Purification and Analytics of pDNA Using Hydrophobic Monoliths in Sample Displacement Mode

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Introduction

Plasmid DNA (pDNA) Purification in Sample Displacement Mode

CIM® chromatographic monoliths enable
1) high productivity of pDNA downstream process (DSP) due to high dynamic binding capacity for pDNA in small elution volumes and short chromatographic runs;
2) high resolution power due to convective-based mass trans.

Sample displacement mode utilizes different relative binding affinities of components in a sample mixture and separates pDNA isoforms under overloading conditions – where sc pDNA isoform acts as a displacer of oc or linear pDNA isoform.

Plasmid DNA analytics

HPLC based PAT methods provide nearly real-time data about the production process and are key enabling technology for efficient process monitoring. Since biological assays for analytics of biopharmaceuticals are usually labor intensive and time consuming, chromatographic analytical methods are excellent alternative due their speed, accuracy and reliability.

CIMac analytical columns can handle complex sample matrices reproducibly and with great resolving capacity. Plug & play CIMac technology handles samples from different feed streams and determines the amount of the target molecule and impurity profile in nearly real time.

Methods

pDNA Polishing DSP Step Using CIMmultus® C4 HLD Preparative Column

Main goal – separate supercoiled (sc pDNA) from other pDNA isoforms (linear, open circular = oc) as well as remains of RNA and gDNA.

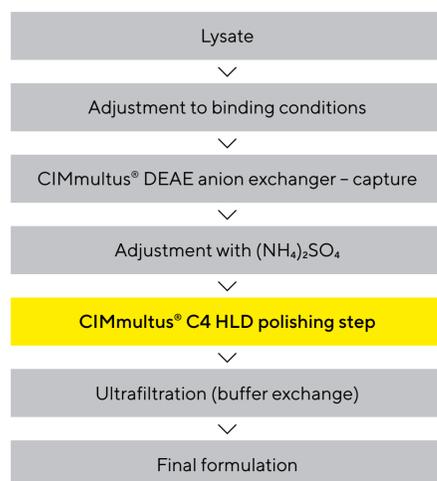**Figure 1:** HiP² Plasmid Process Pack™ Platform for pDNA Purification

Lysate
⌄
Adjustment to binding conditions
⌄
CIMmultus® DEAE anion exchanger – capture
⌄
Adjustment with $(NH_4)_2SO_4$
⌄
CIMmultus® C4 HLD polishing step
⌄
Ultrafiltration (buffer exchange)
⌄
Final formulation

**Figure 2:** CIMmultus® C4 HLD-1

Note. Column name: CIMmultus® C4 HLD-1 (1 mL) advanced composite column
Description: Hydrophobic column with neutral surface optimized for plasmid DNA polishing

Table 1: Chromatographic Conditions for Preparative Run In Sample Displacement Mode

Mobile phases	**Washing buffer** 50 mM TRIS, 10 mM EDTA, 2 M ammonium sulfate (AS), pH 7.2 **Loading buffer** 50 mM TRIS, 10 mM EDTA, 1.7 M AS, pH 7.2 **Elution buffer** 50 mM TRIS, 10 mM EDTA, 1.0 M AS, pH 7.2 **Stripping buffer** 50 mM TRIS, 10 mM EDTA, pH 7.2
Sample	pTURBO (Generi Biotech, Czech Republic), a commercially available 4.7 kbp plasmid
Loading material	pDNA (43 µg/mL) in loading buffer

Table 2: Monitoring Recovery and Homogeneity of sc pDNA From Different Fractions

Monitoring @ 260 nm		sc pDNA	oc pDNA		
Fraction name	Volume (mL)	Mass (ug)	Mass (ug)	sc pDNA (%)	sc pDNA homogeneity (%)
FT	34.0	54.4	96.9	4.8	78.7
EL	8.0	978.4	40.5	86.2	96.0
STRIP	7.0	19.5	5.6	1.7	77.6
LOAD	34.0	1134.5	157.9	100.0	87.8

pDNA Polishing DSP Step Using CIMmultus® C4 HLD Preparative Column

Figure 3: Preparative Run In Sample Displacement Mode
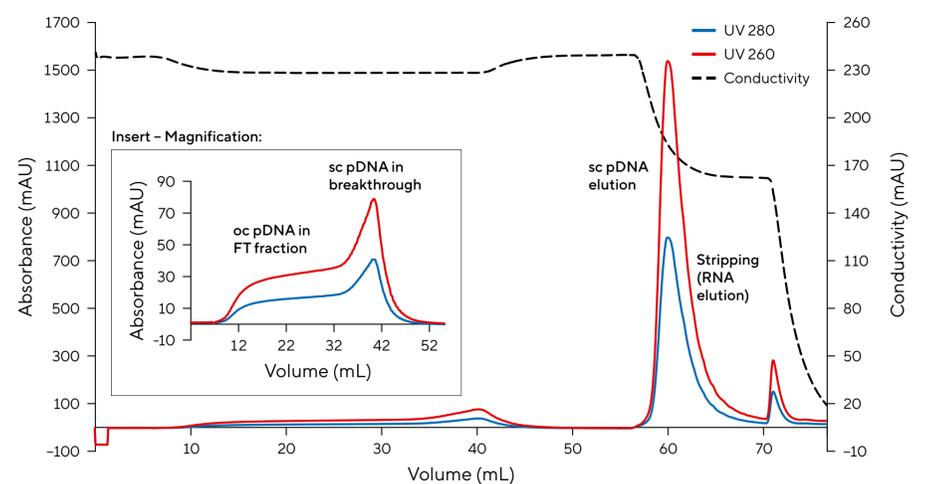
- Chromatographic run completed in less than 15 min.
- Recovery of the sc pDNA in elution fraction 86%
- sc pDNA homogeneity increase from 88% in starting material to 96% in elution fraction
- More than 1 mg of sc pDNA purified per mL of column in a single run

In-Process Analysis of pDNA Purification Using CIMac pDNA Analytical Column

Figure 4: CIMac pDNA Analytical Column

Note.
Column name: CIMac pDNA Analytical Column
Description: Weak anion – exchange column optimized for the HPLC analytics of plasmid DNA

Table 3: Chromatographic Conditions for pDNA Analysis of Two Fractions (pTURBO Load and Strip)

Mobile phases	**Buffer A:** 100 mM TRIS, pH 8.0 **Buffer B:** 100 mM TRIS + 1 M NaCl, pH 8.0
Gradient method	**Wash:** 3 CV Buffer A **Elute:** 24 CV from 65–75% Buffer B **Hold:** 3 CV Buffer B **Equilibrate:** 6 CV Buffer A
Injection volume	50 µL

Figure 5: Analytical Chromatogram for Load pTURBO and Stripping-pTURBO (See the Preparative Peak Above)
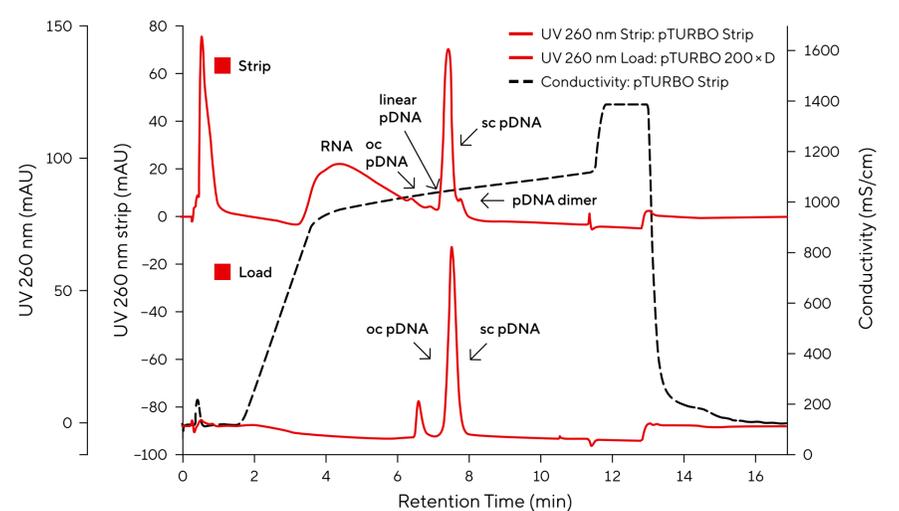
- Chromatogram of load pTURBO and stripping-pTURBO on analytical column showed excellent separation of **oc** and **sc** conformation of plasmid
- In stripping fraction RNA, as well as linear pDNA and pDNA dimer were detected
- Linearity was confirmed with R^2 of 0.9966 for **oc** pDNA and 0.9973 for **sc** pDNA
- RSD for both conformations was less than 5%

4. Conclusions

- Chromatography on CIM® monoliths results in the pDNA of a high purity and homogeneity
- Sample displacement mode enables higher productivity and higher product yield compared to classical bind-elute mode
- The purification process is cost-effective and scalable
- CIMac pDNA column enables a fast, accurate and reliable analysis of pDNA as well as impurities during the development of an up- and/or downstream process in less than 20 min per sample
- CIMac pDNA column enables quantification as well as purity assessment of the product intended for human use