

SUPERCRITICAL FLUID GENERATED SILICA-BONDED PHASES FOR CHROMATOGRAPHY

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In this research, a new generation of chromatographic silica-bonded stationary phases has been prepared using supercritical carbon dioxide (sc-CO₂). By exploiting properties of sc-CO₂ such as solvating power, lower toxicity and enhanced diffusivity, organosilanes can be reacted with surface silanol groups, for the clean, solvent-free synthesis of highly efficient silica bonded phases for liquid chromatography and capillary electrochromatography. Spectroscopic analysis and chromatographic performance testing is used to characterise silica bonded phases generated, such as fluorinated octylsilica (fluoro-sc-C₈) and octadecylsilica phase (sc-C₁₈) phases

INTRODUCTION

Prepared by a "green" chemistry bonding approach that avoids the use of problematic organic solvents, a new generation of high performance liquid chromatographic phases has been developed. The usual manner in which chromatographic phases are synthesised is to introduce a reactive form of the ligand to the support, thereby forming covalent bonds to ensure a stable structure. Silica is the most widely used support phase due to the relative ease with which it can be modified [1]. A wide range of ligands have been successfully immobilised ranging from straight chain hydrocarbons, of which C₈ and C₁₈ chain lengths are the most popular [2], to complex macrocycles such as cyclodextrins [3-6], calixarenes [7-9] and antibiotics [10].

By exploiting the properties of supercritical carbon dioxide (sc-CO₂) such as solvating power, lower toxicity and higher diffusivity (when compared to the organic solvents toluene and dichloromethane traditionally used), highly efficient silica bonded phase for LC and CEC are produced [11]. In addition, sc-CO₂ removes surface adsorbed water prior to reaction, provides gas-like diffusivity for reagent access to residual silanol groups and is compatible with endcapping reactions.

MATERIALS AND METHODS

Silica (Hypersil, 3µm) was obtained from Thermo Hypersil-Keystone (Runcorn, Cheshire, UK). HPLC grade acetonitrile, methanol and isopropyl alcohol were obtained from Labscan (Dublin, Ireland). All water used was distilled and de-ionised to a resistivity of 18.2 MΩ.cm. Test solutes including aniline, N,N-dimethylaniline, fluorene, uracil, biphenyl, benzamide, dimethylphthalate, anisole and diphenylamine were obtained from Fluka (Dublin, Ireland). Benzophenone was obtained from Aldrich (Steinheim, Germany). CO₂ was obtained from BOC gases (Ireland). 1H,1H,2H,2H-perfluorooctyl-triethoxysilane was obtained from Lancaster Synthesis (Morecambe, England). Reactive organosilanes (n-octadecyl-dimethylmethoxysilane, n-octadecyltriethoxysilane) and hexamethyl-disilazane were obtained from Fluka (Dublin, Ireland).

Reaction Procedure. Fluorinated $sc\text{-}C_8$ phase: The reaction was performed using an ISCO model 260D syringe pump with an external stainless steel reaction cell. The reaction cell (internal volume 50ml, i.d. of 2.5cm) was custom made using stainless steel by High Pressure Limited (Pennsylvania, USA). Silica (2.21g, Hypersil, $5\mu\text{m}$) was added, along with 1H, 1H, 2H, 2H-perfluorooctyl-triethoxysilane (0.359 ml), and a magnetic stirrer bar. The cell was filled with 50 ml of CO_2 , the temperature raised to $60\text{ }^\circ\text{C}$ and the pressure to 450 atm. The stirrer plate was switched on, ensuring agitation of the reactants in the supercritical CO_2 and the reaction allowed to proceed for three hours. The system was then allowed to cool by removing the cell from the water bath, depressurised and the modified silica recovered. Elemental analysis of the fluorinated $sc\text{-}C_8$ phase gave % C = 5.54, % H = 0.78.

Non-encapped $sc\text{-}C_{18}$ phase: A C_{18} silica phase was also synthesised using the same apparatus. Silica (2.24g, Hypersil, $3\mu\text{m}$) was added along with 0.387 g of *n*-octadecyl-triethoxysilane. The reaction was allowed to proceed for three hours, under the same conditions as above, again with stirring. The system was then allowed to cool, depressurised and the modified silica recovered. Elemental analysis of the $sc\text{-}C_{18}$ silica gave % C = 20.58 , % H = 1.44.

$sc\text{-}endcapped\ sc\text{-}C_{18}$ phase: To produce an end-capped $sc\text{-}C_{18}$ silica phase using $sc\text{-}\text{CO}_2$, an $sc\text{-}C_{18}$ silica batch was first prepared as described above. After the three hour reaction, 1.5ml of hexamethyldisilazane (HMDS) was added to the reacted silica. The system was again pressurised to 450 atm and the temperature raised to $60\text{ }^\circ\text{C}$. The endcapping reaction was allowed to proceed for three hours, before recovering the end-capped $sc\text{-}C_{18}$ phase.

CONCLUSION

Supercritical fluid generated silica bonded stationary phases have been fully characterised by solid state NMR, showing surface bound species resonances, and through chromatographic performance testing by LC and CEC [11]. This clean technology is particularly suited for the rapid preparation of reversed phase materials, including selective fluorophases. A fluorinated octylsilica (C_8) phase is produced, by reacting 1H, 1H, 2H, 2H-perfluorooctyl-triethoxysilane with silica particles ($3\mu\text{m}$) in $sc\text{-}\text{CO}_2$ at 60°C and 450 atm for just three hours. Similarly, an octadecylsilica phase ($sc\text{-}C_{18}$ silica), prepared by reaction of *n*-octadecyl-triethoxysilane in $sc\text{-}\text{CO}_2$, yielded high column efficiency (up to 141,000 N/meter (fluorene)) and excellent asymmetry factors when column packed for LC.

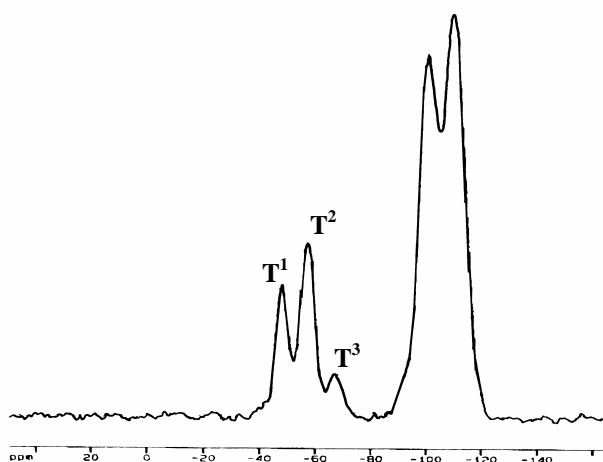


Figure 1 ^{29}Si CP-MAS-NMR spectrum of a $sc\text{-}C_{18}$ silica phase (non-encapped).

Figure 1 shows a ^{29}Si solid state NMR spectrum of the sc-generated octadecylsilica bonded phase, with important resonances indicated for bonded surface silicon species T^1 , T^2 , and T^3 , alongside unreacted silica species Q^3 and Q^4 .

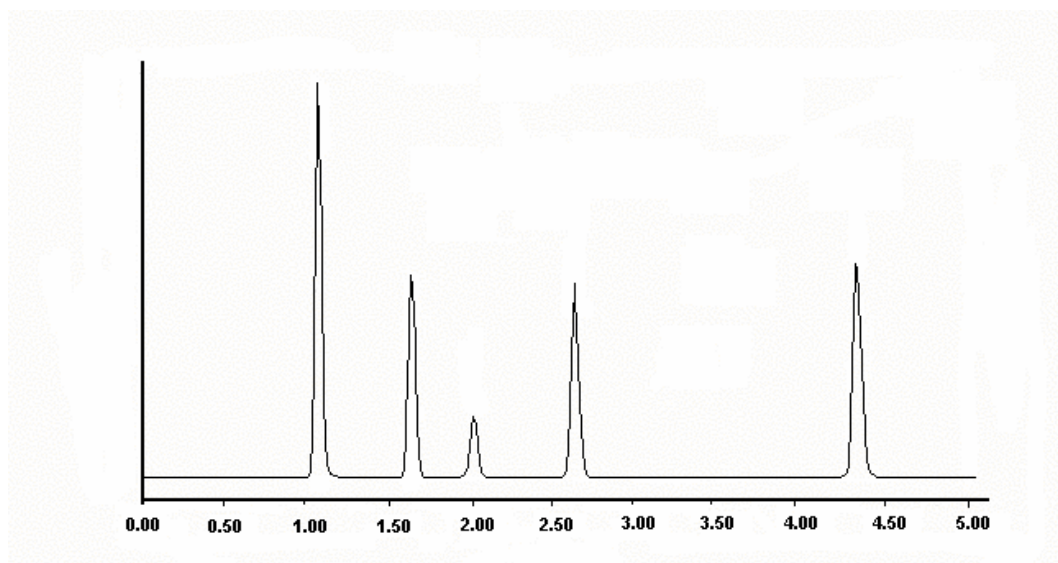


Figure 2 Chromatogram showing a five component test mixture elution on a non-endcapped sc- C_{18} column (4.6 mm i.d. x 150 mm). Peaks in order of increasing retention: uracil, dimethylphthalate, anisole, diphenylamine and fluorine, mobile phase 50% AcN in water (v/v), flow rate of 1.0 ml/min.

The technology is particularly suited for the efficient preparation and end-capping of reversed phase materials, including selective fluorinated and octadecylsilica phases. Importantly, the resulting bonded phase silicas yielded high theoretical plate numbers and good asymmetry factors, even as non-endcapped phases. The method is also applicable to the preparation of silica intermediates and chiral stationary phases. Example of these and other phases prepared, and characterized, will be published in the near future.

Acknowledgements:

We gratefully acknowledge Waters Technologies Ireland, Drinagh, Co. Wexford, Ireland, for assistance with column packing and Enterprise Ireland for grant IF/2001/342 from their Research Innovation Fund. We sincerely thank Professor Klaus Albert and co-workers, Institut für Organische Chemie, Universität Tübingen, D-7207 Tübingen, Germany, for solid state NMR analysis. Our thanks is also due to Thermo Hypersil-Keystone (Runcorn, Cheshire, UK) for supplying $3\mu\text{m}$ silica.

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