

July, 2017

Keywords or phrases

Hydrophobic interaction chromatography, monolith, sample displacement, pDNA, pDNA isoform separation, plasmid DNA, C4 HLD

Comparison of HIC Monolithic Support for Sample Displacement Chromatography of pDNA

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Abstract

This application note investigates three monolithic chromatographic supports with different hydrophobicities regarding their applicability for sample displacement of pDNA. CIMac™ C4 HLD (butyl, high ligand density) as a commercial product and pyridine and histamine as custom immobilised columns are compared.



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Introduction

In pharmaceutical applications, the purity and efficacy of plasmid DNA (pDNA) as a therapeutic product are stringent. The separation of linear, supercoiled (sc), and open-circular (oc) pDNA isoforms has already been established on CIM[®] butyl (C4 HLD) monolithic columns at a preparative scale. This process requires a high concentration of ammonium sulfate for loading, which increases the overall production requirements. Competing adsorption in sample displacement chromatography utilizes the chromatographic resin's binding capacity more efficiently and increases the chromatographic step's productivity.

Materials

All chromatographic runs were performed on CIMac[™] Analytical columns with 0.1 mL bed volume. CIMac[™] C4 HLD (Butyl) is commercially available; pyridine and histamine are custom immobilized columns.

Two different plasmids were used for all experiments: pEGFP-N1, 4700 bp and pMD204, 2345 bp, and they were purified from the clarified lysate using an 8 mL HiP² Plasmid Process Pack[™].

Analytics were performed using CIMac[™] pDNA-0.3 Analytical Column (1.4 μm channel size).

Methods

All chromatographic runs were performed on three different CIMac[™] Analytical columns in sample displacement method. This type of chromatography exploits the different relative binding affinities of components in a sample mixture to achieve accumulation of a desired substance on the column before elution.

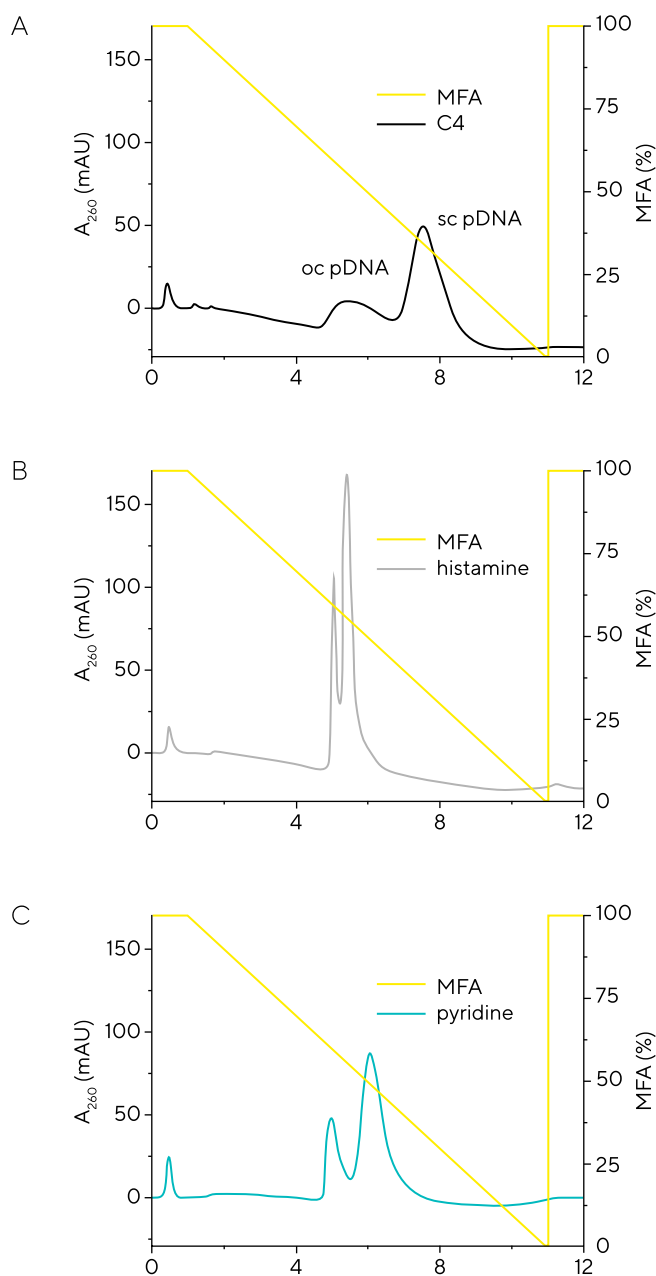


Figure 1: Separation of sc and oc isoforms on three hydrophobic supports..

Results

Sample Displacement of oc pDNA by sc pDNA

Regardless of the support, sample displacement under optimized conditions yields an enrichment factor of at least 1.05 with a capacity of at least 0.03 mg/mL and a homogeneity $\geq 98\%$.

Optimal ammonium sulphate loading concentration needs to be determined to achieve the highest homogeneity or capacity of the supercoiled isoform (Figure 2, right).

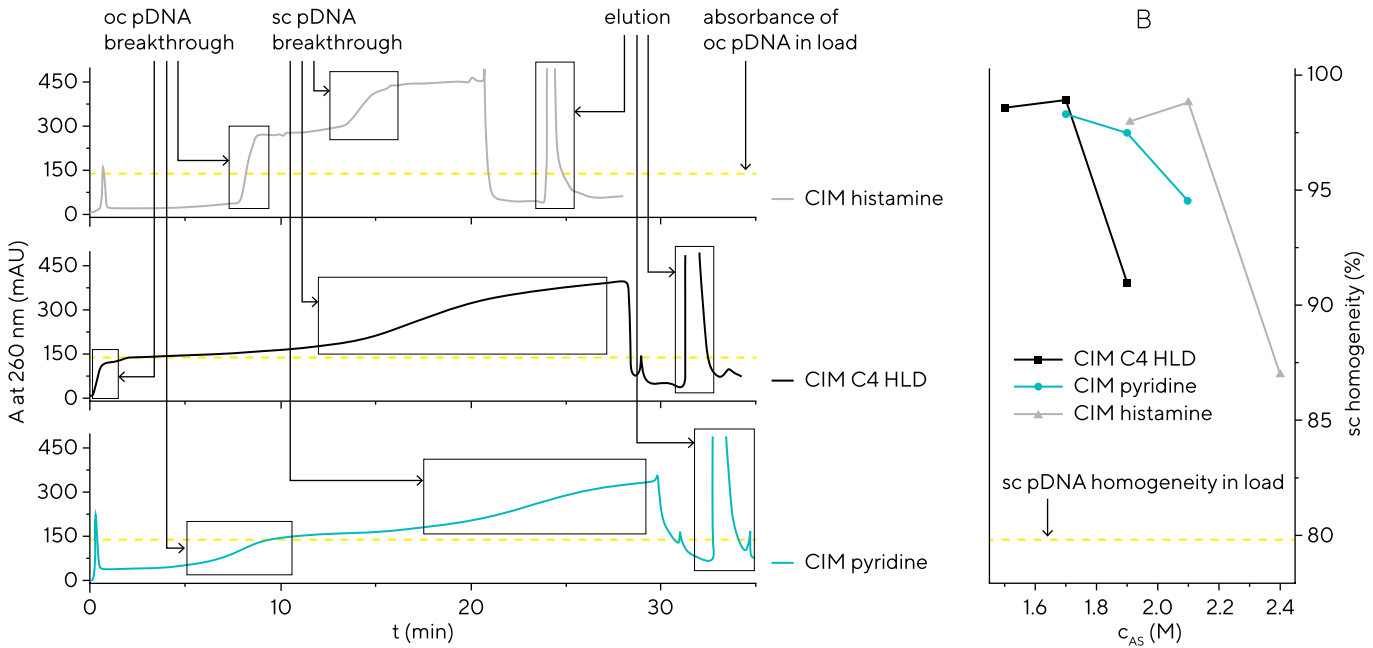


Figure 2: (left) Representative chromatogram for each support. Loading of a mixture of oc and sc pDNA (pEGFP) with sc pDNA homogeneity of 80% on different CIMac™ columns in buffers containing specific concentrations of AS. (right) Homogeneity of sc pDNA in the elution fraction at different AS loading concentration.

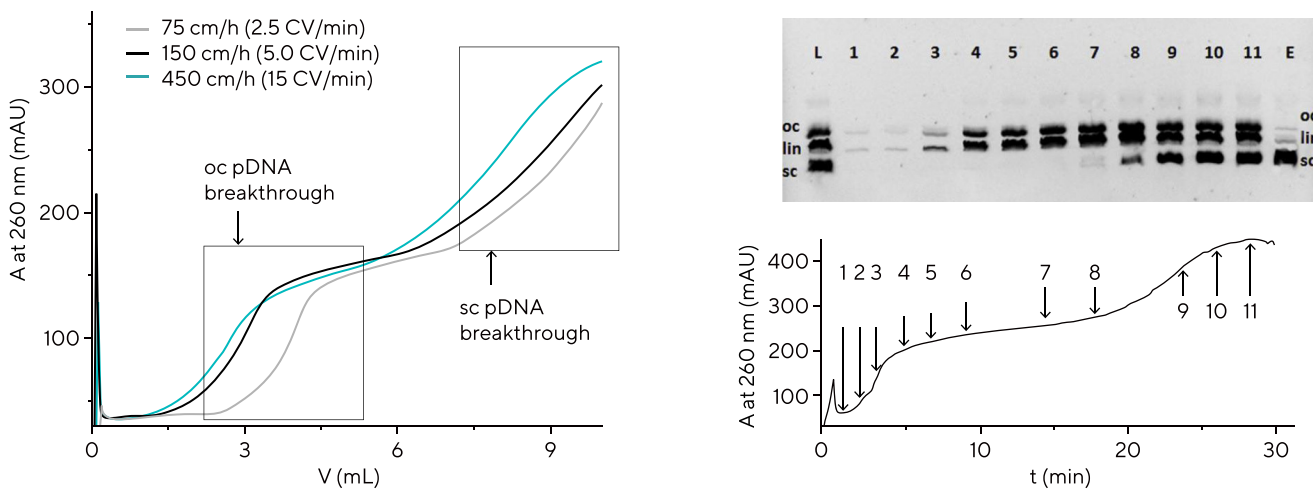


Figure 3: (left) elution of pDNA mixture at different flow rates normalized for column volumes. (right) agarose electrophoresis (AGE) picture of SDC samples from a mixture of three pDNA isoforms. 1-11: loading fractions, E: elution fraction

Q Discussion

The homogeneity of sc pDNA (approx 97.5%) and dynamic binding capacity (1.30 mg/mL support) are preserved at flow rates up to 450 cm/h (15 CV/min, 1.5 mL/min). In addition, a 5-fold increase in the oc isoform ratio has a minimal effect on the homogeneity of sc pDNA in the elution (2% decrease, Table 1). Finally, with up to 30% linear isoform in the sample, the elution fraction still reaches 98% sc homogeneity, showing displacement of linear and open circular isoform by supercoiled pDNA.

% of oc isoform in load	ratio between oc pDNA concentration in breakthrough fractions and in load	amount (mg) of eluted pDNA per mL of column	sc pDNA homogeneity in elution fraction
10%	1.10	1.12	97
25%	1.21	1.15	96
50%	1.05	1.09	95

Table 1: pDNA isoforms analysis from preparative SDC chromatography – pEGFP loading of samples containing different ratios between oc and sc pDNA isoform on CIMac™ pyridine column. Load AS concentration: 1.95 M

💡 Conclusion

Sample displacement allows enrichment of the supercoiled isoform prior to elution. Choosing the optimal loading conditions (ammonium sulfate concentration) affects the purity of the elution fraction, with lower AS concentration resulting in higher purity. The method is robust towards different flow rate, sample compositions (percentage of each isoform) and plasmids.

📖 References

Černigoj, Urh, et al. "Sample displacement chromatography of plasmid DNA isoforms." *Journal of Chromatography A* 1414 (2015): 103-109.

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