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Optimization of Preparative-scale mRNA Capture using Small-scale Oligo dT Affinity Monoliths

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1. Introduction

Sample availability during early process development is often limited and reducing sample consumption enables screening of a broader range of experimental conditions during the process optimization phase. In chromatography, the bed volume of the column is inversely proportional to the number of realisable experiments with a fixed quantity of sample. For chromatographic monoliths, different screening formats are available (Figure 1).

Figure 1: Monolithic chromatographic screening formats: high-throughput (CIM Multi-well Plates), flexible and easy to use (CIMmic discs) and automation-compatible (CIM Octa Columns) solutions.

Table 1: Description of CIMmic family as chromatographic screening tools.

CIMmic discs	
Features	**Applications**
▪ 100 µL bed volume chromatographic monoliths; ▪ properties comparable to the preparative CIMmultus line; ▪ easy-to-handle screening format, combining the advantages of the monolithic stationary phases with a flexible housing design; ▪ broad chemistry portfolio; ▪ can be operated with low pressure pumps, LC systems or even manually with syringes.	▪ Rapid screening of samples or chromatographic conditions; ▪ scale-down of bioprocessing steps; ▪ minimal sample consumption; ▪ simple handling.

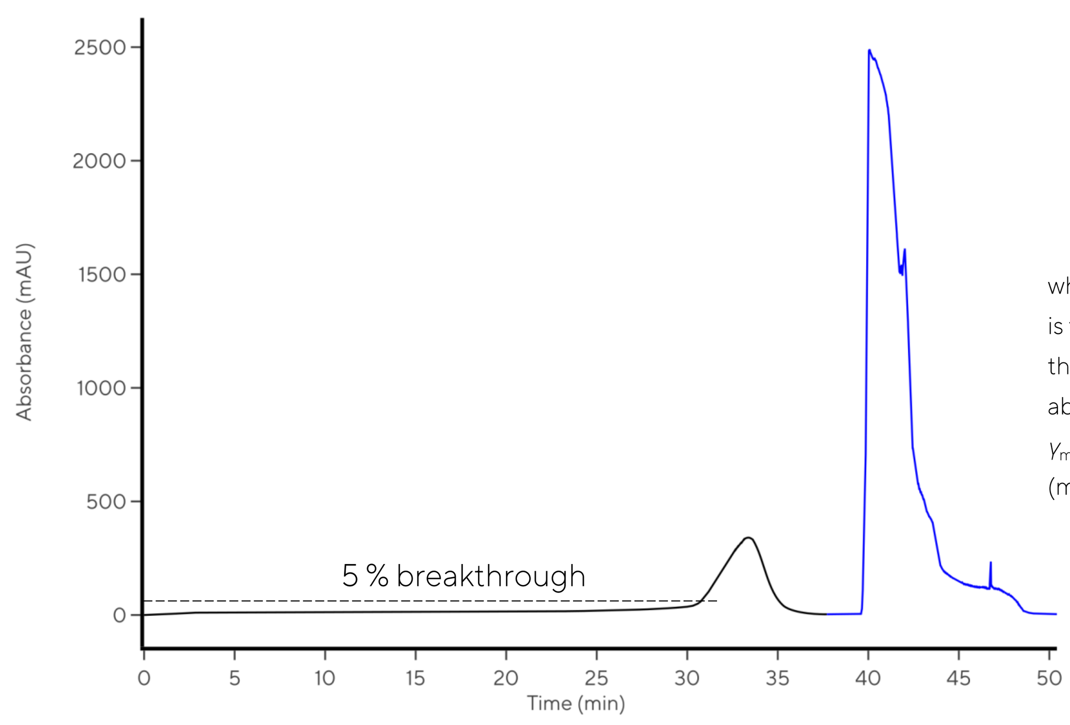
Coupling oligo-deoxythymidilic acid (Oligo dT) probes to monolith surface yields affinity chromatographic columns, capable of selectively binding messenger RNA (mRNA) via hybridization between Oligo dT and mRNA's poly-adenylic acid (poly A) tail. The aim of the presented research is to demonstrate the optimization of mRNA dynamic binding capacity (DBC) via the CIMmic Oligo dT platform.

2. Experimental approach

Dynamic binding capacity (DBC) measurements for mRNA using CIMmic Oligo dT monoliths

0.1 mL CIMmic Oligo dT18 (C12 linker) discs (Sartorius BIA Separations) were used to screen the influence of residence time, salt concentration and salt type on mRNA dynamic binding capacity at 5% breakthrough (DBC_5), see Table 2. The size of the model mRNA was 995 nt and feed concentration was 100 µg/mL. Experiments were performed on a PATfix analytical liquid chromatography system (Sartorius BIA Separations), where UV absorbance at 260 nm and 280 nm was monitored. Binding of mRNA to the columns was achieved in 50 mM phosphate buffer, pH 7.4 with added 0.5-1.0 M salt. Elution of bound mRNA was performed with 10 CV of 10 mM Tris, pH 9.0. A typical chromatogram of mRNA DBC on Oligo dT columns is presented in Figure 2.

For validation of screening results, mRNA DBC was measured on a 1 mL CIMmultus Oligo dT18 (C12 linker) column (Sartorius BIA Separations) with 100 µg/mL mRNA feed concentration in 50 mM phosphate buffer, 0.5 M NaCl, pH 7.4 or 50 mM phosphate buffer, 0.5 M guanidine-HCl (GdnHCl), pH 7.4 at 1 mL/min (1 min residence time). After loading, mRNA was eluted from the column in 10 mM Tris, pH 9.0 and the amount of eluted mRNA was measured by NanoPhotometer NP80 (Implen).



$$DBC_5 = \frac{(t_{5\%} \cdot \Phi - V_d) \cdot \gamma_{mRNA}}{V_{col}}$$

where Φ represents the volumetric flow rate (mL/min), $t_{5\%}$ is the time where the breakthrough signal during loading at the end of the column reaches 5% of the maximum absorbance, V_d is the dead volume of the system (mL), γ_{mRNA} is mRNA concentration in the loading buffer (mg/mL) and V_{col} is the column volume (mL).

Figure 2: A typical mRNA DBC chromatogram on CIM Oligo dT columns with elution (loading in black, elution in blue), and equation to calculate DBC_5 (right). Dashed line demonstrates the threshold for determining the 5 % breakthrough.

Table 2: Chromatographic parameters during optimization of mRNA chromatographic capture using CIMmic Oligo dT18 monoliths. Column volume was varied by packing one (0.1 mL) or three (0.3 mL) CIMmic discs into one chromatographic housing.

Column	Column volume [mL]	Flow rate [CV/min]	Residence time [min]	Type of salt in binding buffer	Salt concentration [M]
CIMmic Oligo dT18 (C12 linker)	0.1 and 0.3	1	1.00	NaCl or GdnHCl	0.5 or 1.0
	0.1 and 0.3	3	0.33		
	0.1 and 0.3	9	0.11		
	0.3	0.33	3.0		

[1] Mencin, N.; Štepec, D.; Margon, A.; Vidič, J.; Dolenc, D.; Simčič, T.; Rotar, S.; Sekirnik, R.; Štrancar, A.; Černigoj, U. Development and Scale-up of Oligo-dT Monolithic Chromatographic Column for mRNA Capture through Understanding of Base-Pairing Interactions. Sep. Purif. Technol. 2023, 304, 122320

3. Results

Screening of residence time

It was previously reported that Oligo dT affinity chromatography is flow-rate dependent even for convective chromatography media, probably due to kinetic limitations in mRNA-oligo hybridization and/or surface packing/orientation of molecules [1]. Screening of mRNA residence time (achieved by varying loading flowrate on 0.1 mL and 0.3 mL columns) in 0.5 M NaCl loading concentration confirmed that maximum DBC_5 (3.9 mg/mL) can be achieved at residence time of 3 min, while DBC_5 is 15% or 30% lower at 1 min or 0.33 min residence times, respectively (Figure 3). Only 3 mg of mRNA was needed for the whole evaluation.

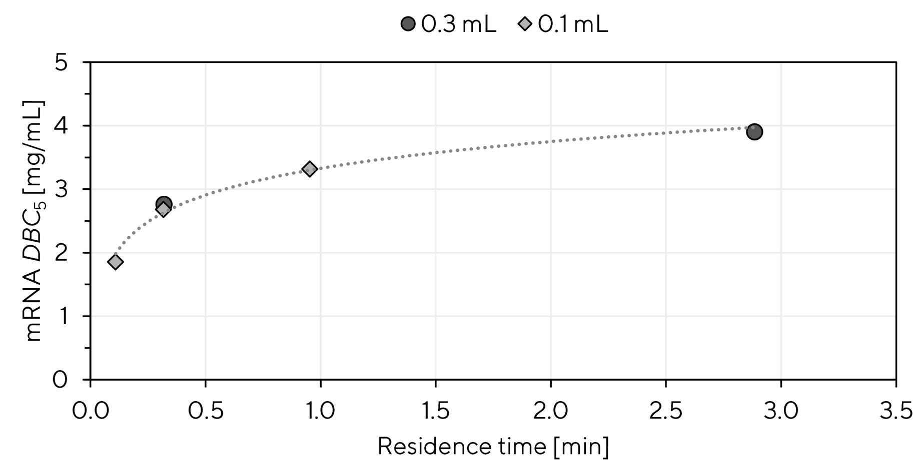


Figure 3: Influence of residence time on mRNA DBC_5 in 0.5 M NaCl using CIMmic Oligo dT discs (0.1 mL or 0.3 mL). Residence times of 0.11-3 min were achieved by varying flow rate in the range of 0.33-9 CV/min. Increasing residence time leads to higher DBC with a plateau around 4.5 mg/mL.

Screening of salt type and concentration

A higher concentration of NaCl (1 M) was evaluated to potentially increase mRNA DBC_5 at shorter residence times. In 1 M NaCl, high DBC_5 (3.7 mg/mL) was achieved already at short residence times (0.33 min), while prolonging the residence time to 3 min increased DBC by 30 %, to 4.8 mg/mL (Figure 4 A).

Changing the salt from 0.5 M NaCl to 0.5 M GdnHCl enabled capacity of 6.8 mg/mL at 3 min residence time, while even at 0.11 min residence time (9 CV/min) the DBC_5 was 3.9 mg/mL (Figure 4 B), similar to the highest DBC_5 in 0.5 M NaCl at 3 min residence time.

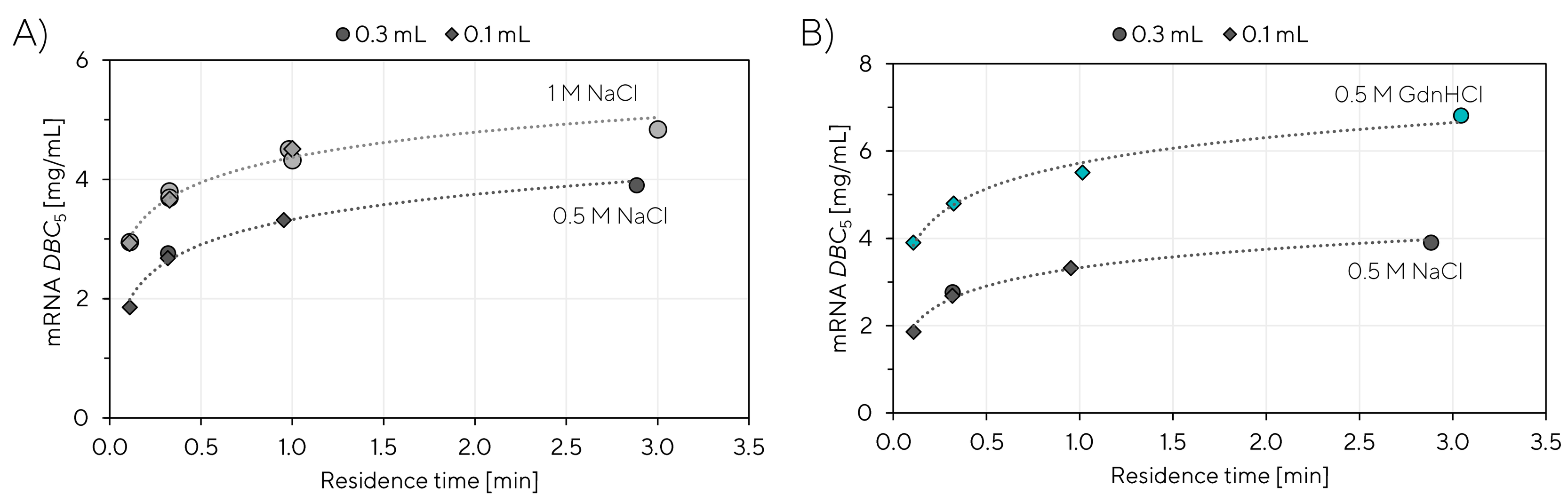


Figure 4: Influence of residence time on mRNA (995 nt) DBC_5 on CIMmic Oligo dT discs (0.1 mL or 0.3 mL) in 0.5 M and 1 M NaCl (A) or in 0.5 M NaCl and 0.5 M GdnHCl (B). Residence times of 0.11-3 min were achieved by varying flow rate in the range of 0.33-9 CV/min.

Confirmation run on 1 mL radial CIMmultus Oligo dT column

To validate the results from screening on CIMmic discs, mRNA DBCs in 0.5 M NaCl and 0.5 M GdnHCl were measured on a 1 mL CIMmultus Oligo dT column at 1 min residence time and 100 µg/mL mRNA feed concentration.

Changing salt concentration from 0.5 M NaCl to 0.5 M GdnHCl increased mRNA DBC_5 for 62 % on 1 mL CIMmultus Oligo dT column, validating the observed increase of 66 % on 0.1 mL CIMmic dics (Figure 5). This confirms the suitability of using CIMmic platform for method development and optimization with smaller sample consumption. However, it is not recommended to use CIMmic discs as the base for industrial scaling-up; instead, the starting column should be a 1 mL CIMmultus column.

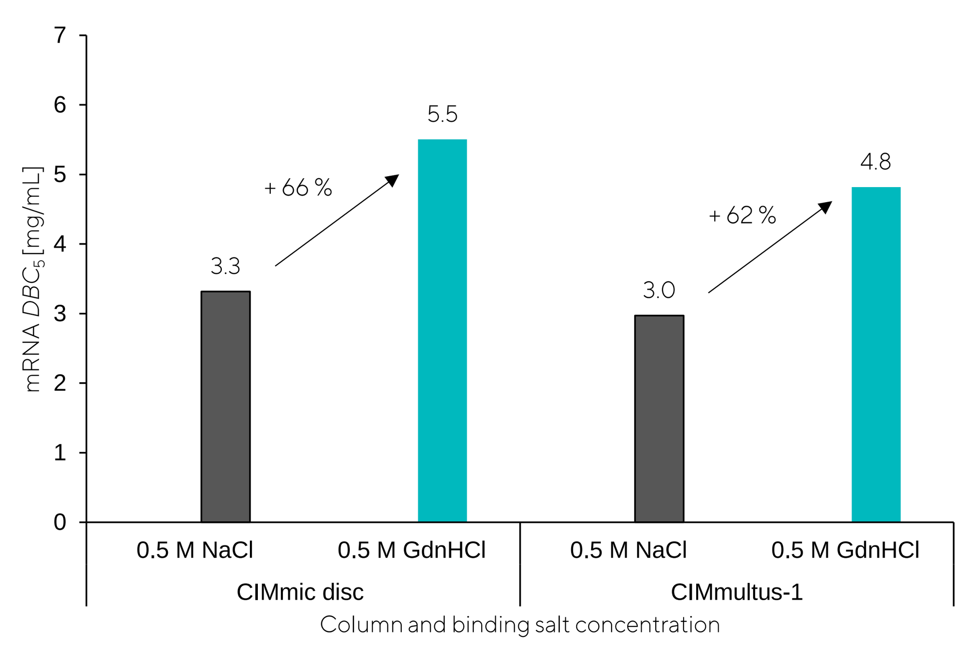


Figure 5: Influence of changing binding salt from 0.5 M NaCl to 0.5 M GdnHCl on mRNA DBC_5 on CIMmic Oligo dT18 (C12 linker) discs and 1 mL CIMmultus Oligo dT18 (C12 linker) columns . Experiment performed with 100 µg/mL mRNA (995 nt) feed concentration at 1 min residence time.

4. Conclusion

- The CIMmic screening platform enables testing of individual small-scale chromatographic units on analytical or FPLC systems, with the advantage of full chromatographic monitoring.
- The CIMmic platform was used for optimization of chromatographic conditions for capturing mRNA molecules using Oligo dT18-modified monolith columns. It enabled the optimisation of chromatographic conditions (flow rate, salt concentration and salt type) to increase DBC_5 from 1.9 mg/mL up to 6.8 mg/mL by utilizing a limited amount of mRNA. DBC_5 trends were additionally confirmed on a ten times larger radial CIMmultus column (1 mL).
- The CIMmic platform enables efficient DoE studies with low sample consumption.

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