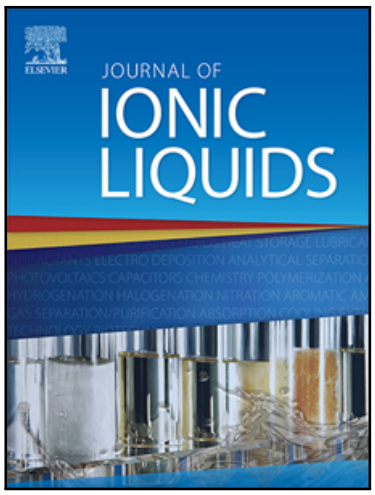

Ionic liquids and deep eutectic solvents: recent advances in the stabilization and functionalization of nucleic acid biopharmaceuticals

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Highlights

- Ionic liquids (ILs) and deep eutectic solvents (DESs) enhance nucleic acid stability.
- ILs and DESs protect nucleic acids by reducing hydrolysis and enzymatic degradation.
- Cholinium- and lipid-based ILs and DESs enhance transdermal delivery of nucleic acid therapeutics.
- Designed ILs and DESs preserve nucleic acid conformation and prolong storage stability.
- ILs and DESs bridge nanotechnology, green chemistry, and gene-based therapeutics.

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Ionic liquids and deep eutectic solvents: recent advances in the stabilization and functionalization of nucleic acid biopharmaceuticals

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Abstract: Nucleic acid-based biopharmaceuticals, including small interfering RNA (siRNA), messenger RNA (mRNA) vaccines, and antisense oligonucleotides (ASOs), represent one of the fastest-growing classes of therapeutics due to their ability to modulate gene expression and treat previously incurable diseases. However, their clinical translation remains constrained by intrinsic instability, susceptibility to enzymatic degradation, and complex storage and delivery requirements. Preserving molecular integrity while ensuring efficient and safe delivery, therefore, remains a central challenge in expanding their therapeutic applications. Ionic liquids (ILs) and deep eutectic solvents (DESs) have recently emerged as versatile media for the stabilization and functionalization of nucleic acid biopharmaceuticals. Their tunable ionic environments, extensive hydrogen-bond networks, and physicochemical versatility enable the preservation of DNA and RNA structures while mitigating hydrolysis, oxidation, and nuclease activity. Over the past decade, biocompatible cholinium- and other ammonium-based ILs, as well as cholinium-derived DESs, have been successfully applied to siRNA, ASO, and mRNA formulations, improving long-term stability and enhancing transdermal or intracellular delivery. Innovative strategies such as IL-robed RNA and surface-active ionic liquids (SAILs) were also proposed, integrating molecular protection with intrinsic delivery capacities, while supporting greener and more scalable formulation approaches. Despite these advances, challenges remain, particularly with respect to cytotoxicity and viscosity issues of ILs and DESs, and the absence of clear regulatory frameworks for pharmaceutical use. Overall, IL- and DES-based media are multifunctional platforms capable of overcoming key limitations of nucleic acid therapeutics and advancing the development of disruptive, safer, and more sustainable next-generation biopharmaceutical formulations. This critical perspective reviews recent progress in the application of ILs and DESs to nucleic acid stabilization and delivery, supported by a SWOT analysis and a personal insight into the future of the field.

Keywords: Ionic liquids; Deep eutectic solvents; Nucleic acids; Biopharmaceuticals; siRNA delivery; Green pharmaceutical formulations.

1. Introduction

Biopharmaceuticals have transformed modern medicine by enabling targeted and effective treatments for cancers, autoimmune disorders, and genetic diseases [1,2]. This class of therapeutics includes proteins, peptides, and nucleic acid-based agents such as messenger RNA (mRNA), small interfering RNA (siRNA), and antisense oligonucleotides (ASO), which directly modulate gene expression to correct or silence disease-associated pathways [3,4]. The clinical success of nucleic acid-based products, particularly mRNA vaccines developed in response to the SARS-CoV-2 pandemic, has validated their potential to reshape preventive and therapeutic medicine [5,6].

Despite these successes, the broader implementation remains constrained by their physicochemical fragility, vulnerability to enzymatic degradation, and stringent cold-chain storage requirements, which collectively hinder large-scale manufacturing and global accessibility [7,8]. These constraints contribute to high manufacturing costs and exacerbate inequities in access to next-generation biologics, particularly in low-resource settings.

In response, extensive research over the past decade has focused on developing innovative formulation strategies to improve nucleic acid stability and delivery efficiency [9,10]. Among these approaches, ionic liquids (ILs) and deep eutectic solvents (DESs) have emerged as highly adaptable and sustainable media capable of preserving molecular conformation, reducing degradation, and facilitating transport across biological barriers [11,12]. Their tunable ionic compositions, rich hydrogen-bonding networks, and increasing evidence of biocompatibility position some IL and DES families as promising alternatives to conventional pharmaceutical excipients [13–15].

The pharmaceutical relevance of ILs began to be considered more systematically after the work of Rogers and co-workers, who introduced the concept of the third evolution of ILs, proposing their deliberate design as active pharmaceutical ingredients (API) rather than solely as solvents or processing media [16]. In this framework, researchers viewed the modular combination of ions as a means to address persistent challenges associated with conventional solid drugs, including polymorphism, limited solubility, stability issues, and constrained delivery options. This perspective has since been refined and consolidated in later critical analyses, which established API-ILs and closely related deep eutectic solvent systems as adaptable pharmaceutical platforms with tunable physicochemical and biological properties [17].

Building on this conceptual shift, early studies evaluated the interaction between ILs and nucleic acids, seeking media able to preserve their structural integrity over extended periods. Foundational work demonstrated that hydrated, biocompatible ILs and cholinium-based DESs can stabilize a range of nucleic acid architectures, including duplex DNA, triplexes, and G-quadruplex structures, in some cases with greater long-term stability than conventional aqueous buffers [18,19]. These observations provided a mechanistic basis for considering ILs not only as stabilizing environments, but also as functional components in nucleic acid formulations.

Subsequent investigations further showed that selected IL systems can facilitate the delivery of large biomolecules by modulating membrane interactions and permeability in a composition-dependent manner. In particular, cholinium-based ILs were able to enhance transdermal transport and intracellular uptake while maintaining acceptable biocompatibility profiles, linking molecular stabilization with practical delivery performance [20,21].

Importantly, the formulation principles established for ILs also extend to DESs, which have emerged as versatile systems for nucleic acid stabilization and delivery. DESs are multicomponent liquid systems formed through cooperative hydrogen-bond interactions, resulting in dense hydrogen-bonding networks, tunable polarity, and composition-dependent physicochemical properties that support the preservation of nucleic acid structure and modulation of biomolecular interactions [22]. These shared physicochemical features enable DESs to adopt roles analogous to those explored for ILs, while offering additional flexibility in composition and formulation design [22–24].

The studies presented in this perspective support the view that ILs and DESs, when rationally designed, can serve as multifunctional formulation components that integrate stabilization and delivery within a single system. This trajectory closely aligns with the original proposal of these compounds as customizable pharmaceutical components, rather than passive excipients, demonstrating their growing relevance in the context of nucleic acid-based biopharmaceuticals.

In this context, the present perspective provides a critical and integrative analysis of advances achieved over the past decade in the use of ILs and DESs for the stabilization and functionalization of nucleic acid-based biopharmaceuticals. Unlike previous reviews on ILs and DESs for stabilization or delivery of nucleic acids [19,25–29], which have predominantly focused on descriptive overviews of emerging applications, this work emphasizes the underlying physicochemical mechanisms governing nucleic acid stabilization, the structure-property relationships of IL/DES systems, and their implications for formulation design. Particular attention is given to the interplay between stabilization and delivery, as well as to the limitations and translational challenges that currently hinder broader implementation. By combining mechanistic insight, comparative analysis with established technologies, and a forward-looking assessment, this perspective aims to provide a more critical framework to guide the rational development of IL- and DES-based platforms for next-generation nucleic acid therapeutics.

2. Nucleic acid biopharmaceuticals

Although no universally standardized definition exists among regulatory agencies, the term *biopharmaceutical* is broadly understood to refer to medicinal products derived from living organisms through biotechnological processes and composed of proteins, peptides, or nucleic acids [1,30]. This category encompasses a wide range of therapeutic classes, including recombinant peptides and proteins such as monoclonal antibodies, cytokines, therapeutic enzymes, fusion proteins, protein-based vaccines, growth factors, and protein hormones, as well as nucleic acid-based biopharmaceuticals, which include mRNA vaccines, siRNA therapies, ASO, gene therapies, and aptamer-based drugs [1,31].

The global market for nucleic acid therapeutics has experienced remarkable growth over the past decade and is expected to continue expanding rapidly. Valued at approximately USD 6.94 billion in 2025, the market is projected to reach USD 12.24 billion by 2029, corresponding to a compound annual growth rate (CAGR) of 15.2% [32]. This expansion is driven by several converging factors, including the rising prevalence of genetic and chronic diseases, the clinical success of RNA-based vaccine platforms, and increased research and development efforts supported by academic and governmental initiatives. The forecast period is anticipated to show further acceleration due to growing demand for precision medicine, the extension of mRNA

technologies beyond COVID-19 vaccines to oncology and rare diseases, and the emergence of next-generation delivery systems for nucleic acid drugs. In parallel, advances in artificial intelligence-assisted drug discovery, modular RNA architectures, and targeted aptamer technologies are reshaping the development landscape.

Nucleic acid biopharmaceuticals comprise several molecular classes that exploit DNA or RNA sequences to regulate gene expression or correct genetic defects at the molecular level [4,33]. **Figure 1** provides a simplified classification of these therapeutics according to the nature of the nucleic acid involved, distinguishing DNA-based, RNA-based, and structure-based systems.

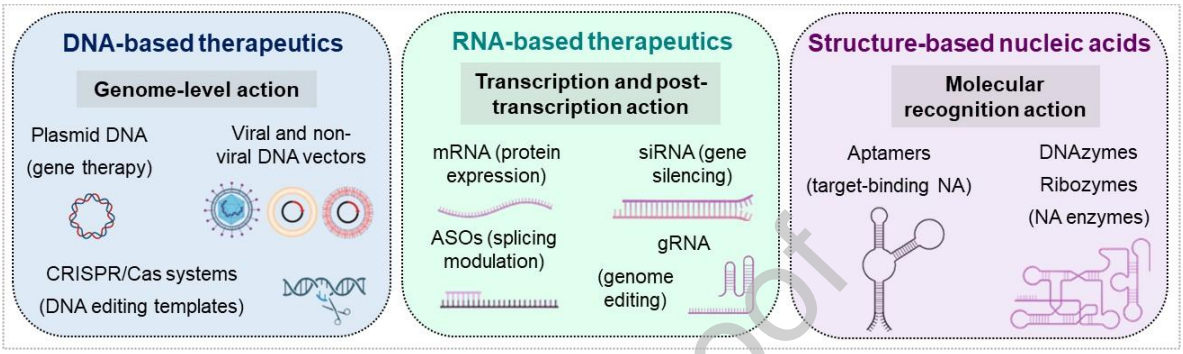

Figure 1. Classification of nucleic acid-based biopharmaceuticals. Abbreviations: CRISPR – clustered regularly interspaced short palindromic; mRNA – messenger RNA; siRNA – small interfering RNA (siRNA); ASOs – antisense oligonucleotides (ASO); gRNA – guide RNA; NA – nucleic acids.

The classes presented in **Figure 1** operate at distinct stages of genetic information processing and underpin different therapeutic strategies, ranging from genome-level interventions to transcriptional, post-transcriptional, and structure-based mechanisms [4,33]. Among these, siRNA mediates post-transcriptional gene silencing by promoting the degradation of complementary mRNA sequences, thereby suppressing the translation of disease-associated proteins [34]. ASOs are short, single-stranded nucleic acids that hybridize with target mRNA sequences to modulate splicing or inhibit translation [35]. In contrast, mRNA therapeutics deliver exogenous transcripts that transiently encode therapeutic proteins, including antigens for vaccination purposes [5]. Guide RNAs (gRNA), although non-coding, play a central role in genome editing by directing RNA-guided nucleases to specific DNA loci, thereby enabling precise genetic modification [36]. Aptamers are single-stranded nucleic acids that fold into defined three-dimensional structures capable of binding specific molecular targets with high affinity, resembling monoclonal antibodies while offering greater tunability and synthetic accessibility [37]. Additional structure-based nucleic acids, such as DNAzymes and ribozymes, exert catalytic activity through defined secondary and tertiary structures, expanding the functional repertoire of nucleic acid therapeutics beyond sequence-based information transfer [38]. Finally, gene therapies and clustered regularly interspaced short palindromic repeats (CRISPR)-based systems utilize plasmid DNA or RNA-guided nucleases to introduce, replace, or correct genetic sequences, thereby offering the potential for long-term or curative therapeutic effects [39,40].

Several nucleic acid-based therapeutics have already received regulatory approval, demonstrating the clinical viability of this class of biologics. siRNA-based drugs such as *Patisiran (*Onpattro®*)* and *Givosiran* (Givlaari®) were among the first to show clinical efficacy in silencing disease-causing genes in hereditary transthyretin amyloidosis and acute hepatic porphyria, respectively [41]. ASO therapies, including *Nusinersen* (Spinraza®) for spinal muscular atrophy and *Eteplirsen* for Duchenne muscular dystrophy, exemplify the therapeutic modulation of

mRNA splicing [42]. The success of mRNA vaccines against SARS-CoV-2, developed by Pfizer-BioNTech and Moderna, further validated RNA-based modalities as scalable and versatile therapeutic platforms [5]. In addition, aptamer-based drugs such as *Pegaptanib* (Macugen®) for age-related macular degeneration and CRISPR-Cas9-mediated therapies currently advancing through clinical trials for sickle cell disease and β-thalassemia underscore the expanding diversity and transformative potential of nucleic acid therapeutics in modern medicine [43]. For a comprehensive and up-to-date discussion of the molecular design principles, clinical progress, and emerging challenges of nucleic acid-based biopharmaceuticals, readers are referred to recent authoritative reviews covering RNA interference [44], oligonucleotide therapeutics [3], and RNA therapeutics more broadly, including mRNA-based platforms [45–48].

The therapeutic efficacy of biopharmaceuticals is closely linked to the structural integrity of their active macromolecules [24]. Nucleic acids are complex biomolecules with hierarchical organization and well-defined three-dimensional conformations that govern their biological activity [49]. They are composed of nucleotides, each consisting of a nitrogenous base, a pentose sugar, and a phosphate group [50]. The nitrogen bases are classified as purines (adenine and guanine) and pyrimidines (cytosine, thymine, and uracil); thymine is unique to DNA, whereas uracil is exclusive to RNA. The sugar moiety also differs between nucleic acids, with DNA containing deoxyribose and RNA containing ribose. Nucleotides are connected via phosphodiester bonds between the 3′-hydroxyl and 5′-phosphate groups, forming the primary structure. Secondary structures arise from hydrogen bonding between complementary bases, resulting in the double helix of DNA and diverse and complex RNA motifs such as hairpins and pseudoknots [49]. The tertiary and quaternary structures involve higher-order folding and intermolecular interactions, including alternative DNA helices (A-, B-, and Z-DNA) and associations with proteins such as histones within chromatin [50]. These structural features are fundamental to both the chemical stability and biological activity of nucleic acid-based therapeutics [24].

Despite their significant therapeutic promise, the clinical effectiveness of nucleic acid biopharmaceuticals critically depends on preserving molecular integrity and stability. These molecules are highly susceptible to physical, chemical, and enzymatic degradation, which can compromise shelf life and biological performance. Moreover, achieving efficient and targeted delivery to specific cells or tissues remains a major challenge. Consequently, the development of strategies to enhance the stability, protection, and delivery of nucleic acids is essential for advancing their therapeutic applications, as discussed in the following section.

3. Current limitations in the therapeutic application of nucleic acids

The therapeutic application of nucleic acid-based drugs remains constrained by their intrinsic physicochemical fragility, susceptibility to enzymatic degradation, and challenges associated with formulation and delivery. DNA and RNA molecules are particularly prone to hydrolysis, oxidation, and deamination processes that disrupt phosphodiester linkages and base pairing, ultimately leading to loss of biological activity [51,52]. In aqueous environments, these macromolecules may also undergo conformational instability and aggregation, particularly in the absence of appropriate stabilizing excipients [8,53].

Enzymatic degradation represents an additional and significant barrier to nucleic acid stability. Nucleases are ubiquitous in biological and environmental settings and can remain catalytically active even under frozen storage conditions, with some retaining activity at −20 °C or even −70 °C [8]. In addition to enzymatic degradation, external factors such as pH, ionic

strength, temperature, and the presence of divalent cations accelerate nucleic acid cleavage and oxidative damage, shortening their functional lifespan [51,52]. Both DNA and RNA are sensitive to these physicochemical conditions, as their structural integrity depends on hydrogen bonding, base stacking interactions, and electrostatic stabilization of the negatively charged phosphate backbone. Disruptions in these interactions can lead to strand destabilization, denaturation, and chemical degradation in aqueous environments [51,52].

Despite these shared vulnerabilities, DNA is generally more stable than RNA due to the absence of the reactive 2'-hydroxyl group in its structure, which reduces susceptibility to spontaneous hydrolysis [19]. However, DNA is still susceptible to degradation mechanisms such as depurination, strand cleavage, and oxidative damage, particularly under non-physiological pH and temperature conditions and in the presence of nucleases [19]. In contrast, RNA is inherently more labile, being highly susceptible to both enzymatic degradation and base-catalyzed hydrolysis, which significantly limits its stability during storage and handling. The first-generation mRNA COVID-19 vaccines exemplify these limitations, as formulations developed by Pfizer-BioNTech and Moderna required ultra-low storage temperatures (−70 °C and −20 °C, respectively) to preserve RNA integrity [8]. Such stringent requirements continue to hinder large-scale manufacturing, global distribution, and equitable accessibility to nucleic acid biopharmaceuticals [54].

Beyond molecular instability, efficient cellular delivery remains a major challenge. The large molecular size and high negative charge density of nucleic acids severely limit passive diffusion across biological membranes, while rapid extracellular and intracellular degradation reduces cytoplasmic availability [45,48]. Viral vectors provide high transfection efficiency but raise concerns related to immunogenicity, insertional mutagenesis, and manufacturing complexity [55]. In contrast, non-viral systems, including lipid nanoparticles (LNPs), cationic polymers, and peptide-based carriers, offer improved safety profiles but still face limitations related to toxicity, biodistribution, and physical instability [8,54].

Among non-viral platforms, LNPs have emerged as the most effective delivery vehicles for nucleic acid therapeutics; however, they remain susceptible to fusion, aggregation, and payload leakage during storage [8]. Their lipid components, particularly phospholipids and ionizable lipids, are susceptible to oxidative hydrolysis, generating reactive degradation products that can form adducts with mRNA and impair translational efficiency [8, 44]. Moreover, repeated administration of cationic materials may trigger inflammatory responses or complement activation, affecting tolerability and safety [54]. These limitations underscore the inherent interdependence between stability and delivery, as nucleic acid integrity must be maintained throughout the formulation, storage, cellular uptake, and intracellular release processes.

To mitigate these challenges, a range of molecular and formulation strategies has been developed and summarized in recent reviews [4,56–60]. Key analyses address stability limitations, delivery barriers, and regulatory aspects of nucleic acid therapeutics [4,60]. The current clinical landscape and major delivery platforms are reviewed elsewhere [59], while advances in nanoscale carriers for improving nucleic acid protection and intracellular delivery are discussed in dedicated reviews [56–58].

Structural optimization of mRNA, achieved by modifying the 5' cap, untranslated regions (UTRs), and poly(A) tail, or by incorporating modified nucleotides such as N1-methylpseudouridine, has proven effective in enhancing stability, increasing translation efficiency, and reducing immune activation [52]. At the formulation level, the composition of LNP

systems critically influences physical stability and delivery performance. Phospholipids such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine enhance membrane fusion and transfection efficiency, while cholesterol contributes to membrane rigidity and reduced permeability [8]. Polyethylene glycol (PEG)-lipids limit aggregation and extend circulation time, whereas ionizable lipids promote efficient nucleic acid encapsulation and pH-responsive endosomal escape, improving intracellular delivery and biocompatibility [53].

Excipients also play a central role in maintaining nucleic acid integrity during storage. Disaccharides such as sucrose and trehalose are widely employed as cryoprotectants to prevent aggregation and ice crystal formation under frozen conditions. Both the Moderna and Pfizer-BioNTech mRNA vaccines incorporate sucrose to preserve LNP structure during storage [8,47]. Sucrose also acts as an antioxidant, improving long-term stability of injectable mRNA vaccines stored for up to six months at 2-8 °C [8]. Buffer selection is another critical parameter: phosphate buffers may undergo pH shifts upon freezing, promoting RNA hydrolysis in certain formulations, whereas Tris-HCl buffers provide improved pH stability and reduced oxidative stress [8,47].

Lyophilization has also proven effective in extending the shelf life of mRNA-LNP formulations by minimizing hydrolytic degradation through water removal. Lyophilized systems containing trehalose or sucrose have demonstrated stability for more than one year at 5 °C, with minimal loss of integrity or immunogenicity [52]. Despite these advances, however, most current nucleic acid therapeutics still rely on cold-chain storage and remain vulnerable to environmental stressors such as humidity, oxidation, and temperature fluctuations. Broader discussions on physical stabilization strategies for nucleic acid therapeutics, beyond conventional cold-chain approaches, are provided in recent reviews addressing solid-state formulations and storage challenges [47,60,61].

To provide a consolidated overview of these interconnected challenges, **Figure 2** summarizes the major physicochemical, formulation, and delivery-related limitations currently associated with nucleic acid-based biopharmaceuticals. The provided scheme shows how intrinsic nucleic acid properties, formulation constraints, and delivery barriers converge into a common formulation bottleneck that impacts stability, storage, and therapeutic performance.

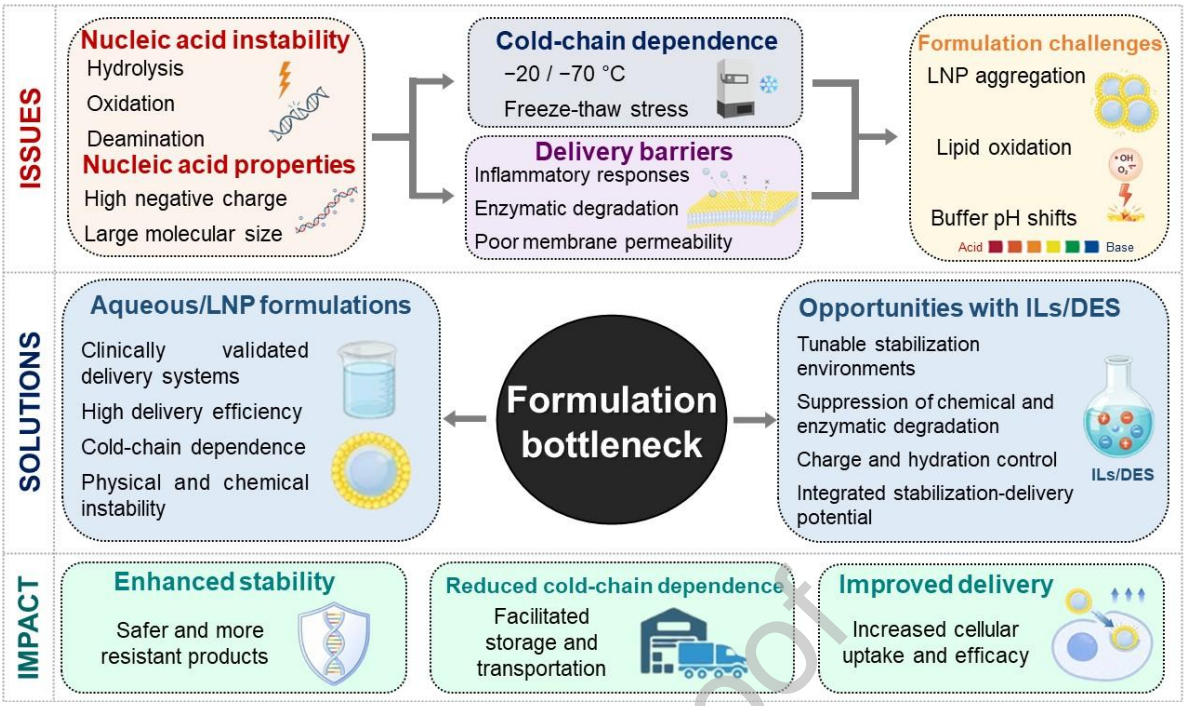

Figure 2. Current limitations in the stabilization and delivery of nucleic acid-based biopharmaceuticals and formulation opportunities with aqueous, lipid nanoparticle (LNP), ionic liquids (ILs), and deep eutectic solvents (DESs).

As depicted in **Figure 2**, existing aqueous and LNP-based formulations have enabled clinical translation by improving protection and delivery efficiency, yet they remain sensitive to physicochemical stress, cold-chain requirements, and formulation instability [10,45,62]. These vulnerabilities reflect the partial nature of current solutions, which often mitigate specific problems while leaving others unresolved, particularly with respect to long-term stability, robustness during storage, and consistent intracellular delivery.

In this context, alternative formulation environments are being investigated to complement conventional approaches. ILs and DESs are of interest because their tunable ionic composition and hydration behavior offer new degrees of control over nucleic acid stability and interfacial interactions [24]. Rather than replacing established delivery platforms, these systems may help address formulation challenges that aqueous and LNP-based technologies do not fully overcome, especially in applications where cold-chain dependence, degradation sensitivity, or formulation robustness remain limiting factors. The next section discusses how IL- and DES-based systems are being explored as emerging tools for the stabilization and functionalization of nucleic acid biopharmaceuticals.

4. Ionic liquids and deep eutectic solvents for the stabilization and functionalization of nucleic acids

ILs and DESs have emerged as versatile and highly tunable solvent classes with increasing relevance in pharmaceutical and biopharmaceutical formulations [12,14,63]. However, despite the growing number of studies reporting their application in biomolecular stabilization, the

mechanistic understanding of IL/DES–nucleic acid interactions remains fragmented and often system-specific. Most available evidence is derived from isolated experimental conditions, making it difficult to establish generalized design principles. This limitation underscores the need to critically examine not only their reported performance but also the physicochemical basis underlying their stabilizing and functionalizing effects. ILs are salts composed entirely of organic cations and organic or inorganic anions, characterized by low lattice energy, resulting in melting points lower than those found in inorganic salts [64]. Their ionic nature confers distinctive physicochemical properties, including high polarity, negligible vapor pressure, a broad liquid temperature range, and a notable thermal and chemical stability. Importantly, these properties can be systematically tailored through judicious ion-pair selection, enabling precise control over viscosity, polarity, hydrogen-bond basicity, and biocompatibility [64,65]. As a result, ILs function as so-called "designer solvents", serving not only as solubilizing media but also as stabilizing co-solvents, excipients, or delivery enhancers capable of preserving biomolecular structure and improving permeability and bioavailability [66]. Recent developments in biocompatible ILs, particularly those based on amino acid, cholinium and other ammonium-based ions, have further expanded their applicability as safe and sustainable alternatives in pharmaceutical contexts [17,64,67,68].

DESs are formed by combining a hydrogen-bond acceptor (HBA), such as cholinium chloride, betaine, and other cholinium-based salts (e.g., cholinium acetate or cholinium dihydrogen phosphate), with a hydrogen-bond donor (HBD), including urea, amino acids, sugars, or organic acids [22,24]. Eutectic mixtures exhibit melting points lower than those of their individual components and share many characteristics with ILs, while offering advantages such as simpler preparation, lower cost, and enhanced environmental compatibility. DES, in particular, deviate from the ideal solid-liquid-phase behaviour, with a significant reduction of the melting point of the mixture. Recent reviews have highlighted DESs as low-cost, easily prepared, biodegradable and sustainable solvents with reduced toxicity compared to traditional organic solvents and ILs [69–74]. DESs display highly tunable polarity, viscosity, and solvation capacity, allowing the rational design of solvent systems optimized to improve the performance of active pharmaceutical ingredients (APIs) [23]. In particular, natural and therapeutic DES, composed of endogenous or bioactive constituents, have demonstrated low toxicity, biodegradability, and excellent biological compatibility [24,75,76]. Their extensive hydrogen-bond networks also impart intrinsic cryoprotective and antioxidant properties, making them attractive media for stabilizing labile biomolecules and integrating into advanced delivery systems [77–84].

Over the past two decades, ILs have become well-established platforms for protein stabilization, supported by extensive evidence across dissolution, storage, purification, and formulation studies [24,64,65,68,85,86]. Depending on composition and concentration, ILs can preserve protein conformation and activity when used as neat solvents, co-solvents, adjuvants, surface-active ionic liquids (SAILs), or phase-forming components in aqueous biphasic systems. Their stabilizing performance depends on ion pairing, hydrogen-bond basicity, hydrophobicity, viscosity, and concentration, as well as intrinsic protein characteristics such as isoelectric point and folding behavior [65]. Cholinium-based ILs generally exhibit superior biocompatibility and aggregation suppression compared with many imidazolium-based systems and, in several cases, outperform conventional excipients in thermal and structural stabilization [36]. In parallel, DES, owing to their similarly hydrogen-bond-rich microenvironments, have gained prominence as IL-like media capable of reducing protein unfolding and aggregation while enabling gentler processing of sensitive biomolecules. Collectively, ILs and DESs constitute a versatile toolbox for next-generation protein stabilization and purification strategies [65,66].

Building on these successes, ILs and DESs have been explored for the preservation and functionalization of nucleic acids [19,25,87]. If properly designed, their unique combination of ionic interactions, hydrogen-bonding ability, and controlled solvation generates microenvironments that mitigate hydrolysis, oxidation, and conformational destabilization of DNA and RNA [19,25,26]. Hydrated cholinium-based ILs, such as cholinium dihydrogen phosphate, have been shown to maintain native duplex, triplex, and G-quadruplex structures while modulating base-pair stability, in some cases even reversing the conventional stability hierarchy in which guanine-cytosine (G·C) pairs are stronger than adenine-thymine (A·T) pairs [19,25]. These ILs can stabilize alternative base-pairing arrangements, such as Hoogsteen interactions, enhance the persistence of higher-order structures, and protect oligonucleotides and plasmid DNA from nuclease-mediated degradation. Moreover, they enable reversible compaction of nucleic acids into ordered nanostructures, a property of increasing relevance for gene delivery and nanobiotechnology applications [25,26].

Beyond stabilization, ILs and DESs have attracted considerable attention for their capacity to functionalize biomolecules and act as multifunctional excipients in advanced formulations. Their ionic and structural tunability enables modulation of biomolecular interactions, surface charge, and hydration, thereby enhancing solubility and delivery efficiency. Cholinium-based ILs, in particular, have been investigated as biocompatible formulation media and adjuvant components capable of improving molecular dispersion, facilitating transmembrane transport, and promoting enhanced biological responses [88]. Similarly, DESs offer fine control over hydration levels and ionic strength through their adjustable hydrogen-bonding networks, directly influencing nucleic acid conformation and dynamics. While DESs can maintain the canonical B-form double helix of DNA, dehydrating or highly ionic conditions may also promote well-characterized conformational transitions to A- or Z-forms or favor the formation of parallel G-quadruplex structures [19]. Notably, the cholinium-geranate-based ILs and DESs have emerged as a leading example of a biocompatible platforms to enable diverse drug delivery routes (e.g., transdermal, oral, buccal, and sustained-release formulations), with translational progress including Phase 1/2 clinical testing [21,89]. These effects have been exploited to enhance nucleic acid solubility, enable long-term storage, and support the design of responsive nucleic acid-based nanomaterials [27].

These attributes position ILs and DESs not merely as passive protective solvents but as active formulation components capable of enabling controlled release, molecular complexation, and even immunomodulatory effects [17]. The same physicochemical principles that allow ILs to tailor protein-solvent or antigen-solvent interactions can be leveraged to engineer the functional behavior of nucleic acids, opening new opportunities in preservation, delivery, and hybrid material design.

From a molecular perspective, the stabilization of nucleic acids in ILs and DESs is possible because of a complex interplay of electrostatic, hydrogen bonding, and hydrophobic interactions between solvent ions/molecules and nucleic acid structures. Unlike simple inorganic cations, ILs ions can interact not only with the negatively charged phosphate backbone but also directly with nucleobases and ribose moieties [19,26]. These interactions, which include Coulombic forces, hydrogen bonding, π–π stacking, and van der Waals contributions, support the modulation of key structural determinants, including hydrogen bonding, base stacking, and conformational entropy, ultimately influencing nucleic acid stability and folding behavior [19,24,26]. Additionally, IL and DES environments alter water activity and disrupt the structured hydration shell surrounding nucleic acids, which can reduce hydrolytic degradation and promote long-term

chemical stability [19]. This partial dehydration effect, combined with reduced nuclease activity in high ionic strength environments and enhanced solvation of nucleic acids in IL-based systems, has been associated with improved preservation of DNA over extended periods compared to aqueous systems [19,26].

At the atomistic level, molecular dynamics (MD) simulations and spectroscopic analyses reveal that IL cations exhibit distinct binding patterns compared to conventional metal ions. Choline and related alkylammonium cations preferentially localize within the minor groove of DNA, particularly in A-T-rich regions, where the groove geometry and electrostatic environment favor stable interactions [90,91]. These cations form multiple hydrogen bonds with DNA, especially with ribose and base functional groups, resulting in longer residence times and more stable binding compared to transient interactions observed with monovalent ions such as Na^+ [91]. This groove-specific localization helps with enhanced electrostatic screening of the phosphate backbone while simultaneously reinforcing base pairing interactions in a sequence-dependent manner. This behavior differs from classical cation-DNA interactions, highlighting the importance of ion/molecule structure in determining nucleic acid stability [90].

These sequence-dependent interactions lead to an inversion of canonical DNA stability trends. While G-C base pairs are typically more stable than A-T base pairs under physiological conditions, the presence of alkylammonium cations can reverse this relationship [90]. Preferential binding of cholinium ions within the minor groove of A-T-rich duplexes stabilizes these regions through favorable enthalpic contributions, while weaker interactions with G-C-rich duplexes and preferential solvation of unpaired guanine bases can destabilize G-C pairing [90,91]. This effect has been consistently observed in both experimental and computational studies and reflects the critical role of localized ion-DNA interactions in modulating duplex thermodynamics [90]. Furthermore, the ability of cholinium ions to bridge strands and interact with multiple structural elements contributes to enhanced stability of higher-order DNA structures, such as triplexes, by stabilizing both Watson-Crick and Hoogsteen base pairing interactions [19].

In addition to DNA, similar principles govern RNA stabilization in IL environments, although RNA presents unique structural and dynamic properties. Spectroscopic and computational studies demonstrate that cholinium amino acid-based ILs interact with RNA through multimodal binding, including electrostatic interactions with the phosphate backbone and insertion into groove regions, without significantly perturbing its global conformation [92]. These interactions are characterized by relatively weak binding energies, allowing preservation of structural integrity while maintaining reversibility. Experimental evidence from fluorescence spectroscopy, circular dichroism, and calorimetry confirms that RNA retains its hydrodynamic radius and secondary structure in IL media, indicating minimal structural distortion even at higher IL concentrations [92].

If properly designed, ILs and DESs are promising and adaptable platforms for both stabilizing and functionalizing nucleic acids, combining molecular protection with opportunities for controlled self-assembly and the formation of multifunctional materials. Their composition-dependent behavior, together with growing evidence of biocompatibility, supports a wide range of applications, from analytical preservation and nanostructure fabrication to advanced drug delivery systems. These interconnected properties position IL- and DES-based systems as versatile platforms in nucleic acid biopharmaceutical formulation, as discussed in detail in the following section.

While these molecular-level insights provide a strong foundation for understanding IL/DES–nucleic acid interactions, their practical relevance ultimately depends on how these effects translate into functional outcomes in real biopharmaceutical systems. In this context, recent studies have begun to explore IL- and DES-based formulations using therapeutically relevant nucleic acids, enabling a more application-oriented evaluation of their potential.

5. Ionic liquids and deep eutectic solvents for the stabilization and functionalization of nucleic acid-based biopharmaceuticals

Recent advances have extended the use of ILs and DESs from model nucleic acids to clinically relevant biopharmaceuticals, including siRNA [93–97] and ASO [98–100] (see **Table 1**). While these studies provide compelling proof-of-concept evidence for the multifunctional role of IL/DES systems, it is important to recognize that most results have been obtained under controlled experimental conditions, often using simplified models. Consequently, their broader applicability to clinically relevant formulations, long-term storage conditions, and large-scale manufacturing remains to be fully established. A critical analysis of these studies is therefore essential to identify consistent trends, limitations, and emerging design principles.

These studies demonstrate that ILs and DESs can function as multifunctional formulation media, simultaneously providing structural stabilization, protection against nuclease-mediated degradation, and enhanced transdermal or intracellular delivery. Their tunable polarity, charge distribution, and hydrogen-bonding capacity enable both physicochemical stabilization and functional modulation, including charge shielding and improved membrane permeability.

In particular, cholinium- and other ammonium-based ILs, as well as cholinium-derived DES, have been successfully employed in topical and transdermal systems for siRNA and antisense therapies [93–99]. These formulations can combine ILs or DESs with lipid components or hydrophobic “robes” (IL- or DES-derived fragments that coat the nucleic acid to enhance stability and membrane permeability) to promote molecular compaction and facilitate transport across biological barriers, while preserving the biological activity of the nucleic acid cargo. Such approaches exemplify how rational solvent design can integrate stabilization and delivery within a single formulation strategy. **Table 1** compiles IL- and DES-based media applied to therapeutic nucleic acids, detailing their clinical or preclinical targets, solvent composition, and concentration. Reported effects on stability, such as structural preservation, thermal resistance, or retained biological activity, are indicated in parentheses ( ), while functional outcomes, including enhanced permeation or cellular uptake, are shown in braces { }. Arrows denote the direction of change (↑ increase, ↓ decrease, = no change). **Figure 3** presents the chemical structure of the IL cations and anions and DES components from the ILs and DESs in **Table 1**.

Table 1. Effect of ILs and DESs on the structural stability and functionalization of nucleic acid-based biopharmaceuticals.

Nucleic acid biopharmaceutical	Clinical use	DESs/ILs	Concentration (M)*	(Stability) {Effect}	Ref.
siRNA					
Cholinium-based ILs					
siRNA against CD45	Autoimmune diseases	[Ch]H_2PO_4	1.0 - 2.5	(↑ Structural, activity, thermal) {Long-term storage}	[95]
siRNA against NFKBIZ	Psoriasis	[Ch][PhC_2H_5COO]	2	(↑ Structural)	[93,94]
		[Ch][$C_9H_{15}COO$]	2	(↓ Structural)	
		[Ch][$C_9H_{15}COO$], [Ch][PhC_2H_5COO]	2	{Skin penetration enhancer}	
		[Ch][$C_9H_{15}COO$]:[Ch][PhC_2H_5COO]	1 (each)	(↑ Structural, simulation, activity) / {Skin penetration enhancer}	
Quaternary ammonium IL-Robed siRNA					
siRNA against GAPDH and MMP12	Skin diseases	[$N_{1,1,(Bz),8}$]-siRNA1, [$N_{1,1,(Bz),14}$]-siRNA1, [$N_{1,1,(Bz),18}$]-siRNA1	0.00005 M	{Skin penetration and cell internalization enhancer}	[96]
Cholinium-based DESs					
siRNA against NFKBIZ	Psoriasis	[Ch]-[$(CH_3)_2CHCH_2COOH$]	2	(↑ Structural)	[93,94]
		[Ch]-[$(CH_3)_2C$=CHCOOH], [Ch]-[PhPhCOOH]	2	(↓ Structural)	
siRNA against GAPDH	Skin diseases	(1) [$N_{1,1,(Bz),8}$] (to rob siRNA) + (2) [Ch][$C_9H_{15}COO$]- [$C_9H_{15}COOH$]	1-2	(= Structural) {↑ Skin penetration and cell internalization}	[97]
Oligonucleotides					
Lidocainum-based IL					
STAT6 decoy oligonucleotide	Skin inflammation	ILTS®	Not disclosed	{Skin penetration enhancer}	[98,99]
Surface-active ILs (SAILs)					
Trabedersen (AP 12009)	Anti-tumor effect	[EDMPC][$C_{18:2}$]	0.03	(↑ Structural and long-term colloidal stability) {↑ Transdermal penetration, cellular uptake, antisense activity, antitumor effect}	[100]
		[EDMPC][$C_{18:1}$]	0.03	(↑ Structural stability) {↑ Skin permeability, intracellular delivery}	
		[EDMPC][$C_{18:0}$]	0.03	(= Structural stability) {↓ Skin penetration}	

* Approximate conversions to molar (M) using MW and density, when available on the manufacturer's site or literature. Abbreviations: [Ch]H_2PO_4 – cholinium dihydrogen phosphate; [Ch][PhC_2H_5COO] – cholinium phenylpropanoate; [Ch][$C_9H_{15}COO$] – cholinium geranate; [Ch][$C_9H_{15}COO$]-[$C_9H_{15}COOH$] – cholinium geranate 1:2 DESs; [Ch]-[$(CH_3)_2CHCH_2COOH$] – cholinium-isovaleric acid DESs; [Ch]-[$(CH_3)_2C$=CHCOOH] – cholinium-dimethylacrylic acid DESs; [Ch]-[PhPhCOOH] – cholinium and biphenyl-3-carboxylic acid DESs; [$N_{1,1,(Bz),8}$] – benzyldimethyloctylammonium; [$N_{1,1,(Bz),14}$] – benzyldimethyltetradecylammonium; [$N_{1,1,(Bz),18}$] –

benzyldimethylstearylammonium; ILTS® – Ionic Liquid Transdermal System (lidocainum-etodolac IL); [EDPC][$C_{18:2}$] – 1,2-dimyristoyl-sn-glycero-3-ethylphosphatidylcholinium linoleate; [EDPC][$C_{18:1}$] – 1,2-dimyristoyl-sn-glycero-3-ethylphosphatidylcholinium oleate; [EDPC][$C_{18:0}$] – 1,2-dimyristoyl-sn-glycero-3-ethylphosphatidylcholinium stearate.

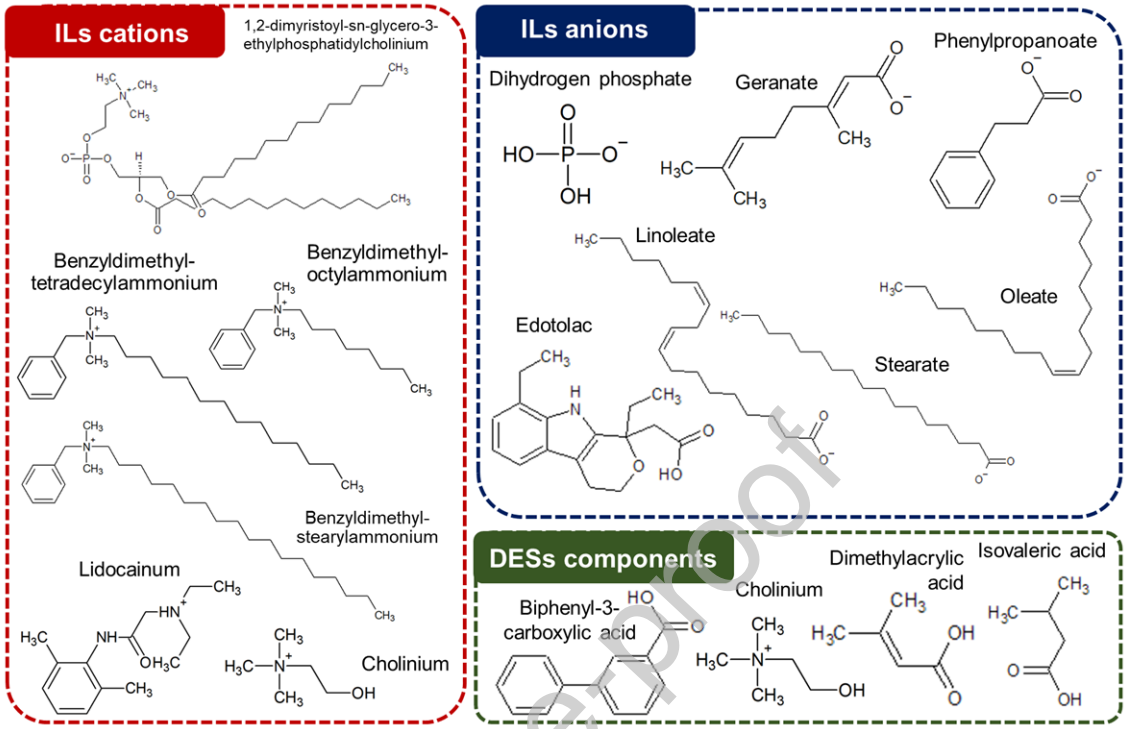

Figure 3. Chemical structures of IL cations and anions and DES components.

The representative examples summarized in **Table 1** illustrate how structural tuning of ILs and DESs governs the balance between molecular integrity and delivery efficiency in nucleic acid-based biopharmaceuticals. Over the past decade, the application of ILs and DESs to nucleic acid therapeutics has advanced substantially, as demonstrated by a series of key experimental studies compiled in **Table 1**. Collectively, these works with the ILs and DESs represented in **Figure 3** show that the physicochemical design of the ionic medium, particularly ion composition, concentration, and hydrophilic–hydrophobic balance, critically determines both the stability and delivery performance of therapeutic nucleic acids such as siRNA and ASO.

The earliest study in this context was conducted by Mazid et al. [95], who investigated the stability of an siRNA targeting CD45 in hydrated cholinium dihydrogen phosphate. High IL concentrations (1.0-2.5 M) markedly enhanced the structural and thermal stability of the siRNA, preserving its activity for up to three months, even in the presence of RNase A. This represented a substantial improvement over aqueous systems, in which degradation occurred within minutes. Importantly, siRNA stored in this IL retained full gene-silencing activity in EGFP-expressing HeLa cells, demonstrating that the ionic environment preserved the native secondary structure required for RNA interference. This study provided the first evidence that hydrophilic ILs can significantly extend the shelf life of siRNA-based therapeutics by suppressing hydrolytic and enzymatic degradation.

The application of ILs was subsequently extended to ASO delivery. Kubota et al. [99] developed the Ionic Liquid Transdermal System (ILTS®), a lidocainum-etodolac IL designed to

enhance the solubility and skin permeability of hydrophilic drugs. Building on this platform, Handa et al. [98] formulated STAT6 decoy oligonucleotides, used to suppress Th2-mediated inflammation in atopic dermatitis, within ILTS® ointments. Compared with conventional Vaseline formulations, ILTS® significantly enhanced cutaneous absorption, reduced IL-4 and IL-13 expression, and improved histological outcomes in murine models. This work represented one of the first demonstrations of clinically relevant IL formulations for nucleic acid–based immunomodulators, confirming the safety and efficacy of IL-enabled transdermal oligonucleotide therapy.

A complementary strategy was introduced by Zakrewsky and Mitragotri [96], who designed "robed" siRNA molecules covalently associated with ammonium-based IL moieties such as benzyldimethyloctyl-, benzyldimethyltetradecyl-, and benzyldimethylstearylammonium ions. These amphiphilic IL-robed siRNAs, targeting GAPDH and MMP12 in skin disease models, achieved remarkable skin penetration and intracellular uptake at extremely low concentrations (≈5 × 10^{-5} M). The IL coating effectively masked the anionic phosphate backbone, increased lipophilicity, and facilitated transdermal transport without compromising RNA integrity. Potent gene-silencing activity and excellent biocompatibility were demonstrated in reconstructed human skin, establishing a scalable and modular prodrug strategy for dermal RNA therapeutics. This "robing" concept introduced a new paradigm in which chemical functionalization with IL fragments confers intrinsic delivery capability to nucleic acids.

In another comprehensive investigation, Mandal et al. [93] evaluated both cholinium-based ILs and DESs for the topical delivery of siRNA targeting NFKBIZ, an inflammatory regulator implicated in psoriasis pathogenesis. The DESs tested were prepared 1:2 cation:anion ratio with cholinium chloride and the following acids: dimethylacrylic acid, isovaleric acid, phenylpropanoic acid, 4-phenolsulfonic acid, phenylphosphoric acid, and biphenyl-3-carboxylic acid. The study revealed that cholinium phenylpropanoate effectively preserved siRNA structural integrity while enhancing skin permeation, whereas cholinium geranate enhanced delivery but caused a minor loss in structural stability. Notably, a 1:1 (v/v) mixture of both ILs (1 M each) produced the most effective formulation, simultaneously improving siRNA stability, biological activity, and skin penetration, and achieving efficient silencing of psoriasis-related pathways, including TNF-α and IL-17A. In contrast, among the DESs tested, only cholinium-isovaleric acid DES preserved siRNA structure, whereas DESs containing dimethylacrylic or biphenyl acids compromised stability. These results emphasize how the fine chemical composition of ILs and DESs governs the balance between molecular integrity and delivery performance. The identification of cholinium-based systems with favorable stability-permeation profiles not only advances the formulation rationale for topical RNA interference but also supports subsequent intellectual property protection (US20240016735A1) [94] for this study, reflecting the translational and application-driven relevance of this approach.

Further refinement of IL-based delivery strategies was reported by Dharamdasani et al. [97], who combined hydrophobic IL-robed siRNA with cholinium geranate 1:2 to enhance dermal penetration. In this dual-IL strategy, hydrophobic cations provided molecular compaction and charge shielding, while cholinium geranate acted as a biocompatible permeation enhancer. The formulation enabled efficient siRNA delivery into both the epidermis and dermis of porcine skin, resulting in significant glyceraldehyde-3-phosphate dehydrogenase (GAPDH) knockdown *in vivo*, with no detectable inflammation or irritation. This synergistic design demonstrated that ILs can simultaneously function as stabilizers, charge modulators, and delivery vehicles in nucleic acid therapeutics.

More recently, Toyofuku et al. [100] expanded the use of biocompatible surface-active ionic liquids (SAILs) for the transdermal delivery of ASO. The researchers developed a solid-in-oil (S/O) dispersion system in which the model ASO Trabedersen (AP 12009), an inhibitor of TGF-β_2 mRNA translation, was coated with lipid-based IL surfactants synthesized from 1,2-dimyristoyl-sn-glycero-3-ethylphosphatidylcholinium paired with fatty acid anions of varying unsaturation degrees: linoleate, oleate, and stearate. At concentrations of approximately 0.03 M (30:1 w/w IL:ASO ratio), these SAILs formed stable nanoparticles (~160 nm) with sustained colloidal stability for up to four weeks. Among the formulations, the linoleate IL yielded the highest enhancement in skin permeation and intracellular uptake of Trabedersen, leading to significant suppression of TGF-β_2 expression and tumor growth in murine models, with efficacy comparable to injectable administration. This work demonstrated that the degree of fatty-acid unsaturation directly influences stratum corneum perturbation and delivery efficiency, establishing lipid-based ILs as promising excipients for non-invasive delivery of nucleic acid biopharmaceuticals.

Building on the molecular-level mechanisms discussed earlier, the studies in **Table 1** show how interactions translate into practical outcomes. Ion-specific effects, such as electrostatic interactions with the phosphate backbone, cation interactions in the grooves, and modulation of base pairing, are key for maintaining nucleic acid structure and activity [19,26], while factors like hydration and hydrophilic-hydrophobic balance influence interactions with biological membranes and, consequently, delivery performance.

Certain trends can be observed from these studies. Hydrophilic systems, particularly cholinium-based ILs and DESs, are consistently associated with enhanced structural preservation and long-term stability, likely due to effective electrostatic screening and maintenance of a hydrated environment [93–95]. In contrast, more amphiphilic systems, especially those containing fatty-acid anions (e.g., geranate, oleate, linoleate), tend to favor membrane interaction and improved transdermal or intracellular delivery, although sometimes at the expense of structural stability [100]. This highlights a tunable trade-off between stabilization and delivery, largely governed by the hydrophilic-hydrophobic balance of the system. In addition, solvent composition and hydration level play an important role, as mixtures of ILs or IL/DES combinations often outperform individual systems by combining complementary properties.

Consistent with these trends, several studies report effective structural preservation and protection against enzymatic degradation, along with improved delivery performance, including enhanced transdermal transport and cellular uptake [24,84]. However, broader applications discussed in the literature, such as reducing cold-chain requirements or enabling large-scale implementation, remain largely prospective and still need validation beyond controlled laboratory conditions. Overall, while the current results are promising, further systematic and translational studies are needed to confirm their practical applicability in biopharmaceutical settings.

It should also be noted that although there is a growing number of studies on IL- and DES-based stabilization of nucleic acids, a definitive understanding of structure-property relationships is still lacking. Factors such as ion identity (cations and anions) and concentration are known to affect stability, influencing interactions like groove binding, base pairing, and electrostatic shielding [19,26]. However, other important aspects, such as the combined effects of temperature on pH and viscosity and the role of nucleic acid length, sequence, and structure, are still poorly explored. This is largely due to the complexity of these systems, which involve many interdependent variables. As a result, most studies focus on specific conditions, making it

difficult to draw broader conclusions. Hence, while the studies summarized in **Table 1** provide useful insights, the field still lacks integrative models linking solvent design to nucleic acid stability, highlighting the need for more systematic and multivariate approaches.

Altogether, these studies reveal a trend that ILs and DESs can be precisely engineered to balance nucleic acid stability with delivery efficiency. Hydrophilic cholinium-based ILs (e.g., cholinium dihydrogen phosphate) favor structural preservation and long-term storage stability, whereas amphiphilic or hydrophobic ILs (such as ammonium- and cholinium-based fatty acid derivatives) enable controlled skin penetration and enhanced intracellular uptake. The emerging integration of IL robing, surface-active ILs, and biocompatible DESs represents a new paradigm for noninvasive RNA-based therapeutics, with significant potential to reduce formulation complexity, replace invasive delivery routes, and expand the clinical applicability of nucleic acid biopharmaceuticals.

6. Comparative analysis of nucleic acid stabilization and delivery strategies

To place ILs and DESs in a broader context, **Box 1** summarizes the main strategies currently used for nucleic acid stabilization and delivery. In addition to IL and DES systems, the comparison includes conventional stabilizers, such as polyols and salt solutions, and widely used delivery platforms, including lipid nanoparticles, polymeric systems, and viral vectors. This overview highlights key differences in terms of stability, delivery performance, and practical limitations. To better contextualize these findings and assess their practical relevance, it is essential to compare IL- and DES-based systems with established strategies for nucleic acid stabilization and delivery.

Box 1. Comparative analysis of nucleic acid stabilization and delivery strategies.

System	Main Role	Stability (Storage)	Delivery Capability	Biocompatibility	Key Advantage	Main Limitation	Ref.
ILs	Stabilization + delivery	Enhances structural integrity; reduces enzymatic degradation; potential RT storage	Improves membrane interaction and cellular uptake (system-dependent)	Depends on ion composition (cholinium-based is generally safer)	Tunable and multifunctional	Limited long-term toxicity and regulatory data; viscosity effects	[19,24,26,27]
DESs	Stabilization + permeation	Preserves structure; reduces water activity (composition-dependent)	Can enhance permeation in topical systems	Generally favorable for natural DESs	Low cost and simple preparation	High viscosity; limited mechanistic understanding	[24,27]
Polyols	Stabilization	Reduces ice formation and slows degradation	No intrinsic delivery function	Well established	Safe and widely used	Limited protection at room temperature	[101,102]
Salt solutions	Stabilization	Provides electrostatic screening; limited nuclease protection	No delivery function	Well established	Simple and low cost	High concentrations may destabilize structures	[103]

LNPs	Delivery + structural protection	Requires controlled (cold-chain) storage	Efficient cellular uptake and endosomal escape	Clinically validated, but may induce an immune response	Gold standard for RNA delivery	Complex formulation; storage constraints	[8,54,62]
Polymeric systems	Delivery + structural protection	Can improve stability depending on formulation	Enables complexation and delivery	Polymer-dependent	Tunable properties	Variable toxicity; lower efficiency than LNPs	[58,62,104,105]
Viral vectors	Delivery + structural protection	Biologically stable	Highly efficient delivery	Immunogenicity concerns	Very high transfection efficiency	Safety and regulatory challenges	[55,106,107]

Abbreviations: ILs – ionic liquids; DESs – deep eutectic solvents; LNPs – Lipid Nanoparticles; RT – Room Temperature.

As shown in **Box 1**, traditional approaches such as polyols and salt solutions are mainly used to improve nucleic acid stability, for example, by reducing water activity or electrostatic repulsion. However, they do not provide any delivery function and are often limited to room-temperature or physiological conditions [101–103]. On the other hand, established delivery systems, such as LNPs, polymeric carriers, and viral vectors, are highly effective for cellular uptake, but typically involve more complex formulations, potential toxicity or immunogenicity issues, and, in some cases, strict storage requirements [8,55,58,62,104–106].

ILs and DESs stand out for combining both stabilization and delivery-related effects in the same system. They can preserve nucleic acid structure, reduce enzymatic degradation, and, depending on their composition, improve interactions with biological membranes [19,24,26,27]. At the same time, their behavior is strongly dependent on the specific ions and formulation conditions, and challenges such as viscosity, limited long-term safety data, and regulatory uncertainty remain. Hence, ILs and DESs are not simply alternatives to existing systems, but may be particularly useful in situations where stability and delivery need to be addressed together.

Importantly, this comparison suggests that ILs and DESs should not be interpreted as direct replacements for existing delivery technologies, but rather as complementary platforms that occupy an intermediate space between stabilizing excipients and delivery systems. Their greatest potential lies in applications where stabilization and delivery must be addressed simultaneously, although this dual functionality also introduces additional complexity in formulation design and optimization.

7. SWOT analysis and perspectives

The integration of ILs and DESs into nucleic acid biopharmaceutical research represents a promising frontier in pharmaceutical formulation. Accordingly, the final section of this perspective considers future directions for their application in the stabilization and functionalization of DNA- and RNA-based therapeutics. To provide a strategic framework, a SWOT analysis is presented in **Figure 4**, summarizing the principal strengths, weaknesses, opportunities, and threats that shape the translational potential of ILs and DES. In addition, a forward-looking assessment (**Box 2**) outlines emerging trends, technological pathways, and research priorities informed by advances over the past decade.

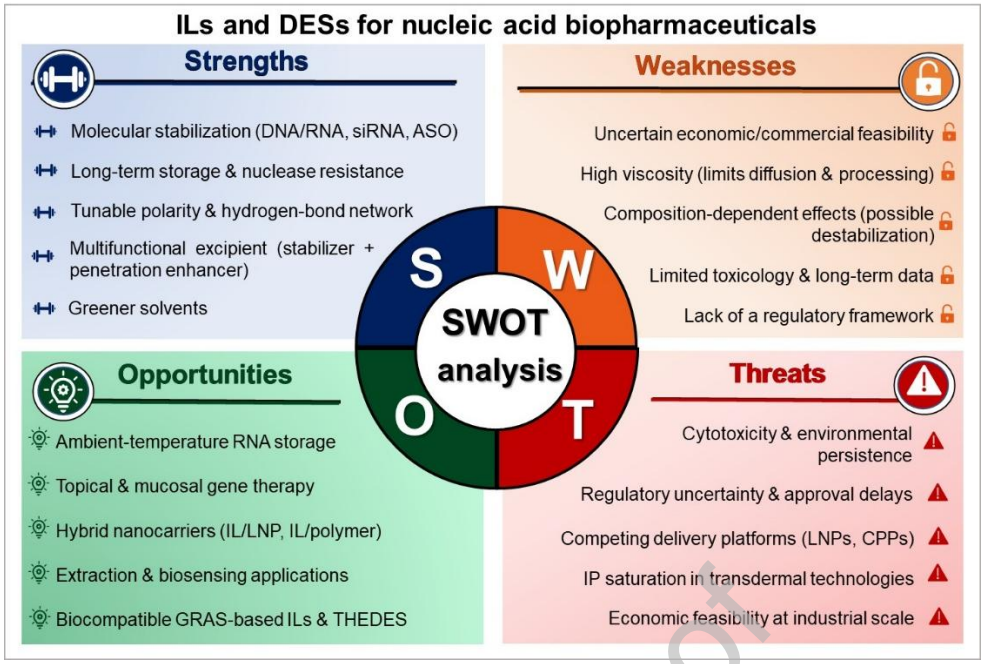

Figure 4. SWOT analysis summarizing the main strengths, weaknesses, opportunities, and threats associated with the use of ILs and DESs in the stabilization and functionalization of nucleic acid-based biopharmaceuticals. Abbreviations: ILs – ionic liquids; DESs – deep eutectic solvents; siRNA – small interfering RNA; ASO – antisense oligonucleotide; LNP – lipid nanoparticle; GRAS – generally recognized as safe; THEDES – therapeutic DESs; CPPs – cell-penetrating peptides.

Figure 4 summarizes the key strategic factors influencing the application of ILs and DESs in nucleic acid-based biopharmaceuticals. Their primary strengths arise from the ability to form structured hydrogen-bonding networks and ionic microenvironments that stabilize nucleic acid conformations, including duplex, triplex, and G-quadruplex motifs [19,24]. Selected cholinium-based ILs and DESs have demonstrated the capacity to inhibit nuclease activity, extend molecular shelf life, and preserve structural integrity even under thermal stress. Moreover, their multifunctional behavior, acting simultaneously as stabilizers, penetration enhancers, and co-solvents, positions them as next-generation excipients capable of improving transdermal or mucosal gene delivery [93,96,97]. These attributes are aligned with global efforts to develop more sustainable formulation media, as many cholinium- and amino acid-derived ILs and therapeutic DESs exhibit potential biocompatibility and biodegradability [24]. These effects are directly linked to ion-specific interactions, including preferential binding of cations within DNA grooves and modulation of base pairing and stacking interactions, as demonstrated in experimental and computational studies [91].

Despite these advantages, several weaknesses remain. Composition-dependent effects may lead to destabilization rather than protection, depending on the selected ion pair, concentration, and hydration state. High viscosity and limited mass transfer, particularly in DESs, can also hinder processability, dosing accuracy, and large-scale manufacturing. In addition, the lack of standardized analytical methodologies and the limited availability of long-term toxicological and regulatory data continue to impede clinical translation [25,26,88]. In addition, a limited understanding of structure-property relationships continues to hinder the rational design of IL/DES systems for nucleic acid stabilization [24]. Moreover, future studies should prioritize the evaluation of *in vivo* pharmacokinetic (PK) and pharmacodynamic (PD) profiles to

better understand the systemic behavior, bioavailability, and therapeutic performance of IL/DES-based formulations [108]. From a translational perspective, practical manufacturing challenges, particularly those associated with high viscosity, may impact key unit operations such as mixing, mass transfer, and sterile filtration, thereby requiring careful consideration during process development and scale-up [109,110].

The identified opportunities highlight the transformative potential of ILs and DESs in nucleic acid therapeutics. These include enabling ambient-temperature storage of RNA-based drugs, expanding topical and localized gene therapy platforms, and integrating ILs and DESs with nanocarriers and hybrid lipid–polymer systems to enhance intracellular delivery and bioavailability [26,88]. Their compositional versatility further supports the development of sequence-specific extraction systems, biosensors, and diagnostic tools, reinforcing the convergence between therapeutic and analytical biotechnology. Advances in molecular modeling and machine learning approaches offer opportunities to establish predictive frameworks linking IL/DES composition to nucleic acid stability and delivery performance [111].

Conversely, key threats include unresolved toxicity concerns, environmental persistence, and the complexity of regulatory approval for novel excipients. Furthermore, the rapid advancement of competing delivery technologies, such as lipid nanoparticles, cell-penetrating peptides, and viral vectors, may limit the industrial adoption of IL- and DES-based formulations unless clear advantages in stability, cost, scalability, or accessibility can be demonstrated [88]. Moreover, the lack of regulatory guidelines for IL/DES-based excipients and the need for standardized safety assessment protocols represent significant barriers to clinical translation [112].

To complement this qualitative assessment, **Box 2** outlines major technological and research perspectives across short-, medium-, and long-term horizons. It highlights priority areas for applying ILs and DESs in nucleic acid therapeutics, encompassing molecular stabilization, delivery enhancement, formulation design, regulatory alignment, and sustainability considerations.

Box 2. Perspectives for the application of ILs and DESs in the stabilization and functionalization of nucleic acid-based biopharmaceuticals.

Area	Short-term perspectives (1-5 years)	Medium-term perspectives (5-10 years)	Long-term perspectives (>10 years)
Molecular stabilization	Screening of biocompatible IL/DES systems for DNA, RNA, siRNA, and ASOs; evaluation of nuclease inhibition and hydration-dependent conformational stability.	Rational design of task-specific ILs and therapeutic DESs combining stabilizing and antioxidant functions.	Establishment of IL-based storage matrices for ambient-temperature RNA preservation and field-deployable formulations.
Delivery enhancement	Exploration of lipid-based ILs and hydrophobic DESs as penetration enhancers in transdermal and mucosal systems.	Integration with polymeric or lipid nanocarriers for co-delivery of nucleic acids and adjuvants.	Development of self-delivering IL-RNA complexes and IL-coated nanoparticles with programmable release kinetics.

Formulation design	Optimization of IL/DES concentration and viscosity for reproducible formulation and scalability.	Development of hybrid formulations (IL-LNPs, IL-polymer gels) with improved loading efficiency and biocompatibility.	Implementation of IL-based excipient platforms in industrial RNA drug production.
***In vivo* studies (PK and PD)**	Inclusion of preliminary in vivo PK and PD studies for selected IL/DES systems.	Establishment of correlations between IL/DES composition and in vivo PK/PD behavior, including bioavailability, biodistribution, and therapeutic efficacy.	Integration of PK/PD-informed design into the development of optimized IL/DES-based biopharmaceutical formulations.
Regulatory and safety aspects	Comprehensive *in vitro* cytotoxicity and genotoxicity screening of IL/DES candidates.	Establishment of standardized protocols and guidelines for IL/DES qualification as pharmaceutical excipients.	Regulatory acceptance by FDA/EMA and integration into official pharmacopeias.
Processability and scale-up challenges	Identification of key physicochemical limitations (*e.g.*, high viscosity, mass transfer constraints) affecting formulation handling and processing.	Development of strategies to mitigate processing challenges, including viscosity reduction, improved mixing, and compatibility with sterile filtration.	Implementation of scalable and robust manufacturing platforms for IL/DES-based formulations, ensuring industrial feasibility and regulatory compliance.
Data-driven design and emerging technologies	Machine learning–assisted screening of IL/DES libraries for biocompatibility, viscosity, and nucleic acid stability.	Integration of AI, molecular modeling, and QbD to guide ion selection and formulation optimization.	Digital platforms combining AI, automation, and predictive toxicology for accelerated discovery and translation.
Sustainability and green chemistry	Use of natural and GRAS components (cholinium, amino acids, sugars, organic acids) for IL/DES synthesis.	Scale-up using low-cost renewable precursors and recyclable synthesis routes.	Full life-cycle assessment and implementation of circular manufacturing processes for IL/DES-based biopharmaceuticals.

Abbreviations: AI – artificial intelligence; ASOs – antisense oligonucleotides; DES – deep eutectic solvents; EMA – European Medicines Agency; FDA – U.S. Food and Drug Administration; GRAS – generally recognized as safe; ILs – ionic liquids; LNPs – lipid nanoparticles; PD – pharmacodynamics; PK – pharmacokinetics; QbD – Quality by Design; siRNA – small interfering RNA.

The perspectives summarized in **Box 2** outline key short, medium, and long-term directions for the application of ILs and DESs in the stabilization and functionalization of nucleic acid-based biopharmaceuticals, covering aspects from molecular design to delivery, formulation, and translational challenges.

In the short term (1-5 years), efforts are expected to focus on the screening of biocompatible IL/DES systems for different nucleic acid classes (*e.g.*, DNA, RNA, siRNA, and ASO), aiming to identify conditions that can inhibit nuclease degradation and preserve structural integrity under stress conditions. Early studies using hydrated cholinium-based ILs and DESs composed of cholinium chloride and urea have already demonstrated a remarkable ability to stabilize Hoogsteen and G-quadruplex structures, even reversing the relative stability of A-T and G-C base pairs observed in aqueous media [19]. These findings highlight the versatility of ILs in modulating nucleic acid conformation and support their use as a platform for rational solvent design, which can be further advanced through the integration of artificial intelligence and machine learning approaches.

At this stage, advances in delivery are also expected through the exploration of lipid-based ILs and hydrophobic DESs as penetration enhancers in transdermal and mucosal systems. In parallel, formulation studies should prioritize the optimization of IL/DES concentration and viscosity to ensure reproducibility and enable initial considerations of scalability. The inclusion of early *in vivo* PK and PD evaluations will also be important to better understand bioavailability, biodistribution, and therapeutic performance, helping to connect in vitro findings with biological outcomes [108]. Moreover, it will be necessary to address physicochemical limitations, particularly those related to viscosity and mass transfer, as these properties can directly affect formulation handling and downstream processing [109,110].

In the medium term (5–10 years), research is expected to move toward the design of task-specific ILs and therapeutic DESs that combine stabilizing, antioxidant, and permeation-enhancing properties [24], alongside the development of hybrid delivery systems integrating ILs/DESs with lipid or polymeric nanocarriers. Lipid-based ILs, such as phosphatidylcholinium-fatty acid salts ([EDMPC][$C_{18:2}$], [EDMPC][$C_{18:1}$], [EDMPC][$C_{18:0}$]), have already shown the ability to promote transdermal and intracellular delivery of ASO with antitumor efficacy comparable to injectable routes, highlighting the potential of IL-mediated solid-in-oil dispersions [93,97]. At the same time, formulation strategies are likely to evolve toward hybrid systems (e.g., IL-LNPs and IL-polymer gels) with improved loading efficiency and biocompatibility [25,26]. As these systems become more complex, a better understanding of structure, property and function relationships will be required, including the correlation between IL/DES composition and in vivo PK/PD behavior, which can support a more predictive approach to formulation design [108]. From a processing perspective, this stage will also require addressing challenges associated with high viscosity, mass transfer limitations, and compatibility with operations such as sterile filtration, which are critical for reproducibility and scale-up [109,110].

In the long term (>10 years), these advances may lead to the development of IL-based storage matrices capable of enabling ambient-temperature preservation of RNA therapeutics, reducing reliance on cold-chain logistics. The emergence of self-delivering IL–RNA complexes and IL-coated nanoparticles with programmable release kinetics may further reshape nucleic acid drug design [26]. These developments are expected to be accelerated by data-driven and quantum-enabled approaches [113,114], as advances in artificial intelligence, machine learning, and emerging quantum computing platforms expand the possibilities for exploring chemical space and optimizing solvent systems [113–117].

Taken together, **Figure 4** and **Box 2** highlight that, although ILs and DESs have already demonstrated significant potential to stabilize and enhance the delivery of nucleic acid–based biopharmaceuticals, their successful translation will depend on integrating improved biological performance with scalable and practical manufacturing strategies. This includes the development of IL-based excipient platforms compatible with industrial RNA production, alongside processes that ensure adequate handling, sterile processing, and regulatory compliance. At the same time, progress will require advances in rational solvent design, toxicity mitigation, regulatory harmonization, and sustainable manufacturing, as well as overcoming current limitations related to toxicological data and cost-effective scale-up [24,88]. Beyond their role as stabilizers, ILs and DESs emerge as multifunctional platforms with the potential to drive innovation at the interface of nanotechnology, precision medicine, and sustainable biopharmaceutical development.

8. Conclusions

Over the past decade, the integration of ILs and DESs into nucleic acid biopharmaceutical research has revealed their considerable potential as both stabilizing and functionalizing media. Their tunable ionic environments, extensive hydrogen-bonding networks, and ability to create protective microenvironments enable the preservation of DNA and RNA secondary structures while mitigating hydrolytic and enzymatic degradation. Experimental evidence, particularly from cholinium-based ILs and DES, demonstrates that these systems can not only enhance molecular stability but also facilitate noninvasive delivery routes, including transdermal and mucosal administration, thereby broadening the formulation landscape for siRNA, ASO, and mRNA therapeutics.

In parallel, lipid-based ILs and surface-active ILs have shown the capacity to simultaneously promote nucleic acid penetration, intracellular uptake, and biological activity, offering innovative solutions to long-standing challenges associated with nucleic acid fragility and membrane impermeability. These advances position ILs and DESs as compelling alternatives or complements to conventional excipients and delivery platforms.

Looking forward, the successful implementation of ILs and DESs in pharmaceutical development will depend on the convergence of rational molecular design, comprehensive toxicological evaluation, and sustainable manufacturing practices. Continued progress in task-specific ILs and therapeutic DESs is expected to enable safer, greener, and more efficient formulations, including systems capable of room-temperature RNA storage and self-delivering nucleic acid complexes. At the same time, the increasing use of data-driven and AI-assisted approaches for molecular screening and formulation optimization may accelerate the identification of ion combinations with improved performance while reducing experimental trial-and-error. Nonetheless, clinical translation will require the establishment of harmonized regulatory frameworks, robust biocompatibility datasets, and economically viable scale-up processes aligned with green chemistry principles.

Overall, ILs and DESs should not be viewed as universal solutions for nucleic acid stabilization and delivery, but rather as adaptable and complementary platforms capable of addressing specific formulation bottlenecks where conventional systems remain limited. Their unique ability to integrate stabilization and delivery within a single medium represents a significant conceptual advance; however, this multifunctionality also introduces challenges related to formulation complexity, reproducibility, and regulatory acceptance. Future progress in the field will depend on establishing robust structure–property relationships, expanding toxicological and long-term stability datasets, and demonstrating clear advantages over existing technologies in clinically relevant settings. If these challenges are successfully addressed, IL- and DES-based systems may play a transformative role in enabling more stable, accessible, and versatile nucleic acid therapeutics.

CRediT authorship contribution statement

Conceptualization, Visualization: NVPV, JFPB; Writing – original draft, Data curation, Formal analysis: NVPV; Writing – review and editing: NVPV, JFBP, MGF.

Declaration of generative AI in scientific writing

During the preparation of this study, ChatGPT was used to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as required and took full responsibility for the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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