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White Paper

Optimizing Poloxamer 188 for Use in Liquid Protein Formulation and Cell Culture Applications

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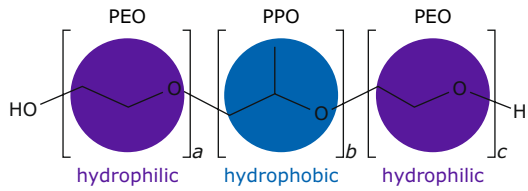
Introduction

Poloxamer 188 can be used as a non-ionic surfactant in therapeutic formulations to stabilize proteins against mechanical and interfacial stresses, and in mammalian cell culture to protect cells from sheer stress.

Poloxamer 188 is a tri-block copolymer consisting of a hydrophobic polypropylene oxide (PPO) chain flanked by two blocks of hydrophilic polyethylene oxides (PEO) (Figure 1). According to the United States and European Union pharmacopeias, the number of units for ethylene oxide and propylene oxide averages 80 and 27 respectively and the average relative molecular mass ranges from 7,680 to 9,510 g/mol. Due to its polymeric nature, every batch of poloxamer 188 can represent a complex mixture of structures with different chain lengths and impurities. As a result, a variance in molecular mass can be observed in batches from different suppliers and between lots from the same supplier.

This white paper describes the stabilization mechanisms of poloxamer 188 and how the molecular weight and hydrophobicity of different poloxamer 188 products dictate which should be used for liquid formulation and cell culture applications.

Poloxamer 188



a, c = 80; b = 27

Poloxamer type	Ethylene oxide units	Propylene oxide units	Content of oxyethylene units [%]	Average rel. molecular mass [g/mol]
188	75–85	25–30	79.9–83.7	7,680–9,510

Figure 1.

Poloxamer 188 structure and characteristics according to European and United States pharmacopeia monographs.

Effect of Molecular Weight and Hydrophobicity on Stabilization

There are different mechanisms by which surfactants can stabilize proteins against mechanical and interfacial stresses (Figure 2).[1] In the absence of a surfactant, proteins can adsorb to interfaces such as air/liquid or ice/liquid, where they can unfold and form protein films covering the surface (Figure 2A). At these surfaces, as well as in solution, hydrophobic patches from the protein core can be exposed to the surface of the protein and then interact with each other via hydrophobic interactions, leading to protein aggregation.

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In competitive surface adsorption (Figure 2B), surfactants cover any hydrophobic surface, preventing proteins from adsorbing onto the surface and keeping them in solution. Because surfactants have a higher surface activity, they can also replace already adsorbed proteins from the hydrophobic surfaces.

Surfactant-protein binding is another mechanism for stabilizing proteins (Figure 2C). In this case, surfactants act as chaperones, stabilizing and solubilizing proteins in solution by binding to them in bulk and covering hydrophobic patches on their surface (Figure 2C).

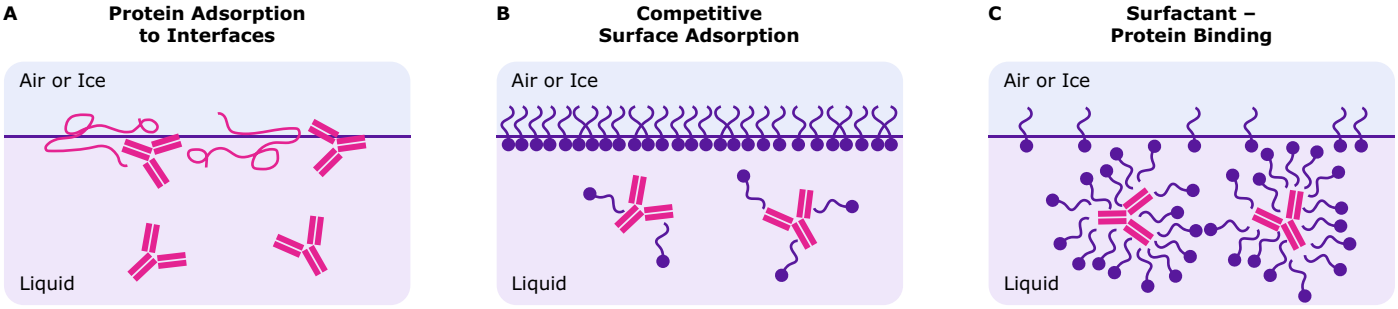

Figure 2.

Protein adsorption to interfaces results in the formation of a protein film and subsequent protein aggregation (A). Addition of surfactants provides protection against interfacial stresses by different protein stabilization mechanisms: competitive surface adsorption with surfactants covering the hydrophobic surfaces (B) or direct binding of the surfactant to the protein molecule, solubilizing the protein (C).

Recent publications have shown that poloxamer 188, with a higher hydrophobicity, can enhance protein stabilization and reduce particle formation in monoclonal antibody (mAb) formulations under stress conditions.[2,3] It has higher surface activity and thus greater coverage of hydrophobic surfaces, causing the protein to remain in solution. In addition, poloxamer 188 with a higher hydrophobicity has a higher affinity to directly interact with exposed hydrophobic patches of the mAb (Figure 3).

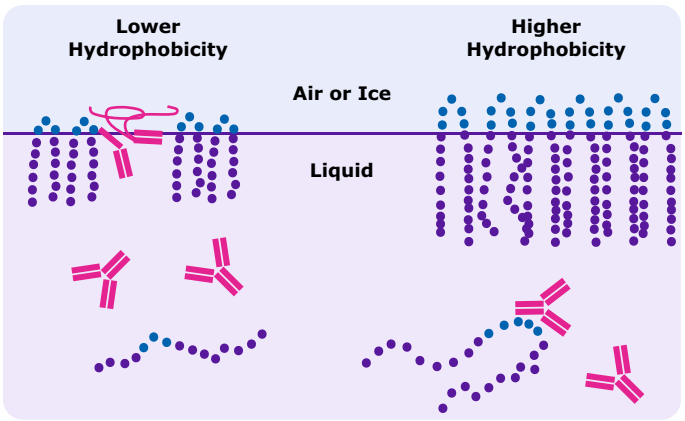

Figure 3.

Comparison of poloxamer 188 with different hydrophobicity and the effect on protein stabilization efficacy. The blue and purple circles represent the hydrophobic polypropylene oxide (PPO) and hydrophilic polyethylene oxides (PEO) blocks, respectively.

Poloxamer 188 can also be used to protect cells from shear stress in bioreactors that results from the mixing and sparging of oxygen. Because cells are hydrophobic, they adsorb to gas bubbles generated by mixing and sparging; these bubbles will eventually burst causing damage to the cells. Cells can be also trapped in the foam and brought to the surface of the bioreactor where there is a lack of nutrients and oxygen.[4,5]

If poloxamer 188 is added to cell culture media, it adsorbs on the surface of the bubbles and with steric hindrance keeps the cells distant.[4,5,6] Poloxamer 188 also decreases the surface tension of the liquid, and the bubble burst will be less powerful, and therefore less damaging to cells. Poloxamer 188 may also adsorb to the surface of CHO cells or insert into their lipid membrane and decrease membrane fluidity, making the cells more resistant to shear stress.[7]

Molecular weight and hydrophobicity have a significant impact on the shear stress performance of poloxamer 188. Poorly performing lots of poloxamer 188 contained high molecular weight and hydrophobic components. We hypothesized that the high molecular weight and hydrophobic component is the root cause of the lack of shear stress protection. In Figure 4, a correlation between percentage of cell viability and PPO block number is shown. An increase of propylene oxide results in a decrease in cell culture performance. If the percentage of ethylene oxide remains constant and equal to 82%, and the number of PPO blocks is varied, the following poloxamer 188 structures are possible:

- PEO_{78}-PPO_{26}-PEO_{78} MW = 8,378 g/mol
- PEO_{84}-PPO_{28}-PEO_{84} MW = 9,022 g/mol
- PEO_{90}-PPO_{30}-PEO_{90} MW = 9,667 g/mol

Lower molecular weight poloxamer 188 with a constant percentage of ethylene oxide results in a lower amount of PPO and higher cell viability and viable cell density. This finding is reported in a patent application filed by S. Wilson and others.[8]

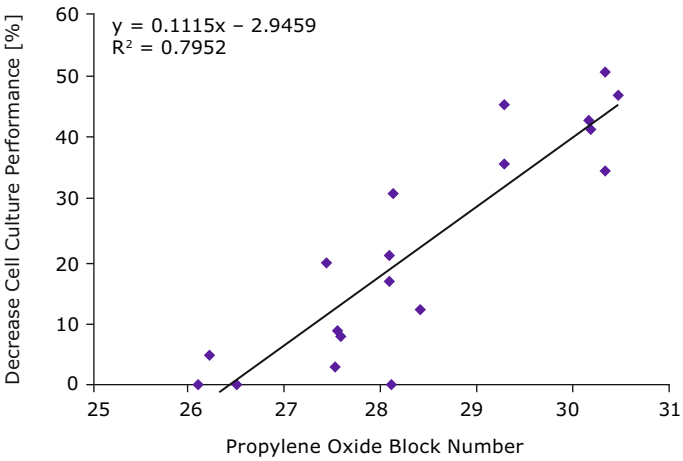

Figure 4.

Poloxamer 188 structure function relationship.

Poloxamer 188 Emprove® Expert Products for Different Applications

Three molecular weight ranges of poloxamer 188 are available as part of the Emprove® Expert portfolio, each optimized for protein stabilization or cell protection.

- 137112 Poloxamer 188 Emprove® Expert Ph Eur, NF is intended for protein stabilization in liquid formulations and all batches evaluated in this study have a molecular weight between 9,100 and 9,400 g/mol, as measured by titration and complies with pharmacopeias.
- The two poloxamer 188 products optimized for cell culture – 137197 Poloxamer 188 Emprove® Expert compendial cell culture grade Ph Eur, NF and 137097 Poloxamer 188 Emprove® Expert cell culture optimized – protect cells from shear stress and have a different range of molecular weight.
- Poloxamer 188 Emprove® Expert cell culture optimized 137097 is intended for cell culture processes without compendial filing restrictions and the molecular weight is determined using size exclusion chromatography (SEC). Poloxamer 188 Emprove® Expert compendial cell culture grade 137197 meets the compendial molecular weight range using the pharmacopeial titration method and is intended for cell culture processes where a compendial product is required.

Table 1.

Poloxamer 188 products optimized to meet different application requirements.

	Poloxamer 188 Emprove® Expert Ph Eur, NF (137112)	**Poloxamer 188 Emprove® Expert compendial cell culture grade Ph Eur, NF (137197)**	**Poloxamer 188 Emprove® Expert cell culture optimized (137097)**
Intended Application	Liguid Formulation	Upstream	Upstream
Molecular weight [g/mol] specifications	7680-9510	7680-9510	7680-9510
Molecular weight [g/mol]*	**9100-9400** (titration acc. to pharma-copeias)	**8500-9200** (in-house SEC method) **7700-8400** (titration acc. to pharma-copeias)	**7800-9000** (in-house SEC method)
Features	**Best protein stabilization; pharma-copoeia compliance**	**Good cell protection; pharma-copoeia compliance**	**Best cell protection**

* Measured and shown in Certificate of Analysis (COA).

Molecular Weight Determination of Poloxamer 188 Emprove® Expert

The number of -OH end groups in poloxamer 188 is determined by titration and this number is used to calculate the number average molecular mass (M_n). SEC measures the molecular mass distribution of the polymer providing different average molecular masses: the number average comparable to the value obtained with titration (M_n), the weight average (M_w), and the peak molecular mass (M_p). Caution should be used when comparing specifications as different values for molecular weight will result from the two methods (Figure 5).

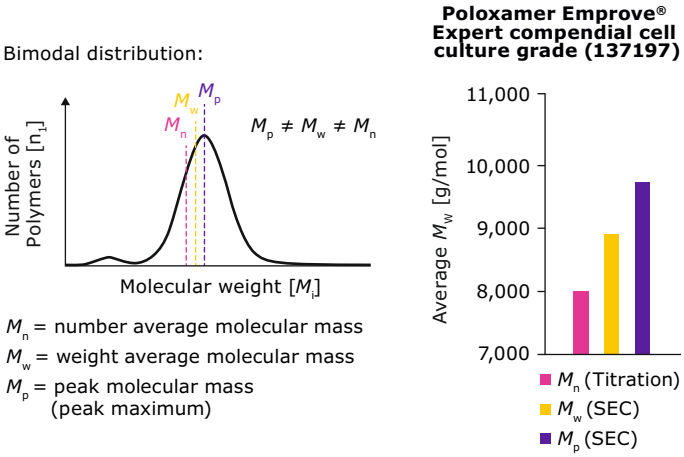

Figure 5.

Average molecular weight determined by titration and SEC cannot be directly compared for a bimodal distribution, showcased using article 137197 Poloxamer 188 Emprove® Expert compendial cell culture grade Ph Eur, NF.

For comparison purposes, the molecular weight M_w of the three Poloxamer 188 Emprove® Expert products was measured by using SEC. Figure 6 shows the distinct M_w values and high batch-to-batch consistency for each of the three Poloxamer 188 Emprove® Expert products optimized for different applications.

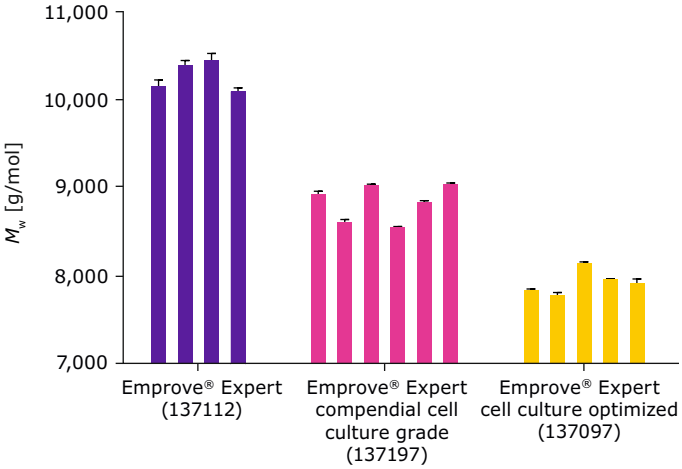

Figure 6.

Batch-to-batch comparison of the molecular weight M_w of three Poloxamer 188 Emprove® Expert products using size exclusion chromatography (SEC).

Composition and Hydrophobicity of Poloxamer 188 Emprove® Expert

A reversed phase (RP)-HPLC method is used to determine the level of hydrophobic species in the Poloxamer 188 Emprove® Expert products as described in literature. Here, the hydrophobic species elute at a longer retention time.[2] 137112 Poloxamer 188 Emprove® Expert Ph Eur, NF for liquid applications has a higher molecular weight and a high amount of hydrophobic species while 137197 Poloxamer 188 Emprove® Expert compendial cell culture grade Ph Eur, NF contains fewer hydrophobic species. 137097 Poloxamer 188 Emprove® Expert cell culture optimized has almost none of these species, and a lower molecular weight (Figure 6 and 7).

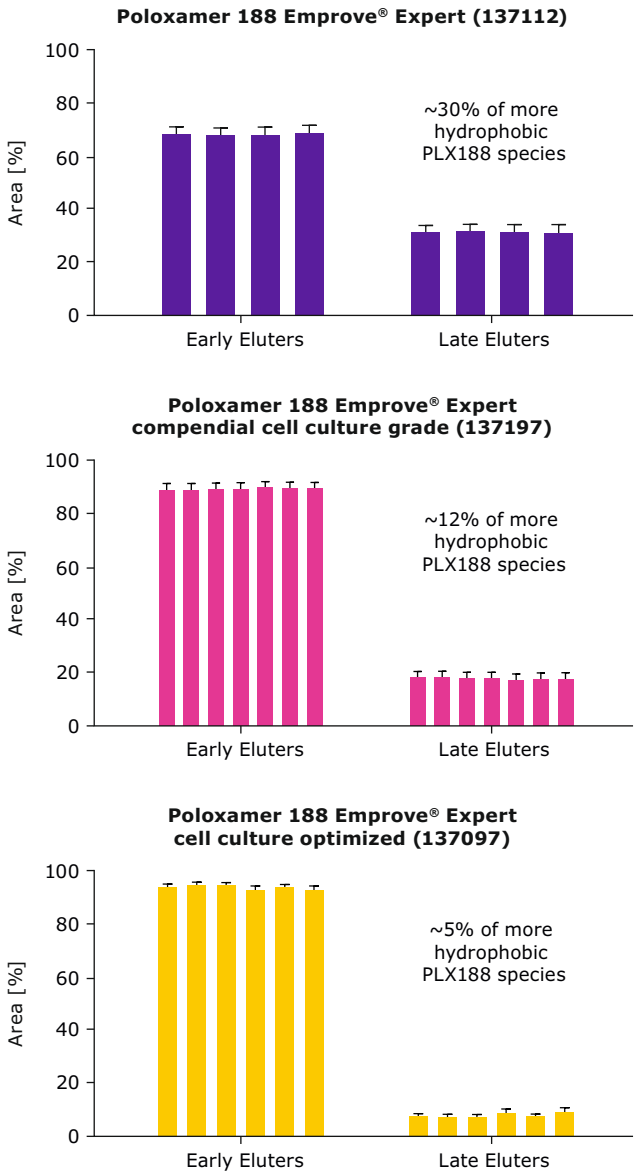

Figure 7.

Reversed phase (RP)-HPLC results comparing the level of hydrophobic species in different Poloxamer 188 Emprove® Expert products: Fraction of "early and late eluters" for different batches of the tested poloxamer products.

Improved Protein Stabilization

According to a recent publication, poloxamer 188 with a higher hydrophobicity offers better protein stabilization due to higher surface activity.[3] To evaluate the surface activity of poloxamer 188, surface tension was measured by using a force tensiometer. In Figure 8A, the general principle of surface tension measurements is shown using an exemplary surfactant. Initially, only a few molecules adsorb to the surface; as more surfactant molecules adsorb to the air-water interfaces over time, the more the surface tension is reduced.

Figure 8B shows the surface tension values for the three Poloxamer 188 Emprove® Expert products. The poloxamer 188 product optimized for liquid formulation, with more hydrophobic species, shows the lowest surface tension values over the measurement time, while the two poloxamer 188 products optimized for cell culture applications have the highest values of surface tension because they are more hydrophilic.

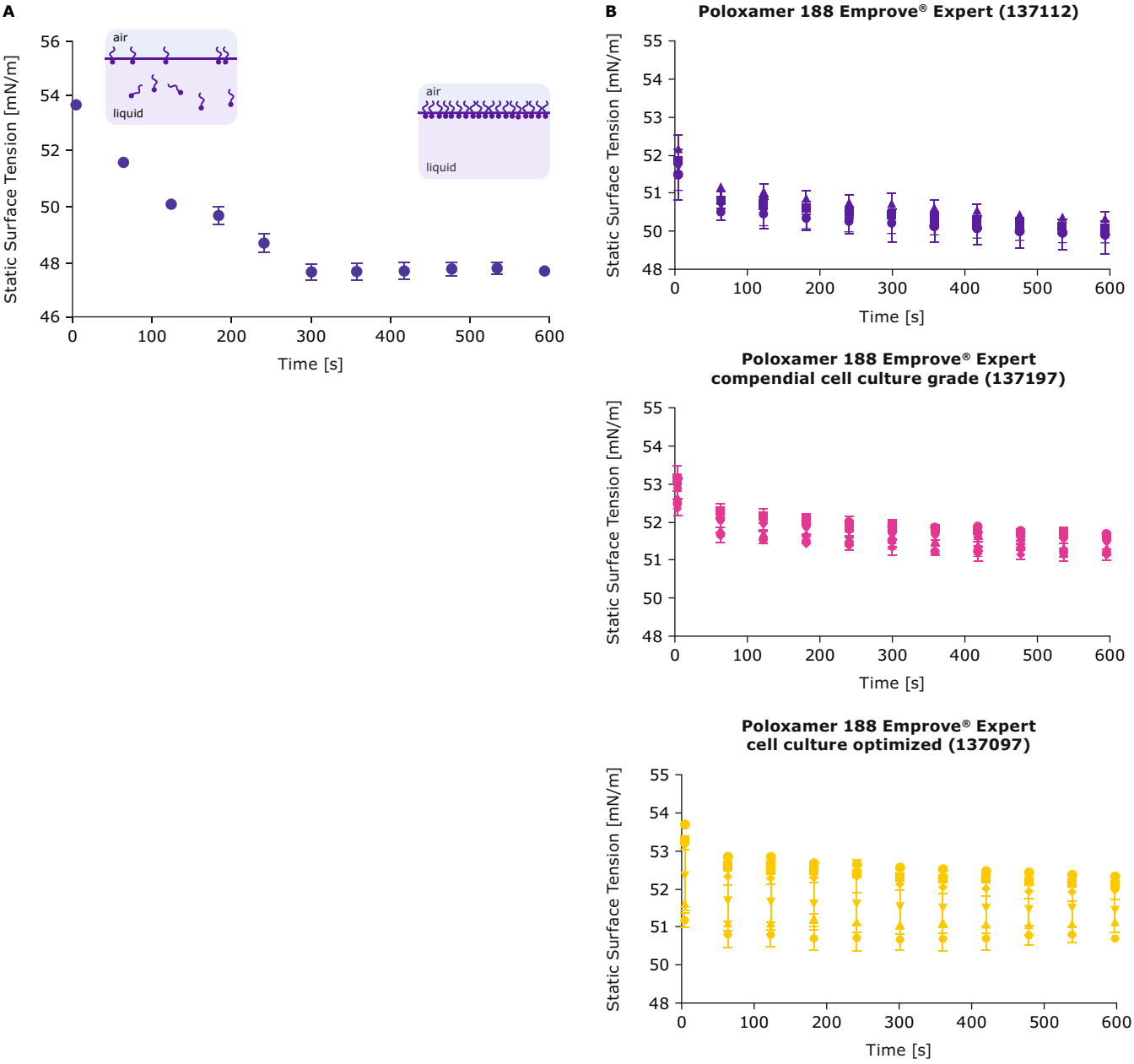

Figure 8.

The principle of surface tension (A) and actual measurements of different batches of the three Poloxamer 188 Emprove® Expert products (B).

Protein stabilization using poloxamer 188 with high hydrophobicity was confirmed using protein degradation studies in which model proteins are stressed by stirring for 24 h at 600 rpm in the absence and presence of surfactants. The resulting level of protein aggregation and particle formation was assessed using turbidity and flow imaging microscopy measurements.

Figure 9 shows the results for the stressed and unstressed controls along with the three different Poloxamer 188 Emprove® Expert products. In comparison to the unstressed sample, high turbidity was observed in the stressed sample without poloxamer 188. The addition of poloxamer 188 resulted in lower turbidity values as the polymer was able to stabilize the protein against stirring stress. When measuring the particle concentration by flow imaging microscopy, a clear trend for the different poloxamer 188 products was revealed. An agitated control without poloxamer 188 was not measured due to its high turbidity indicating that many protein aggregates have been formed potentially causing the clogging of the system.

In the presence of poloxamer 188, particle numbers in the protein sample after stressing were comparable to an unstressed control without poloxamer 188. The lowest particle concentration was observed in the stressed sample that included Poloxamer 188 Emprove® Expert for liquid formulation (137112). This indicates that the poloxamer 188 with a higher hydrophobicity and higher molecular weight can stabilize proteins under stressed conditions more effectively than poloxamer 188 (137097 and 137197) with a lower hydrophobicity.

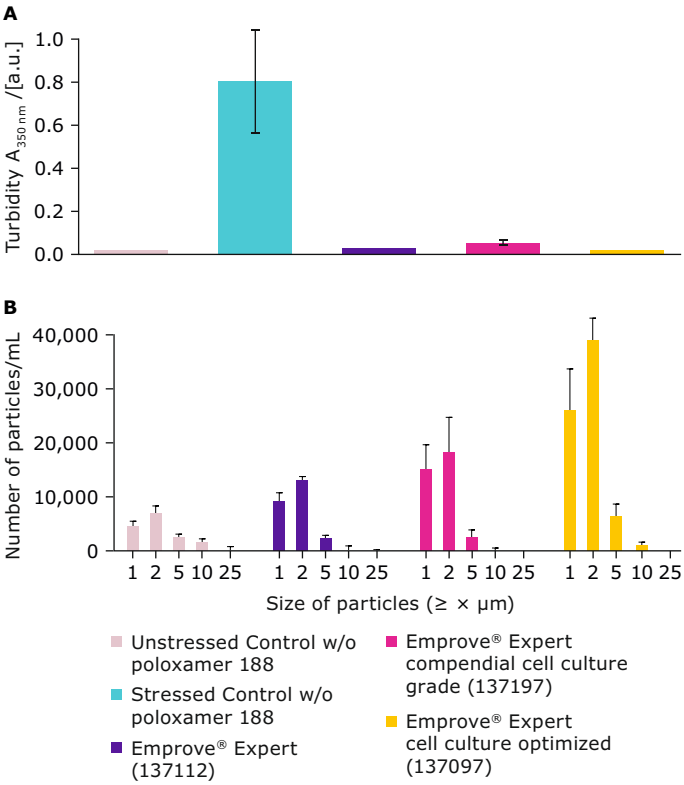

Figure 9.

Formation of protein aggregates and particles under agitation stress (stirring for 24 h at 600 rpm) as shown through turbidity measurements (A) and flow imaging microscopy (B) with different poloxamer 188 products.

Cell-based Shear Protection

A cell-based shear protection assay was performed to assess and classify the protective effect of the three Poloxamer 188 Emprove® Expert products. CHO cells were cultured in baffled shake flasks and vigorously shaken. Different poloxamer 188 lots were added to a proprietary, chemically defined cell culture medium and viable cell density and cell viability were measured for each condition.

The two Poloxamer 188 Emprove® Expert products with low molecular weight and low hydrophobicity (137097 and 137197) were best suited for cell protection as shown in Figure 10.

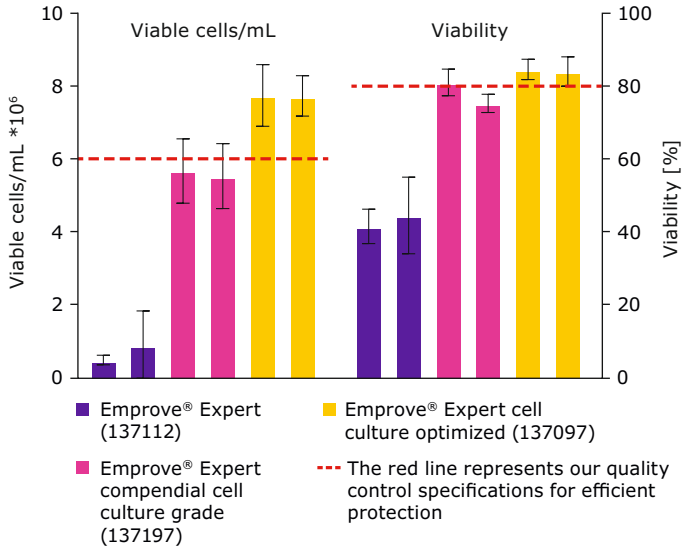

Figure 10.

Comparison of Poloxamer 188 Emprove® Expert products for shear stress protection using shake flask method.

Conclusion

Poloxamer 188 is an important additive in therapeutic liquid formulations to stabilize proteins and for protection of cells from shear stress during mammalian cell culture. Molecular weight and hydrophobicity vary among poloxamer 188 products, and these attributes should be used to select the best option for liquid formulation and cell culture applications. This variability can be present in batches from different suppliers and between lots from the same supplier; as such, batch-to-batch consistency is essential and ensures reliable performance.

To address a range of customer needs, we offer a portfolio of Poloxamer 188 Emprove® Expert products:

- Poloxamer 188 Emprove® Expert Ph Eur, NF (137112) with a high molecular weight and hydrophobicity enables robust protein stabilization in liquid formulations.
- Poloxamer 188 Emprove® Expert cell culture optimized (137097) and Poloxamer 188 Emprove® Expert compendial cell culture grade Ph Eur, NF (137197) with a lower molecular weight and hydrophobicity are best suited for shear protection in cell culture processes. The most suitable option for cell culture applications can be chosen based on whether a compendial product is needed.

The efficacy and reliability of all Poloxamer 188 Emprove® Expert products are assured by high batch-to-batch consistency in terms of molecular weight and hydrophobicity. In addition, all Emprove® Expert products are designed for higher-risk applications where the lowest microbiological and endotoxin levels are needed, supporting a robust risk mitigation strategy.

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