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Ion-Exchange Monolithic Capillary Columns Prepared by Ring-Opening Metathesis Polymerization

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Introduction

Miniaturization has been one of the main trends in HPLC over the past 10 years. The main advantage of monolithic columns is that they provide lower back pressure in combination with enhanced diffusional mass transport and a unique structure and pore distribution, leading to shorter separation times. The concept of monolithic stationary phases is especially favorable for the fabrication of capillary columns[1], due to their ease of preparation without the requirement of sophisticated packing procedures or the manufacturing of end frits.

In recent years, there has been considerable interest in developing monolithic columns bearing functional groups. ROMP offers the unique possibility to prepare such functionalized monolithic columns by applying an *in-situ* derivatization process[2]. The formation of the monolithic column and functionalization can thus be separately optimized in a two step process.

Methods

Monolithic capillary columns were prepared from silanized fused silica capillaries of 200 µm inner diameter by Ring-Opening Metathesis Polymerization (ROMP). The polymerization mixture for the preparation of monolithic columns consists of norborn-2-ene (NBE) and 1,4,4a,5,8,8a-hexahydro-1,4,5,8,-*exo,endo*-dimethanonaphthalene (DMN-H_6), isopropanol (macroporogen), toluene (microporogen) and $Cl_2(PCy_3)_2Ru=(CHPh)$ as ROMP-active initiator[3]. The mixture is filled into norbornenesilane surface-derivatized fused silica capillaries where polymerization takes place (Figure 1). Using the active initiator for the preparation of ion-exchange monolithic capillary columns, the monoliths were flushed with 7-oxanorborn-5-ene-2,3-dicarboxylic anhydride.

Results

Synthesized non-functionalized capillary monoliths show a good separation performance for proteins and peptides (Figure 2).

Furthermore, excellent results for reproducibility in terms of chromatographic parameters were observed. The relative standard deviation of retention time was around 1.8 %. However, in terms of column back pressure versus flow rate, a high variability of the standard deviation of the slope was observed. The reproducibility of the preparation process was significantly improved by cooling of the capillary monoliths during the polymerization process. Although there were no significant changes in morphology (Figure 3), the variation of retention times was enhanced (from 1.8 % to 1.2 %) and the variation of the slope was reduced from 31 % to 8 % (Figure 4).

Initial results for functionalized monoliths were obtained for weak cation-exchange capillary columns. Monoliths bearing carboxylic acid functional groups were prepared using an *in-situ* grafting technique. The functionalization was proved by breakthrough curves using copper sulfate (Figure 5). The proof of principle was thus demonstrated and further work will be dedicated to investigating and improving the synthesis and capacity of functional monoliths.

Conclusion

ROMP-derived non-functionalized monoliths allow rapid and highly efficient separations of proteins and peptides. Although synthesized capillary monoliths already exhibit a high degree of column-to-column reproducibility, the reproducibility was further enhanced by a modification of the preparation process. Initial functionalization studies revealed good ion-exchange capacities. However, the grafting process has to be further optimized in order to achieve higher capacities.

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2 Sinner, F. M., Buchmeiser, M. R.; Angew. Chem. **112** (2002) 1491
3 Sinner, F. M., Buchmeiser, M. R.; Macromolecules **33** (2000) 5777

Preparation of monoliths

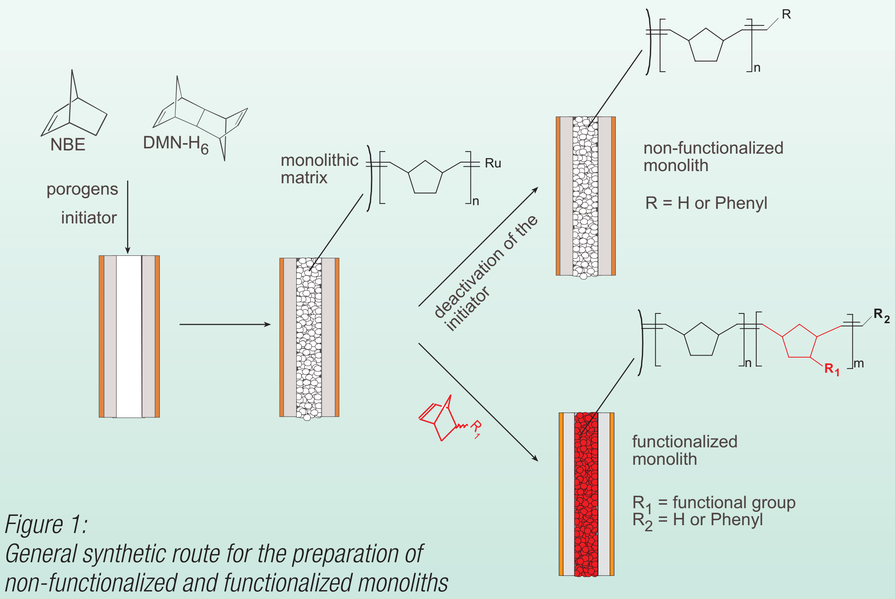


Figure 1: General synthetic route for the preparation of non-functionalized and functionalized monoliths

Reversed phase application

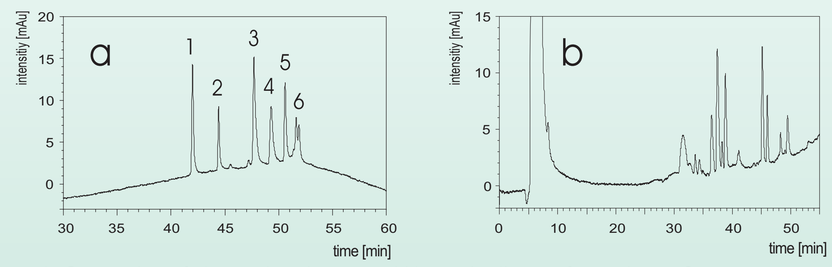


Figure 2: Separation of a) protein mixture and b) tryptic digest of cytochrom C. Chromatographic conditions: flow 1 µl/min; 25 °C; detection: UV, 190 nm; mobile phase A: 95 % water, 5 % acetonitrile, 0.05 % TFA; B: 20 % water, 80 % acetonitrile, 0.04 % TFA; gradient: a) 0–30 min 0–60 % B; 60–90 % B within 5 min; b) 0–30 min 0–50 % B; 50–90 % B within 5 min. (1) ribonuclease A (2) insulin (3) lysozym (4) albumin (5) myoglobin (6) ß-lactoglobulin

Monolithic structure

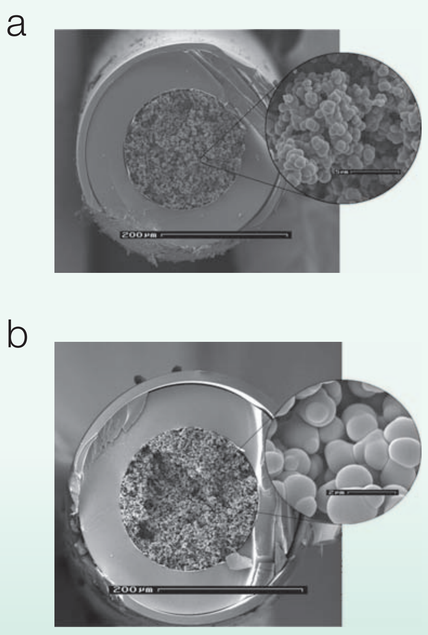


Figure 3: Electron micrographs of monoliths. Polymerization temperature a) 25 °C b) 0 °C.

Reproducibility of monoliths

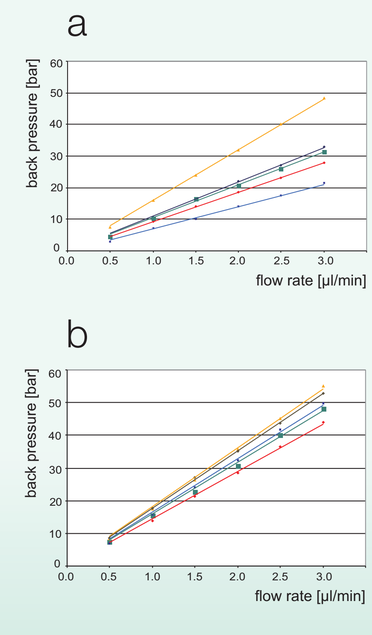
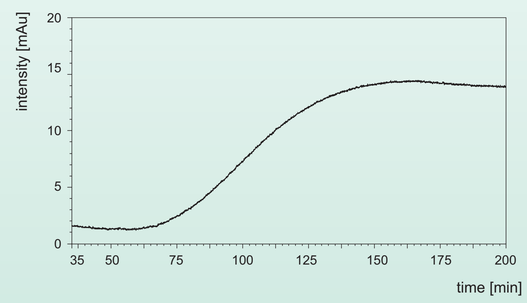


Figure 4: Effective back pressure vs. flow rate. Polymerization temperature a) 25 °C b) 0 °C. Mobile phase: water

Capacity of weak cation-exchange monoliths



monolithic bed
NBE: 10 %
DMN-H_6: 10 %
toluene: 10 %
isopropanol: 70 %

in-situ grafting
7-oxanorborn-5-ene-2,3-dicarboxylic anhydrid: 0.5 %
in methanol

Figure 5: Determination of the ion-exchange capacity of cation-exchange monoliths. Representative breakthrough curve: flow 1 µl/min; 25 °C; detection: UV, 220 nm; mobile phase: copper sulfate 1 mM.

Acknowledgement

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