Variability of Triton X-100 impacts product solution clarity during solvent/detergent treatment

David Briggs, Jonathan Liu, Tom Tomzynski, Kevin Lin, and Steven M. Chamow
Manufacturing Sciences and Technology, Avid Bioservices, Inc., Tustin, CA

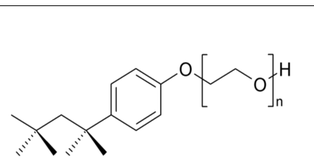
ABSTRACT

Ensuring that high quality raw materials are used in cGMP manufacturing is critical to achieving reproducible process performance. Viral inactivation using solvent-detergent is common in production processes for biologics. The solution clarity of a solvent-detergent treated product pool can be variable after addition of a Triton X-100/TnBP mixture. To determine the cause of this variable appearance, Avid focused on the role of Triton X-100 in preparing this mixture. Avid scientists established that the cloud point of the detergent is lowered substantially by the presence of TnBP and buffer components and, as a result, the Triton X-100/TnBP mixture specified for this viral inactivation step becomes opaque near ambient temperature. Avid scientists concluded that the variation in the quality of Triton X-100 is an important factor in solution clarity. In order to assure solution clarity consistency in manufacturing, a high grade of Triton X-100 with traceability and extensive analytical characterization was identified and implemented. This measure has resulted in consistent solution clarity at the Triton X-100/TnBP step in cGMP manufacturing.

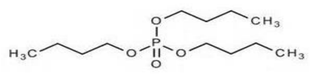
BACKGROUND

Triton X-100 (TX-100) is a polyethylene oxide non-ionic surfactant used as a component in viral inactivation in bioprocessing (1). A physical characteristic of non-ionic surfactants is it's cloud point. Below the cloud point, a clear micelle-based single liquid phase exists. Above the cloud point, the solution becomes opaque due to aggregation of micelles that leads to liquid-liquid phase separation. Different additives may change the cloud point (2). The aim of this study was to evaluate the cloud point of different lots of TX-100 in a TnBP/Tris-sodium sulfate solution with or without protein.

Figure 1. Structures of TX-100 and TnBP



Triton X-100



Tri-n-butyl phosphate

MATERIALS AND METHODS

Four different lots of TX-100 (A-D) were sourced from two different vendor. The cloud point of each lot is shown in Table 1. The TnBP used in these experiments was from Sigma Aldrich (Catalog#90820; Lot#BCBB7359). The test solution consists of Tris, sodium sulfate, without protein or Tris, sodium sulfate, with protein. The final Triton X-100 and TnBP concentration for each experiment were 1% (w/w) and 0.3% (w/w), respectively. To measure turbidity, a Hach 2100P turbiditimeter was used to determine Nephelometric Turbidity Units (NTU). To measure absorbance, a Thermo Scientific Genesys 10 UV monitor was used.

Table 1. Vendor-reported cloud points for the TX-100 used in this study.

Triton X-100 Lot	Vendor-Reported Cloud Point (˚ C)
A (Vendor 1)	66
B (Vendor 1)	63*
C (Vendor 1)	66
D (Vendor 2)	65*

*** As reported on the initial vendor Certificate of Analysis.**

RESULTS

Table 2. 1% TX-100/0.3% TnBP solutions were prepared with buffer only and NTU readings were recorded at 22˚ C.

TX-100 Lot	NTU at 22˚ C
Lot D (Control)	2
Lot A/TnBP	42
Lot B/TnBP	147
Lot C/TnBP	63
Lot D/TnBP	47

Figure 3. Turbidity increases differently with temperature for the various TX-100 lots in solution. A 10% TX-100/ 3% TnBP stock mixture was diluted in buffer (without protein) at 22˚ C and chilled to the temperatures indicated. At each temperature, the turbidity was measured. The reported cloud point for 1% TX-100 in water is 63 – 69˚ C.

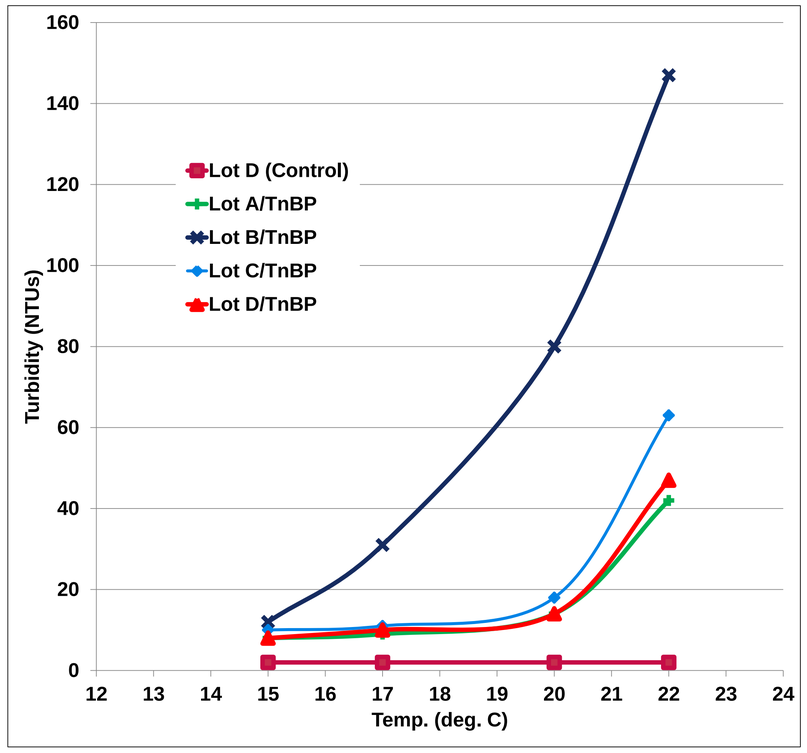


Figure 4. Differences in clarity are independent of protein. The clarity of the 1% TX-100/0.3% TnBP/Protein solution differs with varying lots of TX-100.

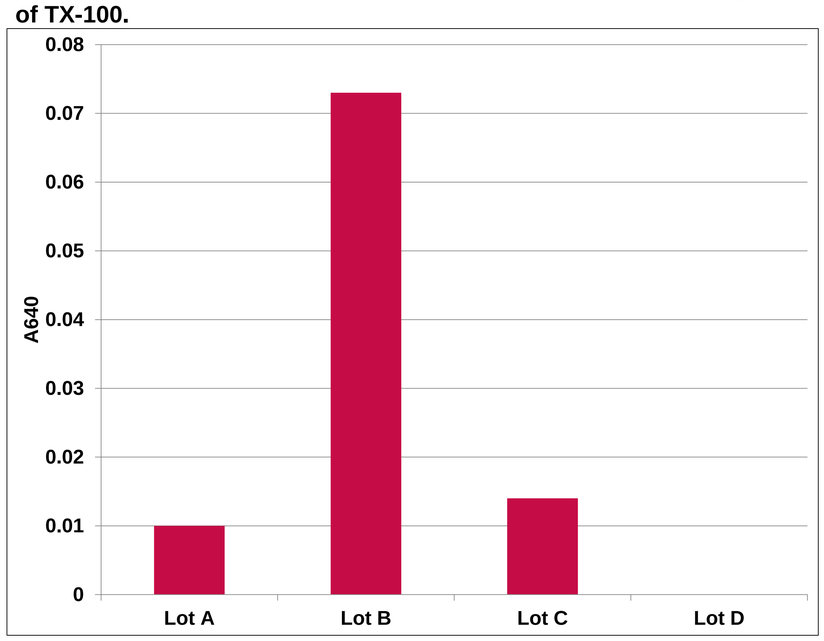


Figure 5. Visual differences of clarity between lots of 1%TX-100/0.3% TnBP solution prepared with buffer only (Refer to Table 2) at 22˚ C. Lot D (Control) was diluted in WFI as a clarity control.

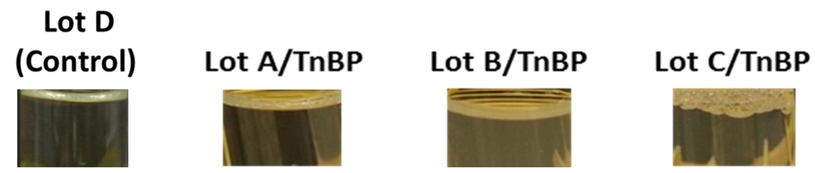


Table 3. Analytical characterization of TX-100 lots is shown below. Based on its extensive analytical characterization, lots D (EP/NF grade) are preferred for cGMP production.

Analytical Criteria	Vendor 1	Vendor 2
Lot ID	A, B, C	D
Grade	R & D	EP/NF
Cloud point (˚ C)	63 – 69 ˚ C	63 – 69˚ C
Identity (IR spectrum)	Conforms to structure	Passes test
Density	1.063 – 1.067 kg/L	1.064 – 1.067 kg/L
Peroxide Value	≤ 10 mg/kg	≤ 10 mg/kg
Assay (HPLC)	Not Reported	90.0 – 110.0%
Identity (HPLC)	Not Reported	Passes test
Avg. # of oxyethylene moieties (H-NMR)	Not Reported	9-10
Hydroxyl Value	Not Reported	85-101
Ethyleneglycol	Not Reported	≤ 620 ppm
Ethylene oxide	Not Reported	≤ 1 ppm
pH Value	Not Reported	6.0 – 8.0

CONCLUSIONS

1. The cloud point of TX-100 is substantially decreased by TnBP in solution that may result in opaque solutions at ambient temperature.
2. This effect is variable; differences in quality of TX-100 can result in opaque solutions.
3. Opaqueness in TX-100/TnBP solutions is independent of protein.
4. Use of EP/NF grade TX-100 is recommended for GMP manufacturing.

REFERENCES

1. Roberts PL, "Virus inactivation by solvent/detergent treatment using Triton X-100 in a high purity factor VIII." *Biologicals* 36(5):330-5 (2008)
2. Koshy L, Saiya AH, Rakshit AK, "The effects of various substances on the cloud point of Triton X-100 and Triton X-114." *Colloid Polym Sci* 274:582-587 (1996)

ACKNOWLEDGEMENTS

We would like to acknowledge Gene Yoshioka, Director of Manufacturing and April Loui, Director of Quality Assurance both at Avid Bioservices as well as both of the Manufacturing and Quality groups at Avid Bioservices.