Monolithic Stationary Phases Prepared by Ring-Opening Metathesis Polymerisation: Influence of Different Polymerisation Parameters on Separation Performance

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Introduction

Miniaturisation is a challenging requirement in the field of proteomics and drug discovery. Therefore, separation media with smaller inner diameter and higher separation performance are needed[1]. Monolithic columns are one attempt to reach this goal. The main advantage of monolithic columns is that they provide lower back-pressure in combination with enhanced diffusional mass transport, leading to shorter separation times. Furthermore, they do not require time-consuming packing procedures or the manufacturing of end frits.

Methods

Monolithic capillary columns were prepared from silanised fused-silica capillaries of 200 µm inner diameter by Ring-Opening Metathesis Polymerisation (ROMP)[2]. The polymerisation 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*-dimethanonaphtalene (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-derivatised fused silica capillaries where polymerisation takes place (Figure 1 and 2).

Preparation of Monoliths

Figure 1: Manufacturing process of monoliths

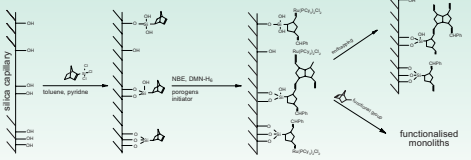

Figure 1: General synthetic route for the preparation of monoliths

Results

Reproducibility of ROMP-monoliths is good for proteins and peptides (Figure 3). The variation of retention times is 1 - 2 % for 5 seperatley prepared monoliths.

Changing triphenylphosphine concentrations as well as different ratios of NBE/DMN-H_6 to porogen have strong effects on monolithic microstructure (Figure 4 and 5). These differences in morphology should lead to differences in separation behaviour. This is the case for cytochrom C and insulin (porcine, bovine, human) but not for proteins with MW > 10 000 Da.

a) Descending triphenylphosphine concentration simplifies the manufacturing process of monoliths (Ph_3P acts as a moderator). As a consequence of the decelerated polymerisation kinetics bigger "particles" are formed (Figure 4), which degrades separation behaviour for peptides.

b) Higher NBE/DMN-H6 amounts lead to higher column back-pressure and better separation performance for peptides.

Influence of Triphenylphosphine concentration on morphology and separation behaviour

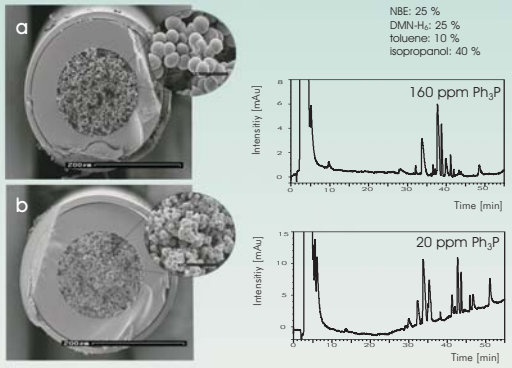

Figure 3: Separation of tryptic digest of cytochrom C on a) monolith 1 b) monolith 2, 120 ng cytochrom C. Chromatographic conditions: flow 1µl/min; 25 °C; detection: UV, 190 nm; mobile phase A: 95% water, 5% acetonitrile, 0,05% TFA; B: 20% water, 80% acetonitrile, 0,04% TFA; gradient: 0-30 min 0-50 %B; 50-90 %B within 5 min

Conclusion

Variation of polymerisation parameters strongly influences the microstructure and therefore separation performance of monolithic columns.

A general tendency could be observed, because in both cases smaller "particles" lead to better separation performance for peptides. This phenomenon can be explained by increased surface area or/and altered microstructures and pore properties. By choosing appropriate polymerisation parameters optimised separation media can be prepared.

[1] Mayr, B., Hölzl, G., Eder, K., Buchmeiser, M. R., Huber, C. G.; *Anal. Chem.* **74** (2002) 6080 - 6087
[2] Sinner, F. M., Buchmeiser, M. R.; *Angew. Chem.* **112** (2002) 1491 - 1494
[3] Sinner, F. M., Buchmeiser, M. R.; *Macromolecules* **33** (2000) 5777 - 5786

Influence of changing ratio NBE/DMN-H_6 to porogen on morphology and separation behaviour

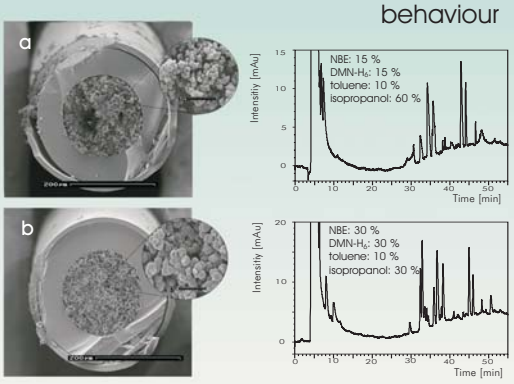

Figure 4: Separation of tryptic digest of cytochrom C on a) monolith 3 b) monolith 4, 120 ng cytochrom C. Chromatographic conditions: flow 1µl/min; 25 °C; detection: UV, 190 nm; mobile phase A: 95% water, 5% acetonitrile, 0,05% TFA; B: 20% water, 80% acetonitrile, 0,04% TFA; gradient: 0-30 min 0-50 %B; 50-90 %B within 5 min

Putting Knowledge to Work