Sample displacement chromatography of plasmid DNA isoforms

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INTRODUCTION

Preparative scale chromatographic separation of open-circular (oc) from supercoiled (sc) plasmid DNA (pDNA) isoforms has been already established on CIM® C4 with high ligand density (C4 HLD) monolithic columns with sample loading in 3.0 M ammonium sulphate (AS)[1]. The process requires high molarity of AS, increasing the overall cost of the process. Sample displacement chromatography (SDC) can be used as an alternative to decrease the AS concentration required during loading onto hydrophobic chromatographic supports. This study compares three chromatographic monoliths with different hydrophobicic ligands on the surface (C4 HLD, pyridine and histamine) for the purification of different pDNA vectors in SD mode[2].

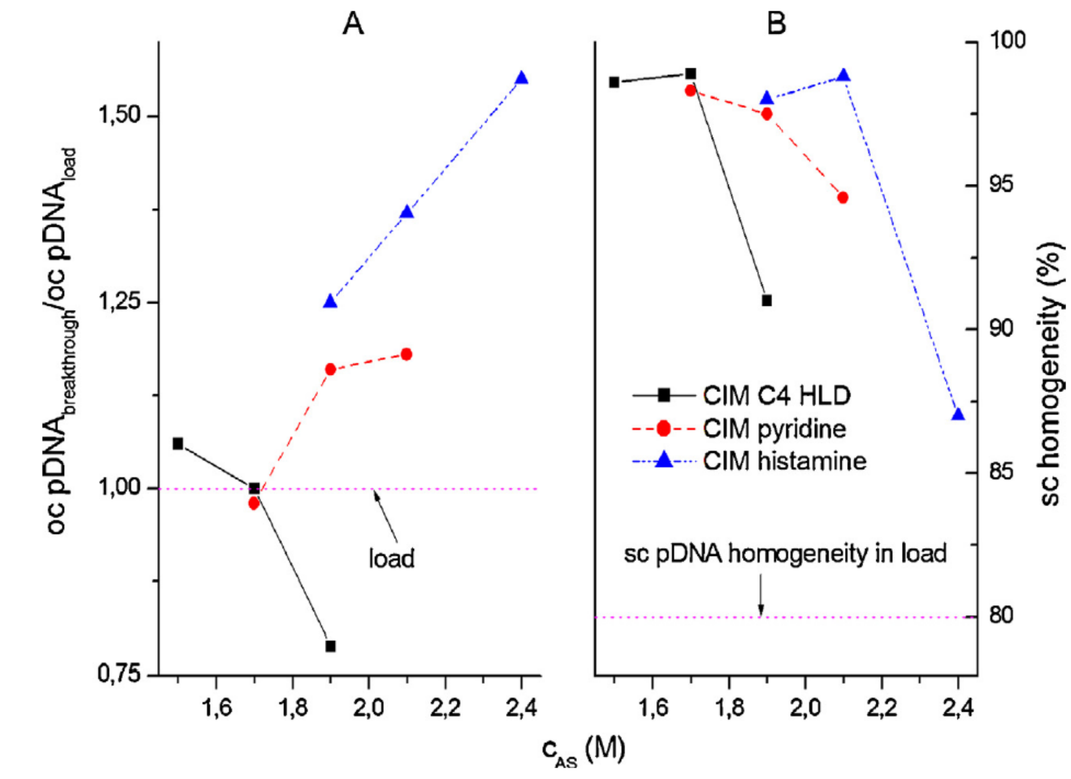
Optimal HIC support for pDNA SDC

Optimal AS loading concentration range was determined by analytical separation of two pDNA isoforms (pEGFP-N1, 4700 kbp, purified using CIM® HiP 8 mL Plasmid process pack, BIA Separations, part number 100.0012-2) in descending AS concentration gradient. Selectivity α (ratio between elution volume of sc and oc), column efficiency (width of chromatographic peak, W_b) and dynamic binding capacites (DBC) are reported in Table 1.

Column chemistry	c_{AS}, oc pDNA M	c_{AS}, sc pDNA M	α	W_b, oc pDNA min	W_b, sc pDNA min	DBC_{10}, oc pDNA mg of pDNA/mL support	DBC_{10}, sc pDNA mg of pDNA/mL support
C4 HLD	1.54	0.82	1.50	6.20	6.60	0.13	1.61
pyridine	1.70	1.32	1.30	1.70	3.00	0.05	0.53
histamine	1.69	1.60	1.07	1.20	1.60	0.01	0.03

Table 1: AS elution concentrations for oc and sc pDNA, column selectivity and efficiency, as well as dynamic binding capacity at 10 % breakthrough for all three supports.

Analytical HPLC analysis parameters	
Column	CIMac™ pDNA Analytical column (part number 150.8501-1.4)
Equilibration buffer	200 mM TRIS, pH8.0
Elution buffer	200 mM TRIS, 1.0 M NaCl, pH8.0
Method	1 min linear gradient from 0 % to 60 % elution buffer, 1 min hold at 60 %, 10 min linear gradient from 60 % to 70 %, step gradient to 100 % elution buffer



A pEGFP isoform mixture (20% oc pDNA) was used for studying the SD behaviour employing frontal analysis experiments on the three supports at different loading AS concentration. Comparison of the oc concentration in the second breakthrough with its corresponding load concentration (Figure 1A) confirms SD in action. The low SD abilities of C4 HLD are likely due to higher selectivity and broader chromatographic peaks (Table 1).

SD effect does not necessarily correlate with homogeneity of the sc pDNA. C4 HLD with the highest selectivity of the three chemistries and the lowest overall SD effect could achieve homogeneity of 98 % sc pDNA (Figure 2B). CIM®-histamine, with the strongest SD effect even in 2.4 M AS improved homogeneity of the sc isoform marginally compared to the load. Different interactions take place and in the case of C4 HLD the oc pDNA isoform was mainly removed by negative mode chromatography.

Figure 1 (left): The detailed analysis of sample displacement effect – influence of AS loading concentration as well as type of the chromatographic support.
A – ratio between oc pDNA concentration in breakthrough vs. its concentration in load; B – homogeneity of sc pDNA in the elution fraction.
The figure is based on the evaluation of the nine different frontal analyses experiments. pDNA isoform concentration was quantified by CIMac™ pDNA.

Effect of flow rate, different oc:sc pDNA ratios in loading sample, and presence of linear isoform

Consistent with convective properties of monoliths, flow rate had small effect on the SD effect (Figure 2A, CIM®-pyridine). Different flow rates (2.5 CV/min, 5 CV/min and 15 CV/min) produced comparable results, with DBC values of 1.3 ± 0.1 mg/mL and homogeneity of the sc pDNA approx 97.5 %. Additionally, linear isoform was successfully removed under SDC conditions from a mixutre of pEGFP 30 % linear, 10 % oc and 60 % sc (Figure 2B). A homogeneity of the elution fraction of 98 % sc pDNA, with an enrichment factor of 1.6 was achieved.

Different oc:sc ratios in the sample had minimal effect on the results. Homogeneity of the elution fractions fell from 97 % to 95 %, however event at 1:1 sc:oc ratio, the enrichment factor remained hight, at 1.9.

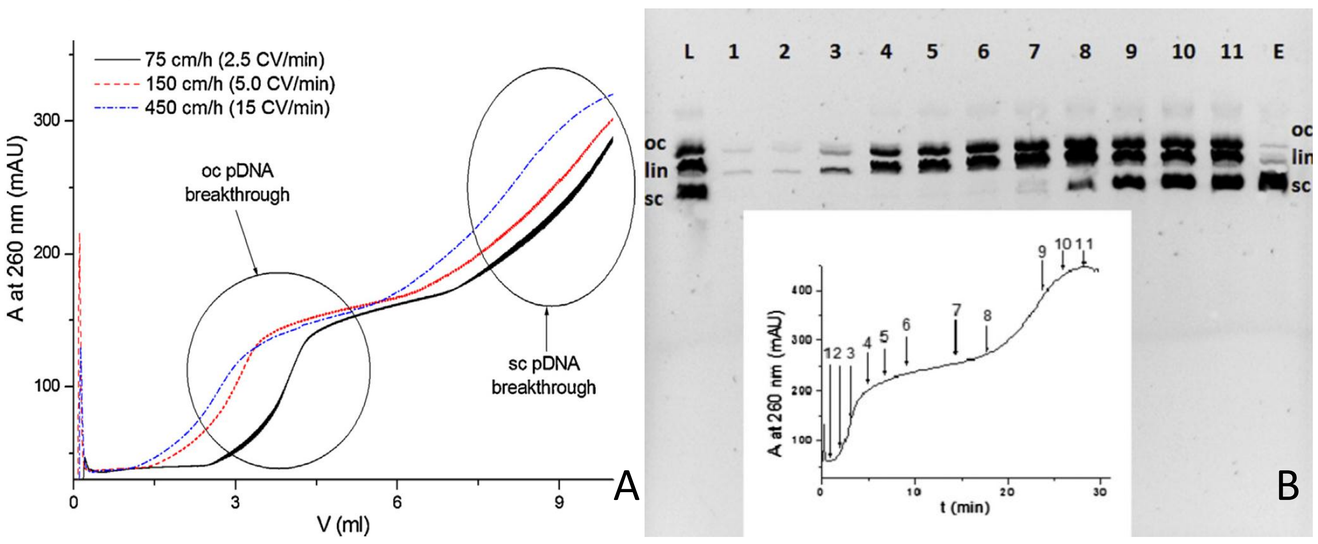


Figure 2 (left): A - Separation of pDNA (80 % homogeneity) under HIC conditions, tested at three flow rates with the x-axis unified. B - Agarose electrophoresis (AGE) picture of SDC samples from mixtures of three pDNA isoforms. The bands 1 to 11 are loading fractions (see inserted chromatogram). Band L is a loading fraction, while band E is an elution fraction from the column (the elution is not shown on the inserted chromatogram). Q = 0.5 mL/min, λ = 260 nm, (oc pDNA) = 3.0 g/mL, (lin pDNA) = 5.0 g/mL, (sc pDNA) = 13 g/mL in 50 mM TRIS, 10 mM EDTA, 1.90 M AS, pH 7.4. Elution buffer: 50 mM TRIS, 10 mM EDTA, pH 7.4.

% 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	1.1	1.12	97
25	1.21	1.15	96
50	1.05	1.09	95

Table 2 (above): 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

CONCLUSIONS

- Sample displacement chromatography of pDNA isoforms under HIC conditions demonstrated for the first time
- The separation efficiency shown to be dependent on the selectivity of the column for different isoform as well as column efficiency
- SD efficiency shown to be independent of plasmid size, presence of linear isoform, for samples with different oc:sc ratios (as high as 1:1)
- SD efficiency independent of flow rate due to characteristics of monolithic chromatographic supports (shown up to linear velocity 450 cm/h)
- The method reduces the concentration of AS required during loading and removes a chromatographic step (wash)
- Continuous, multicolumn chromatography systems can be used to compensate for lower DBC

References: 1) Smrekar F. et al. „Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns.“ *Vaccine* 28 (2010) 2039–2045.
2) Černigoj U. et al. "Sample displacement chromatography of plasmid DNA isoforms." *Journal of Chromatography A* 1414 (2015) 103-109.