

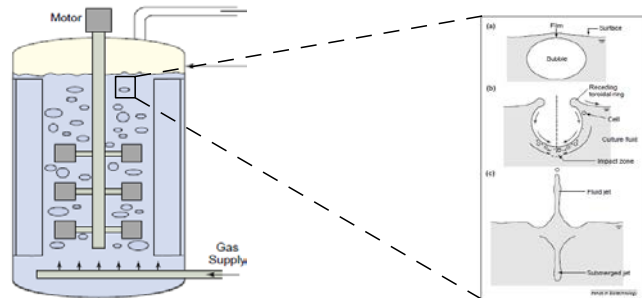
Predictable Protection and Performance

With Poloxamer 188 EMPROVE® EXPERT cell culture grades

Introduction

Poloxamer 188 is a surface-active nonionic polymer used in cell culture media as shear protectant. With time it became a standard ingredient in cell culture media for commercial production processes. It was demonstrated to increase the robustness of mammalian cells to shear from sparging, which probably is the strongest contributor to the hydrodynamic stress in bioreactors.

The new Poloxamer 188 EMPROVE® EXPERT cell culture optimized has been developed to achieve reliable quality and consistency providing shear stress protection for large scale cell culture processes.



- Reduces cell-bubble attachment
- May slightly alter bubble velocity and frequency
- Strengthens cell membranes
- Protective effect on cell surface receptors
- Lower energy level during bubble breakage

Animal-cell damage in sparged bioreactors, Yusuf Chisti, Tibtech, Oct 2000, vol 18

As mammalian cell culture technology improved, e.g., with process intensification through increasing cell densities and productivities in fed-batch and perfusion, issues with Poloxamer started to be reported. Several biopharmaceutical manufacturers reported unexpected loss of cell density and viability in their manufacturing operations which they could correlate to lot-to-lot variation in Poloxamer 188.

This variation became a major pain point in the biopharmaceutical industry and extensive investigations

were initiated to understand the source of lot-to-lot inconsistencies of Poloxamer 188.

The development and validation of proprietary analytical and biological methods based on a reference library of 100+ blinded customer and supplier samples has created the expertise to identify the critical properties of Poloxamer 188.

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Benefits

- **Consistent quality** — With our in-house developed methods we are able to predict the performance and ensure lot-to-lot consistency.
- **Proven functionality** — We test and certify shear stress protection on the Certificate of Analysis.
- **Superior performance** — Our products perform superior to lots across different suppliers, batches and quality grades.
- **Reliable supply** — Large manufacturing capacities ensure reliable supply for our customers.
- **Full transparency** — We provide comprehensive regulatory documentation with our EMPROVE® dossiers.
- **New** — Two different grades available for the use in cell culture applications; both tested with our reliable combination of chemical and biological assays:
 - Accommodating demands for a fully compendial Poloxamer 188 with our new “compendial cell culture grade” (Reference #137197)
 - Focusing on maximum shear stress protection ability with our legacy “cell culture optimized” Poloxamer (Reference #137097) to be used in processes without compendial requirements

Methods

To investigate the reasons for Poloxamer 188 failure, a series of tests and analyses was performed. With the input from industry leading biopharmaceutical manufacturers, various analytical methods have been assessed to identify correlations between physical and chemical properties and cell culture performance of Poloxamer 188. The goal was to find a procedure to reproducibly obtain Poloxamer 188 of reliable protective quality.

Applying a biological as well as an analytical assay, two orthogonal methods have been selected to demonstrate reproducible performance at larger production scales.

Development of a rapid cell-based shear protection assay

A cell-based method to assess and classify the shear stress protective effect was needed.

It has been decided to set up a cell-based test based on vigorous shaking of CHO cells in baffled shake flasks. To increase throughput and shorten time to read-out, it was targeted to stress the cells for a couple of hours only. Shaking speed of the incubator was modified several times to increase selectivity of the assay. A chemically defined cell culture medium was used adding different Poloxamer 188 lots during media preparation. A parental CHO-S served as test cell line. Results could be reproduced with various CHO cell lines (e.g., CHOZN, CHO-K1).

Classification of lots using the rapid cell test

Based on a rapid test, three groups of material were arbitrarily classified for further investigation testing viable cell density (VCD) and viability.

It was observed that a first group of Poloxamer 188 samples had already decreased below 90% viability after 1 hour. This decreased further, with ongoing stress exposure, to values between 70% and 30% after 4 hours. The other groups were relatively close after 1 hour, but spread out over time. Only a few lots remained at very high viability close to 100%*. Control lots for either group had been defined.

This classification was then used to further study the chemical properties of these groups and identify common traits of well performing lots. In parallel, an improved assay was developed to better separate well against poorly performing lots.

*The cell test implemented for standard product release differs from the test described in this section. Reason: A rapid test as above is a high throughput method of relatively low resolution.

Development of an analytical method

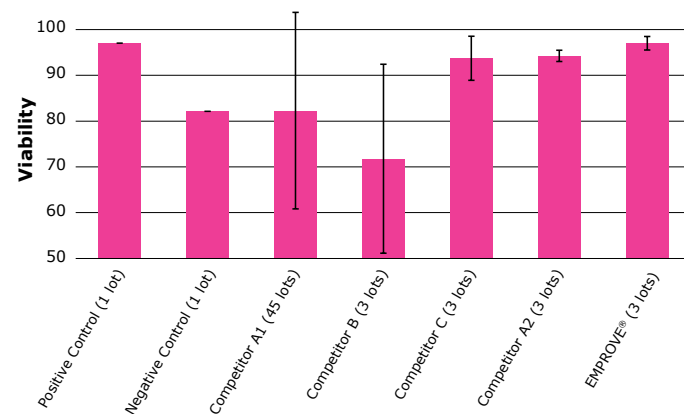
In addition to the biological assay, analytical methods have been developed to compare the molecular weight distribution of different Poloxamer 188 lots. We identified high and low molecular weight impurities with size exclusion chromatography (SEC) as well as hydrophobic impurities with liquid chromatography-mass spectrometry (LC-MS). Based on these results we have established an analytical method to reliably discriminate between well and poorly performing lots.

Performance

Based on results discussed above, Poloxamer 188 properties that positively correlated with shear protective performance could be identified. Several lots of Poloxamer 188 have been produced and compared to the performance of commercially available material.

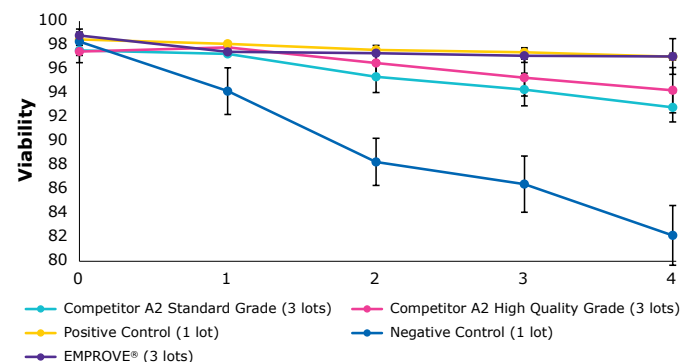
Figure 1 shows a benchmark comparison with other suppliers. The error bars indicate performance variations between tested lots. These show significant lot-to-lot variabilities between multiple lots of the same manufacturer. Each lot was tested in triplicate.

Figure 1. Supplier Benchmark



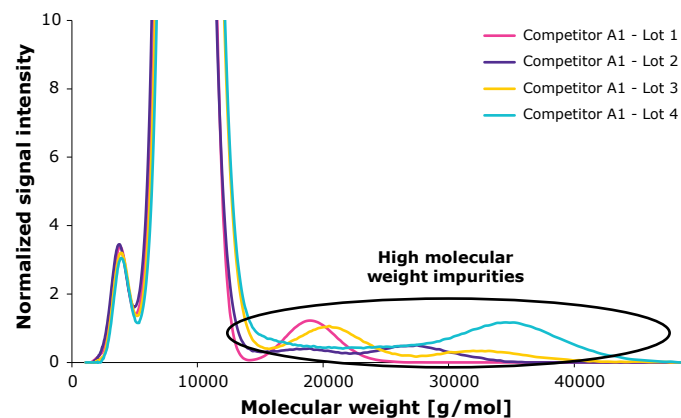
Performance issues in the biopharmaceutical industry were recognized by different manufacturers. This is why new quality grades have been developed. **Figure 2** shows a benchmark comparison between different quality grades. Each lot was tested in triplicate.

Figure 2. Quality Grade Benchmark



High molecular weight components have proven to impact Poloxamer 188 performance negatively, especially when they are hydrophobic. **Figure 3** shows molecular weight distribution of multiple lots of one competitor and the respective viability.

Figure 3. High Molecular Weight Impurities Competition

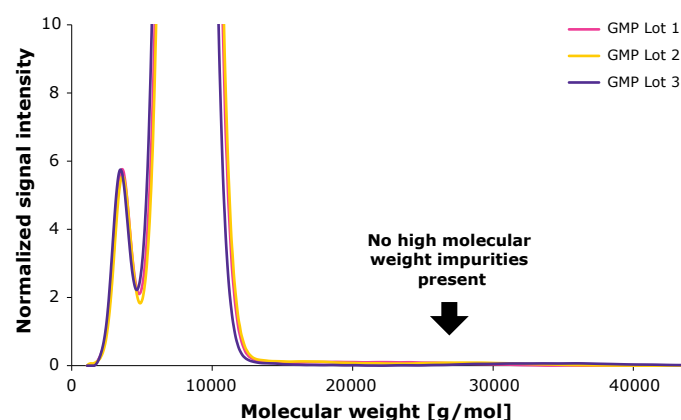


Suspect Lot	Cell Viability
Lot 1	36%
Lot 2	31%
Lot 3	49%
Lot 4	70%

Based on the extensive knowledge gained from our studies we were able to develop Poloxamer 188 with no detectable high molecular weight impurities together with our collaboration partner.

SEC data across multiple lots (**Figure 4**) shows that Poloxamer 188 EMPROVE® does not contain any high molecular weight impurities.

Figure 4. High Molecular Weight Impurities Poloxamer 188 EMPROVE®

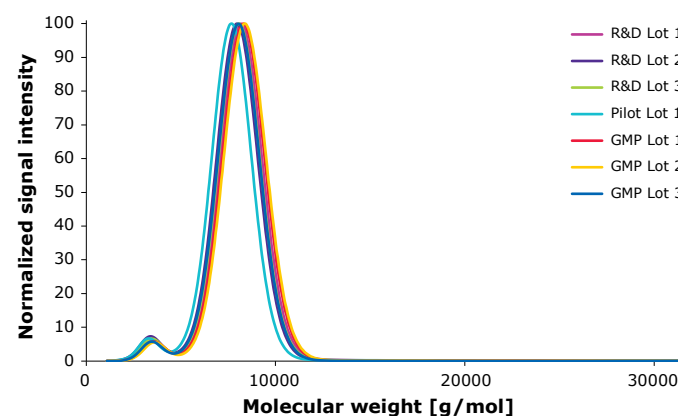


Consistency & Scalability

Biological drug manufacturers have stringent requirements with regard to process consistency and scalability.

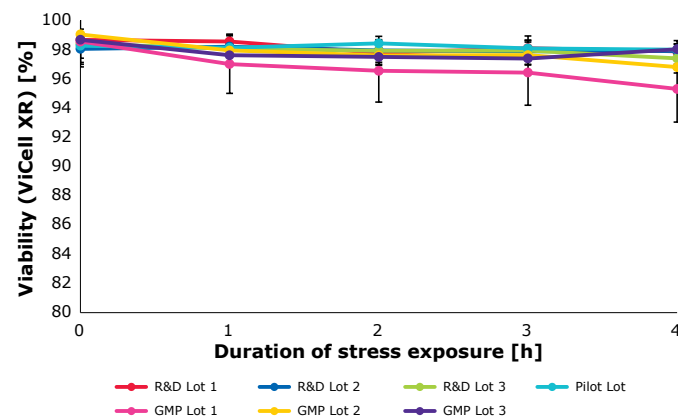
During product development all manufactured Poloxamer 188 lots were tested for lot-to-lot consistency by our analytical assay. **Figure 5** demonstrates very consistent molecular weight distribution for small scale R&D (<10 kg), non GMP (<1000 kg) and commercial scale GMP lots (~ 1000 kg).

Figure 5. Scalability Poloxamer 188 EMPROVE® – Analytical Assay



Also the biological assay proved a constant protective effect of the new Poloxamer 188 EMPROVE® over several production scales (**Figure 6**). Each lot was tested in triplicate.

Figure 6. Scalability Poloxamer 188 EMPROVE® – Biological Assay



Based on the extensive knowledge gained from our studies, we are able to reliably predict the performance of Poloxamer 188 lots. In collaboration with a trusted polymer manufacturer we developed a suitable process, which enables us to offer Poloxamer 188 of consistent quality and proven functionality for cell culture. Poloxamer 188 EMPROVE® EXPERT has been optimized for your biopharmaceutical process from small to large scale production. All presented data referring to “Emprove” or “GMP” Lots is derived from our legacy article #137097, Poloxamer 188 Emprove® Expert cell culture optimized.

Emprove® Program

We market Poloxamer 188 as part of our Emprove® Program, which combines outstanding quality with comprehensive documentation and excellent service.

Each product in the portfolio is complemented with three types of dossiers to help facilitate your qualification, risk assessment and process optimization efforts: Material Qualification Dossier, Quality Management Dossier and Operational Excellence Dossier. They provide information on the manufacturing process, stability data, elemental impurity information, product quality reports, analytical procedures, and much more. The Emprove® Program includes 400 pharma raw and starting materials and a selection of filtration and single-use products.

The dossiers can be accessed online in our new Emprove® Suite. All our subscribers to the Emprove® Suite get 24/7 access to all dossiers of the entire Emprove® portfolio for two years, with up to five accounts per company. The Material Qualification Dossier continues to be available free of charge at [MerckMillipore.com](https://www.MerckMillipore.com)

For more information about the Emprove® Program, visit [MerckMillipore.com/emprove](https://www.MerckMillipore.com/emprove) or ask your local sales representative.

Specifications

Poloxamer 188 Emprove® Expert cell culture optimized (Reference #137097)

The specification has been set in accordance with European Pharmacopeia (Ph. Eur.) and United States Pharmacopeia (NF), all required specification limits and methods are applied except for the average relative molecular mass. In accordance with recommendations by USP, also the lower limit for the parameter "Unsaturation" will be removed, as it does not make sense to define a lower limit for a process impurity.

Due to the nature of our product, the wet chemical determination of the average relative molecular mass gives no convincing information on the real mass distribution/composition of the product. Therefore, we use a fully validated in-house SEC method (Size Exclusion Chromatography) which allows to have information on the real mass distribution. By this method we can ensure that the main component of our product meets the required mass range for Poloxamer 188 (7680-9510 Dalton). As a result of the use of this more robust method, we do not claim any pharmacopeia as part of the product name.

A biological as well as a chemical analytical assay have been defined as release criteria to prove functionality in the biopharmaceutical manufacturing process.

For additional information, visit [SigmaAldrich.com](https://www.SigmaAldrich.com)

To place an order or receive technical assistance, visit [SigmaAldrich.com/customer-services](https://www.SigmaAldrich.com/customer-services)

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Poloxamer 188 Emprove® Expert compendial cell culture grade (Reference #137197)

To offer our customers a fully compendial alternative, complying to European Pharmacopeia (Ph. Eur.) and United States Pharmacopeia (NF), this Poloxamer 188 grade is tested using the compendial wet chemical determination for average relative molecular mass.

Additionally, the parameter "Viscosity" has been added to account for the most current version of the European Pharmacopoeia. All other parameters are the same as for cell culture optimized #137097.

Even though for best possible cell performance we recommend our cell culture optimized grade, for processes restricted by compendial filings for Poloxamer 188, our new compendial cell culture grade #137197 is a safe alternative, ensured by our proven combination of biological and chemical assays.

SEC testing is applied to prove absence of high molecular weight impurities for all grades, likewise a shear stress protection assay is applied to ensure performance in cell culture.

The full product specification can be found at [MerckMillipore.com](https://www.MerckMillipore.com)

Safety Data Sheet

The current safety data sheet can be retrieved from the website: [MerckMillipore.com](https://www.MerckMillipore.com)

Storage and Shelf Life

Product should be stored between +2°C and +25°C.

Minimum 24 months shelf life when stored in unopened original packs in this way.

Packaging and Ordering Information

Our primary recommendation:

Poloxamer 188 Emprove® Expert cell culture optimized

Packaging Unit	Packaging Material	Cat. No.
3 x 1 kg (Sample Kit)	3 x 1,8 L PE wide mouth bottle	1.37097.0003
1 kg	1,8 L PE wide mouth bottle	1.37097.1000
10 kg	35 L Darbox with inner PE bag	1.37097.9010
25 kg	52 L Darbox with inner PE bag	1.37097.9025

Our compendial alternative:

Poloxamer 188 Emprove® Expert compendial cell culture grade Ph. Eur., NF

Packaging Unit	Cat. No.
3 x 1 kg (Sample Kit)	1.37197.0003
1 kg	1.37197.1000
10 kg	1.37197.9010
25 kg	1.37197.9025

