

August, 2016

Keywords or phrases

Plasmid DNA, pDNA purification, isoform separation, supercoiled pDNA, plasmid process pack, polishing step, sample displacement, CIMmultus® C4 HLD

Increasing Productivity of pDNA DSP Using Sample Displacement Chromatography

BIA Separations d.o.o., A Sartorius company, Mirce 21, 5270 Ajdovščina, Slovenia

Correspondence

E-Mail: monolith-purification@sartorius.com

Abstract

Plasmid DNA (pDNA) as a pharmaceutical product has stringent purity and efficacy requirements, 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™) is currently employed in the polishing step of a pDNA purification process. It is mainly used for separation of supercoiled (SC) pDNA separation from open circular (OC) and linear pDNA isoforms and the 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 sample displacement purification (SDP).



For More Information, Visit

www.sartorius.com/monoliths

Introduction

Cell lysate containing 9.1 kbp pKLAC (Generi Biotech, Czech Republic) was captured by 8 mL CIMmultus® DEAE Advanced Composite Column, part of CIMmultus® HiP² Plasmid Process Pack™. Following the capture step on DEAE, the collected sample was divided into two parts subjected to the before-mentioned polishing methods on a 1 mL CIMmultus® C4 HLD Advanced Composite Column.

BE purification requires a 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).

SDP 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. 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).

Two different analytical techniques analysed elution fractions: 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 and Figure 2).

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

Materials

- CIMmultus® DEAE-8 Advanced Composite Column
- CIMmultus® C4 HLD -1 Advanced Composite Column
- CIMac™ pyridine-0.1 Analytical Column

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



Methods

Conditions for capture step

Column	CIMmultus® DEAE-8 Advanced Composite Column
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
Detection	UV at 260 nm
Flow rate	80 mL/min, elution with 10 mL/min
Sample	Elution of pDNA was performed with 1.0 M NaCl

Conditions for polishing step in bind-elute method

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

Conditions for polishing step in sample-displacement method

Column	CIMmultus® C4 HLD-1 Advanced Composite Column
Conditions	Binding 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

Chromatographic conditions for pDNA analytics

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

Results

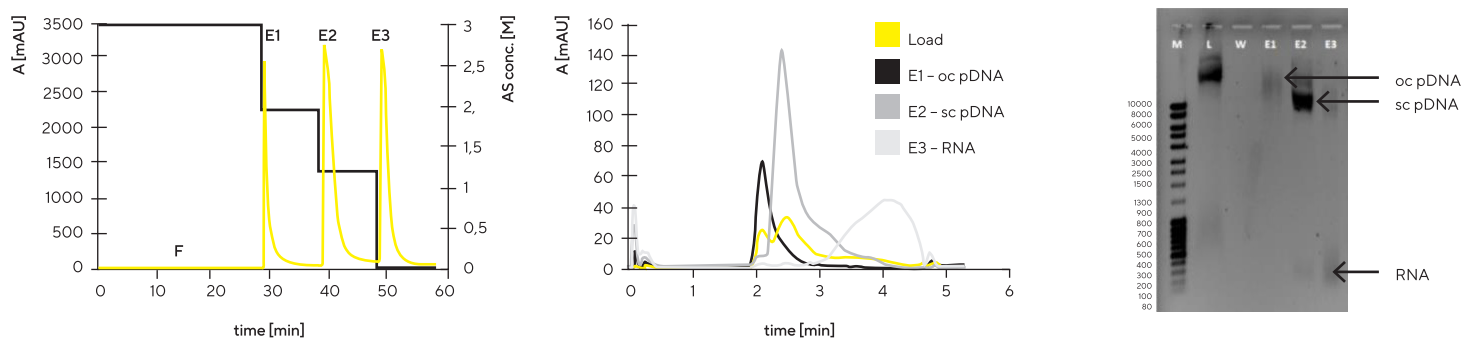


Figure 1: Bind-elute polishing step – left: preparative chromatographic run (load in 3.0 M AS, E1: 1.95 M AS (mainly oc pDNA isoform), E2: 1.2 M AS (mainly sc pDNA isoform), E3: 0 M AS (mainly RNA)); center: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: Agarose electrophoresis (AGE)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

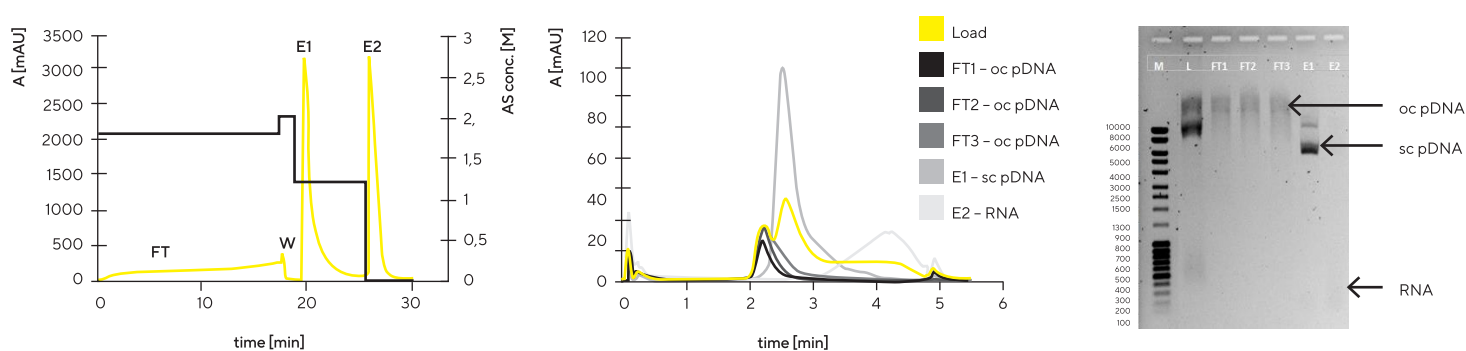


Figure 2: SDP polishing step – left: preparative chromatographic run (load in 1.8 M AS; FT (mainly oc pDNA isoform), W: 2.0 M AS, E1: 1.2 M AS (mainly sc pDNA isoform), E2: 0 M AS (mainly RNA)); middle: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: AGE analysis of elution fractions.

Method	pDNA isoform ratio		RNA presence
	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	

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

Discussion

High homogeneity (98%) of sc pDNA in the main elution fraction was achieved in both methods, 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.

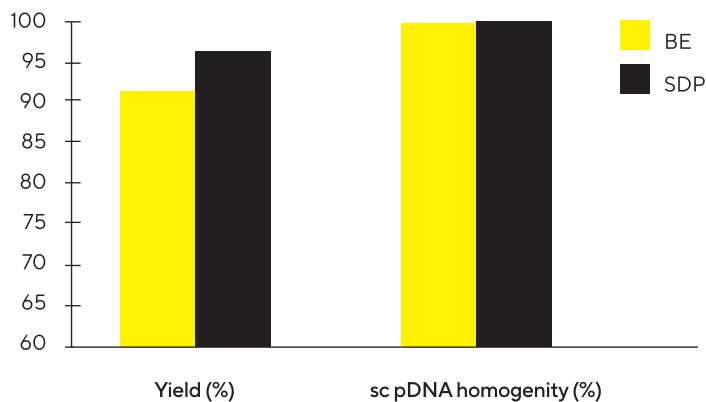


Figure 3: Yield of purified sc pDNA isoform and homogeneity of sc pDNA isoform for BE and SDP

Method - single run	Loaded amount of sc pDNA [mg]	sc pDNA in final elution fraction	m [g] AS / mg sc pDNA	m [g] H ₂ O / 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

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

Conclusion

Both chromatographic methods – classical bind-elute purification (BE) as well as sample displacement purification (SDP), are suitable for polishing purification step of plasmid DNA.

References

- 1) F. Smrekar, A. Podgornik, M. Ciringar, 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.

Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Slovenia

BIA Separations d.o.o.
A Sartorius company
Mirce 21
5270 Ajdovščina

 **For More Information, Visit**
www.sartorius.com