Technical note

J.T.Baker® Cell Lysis Solution Low Foaming

Cells have a lipid bilayer membrane that protects and regulates permeation into the cell. Cell lysis is commonly used in downstream processing of biologic therapeutics to isolate or release intracellular materials such as DNA, RNA, proteins and viral particles. In the case of viral vector production for cell and gene therapy and viral vector vaccines, some viral vector subgroups are not easily released from the cell, thus the cells must be ruptured to release the viral vector particles for further purification.

There are several mechanical and chemical methods for cell lysis [1]. The rudimentary mechanical technique to liberate recombinant adeno-associated virus (AAV) vectors from cells is freeze/thaw cycling followed by a low-speed centrifugation clarification step. However, this technique is not appropriate for large scale purification of AAVs because it is difficult to scale-up. Mechanical homogenization can be used to disrupt the membrane through high shear forces. Although this method is scalable, it has a disadvantage of product loss due to shear stress-induced aggregation and precipitation of viral vectors. Chemical methods such as Octoxynol-9 (also sold as Triton™ X-100,) sodium deoxycholate, and sodium dodecyl sulfate (SDS) have been used as primary detergents for cell lysis 2 as the cell disruption is less harsh than mechanical methods on the protein or viral vector particles being purified.

The use of detergents for cell lysis creates a separate challenge in the generation of foam while processing, which can lead to product loss and manufacturing floor adverse events. Among chemical methods, Triton X-100 has been the preferred detergent for viral vector purification processes due to its superior performance [2]. However, research has shown that Triton X-100 causes acute oral toxicity, eye damage, skin irritation, and chronic aquatic toxicity [3]. As a result, the detergent has been designated a "substance of very high concern" by the European Chemicals Agency's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations, in December 2012 [4]. REACH is a European Union regulation enacted in December 2006 to address chemical safety on both human health and the environment. The detergent has been banned for use in Europe since January 2021 and other regions of the globe may follow [5]. In this study, the foam height generation of the J.T.Baker® Cell Lysis Solution was compared to Triton X-100 to determine whether J.T.Baker® Cell Lysis Solution may potentially create adverse foaming conditions during viral vector bioprocessing.

MATERIALS AND METHODS

In order to assess the foaming characteristics of the J.T.Baker® Cell Lysis Solution compared to Triton X-100, foam height determinations were carried out according to ASTM D1173, Standard Test Method for Foaming Properties of Surface-Active Agents [6]. All foam height measurements were carried out at 25 °C (ambient temperature) using a Ross-Miles Foam Apparatus (VWR Cat# 14007-876) by Wilmad-Labglass. The foam height of J.T.Baker® Cell Lysis Solution and Triton X-100 was measured at 0.1% detergent concentration at time zero and after 5 minutes of hold.

J.T.BAKER® CELL LYSIS SOLUTION FOAM HEIGHT GENERATION

The foam height results at time zero and after 5 minutes of hold time are shown in Figure 1. The foam height generated by J.T.Baker® Cell Lysis Solution was about 28% and 33% lower at time zero and after 5 minutes of hold compared to Triton X-100, respectively. The foam dissipation rate was also quicker for novel cell lysis solution (2.9% per minute) than Triton X-100 (1.6% per minute). The foam dissipation rate was calculated as:

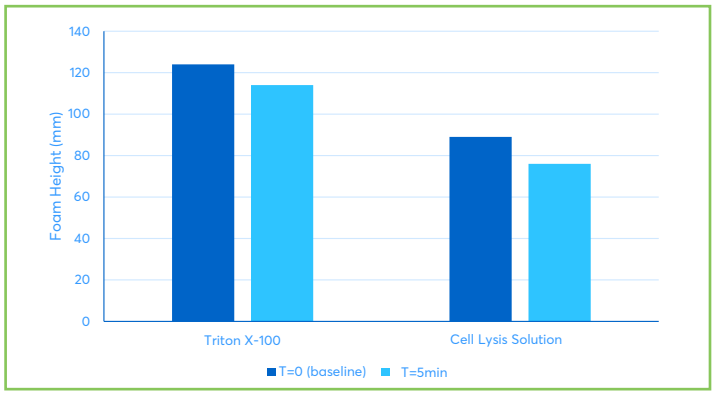

Figure 1: Foam development assessed by Ross Miles foam height analysis at two time points.

$$\textbf{Foam dissipation rate} = \frac{\text{Foam height at t=0 - Foam height at t=5 min}}{\text{time (5 min.)}}$$

CONCLUSIONS

Foaming of detergent-based cell lysis solutions is a manufacturing challenge that must be managed carefully. Excessive foam formation not only creates a mess on the manufacturing floor, but it is also difficult to clean. Generation of foam can also result in loss of yield of the protein or viral vector that is being isolated. Ideally, solutions used for cell lysis should be able to lyse cells without creating too much foam. J.T.Baker® Cell Lysis Solution foams about 30% less and dissipates quicker than Triton X-100. This low foaming makes J.T.Baker® Cell Lysis Solution easy to use. J.T.Baker® Cell Lysis Solution is also ready to use and meets the OECD guideline for readily biodegradable material according to OECD 301.

REFERENCES

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