EXPANDING THE TOOLBOX OF SURFACTANTS AVAILABLE FOR BIOLOGICS FORMULATIONS WITH KOLLIPHOR® HS 15 AND KOLLIPHOR® ELP

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PURPOSE

The purpose of this study was to evaluate the performance of the surfactants Polyoxyl 15 Hydroxystearate (Kolliphor® HS 15) and Polyoxyl-35-castor oil (Kolliphor® ELP) in stabilizing proteins formulations (Table 1). Surfactants are an essential component of biologics formulations, as they stabilize protein therapeutics such as monoclonal antibodies and fusion proteins against destabilizing interfacial and hydrophobic interactions. The current toolbox of surfactants used in approved biologics formulations is limited to polysorbate 80, polysorbate 20, and poloxamer 188, with polysorbate 80 being the most commonly used surfactant. However, it is well established that polysorbates suffer from degradation issues that can cause safety concerns and damage to the active pharmaceutical ingredients (APIs). Therefore, there is an unmet need within the biopharmaceutical industry for additional surfactants that can be used to stabilize biologics formulations for parenteral administration.

METHODS

To study the effects of oxidative stress on protein integrity, we studied α-amylase (BASF SE) as a model protein and evaluated the effects of autooxidation by measuring enzyme activity. Briefly, α-amylase solutions were prepared at 0.5 mg/mL in 10 mM Tris buffer pH 7.1 and incubated in the absence and presence of surfactants for 60 days at RT to study the effects of autooxidation. Kolliphor® HS 15, Kolliphor® ELP, and polysorbate 80 were added to the solutions at concentrations below (0.01% v/v) and above (1% v/v) their critical micelle concentration (CMC). Subsequently, the enzymatic activity of the α-amylase solutions were measured by diluting the samples 1:100 in 50 mM MOPS pH 7.1, 50 mM NaCl, 3 mM $CaCl_2$ and adding a 1:1 solution of ethylidene-blocked-pNG7 (Roche) and α-glucosidase (Roche), and measuring absorption at 405 nm on a SpectraMax M3 photometer (Molecular Devices) for 10 min at 30ºC, and calculating activity using the program SoftMaxPro 5.4.4.

To study the effects of mechanical stress on biologics formulations, we used bovine serum albumin (BSA, VWR Life Sciences) as a model protein and evaluated stability under accelerated stress conditions. Briefly, BSA solutions were prepared at 40 mg/mL in 20 mM Tris-HCl buffer at pH 7.4 containing no surfactant, 0.5% (v/v) Kolliphor® HS 15, 0.5% (v/v) Kolliphor® ELP, and 0.5% (v/v) polysorbate 80 (PS80), where the surfactant concentrations were above the CMC. Samples were then filter sterilized and stressed with a magnetic stirrer at 200 rpm for 168 h at 40ºC, and analyzed at various timepoints using a spectrometer (Infinite® M1000 Reader, Tecan) at 600 nm for visible particle formation, and dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments) for sub-visible particle formation.

RESULTS

Polysorbate 80 is the most commonly used surfactant in biologics formulations for stabilizing proteins against common stresses. Thus, we evaluated the performance of Kolliphor® HS 15, Kolliphor® ELP, and polysorbate 80 in protein formulations by monitoring chemical and mechanical stresses. Excipients used in protein formulations can contain impurities that cause oxidative damage to proteins, thereby reducing their biological activity. Accordingly, we assessed the activity of a model enzyme after 60 days of incubation in the absence and presence of surfactants. In the absence of surfactants, we observed a more than 50% reduction in enzyme activity, however α-amylase maintained its enzymatic activity in the presence of all three surfactants (Figure 1). Additionally, we evaluated the stabilizing effects of the three surfactants in the presence of mechanical stress. When BSA solutions were stressed by vigorously stirring solutions at elevated temperatures, all three surfactants were able to significantly reduce the formation of visible protein aggregates with aggregate formation remaining consistently low for the duration of the 168 h study (Figure 2). Similar results were observed when viewing the formation of sub-visible aggregates with all three surfactants demonstrating comparable performance (Figure 3). Therefore, Kolliphor® HS 15, Kolliphor® ELP, and polysorbate 80 demonstrate similar performance, where they do not contribute to oxidative damage and can stabilize protein formulations against mechanical stress.

CONCLUSIONS

The ever-increasing structural complexity of biologics is a trend that will continue within the industry as we try to treat complex diseases with innovative medicines. Additionally, every biologic has a unique and complex chemical makeup, which necessitates the development of custom-made formulations. With this comes formulation challenges to ensure safe and efficacious medicines can be delivered to patients. To meet these challenges, formulators need access to a larger variety of excipients to screen and identify the optimal formulations. Here we present Kolliphor® HS 15 and Kolliphor® ELP as new tools within the formulator's toolbox to tackle biologics formulation challenges. Both surfactants can stabilize protein solutions against common stresses demonstrating similar performance to polysorbate 80, and they have a history of prior use in FDA-approved parenteral drugs.

Kolliphor® HS 15 and Kolliphor® ELP: Potential alternatives to polysorbates for biologics parenteral formulations

KOLLIPHOR® HS 15 & KOLLIPHOR® ELP

Table 1

The surfactants Kolliphor® HS 15 and Kolliphor® ELP are intended for use in parenteral biologics formulations. They are listed on the FDA IID and have previously been used in FDA-approved parenteral drugs.

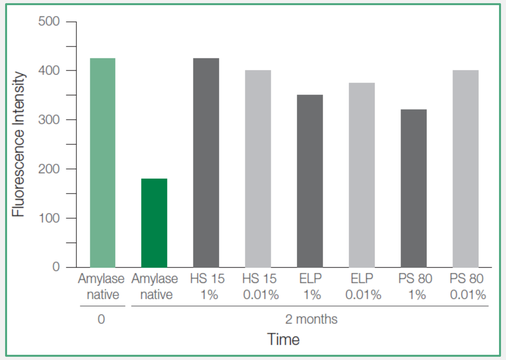
Product	Functionality	Monograph title	Chemical category	FDA IID listing
Kolliphor® HS 15	Nonionic solubilizer and emulsifier (surfactant; HLB = 15)	Macrogol 15 Hydroxystearate (Ph. Eur.) Polyoxyl 15 Hydroxystearate (USP/NF)	Polyethoxylated 12-hydroxystearic acid	Yes[1]
Kolliphor® ELP	Nonionic solubilizer and emulsifier (surfactant; HLB = 12–14)	Macrogolglycerol ricinoleate (Ph. Eur.) Polyoxyl-35-castor oil (USP/NF)	Polyethoxylated castor oil	Yes

[1]Kolliphor® HS 15 is used in a recently FDA-approved parenteral drug. For over ten years Kolliphor® HS 15 has been used in injectable drug formulations in both Canada and Europe.

CHEMICAL STRESS: OXIDATIVE DAMAGE

Figure 1

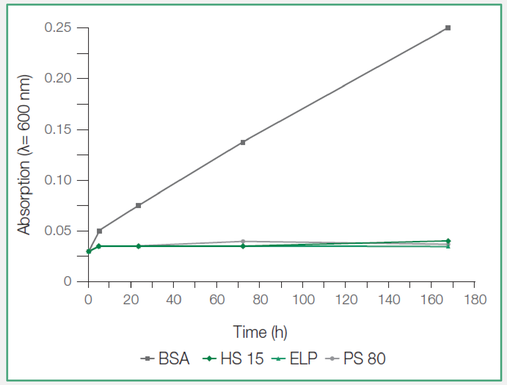
The α-amylase activity assay indicates that the surfactants are not contributing to oxidative damage after 2 months of incubation with Kolliphor® HS 15, Kolliphor® ELP, and polysorbate 80 (PS80).



MECHANICAL STRESS: VISIBLE AGGREGATES

Figure 2

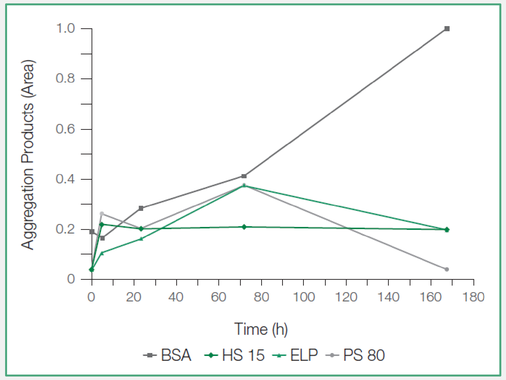
Mechanical stress is modeled by stirring BSA for 7 days with and without surfactants. Absorption measurements show a decrease in the formation of visible aggregates.



MECHANICAL STRESS: SUB-VISIBLE AGGREGATES

Figure 3

Mechanical stress is modeled by stirring BSA for 7 days with and without surfactants. DLS measurements show a decrease in the formation of sub-visible aggregates.



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