





Research paper

# Biochemical Analysis of Solute–Solvent Interactions of Aromatic Amino Acids in Denaturing Environments Relevant to Protein Folding

Naseem Ahmed <sup>a\*</sup>

<sup>a</sup> PG Department of Chemistry, Govt. Degree College Rajouri, Jammu and Kashmir, India

ARTICLE INFO	ABSTRACT
<p><b>Keywords</b></p> <p>DL-phenylalanine L-tryptophan L-tyrosine adiabatic compressibility phosphate buffer comparative thermodynamics</p>	<p>A systematic and comparative thermodynamic investigation of three aromatic amino acids — DL-Phenylalanine, L-Tryptophan, and L-Tyrosine — in phosphate buffer solutions at pH 6, 7, and 8 with 0.1 m aqueous urea has been conducted at concentrations ranging from 0.01 to 0.09 mol kg<sup>-1</sup> and temperatures from 303.15 to 328.15 K. Density and ultrasonic velocity data were used to calculate adiabatic compressibility (<math>\beta_s</math>), specific acoustic impedance (<math>Z</math>), compressibility lowering (<math>\Delta\beta_s</math>), relative change in adiabatic compressibility (<math>\Delta\beta_s/\beta^0</math>), relative association (RA), apparent molal volume (<math>\varphi_v</math>), and partial molal volume at infinite dilution (<math>\varphi_v^0</math>) for all three amino acids. This work provides a unified analysis that highlights how the chemical nature of the aromatic side chain governs the solvation thermodynamics. Phenylalanine, with a purely hydrophobic benzyl side chain, shows negative <math>S_v</math> values indicative of non-polar-polar type interactions; tryptophan and tyrosine, with polar or amphiphilic aromatic side chains, show positive <math>S_v</math> values consistent with polar-polar interactions. Partial molal volumes follow the order L-Tryptophan &gt; DL-Phenylalanine <math>\approx</math> L-Tyrosine. Adiabatic compressibility decreases with concentration for all three amino acids, and all show RA values greater than unity, but the magnitude of these effects varies systematically with side-chain character. The influence of pH (6–8) and temperature (303.15–328.15 K) on the thermodynamic parameters is analysed and rationalized in terms of the ionization state of the amino acid, the buffer ion interactions, and the thermal disruption of hydration structures. Collectively, these results establish a comprehensive thermodynamic framework linking molecular structure to solution thermodynamic properties of aromatic amino acids in physiologically relevant mixed aqueous media.</p>
 DOI <a href="https://doi.org/10.5281/ib-2422526">10.5281/ib-2422526</a>	
<b>*Corresponding author</b> <b>Naseem Ahmed</b>	
✉ <b>Email</b> <a href="mailto:drnaseem123@gmail.com">drnaseem123@gmail.com</a>	
	

## 1. Introduction

The three aromatic amino acids — phenylalanine, tryptophan, and tyrosine — are united by the presence of an aromatic ring system in their side chains, yet they differ fundamentally in the polarity, size, and hydrogen-bonding capability of that side chain. Phenylalanine carries a simple benzyl group that is purely hydrophobic and devoid of any hydrogen-bonding functionality. Tyrosine bears a para-hydroxyphenyl group in which the aromatic hydrophobicity is tempered by the strongly

hydrophilic phenolic hydroxyl, making it amphiphilic. Tryptophan carries an indole group that combines the hydrophobicity of a fused bicyclic aromatic system with the weak hydrogen-bond donating capability of an NH group.

These structural differences have profound consequences for the solvation behaviour of these amino acids in aqueous and mixed-aqueous media. The solvation of an amino acid in water involves contributions from the hydration of the charged zwitterionic termini ( $+NH_3$  and  $COO^-$ ), from the hydrophobic hydration of nonpolar side chains, from

the specific hydrogen bonding of polar side-chain groups with water, and from cooperative and competitive effects among these contributions (Cohn & Edsall, 1943). Comparing the thermodynamic properties of the three aromatic amino acids under identical experimental conditions is therefore an especially effective approach to isolating the contribution of the side chain to overall solvation thermodynamics.

Volumetric and compressibility measurements provide complementary thermodynamic information about solvation. The partial molal volume at infinite dilution  $\varphi v^\circ$  encodes information about the intrinsic volume of the solute and the volume change arising from electrostriction of the solvent by the charged groups. The volumetric interaction parameter  $S_v$  (the slope of the  $\varphi v$  versus  $m$  relationship) characterizes pair-wise solute-solute interactions as mediated by the solvent and carries information about the nature of the dominant intermolecular forces in solution. The adiabatic compressibility and its concentration dependence sense the structural organization of the hydration water around the solute.

The presence of urea as co-solvent introduces a further variable of biological relevance. Urea is a classical protein denaturant that interacts with proteins through a combination of direct hydrogen bonding with the protein backbone and polar groups, and indirect disruption of the hydrophobic effect by weakening the hydrophobic hydration around nonpolar residues. Comparative studies of the three aromatic amino acids in the presence of urea therefore provide insight into how urea differentially affects the hydration of amino acids that differ in the hydrophobic versus amphiphilic character of their side chains.

The phosphate buffer system at pH 6, 7, and 8 provides a physiologically relevant and chemically stable medium. The buffer pH influences the thermodynamic parameters both indirectly (through the ionic composition of the buffer) and directly (if pH changes alter the protonation state of ionizable groups). For the three aromatic amino acids, the terminal amino group ( $pK_a \approx 9.1$ – $9.4$ ) and carboxyl group ( $pK_a \approx 2.2$ – $2.4$ ) remain in their fully ionized zwitterionic state throughout the pH 6–8 range, ensuring that pH effects reflect buffer-amino acid interactions rather than changes in ionization.

The present work reports a unified comparative analysis of all three aromatic amino acids under identical experimental conditions of concentration (0.01–0.09 mol kg<sup>-1</sup>), temperature (303.15–328.15 K), pH (6, 7, and 8), and urea concentration (0.1 m). The principal aim is to delineate the role of aromatic side-chain character in determining solution thermodynamic properties and to provide a comprehensive thermodynamic framework for

aromatic amino acid solvation in biologically relevant mixed aqueous media.

## 2. Materials and Methods

### 2.1 Materials

DL-Phenylalanine, L-Tryptophan, and L-Tyrosine (all  $\geq 99.0\%$  purity, Sigma-Aldrich) were used without further purification. Urea and phosphate buffer salts (all AR grade, Merck India) were used as received. All solutions were prepared using triple-distilled water (specific conductance  $< 1.0 \times 10^{-6}$  S cm<sup>-1</sup>). Phosphate buffer solutions at pH 6, 7, and 8 were prepared from 0.1 M solutions of NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> in appropriate proportions, with pH verified by a calibrated digital pH meter (Systronics Model 335,  $\pm 0.01$  pH units). Aqueous urea solutions (0.1 mol kg<sup>-1</sup>) were prepared in each buffer. Amino acid solutions at molalities 0.01, 0.03, 0.05, 0.07, and 0.09 mol kg<sup>-1</sup> were prepared gravimetrically in the urea-buffer solvent.

### 2.2 Instrumentation and Measurement Protocol

Densities were measured using a calibrated 25 mL bicapillary pycnometer ( $\pm 4 \times 10^{-3}$  kg m<sup>-3</sup>). Ultrasonic velocities were determined at 2 MHz using a multifrequency ultrasonic interferometer (Mittal Enterprises Model M-82,  $\pm 0.1$  m s<sup>-1</sup>). Temperature was controlled by a Julabo circulating thermostat ( $\pm 0.01$  K). Measurements were performed at 303.15, 308.15, 313.15, 318.15, 323.15, and 328.15 K. All measurements were conducted in triplicate and mean values were used. The instruments were calibrated with double-distilled water before each measurement session.

### 2.3 Computation of Thermodynamic Parameters

From the experimental  $\rho$  and  $U$  data, the following thermodynamic parameters were derived: (1) adiabatic compressibility  $\beta_s = 1/(U^2\rho)$ ; (2) specific acoustic impedance  $Z = U \times \rho$ ; (3) compressibility lowering  $\Delta\beta_s = \beta_s^\circ - \beta_s$ ; (4) relative change  $\Delta\beta_r = \Delta\beta_s/\beta_s^\circ$ ; (5) relative association  $RA = (\rho/\rho_0)(U^\circ/U)^{(1/3)}$ ; (6) apparent molal volume  $\varphi v = 1000(\rho_0 - \rho)/(m\rho_0\rho) + M/\rho$ ; (7) partial molal volume at infinite dilution  $\varphi v^\circ$  from least-squares fitting of  $\varphi v = \varphi v^\circ + S_v\sqrt{m}$ . The molar masses used were 165.19, 204.23, and 181.19 g mol<sup>-1</sup> for DL-Phenylalanine, L-Tryptophan, and L-Tyrosine, respectively.

## 3. Results

### 3.1 Comparative Density and Ultrasonic Velocity

For all three amino acids, density increases with concentration and decreases with temperature, while ultrasonic velocity increases with both concentration

and temperature, behaviour that is entirely consistent across pH 6, 7, and 8. At pH 7 and 303.15 K, the density at 0.01 mol kg<sup>-1</sup> follows the order: L-Tryptophan (0.9998 g cm<sup>-3</sup>) ≈ L-Tyrosine (0.9998 g cm<sup>-3</sup>) > DL-Phenylalanine (0.9996 g cm<sup>-3</sup>). At 0.09 mol kg<sup>-1</sup> and the same conditions, densities are L-Tryptophan (1.0044 g cm<sup>-3</sup>) > L-Tyrosine (1.0042 g cm<sup>-3</sup>) > DL-Phenylalanine (1.0028 g cm<sup>-3</sup>). The larger density increment for tryptophan and tyrosine compared to phenylalanine reflects their larger molar masses and the contributions of their polar side chains to the solution density.

Ultrasonic velocities at pH 7 and 303.15 K at 0.01 mol kg<sup>-1</sup> are: DL-Phenylalanine (1515.4 m s<sup>-1</sup>), L-Tryptophan (1512.9 m s<sup>-1</sup>), L-Tyrosine (1514.2 m s<sup>-1</sup>). The small differences in ultrasonic velocity between the amino acids at the same concentration reflect differences in solute-solvent interactions and molecular packing. The increment in ultrasonic velocity per unit concentration increase is similar for all three amino acids but slightly larger for tryptophan, consistent with its stronger solute-solvent interaction network.

### 3.2 Comparative Adiabatic Compressibility

Adiabatic compressibility values decrease with concentration for all three amino acids. At pH 6 and 303.15 K, the β<sub>s</sub> values at 0.01 mol kg<sup>-1</sup> are: DL-Phenylalanine (4.3780) > L-Tyrosine (4.3495) > L-Tryptophan (4.3512) × 10<sup>-7</sup> cm<sup>2</sup> dyne<sup>-1</sup>. At 0.09 mol kg<sup>-1</sup>, the order changes slightly to: DL-Phenylalanine (4.2936) > L-Tyrosine (4.2566) > L-Tryptophan (4.2669) × 10<sup>-7</sup> cm<sup>2</sup> dyne<sup>-1</sup>. The higher compressibility of DL-Phenylalanine solutions at all concentrations compared to the other two amino acids reflects the predominantly hydrophobic character of its side chain, which does not form strong hydrogen bonds with water and therefore contributes less to electrostriction.

The rate of decrease in β<sub>s</sub> with concentration (i.e., the compressibility increment per unit molality) is largest for L-Tryptophan and L-Tyrosine compared to

DL-Phenylalanine, consistent with stronger and more extensive hydration around the polar side chains of the former two amino acids. This difference in the concentration dependence of β<sub>s</sub> provides a direct measure of how the nature of the side chain modulates the overall hydration structure of the solution.

### 3.3 Relative Association

Relative association values exceed unity for all three amino acids at all conditions, indicating solvation dominance. The magnitude of RA values increases in the order L-Tryptophan > L-Tyrosine > DL-Phenylalanine at corresponding concentrations and temperatures, consistent with the interpretation that the stronger and more extensive hydration networks around the polar side chains of tryptophan and tyrosine provide greater resistance to compression and more effective solvation of the amino acid molecules.

### 3.4 Comparative Partial Molal Volume

Partial molal volumes at infinite dilution (φ<sub>v</sub><sup>o</sup>) at 303.15 K for the three amino acids at pH 6 are: L-Tryptophan (144.42 cm<sup>3</sup> mol<sup>-1</sup>) > DL-Phenylalanine (126.19 cm<sup>3</sup> mol<sup>-1</sup>) ≈ L-Tyrosine (122.08 cm<sup>3</sup> mol<sup>-1</sup>). The larger φ<sub>v</sub><sup>o</sup> for tryptophan reflects its significantly larger molecular volume. The comparable φ<sub>v</sub><sup>o</sup> values for phenylalanine and tyrosine, despite tyrosine's higher molar mass by 16 g mol<sup>-1</sup> (one oxygen atom from the phenolic OH), suggest that the hydrophilic OH group leads to stronger electrostriction that partially offsets the larger molecular volume.

A critical distinction emerges from the S<sub>v</sub> values: DL-Phenylalanine consistently shows negative S<sub>v</sub> values (≈ -41 to -54 cm<sup>3</sup> mol<sup>-3/2</sup> kg<sup>1/2</sup> at pH 6), while L-Tryptophan and L-Tyrosine show strongly positive S<sub>v</sub> values (tryptophan: ≈ 53–71; tyrosine: ≈ 43–55 cm<sup>3</sup> mol<sup>-3/2</sup> kg<sup>1/2</sup> at pH 6). This distinction is one of the most significant findings of the comparative study and its molecular basis is discussed in detail below.

**Table 1** Comparative Adiabatic Compressibility β<sub>s</sub> (×10<sup>-7</sup> cm<sup>2</sup> dyne<sup>-1</sup>) of DL-Phenylalanine, L-Tryptophan, and L-Tyrosine in Phosphate Buffer pH 7 + 0.1 m Aqueous Urea Solution at Selected Temperatures

Molality (mol kg <sup>-1</sup> )	DL-Phenylalanine			L-Tryptophan			L-Tyrosine		
	303.15 K	313.15 K	328.15 K	303.15 K	313.15 K	328.15 K	303.15 K	313.15 K	328.15 K
0.01	4.3563	4.2809	4.1956	4.3698	4.2823	4.1963	4.3623	4.2851	4.1936
0.03	4.3414	4.2557	4.1833	4.3445	4.2565	4.1783	4.3426	4.2514	4.1793
0.05	4.3185	4.2407	4.1716	4.3158	4.2286	4.1656	4.3151	4.2368	4.1538
0.07	4.3004	4.2335	4.1543	4.2982	4.2164	4.1483	4.2979	4.2250	4.1401
0.09	4.2879	4.2125	4.1408	4.2833	4.2025	4.1294	4.2842	4.2083	4.1260

All three aromatic amino acids show decreasing β<sub>s</sub> with increasing concentration at pH 7. L-Tryptophan exhibits slightly lower compressibility than L-Tyrosine and DL-Phenylalanine at equivalent concentrations, reflecting stronger solute-solvent interactions associated with the larger indole side chain

**Table 2** Partial Molal Volume at Infinite Dilution  $\varphi_v^\circ$  ( $\text{cm}^3 \text{mol}^{-1}$ ) of Three Aromatic Amino Acids in Phosphate Buffer + 0.1 m Aqueous Urea Solution Across pH 6, 7, and 8 and Selected Temperatures

Amino Acid	pH	303.15 K	313.15 K	323.15 K	328.15 K
DL-Phenylalanine	pH 6	126.19	126.95	127.35	127.54
DL-Phenylalanine	pH 7	125.26	125.55	125.86	126.02
DL-Phenylalanine	pH 8	125.48	125.76	126.05	126.20
L-Tryptophan	pH 6	144.42	145.22	145.75	145.80
L-Tryptophan	pH 7	143.94	144.24	144.56	144.68
L-Tryptophan	pH 8	145.23	145.22	145.84	145.64
L-Tyrosine	pH 6	122.08	122.37	122.65	122.79
L-Tyrosine	pH 7	121.99	122.45	122.42	122.54
L-Tyrosine	pH 8	122.16	122.37	122.57	122.69

The order of partial molal volumes follows  $L\text{-Tryptophan} > DL\text{-Phenylalanine} \approx L\text{-Tyrosine}$ , consistent with the relative molecular sizes of the three amino acids.  $\varphi_v^\circ$  increases with temperature for all systems, reflecting progressive disruption of the electrostricted hydration shells at elevated temperatures

**Table 3** Relative Change in Adiabatic Compressibility ( $\Delta\beta_s/\beta^\circ \times 10^{-3}$ ) at 303.15 K for All Three Aromatic Amino Acids in Phosphate Buffer + 0.1 m Aqueous Urea Solution at Different pH and Concentrations

System	m = 0.01 (mol kg <sup>-1</sup> )	m = 0.03 (mol kg <sup>-1</sup> )	m = 0.05 (mol kg <sup>-1</sup> )	m = 0.07 (mol kg <sup>-1</sup> )	m = 0.09 (mol kg <sup>-1</sup> )
DL-Phenylalanine (pH 7)	3.96	7.36	12.60	16.74	19.60
DL-Phenylalanine (pH 6)	-5.81	0.28	4.23	10.32	13.58
DL-Phenylalanine (pH 8)	2.63	6.32	9.20	14.87	18.56
L-Tryptophan (pH 7)	0.87	6.65	13.22	17.24	20.65
L-Tryptophan (pH 6)	0.34	5.65	9.47	15.88	19.71
L-Tryptophan (pH 8)	3.11	5.77	3.25	18.33	21.88
L-Tyrosine (pH 7)	2.58	7.09	13.38	17.31	20.44
L-Tyrosine (pH 6)	0.74	11.23	13.76	18.20	22.08
L-Tyrosine (pH 8)	4.94	8.90	13.46	18.42	22.08

The relative change in adiabatic compressibility increases with concentration for all three amino acids and at all pH values, confirming the progressive increase in solvation with amino acid content. L-Tyrosine consistently shows the highest  $\Delta\beta_s/\beta^\circ$  values, attributable to enhanced water structuring by the amphiphilic phenolic hydroxyl group of its side chain

## 4. Discussion

### 4.1 Side-Chain Effects on Thermodynamic Parameters

The comparative analysis reveals a clear and consistent pattern linking aromatic side-chain character to solution thermodynamic behaviour. The purely hydrophobic benzyl side chain of DL-Phenylalanine imposes hydrophobic hydration on the water molecules it contacts. These hydrophobically hydrated water molecules are arranged in a cage-like structure around the nonpolar group, with reduced translational and rotational mobility compared to bulk water but without strong energetic attraction to the solute. The resulting hydration shell is characterized by moderate electrostriction and a tendency for the hydrated amino acid molecules, at higher concentrations, to undergo hydrophobic association that reduces the effective volume and produces negative Sv values.

In contrast, the phenolic hydroxyl of tyrosine and the indole NH of tryptophan can form direct hydrogen bonds with water molecules. These hydrogen bonds are energetically stronger than the weak van der Waals interactions that constitute hydrophobic hydration, and they produce a more tightly held hydration shell around the polar groups of the side

chain. When two solvated tryptophan or tyrosine molecules approach each other in solution, their hydration cospheres overlap and release electrostricted water to bulk water. Since electrostricted water has a smaller molar volume than bulk water, this release is accompanied by a volume increase, manifesting as positive Sv values.

### 4.2 pH Effects and Their Molecular Basis

The pH dependence of the thermodynamic parameters studied reveals that while the qualitative patterns (increase of density and ultrasonic velocity with concentration; decrease of compressibility with concentration) are the same at all three pH values, quantitative differences exist. The partial molal volumes at infinite dilution are generally highest at pH 6 and lowest at pH 7, with pH 8 values intermediate, for all three amino acids. This non-monotonic pH dependence may reflect changes in the hydration shell of the terminal groups as a function of the buffer ionic composition, since the ratio of  $\text{HPO}_4^{2-}$  to  $\text{H}_2\text{PO}_4^-$  increases substantially from pH 6 to pH 8. The divalent  $\text{HPO}_4^{2-}$  ion interacts more strongly with the positive charge of the amino terminus than the monovalent  $\text{H}_2\text{PO}_4^-$ , potentially modifying the electrostriction around the  $+\text{NH}_3$  group.

The relative change in adiabatic compressibility ( $\Delta\beta_s/\beta^\circ$ ) shows more complex pH dependence, with values generally being largest at pH 6 for DL-Phenylalanine but at pH 8 for L-Tyrosine. This difference may reflect a specific interaction between the phenolic group of tyrosine and hydroxide ions or dihydrogen phosphate buffer anions that is enhanced at higher pH, leading to stronger electrostriction and greater compressibility reduction.

#### 4.3 Temperature Effects and Hydration Stability

All three amino acids show qualitatively similar temperature dependence: density decreases, ultrasonic velocity increases, and compressibility decreases with rising temperature. Quantitatively, however, the rate of change of the thermodynamic parameters with temperature differs among the three amino acids and provides information about the thermal stability of their respective hydration structures. The temperature coefficient of adiabatic compressibility is largest for DL-Phenylalanine, indicating that the hydrophobic hydration cage around the benzyl group is more thermally labile than the direct hydrogen-bonding interactions that stabilize the hydration of tryptophan and tyrosine side chains.

Partial molal volumes at infinite dilution increase with temperature for all three amino acids. This increase reflects the thermal disruption of electrostriction around the charged zwitterionic termini, causing hydration water to expand toward bulk water volume. The rate of this thermal expansion (i.e.,  $d\varphi_v^\circ/dT$ ) is largest for DL-Phenylalanine at pH 6 ( $\approx 27 \text{ cm}^3 \text{ mol}^{-1}$  per 25 K increase) and smallest for L-Tyrosine ( $\approx 71 \text{ cm}^3 \text{ mol}^{-1}$  over 25 K for tryptophan). The smaller thermal expansion for tryptophan may reflect additional contributions from the temperature-dependent conformational changes of the large indole side chain.

#### 4.4 Role of Urea in the Comparative System

The presence of 0.1 M urea in all solutions provides a common perturbation to the hydration environment of all three amino acids, enabling direct comparison of urea effects. Since urea does not interact selectively with hydrophobic or hydrophilic groups but rather participates in the overall hydrogen bonding network of the solution, its influence on the thermodynamic parameters is expected to be non-specific. The slight reduction in electrostriction-related quantities (such as  $\Delta\beta_s$ ) compared to amino acid solutions in pure water is consistent with urea's known structure-breaking properties, which reduce the effective organization of water around all types of solute groups.

An interesting observation from the comparative analysis is that the effect of urea on  $\varphi_v^\circ$  appears to be

slightly larger for L-Tyrosine than for the other two amino acids. This suggests that the hydroxyl group of tyrosine may engage in direct hydrogen bond competition with urea, analogous to the competition between urea and polar groups in protein denaturation. If urea partially displaces water from the first coordination shell of the tyrosine phenolic OH, the effective electrostriction around this group would decrease, leading to an increase in  $\varphi_v^\circ$  relative to that in pure buffer.

#### 5. Conclusion

This comparative thermodynamic study of DL-Phenylalanine, L-Tryptophan, and L-Tyrosine in phosphate buffer (pH 6–8) with 0.1 M aqueous urea has revealed systematic and interpretable differences in solution thermodynamic properties linked to the structural character of the aromatic side chain. The principal conclusions are as follows.

All three amino acids show density increases with concentration, decreases with temperature, and ultrasonic velocity increases with both variables. Adiabatic compressibility decreases with concentration for all three amino acids, but the rate of decrease is largest for L-Tryptophan and smallest for DL-Phenylalanine, reflecting the strongest and weakest side-chain-mediated electrostriction, respectively. Relative association values exceed unity throughout, but are highest for tryptophan and lowest for phenylalanine, consistent with the order of hydration network strength.

The critical distinguishing feature is the sign of  $S_v$ : negative for DL-Phenylalanine (non-polar-polar interactions dominate) and positive for L-Tryptophan and L-Tyrosine (polar-polar interactions dominate). This structural-thermodynamic correlation provides a clear molecular basis for the observed differences and has direct relevance to understanding how side-chain polarity governs protein-solvent interactions. Partial molal volumes follow the order tryptophan > phenylalanine  $\approx$  tyrosine, and all three increase with temperature, reflecting progressive reduction in electrostriction. pH effects on thermodynamic parameters primarily reflect buffer ionic composition changes rather than amino acid protonation state changes. These comprehensive comparative data establish a rigorous thermodynamic framework for aromatic amino acid solvation in biologically relevant mixed aqueous media.

#### References

1. Buckin, V. A. (1988). Hydration of nucleic acid bases in dilute aqueous solutions: Apparent molar adiabatic compressibilities and volumes. *Biophysical Chemistry*, 29, 283–292.
2. Cabani, S., Conti, G., Matteoli, E., & Tine, M. R. (1981). Thermodynamics of solution of amino acids and related compounds in water. *Journal of the Chemical Society*,

- Faraday Transactions I, 77, 2377–2395. <https://doi.org/10.1039/f19817702377>
3. Chalikian, T. V., Sarvazyan, A. D., & Breslauer, K. J. (1993). Partial molar volumes, expansibilities, and compressibilities of alpha,omega-aminocarboxylic acids at 18–55°C. *Journal of Physical Chemistry*, 97, 13017–13026.
  4. Cohn, E. J., & Edsall, J. T. (1943). *Proteins, amino acids and peptides as ions and dipolar ions*. Reinhold Publishing Corporation.
  5. Enea, D., & Jolicoeur, C. (1982). Heat capacities and volumes of several oligopeptides in urea–water mixtures at 25°C. *Journal of Physical Chemistry*, 86, 3870–3881.
  6. Frank, F. (1968). Hydrogen-bonded solvent systems. In A. K. Covington & P. Jones (Eds.), *Taylor and Francis*, London (p. 31).
  7. Gekko, K., & Hasegawa, Y. (1986). Compressibility-structure relationship of globular proteins. *Biochemistry*, 25, 6563–6571.
  8. Greenstein, J. P., & Wibitz, M. (1961). *Chemistry of the amino acids (Vol. I)*. Wiley-Interscience.
  9. Hedhig, G. R., & Hoiland, H. (1991). Partial molar heat capacities and volumes of transfer of amino acids from water to 7.228 m urea at 25°C. *Journal of Chemical Thermodynamics*, 23, 1029–1036.
  10. Hedhig, G. R., & Hoiland, H. (1993). Volumetric properties of amino acids in 7.228 m aqueous urea solutions at 298.15 K. *Journal of Chemical Thermodynamics*, 25, 349–354.
  11. Hershey, J. P., Sotolongo, S., & Millero, F. J. (1979). Densities and compressibilities of aqueous sodium carbonate and bicarbonate. *Journal of Solution Chemistry*, 12, 233–254.
  12. Iqbal, M., & Mateeullah, M. (1990). Partial molar volumes of some amino acids in aqueous 1-propanol solutions at 25°C. *Canadian Journal of Chemistry*, 68, 725–728.
  13. Iqbal, M., & Verrall, R. E. (1987). Implications of urea and protein denaturation in terms of volumetric and compressibility behaviour. *Journal of Physical Chemistry*, 91, 967–975.
  14. Jolicoeur, C., & Boilieu, E. J. (1978). Apparent molal volumes and heat capacities of low molecular weight polyethylene glycols in water. *Canadian Journal of Chemistry*, 56, 2707–2713.
  15. Kharakoz, D. P. (1989). Volumetric properties of proteins and their analogues in diluted water solutions. *Biophysical Chemistry*, 34, 115–125.
  16. Kharakoz, D. P. (1991). Volumetric properties of proteins and their analogues in diluted water solutions. *Journal of Physical Chemistry*, 95, 5634–5642.
  17. Laliberte, L. H., & Conway, B. E. (1970). Partial molal volumes of electrolytes in nonaqueous solvents. *Journal of Physical Chemistry*, 74, 4116–4125.
  18. Leslie, T. E., & Lilley, T. H. (1985). Aqueous solutions containing amino acids and peptides. *Biopolymers*, 24, 695–710.
  19. McMillan, W. G., & Mayer, J. E. (1945). The statistical thermodynamics of multicomponent systems. *Journal of Chemical Physics*, 13, 276–305. <https://doi.org/10.1063/1.1724036>
  20. Millero, F. J., Losurdo, A., & Shin, C. (1978). The apparent molal volumes and adiabatic compressibilities of aqueous amino acids at 25°C. *Journal of Physical Chemistry*, 82, 784–792.
  21. Mishra, A. K., & Ahluwalia, J. C. (1984). Apparent molal volumes and heat capacities of some amino acids in aqueous urea at 25°C. *Journal of Physical Chemistry*, 88, 86–92.
  22. Mishra, A. K., Prashad, K. P., & Ahluwalia, J. C. (1983). Thermodynamics of transfer of amino acids from water to aqueous urea solutions. *Biopolymers*, 22, 2397–2412.
  23. Natarajan, M., Wadi, R. K., & Gaur, H. C. (1990). Apparent molal volumes and viscosities of some amino acids in aqueous ammonium chloride solutions. *Journal of Chemical and Engineering Data*, 35, 87–93.
  24. Pandey, J. D., Mishra, R. L., & Bhatt, T. (1977). Ultrasonic velocities and compressibilities of aqueous amino acid solutions. *Acustica*, 38, 83–90.
  25. Reading, J. F., & Headwig, G. R. (1989). Partial molar isobaric heat capacities and volumes of glycine, dl-alanine, and dl-serine in aqueous urea solutions. *Journal of Solution Chemistry*, 18, 159–175.
  26. Talukdar, H., Rudra, S., & Kundu, K. K. (1988). Thermodynamics of transfer of glycine, dl-alanine, and dl-serine from water to aqueous urea solutions. *Canadian Journal of Chemistry*, 66, 461–468.
  27. Tanaka, H., Touhara, K., Nakanishi, K., & Watanabe, N. (1984). Computer experiment on aqueous solution: IV. Molecular dynamics calculation on the hydration of urea. *Journal of Chemical Physics*, 80, 5170–5186.
  28. Tiwari, V., & Pandey, J. D. (1980). Ultrasonic velocity in non-aqueous and aqueous solutions. *Indian Journal of Pure and Applied Physics*, 18, 51–58.
  29. Traube, I. (1899). Ueber den Zusammenhang zwischen Löslichkeit und Giftigkeit. *Sammlung Chemischer Vorträge*, 4, 255–290.
  30. Wadi, R. K., & Goyal, R. K. (1992). Densities, viscosities, and application of transition-state theory for water + potassium thiocyanate + amino acid solutions. *Journal of Chemical and Engineering Data*, 37, 377–386.