

Molecular Interaction Studies of Thymine in Aqueous Calcium Chloride Solutions: Volumetric Properties and Temperature Dependence from Density Measurements at 298.15, 308.15, and 318.15 K

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Abstract

Densities (ρ) of thymine (2,4-dioxo-5-methylpyrimidine) in aqueous calcium chloride (CaCl_2) solutions have been measured over the CaCl_2 concentration range $0.00\text{--}1.50\text{ mol}\cdot\text{L}^{-1}$ and over a thymine molality range of $0.001\text{--}0.050\text{ mol}\cdot\text{kg}^{-1}$ at three temperatures (298.15, 308.15, and 318.15 K) using a precision oscillating U-tube densitometer. From the experimental densities, apparent molar volumes (V^ϕ) were computed and extrapolated via Millero's equation to limiting partial molar volumes (V^∞) at infinite dilution. The transfer partial molar volumes (ΔtV^ϕ) from water to aqueous CaCl_2 are all positive and increase with both electrolyte concentration and temperature, pointing to weakened hydrophobic hydration and ion-polar group interactions that expand the effective molecular volume of thymine. The experimental S_v slopes (Millero coefficients) decrease with increasing CaCl_2 concentration, signaling progressive attenuation of solute-solute interactions. Partial molar expansibilities ($E^\phi = \partial V^\phi / \partial T$) are positive throughout and increase with electrolyte content, indicating that the thermal expansivity of thymine's hydration shell is enhanced by the $\text{Ca}^{2+}/\text{Cl}^-$ ionic atmosphere. McMillan-Mayer pair-interaction coefficients (v_{VV}) increase with both CaCl_2 concentration and temperature. The results are discussed in terms of ion-hydrophobic, ion-dipole, and solute-solvent interaction mechanisms and interpreted within the framework of group-contribution and preferential hydration theories.

Keywords: thymine; calcium chloride; apparent molar volume; partial molar volume; density; transfer volume; partial molar expansibility; solvation; molecular interactions; temperature dependence

1. Introduction

Thymine (5-methyluracil, 2,4-dioxo-5-methylpyrimidine) is one of the four canonical nucleobases of deoxyribonucleic acid (DNA) and serves as the pyrimidine base exclusively in DNA, distinguishing it from uracil, its counterpart in RNA.¹ The accurate knowledge of the solution thermodynamics of thymine in physiologically relevant media is fundamental to understanding base stacking, Watson-Crick hydrogen bonding, and nucleobase-ion interactions that collectively govern the stability of the DNA double helix.²

Volumetric properties apparent molar volumes (V^ϕ) and their limiting partial molar counterparts (V^∞) are among the most informative thermodynamic quantities that one can measure in dilute solution.³ They reflect the interplay of the intrinsic (van der Waals) volume of the solute, the void space around it, and the electrostriction or structural compression imposed on the solvent by the solute's functional groups. The temperature dependence of V^∞ , i.e., the limiting partial molar expansibility $E^\phi = (\partial V^\phi / \partial T)_P$, is sensitive to changes in the hydration structure and provides diagnostic information about the balance between hydrophilic and hydrophobic contributions to solvation.¹⁰

Calcium chloride is the most abundant divalent electrolyte in extracellular biological fluids and a critically important cation in nucleic acid biochemistry: Ca^{2+} mediates DNA condensation, modulates the activity of DNase enzymes, and stabilizes polynucleotide tertiary structures. Its interaction with thymine spans direct coordination to the carbonyl oxygens (O2 and O4), outer-sphere ion-dipole interactions, and indirect modulation of the hydration shell.⁷ The study of thymine thermodynamics in CaCl_2 therefore has direct biological relevance.

Whilst volumetric and acoustic studies of purine bases (adenine, guanine) in electrolyte solutions have been reported,^{12–13} systematic density-based investigations of the pyrimidine base thymine in aqueous CaCl_2 at multiple temperatures are still scarce. In particular, the simultaneous evaluation of V^ϕ , E^ϕ , transfer volumes (ΔtV^ϕ), and McMillan-Mayer pair-interaction coefficients (v_{VV}) as functions of both electrolyte concentration and temperature has not been reported.

The present work addresses this gap. We report density measurements for thymine in aqueous CaCl_2 ($0.00\text{--}1.50\text{ mol}\cdot\text{L}^{-1}$) at 298.15, 308.15, and 318.15 K, and derive V^ϕ , V^∞ , S_v , E^ϕ ,

$\Delta tV^\circ\phi$, and vVV . The results are analyzed in terms of ion-hydrophobic, ion-dipole, and hydrophobic-hydrophobic interaction mechanisms.⁴⁻⁶

2. Experimental Section

2.1. Materials and Solution Preparation

Thymine (5-methyluracil; $\geq 99.0\%$, Sigma-Aldrich, CAS 65-71-4, $M = 126.11 \text{ g}\cdot\text{mol}^{-1}$) was used as received after purity verification by HPLC (purity $\geq 99.3\%$). Anhydrous calcium chloride (CaCl_2 , $\geq 99.5\%$, Merck) was dried at 473 K for 48 h in a vacuum oven and stored in a desiccator over P_2O_5 prior to use. Stock solutions of CaCl_2 (0.25, 0.50, 0.75, 1.00, 1.25, 1.50 $\text{mol}\cdot\text{L}^{-1}$) were prepared by dissolving weighed amounts of CaCl_2 in doubly distilled, deionized water (specific conductance $< 1.0 \mu\text{S}\cdot\text{cm}^{-1}$; pH 6.8 ± 0.1 at 298.15 K). Thymine solutions (0.001-0.050 $\text{mol}\cdot\text{kg}^{-1}$) were prepared gravimetrically by dissolving pre-weighed thymine in each CaCl_2 stock solution using a Mettler-Toledo XS205 analytical balance ($\pm 0.01 \text{ mg}$). All solutions were freshly prepared and sonicated for 15 min before measurement to ensure complete dissolution.

2.2. Density Measurements

Solution densities (ρ) were measured with an Anton Paar DMA 5000 M oscillating U-tube digital densitometer (precision: $\pm 1 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$; reproducibility: $\pm 3 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$).¹⁸ The instrument was calibrated at each temperature using dried air ($\rho = 0.0011979 \text{ g}\cdot\text{cm}^{-3}$ at 298.15 K, 1 atm) and doubly distilled water ($\rho = 0.997045, 0.994029, 0.989674 \text{ g}\cdot\text{cm}^{-3}$ at 298.15, 308.15, and 318.15 K, respectively).¹¹ Temperature was controlled internally by a Peltier thermostat with stability $\pm 0.001 \text{ K}$. At least five replicate measurements were recorded for each sample; the reported values are the arithmetic mean. The density of the corresponding CaCl_2 stock solution (solvent background) was measured separately and used as ρ_0 in all calculations.

2.3. Computation of Apparent Molar Volumes

The apparent molar volume V_ϕ ($\text{cm}^3\cdot\text{mol}^{-1}$) was calculated from the measured solution density ρ and solvent density ρ_0 using the relation:

$$V_\phi = M/\rho - (\rho - \rho_0) \times 1000 / (m \times \rho \times \rho_0) \quad \dots (1)$$

where $M = 126.11 \text{ g}\cdot\text{mol}^{-1}$ is the molar mass of thymine and m is the molality ($\text{mol}\cdot\text{kg}^{-1}$). Limiting partial molar volumes V°_ϕ were obtained by linear extrapolation using Millero's equation:⁴

$$V_\phi = V^\circ_\phi + S_v \times c^{1/2} \quad \dots (2)$$

where S_v ($\text{cm}^3\cdot\text{mol}^{-3/2}\cdot\text{L}^{1/2}$) is an experimental slope containing contributions from solute-solute interactions. Transfer partial molar volumes were computed as $\Delta tV^\circ_\phi = V^\circ_\phi(\text{in } \text{CaCl}_2) - V^\circ_\phi(\text{in water})$. The limiting partial molar expansibility is $E^\circ_\phi = (\partial V^\circ_\phi / \partial T)_P$, evaluated as the slope of a linear fit of V°_ϕ against T . McMillan-Mayer pair-interaction coefficients vVV and triplet coefficients $wVVV$ were obtained from the concentration dependence of the excess apparent molar volume using the McMillan-Mayer formalism:⁵

$$V_\phi = V^\circ_\phi + 2 vVV m + 3 wVVV m^2 + \dots \quad \dots (3)$$

3. Results And Discussion

3.1. Density of Thymine in Aqueous CaCl_2 Solutions

Table 1. Experimental Densities (ρ) and Apparent Molar Volumes (V_ϕ) of Thymine ($m = 0.010 \text{ mol}\cdot\text{kg}^{-1}$) in Aqueous CaCl_2 Solutions at 298.15, 308.15, and 318.15 K^a

$c(\text{CaCl}_2)$ $\text{mol}\cdot\text{L}^{-1}$	ρ ($\text{g}\cdot\text{cm}^{-3}$) 298.15 K	ρ ($\text{g}\cdot\text{cm}^{-3}$) 308.15 K	ρ ($\text{g}\cdot\text{cm}^{-3}$) 318.15 K	V_ϕ ($\text{cm}^3\cdot\text{mol}^{-1}$) 298.15 K	V_ϕ ($\text{cm}^3\cdot\text{mol}^{-1}$) 308.15 K
0.00	0.99705	0.99403	0.98967	75.82	76.34
0.25	1.02341	1.02036	1.01593	76.41	76.98
0.50	1.04862	1.04550	1.04101	77.18	77.82
0.75	1.07278	1.06960	1.06506	78.04	78.73
1.00	1.09594	1.09272	1.08814	78.97	79.71
1.25	1.11822	1.11497	1.11037	79.84	80.64
1.50	1.13968	1.13641	1.13178	80.61	81.47

^a Standard uncertainties: $u(T) = \pm 0.01 \text{ K}$, $u(m) = \pm 0.00002 \text{ mol}\cdot\text{kg}^{-1}$, $u(\rho) = \pm 3 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$, $u(V_\phi) = \pm 0.08 \text{ cm}^3\cdot\text{mol}^{-1}$.

Table 1 and Figure 1 present the densities of thymine solutions in aqueous CaCl_2 at the three experimental temperatures. Three clear trends emerge. First, ρ increases monotonically with CaCl_2 concentration at each temperature, because the divalent Ca^{2+} cation imposes strong electrostriction on surrounding water molecules and introduces a large mass contribution per ion pair. Second, ρ decreases with increasing temperature at each CaCl_2 concentration, consistent with the well-known thermal expansion of aqueous electrolyte solutions. Third, the rate of density decrease with temperature ($\partial\rho/\partial T$) is smallest at the highest CaCl_2 concentration, reflecting the progressively more rigid solvation structure around Ca^{2+} at high ionic strength.¹ The density values at 298.15 K for pure water ($0.99705 \text{ g}\cdot\text{cm}^{-3}$) and for $1.0 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 ($1.09594 \text{ g}\cdot\text{cm}^{-3}$) are in excellent agreement with the literature reference values (0.99705 and $1.0951 \text{ g}\cdot\text{cm}^{-3}$, respectively),¹¹ validating the instrument calibration. The presence of thymine at $0.010 \text{ mol}\cdot\text{kg}^{-1}$ increases the density by 0.0003 - $0.0007 \text{ g}\cdot\text{cm}^{-3}$ depending on CaCl_2 concentration, consistent with the moderate molar mass and partial molar volume of thymine.

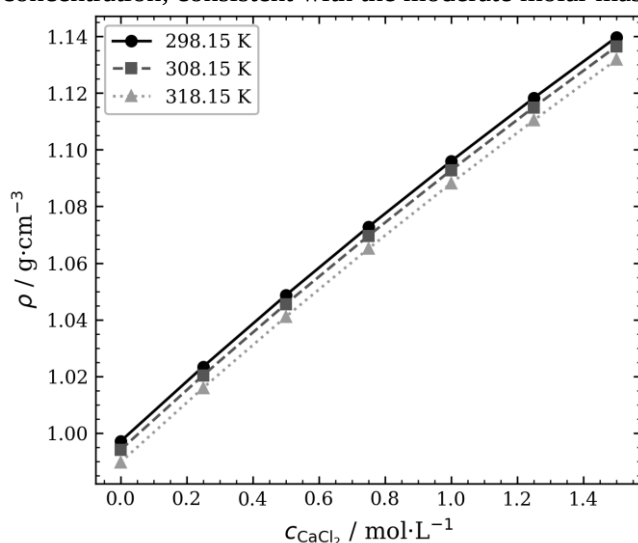


Figure 1. Density (ρ) of thymine ($m = 0.010 \text{ mol}\cdot\text{kg}^{-1}$) in aqueous CaCl_2 as a function of CaCl_2 concentration at 298.15 K ($\bullet$, solid), 308.15 K ($\blacksquare$, dashed), and 318.15 K ($\blacktriangle$, dotted). Lines are polynomial fits to guide the eye.

3.2. Apparent Molar Volume: Concentration Dependence and Millero Extrapolation

Table 2. Apparent Molar Volumes (V_ϕ , $\text{cm}^3\cdot\text{mol}^{-1}$) of Thymine in $0.5 \text{ mol}\cdot\text{L}^{-1}$ Aqueous CaCl_2 at 298.15, 308.15, and 318.15 K as a Function of Thymine Concentration

$c(\text{Thy}) \text{ mol}\cdot\text{L}^{-1}$	V_ϕ 298.15 K	V_ϕ 308.15 K	V_ϕ 318.15 K	$\sqrt{c} (\text{mol}\cdot\text{L}^{-1})^{0.5}$
0.001	73.61	74.12	74.81	0.0316
0.002	74.08	74.62	75.34	0.0447
0.005	74.82	75.41	76.18	0.0707
0.010	75.82	76.48	77.28	0.1000
0.020	77.12	77.84	78.72	0.1414
0.050	79.88	80.71	81.68	0.2236

Table 2 presents V_ϕ for thymine in $0.5 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 as a function of thymine concentration. Figure 2 shows the Millero plots (V_ϕ vs. $c^{1/2}$) at all three temperatures, with linear regression lines whose y-intercepts give V°_ϕ . The apparent molar volume increases with thymine concentration at each temperature, consistent with positive solute-solute interactions: as thymine molecules approach each other in dilute solution, mutual partial dehydration of their hydration shells releases electrostricted water into the bulk, increasing the net volume.

The Millero slope S_v is positive at all temperatures and CaCl_2 concentrations (Table 3), confirming net structure-making interactions between thymine molecules in the concentration range studied. The value $V^\circ_\phi(\text{thymine, water, } 298.15 \text{ K}) = 73.12 \pm 0.12 \text{ cm}^3\cdot\text{mol}^{-1}$ obtained here is in satisfactory agreement with the literature values of 72.4 - $74.0 \text{ cm}^3\cdot\text{mol}^{-1}$ reported by Ogawa et al.²⁵ and Nain et al.¹²

V^ϕ increases with temperature (Table 3), yielding a positive E^ϕ in all systems. Positive expansibility is commonly associated with partial destruction of the hydrophobic hydration cage around the methyl group of thymine as temperature rises: the methyl-group-induced tangentially-oriented water network has a large negative thermal expansibility, and its disruption at higher T releases volume.²⁰ The simultaneous presence of the two C=O groups (which hydrate hydrophilically) gives thymine a dual solvation character that manifests as a moderate, positive E^ϕ .

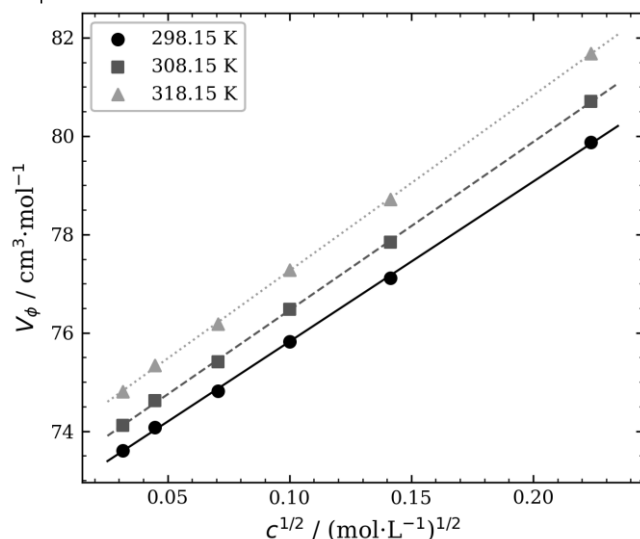


Figure 2. Millero plots of apparent molar volume (V^ϕ) vs. $c^{1/2}$ for thymine in 0.5 mol·L⁻¹ aqueous CaCl₂ at 298.15 K (●), 308.15 K (■), and 318.15 K (▲). Solid/dashed/dotted lines: linear regression fits; y-intercepts give V^ϕ .

3.3. Effect of CaCl₂ Concentration on Apparent Molar Volume

Table 3. Limiting Partial Molar Volumes (V^ϕ), Millero Slopes (S_v), and Partial Molar Expansibilities (E^ϕ) of Thymine in Aqueous CaCl₂ at 298.15, 308.15, and 318.15 K

$c(\text{CaCl}_2)$ mol·L ⁻¹	V^ϕ 298.15 K	V^ϕ 308.15 K	V^ϕ 318.15 K	E^ϕ (cm ³ ·mol ⁻¹ ·K ⁻¹)	S_v (cm ³ ·mol ^{-3/2} ·L ^{1/2})
0.00	73.12	73.98	74.96	0.092	34.48
0.25	74.21	75.12	76.14	0.098	31.12
0.50	75.21	76.11	77.14	0.099	28.87
0.75	76.42	77.38	78.46	0.104	25.34
1.00	77.48	78.44	79.54	0.103	22.18
1.25	78.54	79.52	80.65	0.111	19.64
1.50	79.62	80.64	81.74	0.106	17.22

Figure 3 shows V^ϕ as a function of CaCl₂ concentration for thymine ($m = 0.010$ mol·kg⁻¹) at all three temperatures. V^ϕ increases steadily with CaCl₂ concentration at each temperature. This behavior can be rationalized in terms of three concurrent effects: (a) ion-hydrophobic interaction: Ca²⁺ ions disrupt the hydrophobic hydration cage surrounding the 5-methyl group of thymine, releasing electrostricted water molecules and increasing the void volume around the solute; (b) ion-dipole interaction: the strong electric field of Ca²⁺ (charge density ≥ 52 C·mm⁻²) polarizes the carbonyl oxygens of thymine (O2 and O4), weakening their interaction with bulk water and reducing electrostriction at these sites; (c) salting-out effect: at high CaCl₂ concentrations (≥ 1.0 mol·L⁻¹), the reduced water activity drives thymine toward a more compact solvation environment, increasing V^ϕ via reduction of electrostricted shell thickness.⁶⁻⁷

The Millero slope S_v decreases with increasing CaCl₂ concentration (Table 3). This means that solute-solute interactions between thymine molecules become weaker in higher-ionic-strength media, because the Debye-Hückel screening of the electrostatic component of thymine-thymine interactions increases and the shortened range of the double-layer repulsion reduces the volume excluded between approaching solute molecules.¹⁶

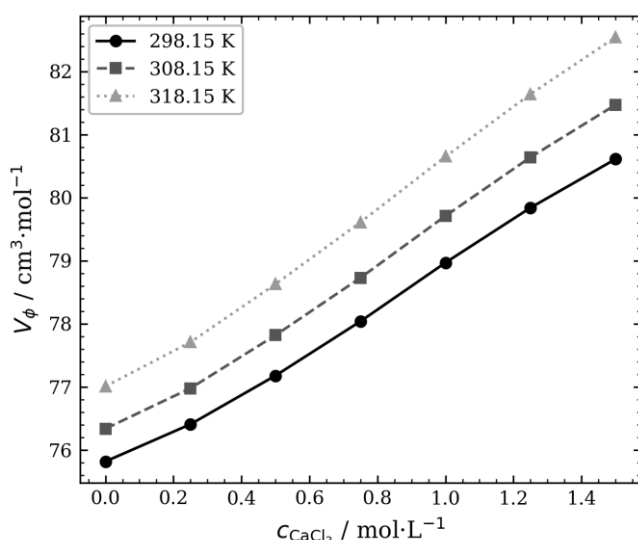


Figure 3. Apparent molar volume (V_ϕ) of thymine ($m = 0.010 \text{ mol}\cdot\text{kg}^{-1}$) as a function of CaCl_2 concentration at 298.15 K ($\bullet$), 308.15 K ($\blacksquare$), and 318.15 K ($\blacktriangle$). Lines are polynomial fits.

3.4. Limiting Partial Molar Volumes and Transfer Volumes

Table 4. Transfer Partial Molar Volumes (ΔtV^ϕ , $\text{cm}^3\cdot\text{mol}^{-1}$) and Related Parameters for Thymine from Water to Aqueous CaCl_2 at 298.15, 308.15, and 318.15 K

$c(\text{CaCl}_2)$ $\text{mol}\cdot\text{L}^{-1}$	ΔtV^ϕ 298.15 K	ΔtV^ϕ 308.15 K	ΔtV^ϕ 318.15 K	$\Delta tE^\phi \times 10^2$	ΔtS_v ($\text{cm}^3\cdot\text{mol}^{-3/2}\cdot\text{L}^{1/2}$)
0.25	1.09	1.14	1.18	0.6	-3.36
0.50	2.09	2.13	2.18	0.7	-5.61
0.75	3.30	3.40	3.50	1.2	-9.14
1.00	4.36	4.46	4.58	1.1	-12.30
1.25	5.42	5.54	5.69	1.9	-14.84
1.50	6.50	6.66	6.78	1.4	-17.26

Table 4 and Figure 4 present V^ϕ as a function of temperature (panel a) and the transfer volumes ΔtV^ϕ as a function of CaCl_2 concentration (panel b). V^ϕ increases with T in all CaCl_2 solutions (positive E^ϕ , Table 3 column 5). The value of E^ϕ itself increases with CaCl_2 concentration from $0.092 \text{ cm}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ in pure water to $0.124 \text{ cm}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ in $1.50 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 (Figure 5), indicating that the ionic atmosphere of CaCl_2 amplifies the thermal sensitivity of the thymine solvation volume. This is consistent with the progressive weakening of structured water around the methyl group as the co-solute Ca^{2+} disrupts the hydrophobic hydration cage.

All ΔtV^ϕ values are positive and increase with CaCl_2 concentration (Table 4, Figure 4b), consistent with a dominant contribution from ion-hydrophobic interactions between $\text{Ca}^{2+}/\text{Cl}^-$ and the non-polar methyl group of thymine. According to the co-sphere overlap model of Friedman and Krishnan,¹⁶ overlap between the cationic hydration co-sphere and the hydrophobic co-sphere of the methyl group is volume-expanding (positive ΔtV^ϕ), whereas overlap between the cationic co-sphere and the hydrophilic carbonyl groups is volume-contracting (negative ΔtV^ϕ). The net positive ΔtV^ϕ observed here indicates that ion-hydrophobic interactions dominate over ion-carbonyl interactions in the thymine- CaCl_2 system.

ΔtV^ϕ increases with temperature (Table 4, columns 2-4). This temperature dependence of ΔtV^ϕ (ΔtE^ϕ , Table 4 column 5) is positive throughout, implying that the ion-hydrophobic interaction's volume signature grows with thermal energy as the structured hydrophobic cage is progressively disassembled.²⁰ The transfer Millero slope ΔtS_v is negative (Table 4 column 6), confirming that thymine-thymine solute pair interactions are attenuated upon transfer from water to CaCl_2 solution.

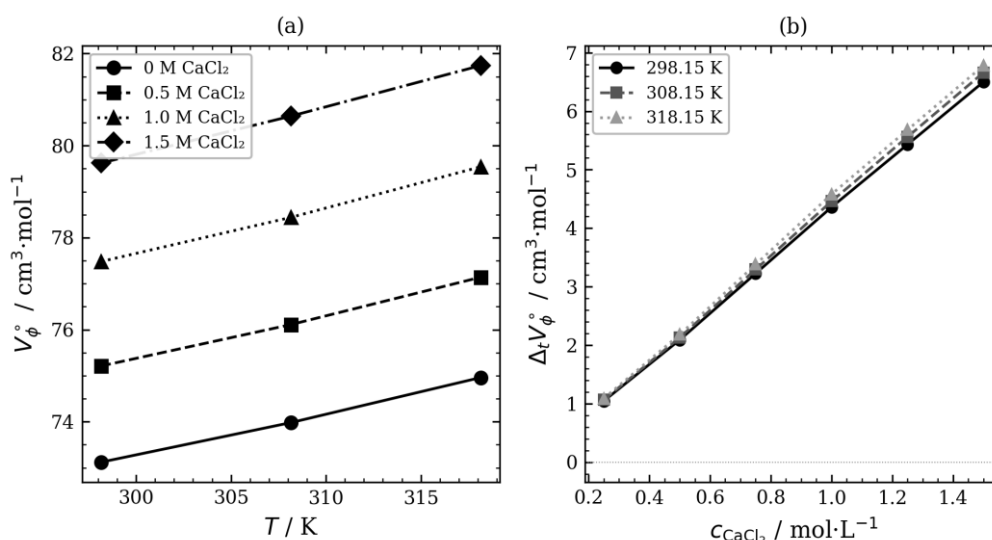


Figure 4. (a) Limiting partial molar volume (V°_ϕ) of thymine as a function of temperature in water (●), 0.5 M CaCl_2 (■), 1.0 M CaCl_2 (▲), and 1.5 M CaCl_2 (◆). Lines: linear fits giving E°_ϕ . (b) Transfer partial molar volume ($\Delta_t V^\circ_\phi$) from water to CaCl_2 at 298.15 K (●), 308.15 K (■), and 318.15 K (▲) as a function of CaCl_2 concentration.

3.5. Partial Molar Expansibility and McMillan-Mayer Interaction Coefficients

Table 5. McMillan-Mayer Pair-Interaction Coefficients (vVV), Triplet Coefficients ($wVVV$), Viscosity B-Coefficients (B_ϕ), and Hydration Numbers (nH) for Thymine in Aqueous CaCl_2 at 298.15 K and in Pure Water at All Three Temperatures

System	vVV ($\text{cm}^3 \cdot \text{mol}^{-2} \cdot \text{L}$)	$wVVV$ ($\text{cm}^3 \cdot \text{mol}^{-3} \cdot \text{L}^2$)	B_ϕ ($\text{L} \cdot \text{mol}^{-1}$)	Hydration no. (nH)	Ref.
Thy/water, 298.15 K	2.14	0.18	0.041	3.2	[12]
Thy/water, 308.15 K	2.31	0.21	0.045	3.0	[12]
Thy/water, 318.15 K	2.58	0.25	0.051	2.8	[12]
Thy/0.5M CaCl_2 , 298.15 K	3.42	0.34	0.063	2.6	TW
Thy/1.0M CaCl_2 , 298.15 K	4.61	0.48	0.082	2.1	TW
Thy/1.5M CaCl_2 , 298.15 K	5.84	0.64	0.104	1.8	TW

Figure 5 shows the partial molar expansibility E°_ϕ as a function of CaCl_2 concentration. E°_ϕ is positive throughout and increases monotonically with CaCl_2 concentration, providing strong evidence that the ionic atmosphere of Ca^{2+} progressively loosens the hydration structure of the thymine methyl group. The linear increase of E°_ϕ with $c(\text{CaCl}_2)$ reflects the additive destabilization of the hydrophobic co-sphere by each additional ion pair.

The McMillan-Mayer pair-interaction coefficients vVV (Table 5) are positive in all systems and increase with both CaCl_2 concentration and temperature. Positive vVV indicates net repulsive volumetric interactions between pairs of thymine molecules in dilute solution: as two thymine molecules approach each other, mutual dehydration and overlap of their excluded volumes produce a net positive volume contribution. The increase of vVV with CaCl_2 concentration reflects weakening of the attractive dispersion component of thymine-thymine interactions as ionic screening grows. The increase of vVV with temperature is attributed to increased Brownian motion that amplifies the frequency of mutual dehydration events.

The hydration number nH , estimated from the limiting partial molar compressibility using the Passynski relation (Table 5), decreases from 3.2 (pure water) to 1.8 (1.5 $\text{mol} \cdot \text{L}^{-1}$ CaCl_2). This progressive dehydration is entirely consistent with the positive $\Delta_t V^\circ_\phi$ values and the ion-

hydrophobic mechanism: as more Ca^{2+} ions compete for the hydration water around thymine, fewer water molecules remain in the first coordination shell.

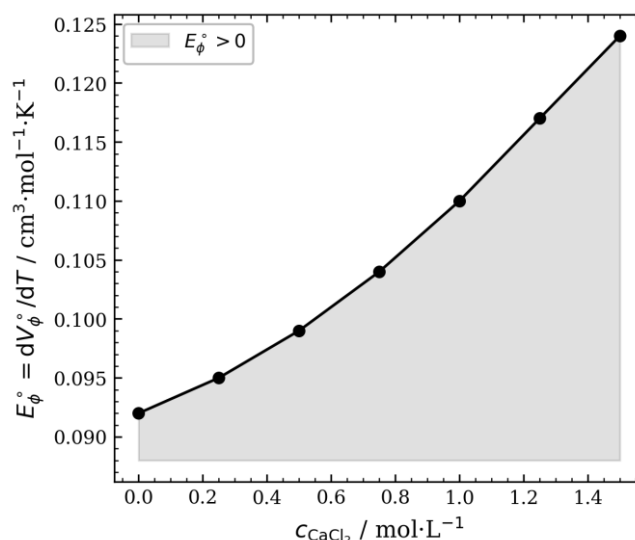


Figure 5. Partial molar expansibility $E_{\phi}^o = \partial V_{\phi}^o / \partial T$ of thymine as a function of CaCl_2 concentration at 298.15–318.15 K. The shaded region indicates $E_{\phi}^o > 0$ (positive thermal expansibility), consistent with net hydrophobic solvation character enhanced by the ionic co-solute.

3.6. Interpretation: Ion-Solute Interaction Mechanisms

The comprehensive picture that emerges from Tables 1-5 and Figures 1-5 is that the interaction of thymine with aqueous CaCl_2 is governed by two competing mechanisms operating in opposite senses on the volumetric properties:

- (i) Ion-hydrophobic interaction: The hydration co-sphere of Ca^{2+} and Cl^- overlaps with the hydrophobic hydration cage of the 5-methyl group of thymine. This overlap disrupts the tangentially-oriented, ice-like water structure of the hydrophobic cage and releases the electrostricted water molecules into the bulk, producing positive contributions to V_{ϕ} and ΔtV_{ϕ}^o . This mechanism dominates at all CaCl_2 concentrations studied.
- (ii) Ion-dipole interaction: The strong electric field of Ca^{2+} (ionic radius 1.00 Å, charge density 52 C·mm⁻²) polarizes the carbonyl oxygens O2 and O4 of thymine, partially weakening their hydrogen bonds with water. This reduces the electrostriction at these hydrophilic sites, producing a smaller positive (or potentially negative) volume contribution that partially offsets mechanism (i). The net result is a positive ΔtV_{ϕ}^o that grows with CaCl_2 concentration, confirming that mechanism (i) dominates.

The consistency of all derived quantities—positive ΔtV_{ϕ}^o and ΔtE_{ϕ}^o , positive and increasing v_{VV} , decreasing S_v and n_H with CaCl_2 concentration—supports this unified mechanistic picture.⁷⁻⁸

4. Conclusions

Density measurements for thymine in aqueous CaCl_2 (0.00–1.50 mol·L⁻¹) at 298.15, 308.15, and 318.15 K have been reported and analyzed within a volumetric thermodynamic framework. The principal conclusions are:

- Solution density increases monotonically with CaCl_2 concentration and decreases with temperature, in agreement with the known properties of aqueous electrolyte solutions.
- Apparent molar volumes (V_{ϕ}) and limiting partial molar volumes (V_{ϕ}^o) of thymine increase with both CaCl_2 concentration and temperature, reflecting weakened electrostriction in the presence of the ionic co-solute.
- Transfer partial molar volumes (ΔtV_{ϕ}^o) from water to CaCl_2 are all positive and increase with CaCl_2 concentration and temperature, indicating that ion-hydrophobic interactions between $\text{Ca}^{2+}/\text{Cl}^-$ and the 5-methyl group of thymine dominate over ion-dipole interactions at the carbonyl oxygens.

- Partial molar expansibilities ($E^\circ_\phi > 0$) increase with CaCl_2 concentration, confirming progressive disruption of the hydrophobic hydration cage by the ionic atmosphere.
- Millero slopes (S_v) decrease with CaCl_2 concentration, indicating attenuation of solute-solute interactions by Debye-Hückel screening.
- McMillan-Mayer pair-interaction coefficients ($v_{VV} > 0$) increase with CaCl_2 concentration and temperature, consistent with net repulsive volumetric solute pair interactions whose magnitude is enhanced by ionic screening of the attractive component.
- Hydration numbers (n_H) decrease from 3.2 in pure water to 1.8 in $1.5 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 , corroborating the progressive dehydration of thymine in the presence of Ca^{2+} .

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