J.T.Baker® Viral Inactivation Solution Process Compatibility

OBJECTIVE

J.T.Baker® Viral Inactivation Solution (JTB VIS) is a ready-to-use, readily biodegradable[1], nonionic detergent-based solution for inactivation of adventitious viruses in biomanufacturing processes. Unlike the substance group 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol, which includes Triton™ X-100[2], JTB VIS is free of alkylphenols, readily biodegradable and EHS (environment, health, and safety) compliant novel viral inactivation solution. An extensive study conducted by a contract research organization (CRO) demonstrated the effectiveness of JTB VIS in achieving ≥6.5 log of viral inactivation (VI) independent of typical manufacturing temperatures ranging from 4°C to 24°C[3]. JTB VIS is compatible with the structurally diverse monoclonal antibodies (mAbs) and analogous proteins, e. g., bispecific antibody and Fc-fusion proteins, with no impact on product stability. Refer to Avantor Technical Note, *Stability of protein therapeutics with J.T.Baker® Viral Inactivation Solution 110*. Moreover, this detergent can easily be removed from the product[4] and be detected by a simple analytical assay. J.T.Baker® Viral Inactivation Solution Analytical method for detection of active ingredient, method 100-300-1001, is available under an active Confidentiality/Non-disclosure Agreement.

A crucial aspect of a detergent mediated VI (DVI) is its compatibility with the subsequent purification process steps. Typically, clarified harvested cell culture fluid (HCCF) is treated with detergent prior to capture chromatography, with Protein A chromatography the most commonly used capture step for mAbs and analogous proteins. Anion exchange chromatography (AEX) has widely been used in biologics purification. In some biologics drug manufacturing, AEX can be used as capture step. In this study, the impact of VIS treatment on the performance of protein A and AEX chromatography was evaluated in purifying mAb, Fab fragment, bispecific antibody (bsAb), and Fc-fusion proteins.

METHOD

Process compatibility of VIS treatment was evaluated through measuring dynamic binding capacity (DBC), recovery and purity. A frontal chromatographic technique was used to determine DBC of mAb (IgG1) at 10% breakthrough. For the DBC experiments, the VIS treated and non-treated (control) samples of purified IgG1 (2 mg/mL) in phosphate buffered saline (PBS) were loaded onto a 1 mL J.T.Baker® BAKERBOND® PROchievA™, Recombinant Protein A column, and the experiments were operated at residence time (RT) 8 min on a AKTA system.

To investigate the impact of detergent on protein recovery and purity in the subsequent purification step, VIS treated and non-treated (control) harvest of IgG1, Fab, and bsAb were processed over the PROchievA™ column. To verify the impact of VIS on the AEX step, Fc-fusion protein treated with VIS was loaded onto a J.T.Baker® BAKERBOND®, PolyQUAT™ Multimode Ion Exchange column, and a control experiment was run in parallel. The process parameters for PROchievA™ and AEX resin are summarized in Table 1.

Table 1: Protein A and AEX chromatography run parameters

Step	Buffer	Volume CV	Residence time, RT
PROchievA™ protein A resin			
Equilibration	50 mM Sodium phosphate, 50 mM NaCl, pH 7.2	10	4 min
Loading	VIS treated and non-treated harvest	NA	
Wash 1	50 mM Sodium phosphate, 50 mM NaCl, pH 7.2	5	
Wash 2	50 mM Sodium phosphate, 300 mM NaCl, pH 7.0	5	
Wash 3	50 mM Sodium citrate, pH 5.0	5	
Elution	50 mM Sodium citrate, pH 3.0	20	
Strip	100 mM Citric acid	6	
CIP	0.3 N NaOH	5	
PolyQUAT™ AEX resin			
Equilibration (Buffer A)	50 mM Tris-HCl, 25 mM NaCl, pH 7.5	10	2 min
Loading	VIS treated and non-treated protein solution	NA	
Wash	50 mM Tris-HCl, 25 mM NaCl, pH 7.5	10	
Elution (Buffer B)	0-100% Buffer B, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0	30	
Strip	50 mM Tris-HCl, 500 mM NaCl, pH 7.0	10	
CIP	1 N NaOH	10	

RESULTS AND DISCUSSION

The DBC of PROchievA™ resin for IgG is represented in Figure 1. DBC values for the VIS treated and non-treated IgG1 were 86.66 and 86.21 g/L respectively. The DBC data demonstrates that VIS treatment does not alter the binding capacity of protein A resin.

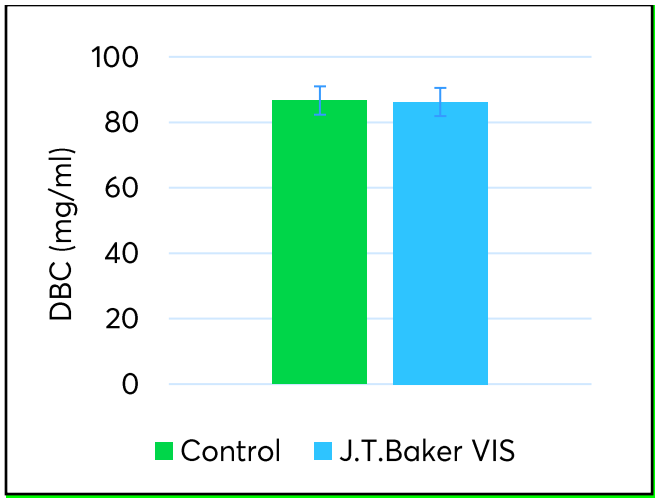

Fig 1: Impact of VIS treatment on binding capacity of Protein A resin

To determine the effect of detergent on product recovery and purity, VIS treated HCCF of IgG1, Fab and bsAb were loaded onto PROchievA™ column, Table 2 represents the summary of product recovery and purity data. No significant difference was observed for protein purity and recovery by PROchievA™ resin.

Table 2: Impact of VIS treatment on product recovery and purity for protein A and AEX chromatography

Process step	Protein molecule	VIS treatment time, minutes	Recovery, %	Purity, %
Protein A (PROchievA™)	IgG1	120	104	97
		Non-treated	100	97
	Fab	120	78	89
		Non-treated	77	89
	Bispecific antibody	120	89	89
		Non-treated	90	89
AEX (PolyQUAT™)	Fc-fusion protein	120	85	91
		Non-treated	86	91

To verify the effect of VIS on the AEX chromatography, the study was conducted using a one-step (protein A) purified Fc-fusion protein. Briefly, the eluate from protein A step was conditioned and treated with VIS prior to processing over a PolyQUAT™ column. A control experiment with PolyQUAT™ was also performed for untreated load. Figure 2 represents the overlay of VIS treated and control PolyQUAT™ chromatograms. The chromatographic profile for both runs were consistent. No significant difference in product recovery and purity was found between VIS treated and control runs (Table 2).

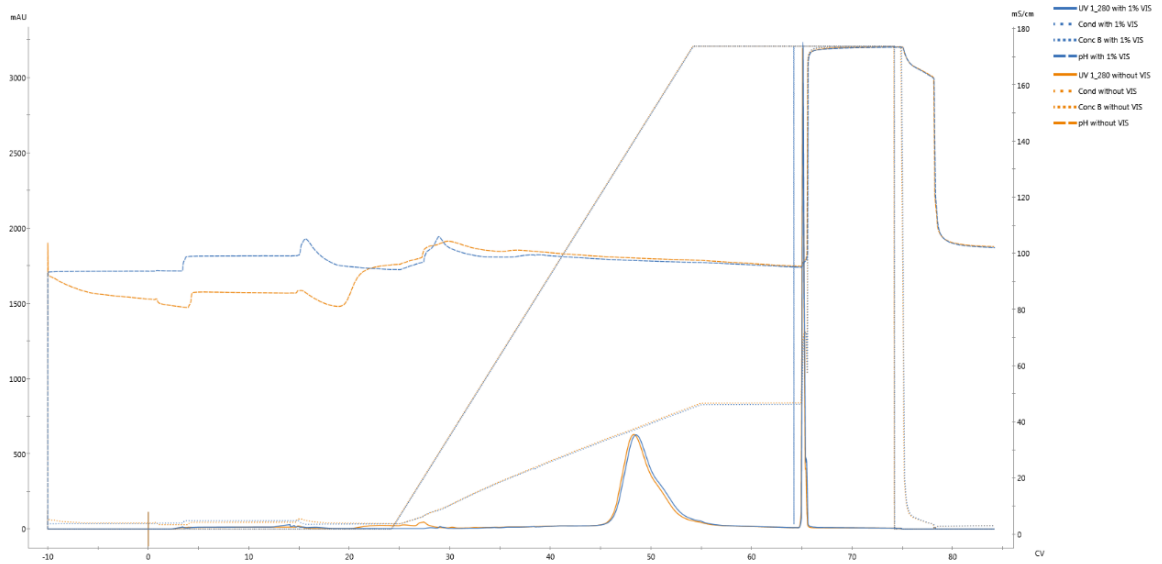

Fig 2: Overlay of the AEX (PolyQUAT™) chromatograms for VIS treated and non-treated load material

CONCLUSION

In this study, process compatibility of the detergent with subsequent chromatography steps was demonstrated by processing the VIS treated load over protein A and AEX chromatography. The experiments were executed using structurally diverse mAbs and analogous proteins. VIS treatment had no notable impact on process performance in terms of binding capacity, recovery, and purity. The experimental data align with the hypothesis that the small, non-ionic detergent having no aromatic ring would be compatible with the most common biologics and manufacturing process steps.

1. ISO 7827:2010, OECD Test Guideline 301F (28d): >60% (10-day window met)
2. Substances restricted under REACH. European Chemicals Agency. Substance Information - ECHA (europa.eu). Accessed on 02 Oct 2023.
3. Alleviating viral safety challenges in biologic manufacturing, Pharma Manufacturing, 04 October 2023.
4. Avantor Lab Report, J.T.Baker® viral inactivation solution (detergent) clearance by purification process. LR 100-200-1002.