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Pharma Solutions

White Paper

Alternative Surfactants for Biologics Stabilization

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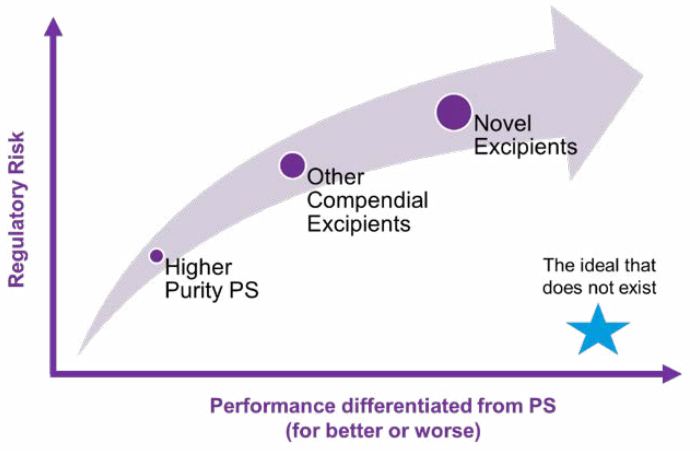
A major challenge in the development of biologic therapeutics is the identification and design of a stable formulation. Due to their complex structure, protein-based biologics can undergo many pathways to unfolding and aggregation, leading to loss of activity and immunogenicity. Excipients are used to help maintain biologics in their native conformation, protecting them from the various forces that lead to destabilization. One of the key mechanisms of destabilization, especially for antibody-based biologics, is interfacial instability, in which the biologic will interact with an interface (air pocket, vial wall, tubing, etc.), unfold to reduce the interfacial tension, and then act as a nucleating point either at the interface or back in solution for further aggregation. To block or mitigate this pathway, surfactants can be added in low level to compete with the biologic for the interface, better keeping the biologic in solution and away from this particular destabilizing force. Polysorbate 80 and Polysorbate 20 are by far the most common surfactants used in parenteral biologic drug products. Originally adopted for parenteral use based on their GRAS status, the polysorbates have gained widespread acceptance through their decades of use. However, in recent years, many concerns about and limitations of the polysorbates have been identified, leading to formulators searching for alternatives to this technology. This white paper summarizes alternatives to polysorbates that are being explored through the industry, highlighting the benefits and drawbacks to each of these technologies.

Polysorbates remain the standard for biologic formulation, working sufficiently well for a majority of formulations that come to market. Regulatory acceptance of polysorbates as an excipient for parenteral use cannot be understated. Given the massive investment required to bring any new drug product to market, anything that can decrease regulatory risk will be viewed favorably by formulators. However, there are challenges to using polysorbates. For some biologics, they simply do not work to stabilize the formulated product, and therefore are not feasible for formulation. Additionally, polysorbates are well known to be subject to oxidative breakdown, and the oxidation can transfer through to the active pharmaceutical, leading to its degradation, loss of activity, and/or increased toxicity. More recently, especially with the development of higher concentration formulations, residual host cell proteins, especially lipase enzymes, have been implicated in hydrolyzing polysorbates into their component hydrophobe and hydrophile. This reaction happens through the ester bond linkage in the polysorbate chemistry and the free fatty acids that are produced can act as nucleating sites for protein aggregation or form oil droplets that increase subvisible or visible particle counts in the formulation. Polysorbates further have a very wide range of acceptable structures and population of hydrophobes based on their monographs in North America and Europe. For example, Polysorbate 80, according to USP, requires only a minimum of 50% oleic

content. Consequently, there can be significant batch to batch variability, leading to product performance variability. To address this particular concern and to be in compliance with the Chinese Pharmacopoeia, several companies have started producing highly purified polysorbates with high content of a single hydrophobe. While these materials do have less batch to batch variability, they are still susceptible to hydrolysis and don't have any improved performance in stabilizing the active pharmaceuticals.[1]

If polysorbates is not effective in your formulation, then consideration must be given to why the polysorbate isn't working and how to best address that challenge. When thinking about alternatives to polysorbates, different excipients will offer different degrees of performance differentiation and different levels of regulatory risk. Unfortunately, there is generally a correlation between higher levels of performance differentiation and higher levels of regulatory risk. The ideal excipient that performs significantly better than polysorbate and carries no addition regulatory risk simply does not exist.

Figure 1. Schematic of how regulatory risk trends with performance differentiation.



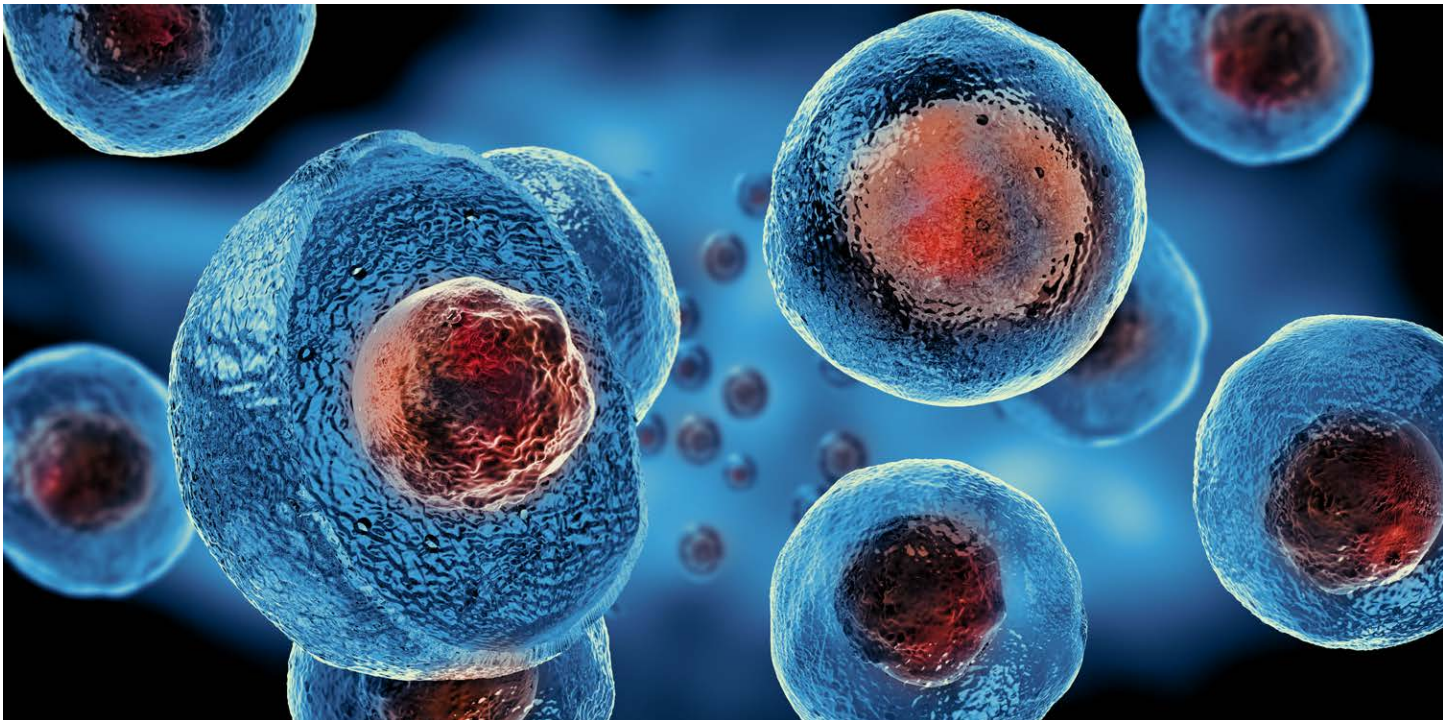
The first excipient that many formulators try when exploring an alternative to polysorbate is poloxamer, specifically, Poloxamer 188 (Px188), a triblock copolymer of polyethylene oxide and polypropylene oxide.[2] While not as commonly used as polysorbates, Px188 is used in several marketed biologic drug products. Additionally, Px188 is frequently used as a cell culture additive during production of the protein. Unlike polysorbate, poloxamers have no ester bond, so they should be stable to host cell proteins. They do have the same polyether structure, however, so oxidation can be an issue. The mechanism of Px188 stabilization is also believed to be different from polysorbate. A significantly weaker surfactant, Px188 is far less efficient at interfacial competition, so for proteins that are particularly interfacially active, Px188 is unlikely to be a viable option. However, Px188 is believed to be able to better stabilize particular regions of instability on a protein itself, providing a different pathway to stabilization. Perhaps because of this mechanism difference, Px188 tends to be used more frequently with non-antibody-based biologics. It is also often used at significantly higher levels (up to an order of magnitude higher) than polysorbates. Understanding the mechanism and pathway of instability is vital for being able to more rationally choose a stabilizing excipient based on the fundamental properties of the excipient.

Borrowing from the small molecule parenteral formulation space, recently substituted β-cyclodextrins (BCD) have been explored for biologic stabilization.[3] Cyclodextrins have been used for decades as solubility enhancers for poorly water-soluble compounds, its mechanism being to form inclusion complexes with the active. A very poor surfactant, the mechanism by which BCD stabilizes biologics is likely very different from the mechanism of polysorbates, and more likely directly interacting with hydrophobic aggregation-prone regions of the protein. While Roquette, the owner of the Kleptose brand-named BCD's, has presented several case studies of biologic stabilization, no data has been published to date exploring the mechanism of stabilization. It is interesting to note that the BCD used in one study showed decreased aggregation both before and after agitation stress. Additionally, while surfactants are typically used at micromolar concentrations, BCD efficacy was shown at tens to hundreds of millimolar concentration.

Continuing to borrow from the small molecule delivery space, a patent was granted to Reform Biologics in 2019 for using hydroxypropyl methylcellulose (HPMC) as a formulation stabilizer for antibodies.[4] A series of different HPMC's were studied, exploring different substitution chemistries and viscosity grades in agitation models of aggregation using both abatacept (fusion protein) and cetuximab (antibody). Low viscosity grades were required, and "E" substitution chemistry was found to perform the best, reducing sub-visible particle formation. However, during agitation, increased foam formation was noted with the HPMC samples and the ability to reduce sub-visible particles was not appreciably differentiated from Polysorbate 80. While HPMC polymers are used extensively in pharmaceutical formulations, especially for modified release solid oral dosage forms, at this time there does not appear to be any injectable products that contain HPMC.

A very common polymer used in pharmaceuticals is polyethylene glycol (PEG). PEG is a water-soluble hydrophilic polymer used in liquid formulations and as a laxative. It is the most common hydrophile in surfactant excipients and, generally, in non-ionic surfactants. It has already been used as a conjugate in parenteral biologic formulations, extending the half-life of the API and shielding the API from immune recognition. While from a regulatory perspective, PEG would be a prime candidate as an alternative to polysorbates, there are several limitations in performance that could minimize its adoption. PEG is an incredibly weak surfactant, in several studies showing that it does not appear to be effective at reducing monomer loss or particle generation.[5] The oxidative breakdown of PEG would also be similar to that of polysorbate. Finally, since PEG is employed to minimize protein interactions in vivo to improve half-lives, it is unlikely that PEG would have an appreciable interaction with a biologic API (unless chemically conjugated) in order to stabilize it.

Similar in structure but more hydrophobic than PEG, polypropylene glycol (PPG), the hydrophobe in Px188, has also



been explored as an excipient by itself.[6] Exploring a range of molecular weights from 425 to 2000 Daltons, PPG was shown to stabilize cetuximab comparably to Polysorbate 80 and better than HPMC or PEG. It stabilized abatacept comparably to HPMC, but also had lower propensity to foam. As an added benefit, PPG also readily passes through ultrafiltration membranes, so it can be added to a formulation ahead of the diafiltration and ultrafiltration concentration step in processing. PPG is still a weak surfactant (though stronger than PEG) and its mechanism of action is more likely one of direct interaction with a protein than through interfacial competition. While the oxidative stability has not been discussed in the pharmaceutical literature, the ether chemistry would suggest that it, too, would have oxidation concerns.

While the aforementioned excipients have been used extensively in pharmaceuticals broadly and may have an easier regulatory path, many of them do not perform the same function as polysorbate and therefore may be limited in their adoption as polysorbate replacements provided the polysorbate is effective for protecting the biologic from the major pathways to aggregation. To explore improvements to polysorbate function, several other surfactants have gained attention by the industry. One series of surfactants are the alkyl polyethers, sold under the Brij tradename by Croda. These surfactants are used commercially in various industrial and personal care applications, but aside from a few topical applications, they are generally not used in pharmaceuticals. As alkylpolyethers contain no ester bond, they address the issue of resistance to host cell proteins, but still may have oxidation concerns due to the polyether. A variety of alkyl chain length/polyether length combinations are available, and several companies have reported exploring these surfactants as alternatives, typically looking at those chemistries that have similar hydrophobes and hydrophilic-lipophilic balance to those of Polysorbate 80 and 20. Brij 58 was reported to reduce monomer loss on agitation similar to polysorbate but was effective at lower concentration,[7] and Brij 92 was found to bind to recombinant human growth hormone comparably to Polysorbate 80.[8] However Brij 35 (also known as L23) was found to increase particle counts during agitation, so effectiveness may be very specific to different surfactant and/or API chemistries.[9] Another potential concern with alkyl polyethers is the relatively low toxicity threshold, NIH TOXNET reporting an LD_{50} in rats of 27 mg/kg, about an order of magnitude lower than was reported for polysorbates.

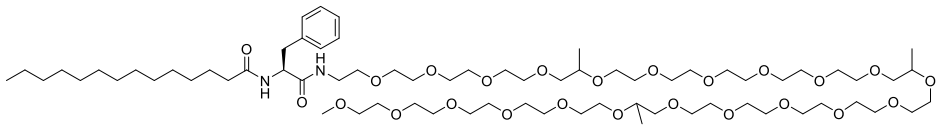
To address concerns about oxidative stability of polysorbates, a series of surfactants utilizing a sugar rather than PEG hydrophile was developed and patented.[10] The main focus of this IP is dodecyl maltoside which was formulated into three different versions for use in nasal, parenteral, and hydrogel formulations. Not previously approved for use in pharmaceuticals, the FDA approved Valtoco, a diazepam nasal spray, which contains dodecyl maltoside in late 2018. In conjunction with this approval, Aegis Therapeutics, the owner of the rights to this surfactant IP was purchased by Neurelis, the owner of Valtoco, and it is unclear if the parenteral formulation platform is still in development.

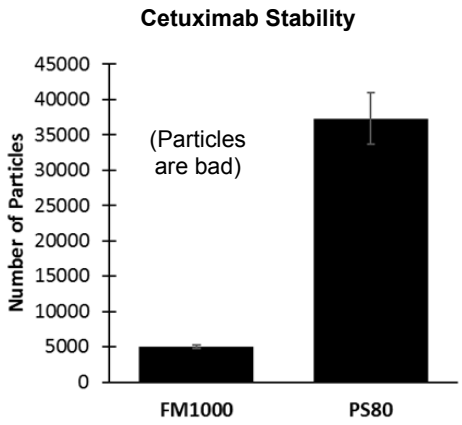
To address several of the limitations of polysorbate and needs in biologic stabilization, a novel chemistry format was developed and patented by DuPont. The N-alkyl amino acid polyether amides are a series of surfactants that link an alkyl tail, amino acid, and polyether hydrophile through amide bonds.[11] The amide bonds resist hydrolysis from host cell proteins (amidases, if residual, would likely attack the API itself before the surfactant present in low levels). Oxidation would be expected to be similar to polysorbates and other polyether chemistries. Because the molecules are chemically defined, there would be very low batch to batch variability. One particular molecule of this series, N-myristoyl phenylalanine Jeffamine M1000 diamide (FM1000), exhibits very rapid interfacial stabilization, coming to an interface an order of magnitude faster than Polysorbate 80 or 20.[12]

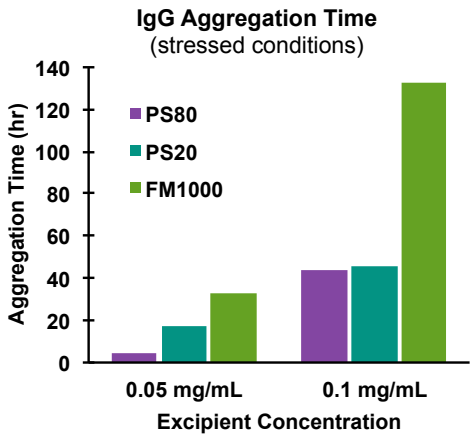
This rapid interfacial stabilization manifests itself through improved biologic stability to agitation and effectiveness at lower surfactant concentrations. FM1000 has also demonstrated that it can improve the thermal stability of some proteins, suggesting that it may additionally have some direct interaction with biologics similar to the mechanism of Px188. This is perhaps due to the small amount of propylene oxide content (the Px188 hydrophobe) present in the FM1000 hydrophile. While as a completely new material, it is not in any drug products today, DuPont is conducting toxicology tests and working with partners to develop it for clinical use.

Figure 2: DuPont's patented & novel experimental surfactant product, "FM1000," offers the Biologics industry a welcomed solution to managing API protein stability issues faced when employing current technologies.

In this white paper, we have reviewed the limited but not insignificant number of novel excipients available and being explored for polysorbate replacement. Analysis of the data shows that different excipients have different mechanisms of stabilization, and knowledge of the dominant mechanisms of biologic destabilization and aggregation for a given API is necessary to help drive the choice of a replacement excipient. The FDA is working on pathways for new excipients. With this forthcoming guidance, the bar to bring new excipients to market should be lowered, enabling innovation to address the challenges facing the formulation community.







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