A051 Increasing productivity of pDNA downstream processing using sample displacement chromatography

Plasmid DNA (pDNA) as a pharmaceutical product has stringent requirements of purity and efficacy and often one or more chromatographic steps are used in the downstream process. High ligand density butyl-modified chromatographic monolith (CIMmultus™ C4 HLD, part of CIMmultus™ HiP² Plasmid Process Pack™ 1-1, product number 100.0011-2) is currently used in a polishing step of a pDNA purification process (1), and is mainly used for separation of supercoiled (sc) pDNA separation from open circular (oc) and linear pDNA isoforms as well as for removal of remaining gDNA and RNA.

This application note presents a comparison of two different polishing processes employing monoliths, namely bind-elute (BE) and the more recently described (2) sample displacement purification (SDP).

CAPTURE STEP

Cell lysate containing 9.1 kbp pKLAC (Generi Biotech, Czech Republic) was captured by CIMmultus™ DEAE-8 Advanced Composite Column, part of CIMmultus™ HiP² Plasmid Process Pack™ 8-8, product number 100.0012-2 (Conditions: Buffer A: 50 mM TRIS 10 mM EDTA pH 7.2; Buffer B: 50 mM TRIS 10 mM EDTA 1 M NaCl pH 7.2; flow: 80 mL/min, elution with 10 mL/min, UV detection: 260 nm). Elution of pDNA was performed with 1.0 M NaCl. Following the capture step on DEAE, the collected sample was divided into two parts which were then subjected to the afore-mentioned polishing methods on a CIMmultus™ C4 HLD-1 Advanced Composite Column.

POLISHING STEP

BIND-ELUTE PURIFICATION

BE purification requires high concentration of ammonium sulphate (AS) during loading. Elution is then achieved by descending AS gradient. After choosing optimal wash and elution mobile phases from screening experiments, optimal run conditions were selected (Figure 1).

Column:	CIMmultus™ C4 HLD-1 Advanced Composite Column
Conditions:	Binding buffer: 50 mM TRIS 10 mM EDTA 3.0 M AS pH 7.2 Washing buffer: 50 mM TRIS 10 mM EDTA 1.95 M AS pH 7.2 Elution buffer: 50 mM TRIS 10 mM EDTA 1.2 M AS pH 7.2 Stripping buffer: 50 mM TRIS 10 mM EDTA pH 7.2
Detection:	UV at 260 nm
Flow rate:	Loading: 4.0 mL/min Wash and elution: 2.0 mL/min
Sample:	Elution fraction from DEAE capture step containing 2.0 mg pKLAC plasmid was diluted with 50 mM TRIS 10 mM EDTA 4.0 M AS pH 7.2 in volumetric ratio 1:3

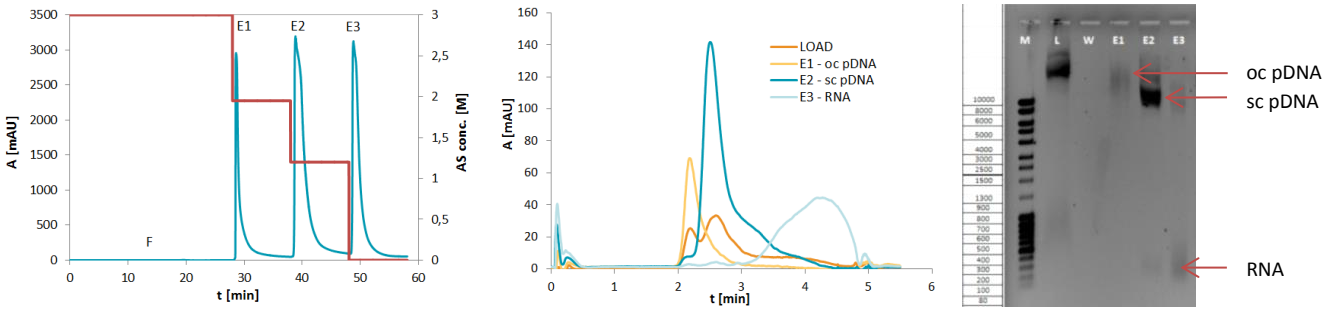

Figure 1: Bind-elute polishing step – left: preparative chromatographic run (load in 3.0 M AS, E1: 1.95 M AS (mainly oc pDNA isoform), E2: 1.2 M AS (mainly sc pDNA isoform), E3: 0 M AS (mainly RNA)); center: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: Agarose electrophoresis (AGE) analysis of elution fractions

CHROMATOGRAPHIC CONDITIONS FOR PDNA ANALYTICS

Elution fractions were analysed by two different analytical techniques: HPLC analytics using CIMac™ pyridine-0.1 Analytical Column (gradient elution from 2.5 M to 0 M AS) and agarose gel electrophoresis (AGE) (Figure 1).

Column:	CIMac™ pyridine-0.1 Analytical Column
Conditions:	Buffer A: 50 mM TRIS 10 mM EDTA 2.5 M AS pH 7.2 Buffer B: 50 mM TRIS 10 mM EDTA pH 7.2
Detection:	UV at 260 nm
Flow rate:	1.0 mL/min
Sample:	Elution fractions from preparative run
Injection volume	200 µL
Method:	Linear gradient from 2.5 M AS to 0 M AS in 4.0 min

SAMPLE DISPLACEMENT PURIFICATION

SDP utilises 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. Optimal AS concentration range was determined from screening runs. Plasmid DNA was loaded in 1.8 M AS and the main elution was collected in 1.2 M AS (Figure 2).

Column:	CIMmultus™ C4 HLD-1 Advanced Composite Column
Conditions:	Loading buffer: 50 mM TRIS 10 mM EDTA 1.8 M AS pH 7.2 Washing buffer: 50 mM TRIS 10 mM EDTA 2.0 M AS pH 7.2 Elution buffer: 50 mM TRIS 10 mM EDTA 1.2 M AS pH 7.2 Stripping buffer: 50 mM TRIS 10 mM EDTA pH 7.2
Detection:	UV at 260 nm
Flow rate:	4.0 mL/min
Sample:	Elution fraction from DEAE capture step containing 1.9 mg pKLAC plasmid was diluted with 50 mM TRIS 10 mM EDTA 4.0 M AS pH 7.2 in volumetric ratio 1:0.82

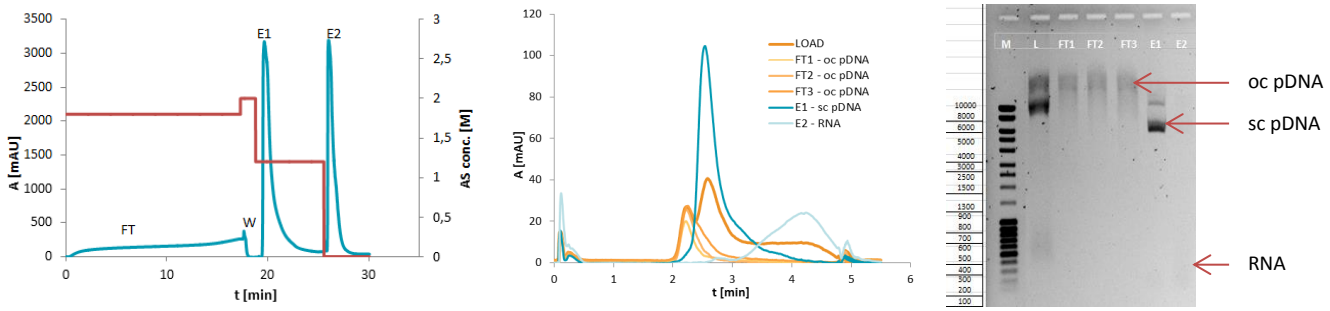

Figure 2: SDP polishing step – left: preparative chromatographic run (load in 1.8 M AS; FT (mainly oc pDNA isoform), W: 2.0 M AS, E1: 1.2 M AS (mainly sc pDNA isoform), E2: 0 M AS (mainly RNA)); middle: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: AGE analysis of elution fractions

Table 1: The composition of loading sample and main elution fraction for both downstream protocols (BE and SDP).

	pDNA isoform ratio		RNA presence
Method	sc pDNA [%]	oc pDNA [%]	RNA [%]
LOAD	59.8	21.1	observed on AGE and in analytical chromatographic run (estimated between 20 % and 30 % of the total nucleic acids amount in loading sample)
Main elution BE	98.4	1.6	Not detected on AGE; estimated below 5 % of the total nucleic acids amount in the main elution sample (from analytical chromatographic run).
Main elution SDP	98.7	1.3	

Supercoiled pDNA production yield and homogeneity of the sc isoform in the main elution fraction were estimated (Figure 3) for both methods. The homogeneity of sc pDNA isoform was determined as the ratio between the area of sc pDNA isoform and the area of both oc pDNA and sc pDNA isoforms in the main elution fraction. Both methods achieved high homogeneity of the sc pDNA (>98%), however the yield in the case of the SDP was 5% better compared to BE.

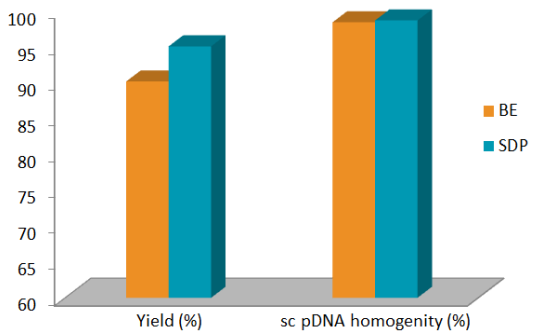

Figure 3: Yield of purified sc pDNA isoform and homogeneity of sc pDNA isoform for BE and SDP

Both methods were compared regarding loaded amount of sc pDNA, mass of reagents, i.e. AS and deionised water, load volume and process time needed for purification of 1 mg of sc pDNA using 1 mL C4 HLD column (Table 2).

Table 2: Comparison of two polishing chromatographic processes – classical bind–elute (BE) versus sample displacement purification (SDP).

Method – single run	Loaded amount of sc pDNA [mg]	sc pDNA in final elution fraction [mg]	m [g] AS / mg sc pDNA	m [g] H_2O / mg sc pDNA	V [ml] load / mg sc pDNA	t [min] method / mg sc pDNA
BE	1.4	1.24	69.1	155.3	58.8	106.2
SDP	1.1	1.06	27.3	84.3	32.3	84.0

CONCLUSIONS

Both chromatographic methods - classical bind-elute purification (BE) as well as sample displacement purification (SDP), are suitable for polishing purification step of plasmid DNA. High homogeneity (98 %) of sc pDNA in the main elution fraction was achieved in both cases, while SDP resulted in 5% higher yield compared to BE.

This 5 % increase in pure product yield (sc pDNA isoform) using SDP was achieved alongside a 60 % reduction in chemicals consumption and a 20 % reduction in processing time per gram of product. These figures represent a significant improvement over bind-elute and could translate into higher profit in an industrial pDNA downstream process.

We gratefully acknowledge Generi Biotech (Machkova, Czech Republic) for providing us biomass containing pKLAC plasmid DNA.

Reference:
1) F. Smrekar, A. Podgornik, M. Ciringer, S. Kontrec, P. Raspor, A. Štrancar, M. Peterka, Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns, Vaccine 28 (2010) 2039–2045.
2) U. Černigoj, U. Martinuč, S. Cardoso, R. Sekirnik, N. Lendero Kranjc, A. Štrancar, Sample displacement chromatography of plasmid DNA isoforms, J. Chromatogr. A 1414 (2015) 103-109.

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