquad (23)$$

The $K_{T,2}$ results presented in Table 6 were analyzed by weighted least-squares using eq 23 to give the following results: $K^{o}_{T,2}$(stack) = $-(3.4_8 \pm 0.2)\cdot 10^{-15}$ m$^3\cdot$mol$^{-1}\cdot$Pa^{-1}; S_k(stack) = $-(197._3 \pm 1)\cdot 10^{-15}$ m$^3\cdot$kg$\cdot$mol$^{-2}\cdot$Pa^{-1}; S_k(mono) = $(27.7 \pm 0.3)\cdot 10^{-15}$ m$^3\cdot$kg$\cdot$mol$^{-2}\cdot$Pa^{-1}. The most notable feature of these results is that the value of $K^{o}_{T,2}$(stack) is less negative than the value of $K^{o}_{T,2}$ for monomeric cytidine ($K^{o}_{T,2} = -(10.35 \pm 0.06)\cdot 10^{-15}$ m$^3\cdot$mol$^{-1}\cdot$Pa^{-1}). Presumably the larger partial molar isothermal compression per cytidine unit within the associated species arises because the distance between the base units is reduced by an increase in pressure.

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