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Ring-opening metathesis polymerization for the preparation of norbornene-based weak cation-exchange monolithic capillary columns

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ABSTRACT

Functionalized monolithic columns were prepared via ring-opening metathesis polymerization (ROMP) within silanized fused silica capillaries with an internal diameter of 200 μm by *in situ* grafting. This procedure is conducted in two steps, the first of which is the formation of the basic monolithic structure by polymerization of norborn-2-ene (NBE) and 1,4,4a,5,8,8a-hexahydro-1,4,5,8-*exo*,*endo*-dimethanonaphthalene (DMN-H6) in a porogenic system (toluene and 2-propanol) using $RuCl_2(PCy_3)_2(CHPh)$ as ROMP initiator. In the second step the still active initiator sites located on the surface of the structure-forming microglobules were used as receptor groups for the attachment ("grafting") of functional groups onto the monolithic backbone by flushing the monolith with 7-oxanorborn-2-ene-5,6-carboxylic anhydride (ONDCA). Functionalization conditions were first defined that did not damage the backbone of low polymer content (20%) monoliths allowing high-throughput chromatographic separations. Variation of the functionalization conditions was then shown to provide a means of controlling the degree of functionalization and resulting ion-exchange capacity. The maximum level of *in situ* ONDCA grafting was obtained by a 3 h polymerization in toluene at 40 °C. The weak cation-exchange monoliths obtained provided good separation of a standard peptide mixture comprising four synthetic peptides designed specifically for the evaluation of cation-exchange columns. An equivalent separation was also achieved using the lowest capacity column studied, indicative of a high degree of robustness of the functionalization procedure. As well as demonstrably bearing ionic functional groups enabling analyte separation in the cation-exchange mode, the columns exhibited additional hydrophobic characteristics which influenced the separation process. The functionalized monoliths thus represent useful tools for mixed-mode separations.

1. Introduction

Monolithic separation media were first introduced to the scientific community about 15 years ago [1–4], since when they have undergone a steady evolution. Through the use of a variety of different precursor materials (silica and polymer-based) [5–8] and column sizes (preparative, analytical and capillary columns and even chips) they have become powerful and widely used chromatographic tools. Originally the domain of specialists who built their own columns, a variety of monolithic columns are nowadays commercially available.

The power of monolithic columns stems from a number of key properties. One of these is their porous structure which combines (i) a high external porosity and resultant low resistance to flow which allows high flow rates at a comparatively low back pressure and (ii) enhanced mass transfer due to convective mass transport [9–11] which allows an increased flow velocity with minimal loss in column efficiency [12–14]. Taken together these properties render monoliths ideal tools for high-throughput separations. A further important feature of monoliths is their ease of preparation by *de*

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novo and chemical polymerization processes *in situ* which neatly avoids the need for the often-sophisticated column packing procedures and the use of end frits associated with particle-packed columns. The use of monoliths for capillary electrochromatography and high-performance liquid chromatography (HPLC) in a variety of chromatographic modes (reversed-phase, ion-exchange, bioaffinity and chiral separation) is the subject of a number of reviews [6,15–22].

Organic polymer-based monoliths have been prepared by polymerization of a wide-range of monomeric precursors, including acrylamide, acrylate and methacrylate, styrene, norbornene and cyclootene [20,23]. Each of these exhibits both advantages and disadvantages, most importantly in terms of ease and duration of synthesis, tailoring of pore size, column stability and ease of alteration of surface chemistry of the resultant monoliths. Functionalized monoliths can be prepared using a number of possible procedures [8,24–26]. The simplest of these involves direct copolymerization with a functionalized monomer. The convenience of this is however offset by a number of associated problems. One of these is that a substantial number of the functional groups are buried within the microstructure-forming polymer and thus unavailable for interaction with analytes. Another key problem is one of column construction flexibility: every time a new monomer is used the column polymerization conditions must be re-optimized according to the properties of the new monomer. These problems are dealt with by two-step, or so-called, "post-polymerization functionalization" techniques. In one of these the copolymerized monomer carries a reactive group which, following the completion of the polymerization procedure is chemically transformed into a specific functional group. The second method, known as "grafting", involves the attachment of chains of functional monomers to these reactive groups. Functionalized monoliths can be prepared using so-called "living" polymerization techniques, such as tetramethylpiperidinyl oxide (TEMPO)-mediated living radical polymerization [27] and ring-opening metathesis polymerization (ROMP) [28,29]. The latter method which is based on a transition metal-catalyzed polymerization mechanism, offers the advantages that both initiation efficiency and control over propagation are well defined. Backbone formation and functionalization conditions can moreover be independently optimized.

The use of ROMP for the preparation of both non-functionalized and functionalized chromatographic supports is already established [30–37], in the latter case through the application of an *in situ* grafting technique using the "living character" of the initiator for surface modification. A common feature of these studies is the use of wide bore columns. The current trend in chromatographic separation is however towards column miniaturization as this offers the advantages of analyzing low sample volumes, low analyte concentrations due to enhanced sensitivity and easier coupling to mass spectroscopy. In accordance with this trend, non-functionalized ROMP-derived monoliths with reduced internal column diameters suitable for microscale separations have been prepared [38–40]. There is great interest in the use of such downscaled monoliths, not least because the problems associated with the production of particle-packed columns alluded to above become increasingly acute when they are miniaturized [6,8,20,41]. Recent, proof-of-principle studies have moreover demonstrated the feasibility of preparing capillary-scale functionalized ROMP-monoliths for anion-exchange chromatography [42]. The present study builds on these promising results and (i) demonstrates for the first time the preparation of cation-exchange capillary monoliths by ROMP, (ii) presents the results of a detailed evaluation of the functionalization conditions and (iii) characterizes the chromatographic properties in weak cation-exchange (WCX) mode of monoliths prepared using different functionalization conditions.

2. Experimental

2.1. Chemicals, reagents and samples

The chemicals used for monolith preparation by ROMP and suppliers were those described previously [38]. 7-Oxanorborn-2-ene-5,6-carboxylic anhydride (ONDCA) were prepared according to a published procedure [43,44] and checked for purity by means of NMR. Copper sulphate, sulfuric acid (96.8%) and sodium chloride (NaCl, 99.5%) were purchased from Fluka (Buchs, Switzerland). Tetrahydrofuran (THF, 99.9%), disodium hydrogenphosphate-2-hydrate (>98.5%) and sodium dihydrogenphosphate-2-hydrate (>98.0%) were purchased from Riedel-de Haën (Seelze, Germany). Dichloromethane (99.5%) and dimethyl sulfoxide (DMSO, 99.5%) were obtained from Merck (Darmstadt, Germany). Dimethylformamide (DMF, 99.9%), water (HPLC-grade), acetonitrile (HPLC-grade) and methanol (HPLC-grade) were purchased from Sigma–Aldrich (Vienna, Austria). The synthetic peptides were obtained from piCHEM (Graz, Austria). These were dissolved in water at concentrations of 50 ng/μl (Peptides 1 and 2) and 500 ng/μl (Peptides 3 and 4) and stored at −80 °C.

2.2. Synthesis of ROMP-based monoliths

Fused silica capillaries (non deactivated, 365 μm O.D., 200 μm I.D.) were purchased from J & W (Agilent, Palo Alto, CA, USA). Surface modification of the capillaries was performed as described previously [36]. Monolith synthesis was carried out according to a published protocol using [2.2.1]bicyclohept-2-ene (norborn-2-ene, NBE), 1,4,4a,5,8,8a-hexahydro-1,4,5,8-*exo*,*endo*-dimethanonaphthalene (DMN-H6), toluene, 2-propanol (10, 10, 10, and 70%, w/w) and (25, 25, 10, 40%, w/w) [33]. Briefly, NBE and DMN-H6 were dissolved in the porogenic system, $RuCl_2(PCy_3)_2(CHPh)$ (0.5%, w/w) and Ph_3P (40 ppm) were added and the resultant mixture was introduced into the capillary. This was sealed at both ends with silicon septa and polymerization of the monolithic matrix was carried out for 30 min, unless stated otherwise. Endcapping was carried out using ethyl vinyl ether and DMSO (non-functionalized monoliths). With the exception of back pressure measurements for which 10 cm columns were used, 15 cm monolithic capillary columns were used for all investigations. Electron microscopy was carried out at the Center for Electron Microscopy, ZFE, Graz, Austria.

2.3. Surface functionalization of ROMP-based monoliths

After structure formation, monoliths were flushed with toluene to remove unreacted backbone precursors. Polymerization was reinitiated by addition of a functional monomer. A solution of 2.5% (w/w) ONDCA in toluene (saturated solution) was infused into the monolith at a constant rate of 0.2 μl/min. After functionalization the monolith was endcapped. Hydrolysis of the anhydride was performed by sequentially flushing the monoliths with water, 0.2 mM sulfuric acid and again with water. Table 1 summarizes the preparation conditions for all non-functional and functional monoliths synthesized in this study.

2.4. High-performance liquid chromatography

All experiments were performed on an Ultimate capillary HPLC system (Dionex, LC-Packings, Amsterdam, The Netherlands) using a UV detection cell with a volume of 45 nl. Chromeleon software (Version 6.70) was used for data acquisition. The mobile phase comprized of 1 mM phosphate buffer (pH 7.0) containing a range of acetonitrile concentrations (0, 10, 20, 30 and 40%). For sepa-