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A narrow residence time incubation reactor for continuous virus inactivation based on packed beds

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ABSTRACT

A narrow residence time distribution (RTD) is highly desirable for continuous processes where a strict incubation time must be ensured, such as continuous virus inactivation. A narrow RTD also results in faster startup and shut down phases and limits the broadening of potential disturbances in continuous processes. A packed bed reactor with non-porous inert beads was developed to achieve narrow RTDs. The performance was defined as the ratio between the onset of the cumulative RTD and the median residence time ($t_{x\%}/t_{50\%}$). Laboratory-scale packed columns were used to study the influence of the column parameters on the RTD. A larger column with a void volume of 0.65 L and a length of 89 cm, packed with beads in a size range of 125 to 250 µm, achieved $t_{0.5\%}/t_{50\%} > 0.93$ across flow rates from 0.1 to 9.8 mL/min. The RTD was significantly narrower than the RTDs of other reactor designs, such as the Coiled Flow Inverter and Jig in a Box. The pressure drop remained under 3 kPa for all tested flow rates. Fluorescent nanoparticles (30 and 200 nm) were used to mimic viruses. These two sizes showed less than 2% difference in terms of $t_{1\%}/t_{50\%}$ and $t_{0.01\%}/t_{50\%}$ scores. These results indicated that viruses travelled through the column at rates independent of size. This proposal of packed beds as incubation chambers for continuous virus inactivation is simple, scalable, and can be realized as single-use devices. Due to the low pressure drop, the system can be easily integrated into a fully continuous process.

Introduction

In the development of continuous integrated biopharmaceutical processes, one of the key challenges is continuous virus inactivation, because a defined, strict incubation time must be ensured [1]. This can be achieved with either a semi-continuous approach, which alternates between two batch reactors, or with a fully continuous approach, where an ideal plug flow reactor is desirable [2].

Continuous biomanufacturing is interesting, due to the potential economic benefits, such as the reduced unit operation size, the reduced footprint, and the possibility of working with disposables, even at full scale [3–5]. To render a process continuous, all unit operations need to be operated in a continuous or semi-continuous manner. A narrow residence time is required to ensure a given reaction time, but also to limit the time for starting up and shutting down. The latter point is of particular interest when the process is interrupted.

Regulatory agencies demand two orthogonal virus inactivation/removal steps for cell-culture derived biopharmaceuticals. The first step is the physical removal of viruses with nanofiltration [1]. The second requires diverse methods that depend on the process and product. The commonest ways to inactivate viruses in processing intermediates include low pH inactivation [6], solvent-detergent treatment [7], and ultraviolet C light inactivation [8]. All of these approaches work by exposing the process fluid to conditions detrimental to viruses, and incubating them in the condition for a given time. In the context of continuous operation, the batch incubation time (value) becomes a time distribution.

Operated in batch, low pH inactivation and solvent-detergent

Abbreviations: λ, wavelength; t, residence time; $t_{x\%}$, residence time at which the normalized signal reaches x %; t_{batch}, batch incubation time; t_{mean}, mean residence time; t_{LOD}, residence time at which the signal reaches the limit of detection; F_{LOD}, cumulative RTD at which the signal reaches the limit of detection; LOD, limit of detection; LRV, log reduction value for virus inactivation; LRV_{batch}, required log reduction value for virus inactivation; RV, reduction value for virus inactivation; $RV_{continuous}$, minimum guaranteed reduction value for a continuous virus inactivation; RV_{LOD}, reduction value for virus inactivation for t_{LOD}; RV_{preLOD}, reduction value for virus inactivation for before t_{LOD} is reached; RTD, residence time distribution; CFI, Coiled Flow Invertor; CV, column volume; JIB, Jig in a Box; PMMA, polymethylmethacrylate

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treatment are performed with exposure times of at least 30 min, but typically 60 min [9,10]. Moving towards continuous operation, a narrow residence time distribution (RTD) is of paramount importance to ensure sufficient incubation time and to prevent excess exposure of the product to harsh virus inactivation conditions. Multiple concepts for continuous or semi-continuous operation have been proposed in recent years.

A semi-continuous approach towards continuous virus inactivation that employs two alternating tanks, which mimics a batch operation is currently on the market (Cadence™ Virus Inactivation system, Pall Corporation). A fully continuous virus inactivation system, based on UV-C radiation, is also currently on the market (UVivatec™, Sartorius Stedim Biotech). Recently, a fully continuous virus inactivation system that employs a size exclusion (SEC) column as the incubation reactor was reported [11]. However, those authors did not discuss the RTD or the characteristics of the reactor. Other fully continuous virus-inactivation reactor designs, with long residence times, have been reported recently in the literature, such as the Coiled Flow Inverter (CFI) [12] and the refined Jig in a Box (JIB) concepts [13,14]. Those designs were based on the induction of secondary radial flow patterns, which narrowed the RTD, compared to straight tubes. The efficiency of those reactors varies with the fluid velocity and tube diameter. The tubes employed in those types of reactors are typically very long relative to their diameter, to achieve a narrow RTD. However, that design can lead to back-pressure limitations at larger scales.

A possible continuous virus inactivation setup (Fig. 1) would start with a static or dynamic inline mixer for adding the inactivation chemicals to the process fluid, and an inline sensor, such as a pH probe or a Fourier transform infrared detector, to ensure conditions remain within defined limits. The mixture then enters the incubation chamber. At the outlet of the incubation reactor, there is another inline mixer for neutralizing virus-inactivation chemicals. A 0.22 µm dead-end filter is placed before and after the inactivation chamber to control the bioburden and to remove aggregates, which could form before or during the incubation [1].

In the present study, an incubation reactor for a fully continuous virus inactivation is developed, based on well-established packed beds of non-porous inert beads. Beads with diameters between 100 µm and 1 mm were considered to achieve a narrow RTD and to avoid high back-pressure and size-exclusion effects (i.e., different flow-through times for larger and smaller viruses). There was no need for custom-designed parts, because well-established chromatographic equipment was already available. In addition, the influence of design parameters on the RTD, such as column dimensions, bead size distribution, operating flow rate and the diameter of tracer nanoparticles, was investigated.

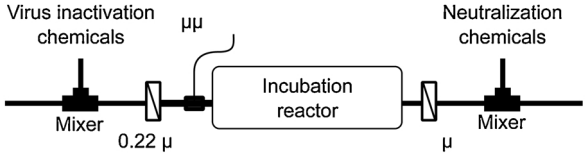

Fig. 1. A diagram of a potential continuous virus inactivation setup. An inline mixer continuously mixes the virus-inactivation chemicals with the process fluid. Next, the mixture passes through a 0.22 µm dead-end filter and the inline sensor (e.g., pH probe or Fourier transform infrared detector), which ensures consistent mixture composition. The mixture then flows through the incubation reactor, and after incubation, it is routed through the second inline filter. The virus inactivation properties of the process fluid are then neutralized by mixing in neutralization chemicals via a second inline mixer.

Materials and methods

Equipment and buffers

Chromatographic columns HR 5, HR 10, HR 16, HS 16, and XK 50 (all GE Healthcare) were packed with non-porous beads. Glass beads (Sigmund Lindner GmbH) of different diameter distributions (0.1 mm – 0.2 mm; 0.2 mm – 0.3 mm; 0.3 mm – 0.4 mm; 0.25 mm – 0.5 mm; and 1.0 mm – 1.3 mm) were used to study the influence of the bead size distribution on column performance. We tested columns with diameters in the range of 5–16 mm and heights between 3.8 and 29.3 cm. Inert beads composed of several different materials were considered for the final continuous virus inactivation application (Table 1). Based on inertness, price, sphericity, density, and robustness, polymethylmethacrylate (PMMA) beads were selected as the most appropriate. A new column was packed with PMMA beads and added to the group of glass-bead columns (Supplementary Table 1).

All RTD characterization experiments were performed on an Äkta Avant™ (GE Healthcare) chromatography system. The outlet concentration was measured by the UV cell of the system. Breakthrough experiments were performed by equilibrating the column with reverse osmosis-H_2O (RO-H_2O), and then switching to a 2% acetone solution. The breakthrough profiles were obtained by measuring the acetone concentration at the outlet of the column, at $\lambda = 280$ nm.

A solvent-detergent mixture/physiological buffer system was used to investigate potential differences in column performance compared to the RO-H_2O/acetone system. The solvent-detergent mixture was prepared with final concentrations of 1% Triton X-100, 0.3% Polysorbate 80, and 0.3% Tri-*n*-butyl phosphate, in the physiological buffer. The content of solvent-detergent chemicals was measured at $\lambda = 300$ nm.

To simulate viruses of various sizes traveling through the column, florescent, virus-sized nanoparticles (Micromod Partikeltechnologie GmbH, Sicastar®-greenF, plain, excitation: 485 nm, emission: 510 nm) of two different diameters (30 nm and 200 nm) were used. A short pulse (< 0.5% of the column volume) of nanoparticles (25 mg/mL) in an aqueous suspension was injected at various operating flow rates. An UltiMate™ 3000 Fluorescence Detector (Thermo Scientific) was used to detect the fluorescent nanoparticle tracers at the outlet of the column. The peak obtained was integrated, and then evaluated as a breakthrough profile.

Column packing

Column packing was performed with a custom-built vibrating column holder. The column was filled with water. Then the column was set to vibrate, as beads were slowly poured in at the top of the column. Column vibration continued, as they immersed in the water. Then, the column was closed and tightened, before vibration was stopped.

Gravimetric testing of the packed columns showed that porosity varied by < 2% for columns packed with the same batch of beads. During 8 months of column usage, we observed no shrinkage of the bed height or decrease in column performance, based on measurements taken in periodic breakthrough experiments, including a prolonged exposure to 1 M NaOH and various aqueous buffers.

Breakthrough profile characterization

Peak-fronting is a primary concern in continuous virus inactivation, because the earliest volume fractions have the shortest incubation times. The narrowness of the RTD was evaluated as the ratio between the time that the concentration of the breakthrough buffer reached a certain small percentage ($t_{x\%}$) and the median RTD ($t_{50\%}$) (Fig. 2a). The closer the $t_{x\%}$ /$t_{50\%}$ ratio is to 1, the closer the onset of a peak is to an ideal plug flow. The Bodenstein number was obtained by fitting a breakthrough curve to the F function, as described previously [12]. In our experience, the F function does not provide a good fit to the