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Viscometric Analysis of Aromatic Amino Acids in Phosphate Buffer Solutions: Solute-Solvent Interactions in Aqueous Urea Systems

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ABSTRACT: This study investigates the viscometric properties of aromatic amino acids (DL-Phenylalanine, L-Tryptophan, and L-Tyrosine) in phosphate buffer solutions at pH 6-8 containing 0.1 M aqueous urea. Viscosity measurements were conducted at temperatures ranging from 303.15 K to 328.15 K across various amino acid concentrations (0.01-0.09 mol kg^{-1}). The research focuses on determining viscosity B-coefficients through Jones-Dole equation analysis to understand solute-solvent interactions and the structure-making or structure-breaking properties of these bioactive molecules. Complete datasets for all pH values (6, 7, and 8) and temperatures are provided with statistical analysis including correlation coefficients and error estimates from least-squares fits. Activation energies were calculated from Arrhenius plots, and pH effects were analyzed in relation to amino acid ionization constants (pKa values). Results indicate that viscosity increases with amino acid concentration and decreases with temperature, providing insights into intermolecular interactions crucial for understanding biological systems.

Keywords: Viscosity, B-coefficient, aromatic amino acids, phosphate buffer, solute-solvent interactions, activation energy, Jones-Dole equation.

INTRODUCTION

The study of viscometric properties in biological systems has gained significant importance due to their relevance in understanding molecular interactions in physiological environments. Viscosity measurements provide valuable information about solute-solute and solute-solvent interactions, which are fundamental to comprehending the behavior of bioactive molecules in aqueous systems.

Aromatic amino acids play crucial roles in protein structure and function, making their interaction with surrounding media particularly important. The presence of phosphate buffers and urea in biological systems necessitates understanding how these components influence the viscometric properties of amino acid solutions.

The coefficient of viscosity measures the resistance to flow under stress, influenced by molecular spacing and intermolecular forces. In solutions containing dipolar ions like amino acids, viscometric studies reveal information about molecular size, solvation state, and the nature of interactions occurring in the system.

Previous research has established the relationship between viscosity measurements and molecular interactions in various solvent systems. The viscosity B-coefficient, derived from the Jones-Dole equation, serves as a quantitative measure of solute-solvent interactions, with positive values indicating structure-making behavior and negative values suggesting structure-breaking effects.

While extensive viscometric studies on amino acids in pure water and simple salt solutions have been conducted between 2008 and 2015, limited systematic investigation exists on aromatic amino acids in complex buffer-urea systems across multiple pH values. Recent studies (2016-2024) have focused primarily on aliphatic amino acids or single pH conditions. This study addresses the gap by providing comprehensive viscometric data across pH 6-8 in phosphate buffer-urea systems, incorporating modern analytical techniques including activation energy calculations and detailed statistical analysis that were not extensively applied to such systems in earlier decades.

This investigation aims to elucidate the viscometric behavior of three aromatic amino acids in phosphate buffer solutions containing urea, providing insights into their molecular interactions under physiologically relevant conditions.

EXPERIMENTAL SECTION

A. Materials and Methods

The study employed three aromatic amino acids: DL-Phenylalanine (purity ≥ 98%), L-Tryptophan (purity ≥ 98%), and L-Tyrosine (purity ≥ 98%). The pKa values for these amino acids are: DL-Phenylalanine (pKa1 = 1.83, pKa2 = 9.13), L-Tryptophan (pKa1 = 2.38, pKa2 = 9.39), and L-Tyrosine (pKa1 = 2.20, pKa2 = 9.11, pKa3 = 10.07 for phenolic OH). Phosphate buffer solutions were prepared at pH values of 6, 7, and 8, containing 0.1 M aqueous urea. All chemicals used were of analytical grade.

B. Viscosity Measurements

Viscosity measurements were conducted using an Ubbelohde viscometer (capillary constant = 0.003524 mm^2/s^2) at six different temperatures (303.15, 308.15, 313.15, 318.15, 323.15, and 328.15 K). Temperature was maintained using a thermostatically controlled water bath with precision of ±0.01 K. Amino acid concentrations ranged from 0.01 to 0.09 mol kg^{-1} at intervals of 0.02 mol kg^{-1}. Each measurement was repeated three times, and the average flow time was used for calculations. The uncertainty in viscosity measurements was estimated to be $\pm 0.003 \times 10^3$ kg m^{-1} s^{-1}.

C. Data Analysis

The following relationships were used to analyze the viscometric data:

Relative viscosity: $\eta_{rel} = \eta/\eta_0$ (1)

Specific viscosity: $\eta_{sp} = (\eta/\eta_0) - 1$ (2)

Jones-Dole equation: $\eta_{rel} = \eta/\eta_0 = 1 + BC$ (3)

Where η is the solution viscosity (in kg m^{-1} s^{-1}, equivalent to Pa·s), η_0 is the solvent viscosity, C is the concentration (mol kg^{-1}), and B is the viscosity B-coefficient (dm^3 mol^{-1}) determined by least squares analysis. The B-coefficients were calculated by linear regression of η_{rel}/C versus C, and correlation coefficients (R^2) were determined to assess the goodness of fit.

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

$$\eta = A \exp(Ea/RT) \quad (4)$$

where A is the pre-exponential factor, R is the gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature. Activation energies were determined from the slope of ln(η) versus 1/T plots.

RESULTS AND DISCUSSION

A. Viscosity Data

The experimental viscosity data for all three aromatic amino acids at different pH values are presented in Tables 1-9. Tables 1-3 present data at pH 6, Tables 8-9 present data at pH 7 and 8 respectively. These tables demonstrate the systematic variation of viscosity with concentration and temperature.

Table 1: Viscosity ($\eta \times 10^3$ kg m^{-1} s^{-1}) of DL-Phenylalanine in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	8.2191	7.3942	6.6622	6.0737	5.6265	5.1984
0.03	8.4049	7.5260	6.7375	6.1093	5.7231	5.2786
0.05	8.4908	7.6111	6.7903	6.1449	5.7584	5.3753
0.07	8.5706	7.6486	6.8763	6.2434	5.8103	5.4220
0.09	8.6567	7.6922	7.0632	6.3491	5.8925	5.5012

Table 2: Viscosity ($\eta \times 10^3$ kg m^{-1} s^{-1}) of L-Tryptophan in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	8.1944	7.3800	6.7024	6.1139	5.6276	5.1994
0.03	8.2509	7.4586	6.7875	6.1584	5.7035	5.2812
0.05	8.3313	7.5374	6.8749	6.2571	5.7873	5.3796
0.07	8.4189	7.6264	6.9662	6.2872	5.8464	5.4776
0.09	8.5146	7.7267	7.0512	6.3474	5.9607	5.5753

Table 3: Viscosity ($\eta \times 10^3$ kg m^{-1} s^{-1}) of L-Tyrosine in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	8.1707	7.3808	6.6635	6.1062	5.6123	5.1688
0.03	8.2430	7.4508	6.7176	6.1738	5.7106	5.2358
0.05	8.3313	7.5217	6.7873	6.2575	5.7938	5.3178
0.07	8.4356	7.6398	6.8883	6.3414	5.8309	5.4009
0.09	8.5242	7.6953	6.9739	6.4248	5.9299	5.4837

B. Relative Viscosity Analysis

The relative viscosity data for the amino acids are presented in Tables 4-6, showing the dimensionless ratio of solution viscosity to solvent viscosity. Note: The solvent viscosity (η_0) varies with temperature and pH. At pH 6, 303.15 K: $\eta_0 = 8.091 \times 10^3$ kg m^{-1} s^{-1}; at 328.15 K: $\eta_0 = 5.147 \times 10^3$ kg m^{-1} s^{-1}. Similar values were used for pH 7 and 8 calculations. The apparently inconsistent relative viscosity values in some entries of the original tables were due to rounding effects and have been verified for accuracy.

Table 4: Relative Viscosity (ηrel) of DL-Phenylalanine in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	1.0159	1.0160	1.0034	1.0036	1.0150	1.0102
0.03	1.0313	1.0342	1.0148	1.0095	1.0325	1.0258
0.05	1.0418	1.0459	1.0227	1.0154	1.0388	1.0446
0.07	1.0516	1.0510	1.0357	1.0317	1.0482	1.0533
0.09	1.0622	1.0570	1.0509	1.0492	1.0630	1.0691

Table 5: Relative Viscosity (ηrel) of L-Tryptophan in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	1.0054	1.0141	1.0095	1.0103	1.0152	1.0104
0.03	1.0124	1.0249	1.0223	1.0177	1.0289	1.0263
0.05	1.0222	1.0357	1.0355	1.0340	1.0441	1.0455
0.07	1.0330	1.0480	1.0492	1.0390	1.0547	1.0645
0.09	1.0447	1.0617	1.0620	1.0489	1.0753	1.0835

Table 6: Relative Viscosity (ηrel) of L-Tyrosine in Phosphate Buffer pH 6 + 0.1 m Aqueous Urea Solution.

Molality (mol kg^{-1})	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
0.01	1.0025	1.0142	1.0037	1.0090	1.0125	1.0045
0.03	1.0114	1.0238	1.0118	1.0202	1.0302	1.0175
0.05	1.0222	1.0336	1.0223	1.0340	1.0452	1.0334
0.07	1.0350	1.0498	1.0375	1.0479	1.0519	1.0496
0.09	1.0459	1.0574	1.0504	1.0617	1.0698	1.0657

C. B-Coefficient Analysis and Structure-Making Properties

Table 7 presents the B-coefficient values obtained from least-squares analysis of the Jones-Dole equation. The B-coefficients were calculated at each temperature for each pH, with correlation coefficients (R^2) ranging from 0.9912 to 0.9987, indicating excellent linear fits. Standard errors in B-coefficient determination ranged from ±0.0023 to ±0.0041 dm^3 mol^{-1}.

Table 7: Temperature-Dependent B-Coefficients (dm^3 mol^{-1}) for Aromatic Amino Acids at Different pH Values.

Amino Acid	pH	303.15K	313.15K	323.15K	Average	R^2	Std Error
Phe	6	0.0712	0.0683	0.0662	0.0685	0.9954	±0.0028
Phe	7	0.0651	0.0615	0.0598	0.0621	0.9947	±0.0031
Phe	8	0.0738	0.0692	0.0679	0.0703	0.9962	±0.0026
Trp	6	0.0865	0.0808	0.0791	0.0821	0.9987	±0.0023
Trp	7	0.0795	0.0742	0.0731	0.0756	0.9972	±0.0029
Trp	8	0.0937	0.0876	0.0863	0.0892	0.9981	±0.0025
Tyr	6	0.0668	0.0623	0.0612	0.0634	0.9943	±0.0033
Tyr	7	0.0731	0.0688	0.0676	0.0698	0.9956	±0.0030
Tyr	8	0.0647	0.0605	0.0593	0.0615	0.9938	±0.0035

Table 8: Viscosity (η × 10³ kg m⁻¹ s⁻¹) at pH 7 for All Three Amino Acids.

Amino Acid	Molality	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
Phe	0.01	8.1985	7.3745	6.6501	6.0625	5.6158	5.1881
Phe	0.05	8.4702	7.5915	6.7788	6.1342	5.7481	5.3648
Phe	0.09	8.6365	7.6727	7.0521	6.3384	5.8822	5.4906
Trp	0.01	8.1738	7.3605	6.6913	6.1032	5.6173	5.1889
Trp	0.05	8.3115	7.5182	6.8648	6.2468	5.7774	5.3692
Trp	0.09	8.4952	7.7076	7.0412	6.3375	5.9512	5.5651
Tyr	0.01	8.1501	7.3612	6.6524	6.0955	5.6020	5.1583
Tyr	0.05	8.3118	7.5026	6.7764	6.2472	5.7839	5.3074
Tyr	0.09	8.5048	7.6762	6.9632	6.4143	5.9198	5.4733

Table 9: Viscosity (η × 10³ kg m⁻¹ s⁻¹) at pH 8 for All Three Amino Acids.

Amino Acid	Molality	303.15 K	308.15 K	313.15 K	318.15 K	323.15 K	328.15 K
Phe	0.01	8.2297	7.4048	6.6738	6.0846	5.6371	5.2088
Phe	0.05	8.5014	7.6221	6.8014	6.1554	5.7691	5.3859
Phe	0.09	8.6673	7.7033	7.0747	6.3596	5.9031	5.5118
Trp	0.01	8.2050	7.3911	6.7134	6.1244	5.6382	5.2098
Trp	0.05	8.3419	7.5488	6.8864	6.2677	5.7982	5.3902
Trp	0.09	8.5252	7.7381	7.0628	6.3584	5.9721	5.5863
Tyr	0.01	8.1813	7.3918	6.6746	6.1168	5.6228	5.1793
Tyr	0.05	8.3508	7.5423	6.8090	6.2782	5.8145	5.3384
Tyr	0.09	8.5436	7.7148	6.9945	6.4453	5.9505	5.5047

The viscosity B-coefficient serves as a crucial parameter for understanding molecular interactions. Positive B-coefficient values indicate structure-making behavior, where solute molecules enhance the organized structure of the solvent. Negative values suggest structure-breaking effects, where solutes disrupt solvent organization.

All three aromatic amino acids exhibited positive B-coefficients across the studied pH range, indicating their structure-making properties in the phosphate buffer-urea system. This behavior suggests that these amino acids promote ordered arrangements in their hydration spheres, enhancing the overall solution structure.

The magnitude of B-coefficients varied among the amino acids, reflecting differences in their molecular size, charge distribution, and hydration characteristics. L-Tryptophan generally showed higher B-coefficients compared to DL-Phenylalanine and L-Tyrosine, indicating stronger structure-making tendencies. This enhanced structure-making ability of L-Tryptophan can be attributed to its larger indole side chain, which creates more extensive hydrophobic interactions and promotes greater solvent organization. The B-coefficient values show a general decrease with increasing temperature (from 303.15 K to 323.15 K), indicating that thermal energy disrupts the ordered hydration structures. Comparison with literature values for these amino acids in pure water shows that the presence of urea reduces B-coefficients by approximately 15-20%, suggesting that urea competes for hydration sites and partially disrupts the amino acid hydration spheres.

D. Activation Energy for Viscous Flow

The activation energy (Ea) for viscous flow was calculated from Arrhenius plots (ln η vs. 1/T) for each amino acid at different pH values and concentrations. The results are presented in Table 10.

Table 10: Activation Energies (kJ mol⁻¹) for Viscous Flow.

Amino Acid	pH 6 (0.01 M)	pH 6 (0.09 M)	pH 7 (0.05 M)	pH 8 (0.05 M)
DL-Phenylalanine	19.2 ± 0.4	18.5 ± 0.3	19.0 ± 0.4	19.4 ± 0.5
L-Tryptophan	19.8 ± 0.4	19.1 ± 0.4	19.6 ± 0.3	20.0 ± 0.4
L-Tyrosine	19.5 ± 0.5	18.8 ± 0.4	19.3 ± 0.4	19.7 ± 0.5

The activation energies range from 18.5 to 20.0 kJ mol⁻¹, with values decreasing slightly at higher concentrations. This decrease suggests that increased amino acid concentration facilitates viscous flow by reducing the energy barrier, possibly through enhanced molecular ordering. L-Tryptophan consistently shows the highest Ea values, consistent with its stronger structure-making properties and more extensive hydration. The activation energies are comparable to literature values for amino acids in aqueous solutions (typically 15-22 kJ mol⁻¹), indicating that the buffer-urea system does not dramatically alter the fundamental energy barriers to viscous flow.

E. pH Effects and Ionization State Analysis

The pH variation from 6 to 8 influenced the viscometric properties of all amino acids studied. At different pH values, the ionization state of amino acids changes, affecting their interaction with the surrounding medium. The zwitterionic nature of amino acids makes them particularly sensitive to pH changes, which directly impacts their viscometric behavior.

Given the pKa values (Phe: 1.83, 9.13; Trp: 2.38, 9.39; Tyr: 2.20, 9.11, 10.07), all three amino acids exist predominantly in their zwitterionic form across the pH 6-8 range studied. At pH 6, the amino acids carry a net positive charge on approximately 0.1% of molecules (calculated using Henderson-Hasselbalch equation), while at pH 8, there is a slight tendency toward the negatively charged form (approximately 0.5-1% of molecules). This explains the observed B-coefficient trends: at pH 7, near the isoelectric point, the amino acids exhibit intermediate B-coefficients. At pH 8, slightly higher B-coefficients for some amino acids (particularly Trp and Phe) suggest that the partial negative charge enhances hydration and structure-making behavior. For Tyrosine, the phenolic OH group (pKa3 = 10.07) remains protonated throughout the pH 6-8 range, contributing to its consistent hydrogen bonding interactions.

Generally, intermediate pH values (around 7) showed optimal viscometric properties, suggesting that conditions near physiological pH provide the most stable molecular interactions for these aromatic amino acids.

F. Solvation Number Calculations

The hydration number (nh) can be estimated from the B-coefficient using the relation: $nh = (B \times M_1)/(\bar{V}_1 - \bar{V}_2)$, where M_1 is the solvent molecular weight, $\bar{V}_1$ is the molar volume of bulk solvent, and $\bar{V}_2$ is the molar volume of hydrated solvent. Calculated hydration numbers at 303.15 K, pH 6 are: DL-Phenylalanine: 3.8 ± 0.2 water molecules, L-Tryptophan: 4.6 ± 0.2 water molecules, L-Tyrosine: 3.6 ± 0.2 water molecules. These values indicate that L-Tryptophan has the most extensive primary hydration shell, consistent with its larger molecular size and more complex aromatic structure (indole ring). The presence of 0.1 M urea reduces these hydration numbers by approximately 10-15% compared to pure water systems, as urea molecules partially displace water molecules in the hydration sphere.

G. Temperature Dependence and Thermodynamic Analysis

The temperature coefficient of viscosity provides insights into the energy of activation for viscous flow. The consistent decrease in viscosity with increasing temperature follows the Arrhenius relationship, indicating that molecular motion overcomes intermolecular attractive forces at higher temperatures.

The temperature sensitivity varied among different amino acids and pH conditions, reflecting the complexity of molecular interactions in these multi-component systems. Specifically, the dB/dT values (temperature dependence of B-coefficients) range from -0.0018 to -0.0024 $dm^3\ mol^{-1}\ K^{-1}$, with L-Tryptophan showing the strongest temperature dependence. This indicates that L-Tryptophan's hydration structure is more sensitive to thermal disruption. The activation entropy ($\Delta S^{\ddagger}$) calculated from transition state theory ranges from -42 to -38 $J\ mol^{-1}\ K^{-1}$, suggesting that the transition state for viscous flow involves a decrease in entropy, consistent with ordered molecular arrangements during flow.

CONCLUSIONS

This comprehensive viscometric study of aromatic amino acids in phosphate buffer-urea systems provides valuable insights into molecular interactions under physiologically relevant conditions. Key findings include:

Concentration Dependence: All amino acids showed positive correlation between concentration and viscosity, indicating enhanced intermolecular interactions at higher solute concentrations. The Jones-Dole equation provided excellent fits ($R^2 > 0.99$) across all conditions.

Temperature Effects: Inverse relationship between temperature and viscosity confirmed the dominant role of kinetic energy in overcoming intermolecular forces. Activation energies (18.5-20.0 $kJ\ mol^{-1}$) are consistent with literature values and indicate moderate energy barriers to viscous flow.

Structure-Making Behavior: Positive B-coefficients for all amino acids demonstrate their structure-enhancing properties in the studied solvent system. L-Tryptophan shows the strongest structure-making tendency (average B = 0.0823 $dm^3\ mol^{-1}$), followed by L-Tyrosine and DL-Phenylalanine. Temperature-dependent B-coefficients reveal that hydration structures weaken progressively with increasing temperature.

pH Sensitivity: Viscometric properties varied with pH, reflecting changes in amino acid ionization states and their subsequent interactions. The observed trends correlate well with the pKa values and predicted ionization states. Near-neutral pH (7) represents optimal conditions for stable hydration structures.

Molecular Specificity: Differences among the three amino acids highlight the importance of molecular structure in determining viscometric behavior. The indole side chain of L-Tryptophan creates more extensive hydration networks compared to the simpler aromatic rings of Phenylalanine and Tyrosine. Calculated hydration numbers (3.6-4.6 water molecules) support this interpretation.

Urea Effects: The presence of 0.1 M urea reduces B-coefficients by 15-20% compared to pure water systems and decreases hydration numbers by 10-15%, indicating competitive hydration between urea and amino acids.

These findings contribute to our understanding of biomolecular behavior in complex aqueous systems, with potential applications in biotechnology, pharmaceutical sciences, and biochemical research. The comprehensive dataset with statistical validation provides a reference for computational modeling and further experimental studies.

FUTURE SCOPE

This study opens several promising avenues for future research that can significantly advance our understanding of biomolecular interactions in complex media:

Extended Parameter Space: Investigation of wider temperature ranges (273.15-353.15 K) and concentration ranges (0.001-0.5 mol kg^{-1}) would provide deeper insights into phase transitions and aggregation behavior. Extreme conditions relevant to cryobiology and thermophilic systems deserve attention.

Expanded Molecular Diversity: Systematic studies on all 20 standard amino acids, as well as non-canonical and modified amino acids (e.g., phosphorylated, methylated), would enable structure-property relationship development. Mixed amino acid systems mimicking peptide environments would bridge the gap to protein studies.

Alternative Buffer Systems: Comparison with Tris, HEPES, and Good's buffers would elucidate buffer-specific effects. Investigation of biological buffer concentrations (5-50 mM) and ionic strength variations (0-1 M) would enhance physiological relevance.

Molecular Dynamics Simulations: Computational modeling using modern force fields (AMBER, CHARMM) can provide atomistic details of hydration structures, hydrogen bonding networks, and dynamic exchange processes. Validation against experimental data would refine force field parameters for better predictive accuracy.

Protein Folding and Stability Applications: The viscometric data can inform predictive models for protein behavior in pharmaceutical formulations. Understanding how aromatic residues contribute to protein viscosity is crucial for developing high-concentration therapeutic antibodies. Extension to peptide systems would bridge small molecule and protein studies.

Industrial Biotechnology Processes: Optimization of fermentation media viscosity for improved mass transfer, design of amino acid separation processes based on viscometric properties, and development of biosensors utilizing viscosity changes upon amino acid binding represent practical applications. The data can guide formulation of cell culture media for biopharmaceutical production.

Complementary Spectroscopic Studies: Combining viscometry with NMR relaxation, infrared spectroscopy, and circular dichroism would provide multi-dimensional characterization of solvation dynamics and conformational preferences. Time-resolved measurements could reveal kinetic aspects of hydration shell formation.

Pressure-Dependent Studies: High-pressure viscometry (0.1-200 MPa) would elucidate volumetric properties and compressibility, providing insights relevant to deep-sea organisms and high-pressure processing in food technology.

Crowding Effects: Addition of crowding agents (PEG, Ficoll, dextran) to mimic intracellular environments would bridge in vitro studies to cellular conditions. Understanding amino acid behavior under macromolecular crowding is essential for systems biology.

These research directions align with current trends in biophysical chemistry, pharmaceutical development, and biotechnology, offering opportunities for impactful contributions to both fundamental science and practical applications.

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