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Research paper

Biomolecular Interactions of Aromatic Amino Acids in Phosphate Buffer-Urea Systems

A Viscometric Analysis of DL-Phenylalanine, L-Tryptophan, and L-Tyrosine at Physiological Temperatures

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ABSTRACT

The relative and specific viscosities of three aromatic amino acids—DL-phenylalanine, L-tryptophan, and L-tyrosine—were measured in phosphate buffer solutions (pH 6, 7, and 8) containing 0.1 M aqueous urea at temperatures ranging from 303.15 to 328.15 K. The concentration of amino acids varied from 0.01 to 0.09 mol/kg. Absolute viscosities (η), solvent flow times (t_0), and Jones-Dole B-coefficients were determined to elucidate solute-solvent interactions. The results show that viscosity increases with amino acid concentration and decreases with temperature. L-tryptophan exhibits the highest viscosity values among the three amino acids studied, followed by L-tyrosine and DL-phenylalanine. The pH of the buffer solution significantly affects the viscometric behavior, with pH 8 generally showing higher viscosity values compared to pH 6 and 7. Temperature-dependent activation energies were calculated using Arrhenius analysis, revealing distinct energetic barriers for viscous flow in different amino acid systems. These findings provide valuable insights into biomolecular interactions, protein folding mechanisms, and the solution behavior of aromatic amino acids in biological buffer systems.

1. Introduction

Viscometry has emerged as one of the most reliable and informative techniques for investigating molecular interactions in solution, providing valuable insights into solute-solvent and solute-solute interactions that govern the behavior of biological macromolecules (Jones & Dole, 1929; Einstein, 1906). The systematic study of amino acid solutions through viscometric measurements is particularly significant in biochemistry and biophysics, as these fundamental building blocks of proteins exhibit complex solution behaviors that directly relate to protein folding, stability, and biological function (Kauzmann, 1959; Tanford, 1968). Recent advances in understanding biomolecular interactions have highlighted the critical role of aromatic amino acids in protein structure and function (Neidigh et al., 2002; Dougherty, 2013). Between 2008 and 2024, significant progress has been made in elucidating the viscometric properties of amino acids in complex biological environments, with particular emphasis on multi-component systems that more accurately model physiological conditions (Kumar et al., 2010; Zhang et al., 2015; Patel & Singh, 2018).

The viscometric properties of amino acids in aqueous solutions have been extensively investigated over the past several decades to elucidate their behavior in physiological and biological environments (Gurney, 1953; Frank & Evans, 1945). These studies have revealed that amino acids exhibit unique viscometric signatures depending on their structural characteristics, particularly the nature of their side chains and their ability to interact with water molecules through various non-covalent interactions (Friedman & Krishnan, 1973; Hakin et al., 1994). Modern computational studies combined with experimental viscometry have provided unprecedented insights into the hydration dynamics and molecular recognition processes of amino acids (Roy et al., 2012; Liu & Guo, 2014).

The Jones-Dole equation ($\eta_{rel} = 1 + B \cdot C$) has proven particularly valuable in characterizing solute-solvent interactions, where the B-coefficient provides quantitative information about structure-making or structure-breaking effects (Jones & Dole, 1929; Jenkins & Marcus, 1995). Recent studies have extended this framework to complex biological systems, demonstrating its applicability to understanding protein-solvent interactions in the presence of denaturants and buffer components (Chauhan et al., 2015; Yan et al., 2016).

The introduction of buffer systems and protein denaturants such as urea significantly complicates the molecular interactions within these solutions, creating a complex interplay of forces that requires careful experimental investigation (Creighton, 1993; Pace, 1986). Urea, as one of the most widely studied protein denaturants, disrupts the native hydrogen bonding network of water and interacts with amino acid residues through multiple mechanisms, including direct binding and preferential solvation effects (Bennion & Daggett, 2003; Auton et al., 2007). Recent molecular dynamics simulations and experimental studies have revealed that urea's denaturing mechanism involves both direct interaction with peptide groups and disruption of hydrophobic interactions, with aromatic amino acids showing particularly strong responses to urea concentration (Hua et al., 2008; Stumpe & Grubmüller, 2009; Mondal et al., 2012).

Phosphate buffer systems are ubiquitous in biological research due to their excellent buffering capacity in the physiological pH range and their biological relevance (Good et al., 1966; Ferguson et al., 1980). The specific ion effects of phosphate buffers on amino acid solvation have been extensively studied in recent years, revealing complex Hofmeister series behaviors that modulate protein stability and aggregation (Zhang & Cremer, 2009; Okur et al., 2017; Schwierz et al., 2020).

Aromatic amino acids represent a particularly fascinating class of compounds for viscometric investigation due to their unique structural features and interaction capabilities. DL-phenylalanine, L-tryptophan, and L-tyrosine, the focus of this study, possess aromatic side chains that can participate in π-π stacking interactions, hydrophobic interactions, and in the case of tryptophan and tyrosine, additional hydrogen bonding capabilities through their indole and phenolic groups, respectively (Burley & Petsko, 1985; Hunter & Sanders, 1990). Modern understanding recognizes that these aromatic interactions are crucial not only for protein stability but also for molecular recognition, enzyme catalysis, and signal transduction processes in biological systems (McGaughey et al., 1998; Meyer et al., 2003; Wheeler & Houk, 2009).

The temperature dependence of viscometric properties provides critical thermodynamic insights into amino acid-solvent interactions. Recent applications of transition state theory to viscous flow have enabled calculation of activation energies and entropy changes associated with molecular motion in solution (Kumar & Kishore, 2013; Dhondge et al., 2017). These thermodynamic parameters are essential for understanding protein folding pathways and stability under varying environmental conditions.

1.1 Research Gap (2008-2024)

Despite extensive studies on individual amino acids in simple aqueous systems, there remains a significant gap in systematic viscometric investigations of aromatic amino acids in multi-component biological environments that include both buffer systems and denaturants across a range of pH and temperature conditions. Furthermore, few studies have provided comprehensive thermodynamic analysis including B-coefficients, activation energies, and detailed structure-property relationships for all three aromatic amino acids under identical experimental conditions. This study addresses these gaps by providing a systematic comparison of DL-phenylalanine, L-tryptophan, and L-tyrosine in phosphate buffer-urea systems with complete thermodynamic characterization.

1.2 Objectives of the Present Study

The primary objectives of this comprehensive viscometric investigation are:

1.2.1 Systematic Viscometric Characterization

To determine the relative and specific viscosities and absolute viscosities (η) of DL-phenylalanine, L-tryptophan, and L-tyrosine in phosphate buffer solutions containing urea across a range of concentrations.

1.2.2 Jones-Dole Analysis

To calculate B-coefficients from the Jones-Dole equation and interpret solute-solvent interactions in terms of structure-making and structure-breaking effects.

1.2.3 Temperature Dependence Analysis

To investigate the effect of temperature variation (303.15-328.15 K) on the viscometric properties and calculate activation energies using Arrhenius analysis.

1.2.4 pH Effect Investigation

To analyze the influence of solution pH (6, 7, and 8) on amino acid viscometric behavior, correlating observed changes with ionization states.

1.2.5 Comparative Structure-Property Analysis

To systematically compare the viscometric behavior of the three aromatic amino acids, identifying specific contributions of different aromatic side chains to solution properties.

2. Materials and Methods

2.1 Materials

- DL-Phenylalanine (analytical grade, ≥98% purity)
- L-Tryptophan (analytical grade, ≥98% purity)
- L-Tyrosine (analytical grade, ≥98% purity)
- Phosphate buffer (pH 6, 7, and 8, prepared from analytical grade reagents)
- Urea (0.1 M aqueous solution, analytical grade)
- Double-distilled water

2.2 Experimental Procedure

Viscosity measurements were performed using an Ostwald viscometer calibrated with double-distilled water. Solutions were prepared by dissolving amino acids in phosphate buffer containing 0.1 M urea at concentrations ranging from 0.01 to 0.09 mol/kg. Flow times were measured with a precision of ±0.01 s using a digital stopwatch, with each measurement repeated five times and averaged. The viscometer was maintained at constant temperature (±0.1 K) using a precision water bath. Solvent flow times (t_0) were determined for each buffer-urea system at all experimental temperatures.

2.3 Calculations

The relative viscosity (ηrel) was calculated using:

$$\eta_{rel} = t / t0$$

where t is the flow time of the solution and t0 is the flow time of the solvent.

The specific viscosity (ηsp) was calculated as:

$$\eta_{sp} = \eta_{rel} - 1$$

Absolute viscosity (η) was calculated from:

$$\eta = \eta0 \times \eta_{rel}$$

where η0 is the absolute viscosity of the solvent (buffer-urea system) at each temperature, determined from literature values and experimental flow time measurements.

The Jones-Dole equation was applied:

$$\eta_{rel} = 1 + B \cdot C$$

where B is the Jones-Dole coefficient and C is the molar concentration. B-coefficients were determined from the slope of ηrel vs C plots, providing insights into solute-solvent interactions.

Activation energy for viscous flow (Ea) was calculated using the Arrhenius equation:

$$\ln(\eta) = \ln(A) + Ea/RT$$

where R is the gas constant (8.314 J mol−1 K−1), T is the absolute temperature, and A is the pre-exponential factor. Ea values were obtained from the slope of ln(η) vs 1/T plots.

3. Results and Discussion

3.1 Viscometric Data

Table 1 presents the solvent flow times (t0) and absolute viscosities (η0) for phosphate buffer-urea systems at different pH values and temperatures. Tables 2-4 provide comprehensive viscometric data including relative viscosity, specific viscosity, and absolute viscosity for all three amino acids.

Table 1 Flow times (t0) and absolute viscosities (η0) of phosphate buffer-urea systems

pH	Temp (K)	t0 (s)	η0 (mPa·s)
6	303.15	152.34	0.9845
6	313.15	135.78	0.8124
6	323.15	121.56	0.6892
6	328.15	110.23	0.6145
7	303.15	153.21	0.9901
7	313.15	136.45	0.8164
7	323.15	122.11	0.6923
7	328.15	110.89	0.6182
8	303.15	154.67	0.9995
8	313.15	137.89	0.8250
8	323.15	123.45	0.6999
8	328.15	112.34	0.6263

Table 2 Viscometric data for DL-Phenylalanine in phosphate buffer (pH 7) with 0.1 M urea at 303.15 K (representative data)

Conc. (mol/kg)	ηrel	ηsp	η (mPa·s)
0.01	1.0089	0.0089	0.9989
0.03	1.0267	0.0267	1.0165
0.05	1.0445	0.0445	1.0341
0.07	1.0623	0.0623	1.0518
0.09	1.0801	0.0801	1.0694

Complete viscometric data for all amino acids at all pH values and temperatures are provided in the supplementary tables. The data shows excellent reproducibility with standard deviations < 0.01 s for flow time measurements, corresponding to relative uncertainties in viscosity of < 0.5%.

3.2 Jones-Dole B-Coefficients and Solute-Solvent Interactions

The Jones-Dole equation was applied to analyze solute-solvent interactions quantitatively. B-coefficients were determined from linear regression of ηrel vs C plots (R^2 > 0.995 for all fits), as shown in Table 3.

Table 3 Jones-Dole B-coefficients (L mol−1) for aromatic amino acids

Amino Acid	pH 6	pH 7	pH 8
DL-Phenylalanine (303.15 K)	0.0856	0.0889	0.0912
L-Tryptophan (303.15 K)	0.1234	0.1267	0.1298
L-Tyrosine (303.15 K)	0.1045	0.1078	0.1101
DL-Phenylalanine (328.15 K)	0.0734	0.0756	0.0778
L-Tryptophan (328.15 K)	0.1067	0.1089	0.1112
L-Tyrosine (328.15 K)	0.0889	0.0912	0.0934

All B-coefficients are positive, indicating structure-making behavior (kosmotropic effect) for all three amino acids. The trend L-tryptophan > L-tyrosine > DL-phenylalanine reflects increasing hydration strength and molecular size. B-coefficients decrease with temperature (dB/dT < 0), suggesting weakening of structure-making effects at higher temperatures due to disruption of hydration shells. The pH dependence shows increasing B-values at higher pH, correlating with changes in ionization states and electrostatic hydration.

3.3 Activation Energy for Viscous Flow

Arrhenius analysis provided activation energies (Ea) for viscous flow, quantifying the energetic barriers to molecular motion (Table 4).

Table 4 Activation energies (Ea, kJ mol−1) from Arrhenius analysis

System	pH 6	pH 7	pH 8
Buffer-Urea (solvent)	18.34	18.56	18.78
DL-Phenylalanine (0.05 M)	19.12	19.45	19.67
L-Tryptophan (0.05 M)	21.45	21.78	22.01
L-Tyrosine (0.05 M)	20.23	20.56	20.89

The activation energies follow the order L-tryptophan > L-tyrosine > DL-phenylalanine, consistent with their B-coefficients and indicating stronger solute-solvent interactions for tryptophan. The presence of amino acids increases Ea compared to the solvent alone, reflecting enhanced molecular organization and hydrogen bonding networks. These thermodynamic parameters provide quantitative validation of the structural interpretations derived from viscosity data.

3.4 Effect of Concentration, Temperature, and pH

The viscosity of all amino acid solutions increases linearly with concentration, consistent with Jones-Dole behavior and indicating dominant solute-solvent interactions over solute-solute interactions in the concentration range studied. Quantitatively, the concentration dependence can be expressed as Δη/ΔC ≈ 0.89 mPa·s·kg/mol for phenylalanine, 1.26 mPa·s·kg/mol for tryptophan, and 1.08 mPa·s·kg/mol for tyrosine at pH 7 and 303.15 K.

Temperature increase causes viscosity decrease across all systems, following Arrhenius behavior. The temperature coefficients (dη/dT) range from -0.015 to -0.022 mPa·s/K depending on amino acid type and pH. This negative temperature dependence reflects reduced intermolecular forces and increased kinetic energy enabling easier molecular motion.

pH effects are significant, with pH 8 showing 3-5% higher viscosities than pH 6 for all amino acids. This pH dependence correlates with ionization state changes: at pH 8 (above pI), amino acids carry net negative charge, enhancing electrostatic hydration and increasing structure-making effects. The pKa values of the aromatic side chains (tyrosine phenolic OH: 10.1; tryptophan indole NH: 16) suggest minimal direct ionization effects, with the observed pH dependence primarily reflecting α-carboxyl and α-amino group ionization

3.5 Data Validation and Quality Assessment

The apparent anomaly of some ηrel values slightly below 1.0 (e.g., phenylalanine at low concentrations and high temperatures) deserves careful consideration. These values, while seemingly unphysical, fall within the experimental uncertainty (±0.5%) and reflect the complex balance of effects in the buffer-urea-amino acid system. Kinetic energy corrections to the Poiseuille equation, non-Newtonian behavior at molecular scales, and competing hydration/dehydration effects may contribute to these observations. All data points were verified through replicate measurements, and the overall trends remain statistically significant and physically meaningful.

3.6 Comparative Analysis and Structure-Property Relationships

The viscosity order L-tryptophan > L-tyrosine > DL-phenylalanine reflects the combined effects of molecular size, aromatic character, and hydrogen bonding capacity. Tryptophan's bicyclic indole system provides both larger molecular volume and additional hydrogen bonding sites (indole NH), explaining its

highest viscosity and B-coefficient. Tyrosine's phenolic OH enables hydrogen bonding but with a smaller aromatic system than tryptophan. Phenylalanine, lacking polar groups on its phenyl ring, shows the weakest interactions despite significant hydrophobic character. These observations align with established understanding of aromatic amino acid behavior in protein folding and stability (Dougherty, 2013).

3.7 Biological Significance

The viscometric behavior observed has direct relevance to protein folding and stability in biological systems. The structure-making effects (positive B-coefficients) of aromatic amino acids contribute to protein stability through enhanced hydration shells and ordered water structure around buried aromatic residues. The temperature dependence of these effects provides insights into cold and heat denaturation mechanisms. The urea-containing buffer system models partially denatured protein environments, and the observed viscometric changes reflect the balance between native-like and denatured conformational preferences. These findings contribute to our understanding of how environmental conditions (temperature, pH, denaturant concentration) modulate protein stability through effects on aromatic residue solvation.

4. Conclusion

This systematic viscometric study of DL-phenylalanine, L-tryptophan, and L-tyrosine in phosphate buffer-urea systems has provided comprehensive insights into aromatic amino acid solution behavior:

1. Complete viscometric characterization including absolute viscosities, Jones-Dole B-coefficients, and activation energies has been achieved across pH 6-8 and 303-328 K temperature range.
2. B-coefficients confirm structure-making (kosmotropic) behavior for all amino acids, with quantitative relationships to molecular structure established.
3. Activation energies (18-22 kJ mol−1) provide thermodynamic validation of interaction strengths, following the order tryptophan > tyrosine > phenylalanine.
4. Temperature and pH dependencies reveal complex molecular interactions relevant to protein stability and folding mechanisms.
5. The multi-component buffer-urea system successfully models biological environments, providing reference data for protein stability studies.

These results contribute significantly to biophysical chemistry and biochemistry, offering both fundamental insights into biomolecular interactions and practical data for pharmaceutical and biotechnology applications.

5. Future Scope

This study opens several promising research directions:

1. **Extension to dipeptides and tripeptides containing aromatic residues** to bridge the gap between individual amino acids and protein behavior, providing insights into cooperative effects and sequence-dependent solvation.
2. **Investigation of other biological buffers** (HEPES, Tris, citrate) and denaturants (guanidinium chloride, glycerol) to establish comprehensive solvent effect databases for computational modeling.
3. **Molecular dynamics simulations** validated against these experimental data to elucidate atomic-level solvation structures and dynamics, particularly water molecule organization around aromatic side chains.
4. **High-pressure viscometry studies** to determine volumetric properties and pressure-temperature-composition phase diagrams relevant to deep-sea organisms and high-pressure biocatalysis.
5. **Application to protein formulation development**, using viscometric data to optimize storage conditions, prevent aggregation, and enhance therapeutic protein stability in pharmaceutical formulations.
6. **Integration with spectroscopic techniques** (fluorescence, NMR, circular dichroism) to correlate macroscopic viscosity changes with molecular-level structural transitions and conformational dynamics.
7. **Investigation of aromatic amino acid behavior in crowded environments** using macromolecular crowding agents (Ficoll, dextran) to simulate cellular conditions and understand in vivo protein behavior.

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