Particle-Loaded Monoliths

for Enantioseparation by Capillary Electrochromatography

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Introduction

Enantioresolution is an important issue in pharmaceutical and biomedical science. The chirality of pharmaceutical drugs significantly affects the biological activity, toxicity and metabolism. In the analysis of chiral pharmaceuticals capillary electrochromatography (CEC) has evolved as a powerful tool. Common designs of capillary columns for CEC are based on open-tubular and packed columns, however, packing of the capillaries and the preparation of frits is a rather complicated procedure. An alternative approach, therefore, is the application of monolithic separation media, due to their ease of preparation without the requirement of packing procedures or the manufacturing of end frits[1]. A recent trend is the preparation of fritless, particle-loaded capillaries utilizing the favorable preparation procedure of monoliths[2,3].

Aims

The applicability of the concept of particle-loaded monolithic columns using polymethacrylamide has already been shown[2]. In order to evaluate the influence of a polymeric backbone prepared by a different polymerization mechanism on chiral separation performance, the separation efficiency of norbornene-based particle loaded monolithic capillary columns was investigated for two different types of chiral selectors.

Methods

Novel particle-loaded monolithic capillary columns were prepared within the confines of fused silica capillaries with 200 µm inner diameter by Ring-Opening Metathesis Polymerization (ROMP). Synthesis of particle loaded-monoliths was accomplished using norborn-2-ene (NBE), 1, 4, 4a, 5, 8, 8a-hexahydro-1, 4, 5, 8,-*exo, endo*-dimethanonaphthalene (DMN-H_6), isopropanol, toluene and $Cl_2(PCy_3)_2Ru=(CHPh)$ as ROMP-active initiator. The silica particles (3 µm) bearing the chiral selector were suspended in the polymerization mixture before adding the initiator. Subsequently, the mixture was filled into norbornenesilane surface-derivatized fused silica capillaries using reduced pressure.

Results

The preparation procedure for norbornene-based particle-loaded monoliths is as simple as for polymethacrylamide-based particle-loaded monoliths. The polymerization mixture containing the suspended silica particles is flushed through the capillary using reduced pressure until the polymerization mixture reaches the detection window. The well-defined transition from the formed monolith to the empty part of the capillary is shown in Figure 1.

Ring-Opening Metathesis Polymerization is much faster than polymerization of methacrylamide, and monoliths are formed within 30 minutes. Figure 2 shows the differences in morphology of particle-loaded monolithic columns based on polymethacrylamide and norbornene.

Although the particle-loaded monoliths differ in their morphology and chemistry, no significant influence on chiral separation performance was observed. Both types of monoliths containing teicoplanin aglycone (TAG) as chiral selector showed good separation performance for a set of glycyl-dipeptides (Figure 3 a and c).

The concept of particle-loaded monolithic columns was also applied to the preparation of monolithic columns using the chiral separation principle of ligand exchange. Particle-loaded monolithic columns containing L-4-hydroxyproline (LHP) immobilized on 3 µm silica particles were used (Figure 3 b and d).

Conclusion

Particle-loaded monoliths containing a chiral selector immobilized on 3 µm silica particles were successfully prepared using Ring-Opening Metathesis Polymerization. Norbornene-based particle-loaded monoliths showed similar separation behavior to particle-loaded polymethacrylamide monoliths. Therefore, the chemical and morphological nature of the monolith used seems to have only a very minor effect on the chiral separation performance of this type of separation media. Significant advantages of particle-loaded monoliths are their easy and inexpensive preparation using any commercially available silica-based chiral HPLC phase.

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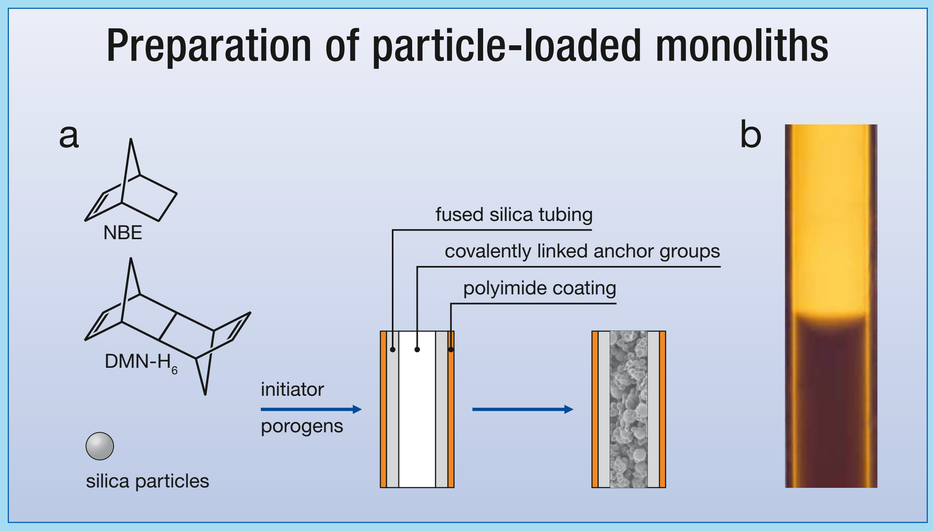

Figure 1:
a) General synthetic route for the preparation,
b) microscopic picture of norbornene-based particle-loaded monoliths

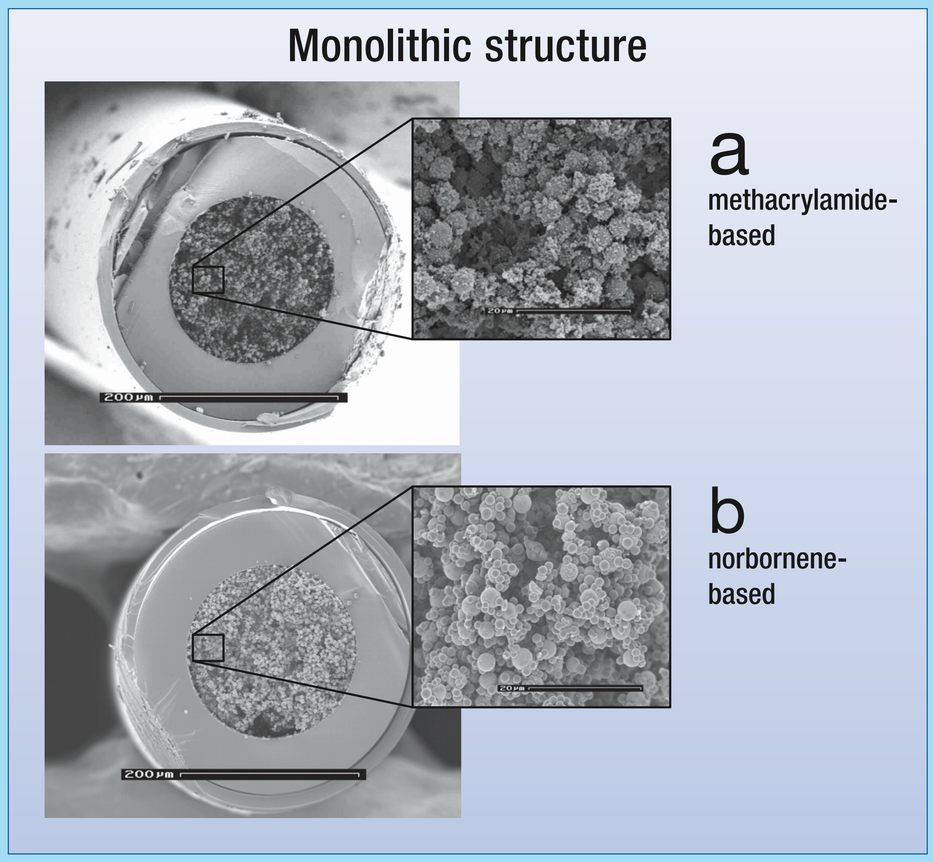

Figure 2: Electron micrographs of
a) methacrylamide-based and b) norbornene-based particle-loaded monoliths.

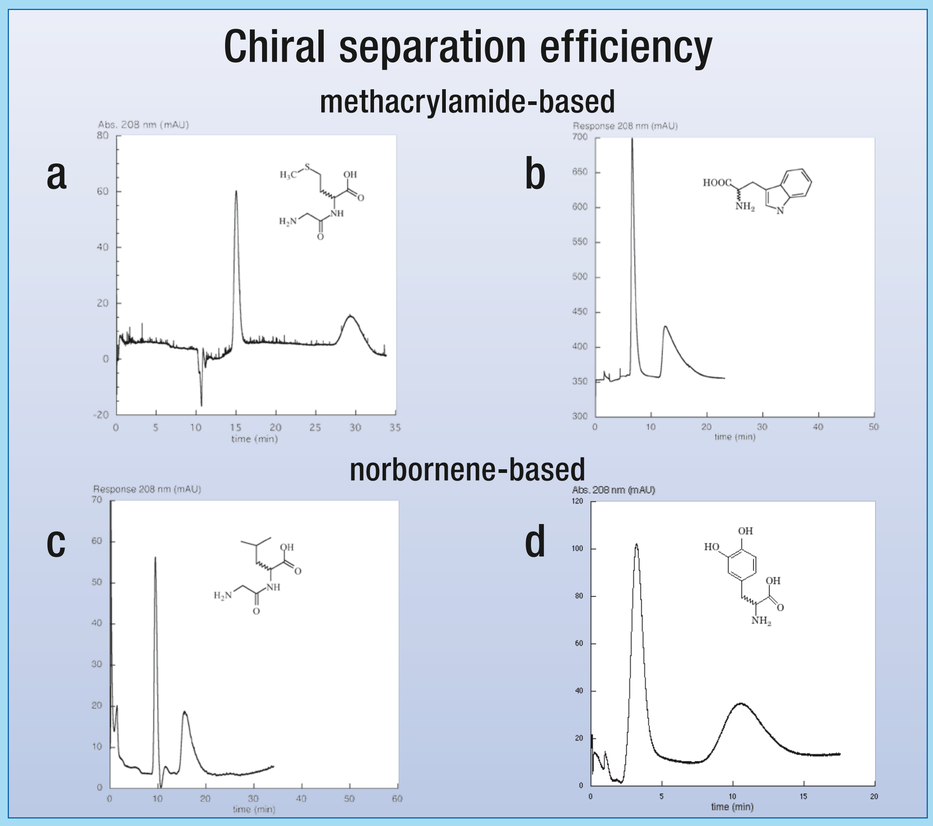

Figure 3: Chiral separation of

a) Gly-Met: 25 % TAG silica, methacrylamide-based (26 cm x 250 µm, 66 cm entire length), mobile phase 50 % aq. TEAA solution (0.2 %, adjusted with acetic acid to pH 4.1)/50 % ACN, 15 kV

b) Trp: 15 % LHP silica, methacrylamide-based, (6 cm x 100 µm, 34 cm entire length), mobile phase: 50 mM phosphate solution, 1 mM Cu(II) pH 4.5 5 kV

c) Gly-Leu: 25 % TAG silica, norbornene-based (27.5 cm x 200 µm, 36 cm entire length), mobile phase 50 % aq. TEAA solution (0.2 %, adjusted with acetic acid to pH 4.1)/50 % ACN, 25 kV

d) DOPA (3, 4, dihydroxy phenylalanine): (20 %) LHP silica, norbornene-based (8 cm x 200 µm, 36 cm entire length), mobile phase: 50 mM phosphate solution, 1 mM Cu(II) pH 4.5, 5 kV, 12 bar pressure support

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