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Review Article

Monoclonal Antibodies Aggregation during Administration to Patients and the Role of Pharmaceutical Excipients

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Abstract: Monoclonal antibodies (mAbs) are powerful therapeutic agents known for their high specificity and effectiveness in treating complex diseases. Yet, one of their major challenges is their tendency to aggregate, which can reduce treatment efficacy and even trigger unwanted immune responses. This review focuses on how pharmaceutical excipients can help prevent antibody aggregation, particularly during the drug administration process, a phase often overlooked. We conducted a systematic literature search using Scopus, PubMed, and ScienceDirect, targeting studies on excipients and aggregation in therapeutic proteins. After applying the selection criteria, six original research articles were analyzed. The findings reveal that several excipients—such as L-arginine, polysorbates, trehalose derivatives, proline analogs, and cyclodextrins—can effectively stabilize mAbs. They work by reducing interfacial stress, minimizing protein–protein interactions, and preserving antibody structure during stress conditions like infusion or inhalation. These insights highlight the importance of choosing the right excipient based on the administration route to ensure antibody stability and therapeutic impact. By shifting the focus from formulation to administration, this review provides a practical perspective that can support the development of safer and more effective mAb therapies.

Keywords: Therapeutic protein, Biopharmaceutical stability, Surfactant agent, Parenteral formulation, Aggregation inhibitor

1. INTRODUCTION

Monoclonal antibodies (mAbs) have become a vital component of modern therapy due to their high specificity, strong therapeutic potential, and ability to target complex biological pathways that small-molecule drugs cannot reach [1-3]. These biologics are widely employed in the treatment of various conditions such as cancer, autoimmune disorders, and genetic conditions [4]. The success of these therapies has revolutionized medicine by offering more effective and targeted treatment options for patients [5,6]. However, despite their significant clinical benefits, monoclonal antibodies face major stability challenges, requiring extensive research and development to optimize their use.

One of the primary obstacles in developing monoclonal antibody drugs is their inherent instability, particularly their tendency to aggregate [7]. Aggregation can be triggered by

environmental factors such as temperature fluctuations, pH changes, interaction with other substances, and mechanical agitation [8-10]. This process not only reduces drug efficacy by decreasing the bioavailability of functional antibody molecules but also raises safety concerns, as antibody aggregates can elicit unwanted immune responses in patients [11]. Studies have shown that aggregation in biopharmaceutical formulations can negatively impact therapeutic outcomes and patient safety, highlighting the critical need for effective stabilization strategies [12].

Beyond formulation and storage, the stability of monoclonal antibodies remains a concern during administration [10,13]. Factors such as dilution, exposure to infusion devices, and interactions with excipients or container materials can exacerbate aggregation issues [14-16]. Different administration routes—whether intravenous, subcutaneous, or inhalation—impose distinct stress conditions, necessitating tailored stabilization approaches [17,18]. Without proper stabilization measures, monoclonal antibodies may lose their structural integrity during administration, ultimately reducing treatment efficacy and increasing the risk of adverse effects for patients.

To address these challenges, various strategies have been developed, including the use of pharmaceutical excipients designed to prevent antibody aggregation [19,20]. Excipients such as polysorbates, amino acids, and sugars play a crucial role in stabilizing MAbs formulations by acting as surfactants, osmolytes, or cryoprotectants [21-24]. These compounds help maintain protein solubility, prevent surface adsorption, and reduce mechanical stress during administration. The choice of excipients depends on the specific antibody and its administration route, making excipient selection a critical factor in formulation development.

While previous reviews have primarily focused on the formulation and storage stability of monoclonal antibodies, this review provides a novel perspective by specifically addressing the often-overlooked challenges of antibody aggregation during drug administration. By systematically examining the role of excipients across different delivery routes, this review aims to fill a critical knowledge gap and support the development of more robust therapeutic antibody formulations.

2. MATERIALS AND METHODS

The next step involved screening the title, abstract, and full articles, resulting in 6 articles relevant to the study being conducted. The literature search flowchart is shown in Figure 1.

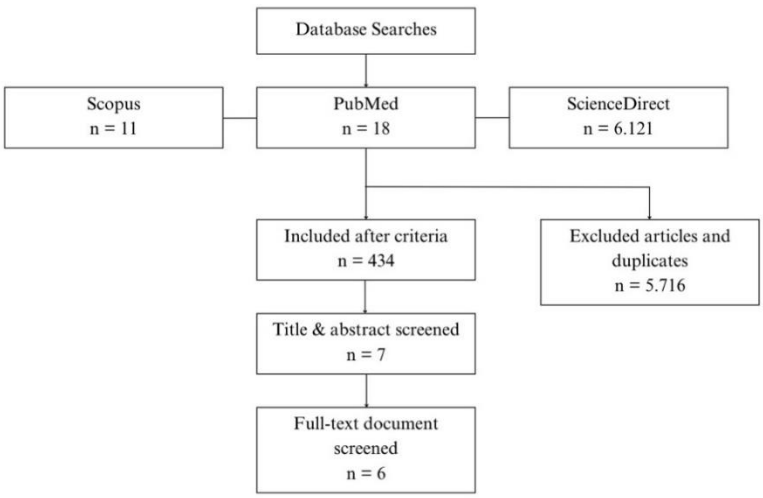

Figure 1. Literature Search Flowchart

The literature search was conducted using the keyword “excipient OR stabilizer AND aggregation AND "therapeutic protein" AND administration” in the Scopus, PubMed, and ScienceDirect databases. The search results from these databases were further screened using inclusion criteria, which consisted of original articles, English language, and open access availability. Articles were excluded if they did not mention or assess the route of administration, used samples that were not in formulation form or the study was not relevant for the review.

3. RESULTS AND DISCUSSION

3.1. Stability Challenges of Monoclonal Antibodies Based on Route of Administration

Administering monoclonal antibodies (mAbs) involves a series of steps that can potentially affect their stability and overall effectiveness. Throughout this process, these delicate molecules are exposed to conditions that may cause them to degrade, ultimately reducing their therapeutic potential. Things like dilution, contact with infusion materials, and specific factors related to the delivery route can all play a role in destabilizing mAbs [25,26]. The sections below explore the key stability challenges faced during administration, specifically focusing on intravenous (IV), subcutaneous (SC), and inhalation routes. Although the FDA has approved at least twelve administration routes for monoclonal antibodies [27], these three were the most frequently reported and discussed in the literature sources extracted for this review, indicating their prominence in current formulation and stability research.

3.1.1. Intravenous (IV)

The intravenous administration of monoclonal antibodies requires high protein concentrations to achieve adequate therapeutic doses within a limited injection volume. A significant challenge in formulating protein-based drugs for IV administration is protein aggregation, which can increase the viscosity of the solution and disrupt manufacturing, storage, and administration processes [28,29]. High-concentration mAbs are prone to self-association due to protein–protein interactions, leading to the formation of dimers, trimers, or larger aggregates [30]. This not only reduces the mAb's bioavailability but also poses a risk of triggering undesired immunological responses.

Antibodies administered via the IV route may also undergo aggregation triggered by several factors, such as pH changes, elevated temperatures, exposure to air–liquid interfaces, interaction with infusion device surfaces, and dilution prior to entering the body [22,31,32]. The study by Hribar-Lee et al. emphasized that high-concentration antibody formulations can significantly increase viscosity due to aggregate formation, thereby hindering the flow of the solution during injection [30]. Protein–protein interactions may lead to either reversible or irreversible self-association of antibody molecules [33,34]. Irreversible aggregation is often associated with partial unfolding, which exposes hydrophobic regions of the protein and initiates the formation of non-native aggregates. Another challenge in IV mAb formulation is maintaining the antibody in a solution with a neutral pH or near its isoelectric point (pI), as the reduction of electrostatic repulsion between molecules can increase aggregation risk [22].

The process of mixing mAbs into an IV bag and diluting them with solvents such as saline or dextrose can significantly alter the original formulation environment [35]. Dilution of mAbs may also dilute their protective excipients, such as polysorbate 20 and polysorbate 80, which function to prevent interfacial aggregation [21]. When the concentration of surfactant in the IV bag decreases, the

air–liquid interface may become insufficiently protected. This condition allows monoclonal antibodies (mAbs) to adsorb onto surfaces, unfold, and subsequently form aggregates. Vargo et al. demonstrated that agitation or mixing within IV bags induces intense interfacial activity, driven by changes in physical orientation and fluid movement [21]. These dynamics create so-called "aggregation rafts"—interfacial regions where protein molecules directly interact in the absence of adequate surfactant protection. Exposure to these interfaces induces conformational changes, particularly the exposure of usually buried hydrophobic regions, which promotes the formation of subvisible particles and large aggregates [36,37].

3.1.2. Subcutaneous (SC)

The subcutaneous route has become a preferred option for mAb administration because it improves patient compliance, enables self-administration, and reduces healthcare costs. The challenges of administering mAbs via the subcutaneous (SC) route share many similarities with the intravenous (IV) route. However, one key difference is the limited injection volume for SC delivery, typically around 1–2 mL, whereas IV administration can tolerate larger volumes when delivered through infusion [38]. To achieve a therapeutic dose, mAb formulations must be prepared at high concentrations, which significantly increases solution viscosity and potentially leads to protein aggregation [39,40]. Additionally, absorption of mAbs through the SC route is slower compared to IV administration, where the drug is immediately available in the bloodstream.

The increase in viscosity is caused by protein–protein interactions (PPIs), both electrostatic and hydrophobic in nature, especially when mAb concentrations approach or exceed 150 mg/mL [41,42]. At a pH near the antibody's isoelectric point (pI), electrostatic interactions become dominant and may actually promote molecular association between antibodies, worsening viscosity and increasing the risk of aggregation [42].

3.1.3. Inhalation

The administration of monoclonal antibodies via the inhalation route offers advantages such as a rapid onset of action and increased local availability in the lungs, achieved with lower doses compared to systemic routes [43,44]. However, inhalation formulation and administration face major challenges in terms of protein stability, particularly related to aggregation induced by spray drying and interactions within the pulmonary environment [45]. During the spray drying process, proteins are subjected to physical stresses such as atomization and heat, which can lead to partial denaturation, unfolding, and aggregate formation, potentially affecting biological activity and increasing the risk of immunogenicity [46]. Antibodies are also highly susceptible to mechanical and thermal stress during aerosolization, which can cause unfolding, conformational changes, and aggregation [23]. Another challenge arises during administration via intratracheal insufflation, where powder distribution in the lungs may be uneven, with deposition of large particles and aggregates in the upper respiratory tract. This can impact absorption and potentially trigger local immune responses [45].

One of the primary causes of protein damage during nebulization is interaction with the air–liquid interface [47]. Due to the amphipathic nature of proteins, when a solution is aerosolized, the surface area of the interface drastically increases, thereby elevating the risk of protein adsorption to the interface. This adsorption often leads to partial denaturation and aggregate formation, particularly if protective excipients such as surfactants are not adequately present to coat the interface

[23,48]. Furthermore, there is the potential for in situ aggregate formation following interaction between the protein and endogenous surfactants or pulmonary fluids, especially in dry powder formulations. This presents an additional challenge in maintaining protein stability after reaching the target site in lung tissues [45].

3.2. Excipients for preventing aggregation in therapeutic protein formulations

To address the challenges of protein aggregation during drug formulation and administration, various pharmaceutical excipients have been employed to enhance the stability of therapeutic proteins. These excipients serve distinct functional roles, such as surfactants, osmolytes, cryoprotectants, and viscosity modifiers, to protect proteins from denaturation, interfacial stress, and undesirable protein–protein interactions [49-51]. Their effectiveness depends not only on the chemical nature of the excipient itself but also on the specific characteristics of the protein and the route of administration. Table 1 presents a compilation of selected excipients used to stabilize monoclonal antibodies (mAbs) across various routes of administration, including intravenous, subcutaneous, pulmonary, and nebulization delivery. The excipients are listed along with their mechanisms of action—such as interfacial protection, colloidal stabilization, water replacement and vitrification, and viscosity modulation. Dosage forms include aqueous solutions, dry powders, and cryoprotectants. This table provides a comparative overview by illustrating how different classes of excipients contribute to enhancing protein stability across various clinical delivery scenarios.

Table 1. Excipients for Preventing Protein Aggregation and Their Mechanisms of Action

mAb Name	Route of Administration	Excipient	Excipient Mechanism of Action	Dosage Form	Reference
Monoclonal antibody (IgG4)	Intravenous (IV)	L-Arginine Hydrochloride	Reduces protein-protein interactions by occupying binding sites on the antibody molecule, thereby reducing aggregation.	Aqueous solution	[30]
Monoclonal antibodies (mAb1–mAb4)	Intravenous (IV)	L-Arginine and L-Glutamate (Arg-Glu)	Arg-Glu enhances colloidal stability and prevents aggregation by increasing the onset aggregation temperature (Tagg) and stabilizing the protein at neutral pH.	Aqueous solution	[22]
Monoclonal antibodies	Intravenous (IV)	Polysorbates (PS 20 and PS 80)	Stabilizes the air-water interface to prevent protein aggregation during mixing and IV dose preparation.	Aqueous solution	[21]
Monoclonal antibodies (mAbs)	Subcutaneous (SC)	Proline and proline analogs	Proline and its analogs reduce protein solution viscosity by forming electrostatic and hydrophobic interactions, preventing aggregation.	Aqueous solution	[42]

continued Table 1…

Anti-IL-4 receptor alpha monoclonal antibody	Pulmonary (inhalation)	2HPβCD and L-Leucine	2HPβCD stabilizes the protein through water replacement, vitrification, and surface activity effects, while leucine improves aerosolization and dispersion.	Dry powder	[24]
Anti-SARS-CoV-2 antibody	Nebulization	Succinylated trehalose (C16TreSuc)	Stabilizes the protein during nebulization by enhancing colloidal stability and antigen-binding ability; acts as a cryoprotectant.	Aqueous solution and cryoprotectant	[23]

3.3. Mechanisms of stabilization of therapeutic protein preparations by excipients

3.3.1. L-Arginine Hydrochloride

L-arginine hydrochloride is a water-soluble salt form of the amino acid L-arginine, containing a positively charged guanidino group at physiological pH. This structure enables L-arginine to interact with protein surfaces through electrostatic and hydrophobic interactions, forming the basis of its function as an excipient [52,53]. In the context of monoclonal antibody formulations, L-arginine hydrochloride acts as a cosolute capable of modulating viscosity and reducing protein aggregation through a competitive mechanism at the antibody binding sites [54,55].

One of the standout features of L-arginine hydrochloride as an excipient is its ability to bind to the same regions on antibody molecules that typically interact with other antibodies [30]. Occupation of these sites helps prevent antibodies from sticking together and forming dimers, trimers, or even larger aggregates. In simulation studies using a binding polynomial model, L-arginine significantly reduced the formation of large aggregates and increased the number of antibodies that remain in a stable, monomeric state. Essentially, L-arginine hydrochloride acts like a competitive blocker. It steps in and binds to interaction-prone areas on the antibody surface before other antibody molecules can, halting aggregation before it even starts. The stronger this interaction, the more effectively it delays the formation of unwanted complexes [30].

What's especially important is that L-arginine doesn't interfere with the antibody's natural structure or its ability to recognize and bind to its intended target [55]. It simply shields the antibody from forming unwanted protein–protein interactions without compromising its biological function. This makes L-arginine a safe and practical choice for stabilizing monoclonal antibody formulations, particularly those at high concentrations [53-56].

Another key advantage of L-arginine hydrochloride is that its protective role continues beyond just formulation and storage. During administration, when the solution may be diluted, shaken, or come into contact with tubing or vials, L-arginine helps keep the antibody stable and in its active form. By reducing the risk of stress-induced aggregation, it helps ensure the antibody remains effective and safe right through to the point of delivery [30].

3.3.2. L-Arginine and L-Glutamate (Arg-Glu)

L-arginine and L-glutamate hydrochloride are charged amino acids that naturally occur in the body and have chemical structures that enable interaction with protein surfaces [57]. L-arginine

contains a positively charged guanidino group, while L-glutamate has a negatively charged carboxylate group. The combination of the two forms a unique ionic pair known as Arg-Glu, which is water-soluble and has a favorable safety profile for use in parenteral formulations [58].

As excipients, Arg-Glu possesses multifunctional properties in protein formulation systems, particularly due to its ability to stabilize proteins against aggregation by modulating intermolecular interactions. The amphoteric nature and high polarity of these amino acids help maintain charge balance around the antibody molecules and form a hydrated environment that supports protein structural stability. This combination also plays a role in reducing viscosity and expanding the pH stability range of antibodies [22].

The primary mechanism of action of Arg-Glu as an anti-aggregation excipient involves hydrogen bonding between arginine and glutamate, which helps to concentrate both ions around the protein surface. This increased local presence creates a crowding effect that effectively reduces interactions between protein molecules, thereby minimizing aggregation [59]. Additionally, the presence of Arg-Glu increases the aggregation onset temperature (Tagg), indicating improved thermal stability of the antibody [22].

Regarding its interaction with the antibody active substance, Arg-Glu does not form covalent bonds or induce significant conformational changes in the antibody structure. Instead, it forms reversible, non-covalent interactions, such as hydrogen bonds and weak electrostatic forces, sufficient to shield the protein surface from undesired association without interfering with its biological activity [22].

3.3.3. Polysorbates

Polysorbate 20 (PS20) and Polysorbate 80 (PS80) are the most commonly used non-ionic surfactants in monoclonal antibody formulations [60-62]. Both are polyoxyethylene sorbitan esters with fatty acids—PS20 contains lauric acid, while PS80 contains oleic acid. This structure enables both surfactants to reduce surface tension and stabilize the air–liquid interface, which is a critical point for protein aggregation [21,61].

The primary property of these surfactants is their ability to coat liquid interfaces and prevent therapeutic proteins from directly interacting with surfaces that may induce denaturation. In antibody-based drug formulations, PS20 and PS80 function to protect antibodies from conformational changes caused by mechanical stress or open interfaces, such as those encountered during mixing in infusion bags [61,63]. The study shows that both PS20 and PS80 significantly reduce aggregate particle formation with increasing concentration, although their performance varies depending on the protein type and solvent environment [21].

The mechanism by which polysorbates prevent aggregation relies on two main principles: (1) reducing interfacial stress by lowering surface tension [64,65]; and (2) competing with proteins for adsorption at interfaces, thereby decreasing the chance of protein aggregation at those sites [66,67]. Observations indicate that PS20 reduces particle formation more quickly and effectively than PS80 due to its faster and more efficient adsorption at the interface [21,65].

The interaction between polysorbates and antibodies occurs non-covalently, without altering the antibody's structure or compromising its biological activity. However, it is essential to note that the use of these surfactants must be optimized, as at specific concentrations, they may promote the formation of soluble aggregates, particularly at critical concentration points, such as the 0.002% level observed with both PS20 and PS80 [21].

The performance of polysorbates in preventing aggregation during administration is especially critical in real-world scenarios, such as the mixing of antibodies into IV bags prior to intravenous delivery. This process creates dynamic interfacial surfaces that can lead to aggregation if unprotected [63]. The study confirms that selecting and calculating the correct polysorbate concentration early in formulation development can provide end-to-end stability, from storage to administration [21].

3.3.4. Proline and Proline Analogs

L-proline is an amino acid characterized by a unique five-membered ring structure (pyrrolidine), which makes it rigid and hydrophobic [68]. Unlike other amino acids, its amino group is integrated into the cyclic structure, giving it a unique stability against denaturation and making it an attractive excipient [69]. Proline and its analogs were used to reduce the viscosity of high-concentration monoclonal antibody (mAb) solutions and to inhibit the formation of aggregates induced by protein–protein interactions (PPIs) [42].

The main property of proline and its analogs that is relevant as an excipient is their ability to form hydrophobic and weak electrostatic interactions with the surface of antibodies [70,71]. Several proline analogs studied also possess aromatic groups or charge distributions that support the formation of π-π and CH-π interactions with the aromatic residues of antibodies [72,73]. These interactions are considered important in reducing viscosity and preventing aggregation, especially in antibodies with predominantly hydrophobic surfaces [42].

The mechanism by which these excipients prevent aggregation is by forming an electrostatic interaction between the mAbs and the excipient, then the viscosity of the mAbs solution can be decreased. Molecular dynamics simulations demonstrated that the greater the level of interaction between the excipient and the antibody surface, the more significant the reduction in viscosity and aggregation observed [42].

The interactions between proline/its analogs and antibodies are specific but reversible, and they do not cause denaturation or major conformational changes in the antibodies. Physical stability tests using DLS and SEC showed that most proline analogs did not cause increased aggregation, even after four months of storage at 4 °C [42]. This indicates good compatibility with antibodies and supports their safety for long-term formulation applications. The ability of proline and its analogs to prevent aggregation is also highly relevant during subcutaneous administration, especially since high-concentration formulations tend to exhibit high viscosity, which can cause injection pain and difficulty. The viscosity reduction these excipients provide helps maintain desirable flow characteristics, improves patient comfort, and preserves antibody stability during injection. Therefore, proline and its analogs are promising excipient candidates for improving the stability and performance of high-concentration monoclonal antibody formulations [42].

In a systematic study, Prašnikar et al. evaluated 35 proline analogs for their effectiveness in reducing viscosity of concentrated subcutaneous mAb solutions and predicted their toxicity [42]. The results in Table 2 demonstrated that 12 of these compounds exhibited equal or greater viscosity-reducing effects compared to native proline, likely non-toxic, and did not destabilize the mAbs.

Table 2. Summary of Proline and Its Analogs as Excipients for Preventing Monoclonal Antibody Aggregation

Group	Excipients	Relative viscosity*
Proline Analog	D,L-Proline	0.77
Dipeptides and Tripeptides with Proline	H-Pro-Pro-OH	0.49
	H-Pro-Gln-OH	0.69
	H-Pro-Ala-OH	0.78
	H-Gly-Pro-OH	0.66
	H-Val-Pro-OH	0.74
	H-Val-Pro-Pro-OH	0.69
Mimetics of Proline and Nicotinic Acid	Piperidine-4-carboxylic acid	0.75
	Nicotinic acid	0.64
	1H-pyrrole-2-carboxylic acid	0.72
	(R)-pyrrolidine-3-carboxylic acid	0.61
	1H-pyrrole-3-carboxylic acid	0.66

*relative viscosities of test compound related to mAbs solution containing proline [42].

Proline and its structural analogs have gained attention as potential excipients for stabilizing monoclonal antibody (mAb) formulations by reducing viscosity and inhibiting aggregation. Analogs with rigid structures and aromatic moieties—along with appropriate charge properties—tended to be more effective in lowering viscosity without compromising the structural integrity of the antibodies. Supporting analyses using dynamic light scattering (DLS) and size-exclusion chromatography (SEC) confirmed that most analogs did not induce significant aggregation or degradation of mAbs, indicating a favorable safety and stability profile [42]. Overall, the use of proline and its analogs not only provides a viable strategy to reduce viscosity in SC formulations but also maintains the physicochemical stability of therapeutic antibodies over clinically relevant timeframes.

3.3.5. 2-Hydroxypropyl-β-cyclodextrin (2HPβCD) & L-leucine

2HPβCD is a derivative of β-cyclodextrin that has been modified with hydroxypropyl groups to improve water solubility and biological compatibility. Its core structure is toroidal, with a hydrophobic cavity on the inside and a hydrophilic surface on the outside. This property enables 2HPβCD to act as an encapsulating agent for hydrophobic molecules or to interact with hydrophobic regions of proteins, including antibodies [74]. In protein formulations, 2HPβCD functions by stabilizing the protein surface through non-covalent interactions, helping to reduce interfacial energy and preventing protein–protein interactions that may lead to aggregation. Additionally, 2HPβCD is known to modify air–liquid or solid–liquid interfaces, which are critical sites for aggregation during processes such as nebulization or storage [75].

L-leucine, on the other hand, is a hydrophobic amino acid commonly used as an excipient in dry powder inhalation formulations [76]. The simple structure of L-leucine enables the formation of a protective layer on the surface of protein particles, preventing direct particle–particle contact and reducing the tendency for aggregation. Its mechanism involves enhancing physical stability by forming a dry matrix that isolates antibody molecules and reduces molecular mobility. L-leucine also

improves particle morphology during spray drying, resulting in particles with better aerosol properties and higher stability [24].

Both excipients can interact with antibodies through hydrophobic bonds or weak electrostatic interactions, without disrupting the tertiary structure or biological activity of the antibodies. In antibody formulations intended for inhalation delivery, as tested by Pan et al., the combination of 2HPβCD and L-leucine was shown to reduce protein aggregate formation after drying and during both rehydration and nebulization. This indicates that both excipients can provide protection against physical degradation of proteins during storage and administration an essential factor in preserving the therapeutic potential of antibodies [24].

3.3.6. Succinylated trehalose (C16TreSuc)

Succinylated trehalose is an excipient derived from trehalose, a non-reducing disaccharide composed of two glucose units, widely known as a biomolecule stabilizer. Based on the study conducted by Noverraz et al., trehalose was chemically modified by the addition of fatty acid chains and a succinyl (carboxylate) group, resulting in an amphiphilic ionic surfactant such as C16TreSuc [23]. This structure gives the excipient a hydrophilic head from the trehalose and a hydrophobic tail from the fatty acid chain, allowing it to interact with protein surfaces and solution interfaces.

The main properties of succinylated trehalose are its high water solubility, ability to form micelles, and good biological compatibility [77]. Compared to conventional surfactants like polysorbate 80, C16TreSuc does not contain PEG and is more environmentally friendly, as its degradation products—trehalose, succinic acid, and palmitic acid—are all naturally occurring compounds [78,79].

As an excipient, the mechanism of action of C16TreSuc in preventing antibody aggregation lies in its ability to stabilize antibody structures through hydrophobic interactions with exposed protein regions caused by physical stress such as nebulization or freezing. This surfactant coats the antibody surface and the air–liquid interface to prevent unfolding and protein–protein interactions, which are the initial steps in aggregate formation. This stability was confirmed through Dynamic Light Scattering (DLS) tests, which showed that the addition of 5.6 mM C16TreSuc nearly eliminated all aggregate formation during the nebulization process [23].

The interaction between C16TreSuc and the antibody is non-covalent, involving hydrogen bonding and hydrophobic interactions, without altering the antibody's affinity for its antigen. This was demonstrated by antigen-binding assays, which showed that sotrovimab stabilized with C16TreSuc remained capable of effectively recognizing the SARS-CoV-2 Spike protein even after nebulization [23].

The ability of C16TreSuc to maintain antibody stability is not limited to storage or freeze-drying stages but has also proven highly effective during the nebulization administration process. This stability is especially critical for pulmonary therapies such as COVID-19, where therapeutic proteins like antibodies are highly vulnerable to denaturation from the mechanical stress of nebulizers [80]. With its ability to preserve the structure and function of antibodies throughout the entire product lifecycle—from storage and preparation to delivery—succinylated trehalose like C16TreSuc, is a highly promising excipient for the development of inhalable antibody formulations [23].

Maintaining the stability of monoclonal antibodies during administration is a critical but often underappreciated aspect of biologic drug development. While much of the research has focused

on formulation and storage stability, the administration phase also presents unique stress conditions that can trigger protein aggregation. The right choice of excipients can serve as stabilizers during storage and protective agents during this vulnerable phase when the drug is delivered to the patient. This highlights the need for a more integrated formulation approach that considers the entire product lifecycle from manufacturing to administration.

This review has shown that each route of administration comes with its own set of stability challenges, requiring tailored excipient strategies. Excipients like L-arginine, modified trehalose, and cyclodextrins have demonstrated strong potential in preserving antibody integrity under administration-related stress. However, long-term safety, biological performance, and regulatory acceptance must also be considered. Developing stable and effective monoclonal antibody formulations, therefore, depends not only on scientific evidence but also on practical and clinical evaluation of the excipients involved.

Based on the findings of this review, future research is encouraged to consider the development of formulation strategies that take into account the drug administration stage, by incorporating excipients that have been proven to reduce aggregation under clinically relevant stress conditions. Additionally, stakeholders in regulatory and industrial sectors are advised to consider route-specific stability testing as a standard component in the evaluation of monoclonal antibody product stability. Interdisciplinary collaboration between formulation scientists, clinicians, and device engineers will also be essential to optimize excipient performance in real-world clinical delivery systems.

4. CONCLUSION

Monoclonal antibodies hold tremendous promise in modern therapeutics, but their stability, particularly against aggregation, remains a key challenge, especially during administration. This review highlights how pharmaceutical excipients are vital in maintaining antibody integrity under various stress conditions associated with intravenous, subcutaneous, and inhalation routes. Excipients such as L-arginine, trehalose derivatives, proline analogs, and cyclodextrins offer targeted mechanisms to prevent aggregation, from shielding hydrophobic interactions to stabilizing interfaces. Beyond formulation, their selection must consider safety, compatibility, and route-specific needs to ensure both efficacy and patient safety. By focusing on the role of excipients during administration, this paper addresses a critical but often underexplored aspect of therapeutic antibody development offering insights that may guide more robust and effective formulations in clinical practice.

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References

[1] T. T. K. Nguyen, K.-Y. Pham, and S. Yook, "Engineered therapeutic proteins for sustained-release drug delivery systems," *Acta Biomater*, vol. 171, pp. 131–154, Nov. 2023, doi: 10.1016/j.actbio.2023.09.018.

[2] L. Yin, X. Chen, P. Vicini, B. Rup, and T. P. Hickling, "Therapeutic outcomes, assessments, risk factors and mitigation efforts of immunogenicity of therapeutic protein products," *Cell Immunol*, vol. 295, no. 2, pp. 118–126, Jun. 2015, doi: 10.1016/j.cellimm.2015.03.002.

[3] N. H. On, P. Kiptoo, T. J. Siahaan, and D. W. Miller, "Modulation of Blood–Brain Barrier Permeability in Mice Using Synthetic E-Cadherin Peptide," *Mol Pharm*, vol. 11, no. 3, pp. 974–981, Mar. 2014, doi: 10.1021/mp400624v.

[4] P. Kiptoo, M. D. Laksitorini, and T. J. Siahaan, "Peptide Delivery," in *Handbook of Biologically Active Peptides*, Elsevier, 2013, pp. 1702–1710. doi: 10.1016/B978-0-12-385095-9.00233-5.

[5] D. B. Singh and T. Tripathi, *Protein-based Therapeutics*. Singapore: Springer Nature Singapore, 2023. doi: 10.1007/978-981-19-8249-1.

[6] M. D. Laksitorini, P. K. Kiptoo, N. H. On, J. A. Thliveris, D. W. Miller, and T. J. Siahaan, "Modulation of Intercellular Junctions by Cyclic-ADT Peptides as a Method to Reversibly Increase Blood–Brain Barrier Permeability," *J Pharm Sci*, vol. 104, no. 3, pp. 1065–1075, Mar. 2015, doi: 10.1002/jps.24309.

[7] J. A. J. Housmans, G. Wu, J. Schymkowitz, and F. Rousseau, "A guide to studying protein aggregation," *FEBS J*, vol. 290, no. 3, pp. 554–583, Feb. 2023, doi: 10.1111/febs.16312.

[8] S. Sreenivasan, C. Schöneich, and A. S. Rathore, "Aggregation of therapeutic monoclonal antibodies due to thermal and air/liquid interfacial agitation stress: Occurrence, stability assessment strategies, aggregation mechanism, influencing factors, and ways to enhance stability," *Int J Pharm*, vol. 666, p. 124735, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124735.

[9] J. Sheng, N. K. Olrichs, B. M. Gadella, D. V. Kaloyanova, and J. B. Helms, "Regulation of Functional Protein Aggregation by Multiple Factors: Implications for the Amyloidogenic Behavior of the CAP Superfamily Proteins," *Int J Mol Sci*, vol. 21, no. 18, p. 6530, Sep. 2020, doi: 10.3390/ijms21186530.

[10] S. Saurabh *et al.*, "Structure and interaction of therapeutic proteins in solution: a combined simulation and experimental study," *Mol Phys*, vol. 121, no. 19–20, Oct. 2023, doi: 10.1080/00268976.2023.2236248.

[11] Y. Tao *et al.*, "Mechanism of Insoluble Aggregate Formation in a Reconstituted Solution of Spray-Dried Protein Powder," *Pharm Res*, vol. 40, no. 10, pp. 2355–2370, Oct. 2023, doi: 10.1007/s11095-023-03524-x.

[12] M. D. Swanson, S. Rios, S. Mittal, G. Soder, and V. Jawa, "Immunogenicity Risk Assessment of Spontaneously Occurring Therapeutic Monoclonal Antibody Aggregates," *Front Immunol*, vol. 13, Jul. 2022, doi: 10.3389/fimmu.2022.915412.

[13] M. R. Nejadnik *et al.*, "Postproduction Handling and Administration of Protein Pharmaceuticals and Potential Instability Issues," *J Pharm Sci*, vol. 107, no. 8, pp. 2013–2019, Aug. 2018, doi: 10.1016/j.xphs.2018.04.005.

[14] M. R. G. Kopp *et al.*, "Surface-Induced Protein Aggregation and Particle Formation in Biologics: Current Understanding of Mechanisms, Detection and Mitigation Strategies," *J Pharm Sci*, vol. 112, no. 2, pp. 377–385, Feb. 2023, doi: 10.1016/j.xphs.2022.10.009.

[15] M. Fukuda, S. Nagae, T. Takarada, S. Noda, S. Morita, and M. Tanaka, "Potential risk factors of protein aggregation in syringe handling during antibody drug dilution for intravenous administration," *J Pharm Sci*, vol. 114, no. 3, pp. 1625–1638, Mar. 2025, doi: 10.1016/j.xphs.2024.12.029.

[16] A. Besheer, "Protein Adsorption to In-Line Filters of Intravenous Administration Sets," *J Pharm Sci*, vol. 106, no. 10, pp. 2959–2965, Oct. 2017, doi: 10.1016/j.xphs.2017.05.028.

[17] L. O. Narhi *et al.*, "Stress Factors in Primary Packaging, Transportation and Handling of Protein Drug Products and Their Impact on Product Quality," *J Pharm Sci*, vol. 111, no. 4, pp. 887–902, Apr. 2022, doi: 10.1016/j.xphs.2022.01.011.

[18] J. Li *et al.*, "Interfacial Stress in the Development of Biologics: Fundamental Understanding, Current Practice, and Future Perspective," *AAPS J*, vol. 21, no. 3, p. 44, May 2019, doi: 10.1208/s12248-019-0312-3.

[19] T. E. King, J. R. Humphrey, C. A. Laughton, N. R. Thomas, and J. D. Hirst, "Optimizing Excipient Properties to Prevent Aggregation in Biopharmaceutical Formulations," *J Chem Inf Model*, vol. 64, no. 1, pp. 265–275, Jan. 2024, doi: 10.1021/acs.jcim.3c01898.

[20] S. H. Lee *et al.*, "Designing a 'Ready-to-Use' powder formulation platform for the inhaled protein therapeutics," *J Drug Deliv Sci Technol*, vol. 76, p. 103820, Oct. 2022, doi: 10.1016/j.jddst.2022.103820.

[21] K. B. Vargo *et al.*, "Surfactant Impact on Interfacial Protein Aggregation and Utilization of Surface Tension to Predict Surfactant Requirements for Biological Formulations," *Mol Pharm*, vol. 18, no. 1, pp. 148–157, Jan. 2021, doi: 10.1021/acs.molpharmaceut.0c00743.

[22] P. Kheddo *et al.*, "The effect of arginine glutamate on the stability of monoclonal antibodies in solution," *Int J Pharm*, vol. 473, no. 1–2, pp. 126–133, Oct. 2014, doi: 10.1016/j.ijpharm.2014.06.053.

[23] F. Noverraz *et al.*, "Novel trehalose-based excipients for stabilizing nebulized anti-SARS-CoV-2 antibody," *Int J Pharm*, vol. 630, p. 122463, Jan. 2023, doi: 10.1016/j.ijpharm.2022.122463.

[24] H. W. Pan, J. Guo, L. Zhu, S. W. S. Leung, C. Zhang, and J. K. W. Lam, "Enhanced powder dispersion of dual-excipient spray-dried powder formulations of a monoclonal antibody and its fragment for local treatment of severe asthma," *Int J Pharm*, vol. 644, p. 123272, Sep. 2023, doi: 10.1016/j.ijpharm.2023.123272.

[25] S. Sreenivasan, C. Schöneich, and A. S. Rathore, "Aggregation of therapeutic monoclonal antibodies due to thermal and air/liquid interfacial agitation stress: Occurrence, stability assessment strategies, aggregation mechanism, influencing factors, and ways to enhance stability," *Int J Pharm*, vol. 666, p. 124735, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124735.

[26] K. T. Pang *et al.*, "Understanding and controlling the molecular mechanisms of protein aggregation in mAb therapeutics," *Biotechnol Adv*, vol. 67, p. 108192, Oct. 2023, doi: 10.1016/j.biotechadv.2023.108192.

[27] M. Prašnikar, M. Bjelošević Žiberna, M. Gosenca Matjaž, and P. Ahlin Grabnar, "Novel strategies in systemic and local administration of therapeutic monoclonal antibodies," *Int J Pharm*, vol. 667, p. 124877, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124877.

[28] C. J. Roberts, "Protein aggregation and its impact on product quality," *Curr Opin Biotechnol*, vol. 30, pp. 211–217, Dec. 2014, doi: 10.1016/j.copbio.2014.08.001.

[29] S. Kanai, J. Liu, T. W. Patapoff, and S. J. Shire, "Reversible Self-Association of a Concentrated Monoclonal Antibody Solution Mediated by Fab–Fab Interaction That Impacts Solution Viscosity," *J Pharm Sci*, vol. 97, no. 10, pp. 4219–4227, Oct. 2008, doi: 10.1002/jps.21322.

[30] B. Hribar-Lee, "The influence of excipients on the viscosity of monoclonal antibody solutions," *J Mol Liq*, vol. 366, p. 120349, Nov. 2022, doi: 10.1016/j.molliq.2022.120349.

[31] H. Wu, R. Kroe-Barrett, S. Singh, A. S. Robinson, and C. J. Roberts, "Competing aggregation pathways for monoclonal antibodies," *FEBS Lett*, vol. 588, no. 6, pp. 936–941, Mar. 2014, doi: 10.1016/j.febslet.2014.01.051.

[32] R. Chaudhuri, Y. Cheng, C. R. Middaugh, and D. B. Volkin, "High-Throughput Biophysical Analysis of Protein Therapeutics to Examine Interrelationships Between Aggregate Formation and Conformational Stability," *AAPS J*, vol. 16, no. 1, pp. 48–64, Jan. 2014, doi: 10.1208/s12248-013-9539-6.

[33] J. Liu, M. D. H. Nguyen, J. D. Andya, and S. J. Shire, "Reversible Self-Association Increases the Viscosity of a Concentrated Monoclonal Antibody in Aqueous Solution," *J Pharm Sci*, vol. 94, no. 9, pp. 1928–1940, Sep. 2005, doi: 10.1002/jps.20347.

[34] B. J. Dear *et al.*, "X-ray Scattering and Coarse-Grained Simulations for Clustering and Interactions of Monoclonal Antibodies at High Concentrations," *J Phys Chem B*, vol. 123, no. 25, pp. 5274–5290, Jun. 2019, doi: 10.1021/acs.jpcb.9b04478.

[35] M. Fukuda, S. Nagae, T. Takarada, S. Noda, S. Morita, and M. Tanaka, "Potential risk factors of protein aggregation in syringe handling during antibody drug dilution for intravenous administration," *J Pharm Sci*, vol. 114, no. 3, pp. 1625–1638, Mar. 2025, doi: 10.1016/j.xphs.2024.12.029.

[36] D. L. Leiske, I. C. Shieh, and M. L. Tse, "A Method To Measure Protein Unfolding at an Air–Liquid Interface," *Langmuir*, vol. 32, no. 39, pp. 9930–9937, Oct. 2016, doi: 10.1021/acs.langmuir.6b02267.

[37] E. D'Imprima, D. Floris, M. Joppe, R. Sánchez, M. Grininger, and W. Kühlbrandt, "Protein denaturation at the air-water interface and how to prevent it," *Elife*, vol. 8, Apr. 2019, doi: 10.7554/eLife.42747.

[38] A. Sreedhara, G. Downing, and K. Rutherford, "Chapter 15: Drug Product Formulation Development and Formulation Robustness Criteria for a mAb in a Pre-filled Syringe," 2020, pp. 373–389. doi: 10.1007/978-3-030-31415-6_15.

[39] D. S. Tomar, S. Kumar, S. K. Singh, S. Goswami, and L. Li, "Molecular basis of high viscosity in concentrated antibody solutions: Strategies for high concentration drug product development," *MAbs*, vol. 8, no. 2, pp. 216–228, Feb. 2016, doi: 10.1080/19420862.2015.1128606.

[40] S. J. Shire, Z. Shahrokh, and J. Liu, "Challenges in the development of high protein concentration formulations," *J Pharm Sci*, vol. 93, no. 6, pp. 1390–1402, Jun. 2004, doi: 10.1002/jps.20079.

[41] M. Proj *et al.*, "Discovery of compounds with viscosity-reducing effects on biopharmaceutical formulations with monoclonal antibodies," *Comput Struct Biotechnol J*, vol. 20, pp. 5420–5429, 2022, doi: 10.1016/j.csbj.2022.09.035.

[42] M. Prašnikar *et al.*, "The search for novel proline analogs for viscosity reduction and stabilization of highly concentrated monoclonal antibody solutions," *Int J Pharm*, vol. 655, p. 124055, Apr. 2024, doi: 10.1016/j.ijpharm.2024.124055.

[43] M. L. Formica, D. A. Real, M. L. Picchio, E. Catlin, R. F. Donnelly, and A. J. Paredes, "On a highway to the brain: A review on nose-to-brain drug delivery using nanoparticles," *Appl Mater Today*, vol. 29, p. 101631, Dec. 2022, doi: 10.1016/j.apmt.2022.101631.

[44] S. Chung, J. M. Peters, K. Detyniecki, W. Tatum, A. L. Rabinowicz, and E. Carrazana, "The nose has it: Opportunities and challenges for intranasal drug administration for neurologic conditions including seizure clusters," *Epilepsy Behav Rep*, vol. 21, p. 100581, 2023, doi: 10.1016/j.ebr.2022.100581.

[45] S. Mahri *et al.*, "Impact of protein aggregation on the immunogenicity of a human monoclonal antibody following pulmonary administration in mice," *Int J Pharm*, vol. 667, p. 124880, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124880.

[46] Y. Tao *et al.*, "Mechanism of Insoluble Aggregate Formation in a Reconstituted Solution of Spray-Dried Protein Powder," *Pharm Res*, vol. 40, no. 10, pp. 2355–2370, Oct. 2023, doi: 10.1007/s11095-023-03524-x.

[47] R. Y. K. Chang, M. Y. T. Chow, D. Khanal, D. Chen, and H.-K. Chan, "Dry powder pharmaceutical biologics for inhalation therapy," *Adv Drug Deliv Rev*, vol. 172, pp. 64–79, May 2021, doi: 10.1016/j.addr.2021.02.017.

[48] H. J. Lee, A. McAuley, K. F. Schilke, and J. McGuire, "Molecular origins of surfactant-mediated stabilization of protein drugs," *Adv Drug Deliv Rev*, vol. 63, no. 13, pp. 1160–1171, Oct. 2011, doi: 10.1016/j.addr.2011.06.015.

[49] S. R. Wlodarczyk, D. Custódio, A. Pessoa, and G. Monteiro, "Influence and effect of osmolytes in biopharmaceutical formulations," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 131, pp. 92–98, Oct. 2018, doi: 10.1016/j.ejpb.2018.07.019.

[50] A. Arsiccio and R. Pisano, "Clarifying the role of cryo- and lyo-protectants in the biopreservation of proteins," *Physical Chemistry Chemical Physics*, vol. 20, no. 12, pp. 8267–8277, 2018, doi: 10.1039/C7CP08029H.

[51] E. Xiao *et al.*, "Formulation Development of a COVID-19 Recombinant Spike Protein-Based Vaccine," *Vaccines (Basel)*, vol. 12, no. 8, p. 830, Jul. 2024, doi: 10.3390/vaccines12080830.

[52] B. Munjal, S. M. Patel, and R. Suryanarayanan, "Role of arginine salts in preventing freezing-induced increase in subvisible particles in protein formulations," *Int J Pharm*, vol. 619, p. 121694, May 2022, doi: 10.1016/j.ijpharm.2022.121694.

[53] I. Seifert and W. Friess, "Improvement of arginine hydrochloride based antibody lyophilisates," *Int J Pharm*, vol. 589, p. 119859, Nov. 2020, doi: 10.1016/j.ijpharm.2020.119859.

[54] A. Tischer, H. Lilie, R. Rudolph, and C. Lange, "Arginine hydrochloride increases the solubility of folded and unfolded recombinant plasminogen activator rPA," *Protein Science*, vol. 19, no. 9, pp. 1783–1795, Sep. 2010, doi: 10.1002/pro.465.

[55] N. A. Kim, S. Hada, R. Thapa, and S. H. Jeong, "Arginine as a protein stabilizer and destabilizer in liquid formulations," *Int J Pharm*, vol. 513, no. 1–2, pp. 26–37, Nov. 2016, doi: 10.1016/j.ijpharm.2016.09.003.

[56] S. Ren, "Effects of arginine in therapeutic protein formulations: a decade review and perspectives," *Antib Ther*, vol. 6, no. 4, pp. 265–276, Oct. 2023, doi: 10.1093/abt/tbad022.

[57] J. Blobel *et al.*, "Protein loop compaction and the origin of the effect of arginine and glutamic acid mixtures on solubility, stability and transient oligomerization of proteins," *European Biophysics Journal*, vol. 40, no. 12, pp. 1327–1338, Dec. 2011, doi: 10.1007/s00249-011-0686-3.

[58] P. Kheddo, A. P. Golovanov, K. T. Mellody, S. Uddin, C. F. van der Walle, and R. J. Dearman, "The effects of arginine glutamate, a promising excipient for protein formulation, on cell viability: Comparisons with NaCl," *Toxicology in Vitro*, vol. 33, pp. 88–98, Jun. 2016, doi: 10.1016/j.tiv.2016.02.002.

[59] D. Shukla and B. L. Trout, "Understanding the Synergistic Effect of Arginine and Glutamic Acid Mixtures on Protein Solubility," *J Phys Chem B*, vol. 115, no. 41, pp. 11831–11839, Oct. 2011, doi: 10.1021/jp204462t.

[60] M. A. Pegues *et al.*, "Effect of Fatty Acid Composition in Polysorbate 80 on the Stability of Therapeutic Protein Formulations," *Pharm Res*, vol. 38, no. 11, pp. 1961–1975, Nov. 2021, doi: 10.1007/s11095-021-03125-6.

[61] A. J. Castañeda Ruiz *et al.*, "Alternative Excipients for Protein Stabilization in Protein Therapeutics: Overcoming the Limitations of Polysorbates," *Pharmaceutics*, vol. 14, no. 12, p. 2575, Nov. 2022, doi: 10.3390/pharmaceutics14122575.

[62] M. P. Zoeller, S. Hafiz, A. Marx, N. Erwin, G. Fricker, and J. F. Carpenter, "Exploring the Protein Stabilizing Capability of Surfactants Against Agitation Stress and the Underlying Mechanisms," *J Pharm Sci*, vol. 111, no. 12, pp. 3261–3274, Dec. 2022, doi: 10.1016/j.xphs.2022.09.004.

[63] S. Rudiuk, L. Cohen-Tannoudji, S. Huille, and C. Tribet, "Importance of the dynamics of adsorption and of a transient interfacial stress on the formation of aggregates of IgG antibodies," *Soft Matter*, vol. 8, no. 9, p. 2651, 2012, doi: 10.1039/c2sm07017k.

[64] S. Samanta and P. Ghosh, "Coalescence of bubbles and stability of foams in aqueous solutions of Tween surfactants," *Chemical Engineering Research and Design*, vol. 89, no. 11, pp. 2344–2355, Nov. 2011, doi: 10.1016/j.cherd.2011.04.006.

[65] J. S. Katz, A. Nolin, B. A. Yezer, and S. Jordan, "Dynamic Properties of Novel Excipient Suggest Mechanism for Improved Performance in Liquid Stabilization of Protein Biologics," *Mol Pharm*, vol. 16, no. 1, pp. 282–291, Jan. 2019, doi: 10.1021/acs.molpharmaceut.8b00984.

[66] A. D. Kanthe *et al.*, "Armoring the Interface with Surfactants to Prevent the Adsorption of Monoclonal Antibodies," *ACS Appl Mater Interfaces*, vol. 12, no. 8, pp. 9977–9988, Feb. 2020, doi: 10.1021/acsami.9b21979.

[67] Y. S. Tein, Z. Zhang, and N. J. Wagner, "Competitive Surface Activity of Monoclonal Antibodies and Nonionic Surfactants at the Air–Water Interface Determined by Interfacial Rheology and Neutron Reflectometry," *Langmuir*, vol. 36, no. 27, pp. 7814–7823, Jul. 2020, doi: 10.1021/acs.langmuir.0c00797.

[68] T. Umumararungu *et al.*, "Proline, a unique amino acid whose polymer, polyproline II helix, and its analogues are involved in many biological processes: a review," *Amino Acids*, vol. 56, no. 1, p. 50, Aug. 2024, doi: 10.1007/s00726-024-03410-9.

[69] M. M. Wright *et al.*, "Stabilization of a miniprotein fold by an unpuckered proline surrogate," *Commun Chem*, vol. 8, no. 1, p. 76, Mar. 2025, doi: 10.1038/s42004-025-01474-6.

[70] V. Kumar, N. Dixit, L. (Lisa) Zhou, and W. Fraunhofer, "Impact of short range hydrophobic interactions and long range electrostatic forces on the aggregation kinetics of a monoclonal antibody and a dual-variable domain immunoglobulin at low and high concentrations," *Int J Pharm*, vol. 421, no. 1, pp. 82–93, Dec. 2011, doi: 10.1016/j.ijpharm.2011.09.017.

[71] M. Zidar, P. Rozman, K. Belko-Parkel, and M. Ravnik, "Control of viscosity in biopharmaceutical protein formulations," *J Colloid Interface Sci*, vol. 580, pp. 308–317, Nov. 2020, doi: 10.1016/j.jcis.2020.06.105.

[72] C. Tilegenova *et al.*, "Dissecting the molecular basis of high viscosity of monospecific and bispecific IgG antibodies," *MAbs*, vol. 12, no. 1, Jan. 2020, doi: 10.1080/19420862.2019.1692764.

[73] Y. Zeng *et al.*, "Caffeine as a Viscosity Reducer for Highly Concentrated Monoclonal Antibody Solutions," *J Pharm Sci*, vol. 110, no. 11, pp. 3594–3604, Nov. 2021, doi: 10.1016/j.xphs.2021.06.030.

[74] T. Serno, R. Geidobler, and G. Winter, "Protein stabilization by cyclodextrins in the liquid and dried state," *Adv Drug Deliv Rev*, vol. 63, no. 13, pp. 1086–1106, Oct. 2011, doi: 10.1016/j.addr.2011.08.003.

[75] V. Ramezani, A. Vatanara, M. Seyedabadi, M. Nabi Meibodi, and H. Fanaei, "Application of cyclodextrins in antibody microparticles: potentials for antibody protection in spray drying," *Drug Dev Ind Pharm*, vol. 43, no. 7, pp. 1103–1111, Jul. 2017, doi: 10.1080/03639045.2017.1293679.

[76] N. Alhajj, N. J. O'Reilly, and H. Cathcart, "Leucine as an excipient in spray dried powder for inhalation," *Drug Discov Today*, vol. 26, no. 10, pp. 2384–2396, Oct. 2021, doi: 10.1016/j.drudis.2021.04.009.

[77] P. J. Naughton, R. Marchant, V. Naughton, and I. M. Banat, "Microbial biosurfactants: current trends and applications in agricultural and biomedical industries," *J Appl Microbiol*, vol. 127, no. 1, pp. 12–28, Jul. 2019, doi: 10.1111/jam.14243.

[78] C. A. Stone *et al.*, "Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized," *J Allergy Clin Immunol Pract*, vol. 7, no. 5, pp. 1533-1540.e8, May 2019, doi: 10.1016/j.jaip.2018.12.003.

[79] G. Guerrini *et al.*, "Monitoring Anti-PEG Antibodies Level upon Repeated Lipid Nanoparticle-Based COVID-19 Vaccine Administration," *Int J Mol Sci*, vol. 23, no. 16, p. 8838, Aug. 2022, doi: 10.3390/ijms23168838.

[80] R. Respaud *et al.*, "Effect of formulation on the stability and aerosol performance of a nebulized antibody," *MAbs*, vol. 6, no. 5, pp. 1347–1355, Sep. 2014, doi: 10.4161/mabs.29938.