Surfactant Selection in Biopharmaceutical Product Development

University of Nottingham

MRes Biomolecular Technology

Genevieve Panton

Supervisors:

Professor Nicholas Darton - AstraZeneca

Professor Stephen Harding - University of Nottingham

Table of Contents

Abstract

Biopharmaceuticals represent one of the fastest growing areas in the biopharmaceutical industry, with monoclonal antibodies (mAbs) dominating recent approvals. However, their inherent instability and high surface activity pose formulation challenges. Throughout their manufacture, transport and administration, mAb products encounter multiple stresses and interfaces that can induce aggregation and visible and sub-visible particles, which are linked to safety risks such as immunogenicity. To address this, non-ionic surfactants such as polysorbates and poloxamers are used due to their effectiveness and history of use. Despite their success, concerns remain around their degradation propensity, heterogeneity and safety. Novel surfactants are under investigation to overcome these issues, with advantages including tunable and modular structures, improved degradation resistance, and potential for rational design. However, their use is limited by complex regulatory pathways and the need for extensive toxicological and compatibility studies. Ultimately, surfactant selection must be guided by a careful, case-by-case risk–benefit evaluation, balancing stability, safety and regulatory considerations. While novel excipients show promise, polysorbates and poloxamers will likely remain central to mAb product formulations, supported by mitigation strategies and ongoing optimisation.

Abbreviations

Antibody-Drug Conjugates (ADCs)

Bispecific Antibodies (BsAbs)

Bovine Serum Albumin (BSA)

Brewster Angle Microscopy (BAM)

Chinese Hamster Ovary (CHO)

Chinese Pharmacopoeia (ChP)

Complementarity-Determining Regions (CDRs)

Critical Micelle Concentration (CMC)

Critical quality attributes (CQAs)

Cyclic Olefin Copolymer (COC)

European Pharmacopoeia (Ph. Eur)

Food and Drug Administration (FDA)

Fourier Transform Infrared (FTIR)

Free Fatty Acids (FFA)

Host Cell Proteins (HCPs)

Human Serum Albumin (HSA)

Hydrogen Exchange-Mass Spectrometry (HX-MS)

Hydrophilic-Lipophilic Balance (HLB)

Hydroxypropyl methylcellulose (HPMC)

Investigational New Drug (IND)

Japanese Pharmacopoeia (JP)

Lauric Acid (LA)

Lipoprotein Lipase (LPL)

Low-Density Polyethylene (LD-PE)

Micro-flow imaging (MFI)

Monoclonal Antibodies (mAbs)

Multicompendial (MC)

Mucor Miehei Lipase (MML)

Neutron reflectometry (NR)

N-Myristoyl Phenylalanine Jeffamine M1000 Diamide (FM1000)

Oleic Acid (OA)

Pediatric Excipients Risk Assessment (PERA)

Poloxamer 188 (P188)

Poly (ethylene oxide) (PEO)

Poly (Propylene oxide) (PPO)

Polyoxyethylene (POE)

Polydimethylsiloxane (PDMS)

Polypropylene (PP)

Polysorbate 20 (PS20)

Polysorbate 80 (PS80)

Polyvinylchloride (PVC)

Progressive pediatric safety factor (PPSF)

Silicon Dioxide (SiO_2)

United States Pharmacopoeia (USP)

Vitamin E, Dimethyl Glutarate (VEDG)

Vitamin E, Dimethyl Succinate (VEDS)

1. Introduction

Antibody-based drugs have experienced some of the most rapid growth in the biopharmaceutical industry (Akbarian & Chen, 2022). As of 2025, the FDA has approved over 200 antibody-based drugs and of these approvals, monoclonal antibodies (mAbs) dominate, with a record number of mAbs approved in 2024 (Crescioli et al., 2024). This success is in part due to their high affinity and specificity for their target, combined with a generally lower incidence of adverse events compared to small molecule drugs (Singh et al., 2023). They have shown efficacy across a range of therapeutic areas, particularly in cancer, making up 50% of mAb targets (De La Torre & Albericio, 2025). Non-canonical formats of mAbs have seen increased development over recent years, including antibody-drug conjugates (ADCs), bispecific antibodies (BsAbs), Fc-fusion proteins and Fab fragments. In 2024, they accounted for approximately 25% of the antibody products approved (Crescioli et al., 2024). High concentration mAb formulations (>100 mg/mL) have also increased, as the industry shifts towards subcutaneous delivery (Ghosh et al., 2023). Due to the inherent instability and surface activity of mAb products, ensuring their stability remains a challenge in biopharmaceutical development (Le Basle et al., 2020).

Throughout manufacture, storage, transport and administration, mAb products are exposed to changes in pH, ionic strength, light, temperature and mechanical stress, which can induce aggregation and may lead to the precipitation of sub-visible and visible proteinaceous particles (Das et al., 2022) (Le Basle et al., 2020). During their life cycle, mAb products encounter a variety of interfaces (air-liquid, solid-liquid, oil-liquid, ice-liquid). MAb product’s high propensity to adsorb to interfaces results in interfacial stress, which is a key factor in particle generation (Koepf et al., 2017) (Vaclaw et al., 2023). Adsorption is influenced by many factors, such as, the properties and interfacial behaviour of the protein, its concentration, excipients, stress conditions and the properties of the interface (Jiménez et al., 2024). Aggregation impacts the quality and efficacy of protein therapeutics by decreasing the drug content, biological activity and purity, which can pose a safety risk to patients (Pham & Meng, 2020). Subvisible and visible particles have been linked to immunogenic responses, such as hypersensitivity (Moussa et al., 2016) (Das et al., 2022) (Wen & Jawa, 2021).

To address these challenges, stabilising excipients, such as non-ionic surfactants, are added to mAb product formulations to reduce their instability. Non-ionic surfactants are amphiphilic and have a high surface activity that allows them to reduce aggregation by two mechanisms; interfacial competition and direct interaction (Aguirre-Ramírez et al., 2021). In biopharmaceuticals, the main surfactants used are polysorbates (Tween®) and poloxamers (Pluronic®) due to their effectiveness and proven safety profiles in various routes of administration (Manning et al., 2024). Polysorbate 80 (PS80) and polysorbate 20 (PS20) are the most extensively used surfactants. They are heterogenous, complex mixtures that are highly effective at preventing interface-induced aggregation and particle formation (Wuchner et al., 2022) (Vaclaw et al., 2023). However, concerns around their safety and stability have emerged as they are susceptible to oxidation and hydrolysis (Katz et al., 2022). These degradation pathways promote protein destabilisation and increase the formation of subvisible and visible particles (Doshi et al., 2015) (Gregoritza et al., 2023). Poloxamer 188 (P188) is a relatively new and effective surfactant that lacks ester bonds, making it resistant to hydrolysis. It is steadily increasing in use but is used much less frequently than PS (Bollenbach et al., 2022). It is a weaker surfactant compared to PS due to lower surface activity; however, it preferentially forms complexes with proteins, making it more effective at reducing thermal instability (Li et al 2024). P188's susceptibility to oxidation and polydispersity pose challenges to formulations and hence, there is an increasing interest in and need for alternative surfactants. Several novel surfactants have been proposed and are currently under investigation, such as ones already established commercially, like Brij®, and novel chemical entities, including N-alkyl amino acid polyether diamides and vitamin-E-derived surfactants (Yue et al 2020) (Katz et al 2019) (Brosig et al 2023). However, due to regulatory limits, developing novel surfactants is challenging and will require industry-wide collaboration and innovation (Thakral et al 2025). The purpose of this review is to discuss the current understanding of mAb product instability, evaluate current surfactants and their challenges, examine novel surfactants, and offer guidance on the selection of a surfactant, along with regulatory and quality considerations required for the production of safe and efficacious drug products.

2. Mechanisms of Action

MAb products are inherently unstable and prone to aggregation, which is primarily driven by exposed hydrophobic patches on their surface (Pham & Meng, 2020). MAb products vary in size, charge and hydrophobicity, which influences its behaviour at interfaces (Downey et al., 2025). Hence, different mAbs and mAb formats will interact differently at the interface depending on their physicochemical properties and conformational stability (Wang et al., 2023). Typically, the more structurally unstable a mAb product is, the higher its surface activity and the faster it adsorbs to the interface (Shieh & Patel, 2015), although this does not always correlate to increased aggregation risk (Singh et al., 2017) (Vaclaw et al.,2021). After adsorption to an interface, mAb products expose their buried hydrophobic residues and can undergo partial unfolding (Kopp et al., 2023). MAb products with higher surface activity and instability have been shown to experience more unfolding (Leiske et al., 2016) (Wang et al., 2023) (Kanthe et al., 2021) (Johann et al., 2024) (Griffin et al., 2024).

The air-liquid interface, commonly formed via headspace in vials or IV bags, have been studied using hydrogen exchange-mass spectrometry (HX-MS) to explore the structural dynamics of proteins (Moorthy et al., 2014) (Xiao & Konermann, 2015) (Leiske et al., 2016) (Gamage et al., 2022). Gamage et al (2022) studied two mAbs at the air-liquid interface. Under agitation stress, there was an increase in hydrophobicity at the interface compared to the bulk solution, which was correlated to partial unfolding and an increase in particle count (Gamage et al., 2022). Similar conclusions have been drawn by other studies (Shieh & Patel, 2015) (Thompson et al., 2025). HX-MS revealed the most destabilised regions were in the complementarity-determining regions (CDRs), suggesting preferential orientation of the Fab tips at the interface (Gamage et al., 2022). Another study inferred, using electron density profile simulations, that mAbs initially adsorb with a flat-on orientation and as bulk concentration increases, reorient vertically and sideways (Kanthe et al., 2021). MAb products can adopt multiple orientations at the interface and unfold in various domains, depending on their structure and the properties of the interface (Zhang et al., 2018). For example, Fab fragments have been found to adsorb in a diagonal orientation at a solid-liquid interface (Peters et al., 2025).

As mAbs adsorb and saturate the interface, there is a measured increase in surface pressure (Pham et al., 2023). This is attributed to the conformational changes and packing that the mAbs undergo as a result of intermolecular interactions (Pham et al., 2023) (Leiske et al., 2016) (Wang et al., 2023). Longer times to reach surface pressure equilibrium is associated with layer reorganisation and further unfolding (Vaclaw et al., 2021) (Leiske., et al 2016) (Hingst et al., 2025). The intermolecular interactions between mAbs leads to the formation of a film with viscoelastic properties. Interfacial rheology can probe the mAb network in terms of their interactions (Pham et al., 2023). High elastic modulus measurements of mAb films suggest that the film consists of relatively strong intermolecular interactions (Pham et al., 2023). An increase in particle count has been associated with this network formation (Pham et al., 2023)(Griffin et al., 2024)(Kannan et al., 2019). Desorption from the interface releases partially unfolded and aggregated mAbs into bulk solution, where they can act as nucleation points and increase the particle count (Kopp et al., 2023). Physical disruption of the film is a key driver of particle formation. Throughout manufacturing processes, transport and administration, such as lyophilisation, fill-finish, accidental dropping, syringe sliding, mixing and pumping, mAb products experience mechanical stress (Das et al., 2022) (Pham & Meng, 2020) (Fukuda et al., 2025). Mechanical stress includes freeze-thaw, agitation, stirring, aeration, cavitation, compression-expansion cycles, or a combination of stresses (Morales et al., 2022). Agitation is a potent driver of aggregation because it refreshes the air-liquid interface continuously, allowing new mAbs to adsorb and eventually aggregate (Gamage et al., 2022). Agitation additionally introduces bubbles to the solution, which increase the available air-liquid interfaces that mAbs can adsorb to (Van Haaren et al., 2024)(Wu et al., 2020). Cavitation caused by mechanical shock, such as dropping a vial, can also cause air bubbles that result in aggregation and particle formation upon expansion and collapse (Wu et al., 2020). Compression of mAb films promotes aggregation and upon expansion, these aggregated layers can be released into bulk solution, thereby increasing particle formation (Kopp et al., 2023).

Solid-liquid interfaces also induce aggregation with similar mechanisms. The adsorption behaviour of mAbs is influenced strongly by the surface properties (Downey et al., 2025). Surfaces found during manufacturing, storage and administration can be complex and hard to characterise (Jiménez et al., 2024). Surface hydrophobicity, charge and roughness all affect mAb adsorption (Downey et al., 2025)(Leiske et al., 2016). Common hydrophobic and hydrophilic surfaces that mAb products may encounter include, stainless steel, glass vials, cartridges, pre-filled syringes, IV bags and filters (Narihi et al., 2022). Hydrophobic interfaces are often found in polymeric materials such as low-density polyethylene (LD-PE) cyclic olefin copolymer (COC), polyvinylchloride (PVC) and polypropylene (PP) or in surfaces coated with silicone oil (Zhang et al., 2018). MAb products have a propensity to absorb to both hydrophobic and hydrophilic surfaces via different mechanisms (Fujita et al 2018). Adsorption to hydrophilic surfaces is driven by electrostatic interactions, whereas hydrophobic interactions are driven largely by hydrophobic interactions (Leiske et al., 2016) (Latour, 2020) (Li et al., 2018) (Hoger., et al 2015).

Primary packaging, such as stoppers, glass vials and pre-filled syringes are often coated with silicone-oil to allow for better gliding, for instance (Narhi et al., 2022). MAb products have been shown to strongly adsorb to the hydrophobic layer of polydimethylsiloxane (PDMS), resulting in aggregation and the formation of PDMS-protein particles (Grapentin et al., 2020) (Soeda et al., 2022). PDMS-protein particles have been shown to be molecule specific and stress specific, although the mechanisms are not fully known due to the complexity of particle formation pathways (Grapentin et al., 2020) (Soeda et al., 2023).

Aggregation at other hydrophobic interfaces is also protein specific. Álvarez-Palencia Jiménez, et al. (2024), compared the adsorption of two mAbs and an ADC to medical surfaces, including IV and manufacturing bags. A higher amount of ADC adsorbed to polymer interfaces (PP, PVC and LD-PE), possibly due to ADC having greater hydrophobicity compared to the mAbs due to their hydrophobic drug load (Johann et al., 2024). It has been reported that absorption to polymer surfaces is driven by hydrophobic interactions, which may be why ADC had a higher affinity to the polymer surface (Álvarez-Palencia Jiménez et al., 2024). Similarly, an Fc-fusion protein with an unstable, hydrophobic domain was more likely to undergo adsorption compared to 4 mAbs, possibly due to increased affinity to a hydrophobic PDMS surface (Grapentin et al., 2020). In both studies, the mAbs studied displayed varying adsorption behaviours to each other based on a combination of hydrophobicity, conformational and colloidal stability, illustrating that interfacial behaviour is determined primarily by intrinsic properties of the specific protein.

The PDMS layer can also release silicone droplets into solution upon agitation or syringe sliding (Fukuda et al 2025). Silicone-oil droplets create an oil-liquid interface, where mAbs can form films in a similar mechanism to air-liquid interfaces (Kopp et al., 2023) (Wu et al., 2020). In some cases, the elastic network is stronger on oil-liquid interfaces compared to air-liquid interfaces, possibly due to higher hydrophobicity of silicone (Kannan et al., 2021). When the viscoelastic film is mechanically perturbed, the silicone droplets release subvisible and visible proteinaceous particles into solution. A high concentration mAb formulation was found to increase the generation of particles at a silicone-oil-liquid interface when mechanically perturbed (Zurcher et al., 2024). The authors hypothesis that the increased propensity to aggregate due to increased protein-protein interactions may explain this observation (Zurcher et al., 2024).

Lypophilisation is used to maintain the stability and improve shelf-life of therapeutic proteins. This is particularly important for particularly unstable modalities such as ADCs, the majority of which are formulated as lyophilised products (Ling et al., 2025) (Wen et al., 2025). However, the process itself can induce aggregation and affect biological potency (Dash & Rathore, 2021) (Arsiccio & Pisano, 2020). One source of aggregation during the lyophilisation process is the introduction of ice-liquid interfaces where proteins can adsorb and unfold (Butreddy et al., 2021) (Strambini & Gabellieri, 1996). Recently Lu et al (2025), investigated the effects of freezing a BsAb and found that hydrophobic interactions with the ice-liquid interface was the main driver of aggregation. Lyophilised proteins are also susceptible to aggregation in response to mechanical stress that may be experienced during transport or handling. Lyophilised mAb products have demonstrated sensitivity to shaking stress, resulting in subvisible particle formation (Fang et al., 2022) (Telikepalli et al., 2015) (Jin et al., 2024). Dropping lyophilised Fc-fusion protein abatacept (Fc-fusion) has also been shown to significantly increase particle formation at -30°C (Torisu et al., 2024). While the relationship between lyophilisation, mechanical stress and aggregation is not fully understood, a localised temperature increase upon mechanical impact has been suggested to contribute to aggregation, similar to cavitation (Torisu et al., 2024).

Throughout its lifecycle, a mAb product will encounter multiple interfaces and stressors, making maintaining mAb product stability difficult to control, particularly during transport and administration where conditions may not be optimal. The type of stress can affect what types of particles predominate. For example, particles may be proteinaceous or non-proteinaceous, such as silicone-oil droplets (Soeda et al., 2023). Therapeutic protein aggregates have been linked to anti-drug antibody production and anaphylaxis, hence it is a critical quality attribute (CQA) and carefully monitored during development (Wen & Jawa, 2021). The US Pharmacopoeia (USP) accepts 6000 particles ≥10 µm and 600 particles ≥ 25 µm in a container of ≤ 100 mL (United States Pharmacopeia, n.d.). The aggregation propensity of mAbs at interfaces is determined by multiple, interconnected factors, including the amount of protein at the interface, its orientation, the strength of intermolecular interactions and the nature of the exposed residues (Wang et al., 2023). Therefore, understanding the structural properties and the interfacial behaviour of each mAb product on a case-by-case basis is necessary.

3. Surfactant Types

Surfactants used in the biopharmaceutical industry are non-ionic, amphiphilic molecules, with high surface activity. They are added to solutions primarily during formulation development (Thakral et al., 2025). They can be pre-added, for instance to pre-coat IV bags and lines, or co-added (Couston et al., 2013). They compete with therapeutic proteins at the interface, blocking them from adsorbing and preventing structural destabilisation due to their higher surface activity (Aguirre-Ramírez et al., 2021)(**Fig. 1**). Their hydrophobic components can form weak interactions with hydrophobic patches on proteins, which prevents self-association of the protein molecules and interactions with the interface, as aggregation prone regions are shielded (Kamerzell et al., 2011). Simultaneously, their hydrophilic component interacts with the solution and increases solubility of the protein. Other than preventing aggregation, surfactants offer several benefits, such as reducing surface tension and viscosity (Downey et al., 2025) (Li et al., 2024). Hence, they are used in the majority of biopharmaceutical formulations to improve protein stability.

3.1 Polysorbates

Polysorbates are the most commonly used surfactants in biopharmaceutical formulations, with PS80 being used the most (Mieczkowski, 2023)(Strickley et al., 2021). Polysorbates consist of a polyoxyethylene (POE) sorbitan core, esterified with fatty acids (Yang et al., 2022). Polysorbates are highly complex and heterogeneous mixtures ranging in composition of fatty acid esters, degree of esterification, arrangement of POE units and variation in the head group (Yang et al., 2022)(Weber et al., 2023). Grades of PS20 and PS80 are determined by the percent of lauric acid (LA) or oleic acid (OA), respectively, present in the raw material (Yang et al., 2022)(Doshi et al., 2020). Multicompendial (MC) grade PS20 and PS80 is commonly used commercially for parenteral formulations and complies with pharmacopeia (i.e. USP/NF, Ph. Eur. and JP), which require that the PS80 oleic acid content is ≥ 58 % and PS20 lauric acid content to be between 40-60% (Cheng et al., 2024). However, the Chinese pharmacopoeia (ChP) requires ≥98% oleic acid content in PS80, which Ultrapure PS80 complies with (99.3% OA) (Brovc et al., 2022). Other compendia compliant grades of PS80 and PS20 with reduced heterogeneity, include High Purity and Super Refined, which have lower levels of impurities (Brovc et al., 2022).

The effective concentration of PS is relatively small, with a critical micelle concentration (CMC) of around 0.001% (w/v). CMC is the concentration above which surfactants saturate the interface and micelles are formed (Gregoritza et al., 2024)(Bollenbach et al., 2022). In biopharmaceuticals, polysorbates are used above the CMC, with the exact concentration depending on the specific protein and formulation conditions but can range between 0.01 - 0.8% w/v (Wang et al., 2023). Polysorbate's hydrophilic-lipophilic-balance (HLB) is around 15-16 (Waters et al 2023). Due to PS80's longer fatty acid tail, it is more hydrophobic than PS20, reflected in its lower HLB (15.7) (Waters et al., 2023).

Polysorbates protect proteins from aggregation and particle formation via two main mechanisms; competitive adsorption and direct interaction, although the exact mechanisms are still under debate (Weber et al., 2025). Direct interaction is driven by non-specific, weak electrostatic forces between hydrophobic patches on proteins and the hydrophilic tails of polysorbates, potentially increasing the protein-surfactant complex's solubility and conformational stability of the protein (Lee et al., 2011). Polysorbates have demonstrated binding to hydrophobic pockets of bovine serum albumin (BSA) (Ruiz-Peña et al., 2010). Hydrophobic interactions also drive polysorbate association with human serum albumin (HSA), along with possible hydrogen bonding (Garidel et al., 2009). Polysorbates can also bind to mAbs although at a lower protein:surfactant ratio, due to mAbs being less flexible and more stable than BSA and HSA, or similar globular proteins (Kanthe et al., 2020). PS20 and PS80 appeared to preferably interact with the Fab region of a mAb, due to exposed hydrophobic residues (Singh et al., 2017). However, some studies have found negligible or no complex formation between polysorbate and IgG (Garidel et al., 2009). If it does occur, direct binding can increase the apparent CMC of polysorbates (Mahler et al., 2009). Competitive adsorption is considered to be the primary mechanism for polysorbate protection of proteins (Khan et al., 2015). As mAbs are much larger and less amphiphilic than polysorbates, they adsorb to interfaces slower than PS20 and PS80 (Hingst et al., 2025). Polysorbate's faster adsorption kinetics drives competitive adsorption and allows them to dominate interfaces. Although, at lower concentrations, particularly below the CMC, coadsorption with mAb products can occur (**Fig. 1**).

Due to their surface activity, both mAbs and polysorbates lower the surface tension of air-liquid interfaces. Kanthe et al (2020) studied the interfacial behaviour of PS80 and two mAbs using tensiometry and X-ray measurements. Below the CMC, mAbs and PS80 coadsorb, where they both contribute to a reduction in surface tension (Kanthe et al., 2020). At the CMC and above, PS80 dominated the interface, forming a monolayer that effectively blocked mAbs from aggregating. When the mAb was already adsorbed to the air-liquid interface, pendant bubble tensiometry revealed that PS80 displaced the mAbs below and above the CMC (Kanthe et al., 2020). This ability was sensitive to the surface activity of the mAb, with the more surface active mAb requiring a greater concentration of PS80 equal to the CMC. Although in some cases polysorbates are not able to completely suppress particle formation, the addition of PS20 and PS80 can significantly mitigate aggregation-induced sub-visible and visible particles by various types of mechanical stress, such as agitation, elevated syringe sliding, cavitation, compression and dilation (Gamage et al., 2022)(Fukuda et al., 2024)(Wu et al., 2020)(Vaclaw et al., 2021). When a mAb film was disrupted by puncturing, PS80 prevented the reconstitution of the film above the CMC (Žuntar et al., 2022). However, PS80 was not able to fully displace the film if it was preestablished, although, after a few days it was observed integrating into the film via Brewster Angle Microscopy (BAM) imaging (Žuntar et al., 2022). In other studies, PS80 and PS20 can fully displace the film, particularly above the CMC (Kanthe et al., 2020)(Hingst et al., 2025). MAb product desorption due to surfactant addition is likely to be dependent on surfactant amount and type, mAb product instability, film properties, and film maturation (Hingst et al., 2025).

Like mAb products, polysorbate's interfacial behaviour and effectiveness can be affected by the properties of interfaces they encounter during development. The hydrophobicity of an interface could enhance the competitive adsorption of polysorbates to mAbs (Li et al., 2018)(Shen et al., 2023). On the other hand, hydrophilic interfaces have been seen to reduce PS80's affinity to interfaces and may decrease effectiveness (Zhuang et al., 2025)(Li et al., 2018)(Zurcher et al., 2023). Understanding how polysorbates interact with different materials across manufacturing, transport and administration is therefore important in the development of a stable biopharmaceutical.

Different polysorbate grades have been studied to determine if their differences in structure result in different interfacial properties and degradation propensity (Doshi et al., 2020)(Vaclaw et al., 2021)(Grabarek et al., 2020). Doshi et al (2020), evaluated Super Refined PS20 and found that it was more prone to oxidation compared to High Purity PS20, which was likely a result of its free fatty acid (FFA) composition, ultimately increasing mAb aggregation. Vaclaw et al (2021) studied Super Refined PS80 (99%) (ChP compliant) and High Purity PS80 (75%) (MC compliant) under dilation stress. In stressed and unstressed samples, they behaved similarly at the interface and both mitigated particle formation. Compared to High Purity PS80, Super Refined PS80 may have had a higher affinity to the interface and better mitigated particle formation in a less stable mAb, making the researchers conclude that PS80's effectiveness is more dependent on mAb type rather than oleic content (Vaclaw et al 2021).

3.2 Challenges with Polysorbates

While polysorbates are efficient at protecting proteins, issues concerning their stability have grown over recent years (Weber et al., 2023). Polysorbates are susceptible to degradation by two main pathways: hydrolysis and oxidation. Degradation compromises the efficacy of surfactants by decreasing effective surfactant concentration and can lead to particle formation (Dwivedi et al., 2018). The ester bond is susceptible to enzymatic and chemically-induced hydrolysis (Weber et al., 2023). At pharmaceutically relevant conditions, chemical hydrolysis is not considered to be the root cause of hydrolysis compared to enzymatic hydrolysis (Dwivedi et al., 2020)(Wuchner et al., 2022). Enzymatic hydrolysis is caused by host cell proteins (HCPs), such as lipases and esterases, that are copurified with the mAb product from Chinese hamster ovary (CHO) cells (Hall et al., 2016). Even at trace amounts, HCPs can catalyse hydrolysis of polysorbates (Hall et al., 2016)(Wuchner et al., 2022). Notably, the level of HCP may increase in proportion to the mAb product concentration, hence can pose a challenge to high concentration formulations (Hall et al., 2016)(Jones et al., 2018).

Enzyme-mediated hydrolysis results in the release of FFAs. As FFAs have limited solubility, they may precipitate and lead to sub-visible and visible particles (Jones et al.,2018)(Doshi et al., 2015). Despite polysorbates being used at low concentrations, it has been shown that as little as 5-10% of PS80 being hydrolysed can lead to oleic acid levels exceeding its solubility limit (Zürcher et al., 2024). The relationship between fatty acid composition (grade) and degradation of PS is not fully understood. Aryal et al (2024) investigated PS20 treated with either esterase or lipase, finding that enzymatic degradation resulted in the formation of FFA, which correlated strongly with the presence of FFA particles, as recorded by micro-flow imaging (MFI). They reported that different PS20 grades (PS20 NF (52.2% LA) PS20 PLA (99.6% LA)) resulted in different hydrolysis patterns and FFA particle formation due to the enzyme varying in its selectivity to the different distribution of esters (Aryal et al., 2024). Gregoritza et al (2024) studied partially hydrolysed PS20 to better represent aged drug product formulation, achieved by bead-immobilised Mucor miehei lipase (MML) and Candida antarctica lipase B (CALB). Hydrolysis of PS20 with higher-order esters and monoesters resulted in different hydrolysis patterns and types of particles. Hence, the exact hydrolysis pattern depends on the ester composition of polysorbate and the type of enzymes present (Wuchner et al., 2022). Due to HCPs consisting of a mixture of enzymes with varying affinities to polysorbates, there are differences in hydrolysis profiles between polysorbate types and grades (Jones et al., 2018)(Doshi et al., 2020). In some cases FFA particles do not impact biological activity of mAbs directly (Dixit et al., 2016)(Aryal et al., 2024)(Roy et al., 2024). However, FFAs can cause degradation of proteins via pathways such as behaving like silicone-oil droplets upon which mAbs can form a viscoelastic film and lead to particle generation (Zurcher et al., 2024).

Oxidation of polysorbates in formulation can be caused by light exposure, temperature and residual impurities, such as peroxides and metal ions (Aryal et al., 2024). H_2O_2 can be left over residue from the manufacturing process or even caused by cavitation (Weber et al., 2023)(Wu et al., 2020). The hydroxyl radicals produced attack the POE units or the saturated double bond of fatty acid ester tail, generating short chain fatty acids, ketones, aldehydes, and peroxides (**Fig.2**). This can also cause oxidation of the mAb product and decrease its biological activity. Degradation can introduce impurities and decrease the effective surfactant concentration, both of which negatively impacts the formulation (Dwivedi et al., 2020). Additionally, challenges include lot-to-lot variability due to heterogeneity, which also makes it hard to characterise analytically (Thakral et al., 2025). Despite being the most widely used surfactant, the challenges associated with it have posed issues to formulators, hence, there has been a drive towards alternative surfactants.

3.3 Poloxamers

Poloxamers are a class of versatile surfactants used in a range of applications including protecting cell culture from shear stress, and in biomedical engineering as hydrogels and blood substitutes (Chang et al., 2017)(Zarrinjaj et al., 2020). In the biopharmaceutical industry, they have been increasingly used as an alternative surfactant to polysorbates (Li et al., 2024). Poloxamers are triblock copolymers consisting of two hydrophilic poly(ethylene oxide) (PEO) units either side of a hydrophobic poly(propylene oxide) (PPO) unit (Bollenbach et al., 2022). Poloxamers, like polysorbates, are heterogeneous in their molecular weight and distribution (Bollenbach et al., 2022). By altering the length of blocks, poloxamers with different properties can be achieved and hence leads to lot-to-lot variability in hydrophobicity (Chen et al., 2022)(Soeda et al., 2022). The most well-known poloxamers are Poloxamer 188 and 407 (Zarrinjaj et al., 2020). Poloxamer 188 consists of 25-30 PPO units, with each PEO chain having 75-85 units (HLB = 27 - 29) (Ruiz et al., 2022). Its CMC is not well defined but can range from around 0.005% to 1% w/v (Dasnoy et al., 2024). In current formulations, P188 is used between 0.01 to 8.0 mg mL^{-1} (Bollenbach et al., 2022). In the literature, the micellication behaviour of P188 is not clear as the CMC will change based on temperature and hydrophobicity. For example, high temperatures not relevant to drug products (65℃) have been seen to be essential to micelle concentration (Bollenbach et al., 2025). P188 is not as surface active as polysorbates. Their differences throughout the studies are likely due to their different hydrophobicities, CMCs and diffusion coefficients (Bollenbach et al., 2022)(Grapentin et al., 2020).

P188 protects proteins via a combination of competitive adsorption and direct interaction. Its primary mode of action is considered to be direct interaction with hydrophobic patches on proteins (Kim et al., 2014a)(Kim et al., 2014b), although some studies have found no interactions with mAbs (Zhang et al., 2021). While polysorbates have higher surface activity and hence are generally more effective at competitive adsorption, P188 can still prevent mAb product adsorption and has demonstrated better thermal stability of proteins (Li et al., 2024). P188 can form intramolecular interactions with each other in solution and at interfaces depending on concentration, surface pressure and temperature (Hingst et al., 2025)(Bollenbach et al., 2022). Hingst et al (2025), compared the adsorption kinetics of P188 and PS20 in the presence of a mAb. At low surface pressures, P188 flattened across the interface. At increasing surface pressures, the PEO blocks protrude further into solution as available space at the interface decreases (Hingst el al., 2025). When injected simultaneously with a mAb, a pharmaceutically relevant concentration of P188 adsorbs to the air-liquid interface faster than the mAb and dominates the interface. This concentration correlates to the protruded or "brush" phase of P188. When P188 is added to a preestablished mAb film, its adsorption is incomplete and was found in patches throughout the film. However, it did not displace the film, unlike PS20, which upon addition resulted in immediate mAb desorption. Compared to P188, PS20 has faster adsorption kinetics, which may partially explain these results. When mAb films were allowed to mature beyond the point where they reach surface pressure equilibrium (after 2 hours), both P188 and PS20 could not displace desorbed mAbs. This is likely due to increased intermolecular interactions between mAbs resulting from film reorganisation and unfolding (Hingst et al., 2025). Thompson et al (2025) found similar results with P188 at the air-liquid interface using BAM imaging and neutron reflectometry (NR). When P188 was added below a pre-established mAb film, it was adsorbed in patches and was only able to rapidly outcompete mAbs when they were pre-mixed (Thompson et al., 2025). Similarly, Zhang et al (2021) used NR and found that while P188 was able to prevent mAb adsorption when it was added to a siliconised solid surface before mAb introduction, after the mAb layer was established, P188 coadsorbed to the surface and altered the layer by changing the orientation of mAbs. This could illustrate that the order that surfactant is important to consider, particularly with P188 (Thompson et al., 2025).

P188 is comparable to PS at protecting proteins against interfacial stress at the ice-liquid interface at 0.1% w/v (Li et al., 2024)(Li et al., 2023). However, the addition of other protectants, such as bulking agents, was required for full protection (Li et al., 2024). Additionally, upon dropping a Fc-fusion in the frozen state, P188 was able to reduce aggregation, although the mechanism of protection is not clear (Torisu et al., 2025). Grapentin et al (2020) tested P188's protective ability in response to various stresses, including agitation, thermal and freeze-thawing in mAb formulations. P188 demonstrated comparable protection to PS (Grapentin et al., 2020). Fourier Transform Infrared spectroscopy (FTIR) microscopy captured PDMS-protein particles in formulations containing P188 but not PS, which may be due to P188's lower interfacial activity and hence decreased effectiveness at protection (Grapentin et al., 2020). However, at a silicone-oil-liquid interface, using a microfluidic droplet platform, P188 was able to prevent mAb adsorption, even at high mAb concentrations (180 mg mL^{-1}) and in response to mechanical perturbation (Zurcher et al., 2024).

3.4 Challenges with Poloxamers

While P188 is not susceptible to enzymatic hydrolysis, its PEO and PPO units can undergo auto-oxidation, due to thermal stress or in the presence of reactive oxygen species, resulting degradation products such as acetaldehyde, acetone and formaldehyde (Chen et al., 2022)(Erlandsson, 2002)(**Fig.2**). This could lead to a loss in P188 efficacy, compromise formulation stability and negatively affect the active pharmaceutical ingredient (API). In addition, excipient grade P188 is polydisperse, which has been linked to renal dysfunction after parenteral injections in animal studies (Emanuele & Balasubramaniam, 2014). The impact of P188's hydrophobic lot-to-lot variation could influence mAb product stability (Soeda et al., 2022). Soeda et al (2022) found that varying hydrophobicity in P188 affected interfacial protective ability. Higher hydrophobicity of P188 demonstrated a higher affinity to hydrophobic air-liquid and PDMS interfaces and therefore offered a greater level of protection against interfacial stress and particle formation (Soeda et al., 2022). Hence, lower hydrophobicity P188 increased particle formation. Another challenge posed may be due to the general lack of studies on tolerability and interfacial activity compared to PS, which makes its behaviour less well-understood, although research is increasing along with its use (Bollenbach et a., 2022).

4. Novel Surfactants

Interest in alternative surfactants has been increasing in recent years due to the instability issues associated with current surfactants. However, developing novel surfactants and subsequently gaining regulatory approval is challenging, costly and time-consuming (Thackral et al., 2025). Hence, most of the candidates that have been proposed have not been approved and still require further studies to establish their potential as a viable alternative to polysorbates and poloxamers. The surfactants discussed here are not an exhaustive list of the alternative surfactants and excipients that are being investigated to improve therapeutic protein stability. Some others include, Kolliphor HS 15 (polyoxyethylene 15 hydroxystearate) Kolliphor ELP (polyoxyethylene 35 castor oil), cyclodextrins and hydroxypropyl methylcellulose (HPMC) (Löw et al., 2025)(Zoeller et al., 2022)(Katz et al., 2022).

4.1 Polyoxyethylene alkyl ethers

Brij ® is a polyoxyethylene alkyl ether and has been used commercially in skincare products and cell lysis buffers (Roy et al., 2024)(Katz et al., 2022). Its CMC ranges from 0.0005 to 0.01% (w/v) (Roy et a., 2024)(Peters et al., 2025)(Yue et al., 2020). Types of Brij include Brij 35, Brij O20 and Brij 58. Brij 35 (also known as Brij L23) is a polyoxyethylene lauryl ether (HLB = 16.7) (CMC = 0.005 - 0.08) (Roy et al., 2024). Brij 35 can make intermolecular interactions with lysozyme and displace it from the air-liquid interface via competitive adsorption (Chernysheva et al., 2018). Brij 35 has been compared to PS20, PS80 and P188 to compare stability against mechanical, thermal, and light stress (Agarkhed et al., 2018). While Brij 35 experienced less oxidation, compared to PS20, PS80 and P188, Brij 35 was less effective at protecting a mAb against mechanical stress, resulting in higher particle counts (Agarkhed et al., 2018). Overall, PS80 provided the most stability against agitation stress compared to all the surfactants. However in other studies, Brij 35 was able to protect a mAb from interfacial stress in thermal and shaking tests with different surfaces (Roy et al., 2024)(Zurcher et al., 2023). Roy et al (2024) investigated the stability of Brij 35 and Brij O20, P188, PS20 and PS80. As expected, the addition of lipoprotein lipase (LPL) resulted in hydrolysis of PS20 and PS80 but this was not seen for the other surfactants due to their lack of ester bonds. Zurcher et al (2023) investigated the effect of surface hydrophobicity and charge on Brij 35, PS20, PS80 and P188. Consistent with the literature, all of the surfactants were unable to protect mAbs at the hydrophilic cellulose interface (Shen et al., 2023). PS20, PS80 and Brij 35 were less efficient at protecting mAbs at the hydrophobic solid-liquid interfaces (COC and a model hydrophobic interface) than the air-liquid interface. However, Brij 35 outperformed PS80 and PS20 at COC interfaces (Zurcher et al., 2023).

Brij 58 is a polyethylene glycol hexadecyl ether (HLB, 16) (CMC = 0.0075)(Roy et al., 2024). Yue et al (2020) investigated 8 excipients, including PS20 and PS80, and proposed Brij 58 as an alternative surfactant for parenteral use. In response to shaking and thermal stress, Brij 58 resulted in reduced particle formation, confirmed by dynamic light scattering (DLS) and visual inspection, at lower concentrations than PS20 and PS80. Brij 58 enhanced the thermal stability of a mAb, suggesting possible binding (Yue et al., 2020). Additionally, Brij 58 could reduce particle formation similar to PS in different mAb isotypes and a BsAb, suggesting that it could be used for other modalities, however, a much wider range of mAbs and modalities is required to confirm this. Similarly to Brij 35, Brij 58 demonstrated less sensitivity to these conditions, whereas PS20 and PS80 likely underwent degradation and resulted in between a 15% to 35% loss of total surfactant content. This is attributed to Brij 58 having an ether bond instead of an ester bond. Preliminary toxicology studies in mice indicated that Brij 58 resulted in no adverse effects based on the acute toxicity test. Although PS80 demonstrated a 10-fold higher LD50 (5000 mg/kg), Brij 58's LD50 still remains in a safe range (343.4 mg/kg) (Yue et al., 2020). Cuzucca et al (2024), compared the safety profiles of alternative surfactants, including Brij58. Interestingly, Brij58 modified with a succinate linker (Brij 58S) demonstrated a better in vitro safety profile than Brij58. The authors hypothesise that a longer linker reduces membrane disruption as it decreases its ability to intercalate into the cell membrane (Cuzucca et al., 2024).

4.2 N-alkyl amino acid polyether diamides

N-myristoyl phenylalanine Jeffamine M1000 diamide (FM1000) has been investigated as an alternative surfactant (Katz et al., 2016)(Katz et al., 2022). FM1000 consists of a polyether head group and a fully saturated 14-carbon alkyl chain, connected via amide bonds. As it lacks ester bonds, it is less susceptible to hydrolysis (Katz et al., 2022). The CMC of FM1000 is much lower than PS20 and PS80 (0.003 mg/ml/0.001% w/v). Initial studies showed that FM1000 reduced thermally-induced aggregation of an IgG and an Fc-fusion protein but not for a commercial mAb, demonstrating that FM1000 may be making specific interactions with IgG and the Fc-fusion protein (Katz et al., 2016). Compared to PS80 and PS20, FM1000 exhibited faster interfacial dynamics at the air-liquid interface (Katz et al., 2016)(Katz et al., 2019)(Kanthe et al., 2022). This may be due to its higher HLB (17), making it more amphiphilic (Katz et al 2019). At silicone-oil interfaces, FM1000, PS80 and PS20 were able to prevent mAb adsorption, with FM1000 inhibiting mAb adsorption at lower concentrations than PS20 and PS80 (Katz et al., 2019)(Kanthe et al., 2022). FM1000 was also able to reduce agitation induced aggregation of mAbs, resulting in lower particle counts compared to PS20 and PS80 (Katz et al., 2019)(Hanson et al., 2020)(Kanthe et al., 2022). FM1000 has also been demonstrated to be compatible in downstream processing operations by showing improved retention at filters, which is potentially an advantage compared to polysorbates (Fares et al., 2023). Dasnoy et al (2024) compared FM1000 and Brij 58 to current surfactants to confirm their protective effects. They both required lower concentrations to protect a mAb derived product against agitation-induced aggregation compared to PS80, confirming previous results. In a survey of industry, 40% of companies responded that they had an interest in FM1000, although most of those respondents currently have no experience with it (Thakral et al., 2025).

4.3 Vitamin-E-derivatives

In a systematic screening of novel surfactants against current surfactants, Brosig et al (2023) screened 40 surfactants, including existing surfactants and synthesised surfactants, using PS20, PS80 and P188 as a benchmark. The surfactants were tested for their biophysical properties, degradation resistance, surface activity, ability to protect against agitation-induced aggregation and surfactant stability, using relevant industry practices (Brosig et al., 2023). The studies resulted in three candidates all of which were synthesised ad hoc for this study. The surfactants were synthesised using PEG1000 as the hydrophilic head-group due to being established in pharmaceuticals, and inserting short linkers, to increase surfactant stability by potentially blocking esterases. This resulted in two candidates: Brij 58S, vitamin E, dimethyl succinate, PEG1000 (VEDS) and vitamin E, dimethyl glutarate, and PEG1000 (VEDG). Vitamin E was chosen due to demonstrating potential in protein stabilisation in the past. These surfactants had a HLB between 12-15, similar to polysorbates. Brij58S, VEDS and VEDG demonstrated a similar decrease in particle count to PS80 with a range of proteins, including mAbs, a Fab fragment, a fusion protein, and a partially disordered protein. VEDS outperformed all the other surfactants in accelerated conditions. VEDS and VEDG were also less prone to oxidation and hydrolysis than PS80, with VEDS being more prone to oxidation than VEDG possibly due to the difference in linker.

The researchers expanded this work on VEDS/VEDG by including other newly synthesised surfactants (Brij58-derived and SPGS-550M) to elucidate their structure-function relationships and safety profile (Cucuzza et al., 2024). From this, the authors determined that VEDG-3.3 had one of the best in vitro safety profiles, comparable to PS80. Additionally, all surfactants demonstrated direct interaction with BSA, although this was relatively weak and did not afford full protection (Cucuzza et al., 2024).

Recently, Peters et al (2025) further investigated VEDS and VEDG-3.3 and found they had different interfacial behaviour than polysorbates and poloxamers. Compared to PS20, PS80, P188 and Brij 58, VEDS/VEDG-3.3 were slower to adsorb to the air-liquid and the silicone-oil-liquid interface, however, it underwent molecular rearrangements which the authors propose results in less reversibility. VEDG-3.3 was not able to fully displace already adsorbed mAb products, however, they adsorbed into gaps between mAbs. The authors proposed that this could facilitate orogenic displacement, which is the process where surfactants displace proteins from an interface by creating areas of high pressure amongst the protein layer, triggering desorption (Peters et al., 2025). VEDG also demonstrated similar prevention of aggregation, compared to polysorbates and poloxamer, in a large-scale particle study

(Strnad et al., 2025). An industry survey has indicated an interest in vitamin-E-derived surfactants, with 27% of respondents stating their interest in VEDS and VEDG (Thackral et al., 2025).

4.4 Regulatory considerations and future perspectives

A novel excipient is considered novel if it has no precedence in an approved medical product or not been previously used via a specific route of administration (FDA, 2005)(IPEC Federation, 2021). If an excipient is not on the FDA Generally Recognised As Safe (GRAS) list or the Inactive Ingredient Database (IID), supporting Chemical, Manufacturing and Controls (CMC) and pre-clinical toxicology data on the new excipient should be included in the drug master file (DMF) Type IV. This DMF is contained within an investigational new drug (IND), new drug application (NDA) or biologics license application (BLA) (FDA, 2005).

Any novel surfactant will carry regulatory risk but will vary depending on how the surfactant is classed. If the novel surfactant has prior use in commercial products such as foods or cosmetics and is included on the GRAS or IID list, it generally requires less safety data than a new chemical entity (IPEC Federation, 2021). For example, tocopherols (vitamin E) is in on the GRAS list (ID: 1406-66-2) and Vitamin E polyethylene glycol succinate is listed in the IID for oral use (CAS No. 9002964), which could potentially reduce the safety data required for VEDS (**section 4.3**), compared to an entity (or components therein) with no prior use. However, due to the complexity, ambiguity and extent of safety data required, many companies avoid this pathway due to concerns about delays to, or even rejections of, drug applications (IPEC Federation, 2021)(Kozarewicz & Loftsson, 2018).

In order to accelerate this process and incentivise innovation, the FDA launched the Pilot Program for the Review of Innovation and Modernization of Excipients (PRIME) (FDA, 2022). The process requires an initial proposal, including a description and the public health need addressed by the excipient. Those selected are required to submit a package containing toxicology and CMC data, similar to the data in a DMF Type IV (FDA, 2022). For example, for parenteral use, an *in vitro* hemolysis study of the excipient may be appropriate (FDA, 2005). Overall, this reduces regulatory risk. However, the safety data required is extensive and still could pose a challenge to industry in terms of time, money and resources (Thakral et al., 2025). Additionally, establishing a reliable supply chain for a novel surfactant is likely to be challenging (Thakral et al., 2025)(Dubey & Giovanni, 2021).

These challenges can inform what is desired in a novel surfactant. Crucially, an alternative surfactant should not be toxic, biologically active, or incompatible with other excipients or manufacturing materials (FDA, 2005). Ideally, the surfactant would have features such as; effectiveness at low concentrations, a well-defined structure, relatively easy synthesis, reliable analytical characterisation, resistance to degradation and a similar HLB to current surfactants (Prossnitz et al., 2025)(Cuzuzza et al., 2024)(Dubey & Giovanni, 2021). A surfactant's effectiveness relies on its interfacial dynamics, however faster interfacial dynamics than current surfactants is not necessarily indicative of a better surfactant. For example, while VEDG-3.3 and VEDS were slower to adsorb to the air-liquid interface compared to PS, P188 and Brij, this did not correlate to a lower protective ability. This was likely due to alternative mechanisms of action and increased molecular rearrangement at the interface, resulting in lower reversibility (Peters et al., 2025). However, this feature should be considered holistically as surfactants should have a degree of quick desorption ability, which is important for stress conditions such as agitation where new interfaces are continuously refreshed (Hanson et al., 2020).

Hence, how surfactants interact with the protein and manufacturing and packaging materials is important in developing novel surfactants. Combined with the current lack of fundamental understanding of how surfactants stabilise surfactants, this explains why there is an interest in modular and tunable surfactants (Prossnitz et al., 2025). A modular structure could lead to more structurally well-defined surfactants, offering improvement on the heterogeneity of current surfactants and potentially reduce analytical challenges. Modular surfactants could lead to better prediction of protein stability and easier elucidation of structure-function relationships (Cucuzza et al., 2024). Tunability of the surfactant may enable the surfactant to be adapted to different types of mAbs and modalities. Overall, this predictive ability could accelerate approval of a new surfactant (Cucuzza et al., 2024).

Studies have been done to elucidate the structure-function relationship of surfactants by changing the type and length of the hydrophobic and hydrophilic components, as well as adding linkers. Hanson et al (2020), studied the effect of FM1000's hydrophobic tail length on protein stabilisation. By studying the behaviour of tails between 8-18 carbons, it was found that a tail of 14 carbons was optimal for reducing aggregation due to its faster interfacial dynamics (Hanson et al., 2020). The authors suggest that a short tail may not be sufficiently hydrophobic, while a longer tail may be too slow to adsorb due to rearranging on the surface, resulting in coadsorption with mAbs. Cucuzza et al, (2024), systemically analysed three types of novel surfactant (VEDG/VEDS, Brij and SPGS) and the impact of changing their components had on interfacial behaviour, protein interactions and *in vitro* safety. The hydrophobic groups investigated included Vitamin E, an alkyl chain and beta-sitosterol (belonging to VEDG/VEDS, Brij and SPGS respectively). Similar to Hanson et al (2020), they found that the hydrophobic group largely defines the properties of the surfactant. Alkyl chain surfactants were faster to reach the interface than those containing vitamin-E. This aligns with findings from Peters et al (2025), where modified Brij-58 displayed faster interfacial dynamics than VEDG/VEDS. Changing the length of the hydrophobic PEG tail, revealed that while shorter tails were slower to migrate to the interface, they exhibited increased hydrophobic interactions with BSA (Cucuzza et al., 2024). However, the authors point out that PEG should ideally be replaced as a hydrophile in practice due to concerns about immunogenicity and susceptibility to autoxidation (Cucuzza et al., 2024)(Katz et al., 2022). Overall, clear structure-function relationships and tunability, could enable the rational design of novel surfactants and holds promise for developing more stable and safer biopharmaceuticals (Prozznitz et al., 2025).

5. Selection of Surfactants

A critical step in developing a therapeutic protein is formulation development (Thakral et al., 2025). As surfactants are a key excipient in maintaining therapeutic protein stability, their selection is essential for assuring safe and efficacious formulations. The choice of a surfactant will depend on knowledge of the therapeutic protein, the route of administration, dosage form, excipients, container closure systems and the patient population (Wen et al., 2025). Since the effectiveness of a surfactant is highly protein-dependent, the process of selecting a surfactant will begin with a thorough understanding of the protein. This evaluation includes a protein's size, charge, structural, solubility, homogeneity, hydrophobicity (spatial aggregation propensity (SAP) map), conformational and colloidal stability (Downey et al., 2025)(Wen et al., 2025). MAb products can vary in their interfacial stability and propensity to aggregate in a way that is often hard to predict. This is exacerbated in other modalities, particularly ADCs, due to its lower colloidal stability and higher aggregation propensity (Wen et al., 2025). For example, ADCs tend to be more unstable than conventional mAbs and may be more sensitive to syringe handling (Fukuda et al., 2024). In terms of formulation and delivery of the mAb product, its concentration, viscosity, dosage form and packaging should be considered and optimised. Furthermore, storage conditions, temperature, impurities (e.g. HCPs) and mechanical stress impacts mAb product stability and can cause particle formation. This is achieved by characterisation through stability studies (Morales et al., 2022).

During formulation, when the surfactant is added, small-scale studies are used to model stress conditions that the formulation may encounter during manufacture, shipping, storage, and administration. Multiple stress conditions are recommended to determine the aggregation propensity of the mAb product and the protective ability of the surfactant at various concentrations (Dasnoy et al., 2024). These could include, shaking, agitation, stirring and aeration, with shaking being the most widely used stress in these studies (Morales et al., 2022). Orbital shaking and horizontal shaking are used, with horizontal shaking able to increase the surface area of air-liquid and increased contact with the stopper (Dasnoy et al., 2024). These studies enable the determination of the type and concentration that is optimal for the formulation (Morales et al., 2022). Concentration is determined with the CMC in mind, and may fall between 0.003% - 0.8% (w/v) (Dasnoy et al., 2024). The ability of surfactants to protect interfaces is dependent on various factors, such as the surfactant concentration, grade, degradation propensity, the mAb products' interfacial properties and external conditions (e.g. light, temperature). Due to their relatively high resistance to unfolding compared to larger, more unstable proteins, mAbs may require smaller concentrations of surfactant (Kanthe et al., 2020)(Waters et al., 2023). This could also apply to differences in non-canonical mAb format stability, with more unstable, surface active or hydrophobic mAb products possibly requiring more polysorbate (e.g ADC). For instance, the same level of PS80 has been seen not being as effective for an Fc-fusion protein compared to a mAb in mitigating particle formation (Griffin et al., 2024). However, this is protein dependent and must be determined on a case-by-case basis. Additionally, the grade of polysorbate can affect effectiveness and particle formation, depending on the impurity profile of the mAb. For example, SR PS20 and HP PS20 undergo different degradation and levels of particle formation depending on enzyme-specificity (Doshi et al 2020). SR PS80 may provide better interfacial protection for a less stable mAb, although this is likely more mAb dependent (Vaclaw et al., 2021).

The route of administration is a critical factor in surfactant selection. Surfactants used in parenteral formulations are subject to rigorous regulatory oversight due to their potential impact on product quality, safety, and stability. Since the risk is so high associated with parenteral administration, a careful risk assessment is required (Salunke et al., 2024). Surfactants must conform to relevant specifications for purity, strength and quality and must be qualified according to Good Manufacturing Practices (GMP) (Kamerzell et al., 2011). This includes raw material control (lot-to-lot), process-related impurity assessments (e.g HCPs, metals, peroxides), degradation profiling, and compatibility testing with packaging components (e.g vials, rubber stoppers, delivery devices) (Kamerzell et al., 2011)(Narhi et al., 2022)(**Table. 3**). Lot-to-lot variation is particularly critical considering the heterogeneity of PS and P188 (Soeda et al., 2022). Additionally, polysorbate raw material can contain impurities, such as unesterified PEO sorbitan derivatives (Doshi et al., 2020).

Surfactants should be able to withstand manufacturing processes at various interfaces, maintain functionality throughout its shelf-life, not affect the drug product or interact with the container or other excipients (IPEC Federation, 2021). Container closure system is another important consideration when selecting a surfactant, as it must be compatible with both the protein and surfactant. Hence, small-scale studies should be done to ensure surfactant and protein stability is unaffected by the materials such as vials, rubber stoppers, prefilled syringes, needles, autoinjectors (Narhi et al., 2022). However, it is worth noting that model surfaces used in many studies are not necessarily representative of medical materials and may yield different results (Jimenez et al., 2024). Additionally, the effect of leachables and stress on surfactant degradation should be characterised to establish the possible effect on critical quality attributes (CQAs) of the formulation, which can be done via accelerated degradation studies (Salnuke et al., 2024). Another consideration is the order when the surfactant is added, which should ideally be before or during mAb product addition. This ensures full saturation of the interface and avoids the potential maturation of the mAb product film, which has been linked to lower surfactant effectiveness at desorbing mAbs (Hingst et al., 2025)(Zuntar et al., 2022).

Choice of surfactant can also be influenced by the target population. The quality attributes of an excipient differ in pediatric populations compared to adults, with safety and tolerability being of greater focus (Salunke et al., 2024). Since pediatric populations have underdeveloped kidneys and livers, they can be particularly sensitive to excipients, with high levels often associated with adverse events (Belayneh et al., 2020)(Salunke et al., 2024). This problem is compounded by off-label use, limited safety data, particularly for neonates, and no industry-wide accepted limits (Kriegel et al., 2020). Polysorbate 80 is considered to be one of the more toxic excipients in pediatric formulations and lacks an established safety profile (Rouaz et al., 2021)(Kriegel et al., 2020). Polysorbates have been linked to serious adverse events, such as cardiovascular diseases, hepatotoxicity, hypertension and even death (Chen et al., 2016)(Bove, 1985)(Saito et al., 2024). This includes the E-ferol incident in the 1980s, where the interaction between vitamin E and mixture of polysorbates caused deaths in neonates (Bove., et al 1985).

To address these risks, other than considering polysorbate-free formulations, predictive tools such as the progressive pediatric safety factor (PPSF) have been proposed that estimate safe dosage based on developmental stage (Kriegel et al., 2020). Standardised assessment tools concerning excipients more broadly, such as Pediatric Excipients Risk Assessment (PERA), aim to improve pediatric formulation safety (Saito et al., 2025). Despite their limitations, polysorbates will continue to be used in pediatric formulation, depending on a risk-benefit evaluation, especially as there are currently no universal alternatives (Rouaz et al., 2021)(Salunke et al., 2024). Novel excipients are not advised as a first choice in pediatric formulations due to their lack of safety data in adults (Kozarewicz & Loftsson, 2018).

When developing a novel surfactant, history of use and route of administration should be considered, along with in-depth studies on mechanisms of action, safety and compatibility with manufacturing processes and packaging (Dubey & Giovanni, 2021). This information will inform a risk-benefit evaluation (Dubey & Giovanni, 2021). Brij, FM1000 and VEDG/VEDS demonstrate promise as alternative surfactants, with protein stabilisation comparable to polysorbates and P188 while having lower propensity to degradation. This could be an advantage for mAb formulations containing high levels of esterases, particularly compared to PS20 as it is more sensitive to hydrolysis than PS80 (Katz et al., 2019). More of these comparative studies to PS and P188 are required to gain a better understanding of the mechanisms of stability and degradation propensity. Additionally, these surfactants will have low lot-to-lot variation due to their synthesis method, which makes them particularly attractive against current surfactants (Prossnitz et al., 2025). Similar to PS and P188, there is currently lack of studies on solid interface compatibility compared to air-liquid interface, however, some of these studies discussed demonstrate compatibility with solid surfaces such as silicone, cellulose and COC, albeit to varying degrees (Katz et al., 2019)(Zurcher et al., 2023). Additionally, more conditions (pH, buffer, ionic strength) and higher concentrations should be studied. For instance, many of these studies discussed (and indeed PS and P188 studies) use low concentrations of mAb product formulations (Peters et al., 2025). While low mAb concentrations (0.5 mg/ml) can fully saturate the surface, bulk concentration has been seen to influence viscosity, colloidal stability and competition between the proteins and surfactants (Katz et al., 2019)(Hingst et al., 2025)(Escobar et al., 2024). While there has been an increase in studies investigating different modalities (Fc-fusion, BsAbs, Fab fragments), historically there has been a lack and therefore continued research into a wider range of proteins would be beneficial, particularly with ADCs, which have yet to be investigated with these novel surfactants. While promising, currently it remains uncertain if these alternative surfactants discussed will be able to replace polysorbates.

Surfactants are essential components for stabilising mAb product formulations. However due to issues concerning their stability and heterogeneity, a careful case-by-case approach is used when developing a formulation, particularly in pediatric populations. Novel surfactants may improve these areas, however, they pose challenges, such as complex regulatory pathways and time-consuming and expensive toxicology studies. More tunable products may be where the industry moves towards as it comes with many benefits, such as well-defined structure and rational design of highly stable formulations. Despite this, polysorbates and poloxamers will continue to be used, using extensive modelling studies, mitigation methods, careful risk-benefit evaluations and a case-by-case approach in order to ensure the formulation of safe and efficacious biopharmaceuticals.

Figures and Tables

Trade name	Active ingredient	Company	Type	Surfactant
Keytruda	Pembrolizumab	Merck & Co.	mAb	Polysorbate 80
Humira	Adalimumab	Abbvie	mAb	Polysorbate 80
Dupixent	Dupilumab	Sanofi/Regeneron	mAb	Polysorbate 80
Stelara	Ustekinumab	Johnson & Johnson	mAb	Polysorbate 80
Opdivo	Nivolumab	Bristol Myers Squibb/Ono	mAb	Polysorbate 80
Skyrizi	Risankizumab	AbbVie	mAb	Polysorbate 20
Eylea	Aflibercept	Regeneron and Bayer	Fc-fusion	Polysorbate 20
Enbrel	Etancercept	Amgen	Fc-fusion	None
Remicade	Infliximab	Johnson & Johnson	mAb	Polysorbate 80

Rituxan	Rituximab	Genetech	mAb	Polysorbate 80

Table 1. Top selling mAb-derived drugs 2019, 2023, 2024 (Verdin, 2024)(Verdin, 2025)(Strickley & Lambert, 2021) Surfactant formulation information obtained from (Drugs@FDA: FDA-Approved Drugs, n.d.).

Trade name	**Active ingredient**	**Company**	**Type**	**Surfactant**
Alhemo	Concizumab-mtci	Novo Nordisk	mAb	Polysorbate 80
Unloxcyt	Cosibelimab-ipdl	Checkpoint Therapeutics	mAb	Polysorbate 80
Bizengri	Zenocutuzumab-zbco	Merus	BsAb	Polysorbate 20
Ziihera	Zanidatamab-hrii	Jazz Pharmaceuticals	BsAb	Polysorbate 20
Vyloy	Zolbetuximab-clzb	Astellas	mAb	Polysorbate 80
Hympavzi	Marstacimab-hncq	Pfizer	mAb	Polysorbate 80
Niktimvo	Axatilimab-csfr	Incyte	mAb	Polysorbate 80
Nemluvio	Nemolizumab-ilto	Galderma	mAb	Poloxamer 188
Kisunla	Donanemab-azbt	Eli Lilly	mAb	Polysorbate 80
Piasky	Crovalimab-akkz	Genetech	mAb	Poloxamer 188
Imdelltra	Tarlatamab-dlle	Amgen	BsAb	Polysorbate 80
Anktiva	Nogapendekin alfa inbakicept-pmln	ImmunityBio	Fc-fusion	None
Winrevair	Sotatercept-csrk	Merck	Fc-fusion	Polysorbate 80
Tevimbra	Tislelizumab-jsgr	BeOne Medicines	mAb	Polysorbate 20

Table 2. Approved novel mAb-derived products FDA 2024 (Novel drug approvals for 2024 FDA, 2025).

Manufacturing step	Parameter to control
Raw material qualification	Storage
	Temperature
	Light
	Heterogeneity
	Compendial vs non-compendial testing
Manufacture	Process related impurities (HCPs, oxidants, solvents, heavy metals, acids, peroxide)
	Choice of container
Release testing	Control of mAb product CQAs
	Surfactant CQAs (Purity %, free FA content)
Stability testing	Characterisation of degradation profile
	Stability indicating assays

Table 3. Key quality considerations for surfactants across the mAb product lifecycle.
Parameters that require control during raw material qualification, manufacturing, release testing, and stability testing are shown. These include both general excipient controls (e.g., storage, temperature, light exposure, container choice) and surfactant-specific critical quality attributes (CQAs) such as purity, free fatty acid content, and degradation profiles. Adapted from (Rao et al., 2020) and sources therein.

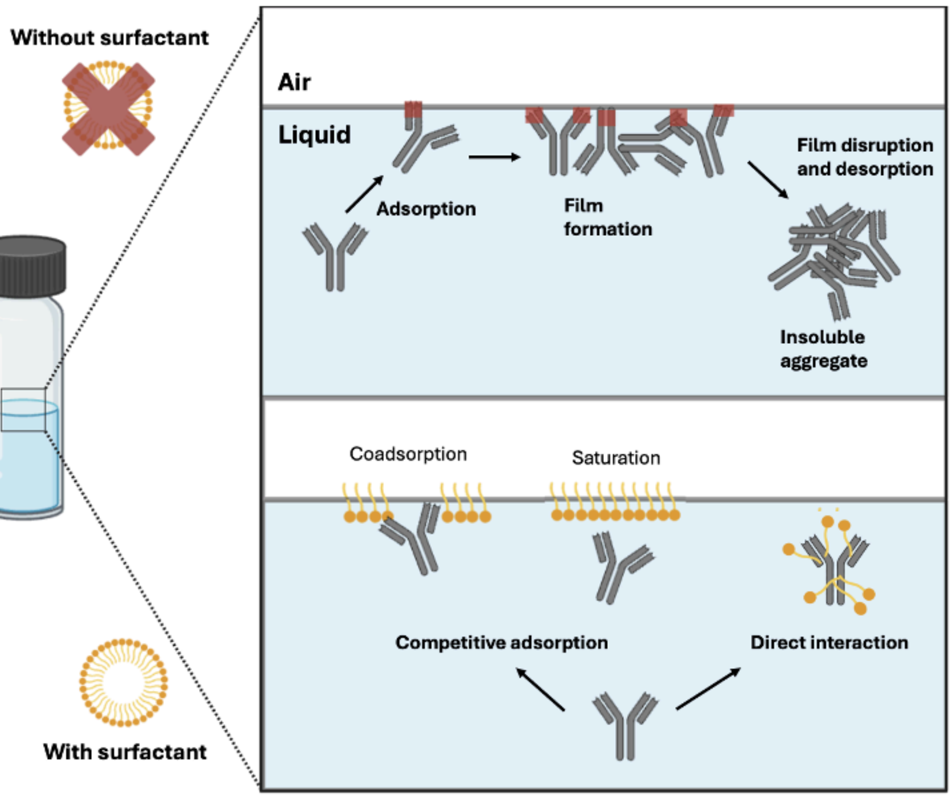

Figure 1. mAb aggregation and surfactant action at an air-liquid interface. Without a non-ionic surfactant, mAb aggregation process involves adsorption to the air-liquid interface (e.g. headspace in a vial), formation of a viscoelastic film via intermolecular interactions (random orientations shown), film disruption, formation of an aggregate and the eventual generation of sub-visible and visible particles (not shown). Red highlight on mAbs indicates exposure of hydrophobic residues and partial unfolding at the interface. When a non-ionic surfactant is added, it can directly interact with the mAb and/or competitively adsorb, which can result in coadsorption or complete dominance of the interface depending on the surfactant concentration, mAb product, stress and order that surfactant was added (not to scale) (Created in Biorender.com).

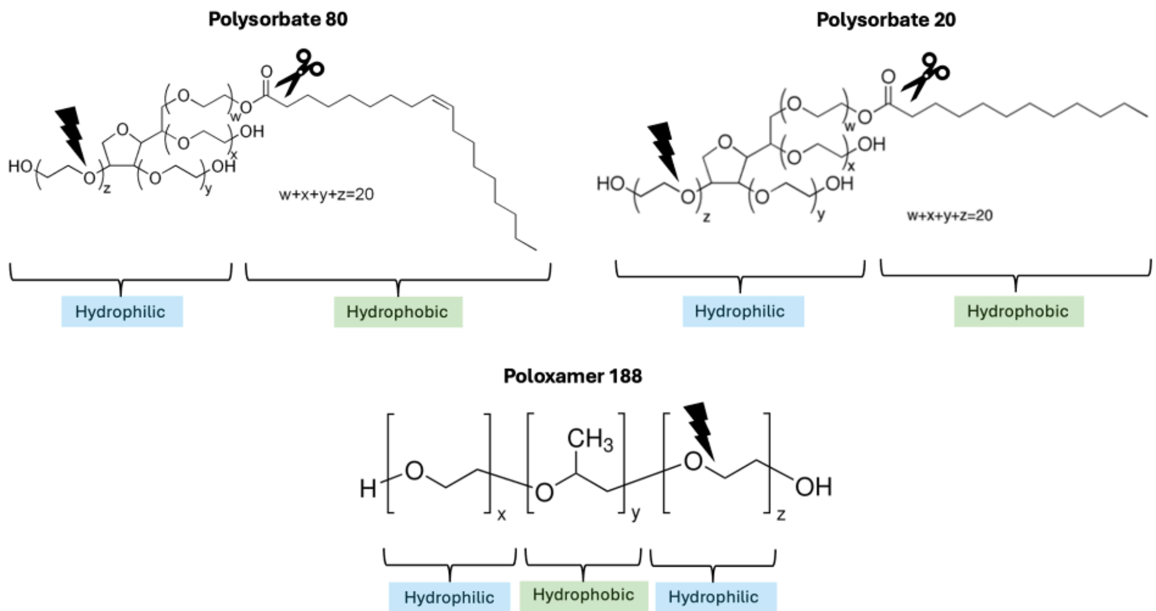

Figure 2. **Polysorbate 80, Polysorbate 20 and Poloxamer 188 structure**. Scissors represent hydrolysis sites at the ester bonds in PS20 and PS80. Bolts represent one of the possible sites of oxidation in PS80, PS20 and P188. Hydrophilic (blue) and hydrophobic (green) portions of each surfactant are indicated. (Images are in the public domain).

Acknowledgements

I would like to thank Nicholas Darton, Sarah Mansfield-Ford and Stephen Harding.

References

Agarkhed, M., O'Dell, C., Hsieh, M., Zhang, J., Goldstein, J., & Srivastava, A. (2013). Effect of Polysorbate 80 Concentration on Thermal and Photostability of a Monoclonal Antibody. *AAPS PHARMSCITECH*, *14*(1), 1-9. https://doi.org/10.1208/s12249-012-9878-0

Agrawal, N., Kumar, S., Wang, X., Helk, B., Singh, S., & Trout, B. (2011). Aggregation in Protein-Based Biotherapeutics: Computational Studies and Tools to Identify Aggregation-Prone Regions. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *100*(12), 5081-5095. https://doi.org/10.1002/jps.22705

Aguirre-Ramírez, M., Silva-Jiménez, H., Banat, I. M., & Díaz De Rienzo, M. A. (2021). Surfactants: physicochemical interactions with biological macromolecules. *Biotechnol Lett*, *43*(3), 523-535. https://doi.org/10.1007/s10529-020-03054-1

Akbarian, M., & Chen, S. H. (2022). Instability Challenges and Stabilization Strategies of Pharmaceutical Proteins. *Pharmaceutics*, *14*(11). https://doi.org/10.3390/pharmaceutics14112533

Arsiccio, A., & Pisano, R. (2020). The Ice-Water Interface and Protein Stability: A Review. *J Pharm Sci*, *109*(7), 2116-2130. https://doi.org/10.1016/j.xphs.2020.03.022

Aryal, B., Lehtimaki, M., & Rao, V. (2024). Stress-mediated polysorbate 20 degradation and its potential impact on therapeutic proteins. *PHARMACEUTICAL RESEARCH*, *41*(6), 1217-1232. https://doi.org/10.1007/s11095-024-03700-7

Bee, J. S., Randolph, T. W., Carpenter, J. F., Bishop, S. M., & Dimitrova, M. N. (2011). Effects of surfaces and leachables on the stability of biopharmaceuticals. *J Pharm Sci*, *100*(10), 4158-4170. https://doi.org/10.1002/jps.22597

Belayneh, A., Tadese, E., & Molla, F. (2020). Safety and Biopharmaceutical Challenges of Excipients in Off-Label Pediatric Formulations. *INTERNATIONAL JOURNAL OF GENERAL MEDICINE*, *13*, 1051-1066. https://doi.org/10.2147/IJGM.S280330

Bollenbach, L., Buske, J., Mäder, K., & Garidel, P. (2022). Poloxamer 188 as surfactant in biological formulations - An alternative for polysorbate 20/80? *Int J Pharm*, *620*, 121706. https://doi.org/10.1016/j.ijpharm.2022.121706

Bollenbach, L., Trutschel, M.-L., Gröger, S., Garidel, P., & Mäder, K. (2025). Interfacial and self-association behaviour of poloxamer 188 in aqueous solutions. *Journal of Molecular Liquids*, *424*, 127119. https://doi.org/https://doi.org/10.1016/j.molliq.2025.127119

BOVE, K., KOSMETATOS, N., WEDIG, K., FRANK, D., WHITLATCH, S., SALDIVAR, V.,…BALISTRERI, W. (1985). VASCULOPATHIC HEPATOTOXICITY ASSOCIATED WITH E-FEROL SYNDROME IN LOW-BIRTH-WEIGHT INFANTS. *JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION*, *254*(17), 2422-2430. https://doi.org/10.1001/jama.254.17.2422

Brosig, S., Cucuzza, S., Serno, T., Bechtold-Peters, K., Buecheler, J., Zivec, M.,…Gallou, F. (2023). Not the Usual Suspects: Alternative Surfactants for Biopharmaceuticals. *ACS Appl Mater Interfaces*, *15*(29), 34540-34553. https://doi.org/10.1021/acsami.3c05610

Brovc, E., Mravljak, J., Sink, R., & Pajk, S. (2020). Rational design to biologics development: The polysorbates point of view. *INTERNATIONAL JOURNAL OF PHARMACEUTICS*, *581*, Article 119285. https://doi.org/10.1016/j.ijpharm.2020.119285

Butreddy, A., Janga, K. Y., Ajjarapu, S., Sarabu, S., & Dudhipala, N. (2021). Instability of therapeutic proteins - An overview of stresses, stabilization mechanisms and analytical techniques involved in lyophilized proteins. *Int J Biol Macromol*, *167*, 309-325. https://doi.org/10.1016/j.ijbiomac.2020.11.188

Chang, D., Fox, R., Hicks, E., Ferguson, R., Chang, K., Osborne, D.,…Velev, O. (2017). Investigation of interfacial properties of pure and mixed poloxamers for surfactant-mediated shear protection of mammalian cells. *COLLOIDS AND SURFACES B-BIOINTERFACES*, *156*, 358-365. https://doi.org/10.1016/j.colsurfb.2017.05.040

Chen, C., & Wu, C. (2016). Acute Hepatotoxicity of Intravenous Amiodarone: Case Report and Review of the Literature. *AMERICAN JOURNAL OF THERAPEUTICS*, *23*(1), E260-E263. https://doi.org/10.1097/MJT.0000000000000149

Chen, W., Klemm, D., Gregoritza, K., Kishore, R., Stracke, J., Wurth, C.,…Oltra, N. (2023). Screening techniques for monitoring the sub-visible particle formation of free fatty acids in biopharmaceuticals. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *190*, 242-247. https://doi.org/10.1016/j.ejpb.2023.07.018

Chen, W., Stolz, S., Wegbecher, V. et al. The degradation of poloxamer 188 in buffered formulation conditions. AAPS Open 8, 5 (2022). https://doi.org/10.1186/s41120-022-00055-4

Cheng, Z., Wang, P., Liu, L., Chen, Q., & Guo, J. (2024). Comparative analysis and mechanistic insights into polysorbate 80 stability differences in biopharmaceutical buffer systems. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *205*, Article 114521. https://doi.org/10.1016/j.ejpb.2024.114521

Chernysheva, M., Shnitko, A., Soboleva, O., & Badun, G. (2018). Competitive adsorption of lysozyme and non-ionic surfactants (Brij-35 and pluronic P123) from a mixed solution at water-air and water-xylene interfaces. *COLLOID AND POLYMER SCIENCE*, *296*(1), 223-232. https://doi.org/10.1007/s00396-017-4240-4

Couston, R., Skoda, M., Uddin, S., & van der Walle, C. (2013). Adsorption behavior of a human monoclonal antibody at hydrophilic and hydrophobic surfaces. *MABS*, *5*(1), 126-139. https://doi.org/10.4161/mabs.22522

Crescioli, S., Kaplon, H., Wang, L., Visweswaraiah, J., Kapoor, V., & Reichert, J. M. (2025). Antibodies to watch in 2025. *MAbs*, *17*(1), 2443538. https://doi.org/10.1080/19420862.2024.2443538

Cucuzza, S., Brosig, S., Serno, T., Bechtold-Peters, K., Cerar, J., Kammüller, M., & Gallou, F. (2024). Modular and tunable alternative surfactants for biopharmaceuticals provide insights into Surfactant's Structure-Function relationship.

INTERNATIONAL JOURNAL OF PHARMACEUTICS, *650*, Article 123692. https://doi.org/10.1016/j.ijpharm.2023.123692

Das, T. K., Chou, D. K., Jiskoot, W., & Arosio, P. (2022). Nucleation in Protein Aggregation in Biotherapeutic Development: A look into the Heart of the Event. *J Pharm Sci*, *111*(4), 951-959. https://doi.org/10.1016/j.xphs.2022.01.017

Dash, R., & Rathore, A. (2021). Freeze thaw and lyophilization induced alteration in mAb therapeutics: Trastuzumab as a case study. *JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS*, *201*, Article 114122. https://doi.org/10.1016/j.jpba.2021.114122

Dasnoya, S., Illartina, M., Queffeleca, J., Nkunkua, A., & Peerbooma, C. (2024). Combined Effect of Shaking Orbit and Vial Orientation on the Agitation-Induced Aggregation of Proteins. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *113*(3), 669-679. https://doi.org/10.1016/j.xphs.2023.08.016

de la Torre, B. G., & Albericio, F. (2025). The Pharmaceutical Industry in 2024: An Analysis of the FDA Drug Approvals from the Perspective of Molecules. *Molecules*, *30*(3). https://doi.org/10.3390/molecules30030482

Dixit, N., Salamat-Miller, N., Salinas, P. A., Taylor, K. D., & Basu, S. K. (2016). Residual Host Cell Protein Promotes Polysorbate 20 Degradation in a Sulfatase Drug Product Leading to Free Fatty Acid Particles. *J Pharm Sci*, *105*(5), 1657-1666. https://doi.org/10.1016/j.xphs.2016.02.029

Doshi, N., Demeule, B., & Yadav, S. (2015). Understanding Particle Formation: Solubility of Free Fatty Acids as Polysorbate 20 Degradation Byproducts in Therapeutic Monoclonal Antibody Formulations. *Mol Pharm*, *12*(11), 3792-3804. https://doi.org/10.1021/acs.molpharmaceut.5b00310

Doshi, N., Fish, R., Padilla, K., & Yadav, S. (2020). Evaluation of Super Refined™ Polysorbate 20 With Respect to Polysorbate Degradation, Particle Formation and Protein Stability. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *109*(10), 2986-2995. https://doi.org/10.1016/j.xphs.2020.06.030

Downey, J. D., Crean, A. M., & Ryan, K. B. (2025). Impact of protein adsorption during biopharmaceutical manufacture & storage. *Eur J Pharm Sci*, *209*, 107071. https://doi.org/10.1016/j.ejps.2025.107071

Drugs@FDA: FDA-Approved drugs. (n.d.). FDA. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm

Dubey, S., & Giovannini, R. (2021). Stability of Biologics and the Quest for Polysorbate Alternatives. *Trends Biotechnol*, *39*(6), 546-549. https://doi.org/10.1016/j.tibtech.2020.10.007

Dwivedi, M., Blech, M., Presser, I., & Garidel, P. (2018). Polysorbate degradation in biotherapeutic formulations: Identification and discussion of current root causes.

INTERNATIONAL JOURNAL OF PHARMACEUTICS, *552*(1-2), 422-436. https://doi.org/10.1016/j.ijpharm.2018.10.008

Dwivedi, M., Buske, J., Haemmerling, F., Blech, M., & Garidel, P. (2020). Acidic and alkaline hydrolysis of polysorbates under aqueous conditions: Towards understanding polysorbate degradation in biopharmaceutical formulations. *EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES*, *144*, Article 105211. https://doi.org/10.1016/j.ejps.2019.105211

Emanuele, M., Balasubramaniam, B. Differential Effects of Commercial-Grade and Purified Poloxamer 188 on Renal Function. Drugs R D 14, 73–83 (2014). https://doi.org/10.1007/s40268-014-0041-0

Erlandsson, B. (2002). Stability-indicating changes in poloxamers:: the degradation of ethylene oxide-propylene oxide block copolymers at 25 and 40 °C. *POLYMER DEGRADATION AND STABILITY*, *78*(3), 571-575, Article PII S0141-3910(02)00233-1. https://doi.org/10.1016/S0141-3910(02)00233-1

Escobar, E., Griffin, V., & Dhar, P. (2024). Correlating Surface Activity with Interface-Induced Aggregation in a High-Concentration mAb Solution. *MOLECULAR PHARMACEUTICS*, *21*(3), 1490-1500. https://doi.org/10.1021/acs.molpharmaceut.3c01125

Fang, W. J., Ingle, R. G., Liu, J. W., Ge, X. Z., & Wang, H. (2022). Freeze-Dried Monoclonal Antibody Formulations are Unexpectedly More Prone to Degradation Than Liquid Formulations Under Shaking Stress. *J Pharm Sci*, *111*(7), 2134-2138. https://doi.org/10.1016/j.xphs.2022.03.002

Fares, H., Carnovale, M., Tabouguia, M., Jordan, S., & Katz, J. (2023). Novel Surfactant Compatibility with Downstream Protein Bioprocesses. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *112*(7), 1811-1820. https://doi.org/10.1016/j.xphs.2023.04.011

FDA (2022, September 14). Pilot Program for the Review of Innovation and Modernization of Excipients (PRIME). U.S. Food And Drug Administration. https://www.fda.gov/drugs/development-approval-process-drugs/pilot-program-review-innovation-and-modernization-excipients-prime

Fujita, R., Nagatoishi, S., Adachi, S., Nishioka, H., Ninomiya, H., Kaya, T.,…Tsumoto, K. (2019). Control of Protein Adsorption to Cyclo Olefin Polymer by the Hofmeister Effect. *J Pharm Sci*, *108*(5), 1686-1691. https://doi.org/10.1016/j.xphs.2018.12.023

Fukuda, M., Nagae, S., Takarada, T., Noda, S., Morita, S., & Tanaka, M. (2025). Potential risk factors of protein aggregation in syringe handling during antibody drug dilution for intravenous administration. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *114*(3), 1625-1638. https://doi.org/10.1016/j.xphs.2024.12.029

Gamage, C. L. D., Weis, D. D., & Walters, B. T. (2022). Identification of Agitation-Induced Unfolding Events Causing Aggregation of Monoclonal Antibodies Using Hydrogen Exchange-Mass Spectrometry. *J Pharm Sci*, *111*(8), 2210-2216. https://doi.org/10.1016/j.xphs.2022.05.002

Garidel, P., Hoffmann, C., & Blume, A. (2009). A thermodynamic analysis of the binding interaction between polysorbate 20 and 80 with human serum albumins and immunoglobulins: A contribution to understand colloidal protein stabilisation. *BIOPHYSICAL CHEMISTRY*, *143*(1-2), 70-78. https://doi.org/10.1016/j.bpc.2009.04.004

Ghosh, I., Gutka, H., Krause, M., Clemens, R., & Kashi, R. (2023). A systematic review of commercial high concentration antibody drug products approved in the US: formulation composition, dosage form design and primary packaging considerations. *MABS*, *15*(1), Article 2205540. https://doi.org/10.1080/19420862.2023.2205540

Grabarek, A., Bozic, U., Rousel, J., Menzen, T., Kranz, W., Wuchner, K.,…Hawe, A. (2020). What Makes Polysorbate Functional? Impact of Polysorbate 80 Grade and Quality on IgG Stability During Mechanical Stress. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *109*(1), 871-880. https://doi.org/10.1016/j.xphs.2019.10.015

Grapentin, C., Müller, C., Kishore, R. S. K., Adler, M., ElBialy, I., Friess, W.,…Khan, T. A. (2020). Protein-Polydimethylsiloxane Particles in Liquid Vial Monoclonal Antibody Formulations Containing Poloxamer 188. *J Pharm Sci*, *109*(8), 2393-2404. https://doi.org/10.1016/j.xphs.2020.03.010

Gregoritza, K., Cai, S., Siketanc, M., Woehr, A., Lebouc, V., Kishore, R.,…Allmendinger, A. (2022). Metal-Induced Fatty Acid Particle Formation Resulting from Hydrolytic Polysorbate Degradation. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *111*(3), 743-751. https://doi.org/10.1016/j.xphs.2021.09.044

Gregoritza, K., Theodorou, C., Heitz, M., Graf, T., Germershaus, O., & Gregoritza, M. (2024). Enzymatic degradation pattern of polysorbate 20 impacts interfacial properties of monoclonal antibody formulations. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *194*, 74-84. https://doi.org/10.1016/j.ejpb.2023.11.024

Griffin, V., Escobar, E., Ogunyankin, M., Kanthe, A., Gokhale, M., & Dhar, P. (2024). Correlating Differences in the Surface Activity to Interface-Induced Particle Formation in Different Protein Modalities: IgG mAb Versus Fc-Fusion Protein. *MOLECULAR PHARMACEUTICS*, *21*(10), 5088-5103. https://doi.org/10.1021/acs.molpharmaceut.4c00488

Hall, T., Sandefur, S., Frye, C., Tuley, T., & Huang, L. (2016). Polysorbates 20 and 80 Degradation by Group XV Lysosomal Phospholipase A2 Isomer X1 in Monoclonal

Antibody Formulations. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *105*(5), 1633-1642. https://doi.org/10.1016/j.xphs.2016.02.022

Hanson, M., Katz, J., Ma, H., Putterman, M., Yezer, B., Petermann, O., & Reineke, T. (2020). Effects of Hydrophobic Tail Length Variation on Surfactant-Mediated Protein Stabilization. *MOLECULAR PHARMACEUTICS*, *17*(11), 4302-4311. https://doi.org/10.1021/acs.molpharmaceut.0c00737

Höger, K., Mathes, J., & Friess, W. (2015). IgG1 Adsorption to Siliconized Glass Vials-Influence of pH, Ionic Strength, and Nonionic Surfactants. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *104*(1), 34-43. https://doi.org/10.1002/jps.24239

Jiménez, R., Maze, A., Bruckert, F., Bensaid, F., El-Kechai, N., & Weidenhaupt, M. (2024). Evaluating surfactant effectiveness in preventing antibody adsorption directly on medical surfaces using a novel device. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *205*, Article 114539. https://doi.org/10.1016/j.ejpb.2024.114539

Jin, M. J., Ge, X. Z., Huang, Q., Liu, J. W., Ingle, R. G., Gao, D., & Fang, W. J. (2024). The Effects of Excipients on Freeze-dried Monoclonal Antibody Formulation Degradation and Sub-Visible Particle Formation during Shaking. *Pharm Res*, *41*(2), 321-334. https://doi.org/10.1007/s11095-024-03657-7

Johann, F., Wöll, S., & Gieseler, H. (2024). "Negative" Impact: The Role of Payload Charge in the Physicochemical Stability of Auristatin Antibody-Drug Conjugates. *J Pharm Sci*, *113*(8), 2433-2442. https://doi.org/10.1016/j.xphs.2024.04.023

Jones, M. T., Mahler, H. C., Yadav, S., Bindra, D., Corvari, V., Fesinmeyer, R. M.,…Singh, S. K. (2018). Considerations for the Use of Polysorbates in Biopharmaceuticals. *Pharm Res*, *35*(8), 148. https://doi.org/10.1007/s11095-018-2430-5

Kamerzell, T., Esfandiary, R., Joshi, S., Middaugh, C., & Volkin, D. (2011). Protein-excipient interactions: Mechanisms and biophysical characterization applied to protein formulation development. *ADVANCED DRUG DELIVERY REVIEWS*, *63*(13), 1118-1159. https://doi.org/10.1016/j.addr.2011.07.006

Kannan, A., Shieh, I. C., & Fuller, G. G. (2019). Linking aggregation and interfacial properties in monoclonal antibody-surfactant formulations. *J Colloid Interface Sci*, *550*, 128-138. https://doi.org/10.1016/j.jcis.2019.04.060

Kannan, A., Shieh, I. C., Negulescu, P. G., Chandran Suja, V., & Fuller, G. G. (2021). Adsorption and Aggregation of Monoclonal Antibodies at Silicone Oil-Water Interfaces. *Mol Pharm*, *18*(4), 1656-1665. https://doi.org/10.1021/acs.molpharmaceut.0c01113

Kanthe, A., Carnovale, M., Katz, J., Jordan, S., Krause, M., Zheng, S.,…Tu, R. (2022). Differential Surface Adsorption Phenomena for Conventional and Novel Surfactants Correlates with Changes in Interfacial mAb Stabilization. *MOLECULAR*

PHARMACEUTICS, *19*(9), 3100-3113. https://doi.org/10.1021/acs.molpharmaceut.2c00152

Kanthe, A., Ilott, A., Krause, M., Zheng, S., Li, J., Bu, W.,…Tu, R. S. (2021). No ordinary proteins: Adsorption and molecular orientation of monoclonal antibodies. *Sci Adv*, *7*(35). https://doi.org/10.1126/sciadv.abg2873

Kanthe, A., Krause, M., Zheng, S., Ilott, A., Li, J., Bu, W.,…Tu, R. (2020). Armoring the Interface with Surfactants to Prevent the Adsorption of Monoclonal Antibodies. *ACS APPLIED MATERIALS & INTERFACES*, *12*(8), 9977-9988. https://doi.org/10.1021/acsami.9b21979

Katz, J., Chou, D., Christian, T., Das, T., Patel, M., Singh, S., & Wen, Y. (2022). Emerging Challenges and Innovations in Surfactant-mediated Stabilization of Biologic Formulations. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *111*(4), 919-932. https://doi.org/10.1016/j.xphs.2021.12.002

Katz, J., Nolin, A., Yezer, B., & Jordan, S. (2019). Dynamic Properties of Novel Excipient Suggest Mechanism for Improved Performance in Liquid Stabilization of Protein Biologics. *MOLECULAR PHARMACEUTICS*, *16*(1), 282-291. https://doi.org/10.1021/acs.molpharmaceut.8b00984

Katz, J., Tan, Y., Kuppannan, K., Song, Y., Brennan, D., Young, T.,…Jordan, S. (2016). Amino-Acid-Incorporating Nonionic Surfactants for Stabilization of Protein Pharmaceuticals. *ACS BIOMATERIALS SCIENCE & ENGINEERING*, *2*(7), 1093-1096. https://doi.org/10.1021/acsbiomaterials.6b00245

Khan, T. A., Mahler, H. C., & Kishore, R. S. (2015). Key interactions of surfactants in therapeutic protein formulations: A review. *Eur J Pharm Biopharm*, *97*(Pt A), 60-67. https://doi.org/10.1016/j.ejpb.2015.09.016

Kim, H., McAuley, A., Livesay, B., Gray, W., & McGuire, J. (2014). Modulation of Protein Adsorption by Poloxamer 188 in Relation to Polysorbates 80 and 20 at Solid Surfaces. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *103*(4), 1043-1049. https://doi.org/10.1002/jps.23907

Kim, H., Mcauley, A., & Mcguire, J. (2014). Protein Effects on Surfactant Adsorption Suggest the Dominant Mode of Surfactant-Mediated Stabilization of Protein. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *103*(5), 1337-1345. https://doi.org/10.1002/jps.23908

Koepf, E., Eisele, S., Schroeder, R., Brezesinski, G., & Friess, W. (2018). Notorious but not understood: How liquid-air interfacial stress triggers protein aggregation. *INTERNATIONAL JOURNAL OF PHARMACEUTICS*, *537*(1-2), 202-212. https://doi.org/10.1016/j.ijpharm.2017.12.043

Kopp, M. R. G., Grigolato, F., Zürcher, D., Das, T. K., Chou, D., Wuchner, K., & Arosio, P. (2023). Surface-Induced Protein Aggregation and Particle Formation in Biologics:

Current Understanding of Mechanisms, Detection and Mitigation Strategies. *J Pharm Sci*, *112*(2), 377-385. https://doi.org/10.1016/j.xphs.2022.10.009

Kozarewicz, P., & Loftsson, T. (2018). Novel excipients - Regulatory challenges and perspectives - The EU insight. *INTERNATIONAL JOURNAL OF PHARMACEUTICS*, *546*(1-2), 176-179. https://doi.org/10.1016/j.ijpharm.2018.05.048

Kriegel, C., Festag, M., Kishore, R., Roethlisberger, D., & Schmitt, G. (2020). Pediatric Safety of Polysorbates in Drug Formulations. *CHILDREN-BASEL*, *7*(1), Article 1. https://doi.org/10.3390/children7010001

Latour, R. A. (2020). Fundamental Principles of the Thermodynamics and Kinetics of Protein Adsorption to Material Surfaces. *Colloids Surf B Biointerfaces*, *191*, 110992. https://doi.org/10.1016/j.colsurfb.2020.110992

Le Basle, Y., Chennell, P., Tokhadze, N., Astier, A., & Sautou, V. (2020). Physicochemical Stability of Monoclonal Antibodies: A Review. *J Pharm Sci*, *109*(1), 169-190. https://doi.org/10.1016/j.xphs.2019.08.009

Lee, H., McAuley, A., Schilke, K., & McGuire, J. (2011). Molecular origins of surfactant-mediated stabilization of protein drugs. *ADVANCED DRUG DELIVERY REVIEWS*, *63*(13), 1160-1171. https://doi.org/10.1016/j.addr.2011.06.015

Leiske, D., Shieh, I., & Tse, M. (2016). A Method To Measure Protein Unfolding at an Air-Liquid Interface. *LANGMUIR*, *32*(39), 9930-9937. https://doi.org/10.1021/acs.langmuir.6b02267

Li, J., Sonje, J., & Suryanarayanan, R. (2023). Role of Poloxamer 188 in Preventing Ice-Surface-Induced Protein Destabilization during Freeze-Thawing. *MOLECULAR PHARMACEUTICS*, *20*(9), 4587-4596. https://doi.org/10.1021/acs.molpharmaceut.3c00312

Li, J., Zeng, C., Guan, J., & Suryanarayanan, R. (2024). Effect of surfactants on lactate dehydrogenase aqueous solutions: A comparative study of poloxamer 188, polysorbate 20 and 80. *Int J Pharm*, *661*, 124374. https://doi.org/10.1016/j.ijpharm.2024.124374

Li, Z., Pan, F., Li, R., Pambou, E., Hu, X., Ruane, S.,…Lu, J. (2018). Coadsorption of a Monoclonal Antibody and Nonionic Surfactant at the SiO2/Water Interface. *ACS APPLIED MATERIALS & INTERFACES*, *10*(51), 44257-44266. https://doi.org/10.1021/acsami.8b16832

Ling, J., Du, Y., Buist, N., Krishnamachari, Y., Xi, H., Templeton, A., & Su, Y. (2025). Molecular mechanisms for stabilizing biologics in the solid state. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *114*(2), 736-765. https://doi.org/10.1016/j.xphs.2024.11.017

Lu, X., Domingo-Yenes, B., Cohen, N., & Grzincic, E. (2025). Freezing-induced protein aggregation in a bispecific antibody: Characterization and mechanistic insights. *J Pharm Sci*, 103711. https://doi.org/10.1016/j.xphs.2025.103711

Löw, N., Schneider, C., Zegota, M., Grabarek, A., Corradini, E., Schuster, G.,…Kellermeier, M. (2025). Physicochemical Comparison of Kolliphor HS 15, ELP, and Conventional Surfactants for Antibody Stabilization in Biopharmaceutical Formulations. *MOLECULAR PHARMACEUTICS*, *22*(8), 4890-4908. https://doi.org/10.1021/acs.molpharmaceut.5c00519

Mahler, H., Senner, F., Maeder, K., & Mueller, R. (2009). Surface Activity of a Monoclonal Antibody. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *98*(12), 4525-4533. https://doi.org/10.1002/jps.21776

Mieczkowski, C. (2023). The Evolution of Commercial Antibody Formulations. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *112*(7), 1801-1810. https://doi.org/10.1016/j.xphs.2023.03.026

Moorthy, B. S., Schultz, S. G., Kim, S. G., & Topp, E. M. (2014). Predicting protein aggregation during storage in lyophilized solids using solid state amide hydrogen/deuterium exchange with mass spectrometric analysis (ssHDX-MS). *Mol Pharm*, *11*(6), 1869-1879. https://doi.org/10.1021/mp500005v

Morales, A. M., Sreedhara, A., Buecheler, J., Brosig, S., Chou, D., Christian, T.,…Singh, S. (2022). End-to-End Approach to Surfactant Selection, Risk Mitigation, and Control Strategies for Protein-Based Therapeutics. *AAPS J*, *25*(1), 6. https://doi.org/10.1208/s12248-022-00773-3

Moussa, E. M., Panchal, J. P., Moorthy, B. S., Blum, J. S., Joubert, M. K., Narhi, L. O., & Topp, E. M. (2016). Immunogenicity of Therapeutic Protein Aggregates. *J Pharm Sci*, *105*(2), 417-430. https://doi.org/10.1016/j.xphs.2015.11.002

Narhi, L. O., Chou, D. K., Christian, T. R., Gibson, S., Jagannathan, B., Jiskoot, W.,…Das, T. K. (2022). Stress Factors in Primary Packaging, Transportation and Handling of Protein Drug Products and Their Impact on Product Quality. *J Pharm Sci*, *111*(4), 887-902. https://doi.org/10.1016/j.xphs.2022.01.011

Nonclinical studies for the safety evaluation of pharmaceutical excipients. FDA (2005). In Guidance for Industry [Report]. http://www.fda.gov/cder/guidance/index.htm

Novel drug approvals for 2024. (2025, July 14). U.S. Food And Drug Administration. https://www.fda.gov/drugs/novel-drug-approvals-fda/novel-drug-approvals-2024

Peters, V., Schneider, L., Brosig, S., Medrano, S. M., & Cucuzza, S. (2025). InterFace/Off: characterization of competitive adsorption of novel surfactants and proteins at the solid-liquid and oil-liquid interfaces. *Colloids Surf B Biointerfaces*, *254*, 114865. https://doi.org/10.1016/j.colsurfb.2025.114865

Pham, K. G., Thompson, B. R., Wang, T., Samaddar, S., Qian, K. K., Liu, Y., & Wagner, N. J. (2023). Interfacial Pressure and Viscoelasticity of Antibodies and Their Correlation to Long-Term Stability in Formulation. *J Phys Chem B*, *127*(45), 9724-9733. https://doi.org/10.1021/acs.jpcb.3c05900

Pham, N. B., & Meng, W. S. (2020). Protein aggregation and immunogenicity of biotherapeutics. *Int J Pharm*, *585*, 119523. https://doi.org/10.1016/j.ijpharm.2020.119523

Prossnitz, A., Nguyen, L., Eckman, N., Borkar, S., Tetef, S., Autzen, A.,...Appel, E. (2025). Defining Structure-Function Relationships of Amphiphilic Excipients Enables Rational Design of Ultra-Stable Biopharmaceuticals. *ADVANCED SCIENCE*, *12*(8). https://doi.org/10.1002/advs.202409604

Rao, A., FDA/CDER, & CASSS CMC Strategy Forum 2020. (2020). Excipients and biotechnology product quality attributes: a regulator's perspective on reducing the interfacial tension. In Pharmaceutical Quality. https://ipq.org/wp-content/uploads/2021/09/Rao-CASSS_SF_2020-v2.pdf

Rouaz, K., Chiclana-Rodríguez, B., Nardi-Ricart, A., Suñé-Pou, M., Mercadé-Frutos, D., Suñé-Negre, J.,...García-Montoya, E. (2021). Excipients in the Paediatric Population: A Review. *PHARMACEUTICS*, *13*(3), Article 387. https://doi.org/10.3390/pharmaceutics13030387

Roy, I., Wuchner, K., Stahl, P., Tran, T., & Yaragudi, N. (2024). A comparison of Polysorbates and Alternative Surfactants for Interfacial Stress Protection and Mitigation of Fatty Acid Particle Formation in the Presence of an Esterase. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *113*(9), 2688-2698. https://doi.org/10.1016/j.xphs.2024.07.010

Ruiz, A., Boushehri, M., Phan, T., Carle, S., Garidel, P., Buske, J., & Lamprecht, A. (2022). Alternative Excipients for Protein Stabilization in Protein Therapeutics: Overcoming the Limitations of Polysorbates. *PHARMACEUTICS*, *14*(12), Article 2575. https://doi.org/10.3390/pharmaceutics14122575

Ruiz-Peña, M., Oropesa-Nuñez, R., Pons, T., Louro, S., & Pérez-Gramatges, A. (2010). Physico-chemical studies of molecular interactions between non-ionic surfactants and bovine serum albumin. *COLLOIDS AND SURFACES B-BIOINTERFACES*, *75*(1), 282-289. https://doi.org/10.1016/j.colsurfb.2009.08.046

Saito, J., Nakamura, H., Akabane, M., & Yamatani, A. (2024). Quantitative Investigation on Exposure to Potentially Harmful Excipients by Injection Drug Administration in Children Under 2 Years of Age and Analysis of Association with Adverse Events: A Single-Center, Retrospective Observational Study. *THERAPEUTIC INNOVATION & REGULATORY SCIENCE*, *58*(2), 316-335. https://doi.org/10.1007/s43441-023-00596-0

Saito, J., Nakamura, H., Akabane, M., & Yamatani, A. (2025). A questionnaire survey on the barriers regarding pharmaceutical excipients during pediatric drug development. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *214*, Article 114775. https://doi.org/10.1016/j.ejpb.2025.114775

Salunke, S., Agrawal, A., Walsh, J., Nunn, A., Hughes, K., Kuehl, P.,…Sherratt, P. (2024). Selecting appropriate excipients for paediatric dosage form - Paediatric excipients risk assessment (PERA) framework - Part 1. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *203*, Article 114458. https://doi.org/10.1016/j.ejpb.2024.114458

Shen, K., Hu, X., Li, Z., Liao, M., Zhuang, Z., Ruane, S.,…Lu, J. (2023). Competitive Adsorption of a Monoclonal Antibody and Nonionic Surfactant at the PDMS/Water Interface. *MOLECULAR PHARMACEUTICS*, *20*(5), 2502-2512. https://doi.org/10.1021/acs.molpharmaceut.2c01099

Shieh, I. C., & Patel, A. R. (2015). Predicting the Agitation-Induced Aggregation of Monoclonal Antibodies Using Surface Tensiometry. *Mol Pharm*, *12*(9), 3184-3193. https://doi.org/10.1021/acs.molpharmaceut.5b00089

Singh, R., Chandley, P., & Rohatgi, S. (2023). Recent Advances in the Development of Monoclonal Antibodies and Next-Generation Antibodies. *Immunohorizons*, *7*(12), 886-897. https://doi.org/10.4049/immunohorizons.2300102

Singh, S., Bandi, S., Jones, D., & Mallela, K. (2017). Effect of Polysorbate 20 and Polysorbate 80 on the Higher-Order Structure of a Monoclonal Antibody and Its Fab and Fc Fragments Probed Using 2D Nuclear Magnetic Resonance Spectroscopy. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *106*(12), 3486-3498. https://doi.org/10.1016/j.xphs.2017.08.011

Soeda, K., Arai, K., Yamamoto, T., Ofuji, K., Fukuda, M., Hashimoto, D., & Yamanaka, Y. (2023). Mechanism of Protein-PDMS Visible Particles Formation in Liquid Vial Monoclonal Antibody Formulation. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *112*(3), 653-664. https://doi.org/10.1016/j.xphs.2022.09.027

Strambini, G. B., & Gabellieri, E. (1996). Proteins in frozen solutions: evidence of ice-induced partial unfolding. *Biophys J*, *70*(2), 971-976. https://doi.org/10.1016/S0006-3495(96)79640-6

Strickley, R., & Lambert, W. (2021). A review of formulations of commercially available antibodies. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *110*(7), 2590-+. https://doi.org/10.1016/j.xphs.2021.03.017

Strnad, U., Zalokar, P., Osterman, N., & Zidar, M. (2025). Small scale model for predicting transportation-induced particle formation in biotherapeutics. *COLLOIDS AND*

SURFACES B-BIOINTERFACES, *245*, Article 114304. https://doi.org/10.1016/j.colsurfb.2024.114304

Telikepalli, S., Kumru, O. S., Kim, J. H., Joshi, S. B., O'Berry, K. B., Blake-Haskins, A. W.,…Volkin, D. B. (2015). Characterization of the physical stability of a lyophilized IgG1 mAb after accelerated shipping-like stress. *J Pharm Sci*, *104*(2), 495-507. https://doi.org/10.1002/jps.24242

Thakral, S., Levesque, C., Cung, K., Li, L., Mould, R. E., Moussa, E.,…Krishnamachari, Y. (2025). A pharmaceutical industry survey results on exploring polysorbate alternatives in biological drug products: poloxamer and other surfactants. *J Pharm Sci*, *114*(9), 103917. https://doi.org/10.1016/j.xphs.2025.103917

The International Pharmaceutical Excipients Council, DeMerlis, C., CCD Consulting Services, Filler, R., Drug Development Consultants, Inc., Hamilton, R., Ashland, Kim, S.-T., Ashland, Kotkoskie, L. A., LAK3 Consulting, Li, Y., Lubrizol, Pitt, J., IFF, Schoneker, D., Black Diamond Regulatory Consulting, Ulman, K., KLU Consulting, Wegesser, T., Amgen, Zawislak, P., IFF, Gronewold, C., Evonik, Schulte, S., BASF, Deore, M., Johnson &. Johnson, Steinberg, M., Borzelleca, J., Enters, E., Kinoshita, F., . . . Plazonnet, B. (2021). Safety Guide for Pharmaceutical excipients. https://ipec-federation.org/wp-content/uploads/2022/02/2021-IPEC-Safety-Guide-F.pdf

Thompson, B., Pham, K., Phan, M., Sanchez-Puga, P., Gutfreund, P., Wang, T.,…Wagner, N. (2025). Competitive Adsorption of Monoclonal Antibodies and Nonionic Surfactants at the Air-Water Interface. *ACS APPLIED MATERIALS & INTERFACES*, *17*(28), 40116-40128. https://doi.org/10.1021/acsami.5c07162

Torisu, T., Maeda, A., Ito, S., & Uchiyama, S. (2025). Protein aggregation in the frozen state induced by dropping stress. *Eur J Pharm Sci*, *205*, 106996. https://doi.org/10.1016/j.ejps.2024.106996

United States Pharmacopeia. (n.d.). Particulate matter in injections. https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisionGeneralChapter788.pdf

Vaclaw, C., Merritt, K., Griffin, V. P., Whitaker, N., Gokhale, M., Volkin, D. B.,…Dhar, P. (2023). Comparison of Protein Particle Formation in IgG1 mAbs Formulated with PS20 Vs. PS80 When Subjected to Interfacial Dilatational Stress. *AAPS PharmSciTech*, *24*(5), 104. https://doi.org/10.1208/s12249-023-02561-4

Vaclaw, C., Merritt, K., Pringle, V., Whitaker, N., Gokhale, M., Carvalho, T.,…Dhar, P. (2021). Impact of Polysorbate 80 Grade on the Interfacial Properties and Interfacial Stress Induced Subvisible Particle Formation in Monoclonal Antibodies. *J Pharm Sci*, *110*(2), 746-759. https://doi.org/10.1016/j.xphs.2020.09.035

van Haaren, C., Byrne, B., & Kazarian, S. G. (2024). Study of Monoclonal Antibody Aggregation at the Air-Liquid Interface under Flow by ATR-FTIR Spectroscopic Imaging. *Langmuir*, *40*(11), 5858-5868. https://doi.org/10.1021/acs.langmuir.3c03730

Verdin, P. (2024). Top companies and drugs by sales in 2023. *NATURE REVIEWS DRUG DISCOVERY*, *23*(4), 240-240. https://doi.org/10.1038/d41573-024-00041-3

Verdin, P. (2025). Top companies and drugs by sales in 2024. *NATURE REVIEWS DRUG DISCOVERY*, *24*(4), 240-240. https://doi.org/10.1038/d41573-025-00049-3

Wang, Y., Wang, T., Chen, Q., Zhou, W., & Guo, J. (2023). Correlation between the Protein Pharmaceutical Surface Activity and Interfacial Stability. *Mol Pharm*, *20*(5), 2536-2544. https://doi.org/10.1021/acs.molpharmaceut.2c01114

Waters, L., Whiteley, J., Small, W., & Mellor, S. (2023). Determining suitable surfactant concentration ranges to avoid protein unfolding in pharmaceutical formulations using UV analysis. *HELIYON*, *9*(11), Article e21712. https://doi.org/10.1016/j.heliyon.2023.e21712

Weber, J., Buske, J., Mäder, K., Garidel, P., & Diederichs, T. (2023). Oxidation of polysorbates - An underestimated degradation pathway? *INTERNATIONAL JOURNAL OF PHARMACEUTICS-X*, *6*, Article 100202. https://doi.org/10.1016/j.ijpx.2023.100202

Weber, J., Pedri, L., Peters, L., Quoika, P., Dinu, D., Liedl, K.,…Garidel, P. (2025). Micellar Solvent Accessibility of Esterified Polyoxyethylene Chains as Crucial Element of Polysorbate Oxidation: A Density Functional Theory, Molecular Dynamics Simulation and Liquid Chromatography/Mass Spectrometry Investigation. *MOLECULAR PHARMACEUTICS*, *22*(3), 1348-1364. https://doi.org/10.1021/acs.molpharmaceut.4c01015

Wen, L., Zhang, Y., Sun, C., Wang, S., Gong, Y., Jia, C., & Luo, J. (2025). Fundamental properties and principal areas of focus in antibody-drug conjugates formulation development. *ANTIBODY THERAPEUTICS*, *8*(2), 99-110. https://doi.org/10.1093/abt/tbaf005

Wen, Y., & Jawa, V. (2021). The Impact of Product and Process Related Critical Quality Attributes on Immunogenicity and Adverse Immunological Effects of Biotherapeutics. *J Pharm Sci*, *110*(3), 1025-1041. https://doi.org/10.1016/j.xphs.2020.12.003

Wu, H., Chisholm, C. F., Puryear, M., Movafaghi, S., Smith, S. D., Pokhilchuk, Y.,…Randolph, T. W. (2020). Container Surfaces Control Initiation of Cavitation and Resulting Particle Formation in Protein Formulations After Application of Mechanical Shock. *J Pharm Sci*, *109*(3), 1270-1280. https://doi.org/10.1016/j.xphs.2019.11.015

Wuchner, K., Yi, L., Chery, C., Nikels, F., Junge, F., Crotts, G.,…Le Brun, V. (2022a). Industry Perspective on the use and Characterization of Polysorbates for Biopharmaceutical Products Part 1: Survey Report on Current State and Common Practices for Handling and Control of Polysorbates. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *111*(5), 1280-1291. https://doi.org/10.1016/j.xphs.2022.02.009

Wuchner, K., Yi, L., Chery, C., Nikels, F., Junge, F., Crotts, G.,…Le Brun, V. (2022b). Industry Perspective on the Use and Characterization of Polysorbates for Biopharmaceutical Products Part 2: Survey Report on Control Strategy Preparing for the Future. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *111*(11), 2955-2967. https://doi.org/10.1016/j.xphs.2022.08.021

Xiao, Y., & Konermann, L. (2015). Protein structural dynamics at the gas/water interface examined by hydrogen exchange mass spectrometry. *Protein Sci*, *24*(8), 1247-1256. https://doi.org/10.1002/pro.2680

Yang, R., Bush, D., DeGraan-Weber, N., Barbacci, D., Zhang, L., Letarte, S., & Richardson, D. (2022). Advancing Structure Characterization of PS-80 by Charge-Reduced Mass Spectrometry and Software-Assisted Composition Analysis. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *111*(2), 314-322. https://doi.org/10.1016/j.xphs.2021.08.036

Yue, L., Yan, Z., Li, H., Liu, X., & Sun, P. (2020). Brij-58, a potential injectable protein-stabilizer used in therapeutic protein formulation. *EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS*, *146*, 73-83. https://doi.org/10.1016/j.ejpb.2019.12.001

Zhang, Z., Orski, S., Woys, A., Yuan, G., Zarraga, I., Wagner, N., & Liu, Y. (2018). Adsorption of polysorbate 20 and proteins on hydrophobic polystyrene surfaces studied by neutron reflectometry. *COLLOIDS AND SURFACES B-BIOINTERFACES*, *168*, 94-102. https://doi.org/10.1016/j.colsurfb.2018.04.036

Zhang, Z., Orski, S., Woys, A. M., Yuan, G., Zarraga, I. E., Wagner, N. J., & Liu, Y. (2018). Adsorption of polysorbate 20 and proteins on hydrophobic polystyrene surfaces studied by neutron reflectometry. *Colloids Surf B Biointerfaces*, *168*, 94-102. https://doi.org/10.1016/j.colsurfb.2018.04.036

Zhang, Z., Woys, A., Hong, K., Grapentin, C., Khan, T., Zarraga, I.,…Liu, Y. (2021). Adsorption of non-ionic surfactant and monoclonal antibody on siliconized surface studied by neutron reflectometry. *JOURNAL OF COLLOID AND INTERFACE SCIENCE*, *584*, 429-438. https://doi.org/10.1016/j.jcis.2020.09.110

Zhonghua Renmin Gongheguo wei sheng bu yao dian wei yuan hui, i. b. c. (2015). *Pharmacopoeia of the People's Republic of China. 2015* ([10th edition]. ed.). China Medical Science Press.

Zhuang, Z., Wu, H., Li, Z., Liao, M., Shen, K., Li, R.,…Lu, J. (2025). Protecting monoclonal antibodies via competitive interfacial adsorption of nonionic surfactants. *JOURNAL OF COLLOID AND INTERFACE SCIENCE*, *684*, 819-830. https://doi.org/10.1016/j.jcis.2024.12.214

Zoeller, M. P., Hafiz, S., Marx, A., Erwin, N., Fricker, G., & Carpenter, J. F. (2022). Exploring the Protein Stabilizing Capability of Surfactants Against Agitation Stress and the Underlying Mechanisms. *J Pharm Sci*, *111*(12), 3261-3274. https://doi.org/10.1016/j.xphs.2022.09.004

Zurcher, D., Caduff, S., Aurand, L., Palmiero, U., Wuchner, K., & Arosio, P. (2023). Comparison of the Protective Effect of Polysorbates, Poloxamer and Brij on Antibody Stability Against Different Interfaces. *JOURNAL OF PHARMACEUTICAL SCIENCES*, *112*(11), 2853-2862. https://doi.org/10.1016/j.xphs.2023.06.004

Zürcher, D., Wuchner, K., & Arosio, P. (2024). Mitigation Strategies against Antibody Aggregation Induced by Oleic Acid in Liquid Formulations. *MOLECULAR PHARMACEUTICS*, *21*(11), 5761-5771. https://doi.org/10.1021/acs.molpharmaceut.4c00754