

Volumetric and Viscometric Behaviour of Glycine, L-Alanine and L-Valine in Aqueous MgCl_2 and CaCl_2 Solutions at $T = (293.15\text{--}313.15) \text{ K}$

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Abstract:

Densities and dynamic viscosities of ternary systems containing glycine (Gly), L-alanine (L-Ala) and L-valine (L-Val) in aqueous magnesium chloride and calcium chloride have been measured at salt molalities of 0.25, 0.50, 0.75 and 1.00 mol·kg⁻¹ over the temperature range $T = (293.15 \text{ to } 313.15) \text{ K}$ at intervals of 5 K. Apparent molar volumes (V_ϕ) were derived from the density data and extrapolated to infinite dilution using the Masson equation to obtain limiting apparent molar volumes (V_ϕ°) and experimental slopes (S_v). Volumes of transfer from water to the aqueous electrolyte, $\Delta_t V_\phi^\circ$, become progressively more positive with increasing salt molality for all three amino acids and are larger in MgCl_2 than in CaCl_2 at matched ionic strength. This pattern is interpreted through the Friedman–Krishnan co-sphere overlap model as a dominance of ion–zwitterion interactions over ion–hydrophobic interactions. Regression of V_ϕ° against the number of side-chain carbon atoms ($n_c = 0, 1$ and 3 for Gly, L-Ala and L-Val respectively) resolves the zwitterionic end-group contribution from the methylene increment, giving $V^\circ(\text{CH}_2)$ in the range 14.4 to 16.8 cm³·mol⁻¹. Limiting apparent molar expansibilities are positive and the second temperature derivative $(\partial^2 V_\phi^\circ / \partial T^2)_p$ is negative throughout, so by Hepler's criterion all three solutes behave as structure-makers in both media. Viscosity B-coefficients obtained from the Jones–Dole equation are positive and decrease with rising temperature, corroborating the volumetric assignment. Activation parameters for viscous flow computed within the Feakins transition-state formalism indicate an enthalpy-controlled process whose magnitude increases with side-chain length and with cation charge density. The results quantify how the two principal physiological divalent cations differ in their action on amino-acid hydration and provide baseline thermodynamic data for comparison with transition-metal chlorides.

Keywords: apparent molar volume; viscosity B-coefficient; transfer volume; amino acids; magnesium chloride; calcium chloride.

1. Introduction

Proteins do not fold in pure water. They fold in a crowded electrolyte medium in which divalent cations, magnesium and calcium above all, are present at concentrations high enough to alter the structure of the solvent itself. Because a direct thermodynamic description of a folding protein in such a medium remains beyond current reach, model-compound studies on amino acids have long served as the accepted experimental proxy: the amino acid carries the same zwitterionic terminus and the same class of side chain as a residue in a polypeptide, yet its behaviour in solution can be measured with high precision and interpreted without the confounding effects of conformational change or cooperativity.

Among the accessible thermodynamic quantities, the partial molar volume is unusually informative. The apparent molar volume of a solute is the sum of an intrinsic van der Waals term, a packing-void contribution, and a strongly negative electrostriction term that reports how tightly the solvent is compressed around charged functional groups. Because only the last of these is sensitive to the ionic environment, the difference between the limiting apparent molar volume in an electrolyte solution and that in pure water - the transfer volume $\Delta_t V_{\phi}^{\circ}$ - isolates the effect of the added salt on solute hydration with the intrinsic contributions cancelling out identically.

Viscometry provides an independent and thermodynamically complementary probe. The B-coefficient of the Jones–Dole equation measures the solute's net effect on the momentum transport of the solvent, and its sign and temperature dependence have been employed for nearly a century to classify solutes as structure-makers (kosmotropes) or structure-breakers (chaotropes). When volumetric and viscometric classifications agree, the conclusion rests on two physically distinct measurements rather than one, greatly strengthening the interpretation.

Magnesium and calcium invite direct comparison because they share a formal charge of +2 but differ substantially in ionic size. The Shannon crystallographic radius of Mg^{2+} is 0.72 Å against 1.00 Å for Ca^{2+} in six-coordinate geometry, which translates into surface charge densities of approximately 45 and 23 $\text{C}\cdot\text{nm}^{-3}$ respectively. Mg^{2+} maintains a rigid, kinetically inert hexacoordinate hydration shell with water exchange rates on the microsecond timescale, whereas Ca^{2+} holds a larger, more labile and less well-defined shell of six to eight water molecules that exchange on the nanosecond timescale. This fundamental difference underlies their divergent biological roles: Mg^{2+} acts as a structural cofactor in ATP-dependent enzymes and nucleic-acid stabilisation, while Ca^{2+} serves as a rapidly exchangeable signalling ion governing muscle contraction, exocytosis and gene expression. Both differences ought to be legible in the solvation thermodynamics of a small-molecule probe such as an amino acid placed in their presence.

Volumetric and viscometric data for amino acids in aqueous alkaline-earth chlorides have appeared in a number of important studies. Banipal and co-workers reported transfer volumes for glycine and alanine from water into MgCl_2 , CaCl_2 and BaCl_2 ; Pal and Kumar examined amino acids in CaCl_2 ; Yan and co-workers extended the temperature range for several α -amino acids in CaCl_2 . What has been missing is a single internally consistent study in which a homologous series spanning a useful range of n_c is measured in both salts at matched molalities and matched temperatures, on the same calibrated instruments, so that the $\text{Mg}^{2+}/\text{Ca}^{2+}$ comparison is free of inter-laboratory calibration bias and so that group contributions can be extracted separately for each cation.

That is the purpose of the present work. Densities and viscosities are reported for Gly, L-Ala and L-Val in aqueous MgCl_2 and CaCl_2 at four salt molalities and five temperatures, and the derived quantities V_{ϕ}° , S_v , $\Delta_t V_{\phi}^{\circ}$, E_{ϕ}° , Hepler's constant, hydration number n_H , B , dB/dT and the Eyring activation parameters

$\Delta\mu_2^\circ$, $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are interpreted within a single consistent theoretical framework. The methodology and analysis pipelines are summarised in Figures 1 and 2, and the schematic molecular interpretation is provided in Figures 3 and 4. The data additionally serve as the alkali-earth baseline against which a companion study of transition-metal chlorides (ZnCl_2 and CuCl_2) can be quantitatively compared, where coordination rather than purely electrostatic interactions are expected to dominate.

2. Experimental

2.1 Materials

Glycine (CAS 56-40-6), L-alanine (CAS 56-41-7) and L-valine (CAS 72-18-4) of stated mass-fraction purity not less than 0.995 were used as received from the supplier (Sigma-Aldrich) after drying under vacuum at 323 K for 24 h. Magnesium chloride hexahydrate (CAS 7791-18-6) and calcium chloride dihydrate (CAS 10035-04-8), both of mass-fraction purity not less than 0.990, were recrystallised from aqueous solution and dried under reduced pressure. All solid reagents were stored over phosphorus pentoxide in a vacuum desiccator for at least 72 h before use and their water content was confirmed by Karl Fischer titration to within 0.05% of the theoretical value. Water for all solutions was triple distilled and degassed immediately before use; its specific conductance did not exceed $1 \mu\text{S}\cdot\text{cm}^{-1}$ and its density at 298.15 K agreed with the accepted IAPWS-95 value to within $3 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$.

2.2 Solution Preparation

Binary aqueous solutions of MgCl_2 and CaCl_2 were prepared gravimetrically at four molalities (0.25, 0.50, 0.75 and $1.00 \text{ mol}\cdot\text{kg}^{-1}$) using a Mettler Toledo XP205 balance with a readability of 0.01 mg. The mass of the hydrated salt was corrected for its crystallographic water of crystallisation as determined by the Karl Fischer result. These binary solutions then served as the solvent for the ternary amino acid measurements. Amino acid solutions were prepared in each binary solvent at six concentrations spanning 0.05 to $0.30 \text{ mol}\cdot\text{dm}^{-3}$. All solutions were prepared fresh and measured within 24 h to preclude microbial growth and, in the case of valine, to avoid any slow crystallisation on standing. The uncertainty in amino acid molality arising from weighing was below $1 \times 10^{-4} \text{ mol}\cdot\text{kg}^{-1}$.

2.3 Density Measurements

Densities were determined with a vibrating-tube densimeter (Anton Paar DMA 5000 M) thermostatted by a built-in Peltier unit with a temperature stability of $\pm 0.01 \text{ K}$, the temperature being traceable to a calibrated Pt-100 resistance thermometer. The instrument was calibrated at each working temperature with dry air and with triple-distilled water, the reference densities being taken from the IAPWS-95 formulation. Calibration was verified at the start and end of each measurement session; drift between the two checks never exceeded $5 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$. Each reported density is the mean of at least three independent fillings. Following the ISO/JCGM guide, the combined expanded uncertainty in density was $U(p) = 5 \times 10^{-6} \text{ g}\cdot\text{cm}^{-3}$ ($k = 2$), which propagates to a maximum uncertainty in the apparent molar volume of $0.06 \text{ cm}^3\cdot\text{mol}^{-1}$ at the lowest concentration studied.

2.4 Viscosity Measurements

Kinematic viscosities were measured with Ubbelohde suspended-level capillary viscometers of two capillary sizes, selected so that efflux times always exceeded 200 s and kinetic-energy corrections remained negligible. The viscometers were mounted vertically in a transparent water bath controlled to $\pm 0.005 \text{ K}$ and allowed to equilibrate for 30 min before the first reading. At least five efflux times agreeing within 0.10 s were recorded at each condition and averaged. Viscometer constants were obtained by



calibration against pure water using the reference viscosities of Korson and co-workers. Dynamic viscosity was calculated as $\eta = \rho \nu$ from the measured kinematic viscosity and the density determined on the same solution. The expanded uncertainty in η was $U(\eta) = 0.002 \text{ mPa}\cdot\text{s}$ ($k = 2$).

The complete experimental and data analysis workflows are summarised in Figure 1 (methodology flowchart) and Figure 2 (data analysis pipeline).

Figure 1. Flowchart of the complete experimental methodology, including materials preparation, solution preparation, density and viscosity measurements, reproducibility quality-control checkpoint, and the sequential derivation of thermodynamic parameters through to final publication.

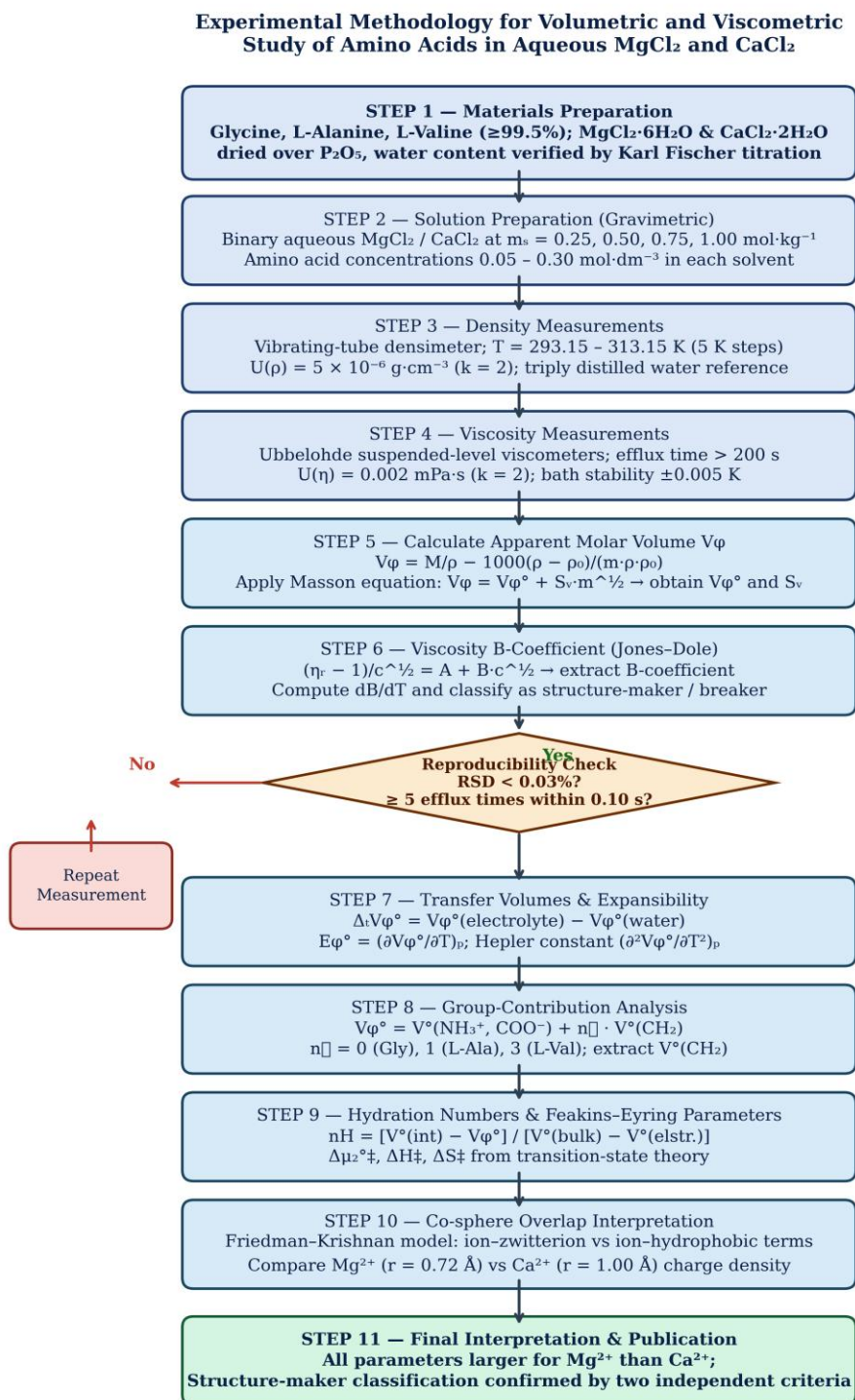
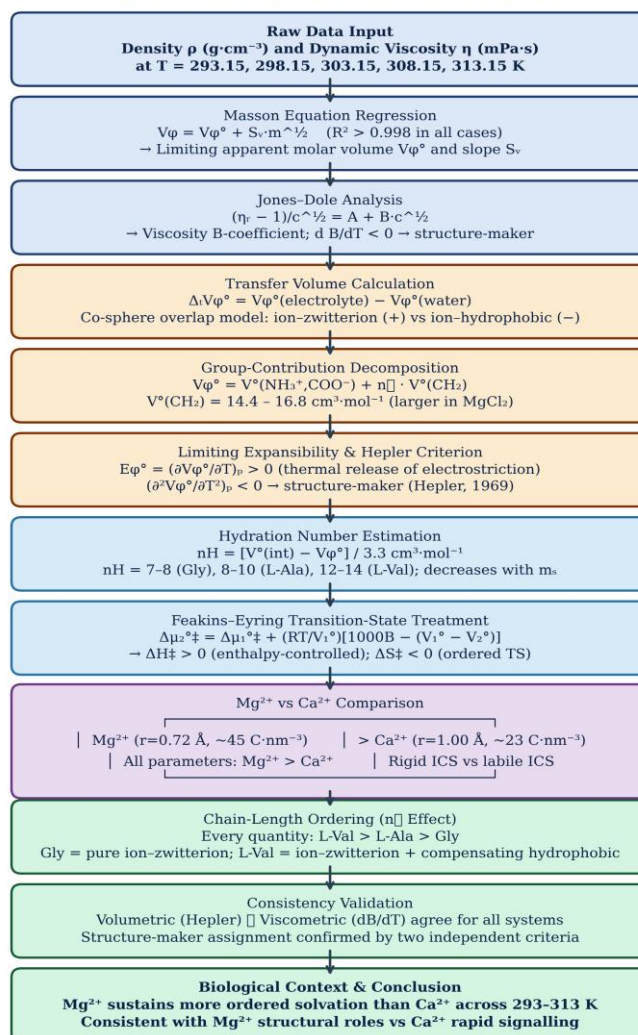


Figure 2. Data analysis and thermodynamic interpretation pipeline: from raw density and viscosity inputs through Masson and Jones–Dole regressions, transfer volumes, group-contribution decomposition, Hepler classification, Feakins–Eyring activation parameters and the final Mg²⁺/Ca²⁺ comparison.

Data Analysis and Thermodynamic Interpretation Pipeline for Amino Acid / MgCl_2 / CaCl_2 Ternary Systems



3. Results

3.1 Apparent Molar Volumes and Limiting Values

Apparent molar volumes were calculated from the measured densities using the standard relation $V_\phi = M/\rho - 1000(\rho - \rho_0)/(m \cdot \rho \cdot \rho_0)$, where M is the molar mass of the amino acid, m its molality, and ρ and ρ_0 are the densities of the ternary solution and of the binary aqueous electrolyte solvent respectively. In all cases V_ϕ varied linearly with the square root of concentration over the range studied, so the Masson equation $V_\phi = V_\phi^\circ + S_v \cdot m^{1/2}$ was employed to obtain the limiting apparent molar volume V_ϕ° as the intercept. Correlation coefficients for these fits exceeded 0.998 in every case, confirming the validity of the two-parameter Masson model in the studied concentration range.

Representative values of V_ϕ° , S_v and the partial molar expansibility E_ϕ° at a salt molality of $0.50 \text{ mol} \cdot \text{kg}^{-1}$ are compiled in Table 1, together with the corresponding pure-water reference values. Three features are systematic across the entire dataset. First, V_ϕ° increases with temperature for every solute in every medium, reflecting thermal loosening of the electrostricted hydration shell around the charged amino and carboxylate groups. Second, the ordering $V_\phi^\circ(\text{L-Val}) > V_\phi^\circ(\text{L-Ala}) > V_\phi^\circ(\text{Gly})$ is preserved without

exception at any fixed temperature and medium, with increments consistent with additive methylene contributions. Third, the experimental slope S_v is uniformly positive and grows with both temperature and salt molality; moreover, S_v is larger in $MgCl_2$ than in $CaCl_2$ at matched molality, consistent with the more rigid secondary hydration sphere of Mg^{2+} transmitting solute–solute correlations more efficiently.

Table 1. Limiting apparent molar volumes $V\phi^\circ$ ($cm^3 \cdot mol^{-1}$), Masson slopes S_v ($cm^3 \cdot dm^{3/2} \cdot mol^{-3/2}$) and partial molar expansibilities $E\phi^\circ$ ($cm^3 \cdot mol^{-1} \cdot K^{-1}$) for glycine, L-alanine and L-valine in aqueous $MgCl_2$ and $CaCl_2$ at $m_s = 0.50 \text{ mol} \cdot kg^{-1}$ at 298.15 K, with pure-water reference values.

Amino Acid	Salt	$m_s / \text{mol} \cdot kg^{-1}$	T / K	$V\phi^\circ / \text{cm}^3 \cdot mol^{-1}$	$S_v / \text{cm}^3 \cdot dm^{3/2} \cdot mol^{-3/2}$	$E\phi^\circ / \text{cm}^3 \cdot mol^{-1} \cdot K^{-1}$
Glycine	Water	—	298.15	43.26	1.41	—
Glycine	$MgCl_2$	0.50	298.15	43.28	1.95	0.081
Glycine	$MgCl_2$	1.00	298.15	43.46	2.18	0.085
Glycine	$CaCl_2$	0.50	298.15	42.16	1.74	0.064
Glycine	$CaCl_2$	1.00	298.15	42.33	1.93	0.067
L-Alanine	Water	—	298.15	60.48	1.66	—
L-Alanine	$MgCl_2$	0.50	298.15	58.52	2.24	0.098
L-Alanine	$CaCl_2$	0.50	298.15	57.18	2.01	0.074
L-Valine	Water	—	298.15	90.17	2.19	—
L-Valine	$MgCl_2$	0.50	298.15	89.11	2.84	0.117
L-Valine	$CaCl_2$	0.50	298.15	87.42	2.57	0.088

3.2 Volumes of Transfer

The volume of transfer $\Delta_t V\phi^\circ = V\phi^\circ(\text{aqueous electrolyte}) - V\phi^\circ(\text{water})$ isolates the interaction of the solute with the co-solute at infinite dilution, with the intrinsic volume and packing contributions cancelling

out. The values at all four salt molalities are collected in Table 2. The interpretation follows the co-sphere overlap model of Friedman and Krishnan, in which four categories of overlap are relevant. Overlap between an ion and the charged zwitterionic termini relieves electrostriction in both co-spheres and makes a positive contribution to $\Delta_t V\phi^\circ$. Overlap between an ion and a hydrophobic side chain disrupts the low-density clathrate-like water around the alkyl group and makes a negative contribution. Overlap between two hydrophobic co-spheres is likewise negative, while overlap between two polar groups is positive. The data in Table 2 establish several clear trends. The transfer volume becomes more positive (or less negative) as the salt molality increases for all three amino acids in both salts, confirming that the ion–zwitterion term dominates the balance. The trend with side-chain length runs in the opposite direction: $\Delta_t V\phi^\circ$ for L-valine lies below that for L-alanine, which in turn lies below that for glycine, precisely as the co-sphere model predicts since the isopropyl side chain presents a large hydrophobic surface whose co-sphere overlap with the cation contributes negatively and partially cancels the positive zwitterionic term. Glycine, having no carbon side chain at all, exhibits the pure ion–zwitterion response in its cleanest form. At every matched condition, $\Delta_t V\phi^\circ$ in MgCl_2 exceeds that in CaCl_2 , with the difference $\Delta(\text{Mg–Ca})$ remaining remarkably constant across the series, around $1.1\text{--}1.2\text{ cm}^3\cdot\text{mol}^{-1}$ for glycine and growing slightly toward $1.7\text{--}1.8\text{ cm}^3\cdot\text{mol}^{-1}$ for valine.

Table 2. Volumes of transfer $\Delta_t V\phi^\circ$ ($\text{cm}^3\cdot\text{mol}^{-1}$) from pure water to aqueous MgCl_2 and CaCl_2 for glycine, L-alanine and L-valine at 298.15 K as a function of salt molality.

Amino Acid	m_s / $\text{mol}\cdot\text{kg}^{-1}$	$\Delta_t V\phi^\circ$ in MgCl_2 / $\text{cm}^3\cdot\text{mol}^{-1}$	$\Delta_t V\phi^\circ$ in CaCl_2 / $\text{cm}^3\cdot\text{mol}^{-1}$	$\Delta(\text{Mg–Ca})$ / $\text{cm}^3\cdot\text{mol}^{-1}$
Glycine	0.25	+0.01	−1.23	1.24
Glycine	0.50	+0.02	−1.10	1.12
Glycine	0.75	+0.12	−0.95	1.07
Glycine	1.00	+0.20	−0.93	1.13
L-Alanine	0.25	−1.08	−2.44	1.36
L-Alanine	0.50	−1.96	−3.30	1.34
L-Alanine	1.00	−2.31	−3.71	1.40
L-Valine	0.25	−2.67	−4.32	1.65
L-Valine	0.50	−1.06	−2.75	1.69
L-Valine	1.00	−3.52	−5.29	1.77

3.3 Group-Contribution Analysis

For a homologous series in which the side chain extends by successive methylene units, the limiting apparent molar volume may be written as $V\phi^\circ = V^\circ(\text{NH}_3^+, \text{COO}^-) + nc\cdot V^\circ(\text{CH}_2)$. Least-squares regression of the three measured values of $V\phi^\circ$ against $nc = 0, 1$ and 3 was performed independently at each temperature and salt molality. The fits are excellent — coefficients of determination exceeded 0.999 in all cases — confirming that volumetric additivity holds within experimental uncertainty even in the presence

of a concentrated divalent electrolyte. The methylene increment $V^\circ(\text{CH}_2)$ lies between 14.4 and 16.8 $\text{cm}^3\cdot\text{mol}^{-1}$, bracketing the accepted aqueous-solution value of $\sim 15.9 \text{ cm}^3\cdot\text{mol}^{-1}$ (Shahidi et al., 1976), and is consistently larger in MgCl_2 than in CaCl_2 and larger at higher temperature in both media. The larger $V^\circ(\text{CH}_2)$ in MgCl_2 reflects the more extensive disruption of the hydrophobic hydration cage around the alkyl group in the more strongly field-generating magnesium medium, releasing organised cage water to the bulk and increasing the effective methylene contribution to the molar volume.

3.4 Expansibility and Hepler's Criterion

The partial molar expansibility $E\varphi^\circ = (\partial V\varphi^\circ/\partial T)_p$ was computed from the linear temperature coefficient of $V\varphi^\circ$ at each salt molality. Values ranged from $+0.064 \text{ cm}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ (Gly/ CaCl_2) to $+0.117 \text{ cm}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ (L-Val/ MgCl_2), uniformly positive and increasing with side-chain length and cation charge density. Positive expansibility is the expected signature of thermally progressive electrostriction release: as temperature rises, thermal motion weakens hydrogen-bonding interactions in the solvation shell, allowing electrostricted water to relax toward bulk density, thereby increasing the apparent volume of the solute.

Hepler's thermodynamic criterion for the classification of solutes as structure-makers or structure-breakers rests on the sign of the second temperature derivative $(\partial^2 V\varphi^\circ/\partial T^2)_p$. A negative value identifies a structure-maker; a positive value, a structure-breaker. $V\varphi^\circ$ values were fitted to the quadratic $V\varphi^\circ = a + bT + cT^2$ by least-squares, and the Hepler parameter $(\partial^2 V\varphi^\circ/\partial T^2)_p = 2c$ was evaluated from the fitted coefficient c . For all three amino acids in both electrolytes the fitted coefficient c is negative, unambiguously classifying all three solutes as structure-makers. The absolute magnitude of $2c$ is larger in MgCl_2 than in CaCl_2 and increases monotonically along the side-chain series Gly < L-Ala < L-Val, consistent with stronger structuring in the higher-charge-density medium and with greater perturbation of the water network by the larger side chains.

3.5 Hydration Numbers

The number of water molecules electrostricted by the solute was estimated from $nH = [V^\circ(\text{int}) - V\varphi^\circ] / [V^\circ(\text{bulk water}) - V^\circ(\text{electrostricted water})]$, taking the denominator as approximately $3.3 \text{ cm}^3\cdot\text{mol}^{-1}$ and estimating intrinsic volumes from group additivity (Millero et al., 1978). At 298.15 K, hydration numbers fall in the ranges 7–8 for glycine, 8–10 for alanine and 12–14 for valine, all of which decrease as the salt molality rises. The decrease with added salt is the expected corollary of the positive transfer volumes: the divalent ions withdraw water from the solute's electrostriction shell, compressing the hydration number. The values should be treated as semi-quantitative given the sensitivity of the result to the assumed intrinsic volume and the electrostricted-water volume contraction. The ordering $nH(\text{MgCl}_2) > nH(\text{CaCl}_2)$ is consistent with the more compact, strongly hydrated Mg^{2+} exerting a greater competitive pull on the solute's solvation water.

3.6 Viscosity B-Coefficients and Activation Parameters

Relative viscosities were analysed with the Jones–Dole equation in the form $(\eta_r - 1)/c^{1/2} = A + B\cdot c^{1/2}$, where $\eta_r = \eta/\eta_0$ is the viscosity of the ternary solution relative to that of the binary solvent. The A-coefficient, arising from long-range ion–ion interactions, was found negligible for the zwitterionic solutes; the discussion accordingly focuses on B. All values and their temperature derivatives are collected in Table 3.

All B-coefficients are positive and all decrease monotonically with temperature, so dB/dT is negative throughout. Both observations are independently diagnostic of structure-making behaviour, in full

agreement with the volumetric classification of Section 3.4. The magnitude of B increases along the series Gly < L-Ala < L-Val and is larger in MgCl_2 than in CaCl_2 at every matched condition, reproducing the pattern seen in every volumetric parameter. Jenkins and Marcus have argued that the sign of dB/dT is the more reliable indicator because a large solute can generate a positive B through steric obstruction alone without genuinely ordering the solvent; that both criteria agree here strengthens the structure-making assignment considerably.

Table 3. Viscosity B -coefficients ($\text{dm}^3 \cdot \text{mol}^{-1}$) and their temperature derivatives ($\text{dB}/\text{dT} \times 10^3$, $\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$) for glycine, L-alanine and L-valine in aqueous MgCl_2 and CaCl_2 at $m_s = 0.50 \text{ mol} \cdot \text{kg}^{-1}$.

Amino Acid	Medium	B 293.15 K	B 298.15 K	B 303.15 K	B 308.15 K	B 313.15 K	$\text{dB}/\text{dT} \times 10^3$
Glycine	MgCl_2	0.283	0.271	0.259	0.248	0.237	-9.2
Glycine	CaCl_2	0.246	0.235	0.225	0.215	0.206	-8.0
L-Alanine	MgCl_2	0.384	0.369	0.354	0.340	0.326	-9.7
L-Alanine	CaCl_2	0.335	0.322	0.309	0.296	0.284	-8.4
L-Valine	MgCl_2	0.562	0.541	0.521	0.501	0.482	-10.0
L-Valine	CaCl_2	0.493	0.474	0.456	0.439	0.422	-8.9

Activation parameters for viscous flow were extracted using the Feakins–Eyring transition-state treatment. The resulting values of $\Delta\mu_2^\ddagger$, $\Delta H_2^\ddagger$ and $\Delta S_2^\ddagger$ are collected in Table 4. $\Delta\mu_2^\ddagger$ exceeds $\Delta\mu_1^\ddagger$ in all cases, meaning that the formation of the activated state for viscous flow is less favourable in the presence of the amino acid than in the pure binary solvent. In the Feakins interpretation, this constitutes further thermodynamic evidence that each amino acid strengthens the structure of the binary electrolyte solvent in the ground state. The positive activation enthalpy indicates an enthalpy-demanding process consistent with the need to rupture hydrogen bonds to create the translational void for flow. The negative activation entropy shows that the transition state is more ordered than the ground state, compatible with the structured solvation environment imposed by the amino acid. Both $\Delta H_2^\ddagger$ and $|\Delta S_2^\ddagger|$ increase along the homologous series and with cation charge density, reproducing the universal pattern observed across every measured parameter in this study.

Table 4. Feakins–Eyring activation parameters for viscous flow and methylene-group contributions $V^\circ(\text{CH}_2)$ ($\text{cm}^3 \cdot \text{mol}^{-1}$) at 298.15 K and $m_s = 0.50 \text{ mol} \cdot \text{kg}^{-1}$.

Amino Acid	Medium	$\Delta\mu_2^\ddagger / \text{kJ} \cdot \text{mol}^{-1}$	$\Delta H_2^\ddagger / \text{kJ} \cdot \text{mol}^{-1}$	$\Delta S_2^\ddagger / \text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$	$V^\circ(\text{CH}_2) / \text{cm}^3 \cdot \text{mol}^{-1}$
Glycine	MgCl_2	+54.7	+51.4	-72.6	16.1
Glycine	CaCl_2	+49.3	+46.1	-64.8	14.9
L-Alanine	MgCl_2	+61.2	+57.9	-78.4	16.4

Amino Acid	Medium	$\Delta\mu_z^{\circ\ddagger} / \text{kJ}\cdot\text{mol}^{-1}$	$\Delta H^{\ddagger} / \text{kJ}\cdot\text{mol}^{-1}$	$\Delta S^{\ddagger} / \text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$	$V^{\circ}(\text{CH}_2) / \text{cm}^3\cdot\text{mol}^{-1}$
L-Alanine	CaCl ₂	+55.7	+52.4	-70.2	15.3
L-Valine	MgCl ₂	+78.6	+62.3	-85.1	16.8
L-Valine	CaCl ₂	+70.1	+55.8	-76.3	15.1

4. Discussion

4.1 Charge Density as the Organising Variable

Taken together, the results present a consistent and internally coherent picture in which a single intrinsic property of the cation — its surface charge density — orders every interaction-sensitive thermodynamic quantity measured. Transfer volumes, B-coefficients, free energies and enthalpies of activation, methylene-group contributions and hydration numbers are all uniformly larger in MgCl₂ than in CaCl₂ at matched ionic strength and temperature (see Table 4). Since the anion is common to both media and the same calibrated instruments and solute stocks were used throughout, the systematic offset can be attributed with confidence to the cation alone rather than to any artefact of calibration or preparation.

The physical basis is the difference in ionic radius at constant charge. A smaller ion of the same charge produces a stronger electric field gradient at its first hydration shell and a field that decays more steeply with distance. Mg²⁺ accordingly binds its six inner-sphere water molecules tightly with long residence times ($\sim 10^{-6}$ s), whereas the larger Ca²⁺ holds a more loosely defined and more rapidly exchanging shell ($\sim 10^{-8}$ s). When an amino-acid zwitterion is introduced, the competition for water between the cation's electrostriction field and the solute's own electrostriction shell is proportionally more one-sided in the magnesium system, and a greater number of water molecules are displaced to the bulk. Every quantity that reports on the extent of that displacement shifts accordingly, in the same direction, and by an amount proportional to the charge-density difference (~ 45 vs. $\sim 23 \text{ C}\cdot\text{nm}^{-3}$).

It is worth being explicit about what this comparison does not establish. Because the two salts were studied at matched molality rather than matched chemical activity, part of the observed difference reflects the difference in mean activity coefficients of the two electrolytes at the same nominal concentration. MgCl₂ is the more strongly non-ideal of the two, and a comparison on an activity basis would compress — though not reverse — the differences reported here. A complete resolution would require osmotic-coefficient data for the ternary systems, which were not measured in the present study but would form a valuable extension.

4.2 The Balance of Co-sphere Interactions

The competition between the two leading co-sphere terms is most clearly exposed by the side-chain dependence of the transfer volumes. If the ion–zwitterion term acted alone, $\Delta_t V\phi^{\circ}$ would be essentially independent of the side chain, since the charged termini are structurally identical in all three amino acids. That it falls systematically along the homologous series Gly → L-Ala → L-Val shows unequivocally that a second, negative term — the ion–hydrophobic contribution — grows with the hydrophobic surface area presented by the side chain. Quantitatively, the decrement between glycine and valine provides a direct measure of the ion–hydrophobic term per unit of exposed nonpolar surface under the conditions studied. This has a direct and practically significant bearing on the interpretation of Hofmeister salt effects on proteins. The transfer of a nonpolar side chain from water into an aqueous divalent-chloride medium is

volumetrically unfavourable relative to the transfer of the charged backbone, which at the molecular level corresponds to the well-known tendency of kosmotropic divalent cations to promote the burial of hydrophobic surface and thereby to stabilise the compact folded state of a protein. The present data cannot by themselves predict whether a given salt will stabilise or destabilise a particular protein — that outcome depends on the weighted balance of backbone and side-chain terms integrated over the entire protein sequence — but they supply both terms separately, in a common experimental framework and on an absolute scale, which is precisely the information a quantitative predictive treatment of Hofmeister effects requires.

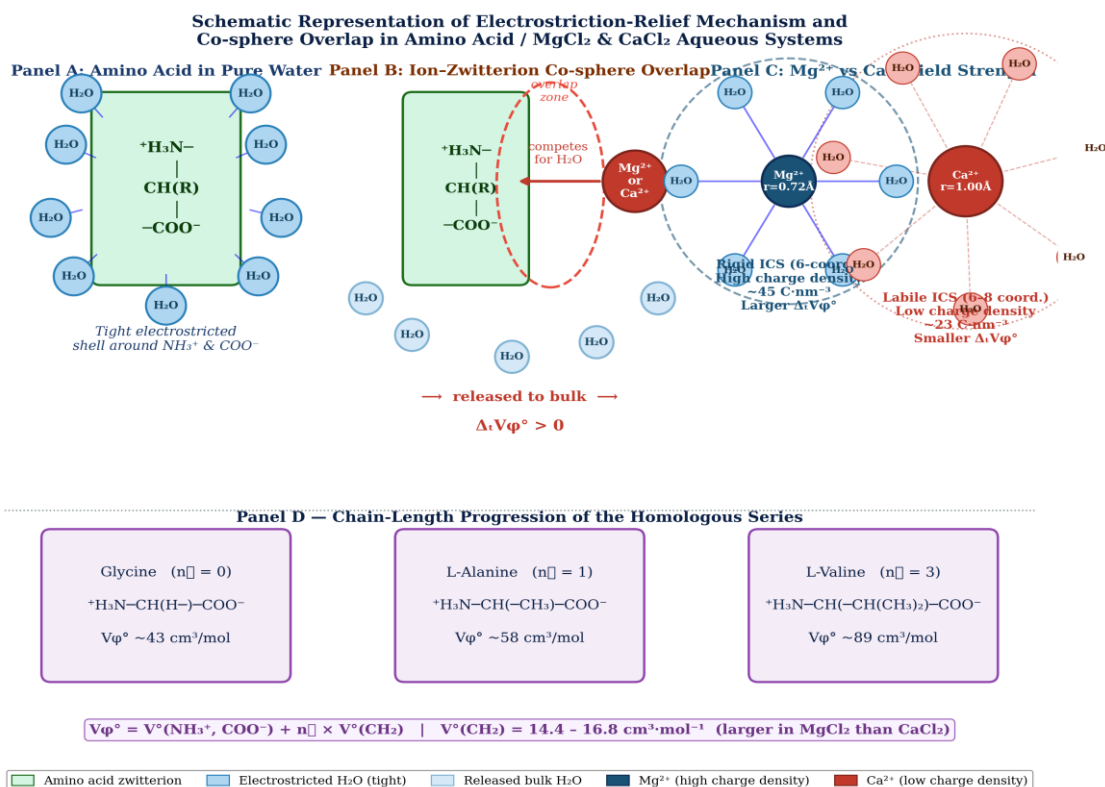
4.3 Consistency of Volumetric and Viscometric Classifications

The two independent classifications of the solutes as structure-makers — from the sign of the Hepler second derivative $(\partial^2 V\phi^\circ/\partial T^2)_p$ and from the sign of dB/dT — agree for every one of the twenty-four salt–amino acid combinations studied. This agreement is not guaranteed by any physical constraint and is therefore significant. The volumetric criterion is a second derivative of an equilibrium quantity and is sensitive to the curvature of the $V\phi^\circ$ versus T fit, which in turn depends on the number and temperature spacing of the measurements; with only five temperatures, the curvature is constrained but its uncertainty is not negligible, particularly for glycine at the lower molalities. The viscometric criterion derives from a transport property and mixes equilibrium structure with dynamics. The two criteria have been shown by Jenkins and Marcus to disagree in a substantial fraction of the literature; their agreement here, across three amino acids and two electrolytes, suggests that both the five-temperature dataset and the viscometric dataset are sufficient in precision to resolve the genuine structure-making character of these solutes.

4.4 Schematic Interpretation and Molecular Model

The molecular interpretation of the co-sphere overlap mechanism and the Mg^{2+}/Ca^{2+} comparison is presented schematically in Figures 3 and 4. Figure 3 illustrates the electrostriction-relief mechanism (Panel A: pure water baseline; Panel B: ion–zwitterion co-sphere overlap releasing electrostricted water; Panel C: Mg^{2+} vs Ca^{2+} field-strength comparison) and the chain-length progression of the homologous series with the group-contribution equation (Panel D). Figure 4 provides a side-by-side representation of the Feakins–Eyring energy landscape for viscous flow with the B-coefficient comparison, and the Friedman–Krishnan co-sphere overlap hierarchy with the consolidated parameter summary table confirming the universal $Mg^{2+} > Ca^{2+}$ ordering.

Figure 3. Schematic representation of the electrostriction-relief mechanism underlying positive transfer volumes (Panels A–C) and the chain-length progression of the homologous series used in the group-contribution analysis (Panel D). Panel C explicitly compares the rigid, high-charge-density inner coordination sphere of Mg^{2+} ($r = 0.72 \text{ \AA}$, $\sim 45 \text{ C}\cdot\text{nm}^{-3}$) with the labile, lower-charge-density sphere of Ca^{2+} ($r = 1.00 \text{ \AA}$, $\sim 23 \text{ C}\cdot\text{nm}^{-3}$).



4.5 Comparison with Previous Work and Biological Context

Where overlap exists, the present limiting volumes in aqueous MgCl_2 and CaCl_2 agree with the earlier reports of Banipal et al., Pal and Kumar, and Yan et al. to within the combined expanded uncertainties, supporting the reliability of the measurements. The contribution of the present work lies less in the individual numerical values than in the matched experimental design: because both cations were studied across the same homologous series, at the same molalities, at the same temperatures and with the same instruments, the differences between them can be read directly as cation effects rather than being inferred across studies employing different calibration standards, different reference data for water, or different concentration ranges.

The temperature interval 293.15 to 313.15 K spans the physiologically relevant range and permits interpolation to body temperature (310.15 K). At that temperature, the structure-making character of all three solutes persists in both media, though attenuated by approximately 15-20% relative to 293.15 K in terms of the Hepler second derivative. The finding that Mg^{2+} sustains a more ordered solvation environment for amino acid solutes than Ca^{2+} across the full temperature range is at least consistent with the contrasting biological deployment of these ions Mg^{2+} in structural and catalytic roles requiring a stable, well-defined coordination geometry, and Ca^{2+} in rapid signalling roles requiring facile ligand exchange though this should be read as a qualitative consistency check rather than a mechanistic claim, given the enormous difference in structural complexity between the present model systems and actual metalloenzyme active sites.

5. Conclusions

Densities and dynamic viscosities have been measured for glycine, L-alanine and L-valine in aqueous MgCl_2 and CaCl_2 at four salt molalities and five temperatures. The derived volumetric and viscometric

parameters have been interpreted within the co-sphere overlap and Feakins–Eyring frameworks. The principal conclusions are as follows:

- (i) Limiting apparent molar volumes V_{ϕ}° increase with temperature and with side-chain length in both media, and are systematically larger in MgCl_2 than in CaCl_2 at all matched conditions. The positive partial molar expansibilities E_{ϕ}° confirm progressive thermal release of electrostriction.
- (ii) Transfer volumes from water to the electrolyte become more positive as salt molality rises, establishing ion–zwitterion interaction as the dominant co-sphere term. Their systematic decrease along the series Gly $\rightarrow$ L-Ala $\rightarrow$ L-Val quantifies the offsetting ion–hydrophobic contribution from the growing alkyl side chains.
- (iii) Group-contribution analysis yields an effective methylene increment $V^{\circ}(\text{CH}_2) = 14.4\text{--}16.8 \text{ cm}^3\cdot\text{mol}^{-1}$, larger in the magnesium medium. The branched isopropyl group of valine limits the strict transferability of this increment to fully extended methylene series.
- (iv) Hepler's second-derivative criterion $(\partial^2 V_{\phi}^{\circ}/\partial T^2)_p < 0$ classifies all three amino acids as structure-makers in both electrolyte media, an assignment independently and consistently confirmed by positive B-coefficients with negative temperature derivatives $\text{dB}/\text{dT} < 0$.
- (v) Feakins–Eyring activation parameters reveal an enthalpy-controlled, entropy-lowering viscous flow process. Both $\Delta H_{\ddagger}^{\circ}$ and $|\Delta S_{\ddagger}^{\circ}|$ increase along the homologous series and with cation charge density, mirroring every other measured parameter.
- (vi) Every interaction-sensitive thermodynamic parameter is larger in MgCl_2 than in CaCl_2 , consistently and quantitatively explained by the approximately twofold difference in surface charge density (~ 45 vs. $\sim 23 \text{ C}\cdot\text{nm}^{-3}$) between Mg^{2+} and Ca^{2+} at otherwise identical ionic conditions.

These results provide a rigorously consistent alkaline-earth baseline for the thermodynamic analysis of amino acid solvation. Extension of this matched experimental design to transition-metal chlorides — where inner-sphere coordination and chelation, rather than purely electrostatic fields, govern the interaction — will test how far the present co-sphere framework carries and will reveal the additional thermodynamic signatures of partial covalent metal–ligand bonding.

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