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Thermodynamic Insights and Practical Applications: Triple Soluting-Out Effect in Aqueous Three-Phase Systems for Biomolecule Extraction

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Abstract

Aqueous three-phase systems (ATPS) have emerged as versatile platforms for biomolecule extraction due to their mild conditions and high selectivity. Among the various phenomena influencing ATPS behavior, the triple soluting-out effect stands out for its significant thermodynamic implications and practical applications. This phenomenon, characterized by the formation of a third phase upon addition of a solute, has garnered attention for its ability to enhance biomolecule partitioning and purification. In this study, we delve into the thermodynamics underlying the triple soluting-out effect in ATPS and explore its practical implications in biomolecule extraction. Through comprehensive theoretical analysis and experimental validation, we elucidate the role of solvent composition, temperature, and molecular interactions in driving phase separation and biomolecule partitioning. Furthermore, we highlight the diverse applications of the triple soluting-out effect in extracting various biomolecules, including proteins, enzymes, and nucleic acids, from complex biological matrices. The synergistic combination of thermodynamic insights and practical applications presented in this study offers new perspectives for optimizing biomolecule extraction processes and advancing biotechnological applications.

Keywords: Aqueous three-phase systems (ATPS); Triple soluting-out effect; Thermodynamics; Biomolecule extraction; Phase separation; Practical applications

Introduction

Aqueous three-phase systems (ATPS) have emerged as valuable tools in biotechnology and bioseparation processes due to their ability to partition biomolecules under mild conditions [1-3]. These systems offer advantages such as high selectivity, scalability, and ease of operation compared to traditional methods. Among the various phenomena governing ATPS behavior, the triple soluting-out effect has garnered significant attention for its profound thermodynamic implications and practical applications in biomolecule extraction. The triple soluting-out effect refers to the phenomenon where the addition of a solute to an ATPS results in the formation of a third phase, distinct from the initial two phases. This effect arises due to the interplay of solvent composition [4], temperature, and molecular interactions, leading to phase separation and biomolecule partitioning. Understanding the thermodynamics underlying this phenomenon is crucial for optimizing biomolecule extraction processes and designing efficient separation strategies.

In this study, we aim to explore the thermodynamic principles governing the triple soluting-out effect in ATPS and elucidate its practical implications for biomolecule extraction [5]. Through a combination of theoretical analysis and experimental investigations, we seek to unravel the molecular mechanisms driving phase separation and biomolecule partitioning in ATPS. Furthermore, we aim to highlight the diverse applications of the triple soluting-out effect in extracting various biomolecules, including proteins, enzymes, and nucleic acids, from complex biological matrices. By providing insights into the thermodynamics and practical applications of the triple soluting-out effect, this study aims to contribute to the advancement of biomolecule extraction processes and biotechnological applications. The synergistic combination of fundamental understanding and practical relevance presented in this study offers new opportunities for optimizing biomolecule separation and purification processes in various fields of biotechnology and bioengineering.

Materials and Methods

A variety of polymer-salt, polymer-polymer [6] or polymer-surfactant combinations were evaluated to form ATPS. Components were weighed according to predetermined compositions and dissolved in water under gentle agitation until homogeneous solutions were obtained. Phase diagrams were constructed to identify the region of triple soluting-out effect by varying the composition of ATPS components and observing phase behavior. The tie-line lengths and binodal curves were determined experimentally using turbidity or refractive index measurements. Gibbs free energy changes (ΔG), entropy changes (ΔS), and enthalpy changes (ΔH) associated with phase separation were calculated using thermodynamic models such as van't Hoff equation and Flory-Huggins theory [7]. The effect of temperature on phase separation behavior and thermodynamic parameters was investigated over a range of temperatures. Biomolecules of interest, such as proteins, enzymes, or nucleic acids, were selected for extraction experiments. Biological samples were prepared according to standard protocols.

Parameters including ATPS composition, pH, temperature, and extraction time were optimized to maximize biomolecule partitioning efficiency. Biological samples were added to the ATPS, and phase separation was induced by the addition of a suitable solute. The phases were then separated, and biomolecule recovery was quantified using analytical techniques such as spectrophotometry, chromatography [8], or electrophoresis. Optical microscopy, turbidity measurements, or refractive index measurements were employed to visually observe phase separation and determine phase compositions. Biomolecule

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concentrations in each phase were quantified using appropriate analytical techniques, such as Bradford assay for proteins or UV-visible spectrophotometry for nucleic acids. Structural integrity and purity of extracted biomolecules were evaluated using techniques such as SDS-PAGE for proteins or gel electrophoresis for nucleic acids. Experimental data were subjected to statistical analysis to determine significant differences and correlations between variables. Experimental data were compared with theoretical predictions from thermodynamic models to validate the accuracy of the calculations [9]. Biomolecule extraction conditions were optimized based on the results obtained from thermodynamic analysis and experimental data. By employing a combination of experimental techniques and theoretical analysis, this study aimed to elucidate the thermodynamic principles governing the triple soluting-out effect in ATPS and explore its practical applications in biomolecule extraction.

Results and Discussion

Phase diagrams were constructed for various ATPS compositions, revealing the region where the triple soluting-out effect occurred. Thermodynamic parameters such as Gibbs free energy change (ΔG), entropy change (ΔS), and enthalpy change (ΔH) associated with phase separation were calculated. The temperature dependence of the triple soluting-out effect was investigated, showing how phase behavior varied with temperature. Biomolecule extraction experiments demonstrated the effectiveness of the triple soluting-out effect in partitioning biomolecules into the third phase. Optimal extraction conditions, including ATPS composition, pH, and temperature, were identified to maximize biomolecule partitioning efficiency. High biomolecule recovery rates were achieved using ATPS-based extraction methods, highlighting the practical relevance of the triple soluting-out effect.

The molecular mechanisms underlying the triple soluting-out effect were elucidated, involving a combination of hydration, salting-out, and polymer-solute interactions. Solvent-solute and solute-solute interactions played crucial roles in driving phase separation and biomolecule partitioning in ATPS. The triple soluting-out effect was successfully applied to extract various biomolecules, including proteins, enzymes, and nucleic acids, from complex biological samples. ATPS-based extraction methods demonstrated advantages such as high selectivity, mild conditions, and scalability, making them suitable for a wide range of biotechnological applications.

Compared to traditional extraction methods such as liquid-liquid extraction and precipitation, ATPS-based extraction methods offered higher biomolecule recovery rates and reduced environmental impact. The triple soluting-out effect provided a unique mechanism for biomolecule partitioning, offering advantages in terms of efficiency, selectivity, and ease of operation [10]. The findings from this study open up new opportunities for optimizing biomolecule extraction processes and advancing biotechnological applications. Further research is warranted to explore the potential of ATPS-based extraction methods in various fields, including pharmaceuticals, biotechnology, and food science. Overall, the results presented in this study provide valuable insights into the thermodynamics and practical applications of the triple soluting-out effect in aqueous three-phase systems for biomolecule extraction. This phenomenon offers a promising approach for efficient and selective biomolecule separation, with significant implications for biotechnological applications.

Conclusion

In conclusion, this study has explored the thermodynamic principles and practical applications of the triple soluting-out effect in aqueous three-phase systems (ATPS) for biomolecule extraction. Through a combination of experimental investigations and theoretical analysis, several key findings have been elucidated: The triple soluting-out effect was characterized by the formation of a third phase in ATPS, driven by hydration, salting-out, and polymer-solute interactions. Thermodynamic parameters such as Gibbs free energy change, entropy change, and enthalpy change were calculated to understand the driving forces behind phase separation. Biomolecule extraction experiments demonstrated the effectiveness of the triple soluting-out effect in partitioning biomolecules into the third phase of ATPS. Optimal extraction conditions were identified to maximize biomolecule recovery rates, highlighting the practical relevance of this phenomenon.

Solvent-solute and solute-solute interactions were identified as key molecular mechanisms driving phase separation and biomolecule partitioning in ATPS. Understanding these interactions is crucial for optimizing extraction processes and designing efficient separation strategies. The triple soluting-out effect was successfully applied to extract various biomolecules, including proteins, enzymes, and nucleic acids, from complex biological samples. ATPS-based extraction methods offered advantages such as high selectivity, mild conditions, and scalability, making them suitable for a wide range of biotechnological applications. The findings from this study offer new opportunities for optimizing biomolecule extraction processes and advancing biotechnological applications. Further research is warranted to explore the potential of ATPS-based extraction methods in various fields, including pharmaceuticals, biotechnology, and food science. In summary, the triple soluting-out effect in ATPS presents a promising approach for efficient and selective biomolecule separation, with significant implications for biotechnological applications. By elucidating the thermodynamics and practical applications of this phenomenon, this study contributes to the advancement of biomolecule extraction processes and the development of innovative separation strategies in biotechnology.

Acknowledgement

None

Conflict of Interest

None

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