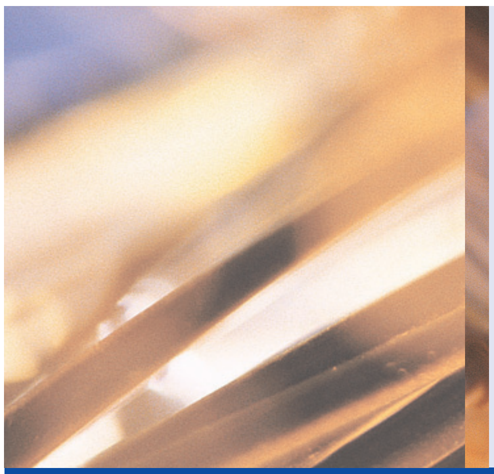

Chromatography Focus

SUPERCRITICAL FLUID GENERATED STATIONARY PHASES FOR LIQUID CHROMATOGRAPHY

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A novel method for the preparation of stationary phases has been developed, using a green chemistry approach that avoids the use of problematic organic solvents. By exploiting properties of supercritical carbon dioxide (sc-CO_2), such as solvating power and enhanced diffusivity, organosilanes are reacted with surface silanol groups, for a clean, solvent-free preparation of highly efficient silica bonded phases for liquid chromatography (LC), such as octadecylsilica, fluorinated octylsilica and pentafluorophenylpropyl phases (1). Spectroscopic and thermal analysis and chromatographic performance testing are used to characterise the silica bonded phases. This supercritical fluid technology is also applicable to the synthesis of important silica intermediates and even to the preparation of chiral stationary phases.

> ***"A CLEAN SOLVENT-FREE PREPARATION OF HIGHLY EFFICIENT SILICA BONDED PHASES FOR LIQUID CHROMATOGRAPHY"***

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SUPERCRITICAL CARBON DIOXIDE

Preparation in supercritical carbon dioxide (sc-CO_2) offers many advantages over current organic solvent reaction media:

- sc-CO_2 is a cleaner, safer solvent than organic solvents and can be easily recycled.
- solvating power of sc-CO_2 is tunable by varying reaction temperature and pressure.
- enhanced diffusivity and rapid mass transfer properties in sc-CO_2 allows greater access to reactive sites and improved coverages for bonded phases prepared in sc-CO_2.

The supercritical state can be achieved at a comparatively low temperature and pressure (critical temperature (Tc) 304.2K; critical pressure (Pc) 72.9atm) *(Figure 1)*. The density and therefore the solubility of compounds in sc-CO_2 can be controlled by varying reaction parameters such as temperature and pressure. McCool and Tripp have demonstrated that hydroxyl groups on silica that are inaccessible in organic solvents, can be fully exchanged with D_2O and can participate in silanisation reactions when sc-CO_2 is used as the reaction solvent (2).

This supercritical fluid technology enables the solvent free functionalisation of silica with organosilanes in sc-CO_2 to generate bonded stationary phases for chromatography, in particular for liquid chromatography (LC) and capillary electrochromatography (CEC) (1).

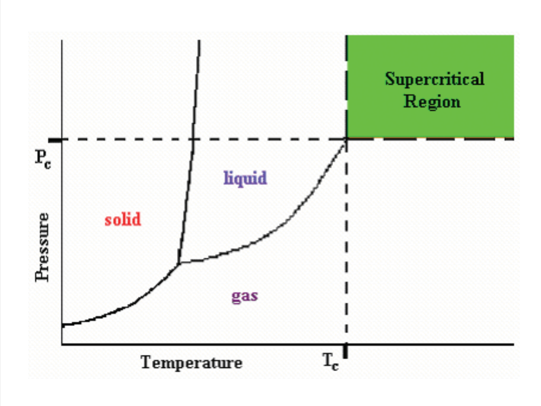

Figure 1. Pressure-Temperature phase diagram for carbon dioxide, which is typically used to define the supercritical region. The dotted line indicates approximately the conditions needed to maintain the critical density at temperatures above Tc and pressures above Pc.

CHROMATOGRAPHIC SILICA BONDED PHASES

Supercritical carbon dioxide (sc-CO_2) is a viable medium for the generation of chromatographic stationary phases (1). Healy *et al.* successfully prepared alkyl and fluorinated alkyl silica bonded phases in sc-CO_2, which, following column packing, exhibited high theoretical plate numbers and good asymmetry factors for the separation of a standard LC test mixture, and of mixtures of analgesics and β blockers.

Silica is widely used as a chromatographic support for many modes of LC including reversed-phase, due to its high mechanical strength, and ease of functionalisation. Stationary phases based on silica generally consist of a non-polar organic ligand (R) covalently attached on the surface via formation of a $Si_{(s)}$-O-Si-R linkage. The general formula of this reaction is:

$$Si_{(s)}\text{-OH} + SiR_nX_{4-n} \longrightarrow Si_{(s)}\text{-O-Si-}R_nX_{3-n} + HX \ (n = 1\text{-}3)$$

where X is usually a chloro or alkoxy group. Three different types of phases can be produced using these silane reagents, including monomeric or "brush" type phases, oligomeric phases, and polymeric or "bulk" type phases. The type of phase produced depends on the functionality of the silane reagent used (mono-, di- or trifunctional) and the presence of water on the silica surface. This silanisation method typically is performed in a high boiling organic solvent, such as toluene (110ºC) or xylene (140ºC), for a reaction time ranging from 3 to 24 hours. Due to the high toxicity and flammability of organic solvents, there has been extensive research into alternative reaction media.

Bonded phases produced can be characterised using many techniques including spectroscopic, thermal and chromatographic methods. Particularly relevant are elemental and BET analysis, thermogravimetric analysis (TGA), diffuse reflectance infrared fourier transform (DRIFT) and cross polarisation magic angle spinning (CP-MAS) NMR spectroscopy. This latter method is particularly powerful as it can differentiate between different forms of silanols (free, geminal and siloxane) and also the various silica surface species bonded on the surface after silica functionalisation (3). Chromatographic testing is one of the primary methods used to evaluate the performance of the packed phase. Tests devised by Engelhardt (5), Walters (5) and Sander and Wise (6) are widely used to characterise column hydrophobicity, residual silanol content and shape selectivity respectively.

OCTADECYLSILICA (ODS) BONDED PHASES

Supercritical modification of a variety of silicas (Hypersil, Exsil-Avanti and Symmetry silica) in sc-CO_2 yields surface coverages comparable to commercially available octadecylsilica (C_{18}) phases *(Table I)*.

Table I. Typical values for C_{18} phases prepared under supercritical conditions using different silica supports.

Supplier	Silica, Particle size, Surface Area	%C	Surface Coverage, μmol/m^2	Theoretical Plates, N/m
Thermo Electron	Hypersil, 3μm, 200m^2/g	20.6	6.0	141,000
Exmere-Alltech	Exsil-Avanti, 3μm, 205m2/g	19.9	5.6	103,707
Waters	Symmetry, 5μm, 315m^2/g	16.0	2.8	91,620

The surface coverage of silica bonded phases can be varied from low to high by selectively tuning the properties of sc-CO_2 such as solubility and density. This is easily carried by varying reaction temperature and pressure. Solid state ^{29}Si and ^{13}C CP-MAS NMR analysis confirmed that surface modification efficiently occurs under supercritical conditions as resonances correlate well with known octadecylsilica resonances for all ODS phases prepared using these different silicas (3). The high chromatographic efficiency of these sc-C_{18} phases is illustrated by the separation of a mixture of analgesics which was achieved with excellent efficiencies and peak asymmetries *(Figure 2)*. Typical theoretical plates for these supercritical fluid generated C_{18} phases are up to 100,000 N/m with peak asymmetries of ca. 1.02.

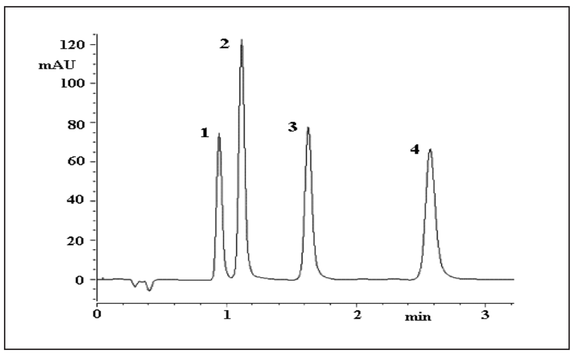

Figure 2. Separation of mixture of analgesics on sc-C_{18} column (C_{18} Hypersil, 5.0μm, 200 m^2/g; 100mm X 4.6mm i.d.) using 25:75 AcN/KH_2PO_4 with a flow rate of 2.00ml/min. Peaks: 1 = Ketoprofen; 2 = Naproxen; 3 = Fentoprofen; 4 = Ibuprofen.

FLUORINATED ALKYL AND PHENYL PHASES

Perfluorinated silica bonded phases exhibit novel separation selectivity as highlighted by the increased retention for fluorinated analytes over non-fluorinated solutes. The preparation of fluorinated phases is particularly favourable in sc-CO_2 as fluorinated reagents show enhanced solubility under supercritical conditions. Fluorinated C_8 phases have been successfully prepared in sc-CO_2 using a variety of silica substrates such as Exsil-Avanti, Kromasil and Hypersil silicas *(Table II)*.

Table II. Typical values for fluorinated alkyl (FC_8) and phenyl (PFPP) phases prepared under supercritical conditions using different silica supports.

Supplier	Silica, Particle Size, Surface Area	Bonded Phase	%C	%F	Surface Coverage, µmol/m^2
Kromasil	Kromasil, 3.5µm, 330m^2/g	FC_8	9.0	20.8	3.1
Exmere-Alltech	Exsil-Avanti, 3.0µm, 205m^2/g	FC_8	4.7	9.6	2.5
Thermo Electron	Hypersil, 5.0µm, 200m^2/g	FC_8	4.6	10.0	2.5
Kromasil	Kromasil, 3.5µm, 330m^2/g	PFPP	9.3	7.2	2.6

Ongoing work involves the further chromatographic testing of these fluorinated phases and investigations into the shape selectivities of the phases prepared from different reagent functionalities. Supercritical fluid generated fluorinated C_8 silica was also utilised for the CEC separation of a series of fluorinated thiourea solutes, for which theoretical plates of up to 154,000N/m were achieved (1).

Pentafluorophenyl (PFP) phases exhibit a unique 'U-shape' relationship between retention and organic modifier content, which allows for the strong retention of basic analytes with good peak shape using highly organic based mobile phases (>90%) (7). This phenomenon results in a substantial increase in the ESI-MS response. Pentafluorophenyl phases have also been proven to be superior to hydrophobic phases for the separation of natural products and taxanes.

Pentafluorophenylpropyl (PFPP) phases were also prepared under optimised supercritical conditions with surface coverages comparable to commercial PFPP phases *(Table II)*. ^{29}Si CP-MAS NMR spectrum confirmed the presence of a monolayer on the surface resulting from the reaction using a monofunctional reagent. The ^{13}C CP MAS NMR spectrum of the sc-PFPP phase is shown in *Figure 3*, showing the PFPP silica surface species and solid state NMR assignments. The viability of this supercritical fluid technology to the preparation of fluorinated phenyl phases is illustrated by PFPP column efficiencies of up to 100,000N/m with peak asymmetries of 1.02 (acenaphthene).

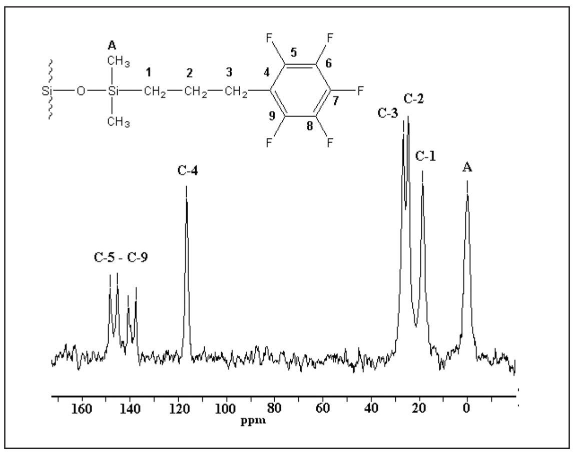

Figure 3. ^{13}C CP MAS NMR spectrum of the supercritical fluid generated phase and proposed silica surface species (PFPP Kromasil, 3.5µm, 330m^2/g, 2.6µmol/m^2).

SILICA INTERMEDIATES

An alternative to the traditional organosilation method of producing silica bonded phases is the preparation of a much more stable direct $Si_{(s)}$-C linkage via a silanisation/hydrosilation approach.

This two step approach firstly involves the formation of a silica hydride intermediate, followed by the catalysed hydrosilation of the desired organic compound onto the hydride modified surface. This approach was successfully employed by our laboratory to modify silica with calixarene macrocycles (8).

Pesek *et al.* devised a methodology for the synthesis of a silica hydride intermediate involving the reaction of a trisubstituted silane such as triethoxysilane (TES) with silica in the presence of water, an acid catalyst in an organic solvent (9). Occasional polymerisation does occur, as H_2O is required for the reaction to proceed.

A supercritical method for the preparation of hydride modified silica in sc-CO_2 has been developed, for which the synthetic limitations are significantly reduced or eliminated. This methodology involves the generation of a Si-H monolayer from the optimised reaction of a monoalkoxysilane – dimethylmethoxysilane ($(OCH_3)Si(CH_3)H$) in sc-CO_2. Hydride loadings were found to be at best comparable to those obtained using organic solvent based methods *(Table III)*. Solid state NMR and DRIFT spectroscopy were used to provide information on surface coverage and the nature and bonding of the different silica surface species. The intense resonance at -1.2ppm (M_H) in the ^{29}Si CP-MAS NMR spectrum corresponds to the Si-H surface species, thus confirming the formation of the desired hydride monolayer on the silica surface.

Mercaptopropyl modified silica is an important silica intermediate as it can act as a reactive site for further modification via a range of reactions including free radical addition, disulfide formation and Michael addition. This supercritical technology has been used to successfully prepare mercaptopropyl modified silicas with superior surface coverages in a shorter reaction time, compared to organic solvent based methods *(Table III)*.

Table III. Typical values for hydride and mercaptopropyl (MPTMS) modified silicas prepared under supercritical conditions.

Silica, Particle Size, Surface Area	Silica Chemistry	%C	Surface Coverage, µmol/m^2
Exsil-Avanti, 3µm, 205 m^2/g	Hydride	1.7	4.9
Exsil-Avanti, 3µm, 205 m^2/g	MPMS	4.0	6.0

The preferential formation of a bidentate T_2 species (-57.9ppm) is evidenced by the ^{29}Si CP-MAS NMR spectrum which indicates a high degree of crosslinking. Resonances due to the monodentate species T_1 (-48.0ppm) and the polymeric species, T_4 +$T_4{}'$ (-67.2ppm) are also present in the ^{29}Si CP-MAS NMR spectrum. The formation of these silica surface species is detailed in *Figure 4*. This extensive crosslinking coupled with high silane coverage indicates the preparation of a highly stabilised silica intermediate. Such stable materials have applications in the separation of polar analytes which may require the use of low and high pH mobile phases.

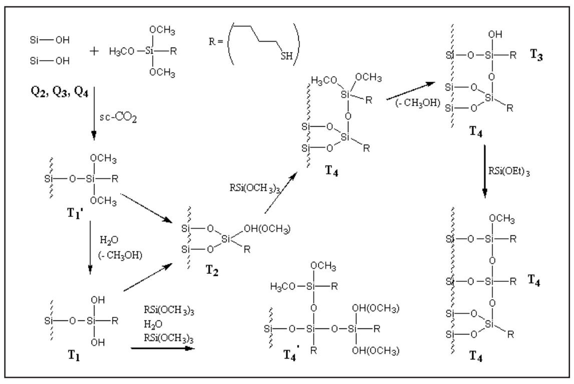

Figure 4. Proposed structures and formation of mercaptopropyl modified silica surface bound species in sc-CO_2.

To further demonstrate the usefulness of the supercritical fluid technology, hydride modified silica prepared in sc-CO_2 was reacted with octadecene in the presence of a free radical initiator, followed by endcapping in sc-CO_2. This sc-C_{18} modified silica material was packed using the supercritical fluid slurry packing technique, thus eliminating the need for organic solvents in the total preparation of a chromatographic column.

In addition a quinine derived chiral selector was also attached to a mercaptopropyl modified surface in a free radical addition reaction in sc-CO_2. This sc-chiral phase displayed good efficiencies and excellent resolution in the direct LC enantioseparation of a series of 3,5-dinitrobenzoyl (DNB) amino acids (10).

CONCLUSIONS

It has been demonstrated that supercritical carbon dioxide is a clean and efficient medium for the generation of chromatographic stationary phases. This "green" approach is applicable to a wide variety of silica bonded phases such as octadecylsilica (C_{18}) and fluorinated alkyl (C_8) and phenyl (PFPP) phases. Important silica intermediates such as hydride and mercaptopropyl modified silica can also be readily prepared and have been successfully modified to yield stable C_{18} phases and a CSP respectively. These sc-generated silica bonded phases yield high column efficiencies and peak asymmetries and the technology has great potential as a "green", efficient method for bonded chromatographic phase synthesis.

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BIOGRAPHY

Norma Scully received her B.Sc. in Chemistry from University College Cork, and is currently completing her Ph.D. research at the Chemistry Department, UCC, in the Innovative Chromatography group, led by Professor Jeremy D. Glennon and Dr. Liam Healy in the Analytical Chemistry Division. She was awarded the Csaba Horváth Memorial award at the 30th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2006), which was held in San Francisco on June 17 – 22, 2006. This award is to honour the memory of Csaba Horváth and recognise his contributions to liquid chromatography. This award also recognises the presenter of an outstanding lecture by a young scientist. Norma has been invited to give an oral presentation at the HPLC 2008 conference in Baltimore, USA.