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Simplifying Progress

The Innovative Flow-Through Solution for Continuous Viral Inactivation

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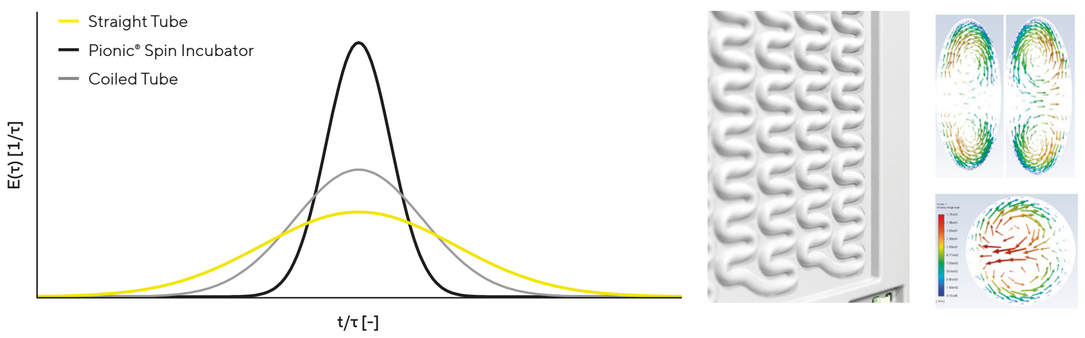
1. Introduction

Virus inactivation (VI) – a regulatory critical step in the downstream processing of active pharmaceutical ingredients (APIs), typically between capture and polishing – reduces active viruses to minimize contamination risk and ensure patient safety. Maintaining viable VI conditions during long-term bioprocessing, particularly in perfusion-feed downstream processing, is a significant challenge. Current methods often struggle to provide the smooth steady state continuous and robust conditions necessary for extended operations, especially at low-pH VI step. This limitation restricts the duration of processes, hindering the efficiency and productivity of biomanufacturing. To overcome this, a solution must enable the autonomous and continuous operation of VI at low-pH for extended periods. The system should be able to titrate the feed in a single step, (e.g., control within ± 0.1 pH) and manage the exposure (incubation) time in a flowing uniform ≥ 30 minute residence time (RT), achieving ≥ 5 Log Reduction Values (LRV) and meeting regulatory expectations for viral safety.

2. Ensuring Reliable and Precise Incubation Time

Pionic® Spin Incubator is a cutting-edge single-use device engineered to enhance VI through a fully closed, irradiated, ready-for-use flow path within intensified continuous bioprocesses. It ensures a minimum RT of 30 to 40 minutes and supports process flow rates ranging from 1 to 22 L/h, operating effectively at viscosities between 1 and 3 mPas.
The incubator's curved design facilitates the formation of Dean vortices in laminar flow, promoting radial mixing of the fluid. This design achieves a narrow residence time distribution (RTD), contrasting with the broad distribution seen in straight laminar pipe flow due to parabolic velocity profiles (Figure 1). Additionally, the device is engineered to minimize pressure drop across the fluid channel's entire length.

Figure 1: *Schematic Representation of the Narrow Residence Time Distribution of Pionic® Spin Incubator Compared With a Coiled Tube and a Straight Tube Based on the Parabolic Velocity Profiles (Left), and Fluid Dynamics Inside a Channel (Right)*

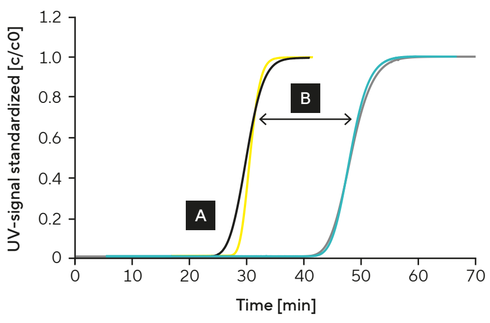


3. Understanding Dean Number: Balancing Flow Rate, Viscosity, and Efficiency

Dean number describes vorticity in a circularly curved pipe and is a function of the flow velocity, pipe geometry and dynamic viscositiy. With an increased flow rate (Dean number should be > 100), the RTD is narrowed, but significant pressure increases are observed. The product portfolio offers various volumes and inner diameters of the fluid channel, accommodating diverse process conditions and ensuring optimal performance.

The effect of the change of process parameters is illustrated in Figure 2: [A] Increased viscosity broadens the RTD and lowers the Dean Number, decreasing here from 125 to 45. [B] Decreased flow extends the overall residence time and broadens the RTD, with the Dean Number decreasing here from 125 to 78.

Figure 2: *Residence Times of a UV tracer in 1.4 L Pionic® Spin Incubator at Different Flow Rates and Viscosities, Highlighting the Change in Residence Time for This Incubator Configuration*



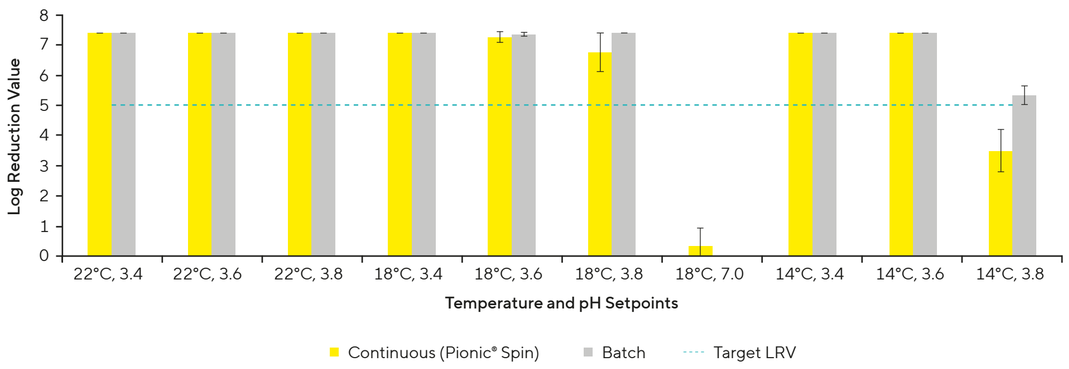
Device	min RT [min]	max RT [min]
1.8 l/h low flow, low viscosity	41.5	56.1
1.8 l/h low flow, high viscosity	41.8	54.7
2.8 l/h high flow, low viscosity	27.3	33.9
2.8 l/h high flow, high viscosity	24.6	35.5

2.8 L/h high flow, low viscosity
2.8 L/h high flow, high viscosity
1.8 L/h high flow, low viscosity
1.8 L/h high flow, high viscosity

4. Effective Inactivation of Bacteriophage: Achieving High Log Reduction Values Under Low-pH Conditions

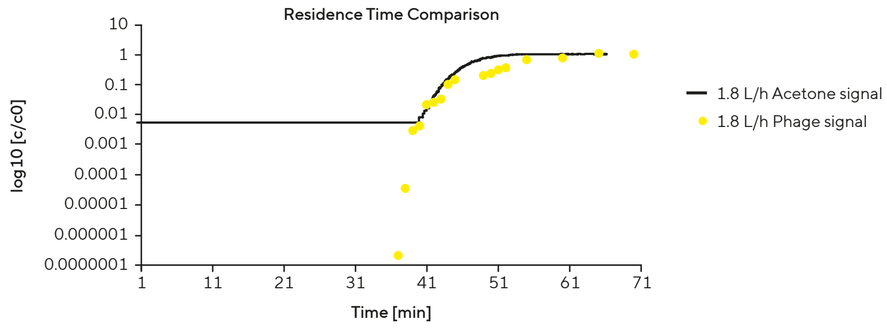
The Bacteriophage Phi 6 stock solution, used as a surrogate model virus for mammalian viruses, was introduced into the incubator (1.4 L) using a syringe pump to achieve a target concentration of 5×10^8 pfu/mL. The study achieved an LRV of > 5 in 30 minutes for most temperature and pH setpoints, indicating effective virus inactivation (Figure 3).
The highest LRV observed was 7.41, with no active phages detected in the plaque assay. Negative controls at pH 7.0 indicated that Pionic® Spin itself does not influence the process of VI. The results were similar between the continuous approach with Pionic® Spin and the batch approach, demonstrating the transferability of the findings. Pionic® Spin effectively inactivated the bacteriophage Phi 6 under low-pH conditions, achieving the target LRV within 30 minutes.

Figure 3: *Log Reduction Values at Different pH and Temperature Setpoints, Comparing Continuous Virus Inactivation Within the Incubator and a Batch Approach as a Reference*



Breakthrough behavior was evaluated in the Pionic® Spin Incubator at a flow rate of 1.8 L/h with an internal volume of about 1.4 L. A Phi 6 phages solution containing 2% acetone was used to generate paired breakthrough curves for acetone and phage. Curves are shown as log-scaled normalized concentration c/c0 versus residence time. The acetone UV tracer establishes the hydrodynamic baseline residence time distribution, and overlaying both curves highlights any shift, broadening, or tailing relative to this baseline. The curves are closely aligned, indicating consistent flow. The Phi 6 Phage solution can be detected at lower concentrations than the UV tracer, which explains the earlier onset at 37 minutes at c/c0 of 0.000035 compared with the UV signal at 38 minutes at c/c0 of 0.01. At high breakthrough, the phage curve shows greater dispersion, with 0.99 c/c0 reached at 65 minutes versus 54 minutes for the UV tracer. Overall, the incubator exhibits comparable residence time characteristics and reproducible performance. Further tests will be conducted across different flow rates, solution viscosities, and incubator sizes to assess scaling effects on residence time and dispersion.

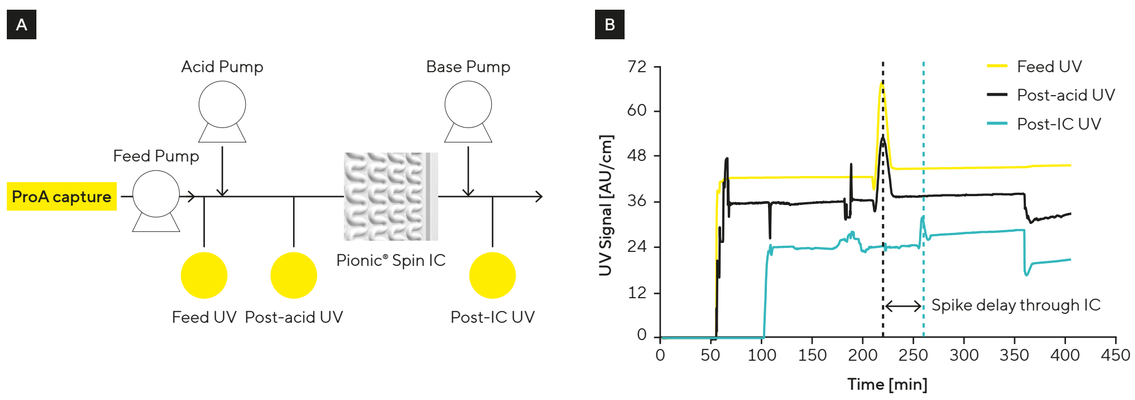
Figure 4: *Breakthrough Curves of an Acetone Solution and a Phi 6 Phage Solution*



5. Meeting Target Residence Time in Continuous Low-pH Virus Inactivation – Sanofi

Inline continuous virus inactivation (cVI) was integrated with continuous Protein A capture and operated for six hours, with two Protein A elution cycles fed into a mixed surge vessel on the Pionic® Spin Incubator (IC) system. Incoming Protein A eluate was continuously titrated to pH 3.5 ± 0.1 using a 5 M acetic acid titrant. The low-pH eluate then flowed through the IC at an average flow rate of approximately 60 mL/min to target an average low-pH inactivation time of 45 minutes, within the target RT (abbreviation for residence time was already introduced) range of 30 – 60 minutes. Spikes in the UV traces in Figure 5 [B] indicate the start of each new Protein A elution cycle. The spike can be traced to approximately 40 minutes after acid addition at Post-IC UV (to be constitent with figure 5 [A]), confirming that the RT of the concentrated pulse matched the calculated RT. Minimal dispersion of the concentration pulse was observed, indicating a narrow residence time distribution (RTD) within the IC. This experiment demonstrates that the IC enabled inline incubation of low-pH-adjusted Protein A chromatography eluate. Future studies will evaluate the longer-term performance of this step.

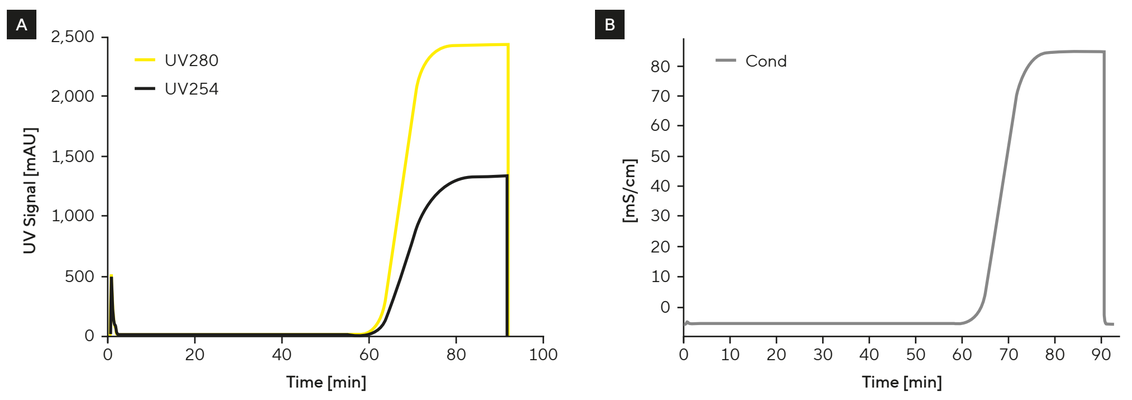
Figure 5: ***(A)*** *Schematic Test Set Up,* ***(B)*** *UV Signals for the Feed, Post Acid Treatment and Post Incubation*



6. Small-Scale Prototype: Flow-Dependent Residence Time – MSD

For applications at low flow rates ranging from 2 to 8 mL/min, small-scale prototypes were developed and provided to MSD for trials focusing on RT (abbreviation was previously used) across various incubator sizes, flow rates, and tracers. This extract from the study presents results from a prototype with an inner volume of approximately 540 mL. The tracers utilized include a 1 M NaCl solution, monoclonal antibodies (mAbs) at 10 g/L with UV280, and mAb at 10 g/L with UV254. Flow rates tested were 8 mL/min and 2.4 mL/min. The findings indicate that, at a flow rate of 8 mL/min, the targeted RT of 60 minutes is achievable. However, at a lower flow rate of 2.4 mL/min, the residence time exceeds the desired duration, which can be attributed to the intrinsic characteristics of the incubator sizes and the resulting flow dynamics.

Figure 6: *Breakthrough Curves for Three Plates in Series With* ***(A)*** *UV Tracer at UV280 and UV254 and* ***(B)*** *Conductivity Tracer 1 M NaCL with 8 mL/min*



Tracer	Flow Rate [mL/min]	3 Plates in Series (540 mL)	
		min RT [min]	max RT [min]
1M NaCl	8	61.82	77.88
mAb@ 10 g/L UV280	8	58.37	76.66
mAb@ 10 g/L UV254	8	58.99	83.80
1M NaCl	2.4	204.01	265.30
mAb@ 10 g/L UV280	2.4	194.09	252.30

7. Conclusion

- Adjustable Residence Time
- Inactivation of ≥ 5 LRV
- Operational Time ≤ 28 Days
- Scalable
- Uniform Residence Time Distributions
- Fully Closed, Irradiated

Last updated: 09 | 2025