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Review Article

Key interactions of surfactants in therapeutic protein formulations: A review

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ABSTRACT

Proteins as amphiphilic, surface-active macromolecules, demonstrate substantial interfacial activity, which causes considerable impact on their multifarious applications. A commonly adapted measure to prevent interfacial damage to proteins is the use of nonionic surfactants. Particularly in biotherapeutic formulations, the use of nonionic surfactants is ubiquitous in order to prevent the impact of interfacial stress on drug product stability. The scope of this review is to convey the current understanding of interactions of nonionic surfactants with proteins both at the interface and in solution, with specific focus to their effects on biotherapeutic formulations.

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1. Introduction

Throughout the past decade, there has been a steady increase in the availability of new biological drugs for patients to treat critical and life threatening diseases in various therapeutic areas from oncology to metabolic diseases [1,2]. Correspondingly, there has also been a surge in drug discovery of therapeutic proteins, especially monoclonal antibodies and their related formats such as antibody drug conjugates and bispecific antibodies. It is critical to preserve the stability of any biologic during bioprocessing, drug product manufacturing, transportation, storage, and administration to the patient. Therefore, formulating the protein suitably and optimally requires significant attention to preserve its stability during all possible stresses until it reaches the patient. There are numerous mechanisms that may trigger the formation of aggregates and particles (e.g. hydrophobic association of partially denatured protein, chemical modifications, and interactions with interfaces) [3]. These events may affect the quality of the product and/or increase the level of visible and sub-visible particles beyond the acceptance criteria. The presence of aggregates also raises a concern as a driving factor for immunogenicity; however, to date only chemically modified aggregates of biologics have shown a clear ability to induce anti-drug antibodies in preclinical models [4,5].

One of the major stresses proteins may encounter is interfacial stress (e.g. from air/water interfaces due to mixing of liquid formulations, or ice/water interfaces during freezing/thawing), which if not suitably stabilized generates aggregates or proteinaceous particles [6]. In the absence of stabilizing surfactants and while under stress or long-term storage, fractions of the therapeutic proteins tend to aggregate and/or generate particles [7–9]. It is in this context that surfactants are prevalently used in the pharmaceutical industry to provide and ensure protein stability, although other excipients (e.g. cyclodextrins) have also shown protection properties [3,10,11]. Certain surfactants, such as polysorbates, have a proven safety profile established by their use as excipients in small molecule drug formulations, acting as solubility enhancers for poorly water-soluble drugs and later used as dispersion stabilizers for nanoparticle formulations [12–18]. The interactions of both ionic and nonionic surfactants with proteins have been reviewed previously [19–21], providing a broad overview. However, the scope of this review is focused on nonionic surfactants and their protection of therapeutic proteins in parenteral formulations, as they play a prominent role as protein stabilizers in commercial formulations, in contrast with ionic surfactants [22,23]. This review will discuss the present state of knowledge of the mode of interaction between proteins and surfactants at air–water and oil–water interfaces, as well as protein–surfactant interactions in solution. Surfactants extensively used in marketed biologics (e.g.

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polysorbate 20, polysorbate 80, and pluronics (P188)) are the primary emphasis for this discussion.

2. Use of nonionic surfactants in protein formulations

Surfactants are amphiphilic molecules with hydrophobic and hydrophilic components. The hydrophobic components drive an interaction with air-water interfaces as well as between the surfactant molecules themselves in solution, driving the assembly of micelles at concentrations above the critical micelle concentration (CMC) [24]. The apparent CMC of a formulated protein solution containing a surfactant may be influenced by other molecules such as excipients or even leachates, as well as the surface active protein drug itself, wherein the CMC of a surfactant typically tends toward higher concentrations, presumably due to competitive interaction with the protein in solution [25].

Nonionic surfactants are widely used in protein formulations, especially parenteral monoclonal antibody formulations in both liquid and dried (e.g. lyophilized) forms. Virtually all marketed parenteral biologics contain varying concentrations of surfactants. In general, nonionic surfactants have been favored for use with biotherapeutics over ionic surfactants due to their proven safety profile, primarily based on prior use in other products [6,26,27]. In contrast, ionic surfactants are known to potentially act as denaturing agents of proteins [28]. The primary role of nonionic surfactants in a protein formulation is to protect the protein against interfacial stresses and related interactions/degradation, including interface-induced protein aggregation [29], protein precipitation (particle formation), and/or surface adsorption [7,29–35]. Surfactants are effective against various stresses such as agitation by shaking or stirring (air/water interfaces) [7,8,36,37], freezing and thawing (ice/water interfaces), and drying stresses that can occur during lyophilization [38,39].

The most extensively used surfactants in biologics formulations are poly-oxy-ethylene (PEO) based surfactants, such as polysorbates 20 and 80 and poloxamer 188 (Fig. 1). Polysorbate 20 (Tween® 20) and Polysorbate 80 (Tween® 80) are not only capable of preventing protein aggregation caused by exposure to air–water interfaces [23,40–44] and freeze–thaw stress [23,29,31,41,45–47], but also inhibit adsorption to various surfaces such as sterilization filters and primary packaging [40,48]. Various reports on polysorbate 20 describe the prevention of aggregation against mechanical stress for various types of proteins, including porcine growth hormone [49], recombinant human growth hormone (rHGH) [9] and recombinant human factor XIII [50]. Similarly, polysorbate 80 has been reported to prevent aggregation induced by vortexing, rHGH [51]; and freeze–thaw, lactate dehydrogenase (LDH) [29], recombinant hemoglobin [41], etc. [23].

Typically, polysorbates are used in the range of 0.001–0.1% (w/v). The choice and concentrations of the surfactant are usually determined by screening for the lowest effective concentration which stabilizes the therapeutic protein upon interfacial stress. These concentrations are determined by stress studies generating air–water and/or ice–water interfaces, such as shaking, stirring or freezing/thawing at varied surfactant concentrations followed by aggregate and particulate analysis. The concentration chosen is usually significantly above the edge of failure to provide a sufficient safety margin and protection during real-time stress such as transportation, stirring, and freeze–thaw. During commercial production, the surfactant is typically added after ultrafiltration–diafiltration (UF–DF) and prior to a bulk freezing step in the bioprocess (drug substance) manufacturing. UF–DF can alter the concentration of polysorbate in an irreproducible manner due to membrane adsorption. Since drug substance bulk is typically stored frozen prior to drug product manufacturing, the presence of surfactant is important to protect the protein from ice–water interfaces formed during freeze–thaw. Thus, the preferential point of surfactant addition is post-UF–DF, but prior to freezing of the drug substance.

While there are obvious benefits from the inclusion of surfactants in protein formulations, as described above, surfactants may present liabilities that could destabilize proteins and/or hinder reproducible drug product manufacturing. Polysorbates are known to degrade via oxidation and hydrolysis [52–54] and cause oxidative damage to the protein via production of reactive oxygen species. The oxidation of polysorbates can lead to a buildup of peroxides that may induce chemical modifications to the therapeutic protein [52,53]. Recently, it has been proposed that trace amounts of host cell protein contaminants, in the form of lipases, may further compromise the stability of polysorbates [55]. Additionally, it is known that polysorbates can adsorb strongly to materials contacted during processing, such as filters and tubing [48].

Due to some of the abovementioned concerns, poloxamers are being used more frequently. Poloxamers are triblock copolymers of PEO–polypropylene oxide (PPO)–PEO, and represent another class of nonionic surfactants used in the pharmaceutical industry. The solution behavior of poloxamers is more complex than that of polysorbates. These surfactants exist with a collapsed PPO block, as unimeric micelles and higher ordered aggregates, the exact states influenced by concentration and temperature. Poloxamer 188 (Pluronic® F68) is commonly employed in fermentation cultures to stabilize cells from shear stresses, reduce aggregation and interactions with air–water interfaces, overall ensuring high viability [56,57]. More recently, it has also been used as a surfactant to stabilize protein formulations.

3. Interactions of nonionic surfactants with proteins

Proteins, being amphiphilic, high-molecular-weight molecules tend to fold and assemble into globular structures in aqueous solutions, in order to expose hydrophilic parts to the exterior and hide the hydrophobic parts in the core of the structure. Their interaction

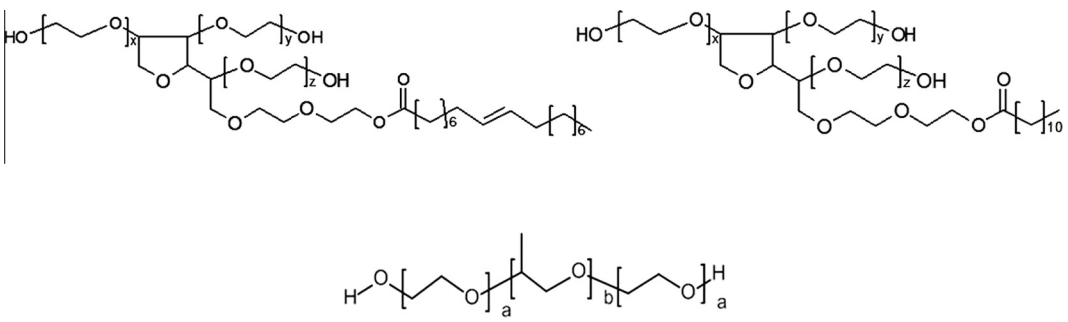

Fig. 1. Chemical structure of commonly used surfactants polysorbate 80 (top left), polysorbate 20 (top right); $x + y + z = 20$ and poloxamer 188 (bottom); $a = 80$ and $b = 27$.

with surfactants is driven by the various forces governing their stability in solution as well as at interfaces. In the following sections, these two aspects are discussed in detail.

3.1. Interactions of protein–surfactant mixtures in solution

Ideally, the nature of interactions between nonionic surfactants and proteins can best be described through thermodynamic drivers, such as changes in enthalpy and entropy, in relation to properties (polar, charged, or hydrophobic) of regions on the surfactant and protein molecules themselves. Unfortunately, detailed thermodynamic and site-specific interaction behavior is difficult to measure and quantify, due to generally low-affinity interactions [58]. Lee et al. [6] recently performed a comprehensive review of the interactions of proteins with polysorbate 20, polysorbate 80, and poloxamer 188 and have summarized the contributions of the protein, the surfactant, and surfactant–protein complexes relating to protein adsorption and aggregation behavior. Table 1 details a fairly inclusive listing of work related to interactions of proteins with surfactants, of which several are highlighted below.

In investigating protein–surfactant interactions, considerable work has been reported with bovine serum albumin (BSA) as a model protein, establishing interactions between BSA and polysorbates 20/80 or other nonionic surfactants. For instance, Perez et al. investigated the interactions between BSA and nonionic surfactants using fluorescence, surface tension measurements, and computational simulations [59]. Their studies demonstrate that upon addition of protein, the surface tension behavior of the surfactants was modified, and the apparent CMC values of polysorbate 20 and 80 increased, compared to the CMC values of the surfactants alone. Their results also suggest that despite structural similarities between polysorbate 20 and polysorbate 80, their binding with BSA in solution seem to be mechanistically different, with potentially varied interaction sites. Distinct aggregation states of the protein in solution seem to govern complexation with the polysorbates. Changes observed in tryptophan fluorescence indicated the surfactants first preferentially associate within cavities in the tertiary structure; once saturated, additional surfactant molecules are able to interact with hydrophobic patches on the protein surface. Nielsen et al. used isothermal calorimetry (ITC) to understand the binding of BSA to nonionic surfactants with C12 acyl chains, along with ionic surfactants [60]. In this study, it was found that nonionic surfactants bind to proteins with association constants several orders of magnitude lower than sodium dodecyl sulfate. It was also concluded that large exothermic enthalpy changes occur along with substantial increases in heat capacity during the binding process. The enthalpy changes pertaining to nonionic surfactants were greater than those observed for anionic surfactants.

Hoffmann et al. [61] reported similar findings when they assessed interactions by ITC and stability in the presence of polysorbates 20 and 80 using differential scanning calorimetry (DSC). Here, ITC provided the binding constants and thermodynamic parameters, while DSC yielded thermal protein stability information. The results showed that both polysorbate 20 and polysorbate 80 bound to BSA with a binding constant of 8–12 $\times 10^{-3}$ M^{-1} and ΔH values ranging from -50 to -60 kJ/mol (25 °C). ITC was also able to determine the stoichiometric ratio: one to two polysorbate molecules binding BSA. The large exothermic enthalpy changes were considered to be an outcome of the interaction of the PEO chain (polyethylene glycol) with the protein by hydrogen bonding. It was also recently shown that PEO chains can also exhibit affinity for proteins such as BSA [62]. Delgado-Magnero et al. [63] support this hypothesis with their molecular dynamics simulation results, which suggest enhanced stabilization of BSA by cooperative self-assembly with polysorbate molecules. However, Hoffman et al. observed the interactions of polysorbates (20 and 80) with a model mAb and lysozyme were negligible based on ITC results, with very little binding observed at highly titrated concentrations.

In a similar analysis performed by Garidel et al. [64], weak interactions between polysorbates 20 and 80 were also found with several immunoglobulins. However, this study did find a measurable interaction with human serum albumin (HSA); binding only mildly increased using a fatty acid-free form of the protein. This finding was interpreted as the fatty acid chains of the polysorbates being a poor steric match for the fatty acid binding pocket of HSA. The results showed that binding constants of polysorbates to human serum albumin were in the range of 10^3 M^{-1}, a rather negligible value, leading to the conclusion that direct surfactant interaction is not the main factor for stabilization of the protein. McAuley et al. [65] also employed ITC to investigate the interaction between polysorbates and lactate dehydrogenase (LDH). Additionally, surface tension measurements and interfacial rheology were used in order to understand the mechanism of prevention of protein adsorption to the air–water interface by the surfactant. No significant interaction between polysorbate 20 and LDH could be found using ITC. It was apparent that surface pressure effects dominated the prevention of LDH adsorption to the air–water interface by polysorbates. Interfacial rheology was used to suggest the concentration of polysorbate 20 needed to displace LDH is well below the CMC. The majority of studies based on ITC have revealed primarily weak binding between polysorbates and therapeutic proteins (in particular mAbs) and those with albumins suggest a significant interaction component driven by van der Waals interactions and hydrogen bonding.

In another study, Chou et al. characterized the binding interactions between the surfactants and Albutropin™ (human growth hormone genetically fused to human albumin) based on fluorescence spectroscopy and ITC [66]. The authors concluded that polysorbates and Albutropin reached saturation with a molar binding stoichiometry of approximately 10:1 (surfactant:protein) and binding of the surfactants to Albutropin led to an increase in free energy of unfolding. This increased free energy of unfolding was thought to be responsible for stabilizing the protein even in concentrations of surfactants well below their CMC. The belief of stabilizing monomeric proteins from aggregation has previously been described in terms of molecular chaperones [40]. Earlier studies used chemically denatured protein to evaluate protein–surfactant interactions, protein structure, and enzymatic activity by electron paramagnetic resonance, circular dichroism spectroscopy, and activity assays [67,68]. These studies suggest polysorbate 20, as well as other surfactants, may temporarily occupy hydrophobic regions exposed on partially denatured proteins, enabling them to displace the polysorbate and refold prior to the formation of an aggregation event.

Kim et al. [69] demonstrated based on optical waveguide light mode spectroscopy measurements that polysorbates 20 and 80 are able to prevent protein adsorption to a hydrophilic surface exclusively by the surfactants sterically inhibiting proteins from associating with the interface, likely governed by preferential mass action kinetics of the polysorbates. It was also concluded that no significant surfactant–protein associations occur in solution. However, poloxamer 188 was thought to have surfactant–protein associations in solution, independent of its affinity for the interface thereby inhibiting protein adsorption to the interface. A variety of techniques have been employed to study surfactant–protein interactions in solutions. Ideal measurement methods based on measuring direct interactions, are capable of providing insight into the mechanistic drivers, and determining the site-specific locations of molecular recognition. However, the weak interactions in play often require more indirect methods to be used, but are still able

Table 1
Example studies researching surfactant-protein interactions.

Sl. no.	Nonionic surfactants investigated	Protein investigated	Nature of investigation	Techniques implemented	Interaction proposed	Authors	Ref.
1	16-Doxyl stearic acid, Brij, PS20, PS40, PS80	rHGH, Inf γ	Binding stoichiometries	Spin labeled partition curves via EPR	N/A	Bam et al.	[22]
2	PS20, Brij 35, Brij 78	α and β-lactoglobulin	Electrophoretic behavior	CE	N/A	Xu et al.	[102]
3	C12E8	rhTF220, 243	Binding stoichiometries	AUC, EPR	N/A	Jones et al.	[103]
4	PS20 and PS80	Albutropin	Albutropin-surfactant interactions	Fluorescence spectroscopy and ITC	Interaction leading to increase of free energy of unfolding	Chou et al.	[66]
5	PS80	LDH	Interfacial behavior at ice liquid interface	DSC and IR	PS competes with protein at interface	Hillgren et al.	[104]
6	PS20 and PS80	IgG and HSA	Interaction and binding	ITC and DSC	Negligible binding with IgG	Garidel et al.	[64]
	PS20 and PS80	BSA, lysozyme, IgG	Interaction and binding	ITC and DSC	Negligible binding with IgG	Hoffmann et al.	[64]
7	PS20, PS40, PS80	LDH	Interfacial behavior at air–liquid interface	ITC, calorimetry, surface tension and interfacial rheometry	Competitive displacement mechanism driven by surface pressure	Kett et al.	[90]
8	Dodecyl dimethyl phosphine oxide	β-Casein	Interfacial behavior at air–liquid interface	Tensiometry and rheometry	Competitive displacement at interface	Kotsmar et al.	[89]
9	PS20, PS40, PS80	LDH	Binding and interfacial behavior	ITC	Weak hydrophobic interaction	McAuley et al.	[65]
10	PS20 and PS80	BSA	Surfactant interaction and binding	Surface tension, fluorescence and computational analysis	Binding model for BSA–surfactant binding	Perez-Gramatges et al.	[59]
11	Triton-X	Gelatin	Surfactant–protein binding	Density, adiabatic compressibility	Hydrophobic binding model	Chauhan et al.	[105]
12	C12EO7, C12EO5	BSA	Surfactant interaction and binding	ITC	Binding model for BSA–surfactant binding	Nielsen et al.	[60]
13	PS20 and PS40	Lysozyme	Adsorption at solid–water interface	CD and adsorption kinetics	Hydrophobicity driven preferential tween adsorption	Joshi and McGuire	[75]
14	PS80	Recombinant factor VIII	Adsorption at air–water interface	Surface tension	Competitive displacement at the interface	Joshi et al.	[106]
15	PS20 and PS40	Fibrinogen	Adsorption and binding	SPR	Adsorption and packing phenomenon	Shen et al.	[107]
16	PS80	BSA	Aggregation prevention	CD and native PAGE	Aggregation inhibition of partially or fully denatured monomers	Arakawa and Kita	[108]
17	P188, PS20, PS80	Recombinant factor VIII	Adsorption to hydrophilic surface	OWLS	Colloidal stabilization and competitive interfacial adsorption	Kim et al.	[69]
18	P188, PS20, PS80	Lysozyme, GCSF	Adsorption at air–water interface	Surface tension	Disruption of surfactant aggregates	Kim et al.	[70]
19	PS80	HSA	Adsorption at air–water interface	Surface tension	Hydrophobic interactions	Dixit et al.	[85]
20	P188, PS20, PS80	Fc-fusion	Adsorption at silicon oil/water interfaces	QCM	Competitive adsorption but, poor displacement of protein	Dixit et al.	[83]
21	P188, PS80	Fc-fusion	Adsorption at silicon oil/water interfaces	QCM and surface tension	Competitive adsorption	Li et al.	[82]
22	PS20, PS80	BSA	Interaction in solution	Computational simulation	Hydrogen bonding, van der Waals, and hydrophobic interactions	Delgado-Magnero et al.	[63]

Note: polysorbate is abbreviated as PS.

to provide valuable insight and offer relative levels of interactions between various molecules.

3.2. Interactions of protein–surfactant mixtures at interfaces

As discussed in the previous section, interactions of nonionic surfactants with proteins vary according to the nature of the protein. It is also evident that the surfactant interaction at interfaces typically dominates (e.g. water–air, water–container, water–silicone oil) as the mechanism stabilizing therapeutic proteins. The complex behavior of surfactant adsorption is governed by many factors: bulk concentration, chemical potentials in the bulk solution and at the interface, adsorption layer thickness, the maximum surfaces excess, and temperature. Upon exposure of a therapeutic protein formulation to a new interface, different species compete to adsorb at the interface which leads to lowering of the surface tension. A dynamic equilibrium is established due to the adsorption and desorption process [70–72]. The ability to adsorb at the interface depends on the relative surface activity as well as the inter- and intra-species interaction in solution [69,73].

Early experiments evaluated the ability of polysorbate 20 to desorb proteins previously adsorbed to hydrophilic and hydrophobic solid surfaces [74]. It was found that the polysorbates had little effect at displacing proteins (fibrinogen and human gamma globulin) preadsorbed on the hydrophilic surfaces. However, polysorbate 20 was effective at removing the protein molecules from a hydrophobic surface. The ability of the polysorbates to desorb protein from hydrophobic surfaces was reduced when the proteins had been incubated for extended periods of time at elevated temperatures. These conditions likely increased the surface area involved in hydrophobic interactions, increasing the entropic barrier for solvation beyond the level the aliphatic chains of the polysorbate could access. Joshi and McGuire [75] later demonstrated that at the liquid–solid interface, the adsorption propensity of nonionic surfactants depends on the hydrophobicity of the surface. They also showed that the pre-treatment of hydrophilic

surfaces with polysorbate 80 did not have an effect on subsequent protein (lysozyme) adsorption, while precoating with polysorbate 80 on hydrophobic surfaces dramatically decreased the ability of a protein film to form on the surface, measured by ellipsometry. The trends for preventing protein adsorption on hydrophobic surfaces held true when both the polysorbate and protein were added in the same solution, compared to protein alone. While the mechanisms involved in protein surface adsorption are complex and are determined by multiple factors (e.g. size, structural stability, and exposed hydrophobic patch distribution [76,77]), literature suggests greater adsorption to hydrophobic surfaces (regardless of protein properties) and by more hydrophobic proteins (regardless of surface properties [74,78]). Polysorbates appear to be most effective at competing and desorbing proteins from moderately hydrophobic surfaces [74,75].

Biologics may also encounter silicone oil, presenting a formidable form of hydrophobic surfaces. Typically, silicone oil is applied to the surface of prefilled syringes and represents an additional pharmaceutically relevant interface and is required in sufficient amount to ensure long-term functionality of syringes. Previous work has evaluated the ability of polysorbate 20 to compete with BSA for interfacial sites on water–oil emulsion droplets [79–81]. These studies relied on front-face fluorescence spectroscopy, based on tryptophan fluorescence, demonstrating a concentration dependent behavior of polysorbates to effectively displace protein from the water–oil interface. Mass adsorption measurements using quartz crystal microbalances (QCM) have been used in evaluating the competition of surfactants and proteins at silicone oil surfaces [82,83]. In one study, it was determined by QCM and surface tension measurements that polysorbate 80 was more effective than poloxamer 188 at inhibiting the adsorption of an Fc-fusion protein to the water–silicone oil interface [82]. This was attributed to polysorbate 80 being more hydrophobic, lower HLB value, than poloxamer 188. In another study, it was found that both polysorbates (20 and 80) and poloxamer 188 were effective at inhibiting the adsorption of an Fc-fusion when preadsorbed, but rinsing the surface after applying the preadsorbed surfactants enabled a greater amount of protein to be adsorbed for the poloxamer 188 case. This effect was attributed to a weaker poloxamer 188–silicone oil interaction creating more unoccupied surface after rinsing. It was also determined that when surfactant and protein were coadsorbed, all surfactants reduced the amount of protein able to be adsorbed to the interface. However, it also showed that all surfactants were not effective at displacing a pre-existing protein adsorbed layer.

A common method for determining the CMC of polysorbates in solution is based upon the stabilization of the surface tension after the concentration of the surfactant exceeds that of the CMC [84]. This is due to the previously mentioned propensity of the polysorbate molecules to associate and orient themselves with the air–liquid interface. This trend of concentration dependent reduction (up to the CMC) of the surface tension remains for polysorbates in the presence of protein solutions, although often altered by the surface activity of the protein itself [25]. A similar study, based on dynamic surface tension, also demonstrated surface tension increases of polysorbate 80 solutions in the presence of protein (HSA); this suggests the lack of surface active molecules present at the air–water interface was attributable to surfactant–protein interactions in solution [85]. These solution phase interactions help to block proteins from associating with the air–liquid interface, much in the same way as the above-described competition at solid–liquid interfaces. Interestingly, the dynamic surface tension may be reduced when interactions take place below the CMC, as seen for solutions of GCSF with polysorbates and poloxamer 188 [70]. This phenomenon is attributed to proteins disrupting surfactant aggregates unable to readily adsorb to the interface, increasing the concentration of surfactant monomer in the subsurface layer below the interface and enhancing the rate of adsorption. Such behavior may improve formulation stability in shake-stress scenarios by enabling more surfactant to compete for the interface. However, the proposed aggregate interaction may also destabilize the protein itself by inducing a partially denatured state.

Mackie et al. [86] propose an "orogenic mechanism" to elucidate the ability of nonionic surfactants to displace proteins from the interface. They explain how small quantities of surfactant added to a protein-adsorbed interface can break away the protein network. This alteration of the interface is thought to be due to the different mechanisms by which surfactants and proteins behave at interfaces. Proteins tend to form strong interactions with one another caused by partial denaturation, due to hydrophobic interactions with large buried surface areas. In contrast, smaller surfactants move in the direction of surface tension gradients, described by the Gibbs–Marangoni effect [87]. The protein network inhibits surfactant lateral mobility and a surface pressure is induced on the protein network, leading to competition and displacement of proteins from the interface [86,88]. Korsmar et al. [89] and Kett et al. [90] demonstrate evidence for polysorbate 20 driven disruption of surface shear viscosity of β-lactoglobulin. While some of the orogenic studies may not be fully translatable to formulation studies with biologics, they provide significant mechanistic insight regarding the need to prevent protein adsorption at interfaces and implications for drug product manufacturing.

4. Discussion

The various studies performed to date provide different perspectives and possible mechanisms by which surfactants contribute to protein stabilization. Although studies conducted with BSA and HSA demonstrate the presence of binding propensity of surfactant to the protein [59,60,64] it could be considered that such binding phenomena are special situations and not broadly

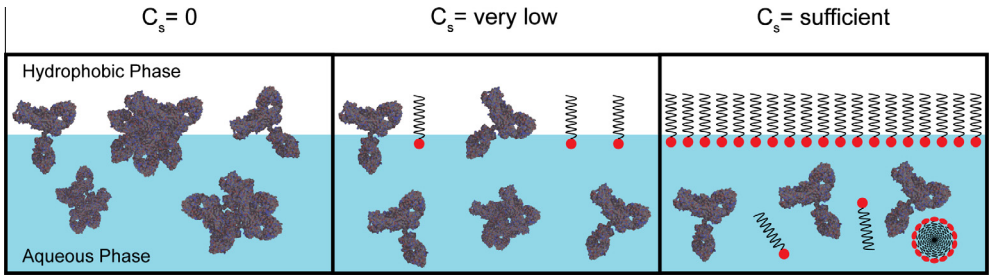

Fig. 2. Basic competitive adsorption mechanism of biologics stabilization via nonionic surfactants: (left) biologic in solution without surfactant ($C_s = 0$) tends to interfaces, denatures, and aggregates; (center) biologic present in the presence of insufficient amount of surfactant (C_s = very low) is not fully stabilized and still reaches the interface in significant amounts, leading to measurable amounts of protein aggregation/particles, (right) biologic is present in solution with a sufficient amount of surfactant, effectively blocking the interface from adsorption.

representative of actual protein formulations. The few studies on IgG, published by Garidel et al. and others, show that the binding energy (from ITC measurements) is weak and likely plays no significant role for stabilization [64]. The presence of surfactant is also not expected to impact the pharmacokinetic parameters associated with biologics, especially in the case for mAbs dominated by FcRn-mediated recycling [91]; this is in contrast to the use of nonionic surfactants in small molecules where the impact on pharmacokinetics can be dramatic [12,92].

There are two main mechanisms by which nonionic surfactants are proposed to stabilize proteins: interfacial competition and surfactant–protein complexation. The interfacial competitive mechanism where the occupancy of the interface by the nonionic surfactant is more thermodynamically favored compared to the protein is well documented, and governed by mass action kinetics [23,51,73,86,93–95]. This competitive adsorption of the surfactant at the interface is correlated to an increased surface pressure by the surfactant [96]. Conversely, surfactant–protein complexation is primarily described by direct binding of the surfactant to the exposed hydrophobic surfaces of the native protein, thereby increasing the protein's colloidal stability [9]. This would lead to thermodynamic stabilization of the native state by hydrophobic patches preferential binding the surfactant [97]. The described "molecular chaperone" function of surfactants stabilizes partially denatured forms of the protein, exposing neo-hydrophobic patches, long enough to enabling refolding of the protein [68].

To date, we believe that based on published data and from our own experience, the primary mechanism of stabilization of proteins, such as mAbs, by nonionic surfactants against aggregation is mainly attributed to competition of (surface active) polysorbate or poloxamer molecules against proteins at interfaces (e.g. air–water), shown in Fig. 2. The competitive adsorption of nonionic surfactants at interfaces would be thermodynamically favored [51,93,94,98] over adsorption of the protein at these interfaces. With the decrease of the protein concentration at the interface, a decrease in localized concentration of proteins is expected, thereby minimizing risk of protein aggregation and/or precipitation (protein particle formation). The connection between protein concentration, collision rate, and their aggregation or self-association is known from studies of non-ideal solutions [99,100]. Surface tension and rheology experiments [89,90] provide evidence that the prevention of adsorption of the protein (LDH in the present case) at the interface is more related to surface energy and surface pressure than to the CMC. Our own studies with mAbs found that surfactants stabilize antibodies against mechanical stress, and found polysorbates and poloxamers have the ability to prevent aggregation and/or particle formation against shaking at concentrations well below their CMC [25,101].

5. Summary

In summary, nonionic surfactants (e.g. polysorbates 20 and 80, and more recently poloxamer (P188)), are typically employed to stabilize proteins in pharmaceutical parenteral products against adsorption to surfaces and interface-induced aggregation/particle formation. Though the mechanism of this stabilization is not clearly established, based on present understanding, competitive interaction of nonionic surface-active components at the interface (such as the air–water or ice–water interface) is thought to be the main mode of interaction between the protein and nonionic surfactants for the majority of commercial products. In certain cases, interaction and colloidal stabilization of the protein by the surfactant is also found to occur. Specifically for therapeutic proteins and mAbs, further work is warranted to evolve a clearer picture of the interaction between biologics and nonionic surfactants.

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