Allyl-functionalized hybrid silica monoliths

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A hybrid organosilica monolith was synthesized containing an allyl functionality. This provided a viable platform for producing silica-based, chromatographic, monolithic columns with the stationary phase bonded through a surface silicon–carbon bond rather than a conventional siloxane bond.

The use of monolithic structures as the chromatographic column in liquid chromatography (LC) has attracted considerable attention in recent years.[1] The use of such structures reduces significantly the pressure requirements to drive the mobile phase through the column, allowing the use of higher-than-usual mobile phase linear velocities. This provides a route for making fast separations while maintaining chromatographic performance.[2] The reduced inlet pressures have allowed the possibility of using inexpensive peristaltic pumps to drive the mobile phase through the chromatographic column in a simple and portable HPLC system.[3] In addition to the low pressure requirements for LC, monolithic columns offer the advantage of improved mass transfer at high flow rates, and are easily fabricated. The monolithic structure simplifies column technology by eliminating the difficulties encountered with frit fabrication in packed capillary columns for capillary electrochromatography (CEC) and capillary liquid chromatography (CLC).[4] In CEC in particular, monolithic columns eliminate a host of problems associated with the retaining frits of packed columns (*e.g.*, non-specific adsorption, bubble formation, and electroosmotic flow discontinuity).

Silica-based monolithic columns for CLC and CEC are typically synthesized *in situ* by sol–gel processing.[2,5] A widely used precursor in the synthetic approach is tetramethoxysilane (TMOS) which is reacted with water under acidic conditions in the presence of a porogenic agent. A capillary tube is then filled with the reaction mixture and submitted to heating routines to promote condensation and evaporate the solvent. After fabrication of the monolithic support, the surface of the silica monolith is functionalized through conventional silane chemistry to anchor the stationary phase in place (*e.g.*, C-18) by means of a siloxane bond. One drawback of this approach is that the poor hydrolytic stability of the bonded stationary phase leads to a limited usability for the separation of solutes—within an approximate pH range of 2–8.[6] At low pH, the siloxane bond that attaches the stationary phase to the surface of the silica support material is prone to nucleophilic cleavage. Therefore, the stationary phase becomes hydrolytically unstable as the pH is lowered. At high pH values on the other hand, the silica dissolves due to hydrolysis of the siloxane backbone. The stability improvements typical of conventional HPLC packing materials (*i.e.* silica beads) can potentially be implemented in monolithic structures (*e.g.*, the use of sterically protected linkages, using bidentate ligands, and synthesizing horizontally polymerized stationary phases).[7] These approaches however, have not been explored in detail for silica monolithic columns. A more hydrolytically stable, bonded phase can however be obtained by silica–carbon (Si–C) or silica–nitrogen (Si–NH–C) bond attachment, rather than by surface O–Si–C linkage.[8] For example, the decomposition of Si–C bonds was not observed after exposing the material to pH 10.5 for 5 d.[8*b*]

One approach to increasing the stability of the stationary phase at the surface through Si–C bonding (used in open tubular columns[9]) is to synthesize a hybrid support structure, in which a silane co-precursor containing a moiety of interest (*e.g.*, octadecyltrimethoxysilane) is incorporated in the reaction mixture. Furthermore, this would produce a monolithic structure containing the stationary phase in a single step. Using such an approach, a hydrolytically-stable amino-silica thin film was fabricated inside a fused silica column and showed a remarkable stability at low pH (<1) compared to the conventional silanization procedure.[10] Although the approach appears to be very attractive for synthesizing monoliths,[11,12] it has not been commonly followed for the fabrication of silica-based monolithic columns for CLC or CEC. This has particularly been the case for precursors with relatively long alkyl chains that are suitable for reverse phase chromatography. Some of the difficulties of using such precursors have recently been discussed.[12] It appears that the hydrolysis and condensation reactions of the dissimilar precursors must be controlled very carefully in order to obtain the desired monolithic structure. Therefore, the synthetic conditions for hybrid monolithic structures containing different functionalities must be optimized independently. This imposes a major problem, as the addition of the co-precursor can affect the integrity and characteristics of the monolithic structure and, at the same time, may compromise the amount of stationary phase that can be added for chromatographic interaction. As is the case with pure silica monoliths, it would be ideal to optimize the synthetic methodology in order to obtain a hybrid monolithic structure that has a given set of physical characteristics. At the same time, it should have a derivatizable moiety attached at the surface through a Si–C bond. This would allow chromatographic selectivity to be varied by derivatizing the attached functionality with a desired moiety—that would then serve as the stationary phase. To this end, we have synthesized hybrid monolithic structures using a silane co-precursor with a short chain allyl group. As outlined in Scheme 1, a functionality that can be further modified to bond desirable phases creates a platform that can be further built upon. In our approach, the surface attachment is through a Si–C bond, while the organoreactive group provides the versatility to allow the surface properties of the final material to be tailored. It is worth mentioning that allyl-silicas have been prepared by conventional silanization to anchor cellulose benzoates as chiral phases for HPLC.[13]

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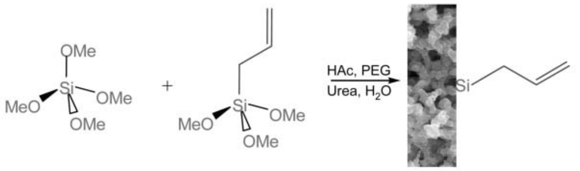

Scheme 1 Overview of hybrid allyl-silica monolith synthesis.

The hybrid allyl-silica monolith was synthesized in a "one pot" approach by mixing allyl-trimethoxysilane (allyl-TrMOS) and TMOS in a solution containing 0.01 M aqueous acetic acid (1 mL), polyethylene glycol (PEG, 10 000 MW) (108 mg) as porogen, and urea (90 mg) to promote mesopore uniformity.[14] The mixture was stirred for 1 h at 0 °C and then heated for 40 h at 50 °C in a sealed container. To fabricate the chromatographic column in a capillary, the solution was introduced into a fused silica capillary tube before heating and the ends of the capillary sealed. After heating, the monolith was flushed with ethanol and reheated for 24 h at 50 °C. The temperature of the monolith was then raised from 50 to 150 °C at a rate of 1.0 °C min^{-1}, allowed to cool to room temperature and flushed with ethanol. IR spectroscopy (Fig. 1) of the final monolithic structure confirmed the incorporation of the allyl group (C(sp^2)–H stretch at 3100, C=C stretch at 1650 and C=C–H out-of-plane bend at $\sim$1000 cm^{-1}).

Fig. 2 illustrates the hybrid monolithic structure prepared inside a 50 μm internal diameter, fused silica capillary tube using a 1 : 4, allyl-TrMOS : TMOS molar ratio. An interconnected, globular, skeleton structure can be seen with flow-through channels of about 2 μm diameter. Nitrogen adsorption analysis performed on the monolith (in pellet form) revealed a surface area of 209 $\text{m}^2\ \text{g}^{-1}$ and mesopores of $\sim$8.2 nm average diameter. The amount of PEG in the reaction mixture affects the morphology, total surface area and mesoporosity of the hybrid structure, allowing for tailoring of the material (see Table 1). By increasing the amount of PEG from 50 to 108 mg, the average mesopore diameter increased and the surface area decreased.

By performing elemental analyses of the hybrid monolithic structure synthesized with a 1 : 4, allyl-TrMOS : TMOS molar ratio and a structure synthesized without the allyl co-precursor, we estimated that about 2.10 mmol of allyl groups were incorporated per gram of material (7.6% C). It is reasonable to expect that the allyl moiety would be distributed throughout the entire material. Chromatographically however, it is the accessible groups at the surface that can be involved in the chromatographic interactions that are most important. To assess the extent of available allyl groups, the hybrid monolith prepared with an allyl-TrMOS : TMOS ratio of 1 : 4 was reacted with Br_2, and proceeded stoichiometrically.[15] The reaction was performed by immersing a piece of the allyl monolith in CH_2Cl_2 containing an excess of Br_2. As control, a piece of a monolith without the allyl functionality was also treated with the Br_2 solution. The products were washed with excess CH_2Cl_2, dried and analysed by elemental analysis.[16] The control experiment did not show any Br_2 incorporation, indicating that the washes with excess CH_2Cl_2 were sufficient to remove any adsorbed Br_2. The product of the hybrid monolith showed a Br content of 23.1 wt %. This corresponds to 1.44 mmol of allyl groups per gram of material that were accessible to react with the Br_2. This indicates that about 68% of the total allyl groups were accessible. Using the surface area of 209 $\text{m}^2\ \text{g}^{-1}$ for the monolithic structure, we obtained a surface coverage of 6.9 μmol m^{-2}. It therefore appears that the allyl group is not homogeneously distributed throughout the monolith. Instead, a surface segregation effect seems to take place in which the allyl-containing moiety is oriented towards the surface of the material. Such effects have been reported for other sol–gel derived hybrid materials.[17]

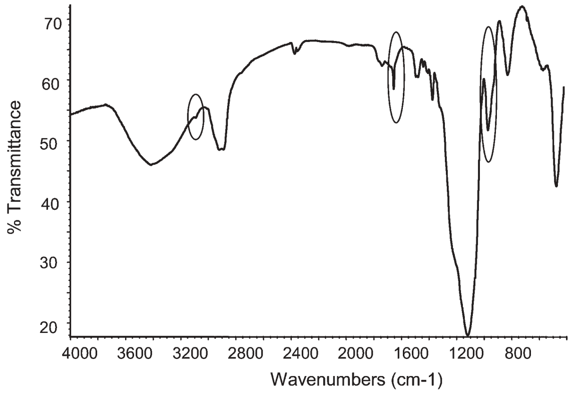

Fig. 1 FTIR of the hybrid allyl-organosilica.

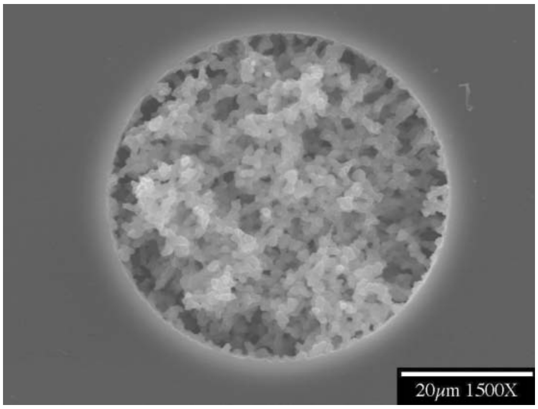

Fig. 2 Scanning Electron Microscopy (SEM) image of the allyl monolith inside a 50 μm internal diameter, 360 μm outside diameter, fused silica capillary. The monolith was fabricated with a molar ratio of 1 : 4, allyl-TrMOS : TMOS.

Table 1 Monolith physical characteristics as a function of PEG quantity in the reaction[a]

Quantity of PEG/mg	Surface area by BET/$\text{m}^2\ \text{g}^{-1}$	Average pore diameter/nm
108	209	8.4
90	225	6.5
70	262	4.3
50	372	2.8

[a] An allyl-TrMOS : TMOS molar ratio of 1 : 4 was used to prepare the monolith. The values for surface area and pore diameter are the average of duplicate measurements.

The allyl functionality of the monolithic silica hybrid can be reacted to alter its surface characteristics and provide chromatographic media with desirable properties. However, the allyl-silica can also be used as a separation medium by itself. A capillary column containing a monolithic structure similar to the one shown in Fig. 2 provided separation of model compounds under

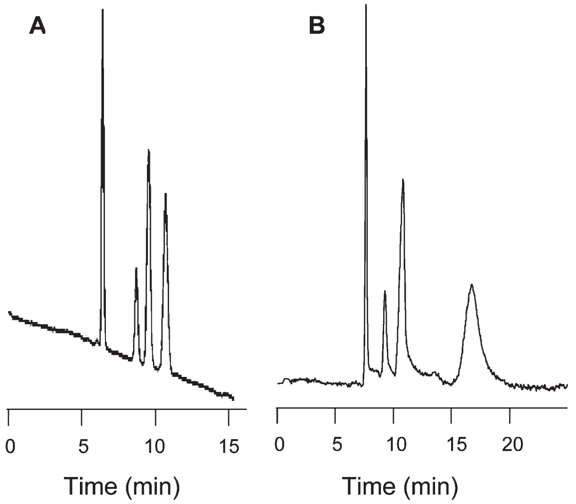

Fig. 3 Separation of model compounds using hybrid monolithic columns: (A) CLC with a hybrid allyl-monolith and (B) CEC with a hybrid allyl-monolith modified with 1-hexene. In (A) the separation was accomplished under 50 psi (3.4 bar) using a mobile phase of acetonitrile and 15 mM TRIS buffer (pH = 7.1) (20 : 80), while in (B) 12 kV was applied ($\sim$385 V cm^{-1}) with a mobile phase of acetonitrile and 15 mM TRIS buffer (pH = 7.1) (30 : 70). The monolithic column was of 50 μm inside diameter and an effective length of 20 cm. UV detection was performed at 214 nm. The peaks are (in order of elution) DMSO, benzene, toluene, and ethylbenzene.

chromatographic conditions for CLC (Fig. 3A). Under such conditions, an average separation efficiency of 37 000 plates m^{-1} was obtained; the permeability (K_o)[18] of the column being estimated as 3.3×10^{-13} m^2. We reacted the allyl moiety of the monolithic structure with 1-hexene in toluene, using 2,2′-azobisisobutyronitrile (AIBN) as catalyst, to attach a six carbon alkyl chain to the monolithic material. After the reaction, the column was conditioned with mobile phase and tested under CEC conditions (Fig. 3B). A non-reacted allyl monolithic column under CEC conditions provided a separation of the model compounds similar to the one in the CLC mode (Fig. 3A). The addition of the alkyl chain provided for an increase in retention time, particularly for the longest-retained compound. The conditions in the chromatograms shown in Fig. 3 are such that retention is more favorable in the column that was not reacted with 1-hexene (*i.e.* with the lower amount of organic modifier in the mobile phase composition). Nonetheless, the retention factor for the last eluting peak was higher for the column under the less favorable retention conditions (*i.e.*, the mobile phase contained higher organic content), indicated by $k = 1.2$ in Fig. 3B compared to $k = 0.7$ in Fig. 3A). This indicates that the reaction with 1-hexene increased carbon loading, despite the less-favorable conditions for retention. It is important to mention that no attempt was made to optimize the separation conditions. Our intention in this communication is simply to demonstrate that our approach is valid and that hybrid monolithic columns are suitable for CLC and CEC.

We conclude that an organosilica hybrid monolith containing allyl groups can be synthesized in a "one pot" reaction, providing allyl functionality at the surface of the monolith through a direct Si–C bond. The hybrid allyl-monolithic structure can be prepared *in situ* inside capillary tubes for chromatographic applications. The synthetic approach can be fully optimized to obtain the desired monolithic properties (*e.g.*, of surface area and pore size). Once the desired properties have been achieved, the characteristic allyl functionality at the surface provides an excellent starting material for many other surface modifications. This allows tailoring of the surface for different chromatographic selectivities. Further experimentation is under way to modify the allyl functionality with different organic moieties for chromatography. Although our application and testing of hybrid materials is primarily for chromatography, we anticipate that the material could be useful for other applications in many different fields.

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