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EXPLORING THE STABILIZATION OF BIOPHARMACEUTICALS WITH IONIC LIQUIDS AND DEEP EUTECTIC SOLVENTS

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ABSTRACT

Biopharmaceuticals have enabled the management of diseases that were once considered untreatable. Nonetheless, challenges related to their solubility and stability continue to obstruct their widespread use, transportation, and preservation. To address these issues, researchers have explored various compounds, including ionic liquids (ILs) and deep eutectic solvents (DESs), to maintain and improve the efficacy of biopharmaceuticals. While the biopharmaceutical sector can utilize a range of substances to enhance drug formulations, the outcomes vary based on the characteristics of the specific biomolecule and the surrounding environmental conditions. Therefore, this review presents a comprehensive overview of the latest research employing ILs and DESs for biopharmaceutical stabilization, examining the attributes of biomolecules, the classifications of ILs and DESs, concentration levels, forms of stability, and impacts. Predominantly, the cholinium-, imidazolium-, and ammonium-based ILs and DESs were the most utilized for biopharmaceutical stabilization, with cholinium ILs notably enhancing delivery. Interestingly, both dilute and concentrated solutions of ILs and DESs showed comparable efficacy in stabilizing biopharmaceuticals. With further research, ILs and DESs could address current formulation challenges in the biopharmaceutical industry.

Keywords: stability of biomolecules, pharmaceutical formulations, biopharmaceuticals, ILs, DESs

1 INTRODUCTION

Biopharmaceuticals have revolutionized the treatment of diseases with traditionally low survival rates, including various cancers, autoimmune disorders, and metabolic diseases.[1] These therapeutic agents encompass a broad spectrum of biomolecules derived from amino and nucleic acids, such as peptides, proteins, and DNA/RNA derivatives.[2] The proven efficacy of biopharmaceuticals has increased their demand, evidenced by a compound annual growth rate (CAGR) of 14.8% from 2023 to 2032, projecting a market value of USD 1.4 trillion by 2032.[3]

Nonetheless, their high cost and susceptibility to degradation at room temperatures and adverse environments make them inaccessible to lower-income populations. For instance, numerous bioactive macromolecules exhibit degradation or reduced absorption and bioavailability *in vivo*, even after showing elevated pharmacological effects *in vitro*.[4] The intrinsic instability of biopharmaceuticals outside their optimal conditions poses significant challenges for their clinical use and their manufacturing, storage, and distribution. Consequently, creating innovative formulations to stabilize and improve the delivery of biopharmaceuticals could broaden their use and accessibility, particularly in low-income and peripheral communities.

Researchers devised various approaches to enhance the stability of biopharmaceuticals, including introducing modern "green" solvents into their formulations. Specifically, various types of ionic liquids (ILs) and deep eutectic solvents (DESs) have been utilized to enhance the stability and delivery of biomolecules like proteins and nucleic acids.[5] Therefore, designing new green solvent formulations for biopharmaceuticals could lead to more efficient, sustainable, and eco-friendly production and use of biological medicines.[6]

With this in mind, this review aimed to organize and analyze the latest research employing ILs and DESs to improve the stability and delivery of biopharmaceuticals. We identified current trends and gaps in knowledge and discussed the potential of biocompatible ILs and DESs as formulation additives and solvents. Moreover, this review presented a comprehensive overview of the latest research employing ILs and DESs for biopharmaceutical stabilization and delivery, examining the attributes of biomolecules, the classifications of ILs and DESs, ILs and DESs concentration ranges, types of stability, and effects.

2 MATERIAL & METHODS

We summarize the literature on ILs and DESs as additives or solvents to improve the stability and delivery of biopharmaceuticals (26 articles).[1] First, we organized the different types of biopharmaceuticals (peptides, proteins, siRNA, and oligonucleotides) and classes of ILs (ammonium-, imidazolium-, phosphonium-, lidocainum-, and cholinium-based ILs) and DESs (Poly(vinyl pyrrolidone-based DESs and cholinium-based DESs). Then, we listed the effect of different concentration ranges of these ILs and DESs on the stability (structural, thermal, activity, aggregation) and delivery of the biopharmaceuticals.

Considering the extensive number of conditions, we evaluated the entries in the tables to determine the amount of ILs and DESs solutions that increased or maintained the stability of the biomolecules according to the stability type, IL class, and concentration range. We presented the results as percentages of the total for each variable. We selected this method to attempt to establish trends regarding the effect of different conditions on insulin stability. We should note that this analysis does not try to be a definitive answer regarding the impact of ILs and DESs on biopharmaceuticals, but it aims to find tendencies and knowledge gaps in this topic and be a guide for future research. Furthermore, we will give our insights regarding the opportunities in the area and the perspectives regarding the expansion of applications of ILs and DESs in the medical field.

3 RESULTS & DISCUSSION

This review summarizes the impact of ILs and DESs on the stabilization and delivery of biopharmaceuticals to discern patterns and highlight research gaps in this field. Our analysis, centered around **Figure 1**, categorizes the effects of ILs and DESs by stability type of the biomolecule, IL and DES class and concentration range, and improved biopharmaceutical delivery, aiming to offer insights for future applications of these compounds in biopharmaceutical formulations.

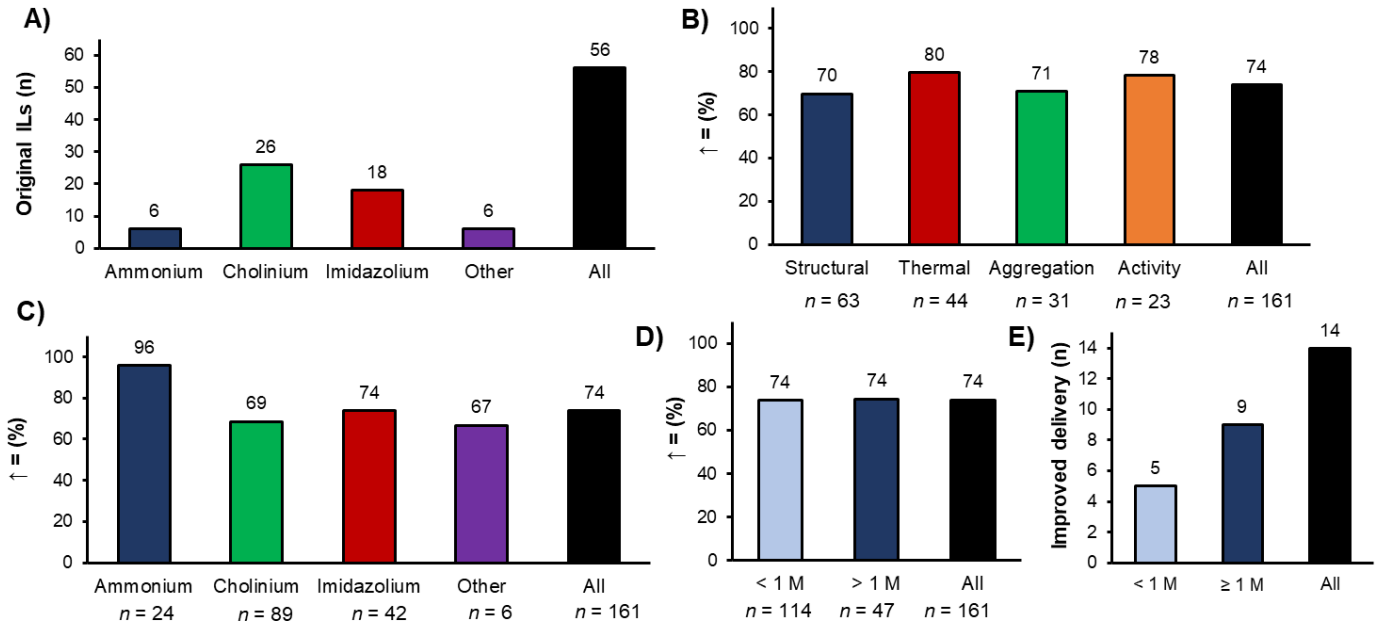

Figure 1 A) Number of original ILs and DESs for each class. **B)** Percentage of IL and DES solutions that maintained or increased the different types of stability of biopharmaceuticals (structural, thermal, activity, and aggregation). For aggregation, improvement of stability represents ILs and DESs that decrease protein aggregation. **C)** Percentage of solutions that maintained or increased the stability of biopharmaceuticals according to their IL and DES class. **D)** Percentage of IL and DES solutions that maintained or increased the stability of biopharmaceuticals according to their concentration (<1 or ≥ 1 M). **E)** Number of cholinium-based ILs and DESs that improved the delivery of biopharmaceuticals at different concentrations. *n* = total number of samples for each condition.[1]

Figure 1 demonstrates that most IL and DES solutions either enhance or maintain biopharmaceutical stability, with **Figure 1.B** showing about 70% success in preserving or maintaining structural and thermal stability, aggregation rate, and activity of proteins, peptides, and nucleic acids. Moreover, **Figure 1.A** and **1.E** reveals a predominance of cholinium-based ILs and DESs, underscoring their biocompatibility and utility in delivery enhancement. This finding aligns with the shift from imidazolium ILs, previously the most utilized class for protein stabilization,[5] towards cholinium-based solvents due to their lower toxicity and better pharmaceutical suitability.

Our findings indicate that low and high concentrations of ILs and DESs are comparably effective, maintaining around 70% stability across different biomolecules. However, the nuanced variations observed in individual macromolecules call for a broader dataset to accurately assess trends.

Among the IL and DES classes, ammonium ILs exhibited the highest compatibility, followed by imidazolium and cholinium variants. Despite limited comparative data on DESs, their application in studies on insulin, immunoglobulin G1, and L-asparaginase has been promising, particularly for delivery improvement. Specifically, cholinium-based solvents have shown potential in improving the thermal stability and delivery of immunoglobulin G1, suggesting their promise for antibody applications, including long-term storage and systemic circulation.

In summary, while ILs and DESs demonstrate significant promise for biopharmaceutical stabilization and delivery, the specific effects vary with the biomolecule and application, highlighting the need for ongoing research. Finally, cholinium-based ILs and DESs stand out for their potential for future pharmaceutical applications, driven by their biocompatibility and delivery enhancement capabilities.

4 CONCLUSION

The evolving field of ILs and DESs for biopharmaceutical applications highlights their crucial role in boosting the stability and delivery of biomolecules. Current findings reveal a generally favorable influence on biopharmaceutical structures and functions.

Yet, the diverse outcomes related to specific IL or DES types, their concentrations, and the targeted biomolecules point to the necessity of continued investigation. In this sense, cholinium-based ILs and DESs have outstanding potential for upcoming pharmaceutical applications due to their biocompatibility and superior delivery features. Therefore, we suggest that the research community intensify efforts in studying biocompatible varieties like cholinium and ammonium-based DESs, known for effectively maintaining biomolecular structure and function and enhancing solubility and delivery. Additionally, there is a call for broadening the range and accessibility of these solvents and delving into their interactions with biopharmaceuticals to illuminate their broader applicability. Advancements in this area could lead to the creation of predictive models and novel biopharmaceutical formulations.

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