Introduction of PAT to improve the Efficiency and Robustness of Vaccine Development and Manufacturing

BIA separations
Leaders in Monolith Chromatography

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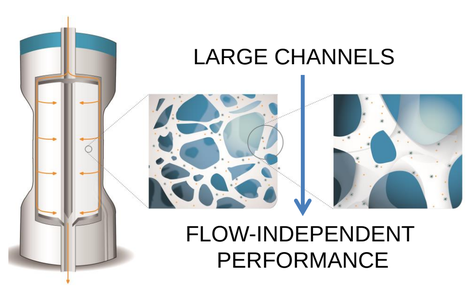
Challenges in monitoring the quality of vaccine production

- Process Analytical Technology (PAT) ensures process reproducibility in bioprocessing
- A mechanism to design, analyze and control pharmaceutical manufacturing processes through the measurement of critical process parameters (CPP) which affect product quality attributes (CQA)
- Initiated by the FDA as part of the 21st Century GMP initiative in 2001 with the goal of increasing productivity
- Application of PAT in vaccine development and manufacturing is challenging due to the sample complexity and batch-to-batch variability.
- During the development of an up- and/or down-stream process of the target biomolecule, a fast, accurate and reliable analytical method is requried for determining the quantity and purity of the product intended for human use..

Solution: Convective Interaction Media Monoliths

- Monoliths are chromatography media cast as a single block, inserted into a housing
- Highly inter-connected network of channels (1-2 μm) containing functionalised binding sites for large biomolecules (viruses, VLPs, pDNA, antibodies)
- Performance unaffected by increasing the flow rate or molecular size

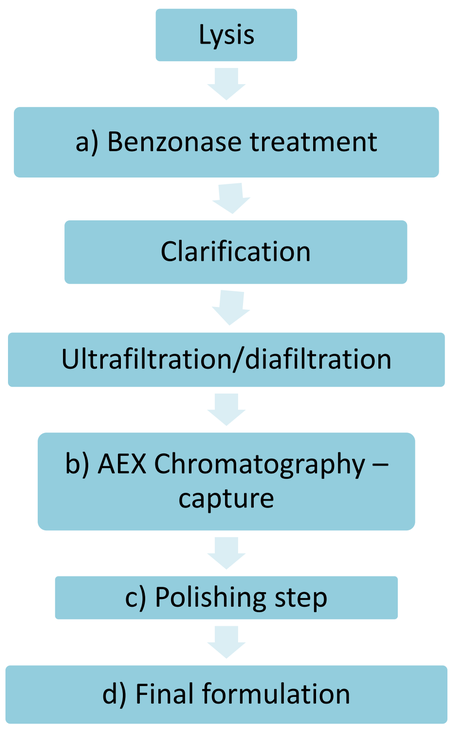


CONVECTIVE MASS TRANSFER

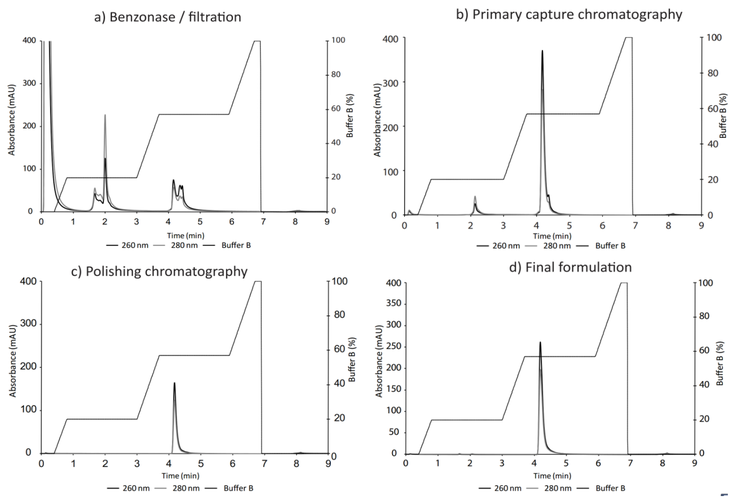
FLOW RATE - INDEPENDENT RESOLUTION

In-process Analysis of Adenovirus production

- Ad5 is the most frequently used adenovirus serotype. Easy to produce in culture, replication deficient, high transduction rate, DNA readily manipulated to insert transgene of clinical importance, does not integrate in production host genome;
- Used in Gene Therapy, Cancer Therapy, Vaccines;
- Non-enveloped, 80-110 nm diameter, ~150 MDa, Icosahedral symmetry → ideal substrate for monolith chromatography with large pores (2 μm);
- **Challenges**: Limited stability, Shear-sensitive, Closely associated impurities →'gentle' and fast chromatographic method required for purification /analysis;
- Current quality control assays: i) **Plaque assay** (measures active viral particles (IU/mL); requires at least three days to develop plaques; ii**) OD_{260} measurement;** provides a Surrogate measure for total particles (TP/mL); can only be applied on purified product; until last steps of downstream process; iii) **qPCR** (measures viral DNA); interference from small amounts of impurities, variable results; iv) Western blot (specific, not quantitative), v) **Bradford Assay** (unspecific, quantitative), vi) **PicoGreen** (measures dsDNA concentration, unspecific)
- **vii) Anion Exchange-High Performance Liquid Chromatography**: Rapid, quantitative method, can be applied at any stage of process development or process monitoring; **suitable for PAT.** Use of **monolith chromatographic support** avoids limitations due to large size of viral particles which limit separation/quantitation of conventional HPLC.
- After method validation, monolith HPLC-based analysis of process development can be applied as an in-process assay to evaluate sample purity within minutes.

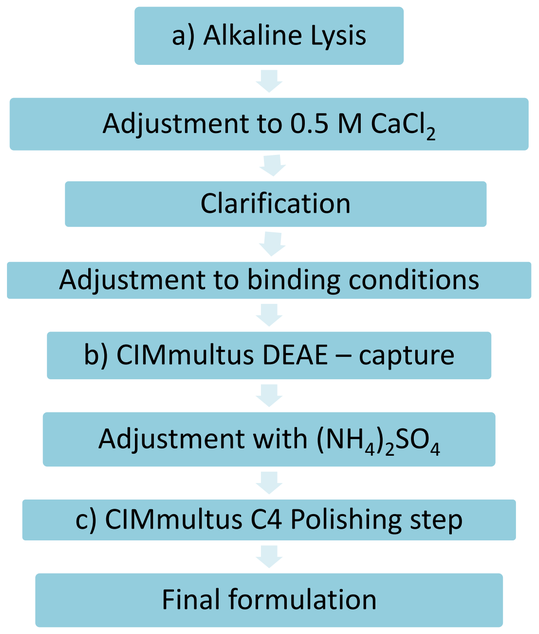


In-process control of Ad5 purification

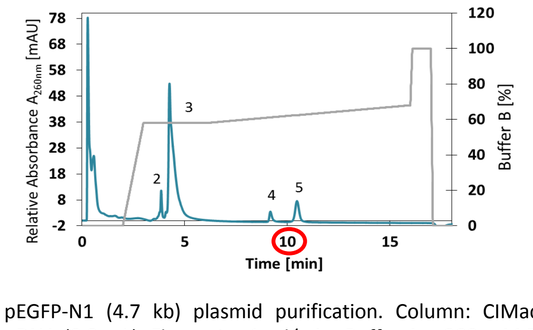


Column: CIMac QA. Column volume: 0.1 mL. Flow rate: 1 mL/min.
Ad5 cellular lysate Injection volume: 25 µL. Mobile phase A: 20 mM Tris-HCl, pH 7.5; Mobile phase B: 20 mM Tris-HCl, 1.5 M NaCl, pH 7.5. Gradient: linear gradient of 0 to 100% buffer B over 19 CV, followed by a 2 CV hold at 100% B. Courtesy of Whitfield *et al* (2009).

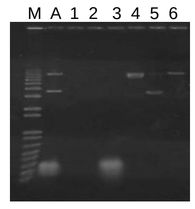
In-process Analysis of pDNA purification



a) After fermentation

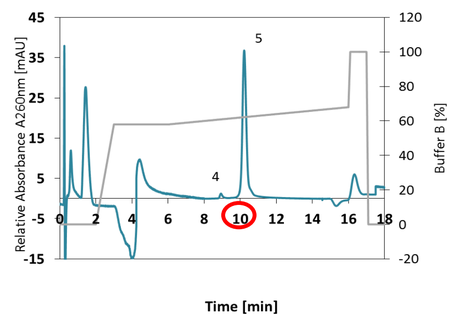


pEGFP-N1 (4.7 kb) plasmid purification. Column: CIMac™ pDNA (0.3 mL). Flow rate: 1 ml/min; Buffer A – 200 mM Tris pH 8.0 and buffer B – 200 mM Tris, 1 M NaCl pH 8.0. Peaks 1 and 2 –impurities, Peak 3 – RNA, Peak 4 – OC pDNA, Peak 5 – SC pDNA.



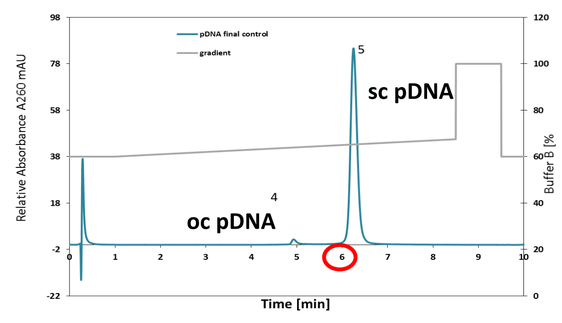
M: Molecular weight marker; A: alkaline lysate plasmid pEGFP-N1; 1: peak 1; 2: peak 2; 3: peak 3; 4: peak 4; 5: peak 5; 6: oc pDNA standard

b) Primary capture chromatography



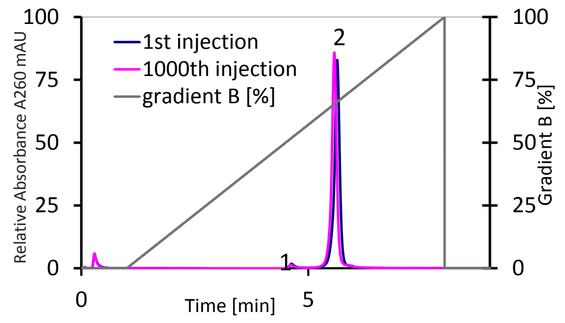
Column: CIMac™ pDNA (0.3 mL). Flow rate: 1 ml/min; Buffer A – 200 mM Tris pH 8.0 and buffer B – 200 mM Tris, 1 M NaCl pH 8.0. Peaks 1 and 2 –impurities, Peak 3 – RNA, Peak 4 – OC pDNA, Peak 5 – SC pDNA.

c) Polishing chromatography



Column: CIMac™ pDNA (0.3 mL). Flow rate: 1 ml/min; Buffer A – 200 mM Tris pH 8.0 and buffer B – 200 mM Tris, 1 M NaCl pH 8.0; Peak 1: OC pDNA form; Peak 2: SC pDNA form.

- Efficient, reproducible and reliable determination of purity and quantity of different pDNA conformations
- Other impurities (proteins, RNA) monitored
- Can be used for PAT during large scale pDNA manufacture
- No loss of signal quality after 1000 injections



Purified pJ plasmid, 0.1 mg/mL; Buffer A: 200 mM Tris + 0.6 M NaCl; Buffer B: 200 mM Tris + 0.7 M NaCl; Gradient: 0 to 100 % B in 7 min. Flow rate: 1 mL/min

CONCLUSIONS

- Monoliths are highly efficient chromatographic columns, able to process samples from different feed streams and determine the amount of the target molecule and the impurity profile in real-time;
- Monoliths enable in-process data to be used for assessing the quality of a batch during manufacturing, significantly reducing the need for finished product testing, and improving lead times;
- Applications in upstream (culture or fermentation) and downstream processing (purification) of viruses, pDNA, phages and antibodies.

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