OPTIMIZATION OF pDNA DOWNSTREAM BIOPROCESSING USING HYDROPHOBIC CHROMATOGRAPHIC MONOLITHS IN SAMPLE DISPLACEMENT MODE

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INTRODUCTION

Since plasmid DNA (pDNA) as a pharmaceutical product has stringent requirements of purity and efficacy, one or more chromatographic steps are often used in the downstream processing train. High ligand density butyl-modified (C4 HLD) monolithic support is currently used in a polishing step of a pDNA purification process (1) and is mainly focused to supercoiled (sc) pDNA isoform separation from the open circular (oc) and linear pDNA isoform as well as for removal of remaining gDNA and RNA.

The goal of the study was to compare the productivities of two variations of the polishing chromatographic process employing monoliths – classical bind-elute (BE) versus recently described (2) sample displacement purification (SDP).

Classical purification requires high concentration of ammonium sulphate (AS) during loading step and elution is then achieved by descending AS gradient. 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 isoform.

EXPERIMENTAL PART and RESULTS

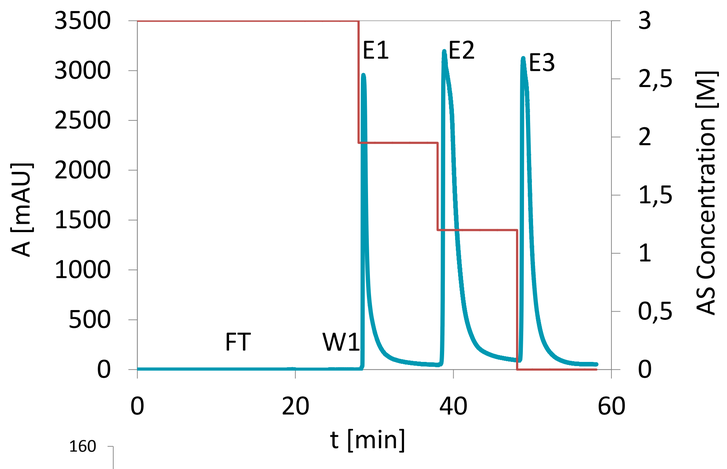
CAPTURE STEP

Cell lysate containing 9.1 kbp pKLAC (Generi Biotech, Czech Republic) was captured by CIMmultus DEAE column (Conditions: Column: CIMmultus DEAE-8; 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 and 280 nm). Elution of pDNA was performed with 1 M NaCl. After DEAE step the sample was divided into two portions and a polishing step using CIM C4 HLD column was done using two different methods, classical bind-elute and SDP.

POLISHING STEP

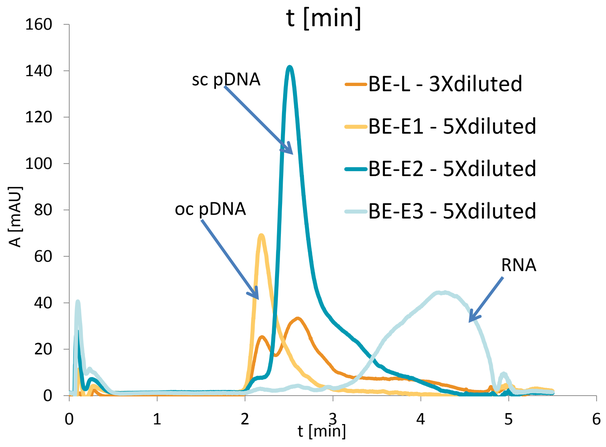
BIND-ELUTE MODE

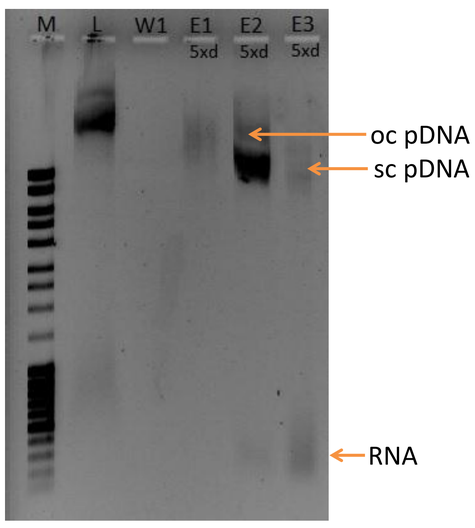
After choosing the optimal wash and elution mobile phases from the screening experiments, the optimal run was implemented (graph below). Conditions: Column: CIMmultus C4 HLD-1; Buffer A: 50 mM TRIS, 10 mM EDTA, 3 M AS, pH 7.2; Buffer B: 50 mM TRIS, 10 mM EDTA, pH 7.2, flow 4 mL/min, UV detection: 260 nm.



Remarks:

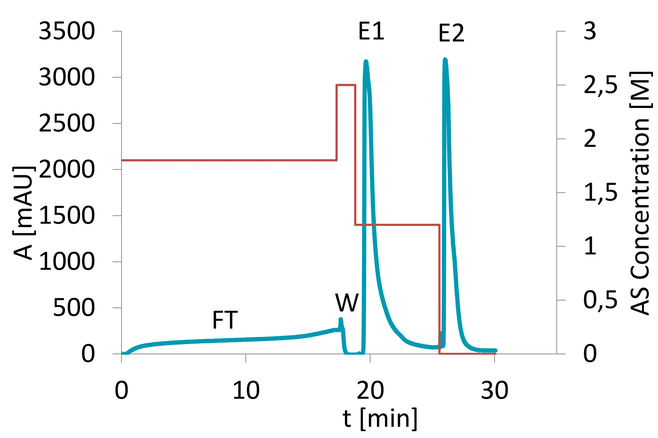
- Load in 3.0 M AS,
- E1: 1.95 M AS (mainly oc pDNA isoform),
- E2: 1.2 M AS (mainly sc pDNA isoform),
- E3: 0 M AS (RNA)





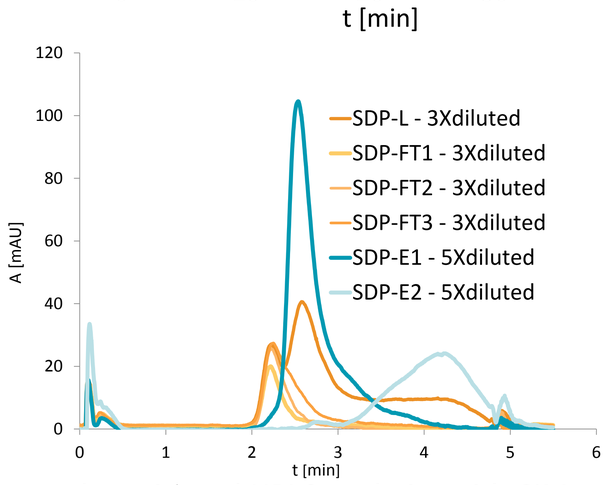
SAMPLE DISPLACEMENT MODE

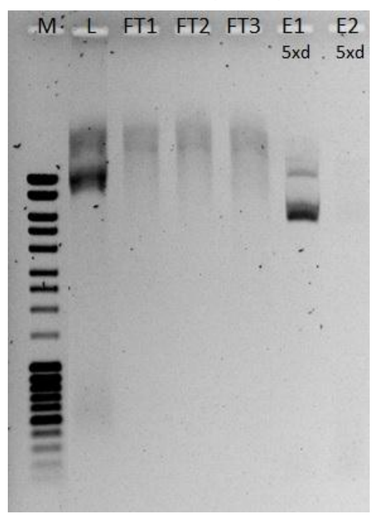
Optimal AS concentration range was determined from analytical run. Plasmid was loaded in 1.8 M AS and main elution at 1.2 M AS (graph below). Conditions: Column: CIMmultus C4 HLD-1; Buffer A: 50 mM TRIS, 10 mM EDTA, 2.5 M AS, pH 7.2; Buffer B: 50 mM TRIS, 10 mM EDTA, pH 7.2, flow 4 mL/min, UV detection: 260 nm.



Remarks:

- Load in 1.8 M AS,
- FT (mainly oc pDNA isoform)
- W1: 2.5 M AS
- E1: 1.2 M AS (mainly sc pDNA isoform)
- E2: 0 M AS (RNA)



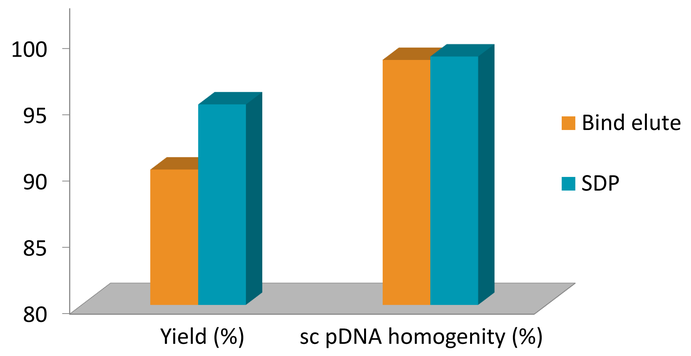


To analyse the elution fractions we used two different analytical technics, i.e. AGE (agarose gel electrophoresis) and HPLC analytics with CIMac pyridine analytical column in the AS gradient from 2.5 M AS to 0 M AS (results above).

Both methods were compared regarding yield of purified sc pDNA isoform, mass of reagents, load volume and process time needed for purification of mg of sc pDNA using 1 mL C4 HLD column.

Method – single run	Loaded amount of sc pDNA [mg]	sc pDNA in final elution fraction [mg]	m [g] AS / mg sc pDNA	V [ml] load / mg sc pDNA	t [min] method / mg sc pDNA
BE	1.7	1.5	56.4	48.0	86.7
SDP	1.4	1.3	22.8	26.9	70.0

We estimated sc pDNA production yield for both methods, as well as homogenity of the sc pDNA isoform in the main elution fraction.



CONCLUSIONS

- For SDP 60 % less chemicals and 20 % less time was needed in polishing step compared to classical bind-elute purification. This was manifested also in lower cost for preparative purification of pDNA with SDP method.
- At the similar homogenity of the sc pDNA in final fraction, the yield in case of the SDP was 5% better compared to BE.

We gratefully acknowledge Generi Biotech (Hradec Králove, Czech republic) for providing us biomass containing pKLAC plasmid DNA .
This research was funded by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n. 312004.

Reference:
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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.