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3M Separation and Purification Sciences Division

Post-Use Installation Validation Technical Brief

3M™ Polisher ST Series Capsules

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1. Executive Summary

This Technical Brief details a post-use bubble point installation validation test[1] for 3M™ Polisher ST single-use capsules. The post-use installation validation test helps identify the existence of mechanical defects in the capsule larger than the pore size of the functional membrane, thereby providing a measure of capsule integrity. Performing this test helps reduce the risk of undetected viral clearance loss prior to further downstream processing due to mechanical damage of the capsule which may have occurred either before or during processing. In virus buffer spiking studies, the “pass/fail” criterion of this post-use installation validation test correlates well with the onset of measurable viral clearance loss due to small mechanical defects in the capsules.

2. Important Safety Information

For important safety information, restrictions on use, warnings, and caution statements, refer to the companion 3M™ Polisher ST Installation Qualification Technical Brief.[2]

3. Introduction

3.1. Background

Flow-through anion exchange (AEX) chromatography is frequently used in biopharmaceutical purification processes for the reduction of negatively charged host cell proteins and viral reduction as part of a validated viral clearance strategy.[3,4] AEX column chromatography is the technology most often used for electrostatic viral clearance, particularly in commercial scale biopharmaceuticals manufacturing, where columns have established a long history of well understood performance. Still, validation of viral clearance by AEX columns in biopharmaceutical processing involves complexities which contribute significantly to operational and regulatory costs. Manufacturers must be concerned with the possibility of micro-channeling in columns which may result from defects in column packing, a concern routinely mitigated by *in situ* measurement of the asymmetry of the elution peak resulting from

the upstream pulse injection of an analyte (e.g., acetone) and quantification of the height equivalent to a theoretical plate (HETP) derived from the elution peak retention time and breadth.[5-7] Another concern is the potential for loss of viral clearance with resin re-use, which may extend over hundreds of use cycles with intervening cleaning procedures that have the potential to cause resin degradation.[7,9] An assessment of the effect of resin re-use on viral clearance is thus generally recommended on a product-by-product basis.[3,7,8]

In recent years, the introduction of single-use AEX technologies has illuminated the potential for reduced regulatory and operational costs associated with flow-through AEX chromatography.[6,10-12] Physically resembling and operated like filters, single-use AEX products benefit from improved specific capacity and enhanced flow rates compared with columns due to the replacement of diffusive kinetics with convective flow. These features have led researchers to note the potential for simpler operation, decreased processing times, and reduced buffer consumption leading to improved economics relative to columns. Additionally, their single-use nature obviates validation costs associated with cleaning and performance over repeat use cycles, including viral clearance performance. 3M's recently commercialized family of 3M™ Polisher ST single-use capsules combine porous media functionalized with two different AEX ligands, a quaternary ammonium ligand and a novel guanidinium ligand that mimics the natural cationic amino acid, arginine.[13] The combination of chemistries leads to high area-specific binding capacity and robustness of performance under fluid conditions that have challenged other single-use AEX technologies,[12] creating the opportunity for routine deployment as an alternative to AEX columns at commercial production scale.

Where they are used as part of a viral clearance strategy, it is important that the seals and other mechanical elements of 3M™ Polisher ST capsules be assessed to help reduce the risk of undetected viral clearance loss, for example, due to capsule damage during assembly or shipping. To this end, 3M recommends using a pre-use installation verification test detailed in the Installation and Operation Instructions for each capsule and a post-use installation validation test based on a bubble point measurement. A step-by-step procedure for the latter test is described in a companion technical brief.[2] The basis for the design and pass/fail criterion of the post-use installation validation test are detailed herein.

3.2. Overview of Post-Use Installation Validation Test

It is recommended that the mechanical seals in each capsule are assessed, prior to use, by a pressure-based installation verification test. This test is performed conveniently during the required pre-conditioning flush as detailed in the Installation and Operation Instructions for each 3M™ Polisher ST capsule. The pre-use installation verification test effectively detects seal leaks that might occur, for example, due to internal damage during shipping. Executing this protocol reduces the risk of processing a product-containing fluid with a damaged capsule.

Importantly, 3M recommends that the overall mechanical structure of each capsule is assessed post-use by following a post-use installation validation test. This test is based on a standard bubble point test[1] that has the capability of detecting mechanical defects larger than the maximum pore size of the nominally 0.8-micron functional microporous membrane contained within the capsule. Performing this test helps reduce the risk of undetected viral clearance loss prior to further downstream processing due to mechanical damage of the capsule which may have occurred either before or during processing. As exemplified in Section 5, the post-use installation validation test is effective in detecting very small mechanical defects and provides results that correlate well with the onset of measurable viral clearance loss in virus spiking studies.

To perform the installation validation test following capsule use, the upstream volume of the wetted capsule is drained, after which an upstream air pressure is applied and then increased at a specified rate while monitoring flow of air through the capsule. Initially, only diffusional flow of air occurs through the fluid-filled pores of the 3M™ Polisher ST functional membrane. When the bubble point of the membrane is reached, the upstream air pressure is sufficient to displace fluid from the largest pores in the membrane, the fluid within which is retained by the weakest capillary force. This results in an inflection in the flow rate of air through the capsule at a pressure known as the bubble point pressure. Observation of a bubble point pressure lower than that expected of an integral capsule indicates a risk of a mechanical defect.

Note that the bubble point test should not be used as a pre-use installation verification test. Wetting and air pressurization of 3M™ Polisher ST capsules prior to use may introduce air bubbles between media layers within the capsule that cannot be reliably removed, resulting in impaired performance of the capsule during use.

4. Post-Use Validation Test Procedure and Basis for Pass/Fail Criterion

For a step-by-step procedure for performing the post-use installation validation test, refer to the companion 3M™ Polisher ST Installation Qualification Technical Brief.[2] The test is a standard bubble point test[1] designed to challenge the capsules with an inlet air pressure up to 1,400 mbar, providing a "Pass" if the measured bubble point pressure of the capsule is greater than or equal to 900 mbar and a "Fail" if the measured bubble point pressure of the capsule is less than 900 mbar. The 900 mbar criterion was selected such that

- Integral (undamaged) capsules pass the post-use validation test when they contain functional membrane having a membrane bubble point pressure at the lower limit of its variation in manufacturing; and
- Test capsules purposely damaged with very small, controlled defects, which pass the post-use validation test, exhibit >5 LRV of clearance in viral spiking studies (see Section 5 for details).

To prevent an explosion hazard, capsules should never be pressurized with an inlet gas pressure exceeding 1,400 mbar.

5. Correlation of Post-Use Validation Test Results with Viral Clearance

5.1. Experimental Procedure

3M™ Polisher ST BC1, BC4, BC25, BC170, BC340, BC1020, and BC2300 capsules were prepared with small, controlled defects by introducing holes through the full media stack using blunt-tipped needles of various diameter. In the case of BC1, BC4, and BC25 laboratory capsules, the holes were introduced in the stacked media prior to capsule assembly. In the case of BC170 capsules, holes were introduced in the media in the internal filter lenticle prior to assembling the capsule. In the case of BC340, BC1020, and BC2300 capsules, holes were introduced through one entire lenticle, including media stacks on both sides of the lenticle, prior to assembling the capsule.

BC1 capsules were assembled with 3 different lots of functional membrane. BC4 capsules were assembled with 2 different lots of functional membrane. The "pinhole area percentage" of each damaged capsule was estimated by dividing the calculated cross-sectional area of the needle by the effective surface area of the capsule and multiplying by 100 percent. Control capsules of each size were manufactured with no defects.

Each capsule was prepared for testing by performing the required pre-conditioning flush as described in the corresponding Installation and Operation Instructions. The capsule was then challenged at a flux of 1 mL/(cm^2-min) with 50 mM Tris-HCl buffer, pH 8.0, containing NaCl such that the buffer conductivity was 20 mS/cm and spiked with Phi-X174 bacteriophage to a target concentration of 1×10^8 viruses/mL. To test viral clearance capability, the virus titer was measured before and after filtration through each 3M™ Polisher ST capsule and the virus log reduction value (LRV) was determined.[14,15] The throughput of the virus-spiked challenge for each capsule type is given in Table 1. Finally, a post-use installation validation bubble point test was performed using a Sartorius Sartocheck® 4 Plus Filter Tester with test parameters as described in the 3M™ Polisher ST Installation Qualification Technical Brief.[2] During the post-use installation validation test, the capsule outlet was immersed in water and the pressure displayed by the Sartocheck® instrument at which bubbles were visually observed at the outlet was noted as the "observed post-use bubble point pressure." Thus, in addition to the PASS/FAIL instrument output, the manually observed bubble point pressure was recorded.

Table 1. Virus Challenge Throughput for Each Capsule

Capsule	Throughput
BC1	15 mL
BC4	30 mL
BC25	250 mL
BC170	1.7 L
BC340	3.4 L
BC1020	8 L
BC2300	5.5 L
BC16000	21 L

5.2. Viral Clearance and Bubble Point Test Results

Viral clearance and bubble point test results for undamaged capsules and capsules damaged using various needle hole sizes for BC1 (6 capsules at each hole size), BC4 (4 capsules at each hole size), BC25 (2 capsules at each hole size); BC170, BC340, and BC1020 capsules (1 capsule at each hole size); BC2300 (7 undamaged capsules, 2 capsules at one hole size); and an undamaged BC16000 capsule are shown in Tables 2-9. Figure 1 is a summary plot of observed post-use bubble point pressure *vs*. viral clearance values for all capsule types. Viral clearance was judged to be "robust" if the LRV was greater than 5, corresponding to 99.999% removal. In nearly every case, a Sartocheck® 4 Plus "PASS" value and an observed post-use bubble point pressure greater than 900 mbar corresponded to robust measured viral clearance greater than 5 LRV.

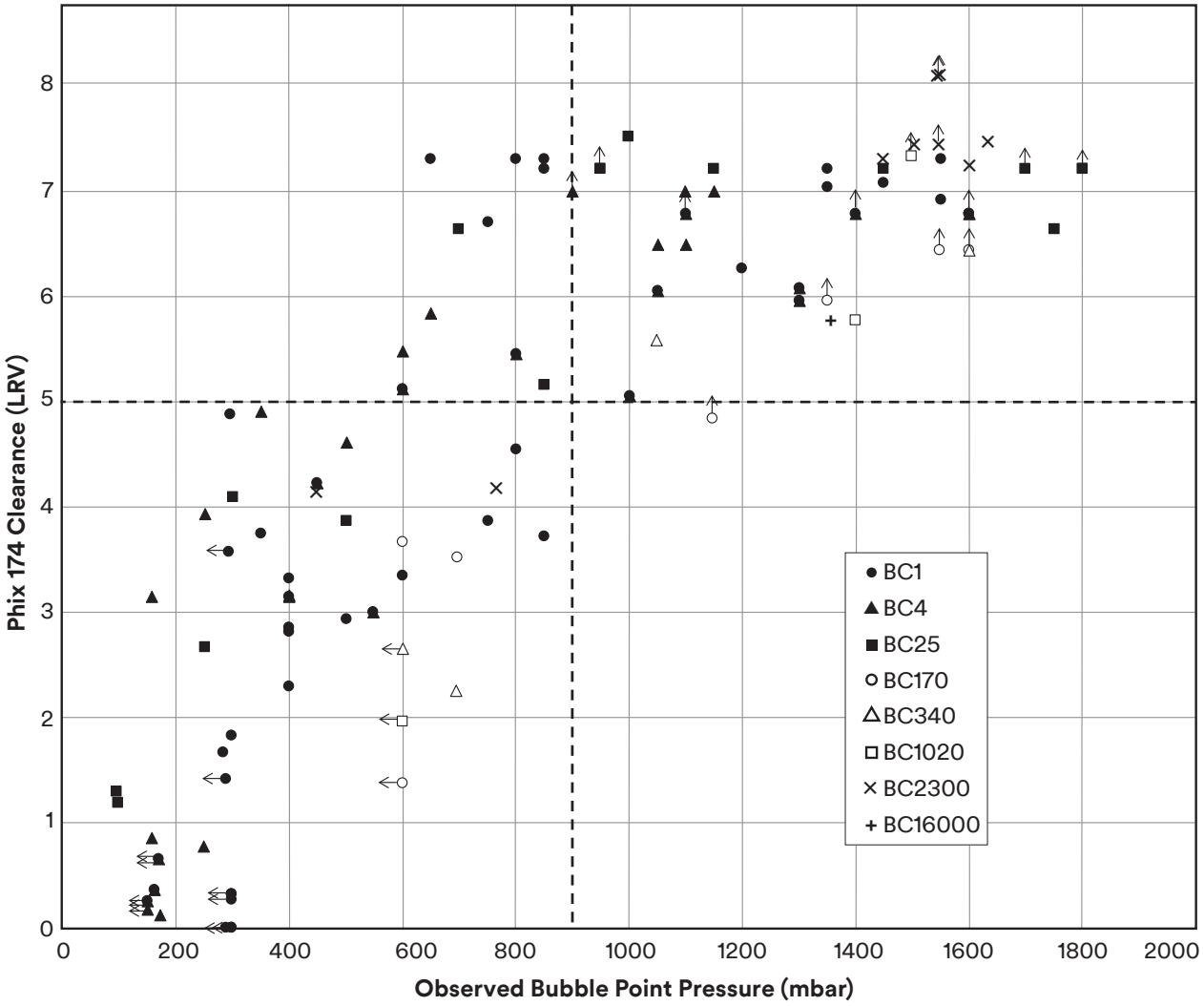

Figure 1. Summary plot of observed post-use bubble point pressure values *vs*. measured Phi-X174 clearance in Tables 2-9. Upward arrows denote viral clearance experiments in which no virus colonies were observed after plating of the filtrate, and the minimum viral clearance was thus defined by the measured concentration of the challenge. Leftward pointing arrows denote bubble point experiments in which bubbles were observed at the outlet before the Sartocheck® instrument displayed a value. Dashed lines highlight the 5-LRV viral clearance level judged to be "robust" as well as the 900 mbar post-use installation validation test criterion.

There were only three exceptions. Two were BC1 capsules, denoted with asterisks in Table 2. In these 2 cases, the viral clearance was less than 5 LRV and, while the manually recorded bubble point pressure was less than the 900 mbar "pass" criterion, as recorded when air bubbles were observed at the capsule outlet, the Sartocheck® 4 instrument returned a "PASS" value. It is thought that these rare occurrences had two contributing factors. First, the resolution of the Sartocheck® 4 instrument with respect to the inflection in air flow at the bubble point is most challenged for the small BC1 capsules. Second, when constructing these damaged laboratory capsules, it was found that the media layers could sometimes shift with respect to one another after introduction of the through-media hole and during capsule welding. This resulted in holes in the various media layers that might be offset from one another. This is not expected to be a likely damage type to occur in manufacturing. In this case, the bubble point of the media stack was higher than in the case where the holes in subsequent layers were "lined up." These results suggest that, for BC1 capsules only, visual observation of the bubble point pressure might occasionally be more conservative than the automated test unit.

In a third case, one BC170 capsule with a 28-gauge hole had a post-use bubble point pressure greater than 900 mbar and had a measured viral clearance of >4.84 LRV. No virus colonies were observed in the plated filtrate in the viral challenge, however, and the value of 4.84 LRV was thus a lower limit of the viral clearance because the concentration of the spike in this particular challenge was insufficient to measure >5 LRV.

With the exception of the above three cases, there was complete correspondence of an automated test "PASS" result with robust viral clearance >5 LRV. Indeed, this test criterion is generally conservative, in that a "FAIL" result may occur from small levels of damage that nevertheless corresponded with robust viral clearance.

The above experiments describe very small, controlled levels of damage in 3M™ Polisher ST capsules. However, seal and capsule damage resulting from manufacturing, shipping, or handling are expected to be much more significant. Thus, the post-use installation validation test described herein is highly effective in identifying mechanical defects correlated with loss of robust viral clearance.

Table 2. Post-Use Validation and Viral Clearance Test Results for BC1 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Functional Membrane Media Lot	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1	1,352	Pass	7.03
				1,351	Pass	7.21
			2	1,551	Pass	7.30
				1,551	Pass	6.91
			3	1,601	Pass	>6.78
				1,601	Pass	>6.78
32	0.108	0.009	1	601	Fail	3.34
				851	Fail	7.21
			2	801	Fail	7.30
				1,451	Pass	7.08
			3	1,401	Pass	>6.78
				1,101	Pass	>6.78
30	0.159	0.020	1	851	Pass*	3.72
				1,201	Pass	6.26
			2	851	Fail	7.30
				401	Fail	3.32
			3	600	Fail	5.12
				1,301	Pass	5.95
28	0.184	0.027	1	501	Fail	2.93
				351	Fail	3.74
			2	651	Fail	7.30
				751	Fail	3.87
			3	451	Fail	4.23
				1,301	Pass	6.08
26.5	0.235	0.043	1	<289	Fail	1.41
				297	Fail	4.88
			2	801	Pass*	4.54
				751	Fail	6.70
			3	1,051	Pass	6.05
				801	Fail	5.45
25	0.260	0.053	1	401	Fail	2.81
				284	Fail	1.67
			2	401	Fail	2.30
				<294	Fail	3.57
			3	401	Fail	3.15
				1,001	Pass	5.05
22	0.413	0.134	1	<300	Fail	0
				300	Fail	1.83
			2	401	Fail	2.85
				<288	Fail	0
			3	<169	Fail	0.65
				550	Fail	3.00
18	0.838	0.552	1	<300	Fail	0
				<300	Fail	0.27
			2	<300	Fail	0.33
				<300	Fail	0.33
			3	164	Fail	0.37
				<150	Fail	0.26

Table 3. Post-Use Validation and Viral Clearance Test Results for BC4 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Functional Membrane Media Lot	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1	1,601	Pass	>6.78
				1,601	Pass	>6.78
			2	1,101	Pass	>7
				1,150	Pass	>7
32	0.108	0.002	1	1,401	Pass	>6.78
				1,101	Pass	>6.78
			2	601	Fail	5.48
				901	Pass	>7
30	0.159	0.005	1	600	Fail	5.12
				1,301	Pass	5.95
			2	501	Fail	4.61
				651	Fail	5.84
28	0.184	0.007	1	451	Fail	4.23
				1,301	Pass	6.08
			2	251	Fail	3.93
				351	Fail	4.91
26.5	0.235	0.011	1	1,051	Pass	6.05
				801	Fail	5.45
			2	1,051	Pass	6.49
				1,101	Pass	6.49
25	0.260	0.013	1	401	Fail	3.15
				1,001	Pass	5.05
			2	401	Fail	3.16
				159	Fail	0.86
22	0.413	0.033	1	<169	Fail	0.65
				550	Fail	3.00
			2	157	Fail	3.14
				250	Fail	0.77
18	0.838	0.138	1	164	Fail	0.37
				<150	Fail	0.26
			2	<150	Fail	0.18
				172	Fail	0.12

Table 4. Post-Use Validation and Viral Clearance Test Results for BC25 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,801	Pass	>7.21
			1,801	Pass	>7.21
32	0.108	0.0004	1,751	Pass	6.64
			701	Fail	6.64
30	0.159	0.0008	1,451	Pass	7.21
			1,701	Pass	>7.21
28	0.184	0.0011	951	Pass	>7.21
			951	Pass	>7.21
26.5	0.235	0.0017	1,151	Pass	7.20
			251	Fail	2.67
25	0.260	0.0021	1,000	Pass	7.51
			851	Fail	5.15
22	0.413	0.005	301	Fail	4.09
			501	Fail	3.86
18	0.838	0.022	98	Fail	1.19
			97	Fail	1.29

Table 5. Post-Use Validation and Viral Clearance Test Results for BC170 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,559	Pass	>6.44
32	0.108	0.0001	1,550	Pass	>6.44
30	0.159	0.0001	1,350	Pass	>5.95
28	0.184	0.0002	1,149	Pass	>4.84
26.5	0.235	0.0003	600	Fail	3.67
22	0.413	0.0008	697	Fail	3.52
18	0.838	0.0032	<600	Fail	1.38

Table 6. Post-Use Validation and Viral Clearance Test Results for BC340 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,559	Pass	>6.44
32	0.108	0.0001	1,050	Pass	5.58
25	0.235	0.0003	695	Fail	2.26
18	0.838	0.0032	<600	Fail	2.66

Table 7. Post-Use Validation and Viral Clearance Test Results for BC1020 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,500	Pass	>7.32
25	0.235	0.0001	1,400	Pass	5.77
18	0.838	0.0011	<600	Fail	1.96

Table 8. Post-Use Validation and Viral Clearance Test Results for BC2300 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,447	Pass	7.31
Undamaged	0	0	1,549	Pass	>8.08
Undamaged	0	0	1,598	Pass	7.28
Undamaged	0	0	1,546	Pass	>8.07
Undamaged	0	0	1,499	Pass	7.44
Undamaged	0	0	1,649	Pass	7.46
Undamaged	0	0	1,548	Pass	>7.46
32	0.108	0.000008	750	Fail	4.21
32	0.108	0.000008	449	Fail	4.14

Table 9. Post-Use Validation and Viral Clearance Test Results for BC16000 Capsules

Pinhole Gauge	Pinhole Diameter (mm)	Pinhole Area (%)	Observed Post-Use Bubble Point Pressure (mbar)	Sartocheck® 4 Test Outcome	PhiX 174 Clearance (LRV)
Undamaged	0	0	1,349	Pass	5.78

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