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Sample Displacement Chromatography for High Purity of Supercoiled DNA Plasmids

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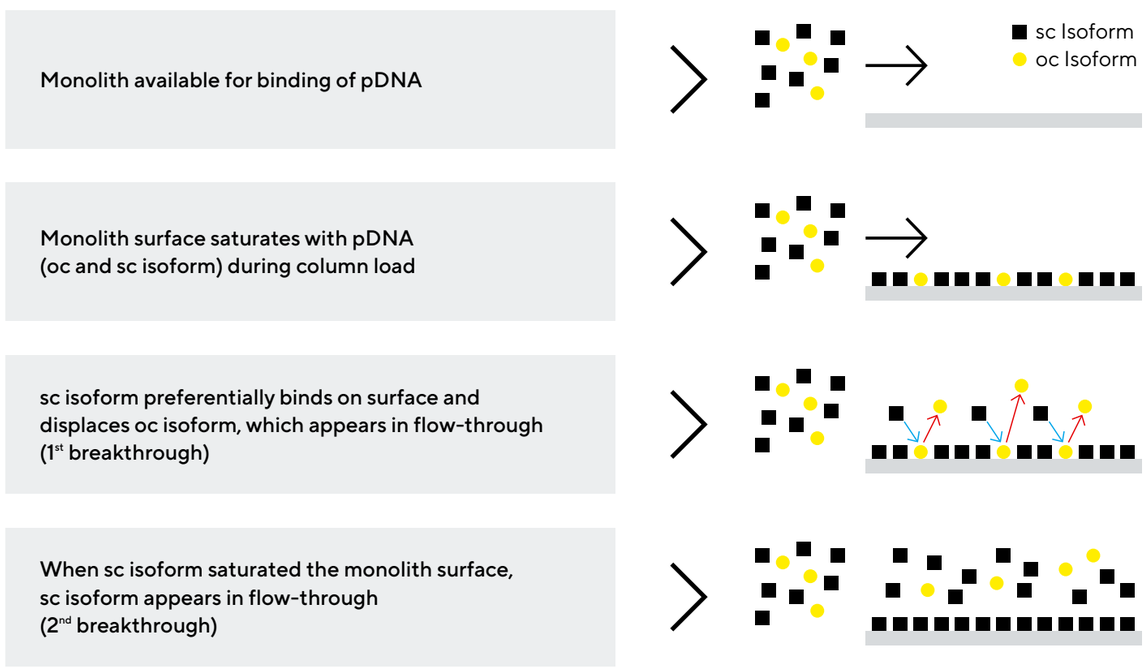
Principle of Sample Displacement Chromatography (SDC)

Sample displacement chromatography (SDC) is a chromatographic technique that utilizes differences in relative binding affinities of components in a sample mixture under chromatographic conditions. Here, we use SDC approach with CIM® C4 HLD monoliths under hydrophobic interaction chromatography (HIC) conditions to separate plasmid DNA (pDNA) isoforms under overloading conditions, where **supercoiled (sc)** isoform acts as a displacer of **open circular (oc)** or linear isoform. High purity of sc isoform is beneficial for use of plasmids as vaccines, transfecting agents for production of gene therapy viral vectors, or as starting material for linearization prior to IVT reaction in production of mRNA vaccines.

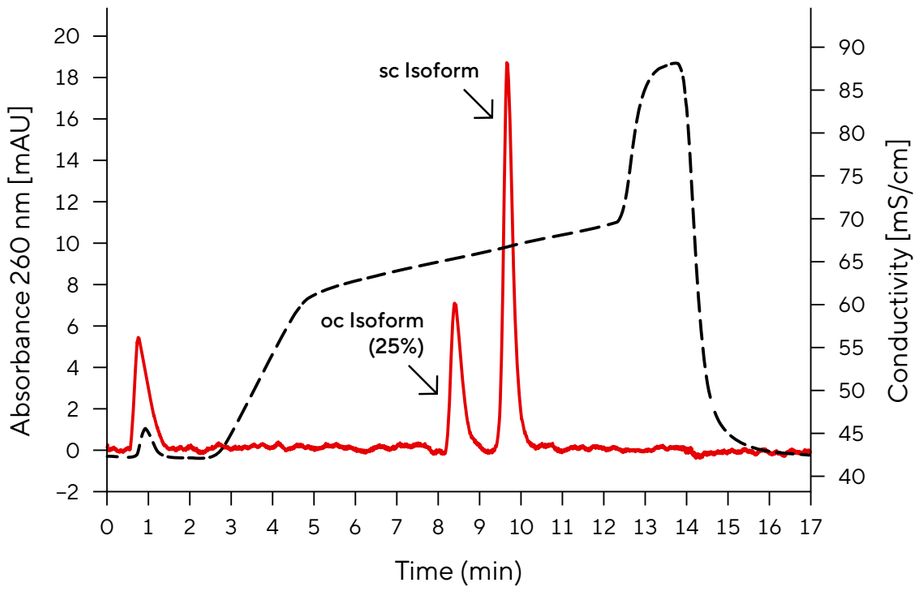
Figure 1: *Scheme of pDNA Loading Under Sample Displacement Conditions.*



Optimization of Ammonium Sulfate (AS) Concentration in Load

Use of higher AS concentrations in loading increases binding capacity for pDNA but also promotes binding of oc isoform, thus optimization of AS conc. is need to achieve optimal binding capacity and high sc isoform purity in elution. AS concentrations tested are given in Table 1.

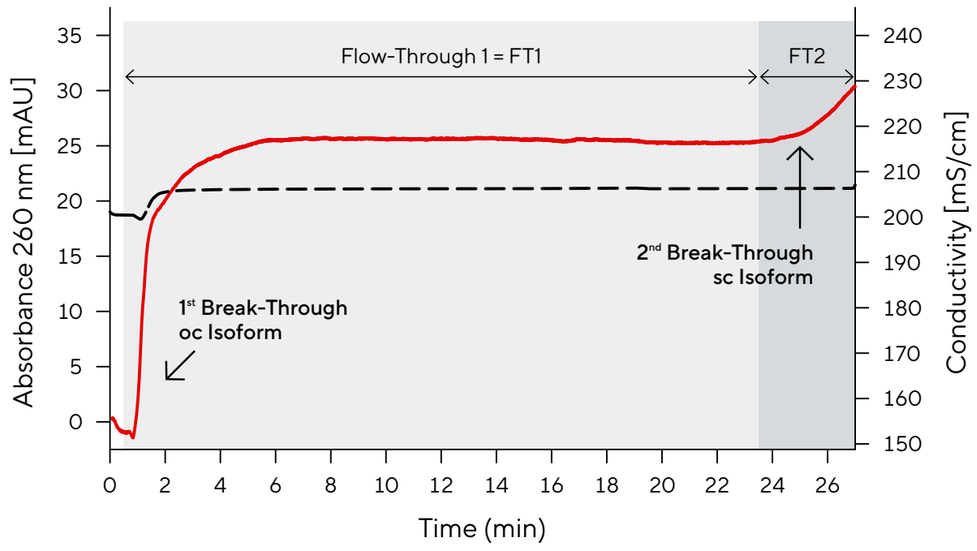
Figure 2: *Representative CIMac pDNA Chromatogram of Starting pDNA (pAAV2/2).*



Note. 600 µg of pDNA (pAAV2/2, containing approx. 25% of oc isoform) in TE buffer + 800 mM NaCl (imitating DEAE eluate matrix) was diluted with 3.7 M AS solution to three AS conc. (1.55 M, 1.7 M, 1.85 M). At 1.7 M AS conc., additional load was prepared including dilution with binding mobile phase (C4 HLD MPA) to minimize the effect of NaCl. pDNA was purified on 200 µL CIM® C4 HLD column (2 µm) and analysed by CIMac pDNA analytical method as described before (Černigoj, U. et al. Electrophoresis 2021 Dec; 42(24):2619-2625) on PATfix® HPLC system, UV absorbance at 260 nm, flow rate 1 mL/min, injection volume 50 µL.

pDNA samples containing a mixture of sc and oc isoforms (Figure 2) were loaded onto 200 µL CIM® C4 HLD column (2 µm) at 1 mL/min (Figure 3). After the second break-through (sc isoform), column wash was performed with corresponding binding mobile phase (1.55 – 1.85 M AS), elution in 0.8 M AS and strip in TE buffer (Table 1).

Figure 3: *Preparative Chromatogram of pDNA Load to 200 µL Cim® C4 HLD Column in SDC Conditions.*



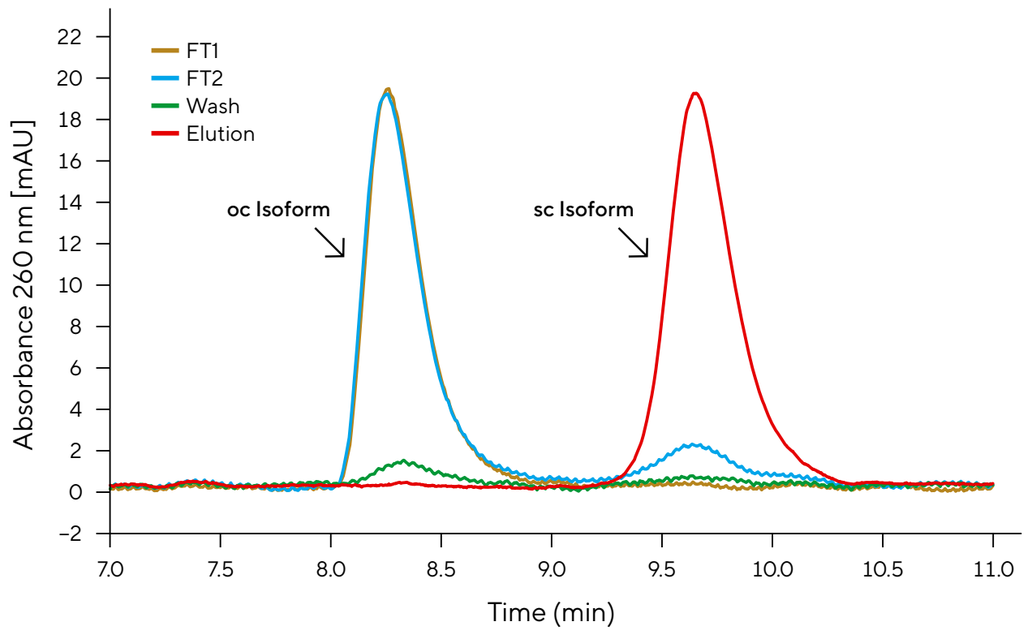
Note. Sample: pDNA in 1.7 M AS, additionally diluted with MPA (volume equal to the volume of initial pDNA sample in 800 mM NaCl). MPA (50 mM TRIS, 10 mM EDTA, 1.7 M AS, pH 7.2), MPB (50 mM TRIS, 10 mM EDTA, pH 7.2). Method 1 – load: 0 – 27 min (100% sample).

Fractions FT1 (oc isoform), FT2 (oc and sc isoform), wash, elution (sc isoform) and strip were collected and analysed by CIMac pDNA for content and purity (Table 1).

Table 1: *Binding Capacity and Purity for sc Isoform vs AS Conc. During Load.*

Exp.	AS Concentration (M)	Capacity (mg pDNA/mL)	Purity of sc Isoform (%)
1	1.55	1.50	100
2	1.70	1.75	96
3	1.85	1.91	90
4	1.70 + additional dilution	1.78	**100**

Figure 4: *Chromatograms of C4 HLD Fractions on CIMac pDNA (Zoomed-in pDNA Elution Window, 7 – 11 Min) From Exp 4.*



Conclusions

- pDNA polishing in SDC mode on C4 HLD monolith results in high purity (>99%) of sc isoform.
- Optimization of AS concentration is needed to achieve optimal performance.
- Lower concentrations of AS used compared to traditional HIC approaches – lower costs of goods.