CHROMATOGRAPHIC PERFORMANCE OF NOVEL POLYMETHACRYLATE-BASED ION-EXCHANGING MONOLITHS

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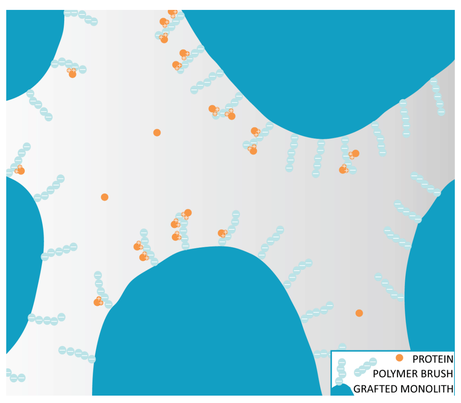
INTRODUCTION

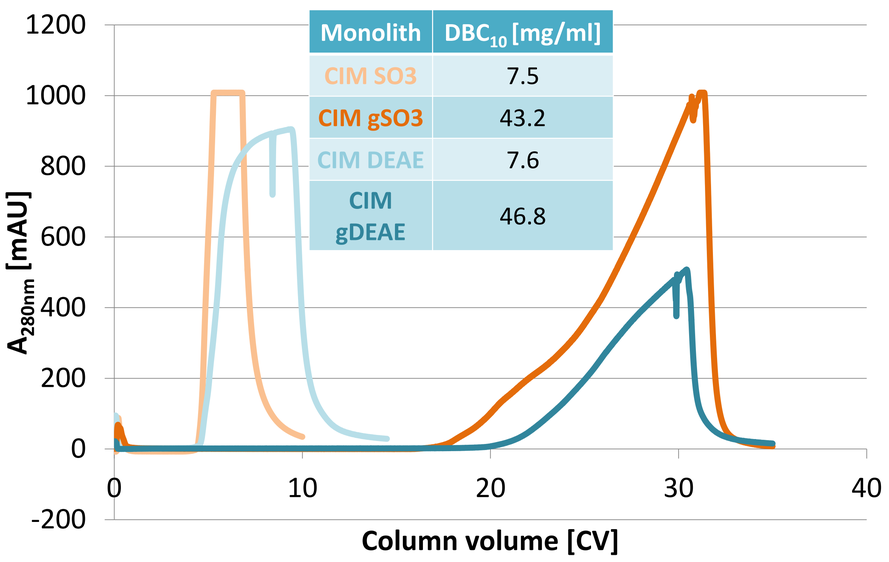
Productivity of the downstream bioprocessing depends among others on the efficiency of chromatographic step. One of the crucial chromatographic parameters is dynamic binding capacity (DBC) for certain biomolecule. DBC could be tailored with changing the surface area of convective pores by tailoring the surface of pre-polymerized monoliths using graft or block polymerization of polymer brushes.
Grafted CIM monoliths have already been prepared via Radical Polymerization (RP) and successfully characterized (1).



Recently, the implementation and optimization of Controlled Radical Polymerization (CRP) for grafting of large pore monoliths (average diameter 6 µm) resulted in polymethacrylate-based ionic exchanger with at least 5 times higher DBC compared to non-grafted 6 µm monoliths, while preserving high permeability.
The main goal of our study was to chromatographically characterize novel grafted ion-exchanging monoliths (CIM gDEAE and CIM gSO3) to see whether novel columns still retain flow independent chromatographic properties of non-grafted monoliths .

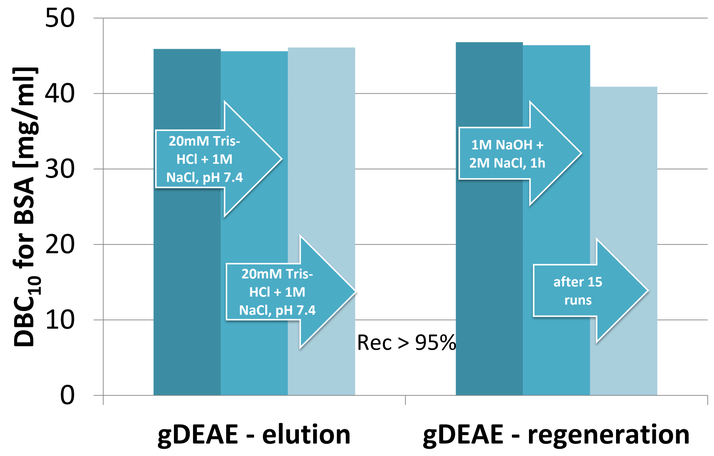
RESULTS

Breakthrough curves for proteins using large pore CIM and novel grafted monoliths



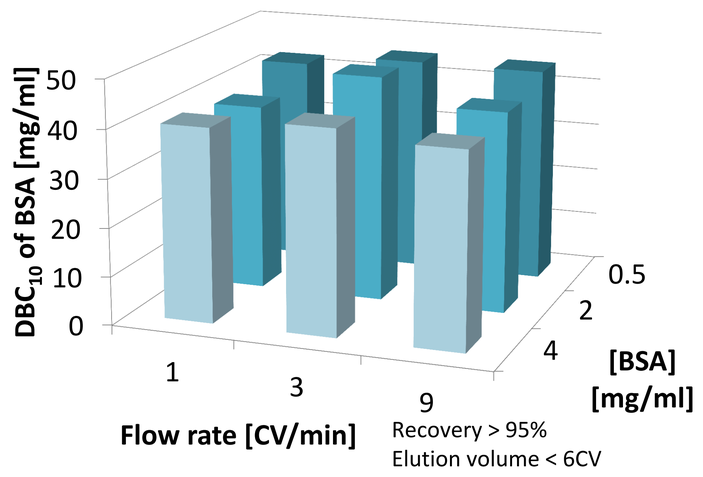
Novel grafted **anion** and **cation-exchanging** monoliths enable at least **5-times higher DBC** for proteins compared to non-grafted monoliths of the same pore size distribution.

Reusability of CIM gDEAE monolith



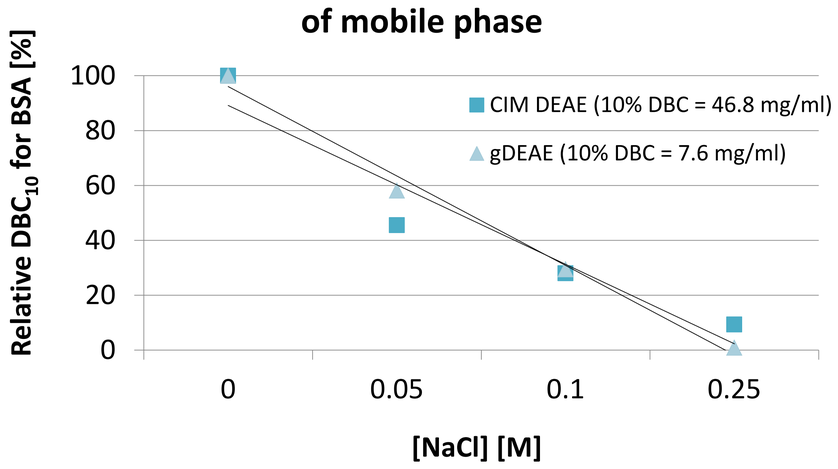
Long-term stability of grafted columns was evaluated. DBC for BSA on CIM gDEAE monolith was determined after 6 months in 20% EtOH, without and with regeneration step and after 15 runs and 5 regeneration steps. DBC value remained constant, only after last set of experiments was 10% lower.

DBC dependence on BSA concentration and flow rate



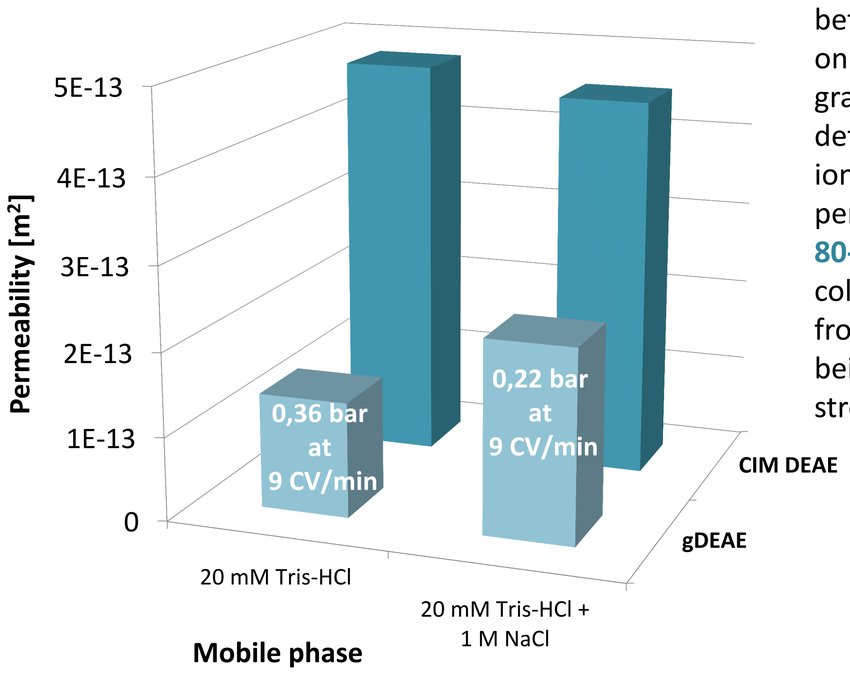
Set of experiments with different loading BSA concentrations and flow rates. DBC is (from perspective of practical applicability) **unaffected** by these two parameters, comparable to results on non-grafted CIM DEAE monolithic disks (see ref. 2).

DBC dependence of different ionic strengths of mobile phase



To investigate the **effect of salt concentration** the DBC measurements were performed using loading buffer with 0.05, 0.1 and 0.25 M NaCl. Results (in term of relative DBC drop) obtained on CIM gDEAE were comparable to the results obtained using large pore non-grafted DEAE.

Permeability of monoliths



As expected for monolithic support, linear relation between flow rate and pressure drop was determined on CIM and grafted monoliths (3). Permeability of grafted monolith was 30% (or 50%) of the value determined for non-grafted monolith in low and high ionic strength buffers, respectively. However, permeability of novel grafted monoliths was at least **80-fold higher** than permeability of previously grafted columns (1). Thickness of grafted layer was estimated from the difference in permeability of monoliths (1), being **280 nm** and **150 nm** in low and high ionic strength buffer, respectively.

Conditions for DBC measurement:
- Columns: CIM DEAE, CIM SO3, CIM gDEAE and CIM gSO3 (pore size: 6µm)
- Loading step: 2 mg/ml of protein (BSA or LYZ) dissolved in 20mM Tris-HCl, pH 7.4. Other BSA concentrations and buffer ionic strengths used are separately specified.
- Wash step: 20mM Tris-HCl, pH 7.4
- Elution step: 20mM Tris-HCl + 1M NaCl, pH 7.4
- Regeneration step: 1M NaOH + 2M NaCl, 1h
- Flow rate: 3 ml/min. Different flow rates used are separately specified.
- UV detection: 280 nm

References:
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2 I. Mihelič, T. Koloini, A. Podgornik, A. Štrancar, J. High Resol. Chromatogr. 23 (2000) 39-43.
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CONCLUSIONS

- At least **5-times higher DBC** for proteins on grafted monoliths of the same pore size distribution for **anion** and **cation-exchangers**.
- Long-term stability was confirmed after 6 months in 20% EtOH, after three following DBC runs and also after 15 DBC runs including 5 regeneration steps.
- The binding capacity for BSA on grafted monolith in presence of 0.1 M NaCl is almost doubled than on non-grafted in absence of NaCl.
- Loading protein concentration and flow rate do not drastically affect binding capacities of grafted monoliths. Together with linear relation between pressure drop and flow rate this indicates convective mass transfer even for grafted monoliths.
- Thickness of grafted layer was estimated – **280 nm** in 20 mM Tris-HCl.

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