Ordered Mesoporous Silica Materials in Liquid Chromatography:

Synthesis and Application

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Isaac Asimov

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Matthias Ide

Preface

Since its discovery more than hundred years ago chromatography has taken on a very important role in the analysis of reaction mixtures, food samples and drugs. In fact HPLC (High Performance/Pressure Liquid Chromatography) equipment has become the third most sold analytical instrument in the world. The development of new stationary phases in liquid chromatography remains a field of research with many opportunities. During the last 10 to 15 years the particle sizes went down to 1.8 µm, superficially porous particles were introduced and monolithic columns became available. The biggest change in column design was seen with the introduction of chromatographic chips where an etched "column" on a "chip" became the holder of the stationary phase instead of a "tube".

Since the efficiency in chromatography is strongly influenced by the properties of the stationary phase, new and improved stationary phases and column designs are researched continuously. The superficially porous particle is a beautiful example of the effect of smaller diffusion lengths on the separation efficiency. This implies that the porosity and the pore structure exhibit a substantial influence on the separation.

Porous materials can be found in a wide range of applications such as catalysis, adsorption, controlled drug release, chromatography, etc. In the research field of porous materials, scientists aim to control pore size, pore shape and pore structure. Zeolites were among the first porous materials to be prepared synthetically to control the pore properties. This especially allowed the design of new cracking catalysts for the petrochemical industry. In 1992 this field was expanded with the discovery of a new type of aluminosilicates, designated M41S materials, by the Mobil Oil Company. The innovation of the described invention comprised the use of assemblies of ionic surfactants as a soft template to direct the polycondensation of the material. Subsequent removal of the surfactant left an ordered mesoporous (2 - 50 nm) pore system behind. The major advantages of these new porous materials were the large surface areas $(\pm 1100m^2/g)$ and the considerably larger pores in comparison with zeolites. The downside was primarily a reduced hydrothermal stability due to thin walls.

During the following years this new class of porous materials was expanded to larger pore systems with the use of the non-ionic Pluronic surfactants, these materials were designated as SBA materials. Depending on the type of surfactant used, different types of pore structures were discovered, such as hexagonally ordered or cubically ordered pore systems. The basic chemicals can be found amongst pure silanes, organosilanes, titania precursors and polymeric monomers.

To improve the hydrothermal stability of the porous silica materials, organic moieties were introduced into the chemical structure of silica. This increased the hydrophobicity of the materials, which in turn improved the hydrothermal stability. This way ordered mesoporous organosilica materials were developed with an alkaline resistance up to pH 14.

This dissertation deals with the application of ordered mesoporous materials as a stationary phase in chromatography. To assess the influences of an ordered pore system on the separation, a comparison has been attempted between home packed hexagonally ordered mesoporous silica spheres and various commercial columns. One of the most interesting results that came up was a strongly increased retention. During a second part of the research an attempt to provide a solution for Open Tubular capillary columns has been researched. For this a thick ordered mesoporous silica layer was deposited on the inside of a capillary, therefore increasing the retention of analytes on the stationary phase enabling successful separations on Open Tubular columns.

Finally an ordered mesoporous organosilica was designed to perform as an ultra stable stationary phase, able to withstand highly alkaline separation conditions.

Outline

This dissertation comprises a theoretical part and a practical part. The first two chapters make up the theoretical segment and aims to provide background so a clear assessment of the practical segment within the literature can be made. The appendices are reserved for a more elaborate explanation on some of the less obvious analysis techniques that were used during the research.

Chapter 1 gives a short in sight into the toolbox of a materials scientist, specialized in the synthesis of porous spherical materials. Hereafter a literary overview is provided of the various techniques used to synthesize spherical ordered mesoporous particles. The focus is put on spray drying techniques because it is the prime synthesis procedure.

Chapter 2 introduces chromatography as the main application for the materials researched during this doctorate. The main aspects and current voids in high performance/pressure liquid chromatography (HPLC) are highlighted. Again a literary overview of all ordered mesoporous materials applied as a stationary phase in HPLC is provided. This gives an insight into what improvements are still to be made to assess the influence of ordered mesoporosity on a chromatographic separation.

Chapter 3 describes the quantification of the silanol sites for the most common mesoporous ordered silicas. In cooperation with the university of Caen we proved to be successful in determining the distribution of surface and bulk silanols, and of isolated, geminal and vicinal silanol sites. The silanol data was aquired with infrared and NMR spectroscopy while the amount of surface accessible groups was probed with three common chromatographic grafting groups (a C3, C8 and C18 silane). The results were linked to the synthesis conditions and the pore properties.

Chapter 4 surveys the initial stages of research to obtain spherical MCM-41 type materials through spray drying. During an experimental design in which six factors were varied on two levels information was obtained on the influence of these factors on the particle and pore properties of the silica spheres. This statistical approach allowed to reduce the amount experiments that had to be performed in order to discover the correct parameters to obtain the correct spherical morphology together with the large uniform pore structure. The main conclusion is that the spherical morphology is mainly dependent on the surface tension of the spray dried solution.

Chapter 5 comprises the synthesis and application of spherical ordered MCM-41 type materials according to the optimized synthesis procedure acquired by the experimental design from chapter 4. It was discovered that the main property that has to be kept under control to obtain spherical particles is the surface tension of the spray dried solution. A higher surface tension allows for a more spherical particle morphology. Since the pore properties are positively influence by the amount of surfactant, which lowers the surface tension, a tradeoff between spherical morphology and high surface areas is eminent. When the materials were packed in a column to be used as a stationary phase it became immediately clear that they increased the retention of the analytes drastically.

Chapter 6 describes the initial research to increase the pore size of spray dried ordered mesoporous materials. For this SBA-type materials have been spray dried. Both hexagonally (SBA-15) and cubically (SBA-16) ordered pore systems have been tried. It was immediately clear that "just" shifting the surfactant from the smaller ionic surfactants (cetyltrimethylammonium bromide) to the much larger triblock co-polymers is not that evident. The larger surfactants strain the reaction conditions in terms of reaction speed. Eventually SBA-15 materials were spray dried but without a spherical morphology.

Chapter 7 focuses on the stability of the new type of stationary phase. By replacing half of the siloxane bridges in a silica structure the entire structure becomes less susceptible to hydrolysis. This improves the stability of the stationary phase. To perform this, the 1,3,5-tris[diethoxysila]cyclohexane precursor has been used to spray dry the first PMO material ever. The material was fitted with a C18 chain and packed into a column for chromatographic evaluation. The packed column exhibited an even higher retention than the pure MCM-41 column. Additionally a mixture of highly polar compounds has been successfully separated. The stability of the column was also tested and found at least as good as commercial columns.

Chapter 8 Illustrates the application of ordered mesoporous silica materials as a thick coating for wall coated open tubular (WCOT) columns. These columns were compared with non-coated capillaries for their separation efficiency and retention in capillary electrochromatography (CEC). The WCOT coumns exhibited a 83 times higher retention than non-coated capillaries. This results could enable the use of WCOT capillaries in LC and CEC.

Chapter 9 gives a general conclusion of the use of ordered mesoporous materials in chromatography and provides a short outlook on how to proceed from here on. It sums up current drawbacks and advantages and how to tackle or exploit these in future research.

Chapter 10 provides a Dutch summary of this PhD work.

List of Abbreviations

A	
AAEISA	Aerosol Assisted Evaporated Induced Self Assembly
ACN	Acetonitrile
ANOVA	Analysis of Variance
APTMS	Aminopropyltrimethoxysilane

В

BET	Brunauer Emmet Teller
BJH	Barret Joyner Halenda
BGE	Background Electrolyte
BSA	Bovine Serum Albumin
BuOH	1-butanol

С

CCD	Charge-coupled Device
CEC	Capillary Electro Chromatography
CMC	Critical Micelle Concentration
CP MAS	Cross Polarisation Magic Angle Spinning
CTAB	Cetyltrimethylamonium bromide
CTAC	Cetyltrimethylamonium chloride
CTES	(chloromethyl)triethoxysilane
CYS	Cystein

D

DA	Dopamine
DAD	Diode Array Detector
DNB-NEA	Dinitrobenzoyl Naphtylethylamine
DOE	Design of Experiments
Dp	Pore Diameter
DRIFTS	Diffuse Reflectance Fourier Transform Spectroscopy
DTGS	Deuterated Triglycine Sulfate

Е

EISA	Evaporation Induced Self Assembly
EO	Ethylene Oxide
EOF	Electro Osmotic Flow
EPA	Estimation Program Interface
EtOH	Ethanol

F

–	
FE-SEM	Field Emission SEM
FTIR	Fourier Transform Infrared

G

-	
GC	Gas Chromatography
GFC	Gel Filtration Chromatography
GPC	Gel Permeation Chromatography
GSH	Glutathione

Н

Hydrophilic Interaction Chromatography
Hexamethyldisilazane
High Performance/Pressure Liquid Chromatography
Human Serum Albumin

Ι

-	
ID	Internal Diameter
IEC	Ion Exchange Chromatography
IPrOH	Isopropanol
IUPAC	International Union of Pure and Applied Chemistry

K KJS

5	Kruk-Ja	roniec-Saya	ri
)	niuk-Ja	romec-saya	I

L	
LC	Liquid Chromatography
LYS	Lysozyme

М	
MES	2-(N-morpholino)ethanesulphonic acid
MCF	Mesocellular Foam
MCM	Mobil Composition of Matter
MP	Mobile Phase
MPS	Mean Particle Size
MPTMS	Mercaptopropyltrimethoxysilane
MSU	Michigan State University
MW	Molecular Weight

N

NMR	Nuclear Magnetic Resonance
NPLC	Normal Phase Liquid Chromatography

Ο

OTLC	Open Tubular Liquid Chromatography
OTCEC	Open Tubular Capillary Electro Chromatography

Р

Polyaromatic Hydrocarbon
Polyethylene Oxide
Periodic Mesoporous Organosilica
Propylene Oxide
Particle Size
Particle Size Distribution

R RPLC

Reversed Phase Liquid Chromatograph	ıy
-------------------------------------	----

S

SA	Surface Area
SAXS	Small Angle X-ray Scattering
SBA	Santa Barbara Amorphous material
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SP	Stationary Phase

Т	
TDSCH	1,3,5-tris[diethoxysila]cyclohexane
TEA	Triethylamine
TEM	Transmission Electron Microscopy
TEOS	Tetraethoxy orthosilicate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TGA	Thermo Gravimetric Analysis
TMB	Trimethylbenzene
TMS	Tetramethylsilane
TP	Thiopurine

U UHI

		/D C T · · 1	
UHPLC	Ultra High Pressure	/ Performance Liquid	Unromatography
0111 10	o rora ringin r robbano	/ I offormanee Bigana	omonavograpny

V

•	
VOAG	Vibrating Orifice Aerosol Generator
Vp	Pore Volume

W

WCOT	Wall (Coated	Open	Tubular
WCOI	wan v	Oateu	Open	Tubulai

X XR(P)D

Λ	
XR(P)D	X-ray (Powder) Diffraction
XRF	X-ray Fluorescence

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1

Ordered mesoporous materials

Porous materials are all around us in our every day lives such as paper tissues, sponges and washing powders. Even cements and ceramics can be considered as porous materials. One of the first porous media ever used by men was charcoal, now named as activated carbon. Because of its adsorptive properties it could serve as an antidote to many poisons. This was already known by the Egyptians more than 5000 years ago.

Porosity means that in a certain structure voids, called pores, can be found. The word pore is derived from the Greek word "poros", which means passage. This indicates that a pore serves as a passage to the interior of a certain structure.

The best know example of ordered porosity are zeolites, in which a crystalline structure leads to micropores in the material. These materials are widely used as washing powders and in crude oil cracking reactors.

It can be easily imagined that taylor-made porous materials would be useful for a lot of industrial applications, ranging from catalysis over chromatography to even controlled drug release and micro-electronics.

The research conducted during the past four years and described over the following chapters is focussed on the application of ordered mesoporous materials in the world of chromatography. Different stationary phases have been developed, from spherical particles to thick layers as an inner lining in capillaries. Organosilicas have been synthesized to improve the hydrothermal stability of the stationary phases.

1.1 Introducing ordered mesoporous materials

1.1.1 What is mesoporosity?

According to IUPAC, porosity is defined in 4 classes: micro-, meso-, macro- and megaporosity.¹ Porous materials are classified according to the pore diameter into one of these 4 classes:

- Micropores: < 2 nm
- Mesopores: 2 nm < 50 nm
- Macropores: 50 nm <7500 nm
- Megapores: > 7500 nm

Of these, the materials with pore sizes between 1 and 1000 nm are also designated collectively as nano porous materials.

1.1.2 A materials scientists toolbox

Porosity

Porosity is generally measured by N_2 -adsorption. This technique is based on the adsorptive properties of materials towards liquid nitrogen. Nitrogen gas is introduced to a porous system in a very controlled and gradual way where it condenses in the pores at 77 K. Molecule per molecule gradually adsorbs to the surface and the volume of N_2 is plotted as a function of the normalized pressure. During several stages the surface area, the pore size distribution and the total pore volume can be calculated. See the appendices for a more elaborate explanation.

Measuring periodicity

Ordered materials possess a repetitive structure which can be visualized by an X-ray diffractogram. The smaller the pores are, the easier it is to take an X-ray diffractogram because lower distance between repeated units means the X-rays are diffracted over higher angles. There are several latices reported for ordered mesoporous materials (see figure 1.1). Most common are the hexagonal 2D (a), cubical 3D (b) and lamellar (c) structures but recently various 3D hexagonal structures (d) were also reported. The crystallographic latices are designated as P6/mm (a), $Ia\bar{3}d$ (b), p2 (c) and P6/mmm (d).



Figure 1.1: A figurative drawing of a hexagonal 2D (P6/mm) (a) (i.e. MCM-41), cubical 3D $(Ia\bar{3}d)$ (b) (i.e. MCM-48), lamellar (p2) (c) (i.e. MCM-50) and a hexagonal 3D (P6/mmm) structure (d) latice. (taken from references^{2,3})

Visualizing internal and external morphology

The particle morphology needs a magnification that shows structures from 10 µm down to a 100 nm. For this kind of magnification SEM (Scanning Electron Microscopy) is used. This technique makes use of an electron gun, scanning the surface of the material. The electrons from the beam interact with the surface and a beam of high-energy electrons is scattered back to the detector via elastic scattering. The signals from this detector are then transformed computationally to images. Morphologies range from loose debris over needles and spheres to the most exotic structures one can think of (see figure 1.2).

Common pore sizes vary from 3 to 10 nm pore diameter. To visualize this, TEM (Transmission Electron Microscopy) is used. This is a technique whereby a high energy electron beam is transmitted through a thin sample, interacting with the sample as it is passing through. The resulting image is captured on a CCD camera to be visualized on a screen. These pictures give support to the structure found by XRD measurements or vice-versa (see figure 1.3).



Figure 1.2: FE-SEM micrographs of ethenylene-bridged periodic mesoporous organosilicas prepared in the presence of various cosolvent and cosolvent concentrations: (A) ethanol, (B) n-propanol, (C) n-pentanol, (D) n-butanol:surfactant = 95:1, (E) n-butanol:surfactant = 115:1 and (F) n-butanol:surfactant = 175:1. (taken from reference⁴)



Figure 1.3: FE-SEM and TEM micrographs of a hexagonal disk with P6/mm symmetry. (taken from reference⁴)

1.1.3 Why nanoporous materials?

The main motive to develop porous materials in general and nanoporous materials in particular is to increase the surface area. Considering surface area, spherical particles have their limits: they can not be made infinitely small without the drawbacks of very small particles (volatile and hard to handle). Porosity offers a solution to this problem. As per definition, the smaller the pores and pore walls the larger the surface area per gram will be. So microporous materials will inherently possess a higher surface area than mesoporous materials, who in turn will have a larger surface area than macroporous materials.

Is this the only advantage? No, the below described nanoporous materials are in fact more or less ordered. Their pore systems possess a crystalline or semicrystalline ordering. This means that the pores can be tuned in directionality, improving for instance the flow through coated materials. Secondly, this means that the pore diameter is also very uniform. Every pore has, when compared to non-ordered porous materials, the same size as the next. This has a positive effect on homogeneous diffusion of components through these materials.

Although porous materials are interesting, they have their drawbacks. Porosity brings instability, which is related to the more open structure towards destructive substances. To counter these problems, the pore walls either are made thicker or they are functionalized. Thicker walls reduce the surface area per gram again and functionalization reduces the pore diameter.

Taking into account all these advantages as well as drawbacks, it is obvious that these kinds of materials should be tuned for the application they are needed for. So to conclude, 'Why nanoporous materials?'. Because they possess advantages which are not even remotely reachable by non-porous materials, but keep in mind that they need to be tuned to the application at hand.

1.1.4 A macro-view on the mesoporous world

1.1.4.1 The family of M41S-materials

In a quest for materials with larger pores, scientists at Mobil Oil Corp. announced in 1992 the direct synthesis of the first family of mesoporous materials, the Mobil Composition of Matter or MCM's.⁵ These materials are build up from Si, O and H and are technically amorphous but possess an ordered pore structure. The synthesis-procedure makes use of templates to form the pore structure. The templates used are ionic surfactants with alkyl chains that are around 16 carbons in length. Depending on what surfactant is used, a hexagonal (MCM-41) or a cubic (MCM-48) pore structure is obtained. Most common used surfactants are CTAB (cetyltrimethylamoniumbromide) and CTAC (cetyltrimethylamoniumchloride) for MCM-41 and the gemini-surfactants (general formula for GEM n-s-m: $[C_nH_{2n+1}N^+(CH_3)_2C_mH_{2m+1}]\cdot 2Br^-)$ for MCM-48.⁶ In general these materials are synthesized through a polycondensation-reaction of TEOS (tetraethoxyorthosilicate) around a lyotropic liquid-crystal phase of surfactant (see figure 1.4). The interaction mechanism between surfactant and precursor varies for every surfactant. Standard MCM-materials have pore sizes between 2 and 4 nm pore diameter, surface areas of around 900 m^2g^{-1} and a pore volume of around 0.85 cm^3g^{-1} . The synthesis procedure has a good reproducibility and these materials are already commercialized, currently 5 g MCM-41 costs 96.30 Eur.



Figure 1.4: A representation of the formation of mesoporous M41S materials. $(taken from reference^2)$

1.1.4.2 The family of SBA-materials⁷

With the pore size limitations of MCM-materials on their minds, researchers at the University of Santa Barbara discovered that larger templates could be used to make mesoporous silicas. The used surfactants were non-ionic alkyl poly(alkyleneoxide) triblock co-polymers with the general formula $EO_x PO_y EO_x$. The two most used surfactants of this family are Pluronic P123 ($EO_{20}PO_{70}EO_{20}$) and Pluronic F127

 $(EO_{106}PO_{70}EO_{106})$. In contrast with MCM-materials, SBA's have thicker walls which are microporous. The mesopores are commonly around 6 nm pore diameter and the entire structure is, due to these thick walls, hydrothermally and mechanically more stable.

Meso cellular foams or MCFs are pore expanded SBA-type materials.⁸ To further increase the pore diameter up to the limit of mesoporosity, a swelling agent can be added to the sol mixture. A common swelling agent is TMB or 1,3,5-trimethylbenzene. Due to its hydrophobicity this group resides in the hydrophobic part of the surfactant micelles, expanding the size of the micelle. These materials exhibit a pore system with a uniform pore size up to 50 nm, however in most cases not ordered.

1.1.4.3 Organically functionalized ordered mesoporous silicas^{2,9}

Modification of silicas with an organic function allows the synthesis of organicinorganic hybrid materials. This makes a symbiosis possible between the vast variation of organic functional groups and the robustness and thermal stability of inorganic systems. This induces eminent advantages for its potential application in heterogeneous catalysis, adsorption techniques, chromatography, sensors, etc. Secondly, this combination leads to materials of which the properties differ greatly with their individually isolated components. There are three ways to modify silica's with an organic function. An organic moiety can be introduced by a post synthesis grafting procedure, a co-condensation and a pure organosilane condensation reaction (figure 1.5).

Grafting¹⁰⁻¹⁴

The first method explored to funcionalize silica-materials is to graft them to the surface. Here the SBA or MCM material is being treated with the wanted organosilane group (i.e. APTMS (aminopropyltrimethoxysilane)). First the methoxygroups of the silane are hydrolyzed to be condensed with the free silanol groups on the surface of the silica. To prevent self condensation of the grafting silane, the grafting reactions have to be performed in a dry non-protic solvent.

However this brings some disadvantages. First of all introducing a group on the surface reduces the pore volume of a porous material. Secondly, if the grafted groups are too large they could condense on the pore mouth and cause pore blocking. Additionally it is not certain that the grafted organic function is homogeneously distributed over the surface due to diffusion problems or even due to total pore blocking.

$Co-condensation^{15-19}$

The introduction of the organic function happens here during the synthesis. This way the functionality is anchored inside the wall. This reduces pore narrowing and blocking as opposed to the grafting method.

But even this method has its disadvantages. First of all the ordering of the pores

drops with the addition of more organically functionalized precursor. This limits the amount of organic function to about 40 mol%. Next to this, homocondensation of both components can occur. This means that, depending on their hydrolysis rates, both components preferentially condense separately, preventing a homogenous distribution of the organic group on the surface.



Figure 1.5: A schematic overview of a grafted (A), co-condensed (B) and fully organosilica (C) material. (taken from reference 20)

Periodic Mesoporous Organosilicas^{3,4,21–38}

PMO materials are a class of hybrid organic-inorganic mesoporous organosilica composites which were developed through direct condensation of a poly(tri-alkoxysilyl) bridged organic component in the presence of a surfactant template. The bridged organosilanes usually have an $(RO)_3 - Si - R' - Si(RO)_3$ structure. The general principal of synthesis is the same as with SBA and MCM materials. In this case a bissilane is polycondensed around a template which is then removed by extraction. Because of the organic nature of the bridging group, this group would be destroyed with the surfactant during calcination, therefore extraction is the only option here.³⁴

PMOs offer a lot of advantages compared to pure silica materials. First of all the organic functionalities are homogeneously divided over the entire surface with a loading of 100%. This, for instance, is not possible when introducing a functionality through grafting or co-condensation. Additionally pore blocking is eliminated, making the pores accessible for further chemical derivatization of these organic functions. These 'soft' organic groups possess different mechanical properties when compared to their inorganic (hard) co-condensates. A second advantage is that the



Figure 1.6: Overview of the organosilica precursors that have been converted into PMOs. Terminal Si atoms: Si = Si(OR)₃ with R = CH₃, C₂H₅ (taken from reference²)

organic precursor can easily be varied, opening up the world of silicas to a very broad series of properties. This leads to new hybrid materials, each with their own interesting electronic, optic, transport, charge or magnetic properties. PMOs are, through their hydrophobic (organic) nature, more resistant towards mechanic and hydrothermal stress than SBAs and MCMs.

Organic functions offer great advantages to these structures. They improve hydrofobicity, rendering the silica more inert towards hydrolysis by water or acidic or alkalic media. Furthermore, these organic functional groups can be derivatized to synthesize materials with non-leachable anchored groups.

PMO precursors Various types of precursors are used to synthesize different PMOs for different applications. The precursors used most frequently are methane, ethane, ethane, benzene and thiophene bissilanes (see figure 1.6). These are in fact the most rigid structures that cause the least problems when synthesizing one of these PMOs. For example strongly branched alkyl groups or long dendrites change the C/Si ratio of the final product extensively. This renders the PMO much more hydrofobic, affecting the dielectric constant of the system. Even with these changes the structural rigidity and the large surface area of the materials are retained.

The ring precursor One precursor used during the course of this PhD was the so-called ring precursor (4th in figure 1.6), synthesized for the first time by Brondani et al.³⁹ The precursor is synthesized from chloromethyltriethoxysilane via a Grignard reaction generating a mixture of 1,3,5 tris[diethoxysila]cyclohexane and a derivative with an extra triethoxysilyl group. This silane has been successfully polycondensed into a periodic mesoporous organosilica by Landskron et al.⁴⁰

1.2 Monodisperse spherical particles

The morphology of the particles is very important for chromatographic purposes, especially a uniformly spherical particle morphology is highly desired. The ideal particles for chromatography exhibit a particle size between 1.5 and 5 µm and very uniform in size. Therefore during the following sections a literary overview is provided of the various methods to produce spherical silica particles, such as the Stöber method, emulsification, pseudomorphic transformation and spray drying. The overview obviously limited to ordered mesoporous particles.

1.2.1 Stöbers method to produce silica spheres.

Ever since the discovery of highly ordered mesoporous materials and their said potential application in chromatography, monodisperse spherical particles are of great interest. There are several methods to produce monodisperse spherical particles first of which the Stöber method is the most renown.⁴¹ The method employs a water/alcohol/ammonia/tetraalkoxysilicate system wherein the alcohol plays the

role of a dispersing agent. By continuous shaking or by use of an ultrasonic bath the formed particles remain in suspension. The dependence of the particle size, shape and monodispersity on co-solvent concentration, the pH value, the reaction time, the stirring procedure and the interactions between the silica source and the co-solvent was extensively investigated. Particles in the size range of 50 nm to 2 µm were easily achieved but larger particle size usually led to a broadened particle size distribution.⁴¹ This is still the most abundantly used methodology to produce silica spheres for HPLC (High Performance/Pressure Liquid Chromatography) columns. The problem with this technique is that when it is modified by adding a surfactant to the mixture, the dispersing power of the alcohol tends to disappear and the final spheres become intergrown.

In 1997 the original Stöber method, which introduces no ordered porosity in the particles, has been modified by the group of Klaus Unger. Surfactants were used to generate mesoporously templated spherical particles in this way. This led mostly to particles of 400 to 1100 nm in size fused to form irregularly coagulated shapes of several tens of micrometers. The particles had high specific surface areas $(\pm 1000\text{m}^2/g)$ and narrow pore size distributions in the 3 nm range.^{42,43} Others replaced the co-solvent with methanol and traded in ammonia for sodium hydroxide. This resulted in very monodisperse particles with average particle size diameters just below 1 µm and comparable pore properties.^{44,45}

In 2007 an extended SEM study was conducted on the influence of the solvent composition on the particle size. An inverse relation was observed between the particle size and the solvent concentration.⁴⁶ From figure 1.7 it is clear that these particles are too small to be used as a packing material in HPLC.

In 1999 the synthesis of cubically ordered mesoporous spheres was published by the Unger group.^{47,48} The particles, with an $Ia\bar{3}d$ space group (see figure 1.1), were synthesized via the modified Stöber method. In 2004 cubically and hexagonally ordered silica spheres with radially oriented pores were reported by Rankin et al.⁴⁹ A thorough TEM study was conducted and a mechanism for the formation of spherical particles with radial pores was proposed.

While a Stöber method is, theoretically, a process in a basic medium, other groups have managed to produce spheres in an acidic medium by using hydrochloric acid as a catalyst. The properties of the particles remained roughly the same except for the particle diameter, these had diameters up to 1 mm.^{50–52}

In 1998 superficially mesoporous ordered spherical particles were reported by Büchel et al.⁵³ The particle cores were only 420 nm in diameter and synthesized according to the method prescribed by Stöber.⁴¹ The cores were subsequently stirred in a solution of water, ammonia, a silica source and a surfactant, generating a porous shell of only 75 nm thick. The surface areas up to $350 \ m^2/g$ were of course lower than fully mesoporous particles, but nevertheless had a narrow pore size distribution around 3.8 nm.



Figure 1.7: SEM images of (a-d, g, j) the as-synthesized and (e, f, h, i, k, l) the calcined particles prepared at the methanol/TEOS ratios of (a) 300, (b) 750, (c) 1125, (d, e, and f) 1500, (g, h, and i) 2250, and (j, k, and l) 3000. Figure (f), (i), and (l) are close up views of figure (e), (h), and (k), respectively. (taken from reference⁴⁶)

In the same year Qi et al. introduced a novel pathway to synthesize spherical mesoporous silica materials by using a mixture of CTAB and the non-ionic surfactant Brij 56 as structure directing agent. Particle diameters of 2 to 6 µm were obtained with pore sizes of 2.6 nm and surface areas up to 1000 m^2/g .⁵⁴

In 2000 Zhao et al. reported the synthesis of spherical SBA-15. The synthesis comprised a modification of the standard SBA-15 synthesis pathway by adding CTAB as a co-surfactant and ethanol as a co-solvent. The particle size was fairly monodisperse around 1 µm but the spherical shape was rather distorted by large humps of silica grown on them like barnacles. The surface area was measured to be around 710 m^2/g and was clearly hexagonal.⁵⁵

Three years later Ma et al. achieved the synthesis of 5 μ m sized SBA-15 particles which were optimal to be used in HPLC.⁵⁶ The only problem was the prevention of particle coagulation. Figure 1.8 shows the original particles produced by Zhao et al. (f) and the updated particles synthesized by Ma et al. (a to d), where both the time and the temperature of the reaction mixtures were varied.



Figure 1.8: SEM pictures of spherical SBA-15 reported by Zhao et al. (f).⁵⁵ SEM images of mesoporous SiO₂ obtained at: (a) 80°C for 5h; (b) 80°C for 5h and 120°C for 12h; (c) 80°C for 5h and 130°C for 12h; (d) 130°C for 12h. Scale bar: 5 µm. (taken from reference⁵⁶)

1.2.2 Emulsions to generate spherical particles

A second method is an emulsing technique, developed by Oh et al.⁵⁷ Herein a biphasic system is used in which an oil (sometimes CCl_4) acts as an emulsifier to disperse the silica condensation cores, preventing them from intergrowing. However, most of these materials either have a poorly ordered pore system or no pores at all. In the original solution an emulsion consisting of n-hexane/water/decanol with TEOS was used. Hydroxypropyl cellulose was added to the decanol phase and polyethylene glycol to the water phase. The resulting material contained mostly disordered macropores with rather low surface areas of around 300 m^2/g . Additionally most of the emulsion approaches, generate spheres in the nanometer or the millimeter range, which is either too small or too large for chromatographic applications.⁵⁸⁻⁶²

However, in 1999 Bossire et al. communicated the synthesis of spherical MSU-X particles.⁶³ In doing so they expanded the field of mesoporous spherical particles to particle sizes in the range of 5 to 15 μ m, the particle size range for application in HPLC. This was achieved under acidic conditions by using the non-ionic surfactant Tergitol 15S12 PEO with sodium fluoride as a catalyst. They successfully controlled the morphology of spherical MSU-X particles by physically separating the assembly step from the hydrolysis step in two separate micro emulsions. This way the final

particle morphology and particle size is function of the respective kinetics of the nucleation and the aggregation processes. At low NaF/TEOS concentrations the low rate of nucleation allows for uniform micrometer sized spheres to be formed. The pore size is still below the 4 nm range because of the rather small non-ionic surfactant that is used $(CH_3(CH_2)_{14}(EO)_{12})$, but due to this method pore swellers can be easily added without disrupting the micro emulsions. Although the particles have the appropriate particle sizes, the morphology is not yet ideal to be used in standard HPLC columns (see figure 1.9)



Figure 1.9: SEM photograph of (A) calcined MSU-1 silica obtained with Tergitol 15S12, a molar ratio TEOS/surfactant = 8 and NaF/TEOS = 1 mol% and (B) calcined MSU-4 silica obtained with Tween 20, a molar ratio TEOS/ surfactant = 4 and NaF/TEOS = 2 mol%. Spherical micrometric particles can be obtained with both surfactants but the mean size depends on various synthesis parameters, especially the TEOS/surfactant and NaF/TEOS ratios. (taken from reference⁶³)

1.2.3 Pseudomorphic transformation retains the spherical morphology

A third method described by Martin et al. is the pseudomorphism route.⁶⁴ Herein commercial silica spheres (Nucleosil 100-5) were bought and stirred in an alkaline solution comprising sodium hydroxide, water and CTAB. After a certain reaction time at 110°C under autogenous pressure the surfactant has templated an ordered pore system inside the spheres without destroying the spherical morphology (see figure 1.10). The change in pore morphology was clearly visible on the X-ray diffractograms and the N₂ adsorption isotherms. Although this seems an interesting approach, it actually leads to an additional step in the production of ordered mesoporous silica spheres.⁶⁵

1.2.4 Spray drying, the route for upscaling to industrial proportions

The final method to induce a spherical particle morphology is an aerosol assisted synthesis, during which the precursor solution is atomized inside a hot air stream. This will be discussed in detail in the section below.



Figure 1.10: SEM pictures of Nucleosil 100-5 silica gel and its transformation by pseudomorphic synthesis into MCM-41 (Nu100-MCM-41). (taken from reference $^{66})$



Figure 1.11: The EISA proces for a thin film with an ionic (A) and a non-ionic template (B).

Aerosol Assisted Evaporation Induced Self Assembly

As the name predicts Aerosol Assisted Evaporation Induced Self Assembly (AAEISA) is based on the pure EISA process. Figure 1.11 shows this process for the formation of thin films of both ionic and non ionic templated mesoporous materials. The process was first described by Brinker et al. in 1999 where it was proven for MCM-41 thin films.⁶⁷

During the process of solvent evaporation the surfactant concentration increases until the critical micelle concentration (CMC) is reached. Above this concentration the surfactant starts forming micelles. CTAB for instance has two CMC values: the first one (0.0009 mol/L in water) for the formation of spherical micelles and a second one for the formation of rod like micelles. The speed of the process depends of course on the used solvent but is inherently a slow process.

Spray drying on the other hand is a fast process, from spray tip to particle collection takes roughly six seconds. It is evident that it is hard to combine a slow EISA process with the speed of an aerosol assisted synthesis such as spray drying. Therefore literature thus far, has reported only materials with the small surfactant CTAB as successfully spray dried mesoporous particles with pore properties equal to those made by a hydrothermal flask synthesis. Figure 1.12 shows the essential spray drying set-up based upon a commercial Büchi system and an M41S type synthesis procedure.



Figure 1.12: The EISA proces applied for the spray drying proces.
M41S type materials

The first report on mesoporous spheres by spray drying was published in 1997 by Bruinsma et al. and dealt with the synthesis of spun fibers and hollow spheres.⁶⁸ The materials were M41S type materials with a surface area of 960 m^2/g and a pore size of 1.9 nm, which is almost mesoporous. The particles had the morphology of collapsed spheres with a crust thickness less than 500 nm.

In 1999 the group of Brinker reported the first spray dried mesoporous material ever.^{69,70} The fully porous mesoporous spheres were produced with a home made aerosol reactor set-up. They made three types of materials based on three different surfactants. The hexagonally structured silica had a surface area of 1256 m^2/g , 1.8 nm pores and was made with CTAB. They also used two larger surfactants, Brij 56 and Pluronic P123 delivering materials with respective surface areas of 514 m^2/g and 478 m^2/g and pore sizes of 4.1 nm and 5.5 nm. The pore structure of these last two materials was vesicular. The particle sizes remained very small with a maximum around 500 nm.

In 2002 Rao et al. published a method remotely linked to an aerosol assisted synthesis which produced very uniform spheres in the size range of 5 to 10 μ m.⁷¹ They used a vibrating orifice aerosol generator (VOAG) to produce the droplets. The only drawback of this procedure is that the vibration of the VOAG tends to break the micelles which leads to inferiorly ordered pore systems.

In 2003 the Unger group reported the first synthesis of spray dried MCM-48 particles by the agglomeration of nanospheres.⁷² First the mesoporous nanospheres were produced according to a modified Stöber method which were then suspended and fed to a spray drier. They were agglomerated to particles between 10 and 25 µm in size with all the characteristics of M41S mesoporous particles. Due to this synthesis pathway both mesopores and macropores are introduced into the spherical particles. Because of this "plural" porosity these are so called hierarchical porous particles.

An in - situ SAXS (Small Angle X-ray Scattering) study of the spray drying process revealed two important influences on the mesostructuring of spray dried mesoporous particles.⁷³ First of all the drying temperature seemed to have an effect on the pore size, as a shift to lower pore sizes occurred when higher temperatures were applied. The second major influence was the water/ethanol concentration on the mesostructuring. It seemed that ethanol rich solutions promoted a better mesostructure formation than a water rich solutions. The explanation for this can be found in the difference in silica condensation rate. Water promoted the kinetically controlled inorganic growth over the self-assembly arrangement hence resulting in less well resolved mesophases.

It was not before 2004 when Alonso et al. published his paper on morphological and textural control of spray dried mesoporous silica-based spheres, that an extensive study of various factors was performed.⁷⁴ The two varied factors were the time during which the siloxane sols were aged and their solvent content. The aging time

had a clear influence on the morphology, very short aging times resulted either in agglomerated spheres or no spheres at all and very long aging times resulted in larger broken spheres suggesting an initial droplet size of about 20 µm. Longer aging times seemed to have a detrimental effect on the mesophase formation. As for the solvent concentration, lower amounts of solvent resulted in an increase of sphericity of the particles. Figure 1.13 shows their proposed scheme of formation.



Figure 1.13: Physico-chemical processes occurring during the drying step. At the micrometric scale (top), droplet fragmentation occurs, followed by droplet agglomeration if the solidification process is slow enough. At the nanometer scale (bottom), the mesophase can be formed if the solidification process is slow (and if the extent of hydrolysis is high). The mesophase extends inside the particles, and even between agglomerated particles if the solidification is very slow. (taken from reference⁷⁴)

SBA-type materials

While the first spray dried SBA-15 type material was reported in 1999 by the group of Brinker, it took five years before a successful follow up communication could be published. In 2004 the group of Sanchez reported the spray drying synthesis of an ordered hierarchical mesoporous core-shell spherical material based on the non-ionic triblock co-polymer Pluronic F127.⁷⁵ The final material exhibited a hexagonal structure with a surface area of 390 m^2/g and particle sizes far below 1 µm.

Various research groups attempted to synthesize SBA type materials via spray drying but none of them managed to produce particles with the same large surface areas or uniform pore systems as can be made by a hydrothermal synthesis. The particle size distributions were also very broad, ranging from sub micron particles up to particles of several micrometers in size. Pluronic F127 seemed to be a better surfactant to successfully obtain templated mesoporous spheres through spray drying.^{76,77} In 2009 the first investigation of the industrial mass-production of spherical mesoporous particles by spray drying was published.⁷⁸ The materials were spray dried from a mixture of TEOS, HCl, Pluronic F127 and ethanol. The largest spheres were 2.2 µm in size with a standard deviation of 0.11 µm. The pore system is guessed to be cubical (the XRDs were not conclusive) with a pore size of 6 nm and a maximum surface area of 327 m^2/g . Around the same time Pitchumani et al. published the spray drying synthesis of mesoporous silica and silica-alumina catalysts using industrial raw materials.⁷⁹ The silica source was nano-sized boehmite (Captal B (Al₂O₃)) and stabilized colloidal silica. There was no information on the particle morphology. The pore system was said to be equal to SBA-15 with a maximum surface area of 292 m^2/g and a pore size of around 10 nm.

PMO-type materials

Up until now there are no spray dried PMO materials reported. However, in 2005 Alonso et al. reported the first co-condensed organically functionalized mesoporous silica spheres.⁸⁰ They described a two step synthesis, in a first step TEOS, HCl, CTAB and isopropanol were mixed and aged. The formed siloxane oligomers were then mixed with methyltriethoxysilane and subsequently spray dried. Non-uniform separated spheres with a hexagonal mesophase were formed. In a subsequent article this procedure was extensively studied in order to trace the effects of various factors on the organization of matter and the formation of perfect and uniform spheres.⁸¹ The best sample had well defined micro metric spheres with an average diameter of 1.2 µm and a standard deviation of 0.74 µm. Furthermore the particles showed a clear core shell structure in a way that the shell had a different pore structure (hexagonal, following the form of the particle) than the core (hexagonal but without clear direction (see figure 1.14). Additionally it became clear that when spray dried at temperatures around 100°C, the solvent (isopropanol or water) played a huge role in the formation of spheres or collapsed spheres. At this temperature, the evaporation of the solvent in the droplets slows down due to the water content and deformation of the droplets occurs, forming collapsed spheres and shells. From this it is clear that solidification has to occur fast in order to form a spherical morphology.

Thiol functionalized mesoporous M41S type materials were spray dried by a cocondensation of TEOS and MPTMS (mercaptopropyltrimethoxysilane) by the group of Alonso.⁸² The materials were subjected to a post-synthesis treatment ranging from calcination (removing every organic function including the mercaptopropyl group), solvent extraction with an ethanol/HCl mixture and a solvothermal and hydrothermal treatment prior to extraction. The sulfonated materials were tested with Ag^+ sorption. This proved, together with NMR data, that the thiol functions were homogeneously distributed over the pore surface and were accessible to other chemicals. Surface areas ranged from 1200 m^2/g for the calcined sample to about 850 m^2/g for the extracted materials. Post-treatments were performed to prevent the particles from losing their long range pore ordering by directly extracting the surfactant from the pores. A post-synthesis curing at 150°C stabilized the texture of the materials enough to allow an extraction procedure. The first SBA type co-condensed mesoporous spheres produced from mixtures of ethane bridged silanes with TEOS, were spray dried by Yamauchi et al.⁸³ Particles had a higher surface area with higher ethane content. They were the first who managed to produce SBA-type pore systems with surface areas up to 673 m^2/g and a pore size of 11 nm. The particles retained a spherical morphology with a mean particle size around 1 µm and a particle size deviation of 0.50 µm.



Figure 1.14: TEM micrograph of an ultramicrotomed sphere. The dashed circle represents the border between the ordered domain found at the surface and the non-ordered domain found in the core of the sphere. The marked spots (from A to E) show clearly the P6/mm structure in the outer layer of the material. (taken from reference⁸¹)

Conclusion

From this overview it is clear that there is still much improvement possible. The number of papers on spray drying ordered mesoporous particles is still very limited. Since the technique first surfaced for nanotemplated materials in 1997, 33 new publications were issued on spray drying of mesoporous materials, most of which on M41S type materials. The reason for this is that the smaller CTAB molecule allows for a faster assembly process in the fast spray drying process. Especially when compared to the larger non-ionic tri-block co-polymers commonly used to make larger pore SBA type materials.

Spray drying is a handy technique to produce large quantities without any scale-up problems arising. Producing monodisperse spherical particles is harder since this pathway inherently leads towards multi-disperse particles, which is a real disadvantage when the particles have to be used as a packing material in chromatography.

2

The importance of order and increased surface area in chromatographic columns

2.1 Introduction

The performance of chromatographic columns is strongly influenced by particle design. Uniform particles allow for a more homogeneous packing of a column and reduce the Eddy diffusion through the column, which is leading to reduced peak dispersion. Since chromatography is in essence a diffusion controlled process, the architecture of the pores in the material plays an important role in both the efficiency and the retention experienced with a particular type of packing material in a column. Current silica based stationary phases exhibit a broad pore size distribution. This means that no two pathways throughout the particle are identical and that not every functional group is equally accessible. To reduce diffusion times through the particle, superficially porous particles were introduced. Superficially porous particles successfully improve the chromatographic performance. However, inherent to this particle design a reduced phase ratio leads to a lower sample loadability and a reduced retention.⁸⁴ To deal with this drawback an ordered pore system with a strongly increased surface area could offer a solution.

To improve the separation efficiency the particle size has been ever reducing over the last four decades from 60 µm to the 1.7 µm particles now available. Two consequences can be attributed to this reduction in particle size: first of all the plate number in liquid chromatography is inversely proportional to two times the particle size in packed columns. Secondly the back pressure of the column will increase because smaller particles will induce smaller interparticle voids leading to an increased pressure necessary to percolate the same flow through the column. To somehow counteract this, superficially porous particles can be used to obtain the same efficiency as small particles without the need for high pressure systems. Therefore an ordered and uniform porosity with a high specific surface area can also increase the retention of packed columns. This increased retention could broaden the scope of reversed phase stationary phases to accommodate the separation of more polar compounds without the need for a different column. Additionally it could give open tubular liquid chromatography and lab on a chip applications the needed push to become a validated technique.

In this chapter an overview is provided of the various strategies which have been followed to introduce increasing degrees of order and surface area in chromatographic columns. In the subsequent chapters the introduction of pore size and particle shape control in chromatography is described. Note that although there are many chromatographic techniques, the emphasis in this work is placed on liquid chromatography, as it is today the most used and the most versatile chromatographic technique.

2.2 High Pressure/Performance Liquid Chromatography (HPLC)^{85,86}

Today HPLC is the most used chromatographic technique world-wide. HPLC equipment is the third most sold analytical instrument after the pH meter and the analytical balance. Liquid chromatography was introduced over a hundred years ago by Tswett with the separation of carotene, xanthophylls and chlorophylls.^{87–89} Over this period the technique underwent many changes and additions including thin-layer chromatography, paper chromatography, ion-exchange chromatography, (capillary) gas chromatography, gel chromatography, HPLC, supercritical fluid chromatography and electrochromatography. In this section some important fundamental aspects of HPLC are introduced, such that the importance of order and surface area in HPLC can be properly assessed in the subsequent chapters.

2.2.1 Principles of HPLC

Chromatography is based on the differential partitioning of analytes between a mobile phase and a nonmiscible stationary phase. When a plug of analytes, dissolved in the mobile phase, is percolated by a constant flow of the latter through a column containing the stationary phase, the solutes with the strongest affinity for the stationary phase will spend relatively more time in the latter. They will therefore be more retained on the column and elute latest. These differences in affinity of the solutes for the stationary phase induce a separation between the different analytes. The stationary phase can be a solid (i.e. underivatized silica) or on immobilized phase exhibiting liquid like characteristics. The mobile phase is usually a pure solvent or a mixture of solvents in liquid chromatography. In HPLC the liquid is forced through a packed bed of stationary phase at high pressures to reach the optimal mobile phase velocity at which the best column performance can be obtained.

2.2.2 The fundamentals of chromatography

In order to effectively assess the influence of the mobile phase velocity on the chromatographic performance, a number of fundamental parameters need first to be introduced.

Efficiency⁸⁶

The efficiency or plate number N is a measure for the quality of the separation, measured during an analysis. It represents the amount of theoretical plates in a column and is based on the ratio of the retention over the dispersion as measured for various analytes eluting from the column. It can be calculated from the peak width and the retention time through the following equations:

$$N = \left(\frac{t_R}{\sigma}\right)^2 = 5.54 \left(\frac{t_R}{W_h}\right)^2 = 16 \left(\frac{t_R}{W_b}\right)^2 \tag{2.1}$$

where t_R , σ , W_h and W_b correspond to the analyte retention time, peak width at 50% of the peak height (2.355 σ) and at the base of the peak (4 σ). In figure 2.1 a chromatogram is represented containing a retained and an unretained peak, eluting at t_R and t_0 , respectively. The effective elution time of a solute (t'_R) is obtained by subtracting the dead time, designated as t_0 , from t_R .



Figure 2.1: Schematic of a chromatogram containing a retained and an unretained signal.

The height equivalent of a theoretical plate H is related to N in such a manner that a column is divided into N segments of length H. A theoretical plate is a hypothetical length of column where equilibrium of distribution of an analyte between the stationary and the mobile phase is just reached. Therefore:

$$H = \frac{L}{N} \tag{2.2}$$

Today a column which is packed with fully porous particles is expected to deliver plate heights which are as low as twice the particle size d_p .

$$N = \frac{L}{2d_p} \tag{2.3}$$

In order to be able to compare different columns under a broad range of particle sizes or column diameters, the reduced plate height h was introduced by Giddings et al.⁹⁰ and can be calculated with the following equation:

$$h = \frac{H}{d} \tag{2.4}$$

with d as a column characteristic, which equals the particle size d_p in packed columns, the internal diameter d_c for open tubular columns or the domain size d_m in monolithic columns.⁹¹

Retention factor⁸⁶

The retention factor k is the ratio of the time spent in the stationary phase compared to the time the analytes reside in the mobile phase. The retention factor is obtained from the chromatogram through the following equation:

$$k = \frac{t_R - t_0}{t_0} = \frac{t_R'}{t_0} \tag{2.5}$$

whereby t'_R corresponds to the effective retention time. The retention factor is directly related to the distribution constant (K) and is, contrary to t_R or t'_R , independent of the column dimensions and flow rate. The retention factor is influenced by the stationary, the mobile phase and the analytes. The higher the eluting strength of the mobile phase the lower the retention and thus the lower the retention factor will be. However, when the amount of stationary phase on the particles increases or becomes more accessible, it is clear that the retention of the column increases and thus the retention factor will increase accordingly. The eluting power of the mobile phase depends on its composition and temperature.

Selectivity factor⁸⁶

The selectivity factor α is defined by the ratio of the retention factors:

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{t_{R2} - t_0}{t_{R1} - t_0} = \frac{k_2}{k_1}$$
(2.6)

The selectivity factor is a very valuable parameter as it depends on the properties of the solutes, the stationary phase, the mobile phase (pH, ionic strength,...), the

temperature and the flow resistance factor φ . It is a complex factor, influenced by many factors therefore it is also a factor that is hard to predict and main the main reason for "trial-and-error"-approach very often applied to optimize a separation.

$Resolution^{86}$

The degree of the separation between two adjacent peaks is defined by the resolution. Empirically it is defined as the ratio of distance between the apex of two peaks compared with their average peak width.

$$R_s = \frac{t_{R2} - t_{R1}}{\left(W_{b1} + W_{b2}\right)/2} \tag{2.7}$$

In figure 2.2 an example is given of two peaks, eluting with retention time $t_R(1)$ and $t_R(2)$, separated with a resolution greater of 3. A resolution of 1.5 is considered as a complete separation as this involves that thereby less than 5% of the two signals are still overlapping.



Figure 2.2: Schematic representation of two peaks separated with a resolution of 3.

Equations 2.1, 2.5, 2.6 and 2.7 can be combined to provide the master resolution equation in chromatography:

$$R_s = \frac{\sqrt{N}}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k}{k+1}\right) \tag{2.8}$$

A closer look at this equation allows to draw some interesting conclusions (see figure 2.3). First of all it can be seen that the resolution is directly proportional to the square root of the plate number. Therefore increasing the plate number by 4 results in only a doubling of the resolution. Secondly the strongest influence is exerted by the selectivity factor. Since the selectivity is also the most arduous parameter to control, this explains the "trial and error" approach which is routinely used during method development in HPLC today. Thirdly it can be seen that the retention factor has little to no effect on the resolution once it is larger than five.



Figure 2.3: The resolution as a function of the plate number, the selectivity factor and the retention factor.

Phase ratio⁸⁶

The distribution process of analytes between the mobile and the stationary phase is controlled by the distribution coefficient, expressed by the distribution constant of Nernst, K:

$$K = \frac{C_S}{C_M} \tag{2.9}$$

with C_S and C_M the concentration of the analytes in the stationary and the mobile phase respectively.

The retention factor k also expresses the ratio of the quantity of the analytes in the stationary and the mobile phase:

$$k = \frac{M_S}{M_M} \tag{2.10}$$

with M_S and M_M the respective quantities of an analyte in the stationary and the mobile phase. K and k are correlated by the phase ratio β :

$$K = k\beta \tag{2.11}$$

hereby β is the phase ratio of the total volume of mobile phase (V_M) over the total volume of stationary phase (V_S) in the column:

$$\beta = \frac{V_M}{V_S} \tag{2.12}$$

van Deemter curves⁸⁶

As the degree of peak dispersion is a result of the interplay of the quality of the column packing (and order in the column in a general way), longitudinal dispersion and of the kinetics of mass transfer between the two phases, and as the latter two affect dispersion in opposing ways as a function of the mobile phase velocity, an optimum can be found in this term for every analyte. In HPLC this optimum typically varies around 1 to 2 mm/s. Note that the "linear" velocity, u, is thereby relevant and to a lesser extend the flow rate, F. (u = F/S, whereby S is equivalent to the cross section of the column times the porosity, ε). The van Deemter equation is thereby the most often used model to describe peak broadening in chromatography.

$$H = A + \frac{B}{u} + C.u \tag{2.13}$$

Whereby

$$A = 2\lambda d_p \tag{2.14}$$

$$\frac{B}{u} = \frac{2\gamma D_M}{u} \tag{2.15}$$

$$C = C_M + C_S \tag{2.16}$$

A is thereby the Eddy diffusion, λ is a term describing packing efficiency and d_p the particle diameter for a packed column. B is a representation of the longitudinal diffusion, D_M the diffusion coefficient in the mobile phase and γ is the obstruction factor.⁹² The C-term, which can be subdivided in a C_M and a C_S term corresponds to the resistance to mass transfer in the mobile and stationary phase, respectively.



Figure 2.4: An illustration of the plate height as a function of the mobile phase (linear) velocity according to the van Deemter model together with the influence of the composing parts.

Figure 2.4 shows the plate height as a function of the mobile phase velocity for equation 2.13 and divided into the 3 composing terms A, B and C. The A term is influenced by the particle size, shape and pore structure; the packing quality and wall effects (material, roughness and column diameter). The B term is influenced by u, D_M , the viscosity, the temperature and the type of analyte. C_M is influenced by d_p , u, D_M , the porosity, the viscosity, the retention factor k and the temperature. C_S is influenced by the quality of the stationary phase, the diffusion coefficient in the stationary phase (D_S) , k, the temperature, d_p and u.

Increasing the efficiency N

From equation 2.2 it is clear that the use of longer columns is the easiest way to increase efficiency. But this also leads to longer analysis times and to increased column back pressures. As an HPLC system can only deliver a limited amount of pressure a physical limit is experienced in extending the column length approach as this inevitably leads to an upper column length whereby one can still operate the optimum flow rate. A second way to improve N is by reducing the plate height. Figure 2.5 depicts the evolution of the particle homogeneity and size over the last 4 decades.⁹³ Accordingly, more powerful pumps had to be developed to deal with the higher back pressures induced by the smaller particles. Again, as with the column

Year of acceptance	Particle size	Nominal size	Plates/5cm
1969	\bigcirc	100µm	170
1973	₹₿₿	$57 \mu m$ (pellicular)	350
1975	*	10µm	2,000
1985		5µm	4,000
1992	9	3.5µm	7,500
2003	•	1.8 µm	14,000

length, this means that there is a physical limit to the particle size.

Figure 2.5: The evolution of the packed particles over the course of time.

An alternative manner to improve N is performing HPLC at higher temperatures. In this way the viscosity of the mobile phase and therefore the column back pressure reduces and higher flow rates can be applied. Since most of the columns are silica based and when water is used as a solvent at higher temperatures, the siloxane bridge of the silica particles starts to hydrolyze leading to a serious reduction in retention due to leaching of the stationary phase and eventually a total collapse of the packed bed. This issue can be partly solved by thoroughly shielding silica from water or by using polymer based columns.

Another and more recent approach reduces the path analytes have to travel trough the particles by using superficially porous particles. Superficially porous particles were first introduced by Kirkland et al. by fusing a layer of nanoparticles around a solid fused silica core.⁹⁴ By fusing these nanoparticles together the layer obtains a porous structure, due to the voids between the particles. These particles efficiently improve the chromatographic efficiency, which is attributed to a reduction in the diffusion length through the particle but also to a reduction in the B-term and to the overall more narrow particle size distributions. A drawback of this approach is that the sample capacity is decreased due to the reduced amount of stationary phase.⁸⁴

To be able to combine the above solutions, a new type of particles that increases particle stability and improves the retention is deemed necessary. This can be performed by replacing the oxygen bridges between silicon atoms by carbon bridges and by improving the pore structure and increasing the surface area of the materials to increase the retention of the materials.

2.2.3 The different separation modes in HPLC⁸⁶

Depending on the properties of the solutes to be analysed, several separation modes have been developped in liquid chromatography. The most popular ones are normal phase liquid chromatography (NPLC), reversed phase liquid chromatography (RPLC), ion exchange chromatography (IEC), chiral chromatography, affinity chromatography, size exclusion chromatography (SEC) and hydrophilic interaction chromatography (HILIC), with respective usage percentages of 5.4%, 44.6%, 18%, 6.8%, 2.4%, 10% and 8.2%.⁹⁵ Reversed phase chromatography is today the most used HPLC mode. An overview of the most relevant modes and of the underlying principles is given below.

Reversed phase liquid chromatography

In reversed phase HPLC the stationary phase is hydrophobic, typically silica functionalised with alkyl groups (C18) but also polymers and graphitic carbon is used. The mobile phase is thereby hydrophilic. Mixtures of water and an organic solvent of which acetonitrile and methanol are most common. The separation itself is mainly based on two effects of which the distribution of the analytes between the mobile and the stationary phase is the most important one. The second effect is the adsorption of analytes between the mobile and the stationary phase. The latter is normally suppressed as much as possible by extensive column deactivation, although complete suppression of adsorption phenomena is normally difficult to achieve. The polarities of the components and the stationary phase are primarily responsible for this. Moreover there are several more properties of the analytes, the mobile and the stationary phase playing a role in the retention mechanism in RPLC. The log k, the saturation degree, the solubility in water, the polarity and the ionic character are some component characteristics that play key role. Furthermore the concentration composition of the mobile phase and the percentage by weight of bonded carbon of the stationary phase are some mobile and stationary phase characteristics which are important for the retention of analytes in RPLC. This mode has the advantage that these interactions are easy to control with a well controlled mobile phase. However the main drawback is that the high viscosity of water in the mobile phase somewhat limits on the maximum applicable flow rate and pressure.

Normal phase liquid chromatography

Historically this was the first chromatographic mode which was introduced. Here the stationary phase is polar, typically bare silica, and the mobile phase is a nonpolar organic solvent. Separation is based on the interaction of polar groups of the analyte with the polar sites of the stationary phase. The affinity of polar groups from the analyzed compounds for the polar hydroxyl groups of the stationary phase are the most important interactions. This means that this technique is particularly useful for the separation of polar compounds. The polarity of the compounds is the main basis of separation, the higher the polarity, the higher the interaction. The main drawback of this mode is caused by an irreproducible water layer on the pure silica particles which sometimes leads to irreproducible separations.

Ion exchange chromatography

IEC is used for the separation of water-soluble ionogenic compounds, typically underivatised amino acids, peptides, proteins and nucleic acids. During the separation process ions from bonded ionogenic groups on the stationary phase are exchanged for the ionic analytes. Ionogenic groups are divided into cation and anion exchangers, which are in turn subdivided into strong and weak groups. The stronger the electrostatic interaction between the ionic analyte and the stationary phase, the more retained a compound will be. The mobile phase is populated with a solution of competing ions. The main advantages of this mode are that no derivatisation of the analytes is required. The main drawback is the need for a regeneration step of the column.

Size exclusion chromatgraphy

SEC is subdivided into Gel Permeation Chromatography (GPC) and Gel Filtration Chromatography (GFC). The former is usually applied for the isolation and the purification of organic polymers while the latter is especially applied for large biomolecules such as proteins. The analytes are separated by their size and form dependant ability to penetrate the pores of the packing. For smaller molecules more pore volume is accessible for diffusion, therefore smaller molecules experience more retention. If the analytes have the same form, elution takes place in order of decreasing size. This means that larger molecules therefore elute faster than the smaller ones which enter the pores. The particles are synthesized in such a way that the diameter of the pores is kept within specific limits. Therefore it can be suggested that current proposed uniform pore systems can be very beneficiary for SEC.

Hydrophilic interaction chromatography

HILIC can be considered a variation of NPLC where both the stationary and the mobile phase are polar. It is therefore also called aqueous normal phase LC. The mobile phase is typically a mixture of water with a high organic modifier content. The water in the mobile phase forms a hydrophilic water-rich layer on the surface of the polar stationary phase due to its interaction with the polar silanol groups. The retention mechanism itself is based on a partitioning of the analytes between a water-rich stationary phase and a water-poor mobile phase.⁹⁶ The more polar analytes will exhibit a much stronger interaction with the aqueous layer than the less polar compounds. Therefore separation is a function of the polarity of the analytes and the degree of solvatation. Any polar chromatographic stationary phase can be used, such as bare silica, amino, amide, cationic or zwitterionic phases. To control the pH and the ionic strengthof the mobile phase, ionic additives are added to the mobile phase. In HILIC they also contribute to the polarity of the analyzed compounds and thus influence the retention mechanism.

Chiral chromatography

Chiral chromatography is used to separate enantiomers with a chiral selector, anchored to the stationary phase. Typical chiral selectors are cyclodextrins, chiral cellulose and chiral enzymes. Most chiral LC methods are operated in the NPLC mode as this promotes the necessary interactions with the chiral cage.

Affinity chromatography

This is a technique that separates (biochemical) mixtures based on the biological function or the individual structure, a custom-made separation method so to speak. Highly specific interactions such as those between antigen and antibody, receptor and ligand or enzyme and substrate are used to obtain a separation. The stationary phase is typically a gel matrix such as agarose with attached analyte specific ligands.

Among these techniques RPLC, IEC, affinity chromatography, SEC and HILIC all require the use of water. Due to the hydrolytical liability of silica the introduction of more stable stationary phases would therefore be very welcome.

2.2.4 HPLC for the analysis of polar compounds

High pH values allow the analysis of more polar compounds. The polarity of molecules analyzed by HPLC is best expressed by the log octanol/water (log P) partition coefficients of those molecules. This value can also be modeled in a fairly accurate way (with the EPA suite free software⁹⁷). Roughly, molecules with a log P > 1 should be retained on a column operated in reversed phase LC mode. The analysis of more polar compounds should currently be performed with alternative modes which promote polar interactions with the stationary phase such as NPLC, HILIC and IEC. For example it is often not possible to analyze (retain) polar peptides and glycopeptides during the same type of analysis. The development of stationary phases with increased retention through increased carbon loading (with RPLC mode) and with increased accessible surface groups should therefore allow to retain even more polar solutes than is possible today by RPLC. This could open the way to an extended performance of reversed phase LC and ever more generic use there off.

2.2.5 The column formats

There are four different column formats, planary columns, packed columns, open tubular columns and monolithic columns.

In planar chromatography the mobile phase is mobilized through capillary action through a sheet of paper or through a thin bed of silica immobilized on a glass plate. It is therefore an open access format whereby after '*developing*' of the plate the various analytes can be detected visually. However, this approach is difficult to automate and less robust compared to the closed column formats.

The other column formats involve closed system into a column shape. A packed column comprises a cylindrical tube packed with spheres. These columns are typically packed at 1.5 times the pressure they are to be used at. The smaller the particles, the lower the plate height (equation 2.3) and the higher the back pressure experienced on these columns will be. A slurry of the packing is mounted on the column and is connected to a pressure pump with the slurry solvent in a reservoir (see figure 2.6). A constant pressure pump drives a slurry with the packing suspended in a solvent in the column at once and this pressure is kept on the column for an additional 30 minutes. The slurry solvent composition depends on the packed spheres in such a way that the final slurry has to be suspended long enough while still being dense enough to ensure an ideally packed column.



Figure 2.6: An impression of the general packing set-up used to pack HPLC columns.

In open tubular columns, the stationary phase is present as a thin layer on the inside of the column wall. Because of the high diffusion rate of the analytes in the gas phase and because of the high phase ratio (equation 2.12) of these columns, these columns are generally used in gas chromatography where columns up to 50 meters long are no exception.

Another closed column format is the monolithic column, these are made up of one

piece of silica or polymeric material with the inclusion of macro and mega pores. This makes the column extremely permeable to the mobile phase and strongly decreases the phase ratio. The main drawback of this type of column is the irreproducibility of these pore systems leading to less reproducible separations (see figure 2.8 for an example).

An alternative to the most commonly used tubular shape of a column are chromatographic chips. Their miniaturized set-up leads to reduced solvent and analyte usage. Chromatographic chips are made up of etched columns in the size range of capillaries. The commercially available chips are 43 mm long with an I.D. of 75 µm. They are packed with spherical particles, which make up the stationary phase, just like standard packed columns. Chromatographic chips are small and fewer connections have to be made between injection, column and detector. These advantages lead to reduced peak dispersion and a better resolution in a shorter timespan. However, these chips still exhibit several drawbacks. First off the packed columns have a pressure limit, which is why the columns cannot be longer than 1m. Furthermore it is far from easy to reproducibly pack these columns. Also external pumping systems have to be used and the needed micro and nano pumps are complex to produce and operate.⁹⁸

A solution proposed by the group of Desmet et al. is the replacement of the packed spheres by etched micropillars to create an ideal packing. This would solve the packing issue and strongly increase the limit on the column length. 99,100

2.2.5.1 Open tubular liquid chromatography (OTLC)

Due to the high diffusivety of molecules in the gas phase open tubular capillary columns are commonly used in gas chromatography (GC). Standard column internal diameters thereby range from 100 to 250 µm. This gives GC a clear advantage in terms of achievable efficiencies, it also improves the temperature stability, the hydrothermal resistance and easy coupling with detectors. Nanograms of material can be analyzed with a good reproducibility. However, GC is limited by its very nature, such that it needs volatile solutes and not every analyte is volatile or can be made volatile without decomposition. Therefore LC, although less efficient, is the most used separation technique today.

Due to the three orders of magnitude slower diffusion in the liquid phase, compared to the gas phase, open tubular columns can not be used unambiguously in LC. Figure 2.7 depicts a separation of several polyaromatic hydrocarbons on an open tubular column in the liquid phase. It shows that the compounds are virtually not retained and therefore not separated. The retention of the analytes in the mobile phase depends on the internal diameter of the capillary and on the amount of stationary phase present on the inner wall of the capillary. A smaller internal diameter reduces the diffusion distance, this and an increased stationary phase ratio improve the retention of analytes. A proposed solution is reducing the internal diameter (I.D.) of the capillaries. However to obtain sufficient retention, the internal diameter of the columns should be reduced with a factor 1000 to about 0.25 µm. The problem that arises with these small columns is that the maximum sample loading capacity is also extremely low. This then again results in severe detection problems in such a way that the analyte can not be distinguished from the background signal. Additionally at these low internal diameters the back pressure of the system becomes an issue again.



Figure 2.7: Electrochromatogram of a test mixture containing five poly aromatic hydrocarbons: (1) naphtalene, (2) biphenyl, (3) fluorene, (4) 2-ethylnaphtalene and (5) 2,6-dimethylnaphtalene. Separation conditions: capillary column, 13 µm I.D. x 50 cm long; mobile phase, MeOH/1mM phosphate buffer (75/25); separation voltage, 30 kV; electrokinetic injection, 2s at 21 kV. (taken from reference¹⁰¹)

2.2.5.2 Monolithic columns

Macroporous monolitic columns grown inside capillaries ranging from 100 to 250 µm diameter (figure 2.8) reduce back pressures due an improved permeability ascribed

to the macroporous structure when compared to packed capillaries. Additionally no column restrictors are needed since the entire structure is intergrown with the capillary fused silica wall. These advantages have lead to some substantial improvements in terms of loading capacity and efficiency in comparison with OTLC. However due to the fact that these are monolitic columns with a disordered macroporous structure, column to column reproducibility is very poor. This is inherent to the production procedure of these columns. A polyethylene oxide polymer is thereby mostly used as porogen to create a porous structure, randomly placed into the seed solution.¹⁰² After polycondensation of the silica structure, the porogen is burned out at high temperature and during this process the core monolith can shrink, a first source of irreproducibility. Secondly since the porogen is randomly mixed with the rest of the sol, the resulting macroporosity is random and not uniform. This means that the pore size distribution of the resulting monolithic column is broad and not equal for each column which is a second source of column to column irreproducibility.



Figure 2.8: SEM figure of a macroporous monolitic capillary column. (taken from reference 102)

2.2.6 Silica hydrolysis: HPLCs heel of Achilles

Particle and bed stability have always been an issue with silica based columns as water is very often and ever increasingly used in (green) chromatography. Hydrothermal stability is related to two chemical properties of silica: silanol groups and siloxane bridges. During the hydrolysis of silica in an aqueous medium, water molecules easily adsorb to the silica surface by means of hydrogen bonding to the surface silanol groups. In a second step these watermolecules attack the surface siloxane bridges, breaking them, resulting into two new silanol groups (see figure 2.9). This process continues exponentially until the entire silica structure has been dissolved.¹⁰³

Due to this susceptibility of silica materials to hydrolysis, conventional column performance is easily affected when using harsh conditions such as elevated pH or higher temperatures in combination with a highly aqueous mobile phase. This instability of silica packing materials is reflected in a reduced column efficiency and a reduced retention as a function of the number of chromatographic runs. Column efficiency is reduced by a dissolution of the particles in the column which leads to a collapsed bed, affecting the analyte flow path (and concomitantly the A-term in equation 2.13). This leads to deteriorated peak shapes and even peak splitting. Chromatographic retention (k) is affected by release and loss of stationary phase. In reversed phase HPLC a hydrophobic stationary phase is currently grafted on the silica particles. The octadecyl group is thereby typically anchored to the silica surface by means of ideally 3 siloxane bridges. Therefore this functional group is as susceptible to siloxane cleavage as the entire structure is.



Figure 2.9: The degradation of silica under the inlfuence of water hydrolysis.

If the problem of column instability, caused by harsh analysis conditions, could be solved, a lot of new opportunities would present themselves on both the economical and the environmental point of view. When elevating the temperature, the dielectric constant of water alters resulting in a reduction in the polarity of water. This leads to an improved miscibility with organic solvents and an increased elutropic strength of water in RPLC. This allows to replace the routinely used gradient in solvent composition in RPLC towards the more hydrophobic solvent at the end of the gradient, by the use of only water as mobile phase in combination with a temperature gradient to increase the eluotropic strength towards the end of the gradient. Current low hydrolytical stability of conventional silica material does hinder proper exploitation of this obvious route towards green sustainable HPLC. The benefits are thereby multiple such as reduced costs for solvent purchase and for disposal after use.

To prevent the occurance of both, several solutions have been proposed. Endcapping was the first solution and more recently researchers started to add more carbon to the stationary phase with semi and full hybrid types of silica, even fully carbonated stationary phases were tried.



Figure 2.10: An illustration of the protective groups at the base of grafted functional groups.

Endcapping to shield residual silanols

After the complete functionalization of the bare silica material, the residual accessible silanol groups are subjected to a methylation process. There are a few groups that are widely used as methylating reagents such as hexamethyldisilazane, chlorotrimethylsilane or N-(trimethylsilyl)imidazole. This way organic umbrellas sterically shield the surface from any attack. Despite these measures and also because these shielding groups are still anchored through a siloxanebridge, hydrolysis of the surface still appears. Depending on the applied conditions (column cloggin and pH) the lifetime of a column varies between 250 and more than 4000 separations.

To improve the stability of the grafted functional groups, such as C18 chains, new types of grafting silanes were used. Silanes with isopropyl groups fitted at the base should prevent an attack of problematic molecules at the base of the anchored group (see figure 2.10).

Semi- and full-hybrid materials to introduce more carbon into the silica structure



Figure 2.11: Example of the use of organosilanes to increase the hydrolitical stability of silica. This is used in commercial columns which are stable from pH 1-12.¹⁰⁴

An example of a commercial semi-hybrid material is shown in figure 2.11. The column is composed of fully porous silica spheres, modified with a multilayer of organosilanes. Two types were introduced, the first made use of $RSiO_3$ groups and the second type added ethane bridged silanes to the multilayer coating. The performance of this type of column runs in line with the expectations of what can be obtained when using conventional silica particles. However they can be used in a broader pH range (1-12). For example the analysis of basic solutes as amines, can greatly benefit from the use of an elevated pH, because in their deprotonated form they tend to depict much less tailing. Note however that, although the enriched organic moieties improve the hydrothermal stability of the material, it is not changing the stability of the grafted functional groups.¹⁰⁴

To further improve the stability of silica materials, an ethane bridged silane has also been mixed with the pure silane precursor with a ratio of one to four. This reduces the amount of siloxane bridges in the final silica material and increases the hydrothermal stability. This material was introduced especially for the UHPLC systems to deal with the high pressure conditions generated on the columns.^{105,106}

Activated carbon used as a column packing

The packed activated carbon particles are made up of hexagonally arranged carbon atoms. These columns exhibits a very high stability over the entire pH range of 0-14. The column is primarily used to separate highly polar compounds and closely related structures. The main disadvantage of this column is that irreversible absorption of the analytes, with irreproducibility as a result, is observed. Additional issues are a suboptimal efficiency compared to theoretical expectations, easy column clogging and limited mechanical stability.¹⁰⁷ Therefore this type of column is not ideal in for example routine analysis in validated pharmaceutical environments.

2.2.7 Flow generating systems

There are two types of systems used to establish a flow in a column. Pressure driven systems are the most applied separation set-up and are commonly used in HPLC. One or more pumps push the mobile phase through the column to generate the flow necessary to enable the separation of analytes.

Electric driven systems on the other hand make use of an electric current to generate an electric osmotic flow. This flow is commonly used in electrophoresis and when performed over a column the induced flow enables the separation of the analytes.

2.3 Capillary electrochromatography (CEC)

Because of the minimal solvent usage, CEC is considered as a very green analysis technique. It combines the broad applicability of HPLC with the high efficiency and speed of electro driven analyses which makes it theoretically the best technique available for the analysis of every type of mixture. Figure 2.12 presents the essential mechanism of electro-osmotic flow (EOF) responsible for the flow of the

mobile phase.



Figure 2.12: Illustration of how the generation of EOF in an open capillary (A) and a packed column (B). (taken from reference 108)

Below are the equations needed to calculate the EOF velocity and the peak capacity of a CEC column.

$$n = 1 + \frac{\sqrt{N}}{4} . ln \frac{V_z}{V_a} \tag{2.17}$$

and

$$V_z = t_z . v_{eof} \tag{2.18}$$

and

$$v_{eof} = \frac{l}{t_{eof}} \tag{2.19}$$

With n the peak capacity, N the plate number of peak z, V_z the retention volume of peak z, t_z the retention time of peak z, v_{eof} the EOF velocity, l the chromatographic column length and t_{eof} the column dead time.

Due to both a chromatographic partition on the stationary phase and differences in electrophoretic migration velocities, the selectivity of a separation can easily be optimized. A very high column efficiency per column length is obtained because of a reduced peak broadening in electro driven techniques. This reduction in peak broadening can be explained by the flat flow profile of an electro-osmotic flow (EOF) in comparison with the parabolic profile of a pressure driven system (see figure 2.13). Because of both these mechanisms in combination with the high efficiencies, the resolution of the separation can easily be maximized. Due to the fact that the flow of mobile phase is generated inside the capillary itself and the size of it is just a function of the applied voltage, the column can be elongated irrespective of the instruments conditions. This because back pressure does not cause an issue in CEC.

Despite these huge advantages, CEC is still a very experimental technique, not used in routine analysis. The reason for this is mainly the lack of reproducible columns.



Figure 2.13: Illustration of the flow profile in electro driven (A) and pressure driven (B) systems. (taken from reference 108)

As of late new CEC instruments were introduced into the market and it is expected that new column technologies will be investigated thoroughly. Currently two major types of capillary columns are available, namely packed and monolithic capillaries. The quality of packed columns is strongly dependent on the quality of the frits. These are short aggregations of immobilized porous material that have to keep the packed bed in place. Obviously they have to be mechanically strong enough to resist the large packing pressure (usually 600 bar). They are made from sintered silica and are easy to make but the process is irreproducible. Variations in the frits lead to changes in EOF, which in turns lead to weak column to column reproducibility.

The second type of columns are the monolithic columns which comprise a network of interconnected silica material (see figure 2.8). The formation of the porous structure cannot be controlled on the pore size level, therefore no two monoliths are the same which again leads to column to column irreproducibility. When their efficiency is compared to packed columns of the same length it becomes immediately clear that this is a huge issue for the monoliths as their efficiency is much lower than that of packed columns.

There is a third type of capillary column, namely the open tubular column. No reports are available on the use of open tubular columns for CEC. However, in nano-LC, Karger et al. coated a 10 μ m I.D. capillary with a porous poly(styrene-divenylbenzene) layer to perform trace level analysis in proteomics. The column was 5 m long and and coated with a 1 μ m thick layer. They separated 4500 peptides with a gradient. However the maximum loading capacity was not more than 50 to 100 fmol.¹⁰⁹

2.4 Ordered porosity in chromatography

The previous sections revealed some deficiencies in liquid chromatography, such as the difficulty of separating polar compounds, the stability of the packings and the problematic phase ratio in OTLC. A solution for these deficiencies can be found in the application of ordered mesoporous materials. Their uniform pore size and greatly increased surface area can lead to an improved retention and phase ratio. This can mean they provide a solution for both OTLC systems as for the separation of highly polar compound with standard reversed phase HPLC. Below is an overview of all the literature on the use of ordered mesoporous materials as a stationary phase in liquid chromatography.

2.4.1 Normal phase HPLC

The first analyte analysis by mesoporous particles was performed under normal phase conditions. The group of Unger et al. compared MCM-41 particles with commercial alumina, titania, zirconia and Lichrosphere Si 100 in NPLC.¹¹⁰ The various stationary phases were tested for the separation of several bases with an n-heptane/2-propanol mixture (99.5/0.5; v/v). The particle sizes were 5 µm for the Lichrospher, alumina and titania materials and $10 \ \mu m$ for the zirconia material. The MCM-41 material was hydrothermally synthesized and ground to obtain particles in a size range between 5 and 10 μ m. This means that these particles are non-spherical and irregularly shaped. Although no SEM pictures were provided, this was concluded from the text. In the article a separation of the same analytes was performed on the MCM-41 column and on the Lichrospher and it was concluded that the latter was performing better. However, the columns had a different length which makes a comparison nearly impossible. Additionally the particle size was different and the MCM material was not spherical. Therefore the different materials could not be compared and the effect of the ordered porosity or the larger surface area could not be thoroughly assessed.

MSU-1 particles (made with a Tergitol 15S12 PEO surfactant) were compared to a commercial Nucleosil material by Boissière et al. for normal phase HPLC. ¹¹¹ The Nucleosil and the MSU-1 particles were packed in 25 cm columns. The chromatographic evaluation was performed under isocratic conditions (dichloromethane/n-hexane; 2/98; v/v) at a flow rate of 1.5 mL/min. It was reported that the retention factors were 4.2 to 6.7 times higher for the MSU material and the plate numbers were 1.6 to 2.1 times higher for the Nucleosil particles. The lower plate numbers were attributed to a larger longitudinal diffusion through the particles but this did not seem to be the only reason. Possibly the broad particle size distribution played a major role in this. The higher retention times were mostly attributed to the larger surface area.

The authors compared the MSU results with the results obtained by Gallis et al.¹¹² As both the MSU and the MCM-41 material (depicted as APMS in figure 2.14) exhibited substantial peak broadening while the MCM-48 and the nucleosil packing did not. Therefore they concluded that in order to obtain sharp elution



Figure 2.14: Left: A SEM micrograph depicting the irregular MSU particles. Top right: A table showing the pore ordering and connectivity for four types of tested materials, MCM-48 and APMS were previously reported by Gallis et al¹¹². Bottom right: The separation of benzene, naphthalene and biphenyl on a Nucleosil column (dashed line) and an MSU column. (taken from reference¹¹¹)



Figure 2.15: Left: SEM images of spherical particles of a) the sph-PMO and b) Nucleosil 50-10. Right: Chromatograms of mixture 1 (benzene (1), naphthalene (2), biphenyl (3), and phenanthrene (4)) separated with n-hexane at a flow rate of 1 mL/min on the Nucleosil 50-10 column or at a flow rate of 2 mL/min on the sph-PMO column. (taken from reference ¹¹³)

peaks there had to be a strong interconnectivity in the pore system. This meant that, according to the authors, cubically ordered systems are the better option for chromatography. In my opinion however, as the column dimensions, particle sizes and chromatographic conditions were different for every type of column, no conclusion other than a higher retention for the mesoporous materials can be drawn from this article. Again the effect of the pore structure cannot be thoroughly assessed.

The first PMO material to be used as a packing in chromatography was reported in 2006 by the group of Fröba.¹¹³ A benzene PMO material (poly- $O_3Si-C_6H_6-SiO_3$) was used without further functionalization and due to the high organic content of the material, both normal phase and reversed phase separations could be conducted on the column, without subsequent functionalization. The benzene PMO material was compared with a commercial Nucleosil 50-10 (5 nm pores, 10 µm particles). Both materials were slurry packed under the same conditions in 25 cm, 4 mm I.D. stainless steel columns. Both columns were chromatographically evaluated under isocratic conditions with n-hexane as eluent. Figure 2.15 shows the SEM micrographs of both the PMO and the Nucleosil material together with the chromatograms of the separation of polyaromatic hydrocarbons. From the chromatograms it is clear that the PMO material exhibits a higher retention than the nucleosil particles.



Figure 2.16: Left: H vs u curves for diethylphtalate and for dibutylphtalate for an adapted composition of the mobile phase allowing to reach similar retention factors for Lichrospher 60 (filled square) and its pseudomorphs MCM-48 (star), MCM-41 (circle), large pore MCM-41 (MTS-TMB) (empty square) and (MTS-TMB/decane) (triangle). **Right**: Evolution of the C parameter of the H vs u equation as a function of the pore diameter. (taken from reference¹¹⁴)

In the same year Galarneau et al reported a comparison between normal MCM-41, MCM-48 and pore expanded MCM-41.¹¹⁴ All the materials were designed by the pseudomorphism syntheses previously reported by the same group. Lichrospher 60 was used as the base material for the pseudomorphic synthesis. It has a particle size of 10 µm, which was retained during the conversion to MCM-41, MCM-48 and two types of large pore MCM-41 (MTS-TMB and MTS-TMB/decane). All the materials were slurry packed into 12.5 cm columns with an internal diameter of 4 mm. With this comparison the authors claimed to have proven that the difference in mass transfer could be attributed to the pore size and pore structure. Lower C values were observed for MCM-48 in comparison with MCM-41 because of the higher connectivity in the mesopore structure for MCM-48. Additionally lower C values were also observed for the large pore MCM-41 materials when compared to the small pore MCM-41 particles (see figure 2.16). Since no chromatograms were presented and the van Deemter curves seem to be incomplete it is hard to comply with the authors on these claims.

This concludes the literary overview of ordered mesoporous materials used in NPLC. From these reported findings the only thing that can be concluded is that higher surface areas induce a higher retention than the classical packing materials. Due to a broad particle size distribution and irregularly shaped particles, the efficiency of the made columns remains below average. The following section gives an overview of the ordered mesoporous materials used in reversed phase HPLC.

2.4.2 Reversed phase HPLC



Figure 2.17: **Left**: A SEM micrograph depicting the irregular particles. **Right**: The separation of uracil (a), benzene (b), naphthalene (c) and biphenyl (d) on a commercial Hypersil-C8 (top) and a C8 modified MCM-41 column (bottom). (taken from reference¹¹²)

The first report on improved retention in RPLC which could be obtained by the large accessible surface area in these porous materials was published in 1999 by Gallis et al.¹¹² However, the separation was poor in terms of plates and resolution because of the non spherical particle shape and because of the broad particle size distribution (see figure 2.17). The ordered mesoporous material was an MCM-41 synthesized hydrothermally under acidic conditions. The material exhibited a surface area of 1186 m^2g^{-1} , a pore diameter of 2.4 nm and a particle size between 4 and 10 µm. This material was fitted with an octyldimethyl group to obtain a reversed phase stationary phase. The material was compared with a commercial Hypersil-C8 column for the separation of uracil, benzene, naphthalene and biphenyl. The separation was performed under isocratic conditions (H₂O/MeOH; 35/65; v/v) at a flow rate of 1 mL/min and a column temperature of 30°C. The MCM-41 type column exhibits a higher retention, but the efficiency is very poor. This can be attributed to the broad particle size distribution.



Figure 2.18: Left: Chromatogram of four small biomolecules with a C18-SBA-15 column. The inset shows a separation on a commercial C18 column. The four peaks represent Cys, GSH, 6-TP and DA, respectively, from left to right. Mobile phase: 0.1 M phosphorate buffer (pH = 3). Right: Chromatogram of myoglobin peptides and four proteins with a C18-SBA-15 2 mm I.D. steel column; mobile phase: H₂O (0.1% trifluoroacetic acid, TFA) and acetonitrile (0.1% TFA). (a) Peptide separation gradient from 5-80 min with acetonitrile (0.1% TFA) changed from 0 to 80%; (b) protein separation. (taken from reference¹¹⁵)

In 2003 the first report of an SBA-15 type material being applied as packing material was published.¹¹⁵ The SBA-15 material was functionalized with dimethyloc-tadecylchlorosilane to obtain a C18 modified SBA-15 with a surface area of 800 m^2g^{-1} , a pore volume of 1.05 cm^3g^{-1} and 8.1 nm pores. Although no SEM mi-

crographs were shown, the materials were claimed to exhibit a non-spherical but uniform particle size. They were slurry packed in a 10 cm, 250 μ m I.D. capillary column. On this column four biomolecules (cystein (CYS), glutathione (GSH), 6thiopurine (6-TP) and dopamine (DA)) were evaluated using a 0.1M phosphorate buffer (pH = 3), no flow rate was given. This analysis is depicted by the chromatograms on the left in figure 2.18. It was compared to a commercial column (inset). Since no separation conditions were provided for this commercial column, no discernible conclusions can be drawn from the comparison. Nevertheless, the authors claim to observe a clear improvement in the retention time and the separation efficiency. In my opinion, the only conclusion that can be drawn here is that the four biomolecules are successfully separated by the C18-SBA-15 material.

Figure 2.18 provides two more chromatograms on the right. These depict a triptic digest of myoglobin (a) and the separation of four proteins (b) on C18-SBA-15. For this the SBA-15 was slurry packed in a 2 mm I.D. steel column (no column length was specified). The chromatogram of the four proteins (lysozyme (1), bovine serum albumin (BSA) (2), myoglobin (3) and ovalbumin (4)) shows six peaks of which the first shoulder peak (labeled *) is the hemo-group dissociated from myoglobin. The last peak was designated to be an impurity from the ovalbumin sample. What this separation shows is that fairly large molecules (entire proteins) are retained on a column with a pore diameter of 8.1 nm, however without a death time marker it is impossible to state whether the molecules enter the pores or not.



Figure 2.19: Left: SEM image of functionalized mesoporous silica spheres C18-SBA-15. Scale bar: 5 µm. Right: Chromatograms of three aromatic molecules (a) and three proteins (b) with a C18-SBA-15 column. Peaks 1-3 in (a) correspond to naphthalene (1), biphenyl (2), and phenanthrene (3), whereas the three peaks in (b) correspond to human serum albumin (HSA), lysozyme (LYS), and bovine serum albumin (BSA), respectively. (taken from reference⁵⁶)

In the same year two other groups reported roughly the same but added a separation of small aromatic compounds and polyaromatic hydrocarbons.^{56,116} Ma et al. reported the synthesis of spherical C18-SBA-15 with a surface area of 522 m^2g^{-1} , a pore volume of 1.06 cm^3g^{-1} and 6.1 nm pores. The particles consisted of coagulated spheres as can be seen from the SEM micrograph in figure 2.19. The particles were slurry packed in a 5 cm stainless steel column with a 4.6 mm internal diameter. The chromatograms on the right depict a separation of naphthalene, biphenyl and phenantrene (a) under isocratic conditions at 0.5 mL/min with a water/acetonitrile mixture (30/70; v/v). The second chromatogram (b) shows the separation of three proteins: lysozyme, human serum albumin (HSA) and bovine serum albumin (BSA). This separation was also performed under isocratic conditions at a flow rate of 0.5 mL/min with water (0.1% TFA)/acetonitrile (0.1% TFA) as the eluent (composition not provided).

Mesa et al. reported the the synthesis of C18 functionalized SBA-15 with a surface area of 811 m^2g^{-1} , a pore volume of 0.91 cm^3g^{-1} and 7.6 nm pores. The material was slurry packed in a 15 cm column with a 4.6 mm internal diameter. The column was evaluated for the separation of a mixture of phenol, benzene, toluene, naphthalene, anthracene and benzopyreen (figure 2.20).



Figure 2.20: Left: SEM micrograph of the packed C18-SBA-15 material. Right: Reverse-phase chromatogram from a column packed with C18-SBA-15 material. Solute: a mixture comprising (a) phenol, (b) benzene, (c) toluene, (d) naphthalene, (e) anthracene, (f) benzopyrene. Conditions of elution: 1 mL/min with methanol/water (70%/30%; v/v) during 4 min; linear increase from 1 to 1.5 mL/min in 2 min with methanol/water (90%/10%; v/v); 1.5 mL/min with methanol/water (90%/10%; v/v) to the end. (taken from reference¹¹⁶)

The next year an MCM-41 type material produced from commercial Nucleosil 100 (Nu100MCM-41, 5µm) and LiChrospher 100 (LiC-MCM-4,15µm) spheres via morphosynthesis was used in reversed phase HPLC after grafting with an octyl chain.⁶⁶ Nu100MCM-41 exhibited a surface area of 913 $m^2 g^{-1}$, a pore volume of $0.79 \ cm^3 g^{-1}$ and a pore diameter of 3.9 nm, while LiC-MCM-41 had a surface area of 911 m^2g^{-1} , a pore volume of 0.78 cm^3g^{-1} and a pore diameter of 3.8 nm. The materials were slurry packed in stainless steel columns of 5 cm with an internal diameter of 4 mm. A convential silica-based stationary phase was compared to the MCM-41 materials, a ZorbaxSBD-C8 with 5 μ m particles in a 15 cm 4.6 mm I.D. column. For the first time van Deemter curves were provided and showed an optimal plate height of 45 µm for the LiChrosphere MCM-41 column (see figure 2.21A) and a plate height of 12 µm for the Nucleosil 100 MCM-41 material (reported in the article). The optimal linear velocities were 0.04 cm/s and 0.10cm/s respectively. The MCM-41 stationary phases were reported to have very low mass transfer coefficients, much like monolithic columns which was attributed to the uniform pore system. Additionally their retention was much higher than the non-ordered commercial materials (see figure 2.21B).



Figure 2.21: A: Experimental plate height H values versus linear velocity u for the LiC-MCM-41 phase on propylbenzene with water/methanol (30/70; v/v) as mobile phase. B: Log(k) versus the number of carbons in the alkylchain of alkylbenzenes on the Nucleosil Nu100MCM-41 phase in water/methanol (30/70; v/v) (full triangle), in water/acetonitrile (40/60; v/v) (hollow triangle) and on a ZorbaxSBD-C8 in water/methanol (30/70; v/v) (full square) and in water/acetonitrile (40/60; v/v) (hollow square) at 30°C. (taken from reference⁶⁶)

In 2008 Yang et al reported the synthesis of spherical SBA-16 particles with a surface area of 632 m^2g^{-1} , a pore volume of 0.70 cm^3g^{-1} and a pore diameter of 8.0 nm. They were used as packing material for the reversed phase separation of aromatic molecules.¹¹⁷ The materials were functionalized with a C8 chain and slurry packed in a 15 cm stainless steel column with an internal diameter of 4.6 mm. The column was evaluated isocratically for the separation of a mixture of aromatic hydrocarbons (benzene, naphthalene, phenanthrene and pyrene). The eluent was methanol/water (65/35; v/v). The maximum obtained plates were 2250 of the theoretical 12500. Again this could probably be attributed to the broad size distribution of the particles (see figure 2.22).

Zhang et al reported the synthesis of highly uniform para and meta oriented phenylene-bridged PMO spheres.¹¹⁸ The materials were slurry packed into 50 x 2.1 mm I.D. stainless steel columns without modification. The phenylene-bridges were the stationary phase for RPLC. The columns were both evaluated for a mixture of 11 compounds under the same gradient conditions. Figure 2.23 shows uniform spherical particles of 2 µm for both the meta and para oriented organosilicas. The synthesis procedure was based on a modified Stöber method. The materials itself have a pore size of 5.2 and 4.3 nm, a surface area of 550 and 750 m^2/g and 0.72 and 0.63 cm^3/g respectively for the para and meta material. The meta oriented material showed more retention for the same analysis. This could be attributed to

the fact that a para oriented organosilane has its functional phenylene group inside the walls whilst the meta oriented phenylene groups tend to stick out, improving the interaction with the analytes.



Figure 2.22: Left: SEM imaging of the packed materials. Right: Chromatogram showing the separation of benzene (a), naphthalene (b), phenanthrene (c) and pyrene (d) using the material on the left as a packing material with a mobile phase (methanol/water; 65/35; v/v) flow rate of 1 ml/min (column dimensions: 15 cm x 4.6 mm I.D.). (taken from reference¹¹⁷)



Figure 2.23: Left: SEM imaging of the packed materials, scale bar = 5 µm. Right: Chromatograms for a mixture of 11 compounds with the (a) p-PHS column (50 mm x 2.1 mm I.D.) and (b) m-PHS column (50 mm x 2.1 mm I.D.). Gradient: 0-4 min, 5%-60% B; 4-5 min, 60% B; mobile phase: A: Water B: acetonitrile; flow rate: 0.5 mL/min; 55°C. Analytes: (1) uracil; (2) cytidine; (3) phenol; (4) acetanilide; (5) benzene; (6) acetophenone; (7) (4-methoxyphenyl)ethanone; (8) 1,2-dinitrobenzene; (9) diphenyl ketone; (10) naphthalene; (11) phenanthrene. (taken from reference¹¹⁸)

In 2009 the same group reported the same phenylene-bridged material but with expanded pores and a C-18 chain grafted to the surface, designated as PHS-C18.¹¹⁹ The pore size of the material was 8.5 nm with a surface area of $354 m^2/g$ and a pore volume of 0.63 cm^3/g . This resulted in uniform particles with a particle size of 2 µm and subsequently in adequate separations with a plate height of 10 µm, the best reported so far. Additionally the retention stability of this material in comparison with the pure silica (S-C18) was reported (see figure 2.24). The columns were exposed to a 50 mM triethylamine (TEA) solution (pH 10) for 60 minutes (0.2 mL/min, 50) and then purged with water and acetonitrile (0.2 mL/min, 50°C) for 10 minutes each. Phenanthrene was used as a probe molecule to test the efficiency after every run (flow rate: 0.2 mL/min, isocratic; acetonitrile/water, 60/40, v/v; 50°C). The authors concluded that the phenyl group reduced bleeding of the C-18 stationary phase into the eluent whilst the retention of the compared pure silica dropped significantly as a function of time.



Figure 2.24: Left: Plot of theoretical plate height (H) vs linear velocity. Column: PHS-C18 (2.1 mm x 50 mm); mobile phase: acetonitrile/water (55/45; v/v). 30°C. Test probe: phenanthrene. **Right**: Loss of retention for columns PHS-C18, PHS, and S-C18 as a function of purge time of 50 mM TEA solution: k' measured for phenanthrene. (taken from reference¹¹⁹)

In 2011 Li et al. reported the synthesis of a co-condensed organosilane with spherical morphology with both an aminopropyl silane and an ethane bridged silane.¹²⁰ The same aging procedure as described by Zhang et al.¹¹⁹ was used, but now anthracene was used as a probe molecule. Due to the fact that the aminopropyl function was incorporated into the wall it did not wash out as easily as the grafted aminopropyl material. Here the resistance to leaching of the function was ascribed to the ethane bridge which makes the silica structure more hydrophobic and thus less susceptible to hydrolysis.

2.4.3 Size exclusion chromatography

The pore size of ordered periodic mesoporous materials is very narrowly defined and their pore size can be tuned within the size range of 2 to 50 nm. Due to this uniform pore size distribution it can be imagined that the family of ordered
periodic materials could offer some benefits in size exclusion chromatography. The narrow pore size imposes a narrow size cut-off in an analysis of e.g. a polymer mixture. At the moment such narrow pore size is not available. With a column consisting of known ratios of different pore sizes, mixtures of polymers could be separated in one chromatogram.

The group of Unger et al. used MCM41 particles for the first time in a chromatographic application in 1996.¹²¹ The columns (25 cm x 8 mm I.D.) were packed with either membranes or non-spherical MCM-41 particles and tested for their ability to perform size exclusion chromatography. The MCM-41 materials performed rather poorly because two pore systems were observed, the primary pore system being the uniform pores through the particle (4.3 nm) and the secondary being the inter-particle porosity (see figure 2.25A). Therefore the polystyrene standards were roughly separated into two batches who partially overlapped each other. The main reason this did not work well was probably due to the fact the particles were not spherical and secondly not uniform causing irregularities in the flow pattern through the columns, leading to peak broadening.



Figure 2.25: A: The SEC curve of an MCM-41 packing by the group of Unger et al.¹²¹; **B** The calibration curve for the separation of polystyrene standards on the Nucleosil column, on the APMS column (**C**, **top**) and the MCM-41 column (**C**, **bottom**). The total permeation (1), the size exclusion (2) and the total exclusion (3) zones are designated. (B and C were taken from reference¹²²)

The first proof of a sharper cut-off in size exclusion chromatography was reported by Nassivera et al. in 2002. ¹²² The main reason for this can be found in more uniform particle packings. Two types of MCM-41 materials (designated as APMS-30 and MCM-41) were prepared according to previously reported methods. ^{112,123} The APMS-30 (SA: 1130 m^2g^{-1} , Vp: 0.6 cm^3g^{-1} , Dp: 2.9 \pm 1 nm, 4-10 µm particles) and the MCM-41 (SA: 1260 m^2g^{-1} , Vp: 1.0 cm^3g^{-1} , Dp: 3.4 \pm 0.5 nm, 1-50 µm particles) were compared with a commercial Nucleosil-50 (SA: 369 m^2g^{-1} , Vp: 0.8 cm^3g^{-1} , Dp: 11.6 \pm 11 nm, 4-6 µm particles) for the size exclusion separation of polystyrene standards of various molecular weight. The materials were packed into 20 cm stainless steel columns with a 4.6 mm internal diameter. The size exclusion experiment was performed at a flow rate of 1 mL/min with tetrahydrofuran (THF) as eluent. Figure 2.25 C shows the SEC curve for the APMS packing with a uniform pore diameter of 2.9 nm and the MCM-41 packing with a uniform pore diameter of 3.3 nm. It shows that there is no total permeation limit for both mesoporous columns for which no apparent explanation is given by the authors.

To further update the range of ordered mesoporous size exclusion stationary phases Han et al. published the use of ultra large pore ordered mesoporous (MCFs or mesocellular foams) packings with uniform pore diameters varying from 20-50 nm.¹²⁴ Three MCF materials were synthesized, designated as MCF-26 ($d_p = 50$ nm), MCF-17 ($d_p = nm$) and MCF-10 ($d_p = 26$ nm). They were slurry packed into 25 cm stainless steel columns with an internal diameter of 4.6 mm.



Figure 2.26: (a,b) TEM micrographs and (c,d) N_2 -sorption isotherms of (a,c) conventional SEC silica packing material used in an Agilent Zorbax PSM 300 column and (b,d) the MCF-26 column. (taken from reference¹²⁴)

Figure 2.26 depicts the difference in pore structure between a commercial Zorbax column and the synthesized mesocellular foams.

The packed materials were evaluated for the size exclusion separation of polystyrene standards with molecular weights ranging from 104 up to 770000 Da. The mobile phase was THF and the flowrate was 1.0 mL/min (see figure 2.27). The materials were compared to a commercial size exclusion column (Zorbax PSM 300) with a

similar molecular weight range as the one of the tested columns (50 nm pores). The MCF column depicted a larger selectivity, which was attributed to the higher pore volume of the material. The column efficiency of the MFC column was lower (9715 plates vs 11210 plates) than the commercial columns which was, by the authors, attributed to a broader particle size distribution.



Figure 2.27: The separation of polystyrene standards by 3 different MCF columns (a = MCF-26, b = MCF-17, c= MCF-10) and the Zorbax PSM 300 column (d). (taken from reference¹²⁴)

Bayram-Hahn reported the pseudomorphical transformation of commercial particles (Lichrospher 60) to ordered mesoporous (MTS) particles.¹²⁵ The original Lichropsher 60 base material had a surface area of 769 m^2g^{-1} , a pore volume of $0.9 \ cm^3g^{-1}$ and a pore size of 4.5 ± 1.3 nm. The pseudomorphic material exhibited a surface area of $1042 \ m^2g^{-1}$, a pore volume of $2.2 \ cm^3g^{-1}$ and a pore size of 5.4 \pm 0.6 nm. The MTS material clearly has a larger pore volume, surface area and a more narrow pore size distribution. The materials were slurry packed into 12.5 cm columns with a 4 mm I.D. before their evaluation for size exclusion chromatography. The SE separation was performed on polystyrene standards with a molecular weight ranging from 162 to 23300000 Da. The flow rate was 0.2 mL/min with THF as the eluent. Due to the fact that the original particles have a uniform particle size distribution, the column efficiency for both types of columns is the same but the size exclusion efficiency is improved.



Figure 2.28: The SEC curves and the H versus u plots for both the original Lichrospher 60 and the pseudomorphic column. (taken from reference 125)

SEC columns seem to exhibit a sharper molecular weight cut-off than the traditional SEC materials. This delivers a cleaner cut in the molecular size distribution of the mixtures but also means that no more than two sizes of molecules can be separated without an array of different pore sizes. This could be a drawback of ordered mesoporous materials as a packing material for SEC.

2.4.4 Chiral HPLC

The first report of chiral HPLC performed on MCM-41 modified with a chiral selector was published by the fellow Belgian group of Pierre Jacobs.^{126,127} The particles were synthesized through a modified Stöber method which resulted in a broad particle size distribution. The material was functionalized with γ -aminopropyltriethoxy-silane and (R)- α -(1-naphtyl)ethylamine as a chiral selector. A commercial Lichrosorb-NH₂ was also fitted with the chiral selector and both materials were slurry packed in 10 cm columns (no I.D. was provided). A chiral separation of dinitrobenzoyl naphtylethylamine (DNB-NEA) was performed with n-hexane/1,2-dichloroethane/ethanol (40/12/3) at a flow rate of 0.6 mL/min. The MCM-41 material exhibited a substantially higher retention and also the calculated resolution was higher for the MCM-41 material ($R_S = 2.6$ vs 3.6). The chromatogram for the MCM-41 material is depicted in figure 2.29, no chromatogram for the commercial material was provided. The poor chromatographic efficiency was attributed to the broad particle size distribution.



Figure 2.29: Left: SEM micrograph of the packed chiral material. Right: Chromatogram for the resolution of DNB-NEA on a column, packed with an MCM-41 material with (R)- α -(1-naphtyl)ethylamine as the chiral selector. Conditions: column 10 cm; mobile phase: n-hexane/1,2-dichloroethane/ethanol (40/12/3) at a flow rate of 0.6 mL/min. (taken from references^{126,127})



Figure 2.30: Left: SEM micrograph of the packed chiral material. Right: Chromatograms for the resolution of DL-valine on columns packed with (a) trans-(1R,2R)-diaminocyclohexane-Kromasil and (b) EM30-4 Conditions: column 100 x 4.6mm I.D.; mobile phase: methanol/0.1mM Cu(OAc)₂ and 50mM KH₂PO₄ aqueous solution = 10/90 (v/v); flow rate: 0.8 mL/min; detection: 254 nm UV; temperature: 25 °C. (taken from reference¹²⁸)

In 2007 Zhu et al. reported a comparison between a grafted commercial material and a co-condensed organosilica for the separation of chiral groups.¹²⁸ The used chiral selector was trans-(1R,2R)-diaminocyclohexane and was grafted on commercial Kromasil material. The co-condensed organosilica was synthesized from a mixture of an ethane bridged silane and N-[3-(triethoxysily1)propy1]-trans-(1R,2R)diaminocyclohexane. This way the loading of the chiral group was greatly enhanced resulting in a superior resolution when compared to the grafted material. The cocondensed organosilica had a surface area of 829 m^2g^{-1} , a pore volume of 0.6 cm^3g^{-1} and a pore size of 2.8 nm. The mesoporous column clearly exhibits a larger retention for the racemates, but the column efficiency was poor. This poor efficiency was attributed to a broad particle size distribution.

2.4.5 Ordered porosity in CEC

In 2006 the first and only report on ordered mesoporous particles used for CEC was published.¹²⁹ Both bare and C-18 functionalized submicron SBA-15 particles were slurry packed into a capillary and used to separate analytes under normal and reversed phase conditions respectively. The SBA-15 material exhibited a surface area of $639 \ m^2 g^{-1}$, a pore volume of $1.39 \ cm^3 g^{-1}$, a pore size of 12 nm and a mean particle size of 0.4 µm. Plate heights of 2 µm were reported but on very few van Deemter points. Figure 2.31 depicts a van Deemter curve for thiourea and a chromatogram of 6 aromatic compounds. Additionally the particles were reported sphere-like instead of spherical, which was proven by SEM images.



Figure 2.31: Left: Plot of the plate height versus the linear velocity for thiourea on a pure SBA-15 column; conditions: capillary column, 8.5/33 cm 50 µm ID; mobile phase, 10 mM Tris-HCl (pH 8.1) containing 95% ACN; applied voltage, from 5 to 30 kV; injection, 2 kV for 5 s (thiourea). **Right**: Separation of aromatic compounds on the C18 modified SBA-15 silica packed column. Experimental conditions: capillary column, 8.5/33 cm 50 µm I.D.; mobile phase, 10 mM Tris-HCl (pH 8.1) containing 80% ACN; separation voltage, 30 kV; injection, 2 kV for 5 s. Solutes: (1) thiourea; (2) aniline; (3) benzene; (4) toluene; (5) dimethylbenzene; (6) 1,3,5-trimethylbenzene; (7) 1,2,4,5-tetramethylbenzene. (Taken from reference¹²⁹)

2.5 Conclusions and Aim

Ordered mesoporous materials have been widely tested for their chromatographic properties and as can be deducted from the literary conducted above, a lot of improvement still is possible. Some early publications mention an apparent increase of retention factors when ordered mesoporous spheres are applied. This could be mainly attributed to an increase in the surface area. The aim of this dissertation is to investigate if substantial improvement can be reached by using ordered mesoporous organosilica materials as a packing material for HPLC and OTCEC. The particle synthesis will be statistically designed from an industrial point of view and new organosilanes will be tried. To accomplish this a spray drying technique will be used, mainly because of its easy scale up properties.

In a smaller second part the improved retention will be applied for open tubular capillary electro chromatography. By coating a layer of ordered mesoporous material on the inside of fused silica capillaries these columns are believed to gain enough retention to make OTCEC possible.

3

Quantification of silanol sites for the most common mesoporous ordered silicas and organosilicas: total versus accessible silanols.

3.1 Introduction

The silanol number (expressed as the number of silanol groups per nm^2) is a topic of interest and discussion since the 1950s until today. A good knowledge of the number of silanols on the silica surface is important in areas such as catalysis and adsorption. Also in emerging disciplines, such as drug delivery, tissue engineering by silica and organosilica scaffolds, thin film technology, etc., the exact knowledge of the silanol number is needed or at least desirable. More specifically, in the field of chromatography, the silanol groups are of major importance for two reasons. In the first place, they are the anchoring point for the stationary phase functional groups. In this way, they have a direct influence on the retention mechanism of a chromatographic packing material. Secondly, once the functional groups are grafted on the silica surface, it is important that the residual silanol groups are removed from the system by an endcapping with a trimethylsilylgroup. Remaining silanols typically cause silanol tailing on the bases of the peaks of the chromatogram in the analyte mixture. On the other hand, in capillary electro chromatography (CEC) the remaining silanol groups help to create the electro osmotic flow, which drives the separation. 130

The determination of the silanol number is complicated by the easy adsorption of water on silica. Therefore the exact quantification with MAS NMR, FTIR, selective chemisorption, deuterium exchange and mass spectroscopy is always complicated by the need for a very controlled atmosphere during sample treatment and measurement. Many models appeared in the 1970s and 80s on the determination of the silanol number of silica gels or fumed silicas.^{131–133} For silica gels, many different datasets on the silanol number are available, with a large number originating from the former USSR. The silanol number is often referred to as the Zhuravlev number, in honor of his pioneering work in the field.^{134,135} In 1995, Van Der Voort et al. published an overview of the silanol number of silica gels as a function of temperature with a specification of the number of isolated, geminal and vicinal silanols.¹⁰³ However for the mesoporous ordered (organo)silica materials, prepared by anionic, cationic or neutral templates at a wide range of pHs and temperatures, this table is no longer applicable.

Several standard methods have been used to characterize and quantify the density of silanol groups of ordered mesoporous silica materials. Thermogravimetric analyses¹³⁶, diffuse reflectance fourier transform infrared spectroscopy (DRIFT), ^{137,138}²⁹Si and ¹H NMR, ^{139,140} microcalometry¹⁴¹ or a combination of these techniques. These were even combined with the use of probing molecules such as silanes, ^{142–144} water^{145–147} or pyridine¹⁴⁸ adsorption. In most cases MCM-41 was analyzed, not always unambiguously due the fact that each paper reports a different silanol number for MCM-41 without considering synthesis and analysis parameters^{149–154}. For example the silanol number is strongly dependent on the temperature of any post synthesis treatment.¹⁰³ However in six different reports where MCM-41 was post treated at the same temperature, silanol values varying from 0.9 up to 3 groups per nm^2 were found.^{136,148,155–158} After a thorough search of literature no systematic determination of the silanol number of periodic mesoporous organosilicas could be found.

Therefore in this chapter, the accessible surface silanols for various chromatographic relevant functional groups together with the total amount of silanol groups, whether they are on the surface or not are reported. This information will be useful for many colleagues in various fields of research and for the chromatographic colleagues in particular. The silanol numbers have been quantified using previously described IR techniques¹⁵⁹ combined with solid state NMR and elemental analysis of grafting procedures.

3.2 Experimental

3.2.1 Synthesis of the supports

SBA-15¹⁶⁰, SBA-16¹⁶¹, MCM-41¹⁶², MCM-48¹⁶³, spray dried MCM-41¹⁶⁴ and ethene PMO¹⁶⁵ were all prepared according to methods found in literature. All syntheses were carried out under hydrothermal conditions, except for a spray dried MCM-41 type material, denoted as MCM-41 (SD). Two types of pore systems were synthesized, a hexagonal and a cubical pore system. MCM-41, MCM-41(SD), SBA-15 and the ethene bridged PMO have a hexagonal pore system. The MCM-48 and the SBA-16 both exhibit a cubical pore system. Nucleosil 300-5 is commonly used as a packing material in chromatographic HPLC applications. MCM-41, MCM-

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41(SD), MCM-48, SBA-15 and SBA-16 were calcined at 550°C for 6 hours with a heating rate of 2°C/min to remove the surfactant. The surfactant of the ethene bridged PMO was removed by a soxhlet extraction with acetone at 120°C.³⁴ After the synthesis all the samples were kept dry and under inert atmosphere.

3.2.2 Grafting procedures

Prior to grafting water was removed from the samples by a heating procedure at 120°C under vacuum conditions for 24 hours. Three different silanes with increasing tail length were used as probing molecules to determine the amount of accessible silanol sites on the surface of the different silica materials. A trimethylsilyl (C3), an octyldimethylsilyl (C8) and an octadecyldimethylsilyl (C18) group were anchored to the surface. The reaction time was 24h for all the grafting reactions and the composition of the reaction mixture was 0.5 g silica/1.4 mmol silane/30 mL solvent. The trimethylsilyl and the octyldimethylsylil group were grafted in dry acetonitrile by means of hexamethyldisilazane (HMDS) and octyldimethylchlorosilane respectively, and left to stir for 24 hours at room temperature. The octadecyldimethylsilyl group was grafted by means of octadecyldimethylchlorosilane in dry toluene under the same conditions as cited above. The reaction scheme is depicted below in figure 3.4. After reaction the materials were filtered off, washed thoroughly with acetonitrile or toluene and acetone, dried at 120°C under vacuum and analyzed with elemental analysis.



Figure 3.1: The reaction schemes depicting the reaction of the silica surface with HMDS (A) and the chlorosilanes (B).

3.2.3 Characterization

Nitrogen sorption experiments were measured at 77K using a Belsorp mini II gas analyzer. X- ray powder diffraction (XRPD) patterns were collected on an ARL X'TRA X-ray diffractometer with $CuK\alpha$ radiation of 0.15418 nm wavelength and a solid-state detector. Elemental analyses (C, H, N) were performed on a Thermo

Scientific Flash 2000 CHNS-O Analyzer.

Solid-state MAS NMR spectra were recorded on a Bruker Avance-400 (9.4T) spectrometer using 4 mm-OD zirconia rotors and a spinning frequency of 12 kHz. Single pulse excitation (30° flip angle) and 20 s recycling delay was used for ²⁹Si MAS NMR experiments. (¹H)-²⁹Si cross-polarization (CP) MAS experiments were performed using a contact time of 6 ms and a recycle time of 3 s. Tetramethylsilane (TMS) was used as chemical shift reference for the ²⁹Si nuclei.

FTIR-analysis with an in-situ IR-cell

For the determination of the silanol number, a special transmission FTIR setup was used. The silica powders were pressed (10^7 Pa) into self-supported discs ($2 cm^2$ area, 7-8 mg/cm^2). They were placed in a cell equipped with KBr windows. A movable quartz sample holder allowed placing the pellet in the infrared beam, for recording spectra, and moving it into a furnace at the top of the cell for thermal treatment (see figure 3.2).

A Nicolet 6700 IR spectrometer equipped with a DTGS detector and an extended-KBr beam splitter was used for the acquisition of spectra recorded in the 400-5500 cm^{-1} range. Spectra were recorded with a resolution of 4 cm^{-1} and 250 scans were co-added for each spectrum.



Figure 3.2: The In-Situ IR-cell used to determine the silanol number of the materials.

3.3 Results and discussion

In order to simplify the discussion on the effect of the synthesis method on the material properties, the main differences between synthesis conditions and template removal are summarized in table 3.1.

Material	Synthesis cond	Template Removal	
SBA-15	Hydrothermal	Acid	Calcination
SBA-16	Hydrothermal	Acid	Calcination
MCM-41	Hydrothermal	Base	Calcination
MCM-48	Hydrothermal	Base	Calcination
MCM-41SD	Spray dried	Acid	Calcination
EthenePMO	Hydrothermal	Acid	Extraction

Table 3.1: Overview of the materials: synthesis and template removal.

As mentioned above in the experimental part, the template removal has been performed by the conventional calcination at 550°C, except the PMO which was activated by soxhlet extraction at 120 using acetone as solvent. A comparison of MCM-4 and MCM-41(SD) allows understanding the effect of a hydrothermal and a spray dried synthesis on the material behavior. Further removal of the surfactant for all pure silica materials has been performed via calcination at 550°C. Only the ethene PMO undergoes an extraction. For comparison, Nucleosil was purchased from Macherey-Nagel in order to be used as reference.

3.3.1 The pore properties of the materials

Two types of pore structures were synthesized, a hexagonal P6/mm structure (MCM-41-HT, MCM-41-SD, SBA-15 and ethene bridged PMO) and a cubical structure (MCM-48 ($Ia\bar{3}d$) and SBA-16 ($Im\bar{3}m$)). Additionally there are substantial differences between MCM type materials and SBA type materials in general. MCM type materials exhibit pore sizes between 2 and 4 nm with a wall thickness of about 1 nm. No significant difference in the pore properties can be discerned between the hydrothermally synthesized and the spray dried MCM-41 material. These pore sizes are small compared to SBA and PMO materials where pore sizes vary between 6 and 10 nm with a wall thickness between 3 and 7 nm. This difference in pore size properties can be assigned to the use of a different surfactant as pore generating agent. An additional property of the materials synthesized with Pluronic surfactants is that next to mesopores also micropores are generated into the walls.

The various pore properties are shown in table 3.2, the surface area $(SA_{(BET)})$, pore volume (V_p) and pore diameter (d_p) were determined with N₂-sorption. The unit cell a_0 was determined from the XRD spectra of the materials and the wall thickness (h) was calculated with the following formulas:

• MCM-41, SBA-15 and EthenePMO:¹⁶⁶

$$h = a_0 - 0.95.d_p \tag{3.1}$$

• MCM-48:¹⁶⁷

$$h = \frac{a_0}{3.0919} - \frac{d_p}{2} \tag{3.2}$$

• SBA-16:¹⁶⁸

$$h = \frac{\sqrt{3}}{2} .a_0 - d_p \tag{3.3}$$

Note that for Nucleosil no pore diameter could be calculated due to its broad pore size distribution and because of the lack of order in this system no XRD pattern was measured either. Therefore no pore wall could be calculated, the value in the table was provided by the supplier.

Table 3.2: The pore properties of the various materials.

Material	$SA_{(BET)}$	V_p	d_p	a_0	h
	(m^2/g)	cm^3/g	(nm)	(nm)	(nm)
SBA-15	656	0.7	6.9	10.7	4.2
SBA-16	738	0.6	6.2	14.6	6.4
MCM-41	1144	0.6	2.9	3.8	1.1
MCM-48	1293	0.8	2.8	7.3	1.0
MCM-41SD	719	0.4	2.9	3.6	1.2
EthenePMO	923	1.0	6.2	9.0	3.1
Nucleosil	88	0.4	~ 30	/	/

3.3.2 The total concentration of silanol-groups from in situ IR

According to the literature¹⁵⁹, adsorbed water species are characterised by a δ -H₂O band near 1630 cm⁻¹ (figure 3.3a). The spectrum of the dried sample (figure 3.3b) presents a narrow band at 3740 cm⁻¹ characterizing isolated silanol groups. The band of inner hydroxyl groups is situated at about 3650 cm⁻¹. The band of H-bonded hydroxyl groups is situated at about 3540 cm⁻¹. From the area of the ($\nu + \delta$) OH band at 4560 cm⁻¹ (insert of figure 3.3), we have estimated the global concentration of Si-OH groups (table 3.3, column 1).

3.3.3 The various OH-species according to MAS ²⁹Si NMR

The OH species were quantified using MAS ²⁹Si NMR and the NMR band was located using CP MAS ¹H ²⁹Si. It is well known that ²⁹Si MAS NMR is a sensitive technique to show the presence of different types of silicon sites in solids. In siliceous zeolite, silicon species are depicted as Q^4 if the silicon is surrounded with four -O-Si groups, Q^3 if one -O-Si group is replaced with one OH group, etc... These species present a very different chemical shift. The CP MAS (¹H)-²⁹Si is a technique that highlights the presence of OH groups which elucidates the ²⁹Si MAS spectrum.

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Figure 3.3: Infrared spectra of SBA-15 sample in 1500-5500 cm^{-1} range. A: spectrum recorded under atmospheric conditions. B: spectrum recorded after degassing.

3.3.3.1 The OH-species for MCM and SBA materials

First the pure silica materials (MCM and SBA-type) are compared. Spectra obtained by direct polarization and by CP MAS are presented in figure 3.4 A and B, respectively. Figure 3.5 depicts the NMR spectra for the silica materials with the Q^2 , Q^3 and Q^4 sites, as well as schematic explanation of these three species. It is interesting to see differences in intensity and width in the three Q^n peaks in the direct spectra (figure 3.4A) as well as in the cross polarized spectra (figure 3.4B). The amount of SiO₂(OH)₂ (geminal silanol), SiO₃OH (single silanol) and SiO₄ species respectively, are then estimated for all samples by using decomposition and comparison with peak intensity in CPMAS as presented in figure 3.4.

From the integration of the three different peaks obtained in each sample it has been possible to estimate the relative amount of each species. The quantification results are then presented in table 3.3. Column 1 depicts the silanol numbers acquired with infrared spectroscopy. This is the total silanol number, so both the surface silanols as the ones in the wall. From this a general trend visible in these data is that thick walled materials (SBA type materials) exhibit a higher number of silanol groups. Columns 2 and 3 depict the separate percentages of $SiO_2(OH)_2$ and SiO_3OH species respectively indicated as geminal and single silanols. The total percentage of hydroxylated silicon atoms ($SiO_2(OH)_2 + SiO_3OH$) is shown by column 4. The last column (5) is the complement of the total percentage of hydroxylated silicon atoms and is designated as the condensation degree. The condensation degree is larger for hydrothermally synthesized SBA materials in comparison with hydrothermal MCM materials. The spray dried MCM material (MCM-41-SD) is not considered at this moment because of the difference in synthesis procedure. The degree of condensation is higher for the SBA materials in comparison with the MCM materials. Because the wall thickness of SBA materials is higher than MCM materials this indicates that the total amount of hydroxylated silicon atoms lowers with the wall thickness. Therefore it can be concluded that the main part of the silanol groups is situated at the pore wall/air interface.



Figure 3.4: The 29 Si MAS NMR (A) and the CP MAS ${}^{1}H{}^{29}$ Si (B) spectrum for MCM-41 (a), MCM-48 (b), MCM-41SD (c), SBA-15 (d) and SBA-16 (e).



Figure 3.5: The allocation of the Q^2 , Q^3 and Q^4 sites on the NMR spectra of the MCM and SBA materials.

	1	2	3	4	5
	(IR)	SiO_3OH	$SiO_2(OH)$	₂ Total SiOH	Condensation
		(%)	(%)	ratio	degree
Material	α_{OH}	$Q^3/$	$Q^2/$	$(Q^2 + Q^3)/$	$Q^4/$
	$(\#/nm^2)$	$(Q^2 + Q^3)$	$(Q^2 + Q^3)$	$(Q^2 + Q^3 + Q^4)$	$(Q^2+Q^3+Q^4)$
SBA-15	3.2	88	12	0.25	0.75
SBA-16	6.8	96	4	0.35	0.65
MCM-41	1.9	94	6	0.40	0.60
MCM-48	2.4	94	6	0.42	0.58
MCM-41SD	2.7	97	3	0.26	0.74

Table 3.3: The silanol number and the Q^2 , Q^3 and Q^4 species in the material.

Remarkably the spray dried MCM-41 has a higher condensation degree than the other MCM-type materials. This higher degree of condensation cannot be correlated to the wall thickness, because this is comparable with the other MCM type materials. However, this can be explained by the synthesis procedure. During the spray drying process, sol droplets are atomized and evaporated initially at the evaporation temperature of the solvent mixture. After this the solids are collecteded by the cyclone into a vessel, the temperature of the materials at this point is 140°C. This is a higher condensation temperature than for the hydrothermal synthesis of MCM and SBA type materials, which typically does not exceed 110°C. Additionally the aging step usually applied to promote further condensation and reordering of the structure after precipitation is not present in spray dried samples. This high temperature of spray drying combined with the rapid and constant evaporation of the solvent and the ethanol produced by the hydrolysis of the ethoxy functions of the silane, will eventually result in a higher degree of condensation.

3.3.3.2 The OH-species for ethene PMOs

	1	2	3	4	5
	(IR)	$\rm SiO_2OH$	$SiO_1(OH)$	₂ Total SiOH	Condensation
		(%)	(%)	ratio	degree
Material	α_{OH}	$T^{2}/$	$T^1/$	$(T^1 + T^2)/$	$T^{3}/$
	$(\#/nm^2)$	$(T^1 + T^2)$	$(T^1 + T^2)$	$(T^1 + T^2 + T^3)$	$(T^1 + T^2 + T^3)$
EthenePMO	11.0	95	5	0.55	0.45

Table 3.4: The silanol number and the T^1 , T^2 and T^3 species in the ethene bridged material.

In comparison with the chemical shifts of the silicon atoms of pure silica materials in ²⁹Si MAS NMR, a significant and distinct shift occurs for organosilica materials. Typically, the signals for the silicon atoms with attached ethene bridges are located between -60 and -90 ppm.²⁰ The spectrum and a schematic overview of the different T sites are depicted in figure 3.6. No Q-sites were measured which indicates that no ethane bridges were broken during the synthesis of the silica material.



Figure 3.6: The allocation of the T^1 , T^2 and T^3 sites on the NMR spectra of the PMO material.

Table 3.5 depicts the quantified silicon species from this spectrum. Column 1 repeats the total silanol number obtained with in-situ IR spectroscopy. Columns 2 and 3 give the respective percentages of single (T^2) and geminal (T^1) silanol groups. Column 4 depicts the total percentage of hydroxylated silicon atoms and column 5 is the complement of this value, designated as the degree of condensation. When the total amount of OH species (columns 1 and 4) is compared to these on pure silica materials (table 3.3: columns 1 and 4), there are more hydroxyl groups present in the organosilica material. This is reflected in a much lower (below 50%) degree of condensation (column 5).



Figure 3.7: The polycondensation of silicates (A) and the autohydrophobisation illustrated for ethene PMOs (B).

Two different mechanisms lead to these differences. First of all the polycondensation reaction of the ethene bridged precursor molecule does not progress to completion because there are six siloxane bridges to be formed per precursor molecule instead of the four of TEOS. (See the T^3 sites in figure 3.6). This is sterically more difficult than the polycondensation reaction with TEOS, used in the synthesis of MCM and SBA type materials. The second and probably the largest influence on the degree of condensation, is the temperature of the post treatment. While pure silica materials are calcined at 550°C, organosilicas are obviously not. The surfactant that acts as the porogen inside the periodic mesoporous organosilicas is typically extracted by soxhlet extraction with acetone or ethanol.³⁴ The materials are never exposed to temperatures above 120°C. When PMOs are treated at temperatures above 200°C, the residual silanols will be further reduced by interaction with the ethene bridge, via an autohydrophobisation reaction, as described by Ozin¹⁶⁹ and Van Der Voort⁹. These reactions are shown in figure 3.7. Finally, as the temperature reaches 300°C, the organic bridges will be destroyed.

3.3.4 Determination of the maximum loading of the probing molecules

The maximum loading of the materials was determined via the grafting procedure of three silanes that differ in carbon chain length. Through the accessible silanol groups, the probing molecules were anchored to the surface. The amount of probing molecules was quantified on the carbon amount determined with elemental analysis.

Table 3.6 depicts the loading capacity of the various (organo)silica materials for a trimethylsilyl (C3), an octyldimethylsilyl (C8) and an octadecyldimethylsilyl (C18) group. The pore diameter has been added for reference. From this table it can be observed that for the same reaction time the amount of grafted groups drops with the length of the aliphatic chain. When the hydrothermally synthesized pure silica materials are compared, the general tendency is that with smaller pores a lower amount of groups are grafted on the surface (MCM vs SBA vs Nucleosil). The same tendency has been shown as a function of the pore size of MCM materials by Waksburg et al.¹⁷⁰

However, when we consider the C18 silane, this logical trend seems no longer valid, as in this case the MCM-41 has a higher loading of the C18 silane $(0.2 \text{ groups}/nm^2)$ than the SBA-15 material $(0.1 \text{ groups}/nm^2)$. This cannot be due to the original amount of silanols, as this amount was much higher for the SBA-15 than for the MCM-41. Due to the fact that this discrepancy is only observed for the longer C18 chains and not for the shorter C3 groups it cannot be explained by fewer silanol groups on the surface as opposed to those inside the silica walls. Nevertheless this can be explained by the typically very long and curvy *spaghetti-like* pore system of the SBA-15 structure. The reason why this pore blocking does not occur in the MCM-41 material can be attributed to the particle morphology (see figure 3.8). The smaller MCM-41 particles do not have a long curvy *spaghetti-like* pore system, but rather short parallel pores. These SBA-15 pore systems run along the entire length of the particles and can be up to hundreds of micrometers long,

inducing important diffusion limitations. As is reflected in the table, these diffusion limitations become more and more important as the length of the aliphatic side chain increases, this, additionally, due to the presence of micro pores in SBA materials. 171



Figure 3.8: SEM micrographs of MCM-41 (A) and SBA-15 (B) materials. TEM micrograph of SBA-15 material (C). Picture (C) was published previously by Van Der Voort¹⁷².

A second interesting observation can be made when SBA-15 is compared with SBA-16 and MCM-41 with MCM-48. The cubical pore systems (SBA-16 and MCM-48) systematically have a larger amount of C18 groups anchored to the surface. This is due to the fact that a cubic pore system is accessible from three dimensions while a hexagonal pore system just from one dimension. Therefore it is clear that the inside of an SBA-16 and an MCM-48 particle can be reached more easily and thus faster than the inside of an SBA-15 and MCM-41 particle. This easy access to the whole of the pore system from multiple dimensions is the reason the carbon loading on cubical pore systems is higher than the loading on hexagonal pore systems.

Material	Pore size	C	3	С	8	C1	8
	nm	mmol/g	$\#/nm^2$	mmol/g	$\#/nm^2$	mmol/g	$\#/nm^2$
SBA-15	6.9	1.8	1.7	0.7	0.7	0.1	0.1
SBA-16	6.2	2.3	1.9	0.8	0.7	0.5	0.4
MCM-41	2.9	2.2	1.2	0.9	0.5	0.4	0.2
MCM-48	2.8	2.5	1.2	1.2	0.6	0.5	0.3
MCM-41SD	2.9	1.9	1.6	1.0	0.8	0.3	0.3
EthenePMO	6.2	1.1	0.7	0.8	0.5	0.4	0.2
Nucleosil	~ 30	0.4	2.8	0.5	3.3	0.3	1.9

Table 3.5: The loading capacity for relevant chromatographic groups for various ordered mesoporous (organo)silica materials.

This theory is confirmed by Nucleosil where the amount of grafted groups per nm^2 is much higher in comparison with the other materials. The Nucleosil material consists of perfect spheres with large disordered pores (~ 30 nm). This much

higher loading for Nucleosil confirms that the grafting procedure for porous systems is strongly diffusion regulated. The influence of diffusion is larger for the longer and more bulky grafting molecules than for the smaller ones and is larger for the hexagonal one-dimensional pore systems than for the cubic three-dimensional pore systems.

However when the molar amount of groups per weight unit (mmol/g) is considered it is immediately clear that the ordered mesoporous materials exhibit a much higher loading than the commercial Nucleosil material.

3.3.5 Computational considerations

To get a better view on the behavior of silane groups on the silica surface, theoretical calculations were performed to determine the geometry of a carbon chain substituent. All results were obtained with the gaussian09 software package¹⁷³ using a B3LYP functional^{174,175} with a 3-21G Pople basis set. Since the primary focus was set on determining geometries this level of theory was chosen. It was chosen to consider a large system on a lower level of theory since the geometry is more important than accurate energies.



Figure 3.9: Random silica surface with 5.3 hydroxy group per nm^2 and one C8 silane substituent. This silane blocks two neighboring silanol groups. Figure a) is without an implicit solvent model, b) is the same starting geometry optimized with such a model. The differences are minimal.

First, a random silica slab was made with approximately 5.3 hydroxyl groups per nm^2 . This model is designed to represent an amorphous silica surface, thus a random cluster was built with the required silanol-density. To calculate the density the total number of hydroxyl groups was divided by the exposed surface of the cluster, hydroxyl groups at the edge are only counted as half since they are also part of neighboring clusters. The proposed approach was chosen since there are no surface models available for silicas with this high silanol loading. The edges of the slab are saturated with hydrogen atoms which were unrestricted during the optimization. One of the hydroxyl groups was exchanged with a C8 silane group. Geometry optimizations with the surface. This is as expected, since the apolar tail cannot interact with the polar surface (figure 3.9).



Figure 3.10: Surface model with about 2.5 hydroxyl groups per nm^2 the surface is based on an ordered silica slab. The silane group is close to a neighboring hydroxyl group, but since the surface is more open this does not block the site. As an upper bound, on this type of surface it is possible to have 2.5 silane substituents per nm^2 . However, when the surface is almost full, the close interaction (2.35Å) will fully shield one of the hydroxyl groups and the theoretical maximum will never be reached.

Similar calculations, where an implicit solvent model (IEFPCM¹⁷⁶) was added, characterized by the dielectric constant of toluene gave comparable results. The geometrical results show that only the $-Si(CH_3)_2$ - moiety imposes a limit on the number of possible substituents per surface area. From the result it can be seen

that the $-\text{Si}(\text{CH}_3)_2$ - moiety blocks at least two extra hydroxyl groups. Since those groups can also be overlapped by another silane group from a more distant position, they are only counted as half. So it can be concluded that, on this dense cluster, at least two groups are needed to accommodate the substituent leading to a maximum possible loading of 2.65 groups per nm^2 . On a less dense surface with about 2.5 hydroxyl groups per nm^2 there are no neighboring positions blocked (figure 3.10).

This means the theoretical density in this case is 2.5 groups per nm^2 . On average, 2.5 groups per nm^2 is the theoretical maximal loading on a silica surface with only single silanols present, this is close to the value that can be estimated from the kinetic diameter of the silane and that was reported previously by Van Der Voort.¹⁷⁷



Figure 3.11: The original cluster is modified to incorporate a geminal silanol, the Si-O bond between the atoms explicitly named on the figure was broken and the marked hydrogen atom was added for saturation, as second silane group was then added to the free oxygen. With two silane groups on a geminal silanol two other silanol groups are blocked. This allows a more efficient stacking of the substituents.

To assess the influence of geminal silanols a slight modification on the random surface was made, an extra hydroxyl group was added on a silicon atom and one Si-O-Si bond on that atom was broken. The other silicon atom was saturated by hydrogen. With this approach the surface contains a single geminal group. Two silane groups were placed on this site and after geometry optimization it appeared that at least two extra silanol groups were blocked by this. (figure 3.11).

For the same reason as before they only count as half. With the extra hydroxyl group from the geminal silanol, the surface silanol density is 6.7 groups per nm^2 (the surface area remains unchanged but an extra hydroxyl group is added). This means that when one extra hydroxyl group is blocked by the silane moiety, the density of substituents on the surface can be calculated as:

$$4.5 \text{ groups}/nm^2 = \frac{2 \text{ silane groups . } 6.7 \text{ groups}/nm^2}{3 \text{ hydroxyl groups}}$$
(3.4)

A fully hydroxylated silica surface (single silanol groups) can have maximum of 4.6 ± 0.5 groups per nm^2 . This means that roughly only half of the single silanol groups is sterically available for grafting which coincides with previous reports on that matter.¹⁰³

The amount of single and geminal silanols (columns 2 and 3 in table 5) are calculated by multiplying the relative amount of silanols with the total number of OHgroups determined by IR spectroscopy (i.e. $\alpha OH \ge (Q^3/(Q^2+Q^3) = \alpha_{OH,single})$). This gives the absolute amount single and geminal silanols, irrespective of their location. They could be on the pore wall or in the bulk silica.

Table 3.6: The silanol number, the amount of single and geminal silanols, the surface and the internal silanol groups, depicted with the pore diameter (d_p) and pore wall (h).

	1	2	3	4	5	6	7
			α_{OH}			h	d_p
	total	single	geminal	surface	internal		
Material			$(\#/nm^2)$			(nm)	(nm)
SBA-15	3.2	2.8	0.4	1.7	1.5	4.2	6.9
SBA-16	6.8	6.5	0.3	1.9	4.9	6.4	6.2
MCM-41	1.9	1.8	0.1	1.2	0.7	1.1	2.9
MCM-48	2.4	2.3	0.1	1.2	1.2	1.0	2.8
MCM-41SD	2.7	2.6	0.1	1.6	1.1	1.2	2.9
EthenePMO	11.0	9.3	1.7	0.8	8.5	3.1	6.2

Column 4 then shows the silanols that were available to the small HMDS (C3 silane). Because none of these values exceed the theoretical maximum values they can be considered as the "surface or reachable" silanols. It can be observed that the amount of surface accessible silanol groups for SBA-15, SBA-16 and MCM-41 (SD) is higher than for MCM-41 and MCM-48. This can be explained by the synthesis procedure, the first materials were synthesized in an acidic medium, whilst MCM-41 and MCM-48 are both synthesized in a basic medium. In both cases the materials

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are formed through the normal sol-gel condensation of the silicic acid $(Si(OH)_4)$, in which 2 silanols condense to form a siloxane bridge: $2SiOH \rightarrow Si-O-Si+H_2O$. This process is very well described and understood in literature.¹⁷⁸

However, when a surfactant is added to form ordered mesoporous silica, most of these silanols interact with the surfactant. It is due to these surfactant-silicate interactions that the porous structure is formed. The reaction medium plays an important role in the interaction between the surfactant and the silicate species during polycondensation. In a basic medium (MCM-41 and MCM-48) the ionic surfactant has a direct interaction with the silanol groups of the condensing silica via an S^+I^- interaction (S being surfactant and I being the inorganic moiety). In the case of an acidic medium (MCM-41 (SD), SBA-15 and SBA-16) a negatively charged ion makes the bridge between the surfactant and the condensing silica via an S^+X-I^+ interaction. In the case of SBA materials non-ionic surfactants are used and anion mediated hydrogen bridges make the bridge between the inorganic moiety and the surfactant ($S^0(XI)^0$). These interactions are illustrated by figure 3.12.^{2,179} As a consequence of this secondary interaction with the surfactant, more silanol groups are pulled to the surface of the pores when an acidic reaction medium is employed.



Figure 3.12: An impression of the three types of interaction between the silica precursors and the surfactant, with S = surfactant, I = inorganic precursor and X a halogen atom. (taken from reference²)

Due to the completely different nature of the ethene PMO material the same explanation does not apply. The addition of the organic moiety will reduce the amount of silicon atoms at the surface of the pores, substantially reducing the amount of surface free silanol groups.

Column 5 depicts the amount of internal silanol groups, calculated from the difference between the total silanol number (column 1) and the surface silanol groups (column 4). Here there is a clear difference between the MCM materials on one side and the SBA and PMO materials on the other side. This difference completely coincides with the difference in pore wall. There is a direct proportional relation between the wall thickness and the amount of internal silanol groups. However this relation is not linear, which can be explained by the fact that the amount of silanol groups in general and the internal silanol groups in particular are influenced by the degree of condensation. This in turn is influenced by the reaction procedure and the post treatment conditions.

3.4 Conclusion

We have established that the amount of silane groups (expressed as groups per nm^2) that can be grafted on the surface of ordered mesoporous silicas (MCM-41, MCM-48, SBA-15, SBA-16) is much smaller than on the surface of the non-porous (commercial) materials, which are typically used as a packing material for HPLC columns. The main reason for this is that the grafting reaction is strongly diffusion limited, as the surface area of these materials is basically internal surface area.

For the mesoporous silicas synthesized under acidic conditions, it can be concluded that the amount of surface silanols is larger than for the mesoporous silicas synthesized under basic conditions. The amount of internal silanol groups is proportional, however not linearly, to the wall thickness. Silica materials with thicker walls (SBA-15, SBA-16 and ethene PMO) have more silanol groups than the thin walled MCM-materials. For thin walled silica materials (MCM-41, MCM-48) this relationship is not that pronounced.

Spray dried MCM-41 exhibits a more condensed silica structure due to the higher synthesis temperature. The PMO material (ethene PMO) on the other hand exhibits a lower condensation degree. This can be attributed to the different condensation behavior of $(EtO)_3$ -Si-CH=CH-Si(OEt)_3 compared to Si(OEt)_4 and to the absence of a high temperature calcinations procedure.

4

An experimental design to screen the parameters.

Spray drying has been chosen as the technique to produce spherical mesoporous silica materials. Multiple synthesis factors have an influence on both the particle morphology and the pore properties. After the scouting experiments 6 factors were designated to have the highest effect on either the particle morphology or the pore properties or both. To asses their effects thoroughly in a short time, the experimental set-up was statistically approached with an experimental design. A design of experiments allows to reduce the amount of experiments significantly without loosing essential information on the effects the factors exhibit on the particle morphology and the porosity. This process has been applied to spray dry spherical MCM-41 particles.

4.1 Design of Experiments or DOE

According to Montgommery et al, design of experiments (DOE) or experimental design is the design of any information-gathering exercise where variation is present, whether under the full control of the experimenter or not.^{180–182} However, in scientific research, DOEs are used to gather information about the influences of two or more factors on the outcome of an experiment. A factor can be varied over several levels, i.e. if you have 3 factors and 2 levels per factor, the total amount of experiments needed to have all possible combination is $2^3 = 8$. This is called a full factorial design and allows to analyze any possible effect any one, two or three factors might have on the outcome of the experiment. So this visualizes effects multiple factors exhibit together on the result. An essential detail of this technique is that all the experiments are conducted randomly to exclude any unwanted factor to become statistically relevant. The outcome of the experiment is defined as one or more response factors. These are measurable factors by which the relevant results of the experiment are defined.

Eight experiments is not that much but when more and more factors are included in the design the number of experiments one has to perform increases exponentially. To reduce the number of experiments in the interest of time and money, one can choose to perform a fractional factorial design of experiments. This can only be done if the experimenter can assume that higher order interactions are negligible. Figure 4.1 illustrates this perfectly.



Figure 4.1: The possible fractions for a DOE, red: significant loss of information; green no significant loss of information.

The software used here is a statistical program called Minitab. It helps in setting up the array of experiments and also in the analysis of the generated results. The array of experiments is set up randomly and one can choose for a full or a fractional factorial design.

4.2 The underlying statistics

An experimental design is analyzed by an iterative ANOVA (Analysis of Variance) procedure. During a first run all possible factors and all possible interactions between factors are included in the analysis. Then a first analysis is performed in which hypothesis testing defines if factors are significant (H_1) or not (H_0) , the student t-test is used to calculate t_0 and the p-value is defined with a level of significance α of 0.05. The p-value is defined as the smallest level of significance that would lead to rejection of the null hypothesis H_0 . A Pareto chart of standardized

effects (see figure 4.2) will be generated in which factors are plotted in the y-axis and the horizontal bars represent their effect. The vertical red line defines the cutt-off; meaning that standardized effects larger than the red line are considered significant. The next thing to do is to remove gradually the bottom factors one by one and each time redo the same analysis, until only the significant factors or combinations thereof remain.



Figure 4.2: The pareto chart of standardized effects.

4.3 Experimental Design 1

4.3.1 The factors, the levels and the response factors

As can be concluded from the section above, it is clear that the choice for the correct levels is very important. If for instance the levels are too close together, it could lead to the conclusion that the factor has no influence on the outcome of the experiment, while in reality it does have an influence. However, if the levels are too far apart, the factor could have an influence, while in fact it does not. Therefore it is imperative to know the experimental set up very well to choose the correct levels.

First the factors that might have an actual influence on either the particle morphology, the pore properties or both are defined and then the levels are chosen. The only factors that have an actual influence are shown in table 4.1. Some initial experiments indicated that six factors have an influence on either the particle morphology, the pore properties or both. Two levels are defined based on our experience with the material and the spray drying technique. This would result in $2^6 = 64$ experiments in case of a full factorial design. Since it is unlikely that more than 3 factors at once would have an influence on the responses, a resolution IV design was chosen. Herein the 64 experiments were reduced to 16 unique experi-

ments. To test for repeatability every experiment was performed twice randomly so the total number of experiments to perform was 32.

]	Factor	Solution f	low H_2O	Stir	ring	Gas flow	CTAB	Solvent
	Level	rate (pump	p%) ratio ^a	time	e (h)	rate (L/min)	ratio	
	Low	10	700	0	.5	6	0.12	1-butanol
	High	17	1400	1	.5	8	0.24	isopropanol
\overline{a}	Mola	r ratio	compared	to a	a T	$EOS:CTAB:H_2$	O:IPrOH	/BuOH:HCl

Table 4.1: The chosen factors for the first DOE.

(1:0.12/0.24:700/1400:10:5) solution.

The responses have to represent the results of the experiments in such a way that they are a good measure for the particle morphology and the pore properties. The chosen responses are summarized in table 4.2. The response morphology, expressed in %, is a subjective number given by visually analyzing the SEM pictures made from the particles and is therefore not so reliable as the particle size distribution (PSD) and its deviation. The particle size distribution is measured by laser diffraction with a Malvern 2600c Series Particle Sizer in suspension.

Table 4.2: The responses defining morphology and porosity.

Property	Response	Unit of measure
Morphology	Mean Particle Size (PSD)	μm
	PSD deviation (σ_{μ})	μm
	Morphology	%
Porosity	Surface area	m^2/g

Figure 4.3 depicts the SEM chart by which the particles were classified according to their morphology. An overview of the actual factors varied is given by table 4.3.

4.3.2 The results and their interpretation

The results are summarized in table 4.4 below. The first column is the standard order and the second column is the run order. The run order is the order in which the experiments were actually conducted and is a completely randomized output of the software. For each response the influence of the six factors is calculated according to the above described method. Figure 4.4 shows the Pareto charts and the main effects plots concerning the response factors. From these it is immediately clear that the porosity is by far only influenced by the amount of surfactant. The main effects plot for the surface area shows that it is directly proportional with the amount of CTAB used. After a regression analysis this results in a prediction formula for the surface area:

$$SurfaceArea(m^2/g) = 344 + 2266.CTAB(g)$$
 (4.1)



Figure 4.3: The subjective SEM chart in accordance with the morphologic percentages.

This equation can only be applied with the used composition, spray drying conditions and only when the amount of CTAB is inserted in grams. Looking closer the equation reveals that there is a basic minimal surface area equal to $344 \ m^2/g$, this is more or less the maximal surface area for non porous perfect spheres. Of course these spheres would be very small.¹⁰³ For a full equation more experiments would be needed to determine a maximum surface area depending on the amount of surfactant. This maximum will depend on the amount of silica source, once this is depleted, the extra amount of surfactant will no longer have an influence on the surface area.

Std	Run	Solution	H_2O	Stirrtime	Gasflow	Solvent	CTAB
order	order	flow $(\%)$	ratio	(h)	(mm)		(g)
23	1	10	11600	1.5	30	IPrOH	0.1981
10	2	17	5800	0.5	40	BuOH	0.3962
2	3	17	5800	0.5	30	BuOH	0.1981
5	4	10	5800	1.5	30	BuOH	0.3962
6	5	17	5800	1.5	30	IPrOH	0.3962
28	6	17	11600	0.5	40	IPrOH	0.1981
17	7	10	5800	0.5	30	IPrOH	0.1981
18	8	17	5800	0.5	30	BuOH	0.1981
8	9	17	11800	1.5	30	BuOH	0.1981
9	10	10	5800	0.5	40	IPrOH	0.3962
24	11	17	11600	1.5	30	BuOH	0.1981
21	12	10	5800	1.5	30	BuOH	0.3962
11	13	10	11600	0.5	40	BuOH	0.1981
4	14	17	11600	0.5	30	IPrOH	0.3962
13	15	10	5800	1.5	40	BuOH	0.1981
16	16	17	11600	1.5	40	BuOH	0.3962
19	17	10	11600	0.5	30	BuOH	0.3962
15	18	10	11600	1.5	40	IPrOH	0.3962
3	19	10	11600	0.5	30	BuOH	0.3962
29	20	10	5800	1.5	40	BuOH	0.1981
31	21	10	11600	1.5	40	IPrOH	0.3962
12	22	17	11600	0.5	40	IPrOH	0.1981
14	23	17	5800	1.5	40	IPrOH	0.1981
1	24	10	5800	0.5	30	IPrOH	0.1981
26	25	17	5800	0.5	40	BuOH	0.3962
7	26	10	11600	1.5	30	IPrOH	0.1981
20	27	17	11600	0.5	30	IPrOH	0.3962
22	28	17	5800	1.5	30	IPrOH	0.3962
27	29	10	11600	0.5	40	BuOH	0.1981
30	30	17	5800	1.5	40	IPrOH	0.1981
25	31	10	5800	0.5	40	IPrOH	0.3962
32	32	17	11600	1.5	40	BuOH	0.3962

Table 4.3: The varied factors

The morphology on the other hand is influenced by three factors: the water amount,

the gas flow and the amount of CTAB. The main effect plots shows that the morphology is directly proportional with the gas flow and the water amount and inversely proportional with the amount of CTAB. There also seems to be a small interaction between the water amount and the gas flow.

Std	Run	SA_{BET}	Mean PSD	PSD (σ_{μ})	Morphology
order	order	(m^2/g)	(μm)	(μm)	(%)
23	1	722	13.8	9.5	70
10	2	1089	9.1	6.5	60
2	3	747	2.6	10.3	80
5	4	1263	21.2	15.1	70
6	5	1297	16.3	10.7	50
28	6	789	19.1	15.5	60
17	7	779	18.9	15.4	40
18	8	796	10.4	9.2	70
8	9	795	10.2	6.9	80
9	10	1288	30.2	19.7	40
24	11	706	3.9	3.9	70
21	12	1187	9.6	11	60
11	13	866	2.2	0.3	90
4	14	1185	6.4	4.7	50
13	15	854	1.8	0.8	70
16	16	1204	4.4	4.4	80
19	17	1291	2.9	2.5	70
15	18	1216	2.2	0.3	90
3	19	1303	3.9	4.2	60
29	20	854	2.2	0.3	90
31	21	1269	7.1	7.7	85
12	22	816	5.2	4.2	95
14	23	801	1.9	1	65
1	24	772	4.5	7.4	55
26	25	1228	6.4	5.6	50
7	26	707	2.7	1.9	60
20	27	1255	2.8	0.5	65
22	28	1237	6.5	4.7	55
27	29	864	1.8	0.2	90
30	30	821	2.2	0.3	70
25	31	1316	9.9	9.3	75
32	32	1690	7.4	7	80

Table 4.4: The results

As can be derived from the Pareto charts for both the morphology and the surface area, the amount of CTAB influences the surface area (and thus the pore properties) positively but it influences the morphology negatively. Both influences can be statistically extrapolated and there is an optimal point where both the porosity properties and the spherical morphology are maximized. This is represented in an optimization plot, presented by figure 4.5. The calculated optimal point for the CTAB concentration is 0.3498 g or 0.21 mol equivalents versus TEOS. This means that the H_2O fraction and the gas flow are the only two factors left to vary.



Figure 4.4: The Pareto chart of standardized effects and the main effects plots for the four response factors.

These two factors are subsequently analyzed in a second experimental design. The amount of CTAB was held constant at the optimum level predicted by the statistical analysis. The surface area remained constant at $1066\pm80m^2/g$, which is within the accuracy range of the N₂-sorption equipment. This confirms the conjecture that the surface area is only influenced by the amount of CTAB used.



Figure 4.5: The optimization plot for the first DOE.

4.4 Experimental Design 2 and then some more...

4.4.1 The factors, the levels and the responses

The first experimental design revealed that the number of significant factors could be reduced to three main interactions and one secondary interaction namely the H_2O fraction, the gas flow, the amount of CTAB and a combination of the first two. The analysis led to a second DOE process in which only two factors were varied on two levels. For this analysis a full factorial experimental design was conducted, so $2^2 = 4$ experiments were done. In order to obtain an estimate of the repeatability, each experiment was performed in duplicate.

This time only the amount of water and the gas flow were altered on 2 levels. The molar composition of the solution was 1:0.21:1400/2800:10:5 for TEOS:CTAB:H₂O: BuOH:HCl. The gas flow was varied between 8 L/min and 10 L/min. The stirring time was kept constant at 0.5 h and the solution flow was held constant at 2.95 mL/min (see table 4.5)

Factor	Solution flow	ΗΟ	Stirring	Cas flow	CTAB	Solvent
ractor	Solution now	$m_2 O$	Dunning	Gas now	OIND	DOIVCII
Level	rate $(pump\%)$	$ratio^a$	time (h)	rate (L/min)	ratio	
Low	10	1400	0.5	8	0.21	1-butanol
High	10	2800	0.5	10	0.21	1-butanol
\overline{a} Molar r	atio compared to	a TEOS	:CTAB:H ₂ O	D:BuOH:HCl (1	:0.21:1400	0/2800:10:5)

Table 4.5: The chosen factors for the second DOE.

 a Molar ratio compared to a TEOS: CTAB:H_2O:BuOH:HCl (1:0.21:1400/2800:10:5) solution.

During this second DOE the responses have been extended with additional characteristics for the porosity.

Table 4.6: The extended response factors defining morphology and porosity.

Property	Response factor	Unit of measure
Morphology	Mean Particle Size (PSD)	μm
	PSD deviation (σ_{μ})	μm
	Morphology	%
Porosity	Surface area	m^2/g
	Pore volume (Vp_T)	cm^3/g
	Pore diameter (d_p)	nm

Table 4.7: The varied factors and the response factors

Std	Run	H ₂ O	Gas Flow	SA (m^2/g)	d_p	$Vp_T(cm^3/g)$
order	order	(g)	(mm)		(nm)	
3	1	112.25	50	1097	3.1	0.75
1	2	112.25	40	1086	3.2	0.78
8	3	224.50	50	1139	3.1	0.74
6	4	224.50	40	984	3.0	0.63
7	5	112.25	50	990	2.9	0.62
5	6	112.25	40	1036	3.1	0.70
4	7	224.50	50	1143	3.3	0.84
2	8	224.50	40	1055	3.2	0.73
Std	Run	H ₂ O	Gas Flow	Morphology	MPS	PS (σ_{μ})
Std order	Run order	$ \begin{array}{c} \mathrm{H_2O} \\ \mathrm{(g)} \end{array} $	Gas Flow (mm)	Morphology (%)	MPS (µm)	$\begin{array}{c} PS & (\sigma_{\mu}) \\ (\mu m) \end{array}$
Std order 3	Run order 1	$\begin{array}{c} {\rm H_2O} \\ {\rm (g)} \\ 112.25 \end{array}$	Gas Flow (mm) 50	Morphology (%) 90	MPS (µm) 1.8	$\begin{array}{c} \text{PS} & (\sigma_{\mu}) \\ (\mu\text{m}) & \\ 0.2 & \end{array}$
Std order 3 1	Run order 1 2	$\begin{matrix} \rm H_2O \\ (g) \\ 112.25 \\ 112.25 \end{matrix}$	Gas Flow (mm) 50 40	Morphology (%) 90 85	MPS (μm) 1.8 1.8	$ \begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ 0.2 \\ 0.2 \\ \end{array} $
Std order 3 1 8	Run order 1 2 3	$\begin{array}{c} \rm H_2O\\ (g)\\ 112.25\\ 112.25\\ 224.50\\ \end{array}$	Gas Flow (mm) 50 40 50	Morphology (%) 90 85 95	MPS (µm) 1.8 1.8 4.4	$\begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ \hline 0.2 & \\ 0.2 & \\ 3.5 & \\ \end{array}$
Std order 3 1 8 6	Run order 1 2 3 4	$\begin{array}{c} \rm H_2O\\ (g)\\ 112.25\\ 112.25\\ 224.50\\ 224.50\\ \end{array}$	Gas Flow (mm) 50 40 50 40	Morphology (%) 90 85 95 90	MPS (µm) 1.8 1.8 4.4 3.0	$\begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ \hline 0.2 & \\ 0.2 & \\ 3.5 & \\ 5.0 & \\ \end{array}$
Std order 3 1 8 6 7	Run order 1 2 3 4 5	$\begin{array}{c} \rm H_2O\\ \rm (g)\\ 112.25\\ 112.25\\ 224.50\\ 224.50\\ 112.25\\ \end{array}$	Gas Flow (mm) 50 40 50 40 50	Morphology (%) 90 85 95 90 85	MPS (µm) 1.8 1.8 4.4 3.0 1.9	$\begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ 0.2 & \\ 0.2 & \\ 3.5 & \\ 5.0 & \\ 1.0 & \\ \end{array}$
Std order 3 1 8 6 7 5	Run order 1 2 3 4 5 6	$\begin{array}{c} \rm H_2O\\ (g)\\ 112.25\\ 112.25\\ 224.50\\ 224.50\\ 112.25\\ 112.25\\ 112.25\\ \end{array}$	Gas Flow (mm) 50 40 50 40 50 40	Morphology (%) 90 85 95 90 85 80	MPS (µm) 1.8 1.8 4.4 3.0 1.9 7.0	$\begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ \hline 0.2 & \\ 0.2 & \\ 3.5 & \\ 5.0 & \\ 1.0 & \\ 6.7 & \\ \end{array}$
Std order 3 1 8 6 7 5 4	Run order 1 2 3 4 5 6 7	$\begin{array}{c} \rm H_2O\\ (g)\\ 112.25\\ 112.25\\ 224.50\\ 224.50\\ 112.25\\ 112.25\\ 224.50\\ \end{array}$	Gas Flow (mm) 50 40 50 40 50 40 50 40 50	Morphology (%) 90 85 95 90 85 80 95	MPS (µm) 1.8 1.8 4.4 3.0 1.9 7.0 1.8	$\begin{array}{c c} PS & (\sigma_{\mu}) \\ (\mu m) & \\ \hline 0.2 & \\ 0.2 & \\ 3.5 & \\ 5.0 & \\ 1.0 & \\ 6.7 & \\ 0.2 & \\ \end{array}$
4.4.2 The results and their interpretation

The results are summarized in table 4.7 and the Pareto charts and the main effects plots are represented by figures 4.6 and 4.7. Both factors have an effect on the mean particle size and the particle size deviation but it is statistically not significant within the factor levels, however they have a significant influence on the morphology.

The last factor to be tested was the influence of the water fraction. In a final experiment two more compositions were spray dried. This time only the fraction of the water was varied, the molar composition of the solution was 1:0.21:5600/8400:10:5 for TEOS:CTAB:H₂O:BuOH:HCl. The gas flow was kept constant at the maximum value of the system, which was of 10 L/min. These results are explained more in detail in the next chapter.



Figure 4.6: The Pareto charts and the main effects plots for the water ratio and the gasflow with the pore properties as response factors.



Figure 4.7: The Pareto charts and the main effects plots for the water ratio and the gaslfow with the morphology as the response factors.

5

Spherical MCM-41 type silica particles by spray drying: doubling the retention factor of HPLC columns

5.1 Introduction

Although MCM-41 type materials were introduced in 1992, the number of applications thereof has been relatively limited. Mesoporous materials show unique properties which can be beneficiary for a wide range of applications. They are fairly easy to functionalize to yield heterogeneous catalysts or adsorbents for gas and liquid waste stream clean ups. An emerging field is the application of mesoporous thin films as low-k insulators, in superconductors and as solar-cells.^{9,183-186} Although these benefits are mentioned in almost every contribution making use of these materials, still very few of them prove that they are in fact a better or a useful alternative to the commercially available materials they are to replace. In this chapter it is demonstrated that mesoporous materials possess distinct benefits as packing materials for high end separations in HPLC (High Performance Liquid Chromatography). The use of particles with a highly uniform spherical morphology is important in a packed column as this results in a more regular flow profile and in minimal chromatographic peak broadening. The specific goal here is to create uniform spheres with a controlled and ordered mesoporosity together with a high surface area.

During the previous chapter an experimental design was described in which the relevant factors for spherical mesoporous particle design through spray drying were designated. In this chapter the results are analyzed and used to synthesize ordered mesoporous silica spheres and use them as a packing material for HPLC.

Experimental 5.2

5.2.1**Synthesis**

The experimental setup comprises a mixing and a spray drying step. Spray drying was performed on a Buchi B290 with a two-fluid nebulizer connected to pressurized air. The heater inlet temperature was held constant at 493K and the outlet temperature stayed constant at 398K. The aspirator ran at a maximum air velocity of 40 m^2h^{-1} . The nozzle gas flow and the solution feeding speed were variable factors during the scouting experiments. During the first step a solution of CTAB, H_2O , TEOS, HCl is mixed with or 1-butanol at room temperature for a certain amount of time (see table 5.1). Subsequently this clear mixture is fed to the spray dryer, resulting in MCM-41 powders which are collected by a cyclone separator. The particles are calcined under air at a heating rate of 1.5K per minute from room temperature to 823K and kept at this temperature for 6 hours. After this the samples were analyzed with XRD, N₂-sorption and SEM to obtain information about the porosity and the morphology.

Level	Solution flo	w H ₂ O	Stirring	Gas flow	CTAB	Solvent
	rate (pump?	%) ratio ^a	time (h)	rate (L/min)	ratio	
Low	10	700	0.5	6	0.12	1-butanol
High	17	1400	1.5	8	0.24	isopropanol
a Mo	lar ratio	compared	to a	TEOS:CTAB:H	2O:IPrOF	H/BuOH:HCl
(1 0 10 /	0.04 000 /1 400	10 5) 1				

Table 5.1: The initial reaction conditions.

(1:0.12/0.24:700/1400:10:5) solution.

Varying the surfactant/TEOS ratio 5.2.1.1

Only the surfactant/TEOS ratio was varied while the rest was kept constant. The composition of the mixture was TEOS:CTAB: $H_2O:BuOH:HCl = 1:x:1400:10:5$ where x equals 0.12, 0.21 and 0.24. The feeding speed and the gas flow were held constant at 3 mL/min and 8 L/min respectively.

Varying the $H_2O/TEOS$ ratio 5.2.1.2

Here only the water/TEOS ratio was varied while the rest was kept constant. The composition of the mixture was TEOS:CTAB:H₂O:BuOH:HCl = 1:0.21:x:10:5 with x equal to 700, 1400, 2800, 5200 or 8400. The feeding speed and the gas flow were held constant at 3 mL/min and 8 L/min respectively.

5.2.1.3Varying the gas flow

During this section only the gas flow was varied while the rest was kept constant. The composition of the mixture was TEOS:CTAB: $H_2O:BuOH:HCl = 1:0.21:1400:$ 10:5. The feeding speed was held constant at 3mL/min and the gas flow was varied between 6, 8 and 10 L/min.

5.2.1.4 Scaling the synthesis to gram levels

The final step involved upscaling the approach and to test the robustness of the synthesis procedure. The molar composition of the solution was thereby: 1:0.21:8400: 10:5 for TEOS:CTAB:H₂O:BuOH:HCl, which was also the final composition used for the manufacturing of the particles that were eventually functionalized and packed. The largest batch was spray dried from a 7.5 L solution, while the batches used during the development as described in the previous paragraphs varied between 63 and 793 mL. With this increased solution volume a larger type of spray drier was used, i.e. a Mobil Minor from GEA Niro A/S with a rotary nozzle. The nozzle pressure was held constant at 4.5 bar, connected to the pressurized air. The solution pump was a Watson Marlow 520U which was pumping at 6 rpm, corresponding to a flow rate of 27 mL/min for this solution. The inlet temperature of the spray chamber was kept constant at 473K and the outlet temperature at 362K. Note that these temperatures are much lower than is the case with a Buchi B290 spray drier, this is because of system limits.

5.2.1.5 C18 Functionalisation

n-Octadecyldimethylchlorosilane was used to functionalize the silanol groups on the surface of the spray dried mesoporous spheres according to a previously described procedure.^{56,187} For every gram of silica, 1.2 g n-octadecyldimethylchlorosilane was dissolved in 60 mL toluene. Commercial anhydrous toluene was used without any additional drying. To this solution, the mesoporous spheres were added and the flask was sonicated for 24 hours. No stirring was applied to prevent the particles from being crushed. The particles were filtered on a nylon filter with $0.45 \mu m$ pores. The fines were removed after functionalization by suspending the material in dichloromethane and collection of the heaviest fraction.

5.2.2 Reversed phase LC evaluation conditions

The particles were then slurry packed at 900 bar with 50/50 hexane/2-propanol as slurry and packing solvent in a (5 cm x 2.1 mm I.D.) column and evaluated on an Agilent 1200 system with an 80 Hz DAD (Diode Array Detector). Subsequently four test mixtures were made: 1) a mixture of parabenes containing uracil, methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate(50 $\mu g/mL$ each dissolved in 50/50 acetonitrile/H₂O); 2) a phenone mixture comprising of uracil, 1-phenyl-1-ethanone, 1-phenyl-1-butanone, 1phenyl-1-pentanone, 1-phenyl-1-hexanone, 1-phenyl-1-heptanone, 1-phenyl-1- octanone, 1-phenyl-1-decanone and 1-phenyl-1-dodecanone (50 $\mu g/mL$ each dissolved in 50/50 acetonitrile/H₂O); 3) a polyaromatic hydrocarbon (PAH) mix with uracil $(50 \ \mu q/mL)$, benzene $(80 \ \mu q/mL)$, naphthalene $(50 \ \mu q/mL)$, antracene $(300 \ \mu q/mL)$, fluoranthene (50 $\mu g/mL$), benzo[k]fluoranthene (300 $\mu g/mL$) diluted in 50/50 acetonitrile/ H_2O ; and 4) a general column test mixture containing uracil (20 $\mu q/mL$), caffeine (100 $\mu q/mL$), phenol (100 $\mu q/mL$), 1-phenyl-1-ethanone (100 $\mu q/mL$), propyl-4-hydroxybenzoate (100 $\mu q/mL$), benzene (500 $\mu q/mL$) and toluene $(500 \ \mu g/mL)$ which was diluted in 40/60 acetonitrile/H₂O.

Mobile phase A was water and B acetonitrile. The following gradient profile was applied for the analysis of mixture 1, 2 and 3: from 50% to 100% B in 7.5 minutes; this composition was kept constant for 2.5 minutes before returning to the initial conditions in 1.5 min and column regeneration for 6 min. The sample volume was $0.5 \ \mu L$. Detection was performed at 210 nm for the phenones and the polyaromatic hydrocarbons and at 254 nm for the paraben mixture. Test mixture 4 was used to evaluate the home packed column (5 cm x 2.1 mm I.D.) and to compare the obtained results with three commercially available C18 columns of the same length (5 cm), namely a Zorbax SB C-18 column from Agilent (I.D. 2.1 mm), a Luna C18 (2) column from Phenomenex (I.D. 4.6 mm) and an X-Bridge C18 column from Waters (I.D. 4.6 mm).Analyses were performed isocratically with 40% acetonitrile/ 60% water. The flow rate used on each column was adjusted such that all void times coincided resulting in the same linear velocity in all cases.Therefore the flow rates used on the MCM-41, Zorbax SB C-18, Luna C-18(2) and on the X-bridge C-18 were 0.1, 0.134, 0.635 and 0.6411 mL/min respectively.

5.2.3 Characterization

Scanning electron microscopy (SEM) images were taken on a FEI Quanta 200FEG microscope with 4 nm resolution operating at 30 kV. Nitrogen sorption experiments were performed at 77 K with a Belsorp-mini II gas analyzer. The different samples were dried overnight under vacuum at 120°C to remove adsorbed water. Diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) was measured on an Equinox 55S from Bruker with a N₂ cooled MCT-B (mercury-cadmium-tellurium) detector. Thermo gravimetric analysis (TGA) was performed on an SDT 2960 from TA Instruments. Particle size distributions were measured with a Malvern 2600c Series Particle Sizer in a H₂O/IPrOH (50/50) suspension. XRD measurements were performed on an ARL X'TRA Diffractometer from Thermo (radiation CuK α generated by 45 kV and 44 mA, with slits of 2, 4, 1.5 and 0.2 for the divergence, scatter, receiving scatter and receiving slit respectively, at 0.25° 2 θ min⁻¹).

5.3 Results and discussion

5.3.1 Control of the surface properties

Initial experiments revealed that there is one major factor affecting the pore and surface properties, which is the CTAB/TEOS ratio. The surface area is proportional to the CTAB/TEOS ratio. This is shown in figure 5.1 and in table 5.2. Next to a rise in surface area, calculated according to the BET theory¹⁸⁸, a small drop in pore diameter from 3.5 nm to 3.2 nm can be seen when the CTAB/TEOS ratio was increased in the studied range. According to Bore et al., this is due to a relatively reduced amount of silica resulting in thinner walls and smaller a_0 values.¹⁸⁹ We also observed these thinner walls and a drop in a_0 , however, contrary to Bore, in our case also the d_p value drops.

The amount of water relative to the other components in the solution has a small influence on the pore properties. When the amount of water is increased from 700 mol

eq to 8600 mol eq the surface area dropped from 1023 m^2/g to 910 m^2/g which is a small decrease taking into account the uncertainty on a BET measurement (here 50 m^2/g).



Figure 5.1: N_2 -sorption isotherms and KJS pore size distributions (inset) as a function of the amount of CTAB.

Table 5.2: The effect of the surfactant concentration on the surface properties, h = wall thickness.

CTAB	H_2O	$SA_{(BET)}$	V_p	$d_{p(KJS)}$	a_0	h
$ratio^a$	$ratio^a$	(m^2/g)	cm^3/g	(nm)	(nm)	(nm)
0.06	1400	390	0.3	3.6	4.7	1.1
0.12	1400	866	0.7	3.5	4.5	1.0
0.21	1400	1086	0.8	3.2	3.9	0.6
0.24	1400	1204	0.8	3.2	3.9	0.6
0.36	1400	739	0.4	/	/	/

^{*a*} Molar ratio compared to a TEOS:CTAB:H₂O:IPrOH/BuOH:HCl (1:0.12/0.24:700/1400:10:5) solution.

Figure 5.2 shows the XRD patterns obtained for the synthesized particles showing the typical pattern of particles with a hexagonal pore structure. It can be seen from figures 5.1 and 5.2, that in all cases hexagonally ordered mesoporous silica spheres are obtained, with a very narrow pore size distribution and the typical isotherm for small mesopores. For this reason, the Kruk-Jaroniec-Sayari (KJS) model is used to calculate the pore size distribution.¹⁹⁰

The other factors (gas flow, flow rate and stirring time) were of little influence on the pore order and surface area.



Figure 5.2: XRD-patterns for spray dried MCM-41 analogues as a function of the CTAB concentration.



Figure 5.3: N₂-sorption isotherm (left) and XRD pattern (right) of a mesoporous silica made with following composition: TEOS:CTAB:H₂O:BuOH:HCl = 1:0.21:9800:10:5.

True EISA or not

To test whether the aerosol assisted EISA is in fact an evaporation induced self assembly, the reaction mixture has been adapted so that the concentration of CTAB was below the CMC of the surfactant. This meant that 9500 equivalents of water had to be added. After spray drying and calcination N₂-adsorption isotherms were measured to determine the porosity of the material. As figure 5.3 shows, the material exhibits the porosity of an MCM-type material with a surface area of 951 m^2/g , a pore volume of 0.6 cm^3/g and a pore diameter of 3.4 nm. This proves that in the case of CTAB the formation of final structure is not based on a liquid crystal templating method, initially introduced by Beck.¹⁶² However it can be either an EISA process or the co-assembly process described by Stucky and his co-workers.^{179,191} As no micelles are present in the spray solution it means that CTAB can form its micelles within the very short time frame of a few seconds.

5.3.2 Control of the particle morphology

Secondly the external morphology of the particles was investigated. This is considered as a critical factor because efficient chromatography is not possible without a spherical morphology. Scouting tests revealed that 3 main factors are affecting the morphology. These are the gas flow, the CTAB fraction, and the amount of H_2O .



Figure 5.4: Particle size distribution measurements as a function of the applied gas flow.

5.3.2.1 The influence of the gas flow

When the atomization force and thus the gas flow is altered from 6 to 10 L/min the particle size distribution shifts to smaller particles as is apparent from the

Chapter 5. Spherical MCM-41 type silica particles by spray drying: doubling the 98 retention factor of HPLC columns

laser scattering experiments shown in figure 5.4. It can also be seen that more uniform particles are obtained when increasing the gas flow from 6 to 8 L/min. Further increase to 10 L/min resulted in a shift of the mean distribution to smaller particle sizes. This is illustrated by the particle size distributions in figure 5.4. An increase in gas flow increases the kinetic energy transferred to the solution flow. This increase in kinetic energy is inversely proportional to the droplet size of the atomized flow. ^{192,193} This means that with increasing gas flow the droplet size has to decrease as well as the particle size, as illustrated in figure 5.4. As a secondary effect, due to the size of the separating cyclone, particles smaller than 1 μm are not collected. Therefore increasing the gas flow does not only produce smaller particles but it also narrows the particle size distribution due to a cut-off effect of the cyclone for sub micron particles.



Figure 5.5: Particle size distribution measurements as a function of the CTAB/ TEOS ratio.

5.3.2.2 The influence of the amount of CTAB

When the amount of CTAB is raised from 0.12 mol eq. to 0.24 mol eq., the uniformity of the particle size distribution decreases. All the remaining factors are kept constant so the changes in particle size distribution could be attributed solely to the change in surfactant. In all cases the 1.78 μm particle size was the highest in abundance and the distribution narrows down to this size with a decreasing amount of CTAB. This leads to suspect that the amount of CTAB influences the degree and the rate of droplet fragmentation in such a way that with a higher surfactant ratio size distribution of the particles broadens. Figure 5.5 shows this evolution clearly. The corresponding scanning electron micrographs (figure 5.6) also show an evolution from spherical to collapsed spheres. Since the spherical morphology collapses this leads to suspect that the surface tension of the solution is

altered. If the solution droplets are to remain perfect spheres during the subsequent drying process the surface tension of these droplets has to withstand the forces it is subjected to on the way to the cyclone. These forces can deform the droplet, unless the surface tension is greater than the deformation forces. The surface tension is inversely proportional with the amount of surfactant in the solution. This would perfectly explain the change in morphology with a higher concentration of CTAB.



Figure 5.6: SEM images of the particles when spray dried with a CTAB/TEOS fraction of 0.12 mol eq., 0.21 mol eq., 0.24 mol eq. from left to right respectively. The scale bar equals 10 μm .

5.3.2.3 The influence of the amount of water and the effect of scaling

When increasing the amount of water from 700 to 2800 mol eq. the particle size distribution shifts to smaller and more uniform particles (figure 5.7). A change in water fraction from 700 to 1400 mol eq. results in a shift from the highest proportion of the particles to smaller particles. Further dilution of the solution with water to 2800 mol eq. results in a narrower particle size distribution. The particle morphology has a comparable evolution as to when the amount of surfactant is varied, but in the case of water the morphology improves when the amount of water is increased (figure 7a, b and c). As can be seen from the SEM micrographs the particles formed from the solution with the lowest amount of water exhibit the same collapsed sphere morphology as when the amount of CTAB is high. This leads to the same conclusion that again the surface tension of the solution is crucial. A larger amount of water leads to an increase in surface tension of the solutions.

One of the advantages of spray drying is the continuous flow set-up, this allows an easy scale-up of the synthesis. This scale-up was necessary to produce enough material to be able to pack an HPLC column. Despite this advantage, the mixing of the products is still a batch procedure. When the amount of solution is multiplied by 10, the morphology changes back to the collapsed spheres (see figure 5.9). Spray drying this solution equally takes 10 times as much time, during which the physical properties of the solutions are changed since the silica precursor continues to polycondensate into oligomers. These oligomers could disrupt the surface tension of the solution, resulting in the observed change in morphology. To overcome this problem the amount of water has been further raised to 5600 and 8400 mol eq. to increase the surface tension of the solution.



Figure 5.7: Particle size distribution measurements as a function of the water/ TEOS ratio.



Figure 5.8: SEM images of the particles when spray dried with a water fraction of (A) 700 mol eq., (B) 1400 mol eq., (C) 2800 mol eq., (D) 5600 mol eq. and (E) 8400 mol eq., respectively The scale bar equals 10 μm .

The SEM pictures (see figure 5.8d and e) show that the thus acquired particles are spherical. Secondly the large batches were spray dried on a larger system, the Mobil Minor from GEA Niro A/S. The system itself had no influence on the particles whatsoever but it allowed to process larger volumes of solution per unit of time.



Figure 5.9: Example of the deformation of the particles from spheres to donut shaped structures as a result of insufficient surface tension. The scale bar equals $10 \ \mu m$.



Figure 5.10: The surface tension of the spray dried solutions as a function of the water content.

In conclusion the formation of spheres through spray drying is strongly dependent on the surface tension of the solution (see figure 5.16). The higher the surface tension of the droplets the less deformation will occur when the particles solidify. Nearly every additive to the solution lowers the surface tension of the droplets, affecting the spherical morphology of the final particles. Therefore, high amounts of water, with its very high surface tension (72.7 mN/m), are beneficial for the formation of spheres.

5.3.3 Porosity versus morphology

A tradeoff has to be made between particle morphology and surface properties because high CTAB concentrations are detrimental for the former and beneficial for the latter. Figure 5.11 represents the surface area and the morphology as a function of the CTAB concentration. The morphology is defined both by the particle shape and the particle size distribution. Since the particle shape is not quantified here, the therm "morphology" has been expressed as the inverse of the standard deviation of the particle size mean in μm (morphology = $1/\sigma_{\mu}$). The ratio of the water versus TEOS in this case is $H_2O/TEOS = 1400$.



Figure 5.11: Plot representing the particle morphology and the surface area as a function of the CTAB/TEOS ratio.

This leads to a study of the other two factors affecting the morphology, i.e. the gas flow (or atomization force), and the $H_2O/TEOS$ ratio. Both of them have a positive influence on the morphology. The atomization force is influenced by system properties and in this case it can be raised to its maximum. The water ratio on the other hand is a solution property and its influence has been discussed in section 5.3.2.3. Figure 5.12 shows the combined influence of the $H_2O/TEOS$ fraction on both the surface area and the morphology, the latter again expressed as the inverse of the particle size deviation of the mean in μm . It is clear that this ratio has a low influence on the pore surface area, especially when compared to the influence of the surfactant on the pore properties. Even when the water ratio is thus high so that the CTAB concentration drops below its CMC (0.92 mM), an ordered mesoporous silica is formed (see 5.3). This suggests that the mechanism to form ordered mesoporous silica spheres through spray drying follows a (cooperative) self assembly rather than a liquid crystal templating method.



Figure 5.12: Plot representing the particle morphology and the surface area as a function of the $H_2O/TEOS$ ratio.

When the morphology itself is regarded, the influence of both the CTAB/TEOS ratio and the $H_2O/TEOS$ ratio follow the same relation as the inverse of the particle size deviation. This is represented by figures 5.6 and 5.9 respectively.

To produce large quantities, higher ratios of water versus the constituents of the solution were needed because of the longer residence time of the stagnant solution in the supply reservoir, affecting the morphology. This of course puts a strain on the daily yield that can be produced. With regard to the discussion above this yield could be enhanced by using a continuous flow set-up. When the solution is not left idle for hours the collapse of the spherical morphology would not occur with lower $H_2O/TEOS$ ratios. So in order to produce with a higher daily yield the mixing step before spray drying should also be in a continuous flow set-up. Current yields are 0.06 g silica per ml TEOS, or 0.12 g silica per hour spray drying. This translates into a molar yield of 22%. The yields are low but the particle size distribution is more important than the yield when the particles are applied as a packing in

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HPLC columns. This is calculated from two large batches of 7 liter solution which was spray dried in 5.6 h each and delivered 1.2331 g of mesoporous silica (weighed after calcination). This amount was sufficient to functionalize with C18, classify and pack in a 5 cm column with an internal diameter of 2.1 mm.

From the above it is clear that the surface tension of the solution is crucial to form spherical particles. Surfactants on the other hand are to be added as a porosity inducing agent. The most promising particles for HPLC applications are therefore a compromise between the spherical morphology and a high surface area. Note that the perfectly spherical materials still possess a surface area of 800 m^2/g . In applications where the morphology is less important, surface areas up to 1200 m^2/g can be obtained in a very reproducible manner.

5.3.4 C-18 Grafting procedure

The optimized particles were grafted with n-octade cyldimethylchlorosilane and characterized by N₂-sorption, TGA and DRIFTS to discover any changes in particle and pore morphology and to reveal the amount of C-18 chains attached to the surface of the particles. Nitrogen adsorption measurements revealed a surface area of 958 m^2/g , a total pore volume of 0.7 ml/g and a pore diameter of 3.3 nm (on the adsorption branch) before grafting. (see figure 5.13)



Figure 5.13: N_2 -sorption isotherm and KJS pore size distribution of the material before and after grafting.

Figure 5.14 A (after grafting) shows that the C-H stretch vibrations of the C18 chain are clearly visible at 2800 cm^{-1} . Furthermore the free silanol peak of the

pure silica at 3746 cm^{-1} has almost disappeared. The peaks below 1500 cm^{-1} are characteristic for silica as such. TGA measurements (figure 5.14 B) revealed 8% w/w of the C18 groups anchored to the surface. With a surface area of 958 m^2/g a number of 0.2 groups per nm^2 is calculated.



Figure 5.14: Drifts measurement (A) and thermogravimetrical measurement (B) of C18 functionalized ordered mesoporous silica spheres.

5.3.5 Evaluation of the column performance and stability by HPLC

To ensure optimal bed stability the columns were packed at a 50% higher pressure (900 bar) compared to the highest operating pressure of the HPLC system (600 bar). In first instance it was thereby important to test the hydrolytical stability of this material as a function of packing pressure and of the column use in HPLC. Therefore, N₂-sorption measurements and scanning electron micrographs were collected of the material before packing and after 300 injections on the column (Figure 5.15). The results attest that neither the porosity nor the morphology was affected (within the systems uncertainty level) by the used packing and HPLC running conditions. This implies that in spite of their high porosity (BET S.A.: $1000 \ m^2/g$) the particles remain stable under the separation conditions used. This was confirmed by particle size distributions.



Figure 5.15: Particle and pore stability as a function of packing conditions, SEM fltr: after grafting and after packing and 300 chromatographic runs. The scale bare equals 5 μm

Subsequently the columns were evaluated with a number of classical test mixtures for reversed phase HPLC. In figure 5.16 A and B the analysis of 4 parabens (and uracil as a void marker) is shown at two flow rates (0.15 and 0.19mL/min, respectively). In figure 5.16 C the separation of a phenone mixture is demonstrated and in figure 5.16 D the analysis of a PAH mix can be seen. Figure 5.16 C was obtained after more than a hundred other analysis corroborating the column stability observation described above. The good peak symmetry visible in figure 5.16 A, B and C suggests that the particle size distribution of the material is sufficiently low such that good columns could be packed.



Figure 5.16: Analysis of a mixture containing uracil (1), methyl-4-hydroxybenzoate (2), ethyl-4-hydroxybenzoate (3), propyl-4-hydroxybenzoate (4) and butyl-4-hydroxybenzoate (5) at 0.15 (A) and 0.19 (B) mL/min, respectively; (C) Analysis of a mixture containing uracil (1), 1-phenyl-1-ethanone (2), 1-phenyl-1-butanone (3), 1-phenyl-1-pentanone (4), 1-phenyl-1-hexanone (5), 1-phenyl-1-heptanone (6), 1-phenyl-1-octanone (7), 1-phenyl-1-decanone (8) and 1-phenyl-1-dodecanone (9); (D) Analysis of a mixture of uracil (1), benzene (2), naphtalene (3), anthracene (4), fluoranthene (5), benzo[k]fluoranthene (6). The flow rate was 0.15 mL/min in A, 0.19 mL/min in B, 0.18 mL/min in C and 0.20 mL/min in D. MP: A: H₂O; B: ACN. Gradient profile: 50% to 100% B in 7.5 min; constant for 2.5 min: return to the initial conditions in 1.5 min and column regeneration for 6 min.

It could be argued that the increasing tailing of the late eluting PAHs is related to exclusion of these large structures from the 3.2 nm pores. This however, is doubtful as a size exclusion mechanism would result in a much reduced retention time of the largest molecules, which was not observed. The size exclusion cut-off of this material was studied injecting polystyrene standards using 100% THF as a non retaining mobile phase. In this way it could be established that the exclusion of

the larger structures was only taking place from a MW of 480 Da and higher. The largest PAH used in these experiments was 0.6 nm in length.



Figure 5.17: Analysis of a mixture containing uracil (1), caffeine (2), phenol (3), 1-phenyl-1-ethanone (4), propyl-4-hydroxybenzoate (5), benzene (6), and toluene (7) on an X-bridge (A), a Zorbax SB C18 (B), a Luna C18 (2) (C) and a home-made (D) column.

A drawback of gradient analysis is that different degrees of peak compression occur depending on the gradient steepness. This makes it difficult to measure important chromatographic parameters such as column efficiency (N) and retention factors (k'). These can be measured from the chromatograms by equations 2.1 and 2.5 on pages 23 and 24.

Figure 5.17D shows the analysis of a test mixture under isocratic mobile phase compositions, which allows to draw a number of conclusions. A distinction is hereby made in the discussion about column performance between efficiency and retention. Providing that the particles show a narrow particle size distribution and that the packing process is optimized, the efficiency is expected to be equal to L/2dp, whereby L corresponds to the column length and dp to the particle size.¹⁹⁴ Hence 13000 plates should be expected on this column and only about 5000 are observed. It is obvious that this aspect needs further improvement by drastic up scaling and extensive size classification, making use of high cost air classifiers followed by fully optimized column packing processes.

The real benefit of the proposed material is not to be found in efficiency improvements, which will at best reach theoretical limits. But it can be found in the much enhanced retention factor which can be achieved with this stationary phase because of a much higher amount of accessible stationary phase compared to conventional silica particles where most of the stationary phase is never reached by the analytes. This opens a lot of possibilities to retain, and therefore separate, a large number of solutes that today are considered too polar to analyse by reversed phase LC such as underivatized amino acids, sugars, glycoproteins and many important metabolites. For these analytes there is much need to be able to analyse them in the same reversed phase analysis together with the other more hydrophobic solutes without the requirement of using another additional HPLC mode, such as hydrophilic interaction LC (HILIC).⁹⁶

Column	k'	$SA_{(BET)}$	Carbon Load	Groups	k/group
		(m^2/g)	m w%	per nm^2	
mesoporous	33	957	8	0.2	0.17
Luna	14.5	400	17.5	1.1	0.03
X-bridge	8.6	185	18	2.3	0.02
Zorbax	12.2	180	10	1.3	0.05

Table 5.3: An extended retention study on the tested columns.

Upon comparing the analysis of the test mixture on the ordered mesoporous column with analyses performed on commercial state-of-the art columns with identical mobile phases, it can be seen that the retention factor is more than double that of the commercial columns (figure 5.17 A-D). This can be coupled to the specific surface area of the packing. The mesoporous packing exhibited twice the surface area of a Luna column while the retention factor also doubles. This apparent direct relation between the surface are is no longer valid for the Zorbax and Xbridge materials, as can be concluded from table 5.3. It depicts the retention factor next to the amount of C18 groups per nm^2 . It shows that the retention per C18 group is greatly increased. For toluene a k' of 33 was obtained on the ordered mesoporous column whereby k' was 14.5, 12.2 and 8.6 on the Luna C-18 (2), Zorbax SB C-18 and on the X-Bridge C-18 column, respectively. Note that this was obtained with only 8% carbon load on the mesoporous silica, whereby e.g. the Luna, Xbridge and the Zorbax columns contain 17.5, 18, and 10%(w/w) of octadecyl groups, respectively. This suggests that if longer grafting conditions are used even higher retention is possible. Note that all columns were of the same length and that for each column the flow rate of the mobile phase was adjusted such as to obtain the same void time in all cases. In this way the same linear mobile phase velocity was obtained in each analysis. This new material can be considered therefore promising for use in HPLC.

5.4 Conclusions

The main factors which, from this study, appeared to influence the spherical morphology of the spray dried hexagonally ordered mesoporous silica spheres are the CTAB concentration, the water fraction and the gas flow. Raising the gas flow and the amount of water results in smaller and more uniform particles with a spherical morphology. None of the instrumental settings, including the gas flow appeared to be affecting the mesopore structure. The amount of water has a mildly negative influence on the surface properties, but only when the amount is very high. The CTAB concentration on the other hand has clearly a positive influence on the surface properties.

All these observations indicated the importance of one critical component, the surface tension of the solution. When the surface tension is high the droplets formed at the tip of the spray drier will not deform easily. Water has one of the largest surface tensions measured, so adding water leads to a better morphology. CTAB is a surfactant which lowers the surface tension, resulting in unfavorable morphology. Hence the synthesis of hexagonally ordered mesoporous silica spheres is a trade-off between excellent surface properties and a spherical morphology.

The particles were used as a packing material in an HPLC column and compared to state of the art commercial columns. The high surface area leads to a doubling of the retention factor. Nevertheless the column allowed the analysis of various test mixtures in less than 5 min and is promising for ultraperformance HPLC applications where such high retentions are necessary.

The next chapter describes how ordered mesoporous silica materials with larger pores can be spray dried.

6

Increasing the pore size of spray dried silica spheres

6.1 Introduction

As shown in the previous two chapters the production of spherical M41S type materials is well defined. The biggest challenge now is enlarging these pores to the size of around 10 nm. SBA type materials have these larger pores but still there are few reports in literature of them being spray dried. Most of the literature reports very large or small or jointed spheres, unsuitable to be used as a packing material. Several additives have been used as a co-solvent or a co-surfactant such as ethanol or CTAB.

The hydrothermal Stöber synthesis method has the advantage that it produces, under the non-modified procedure, perfectly uniform spheres. It has the disadvantage that upscaling could be subjected to concentration problems or heat dissipation problems. But foremost the addition of surfactant molecules to the classic Stöber method reduces the ability to control the particle size and particle aggregation because it interferes with the dispersing effect ethanol has in the method. Therefore an aerosol assisted synthesis could bring a solution for all of these problems.

As demonstrated in the previous chapter this is a fairly easy technique to produce spherical M41S type materials. However the larger, more slowly assembling triblock co-polymers on the other hand, take more time to assemble into an ordered mesoporous system. Because these surfactants are large, it strains the micelle formation and co-assembly process, necessary to form ordered mesopores when the particle formation is sped up by a spray drier. As demonstrated in the previous chapter this obstacle is not present with the smaller ionic surfactants (such as cetyltriammonium bromide), even with a surfactant concentration below the critical micelle concentration (CMC) it still forms an M41S type material and within a few seconds the solution dries and solidifies. This of course has consequences for their application in a spray drying set-up. An aerosol assisted (or spray drying) set-up has two advantages over a hydrothermal synthesis. Firstly, it produces particles through the formation of spherical droplets making it easier to retain this spherical morphology in the dried particles. Secondly, it allows for an easy scale up of the entire synthesis procedure since the essential reaction vessel remains the same regardless of the amount to be spray dried, namely the size of a droplet.

6.2 The hexagonal SBA-15 pore system

6.2.1 Experimental

The synthesis of these materials is based on a modified Stöber method reported by Pitchumani et al. (composition of SBA15-SD-1).¹⁹⁵ The composition and the optimization procedure are summarized in table 6.1 and 6.3. The solution was stirred for a certain amount of time at a certain temperature and then fed to the spray dryer. It is imperative that there is no precipitation because this can block the spray head and it can distort the final particle morphology. The acquired powders were collected from the cyclone, calcined and characterized with SEM and N₂-sorption.

SBA-15	TEOS	P123	CTAB	H_2O	HCl	EtOH
						BuOH
SBA15-SD-1	1	0.009	0	10	0.012	4.5
SBA15-SD-2	1	0.012	0.021	450	3.5	0
SBA15-SD-3	1	0.012	0.021	450	3.5	1
SBA15-SD-4	1	0.009	0	10	0.012	4.5
SBA15-SD-5	1	0.009	0	10	0.012	3
SBA15-SD-6	1	0.009	0	10.5	2	4.5
SBA15-SD-7	56	1	0	1562	220	490
SBA15-SD-8	56	1	0	1184	110	490
SBA15-SD-9	42	1	0	1562	220	490
SBA15-SD-10	42	1	0	1184	110	490
SBA15-SD-11	35	1	0	976	92	403
SBA15-SD-12	42	1	0	1562	220	490

Table 6.1: The composition of the spray dried solutions.

SBA-15	Stirrtime	Stirrtemp	Agetime	Agetemp
	(min)	(°C)	(min)	(°C)
SBA15-SD-1	60	20		
SBA15-SD-2	5	25	1440	95
SBA15-SD-3	5	25	40	95
SBA15-SD-4	30	20	80	95
SBA15-SD-5	60	20	200	95
SBA15-SD-6	5	90		
SBA15-SD-7	30	90		
SBA15-SD-8	30	90		
SBA15-SD-9	30	90		
SBA15-SD-10	30	90		
SBA15-SD-11	30	90		
SBA15-SD-12	60	90		

Table 6.2: The synthesis conditions of spray dried SBA-15

6.2.2 Results and discussion.

Avoiding a mixed pore structure.

Figure 6.1 depicts the isotherms for SBA15-SD-1, 4 and 5 and table 6.3 gives the pore properties. The isotherms show a shift from a mixed pore structure (presence of a second desorption step) to a single pore structure when the stirring step at 20 °C is reduced to 30 minutes and an aging step (80 minutes at 95 °C) is added. However the surface area and the pore volume are still strongly reduced, suggesting a lot of non porous material is present. It has been previously reported in literature that the aging time and temperature improve the pore structure of the materials. The reason for this can be found in the fact that micelles of triblock co-polymers are first disk shaped with a quick transition to a spherical shape. The subsequent aggregation to rod-like micelles occurs within the next 1.5 hours, this is called a sphere-to-rod transition. The addition of TEOS accelerates this process with the first cylindrical micelles occurring after 30 minutes.^{196,197} When the stirring time was doubled again to 1 hour, the aging time was raised to 200 minutes and butanol was added an SBA-type material is no longer obtained. The reason for this is still unclear but it is suspected that the surfactant is destroyed. The obtained material exhibits smaller pores, a lower pore volume and thus a higher surface area.

Material	$SA_{(BET)}$	V_p	d_p
	$(m^2/g)^{'}$	(cm^3/g)	(nm)
SBA15-SD-1	264	0.39	8.1 (BJH)
SBA15-SD-4	182	0.25	6.24 (BJH)
SBA15-SD-5	417	0.19	2.3 (KJS)

Table 6.3: The pore properties of material 1, 4 and 5



Figure 6.1: The N₂-sorption isotherms of SBA15-SD-1, 4 and 5

Figure 6.2 shows the particle morphology of material 4 and 5 measured on SEM. Material 4 has a very broad particle size distribution ranging from 25 down to a few µm. When the conditions are shifted to the ones for SBA15-SD-5 small non spherical silica seed particles grow on the larger spherical like particles. The morphology itself ranges from perfectly spherical to collapsed spheres. As explained in chapter 5 this can be attributed to a lower surface tension of the feed solution. This leads to the conclusion that more water will be needed during the synthesis, both to increase the surface tension of the solution and by dilution of the sample to obtain smaller particles with more narrow particle size distributions.

The influence of HCl and TEOS

The HCl concentration was raised to improve the interaction between TEOS and the surfactant because both the H^+ and the Cl^- ions play a role in the anion mediated interaction between TEOS and the surfactant. The stoichiometrics of the interaction predicts there are two HCl molecules needed for a perfect interaction. However, when this was tried (SBA15-SD-6) the solution formed a gel, whilst the early stages of a sol were needed to be able to successfully spray dry a mesoporous material. To prevent this from happening, half of the HCl was added at the start and half was added just before the solution was spray dried. This way the structure can already partly polycondensate around the surfactant molecules. This second hydrochloric acid amount was not added in solution 8 and 10 to test the influence of this second amount of acid to the pore properties.



Figure 6.2: The particle morphology for SBA15-SD-4 (A) and 5 (B)

A second modification to the solution feed was lowering the amount of TEOS as this has been reported to increase the pore size.¹⁹⁸ The final modification performed on all four samples equally was an increase of the stir temperature to 90 °C because from the former section it was apparent better pore properties were acquired.

So to see the effect of the HCl concentration sample 7 has to be compared with 8 and 9 has to be compared with 10. To asses the effect of TEOS, sample 7 has to be compared with 9 and 8 with 10.

Figure 6.3 and table 6.4 represent the pore properties of the obtained materials when these modifications were applied. Firstly it is immediately clear that these modifications have little to no effect on the pore properties, except for the pore size. The pore size increases when the amount of hydrochloric acid is halved. This can be explained by a reduced interaction between TEOS and the surfactant due to less HCl in the solution. The final modification performed on all four samples was increasing the temperature of the stir step. The largest influence on the pore properties, in comparison with SBA15-SD-1, 4 and 5, is suspected to be the augmented stir temperature.

Material	$SA_{(BET)}$	V_p	$d_{p(BJH)}$
	(m^2/g)	(cm^3/g)	(nm)
7	405	0.57	5.5
8	423	0.67	7.1
9	461	0.61	5.5
10	438	0.60	6.2

Table 6.4: The pore properties of SBA15-SD-7, 8, 9 and 10

In terms of morphology there was no improvement, on the contrary, it was further from a spherical morphology than samples 4 and 5. Additionally, the HCl and





Figure 6.3: The N_2 -sorption isotherms of SBA15-SD-7, 8, 9 and 10. The offset of 8, 9 and 10 was 100, 200 and 300 respectively.

The influence of the surfactant and the stirring time

By increasing the amount of surfactant, the pore size should also increase. To assess this effect sample 9 is compared with sample 11. Also by doubling the stirring time, the TEOS/surfactant assembly gets more time to improve the structure. Important here is to keep the spray dried solution clear so that no precipitates can clog up the spray head. Figure 6.4 and table 6.5 show that an increase in surfactant has an influence only on the pore size (an increase with almost 30%), it has no significant influence on the rest of the pore properties.

 $\overline{\mathbf{V}}_p$ Material $SA_{(BET)}$ $d_{p(BJH)}$ $(cm^{3}\underline{/g})$ (m^2/g) (nm)9 4610.615.511 7.14380.61125020.574.8

Table 6.5: The pore properties of material 9, 11 and 12

The stirring time on the other hand has an influence on all the pore properties (compare sample 9 with sample 12). The surface area increases slightly with 9% and the pore volume and pore diameter decrease slightly with 7% and 13% respectively. The N_2 -isotherm shows a broader pore size distribution.



Figure 6.4: The N_2 -sorption isotherms of SBA15-SD-9, 11 and 12. The offset of 11 and 12 was 50 and 100 respectively

The influence of a co-surfactant

A different approach is to add a second surfactant to the mix, the surfactant commonly added in literature is CTAB.⁵⁶ This surfactant is normally used to synthesize MCM type materials. As reported in literature this was initially a hydrothermal synthesis procedure. The solutions were first stirred at 25°C for 5 minutes and then aged for either 24 hours or 40 minutes respectively at 95°C. A second difference between solution 2 and 3 is the addition of butanol to solution 3 as this seems to improve the pore structure in most cases. However in this case the addition of BuOH seemed to reduce the surface area and a second desorption step occurred (see figure 6.5 and table 6.6). As both the aging time and the co-solvent have been changed, no conclusion on the influence of either can be made.

Material	$\begin{array}{c} \mathrm{SA}_{(BET)} \\ (m^2/g) \end{array}$	$\frac{\mathbf{V}_p}{(cm^3/g)}$	$\begin{array}{c} \mathbf{d}_{p(BJH)} \\ \text{(nm)} \end{array}$
2	742	0.57	6.2
3	436	0.59	7.1

Table 6.6: The pore properties of materials 2 and 3



Figure 6.5: The N_2 -sorption isotherms of SBA15-SD-2 and 3

The aging time seems to have the largest influence on the morpholgy. The spherical morphology is better retained when the aging time is shorter. This seems to be in relation to what Alonso et al. discovered for M41S type materials.⁷⁴ Figure 6.6 could show a more uniform spherical morphology at lower aging times. However since this can also be attributed to the addition of butanol no solid conclusion can be drawn here either.



Figure 6.6: The particle morphology for materials 2 (A) and 3 (B).

6.3 The cubical SBA-16 pore system

6.3.1 Experimental

The synthesis conditions are based on the composition of a standard hydrothermal SBA-16 synthesis. The difference is that the solutions are fed to a spray drier and not left to age for two days. Table 6.7 summarizes the synthesis conditions of the spray dried SBA-16 material. For the last two compositions the amount of hydrochloric acid was added in two batches, one half immediately and the rest just before feeding it to the spray drier.

SBA-16	TEOS	F127	H_2O	HCl
SBA16-SD-1	1	0.006	320	4,23
SBA16-SD-2	1	0.006	320	4,23
SBA16-SD-3	1	0.006	320	4,23
SBA16-SD-4	1	0.006	320	4,23
SBA16-SD-5	1	0.008	430	$5,\!64$
CDA 1C	C1:	d 1. 1	A	A 4
5BA-10	Surrume	Stirrtemp	Agetime	Agetemp
5DA-10	(min)	(°C)	(min)	Agetemp (°C)
SBA-10 SBA16-SD-1	(min) 5	(°C) 45	Agetime (min) 200	Agetemp (°C) 90
SBA-10 SBA16-SD-1 SBA16-SD-2	Stirrtime (min) 5 5	Stirrtemp (°C) 45 45	Agetime (min) 200 120	Agetemp (°C) 90 90
SBA16-SD-1 SBA16-SD-2 SBA16-SD-3	Stirrtime (min) 5 5 30	Stirrtemp (°C) 45 45 90	Agetime (min) 200 120 /	Agetemp (°C) 90 90 /
SBA16-SD-1 SBA16-SD-2 SBA16-SD-3 SBA16-SD-4	5 5 30 5	Stirrtemp (°C) 45 45 90 45	Agetime (min) 200 120 / 30	Agetemp (°C) 90 90 / 90

Table 6.7: The synthesis conditions of spray dried SBA-16

6.3.2 Results and Discussion

Table 6.8: The pore properties of spray dried SBA-16 materials

Material	$SA_{(BET)}$	V_p	$d_{p(BJH)}$
	(m^2/g)	(cm^3/g)	(nm)
SBA16-SD-1	517	0.28	4.3
SBA16-SD-2	354	0.24	4.3
SBA16-SD-3	272	0.34	8.1
SBA16-SD-4	246	0.34	8.1
SBA16-SD-5	326	0.50	9.26

The influence of the aging time

Because the first solution became clouded after 200 minutes, the aging time was gradually reduced until a clear solution was obtained. Clouding in the solution indicates that precipitates are being formed which have to be avoided to ensure optimal control over the final particle morphology and to prevent the spray head from clogging up. Nevertheless, the solution was spray dried, with caution with SBA16-SD-1 as a result. Figure 6.7 and table 6.8 show that as a function of the

aging time the surface area drops and the mean pore diameter rises. Moreover a second desorption step is observed as the aging time drops, indicating the deposition of non porous silica material into the pores. With a reduction in aging time the sols do not have enough time to fully co-assemble with the surfactant, leading to large portions of non porous silica material. These reduce the surface area per gram and can also lead to plugged systems, reflected by the secondary desorption step. These plugged systems were primarily described by Van Der Voort.^{172,199}



Figure 6.7: The N_2 -sorption isotherms of SBA16-SD-1, 2 and 4 as a function of aging time (1: 200 min, 2: 120 min and 4: 30 min.)

The SEM pictures in figure 6.8 show that the morphology is closer to a spherical morphology than the SBA-15 materials but still is not what it should be. It seems that with a longer aging time mainly collapsed spheres are formed, while the shorter aging times show more coagulated near spherical particles. This observation has also been made with spray dried SBA-15 and MCM-41 materials.

The longer aging time mainly improve the pore properties of the spray dried material. This indicates that the co-self assembly process for the larger Pluronic surfactants requires more time than for the smaller CTAB family of surfactants. Several reports on the assembly process of Pluronic based ordered mesoporous materials state that the first evidence of an ordered pore structure appears after 30 minutes of reaction time. After that the pore structure is reinforced while it grows. So after 30 minutes more and more material obtains the ordered pore structure. ^{196,197,200,201}



Figure 6.8: The particle morphology as a function of the aging time (A: 200 min (SBA16-SD-1); B: 30 min (SBA16-SD-4)).

The influence of the amount of precursor

When the amount of TEOS is reduced the surface area, the pore volume and the pore size strongly increase. This can be attributed to the fact that the surfactant molecules are given more space to create larger micelles.¹⁹⁸ Figure 6.9A and table 6.8 clearly show this evolution. The secondary desorption step is not present when the amount of TEOS is reduced. This indicates that no excess silica material is deposited inside the pores, which would lead to a so-called plugged system.



Figure 6.9: The N₂-sortion isotherms of SBA16-SD-4 and SBA16-SD-5.

The influence of the pre spray drying conditions

When the same stirring regime is applied as for the SBA-15 materials (no aging time, stirring at 90°C), the second desorption step appears on the N₂-sorption isotherms (see figure 6.10). This indicates that stirring or aging delivers the same result as long as the temperature is 90 °C.



Figure 6.10: The N₂-sorption isotherms of SBA16-SD-3 and SBA16-SD-4.

6.4 Conclusion

To increase the pore size of ordered mesoporous materials a larger surfactant has to be used, therefore triblock co-polymers of the Pluronic family are applied. In a first step the emphasis of the synthesis was put on obtaining an ordered SBA structure which is not evident due to speed of the spray drying process. The research is at the point that it is possible to spray dry an ordered mesoporous SBA-15 material with good pore properties but the morphology of the particles still has to be tuned to obtain spherical particles. For SBA-16 materials the research is still on going to obtain a well structured material.

The main obstacle to obtain ordered mesoporous SBA-type materials is the speed of the reaction time. The co-assembly process to form these materials takes minimum 30 minutes while spray drying takes about 6 seconds. This means that SBA materials need to be spray dried right at the moment right before precipitation. At that point silica/surfactant aggregates have been formed which can still remain in solution but show the structure of an ordered mesoporous SBA-type material. It seems that the cubically structured mesoporous SBA-16 material will impose less problems to obtain a spherical particle design. This can maybe attributed to the fact that the used surfactant prefers to form spherical micelles instead of cylindrical ones. Nevertheless before these materials can be tested for their chromatographic usefulness a lot of work still has to be done.

During the next chapter the hydrothermal stability of the packing materials will be improved.
7

Hybrid silica particles with ordered porosity: chromatographic packing materials with increased stability

7.1 Introduction

To counteract the hydrolysis of silica structures, the oxygen bridges in silica can be replaced by carbon bridges. First off, these are impervious to the attack of water and secondly they render the complete material more hydrophobic, reducing the water adsorption to the silica surface. With this philosophy in mind the best available organosilane to use as a silica precursor is 1,3,5-tris[diethoxysila]cyclohexane (TDSCH) (see figure 7.1 on page 126), firstly used to prepare a PMO by Landskron et al.^{39,40} Standard silica has 4 oxygen atoms connecting each silicon atom. With the aforementioned silane this number is reduced to 2 oxygens per silicon atom. As has been shown in previous work in the group, the resulting PMO material possesses a pH stability up to pH 14.²⁰²

This chapter demonstrates how PMOs have been spray dried for the first time generating a superiorly ordered mesoporous organosilica sphere for high end separations in HPLC (High Performance Liquid Chromatography). The use of 1,3,5tris[diethoxysila]cyclohexane as a silica precursor reduces the number of hydrolysable siloxane bridges from four to two, greatly improving its hydrothermal stability. This way a column packing was produced with a very high retention factor and a pH stability ranging from 0 to 14. The pore structure of this ring PMO is similar to the M41S type materials. Our previous experience with spray drying spherical M41S type materials was the basis on which this new material was based. It is shown that ordered mesoporous organosilica materials with high organic content are superior in terms of column and particle stability when applied as a packing material for high end separations in HPLC (High Performance Liquid Chromatography). The use of a highly uniform spherical morphology is essential in a packed column because it results in a significant reduction of Eddy diffusion. The synthesized prototype column still had a rather broad particle size distribution, which means that the plate numbers were substantially lower than the theoretical plates. Additionally, the highly ordered and uniform pore size results in superior retention effects, as previously evidenced.

After the synthesis of the particles an in depth analysis of the columns was performed. The column was tested for various mixtures to screen the stability of the column, the ability to retain polar compounds, the packing density and the standard performance of the column.

7.2 Experimental

7.2.1 Synthesis

7.2.1.1 Synthesis of the ring-precursor.

The synthesis of the ring-precursor has been slightly up-scaled from the synthesis reported by Goethals et. al.²⁰² In a first step 0.5 g of iron(III)chloride is dissolved in 200 mL commercially anhydrous tetrahydrofuran (THF). Separately in a 2 L round bottom flask, 14 g of magnesium chips are introduced under an argon atmosphere with a reflux-cooler attached to it. To this the iron(III)chloride solution is added drop-wise, hereafter the mixture is stirred for an hour until the yellow color has disappeared. During this 40 mL of (chloromethyl)triethoxysilane (CTES) is mixed with 140 mL of THF and stored under argon. Once the yellow color has disappeared the silane mixture was added drop-wise in 30 minutes. The entire mixture was subsequently stirred for 32 hours at 50°C. After the reaction mixture was cooled down, the magnesium-iron pulp was filtered off and a mixture of THF with the silicon oil was recovered. THF was evaporated and 100 mL n-pentane was added to the silicon oil. A white precipitate (MgCl) was formed and filtered off. Once all precipitate was removed pentane was evaporated until only a clear silicon oil remained. If the remaining oil is not clear, heavier derivatives have been formed. These heavy fractions can be distilled under vacuum at 160°C. See figure 7.1 below for the reaction scheme.

Figure 7.1: The formation of the ring precuror, R1 is an ethoxy group.

7.2.1.2 Particle synthesis.

The experimental setup comprises a mixing and a spray drying step. Spray drying was performed on a Buchi B290 with a two-fluid nebulizer connected to pressurized air. The heater inlet temperature was held constant at 493K and the outlet temperature remained constant at 398K. The aspirator ran at a maximum air velocity of 40 m^3/h . The nozzle gas flow and the solution feeding speed were held constant at 9 L/min and 10% of maximum solution flow respectively. First an optimization of the solution composition had to be performed in order to obtain spherical PMO materials with good pore properties. The primary spray solution was based on the first publication of the ring PMO by Landskron et al.⁴⁰ Table 7.1 shows the composition of the solutions during the optimization procedure.

Material	TDSCH	CTAB	H_2O	BuOI	H HCl
rPMO-SD1	1	0.71	4663	27	20
rPMO-SD2	1	0.32	2055	12	7.4
rPMO-SD3	1	0.32	3000	12	7.4

Table 7.1: The compositon of various spray dry solutions.

The last composition (rPMO-SD3) yielded spherical ring PMOs with good pore properties, this composition was used to produce the particles for the final column. During the first step a solution of CTAB, H₂O, 1,3,5-tris[diethoxysila]cyclohexane, HCl and 1-butanol were mixed at room temperature for 15 minutes. Subsequently this mixture was fed to the spray dryer, resulting in inorganic-organic hybrid M41S type powders, which were collected by a cyclone separator. To prevent that precipitates are introduced into the spray-tip, a 0.45 µm nylon syringe filter was fitted before the spray-tip. To obtain a large amount of particles six subsequent solutions of 160 mL were fed to the spray drier. The particles were washed with an acidified ethanol solution (ethanol-HCl²¹) to remove the surfactant. After this the samples were analyzed with XRD, N₂-adsorption, elemental analysis and SEM to obtain information about the porosity, the purity and the morphology.

7.2.1.3 Post-synthesis functionalization.

n-Octadecyldimethylchlorosilane was used to functionalize the silanol groups on the surface of the spray dried mesoporous spheres according to a previously described procedure (see chapter 5) For every gram of silica, 1.2 g n-octadecyldimethylchlorosilane was dissolved in 60 mL toluene. Commercial anhydrous toluene was used without any additional drying. To this solution, the mesoporous spheres were added and the flask was sonicated for 24 hours. No stirring was applied to prevent the particles from being crushed. The particles were filtered off on a nylon filter with 0.45 μ m pores.

This material was end-capped with hexamethyl disilazane (HMDS). For every gram of silica 1.6 mL of HMDS was added and the mixture was sonicated for 5 hours. Hereafter the particles were filtered off on a 0.45 μ m nylon filter. After functionalization the particles were used without further classification.

7.2.2 Reversed phase LC evaluation conditions

The particles were slurry packed at 900 bar with hexane as slurry and packing solvent in a (5 cm x 2.1 mm I.D.) column and evaluated on an Agilent 1200 system with a diode array detector.

7.2.2.1 Total pore blocking method.

The total pore blocking method was used to determine the external porosity of the column.²⁰³ First the column was flushed for an hour with isopropanol at 0.2 mL/min to remove all aqueous and organic compounds. After this the column was flushed with decane for 1.5 hours at 0.2 mL/min to ensure that all the pores are completely filled with decane. Decane thereby plays the role of pore blocking solvent. Hereafter the column was removed from the system and a zero dead-volume connection was fitted. Subsequently the system was flushed with isopropanol at 0.2 mL/min for five minutes. After this the system was flushed with a 10 mM ammonium acetate (pH 3) buffer at 0.2 mL/min for five minutes. Then the column was fitted back in the system and flushed with buffer at 0.1 mL/min for 600 minutes, injecting 0.5 µl of a 1 mg/mL NaI solution. Here the Γ ion was detected by the UV detector at 210 nm and acts as a dead time marker.

7.2.2.2 Standard HPLC testing.

For the standard column test four test mixtures were made: 1) a mixture of parabenes containing uracil, methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate (50 µg/mL) each dissolved in 30/70 acetonitrile/H₂O); 2) a phenone mixture comprising uracil, 1-phenyl-1-ethanone, 1-phenyl-1-butanone, 1-phenyl-1-pentanone, 1-phenyl-1-hexanone, 1-phenyl-1-hexanone, 1-phenyl-1-heptanone, 1-phenyl-1-decanone and 1-phenyl-1-do-decanone (50 µg/mL) each dissolved in 50/50 acetonitrile/H₂O); 3) a polyaromatic hydrocarbon mix with uracil (50 µg/mL), benzene (80 µg/mL), naphthalene (50 µg/mL), fenantreen (300 µg/mL), fluoranthene (50 µg/mL), 7,12 dimethyl-benz[a]antraceen (300 µg/mL) diluted in 50/50 acetonitrile/H₂O; and 4) a general column test mixture containing uracil (20 µg/mL), caffeine (100 µg/mL), phenol (100 µg/mL), 1-phenyl-1-ethanone (100 µg/mL), propyl-4-hydroxybenzoate (100 µg/mL), benzene (500 µg/mL) and toluene (500 µg/mL) which was diluted in 40/60 acetonitrile/H₂O.

Mobile phase A was water and B acetonitrile. The following gradient profile was applied for the analysis of mixture 1: 0.5 minutes constant flow at 30% B, then from 30% to 100% B in 9.5 minutes; this composition was kept constant for 1.5 minutes before returning to the initial conditions in 0.5 min and column regeneration for 3 min, the flow rate was 0.15 mL/min; mixture 2: from 50% to 100% B in 5 minutes; this composition was kept constant for 2.5 minutes before returning to the initial column regeneration for 6 min, the flow rate was 0.20 mL/min; mixture 3: from 50% to 100% B in 10 minutes; this composition was kept constant for 1 minute before returning to the initial conditions in 0.5 min and column regeneration for 5 min the flow rate was 0.20 mL/min; mixture 3: from 50% to 100% B in 10 minutes; this composition was kept constant for 1 minute before returning to the initial conditions in 0.5 min and column regeneration for 5.5 min and column regeneration for 5.5 min and column regeneration for 5.5 min and column regeneration. The sample

volume was 0.5 µl. Detection was performed at 210 nm for the phenones and the polyaromatic hydrocarbons and at 254 nm for the paraben mixture. Test mixture 4 was used to compare the home packed column (5 cm x 2.1 mm I.D.) with three commercially available C18 columns of the same length (5 cm), namely a Zorbax SB C18 column from Agilent (I.D. 2.1 mm), a Luna C18 (2) column from Phenomenex (I.D. 4.6 mm) and an X-Bridge C-18 column from Waters (I.D. 4.6 mm). Analyses were performed isocratically with 40% acetonitrile/ 60% water. The data for the commercial columns is depicted in figure 5.17 on page 108 of chapter 5. The used flow rate was 0.15 mL/min.

For the van Deemter analysis of the column a test mixture containing uracil (20 μ g/mL) and 1-phenyl-1-ethanone (100 μ g/mL) diluted in 40/60 acetonitrile/H₂O was injected (0.5 μ L) at different linear velocities between 0.010 ml/min and 0.200 ml/min.



Figure 7.2: The chemical structure of desethyl-2-hydroxyatrazine (1), desethyldesisopropylatrazine (2), desisopropylatrazine (3), aldicarb (4), cyanazine (5), simazine (6), prometryne (7), atrazine (8), propazine (9) and diphenhydramine (10).

7.2.2.3 Polar compounds.

To test the retention capacity for polar compounds a test mixture with polar pesticides was made containing NaI (1 mg/mL), desethyl-2-hydroxyatrazine (200

 μ g/mL), desethyl desisopropyl atrazine (400 μ g/mL), desisopropyl atrazine (50 μ /gram/mL), aldicarb (50 μ g/mL), cyanazine (50 μ g/mL), simazine (400 μ g/mL), prometryne (50 μ g/mL), atrazine (50 μ g/mL) and propazine (50 μ g/mL) which was diluted in 50/50 acetonitrile/water (see figure 7.2 for the structures). The analysis was performed isocratically at 0.1 mL/min with 20% acetonitrile/ 80% water with a sample volume of 0.5 μ l and detection at 254 nm both on the home made column and on a commercial Kinetex column with 2.6 μ m C18 particles (5 cm, 2.1 mm I.D.).

7.2.2.4 Column stability.

To test the column stability a test cycle was set up with acid and base flushes. The following mobile phases were prepared: A1: 1 L of a 10 mM ammonium bicarbonate at pH 10.5 solution in water; B1: 2.5 L acetonitrile; A2: 2.5 L water; B2: 1L of a 50/50 water/acetonitrile with 0.5% of formic acid at pH 2 solution.

The following cycle of flushes and analyses were performed on the column, the six steps were repeated 13 times.

Step 1: 24 times high pH flush procedure: (mobile phases A1 and B1)

The following gradient profile was applied: from 5% to 95% B1 in 6 minutes; this composition was kept constant for 2 minutes before returning to the initial conditions in 1 min and column regeneration for 2 min, the flow rate was 0.15 mL/min.

Step 2: high pH testing: (mobile phases A1 and B1)

The analysis was performed isocratically with a 50/50 composition of A1 and B1 at a flow rate of 0.15 mL/min with detection at 230 nm of a sample mixture of uracil (50 µg/mL) and diphenhydramine hydrochloride (300 µg/mL) diluted in 50% A1 and 50% B1 and a 0.5 µL injection volume. The injection was performed 3 times and the analysis ran for 10 minutes.

Step 3: 2 times neutral pH flush procedure: (mobile phases A2 and B1) The following gradient profile was applied: 2 minutes constant flow at 5% B1, then from 5% to 100% B1 in 3 minutes; this composition was kept constant for 5 minutes before returning to the initial conditions in 1 minute, the flow rate was 0.15 mL/min.

Step 4: neutral pH testing: (mobile phases A2 and B1)

The analysis was performed isocratically with a 40/60 composition of B1 and A2 at a flow rate of 0.15 mL/min with detection at 210 nm of a sample mixture 4 described two sections above. The injection was done 3 times with an injection volume of 0.5 μ l and an analysis time of 20 minutes.

Step 5: 24 times low pH flush procedure: (mobile phases A2 and B2)

The following gradient profile was applied: from 5% to 95% B1 in 6 minutes; this composition was kept constant for 2 minutes before returning to the initial conditions in 1 minute and column regeneration for 2 min, the flow rate was 0.15 mL/min.

Step 6: 2 times neutral pH flush procedure: (mobile phases A2 and B1) The following gradient profile was applied: 2 minutes constant flow at 5% B1, then from 5% to 100% B1 in 3 minutes; this composition was kept constant for 5 minutes before returning to the initial conditions in 1 minute, the flow rate was 0.15 mL/min.

7.2.3 Characterization

Scanning electron microscopy (SEM) images were taken on a FEI Quanta 200FEG microscope with 4 nm resolution operating at 30 kV.

Nitrogen adsorption experiments were performed at 77 K with a Belsorp-mini II gas analyzer. The different samples were dried overnight under vacuum at 120°C to remove adsorbed water.

CHN-elemental analysis was performed on a Thermo Flash 2000 CHNS-O analyzer. XRF elemental analysis of both the powders and the solutions was performed on a Rigaku NEX CG Energy Dispersive analyzer with an XRay tube and Pd anode. The maximum power was 50 W and 50 kV and the detectors were a high performance SDD and a cooled peltier counter.

XRD measurements were performed on an ARL X'TRA Diffractometer from Thermo (radiation CuK α generated by 45 kV and 44 mA, with slits of 2, 4, 1.5 and 0.2 for the divergence, scatter, receiving scatter and receiving slit respectively, at 0.25° 2θ min⁻¹).

7.3 Results and discussion

7.3.1 Obtaining spherical PMO materials through spray drying.

Pore properties

The initial solution composition was based upon the composition of the first report on ring PMOs by Landskron et al.⁴⁰ After N₂-adsorption analysis it became clear that the CTAB/precursor composition was far from ideal which meant that this composition can not be used in an aerosol assisted synthesis.

To counteract the loss in pore structure (see rPMO-SD1 in figure 7.3), the amount of precursor was more than doubled and the amount of hydrochloric acid was decreased. This was necessary to reduce the oligomerization rate of the precursor molecule in order to obtain a better interaction between the surfactant and the precursor molecules. This evolution is depicted by figure 7.3 and table 7.2.

The pore size of the final material that was used as a the chromatographic packing material was 2.9 nm, which is smaller than the reported materials in chapter 5. Nevertheless if two C18 chains would be grafted opposite each other a gap of 0.5 nm would still be present, but it is unlikely this would be the case since the actual loading is $0.22 \text{ groups}/nm^2$ as can be seen from the section 7.3.2 on page 133.



Figure 7.3: N₂-sorption isotherms for rPMO-SD1, rPMO-SD2 and rPMO-SD3.

Table 7.2: The pore properties of the various products.

Material	$SA_{(BET)}$	V_p	$d_{p(KJS)}$
	(m^2/g)	cm^3/g	(nm)
rPMO-SD1	421	0.20	/
rPMO-SD2	892	0.55	3.1
rPMO-SD3	903	0.51	2.9



Figure 7.4: SEM micrographs for rPMO-SD1 (A), rPMO-SD2 (B) and rPMO-SD3 (C).

The particle morphology

A second small optimization was performed to produce uniform loose spheres. The particle morphology is influenced by the surface tension of the spray dried solution. This surface tension is positively influenced by the amount of water and negatively influenced by the amount of CTAB. The CTAB concentration has been optimized in relation to the precursor, this was kept constant. Instead the amount of water was raised to raise the surface tension of the solution to produce more uniform spherical particles. Figure 7.4 shows the SEM micrographs of this evolution. From these SEM pictures a mean particle size of 2.3 μ m was derived.

7.3.2 Carbon loading and particle purity analysis

7.3.2.1 Carbon loading

Since a new hybrid silane precursor was used to generate a chromatographic packing material the eventual carbon loading will be much higher than can be expected from a standard silica packing. Because this organosilane has been used as the only precursor molecule, it will be homogeneously distributed throughout the entire structure. This means that theoretically the structural formula should change from SiO₂ to SiOC, replacing half of the siloxane bridges with carbon bridges. The elemental analysis in table 7.3 suggests Si₂O₃C₃ as a structural formula. This discrepancy could be caused by either one of two factors, first off it could be that since XRF is not extremely sensitive for silicon the measurement has been under estimated, although it is unlikely that this would cause a difference of 1 to 3. Secondly it is more likely that since the organosilica structure is far from fully condensed, which would lead to relatively more oxygen and carbon atoms per silicon atom. After C18 grafting there are 0.22 groups/nm² on the surface which is the same as for the pure silica analogue described in chapter 5.

Si Loading (XRF)	O Loading (δ)	C Loading before	
		C18 (CHNS)	
14 mmol/g	21 mmol/g	21 mmol/g	
$16 \ \mu mol/m^2$	$24 \ \mu mol/m^2$	$24 \ \mu mol/m^2$	
$9.5 \ groups/nm^2$	$14.2 \ groups/nm^2$	$14.5 \ groups/nm^2$	
		C Loading after	C18 loading
		C18 (CHNS)	
		28 mmol/g	0.35 mmol/g
		$31.5 \ \mu mol/m^2$	$0.37 \ \mu mol/m^2$
		$19 \ groups/nm^2$	$0.22 \ groups/nm^2$

Table 7.3: Elemental analysis of the organic groups.

7.3.2.2 Particle impurity analysis.

Impurities in packing materials for chromatography influence the efficiency of the separations. Small amounts of metal inclusions interact with basic compounds causing band broadening. Therefore an elemental analysis was performed on both the synthesized powders and the spray dried solutions.

Element	Solution	Powder	Difference	Load
	$(w\%)^a$	(w%)	(m w%)	$(\mu mol/m^2)$
Cl	8.44	0.762	-7.678	0.2380
Br	1.0167	0.0416	-0.9751	0.0058
Fe	0.0283	0.0461	+0.0178	0.0091
\mathbf{Ca}	0.0389	0.0387	-0.0001	0.0107
Κ	0.1328	0.02	-0.1128	0.0057
Ti	0	0.0025	+0.0025	0.0006
V	0	0.0019	+0.0019	0.0004
Cr	0	0.0161	+0.161	0.0034
Ni	0	0.0026	+0.0026	0.0005
Cu	0	0.0011	+0.0011	0.0002
Zn	0	0.002	+0.002	0.0003

Table 7.4: Elemental analysis on XRF) of the impurities of the spray dried solution and the resulting powders.

^a normalized to the weight percentage powder resulting from the solution.

Summarized in table 7.4 are the results of the elemental analysis which show that the process introduces metals in the material. Since all of these metals, except for iron, are not present in the initial solution it is suggested that they originate from the spray drier itself. The solution contains hydrochloric acid which has the ability to dissolve steel, since the spray head is made up from steel it is evident these metal inclusion originate from the spray head. Titanium, vanadium, chrome, nickel, copper and zinc are common metals to be found in steel, apart from iron.

The rest of the elements that are found in the solution such as bromine, chlorine, calcium and potassium are reduced in amount during the spray drying process, but they are still present. This means that a thorough washing step with acids will be necessary.

7.3.3 Evaluation of the column.

To ensure optimal bed stability the column has been packed at a 50% higher pressure (900 bar) than the highest operating pressure of the used HPLC system. Subsequently the column was evaluated with a broad range of different test mixtures to obtain information about the packing density, common column efficiency, column retention behavior and column stability under stress conditions.

7.3.3.1 Packing density testing.

A measure for the packing density of a column is the external porosity of a column. The total pore blocking method was used to determine the external porosity (ε_e) of our packed column.²⁰³ The principle is based on the repulsion of polar and apolar compounds. First the column is completely filled with decane. In a second step the column is continuously flushed with an ammonium acetate solution while injecting NaI every three minutes as a dead time marker. The external porosity is calculated with the following two formulas:

$$\varepsilon_e = F t_0 V_q \tag{7.1}$$

and

$$V_q = \pi r 2L \tag{7.2}$$

Whereby ε_e , F, t_0 , V_g , r and L are the external porosity, flow rate, retention time of NaI, total column volume, column radius and the column length respectively. With a length of 5 cm and an internal diameter of 2.1 mm, the total column volume is calculated to be 173.2 µL. The t_0 -times have been corrected by the system t_0 -time, which was 0.05 min. Figure 7.5 shows that an external porosity of 45% could be reached. This is 5 to 7% more than the porosity value typically obtained for packed columns. The theoretical lowest value can be calculated out of the hexagonal closest packing and is 35%. This high value could explain the rather low plate height we experience for our packed column and might be a measure for the remaining polydispersity of the packed spheres.

The total column porosity can be calculated from the devision of the retention volume of a retained compound by the total geometrical volume of a column. For this column this was calculated to be 73%. The internal volume of the particles, or the pore volume, is then the difference between the total and the external porosity. This was calculated to be 28%.



Figure 7.5: The external column porosity as a function of time.

7.3.3.2 Standard column evaluation and comparison.

Figure 7.6A represents the separation of 4 parabens at a flow rate of 0.15 mL/min, figure 7.6B depicts the separation of 8 phenones at a flow rate of 0.2 mL/min and figure 7.6C shows the separation of 5 polyaromatic hydrocarbons at a flow rate of

0.2 mL/min. These analyses were performed under gradient conditions, with the drawback that different degrees of peak compression are occurring depending on the gradient steepness. Therefore important chromatographic parameters such as column efficiency (N) and retention factors (k') become distorted. To be able to compare this column with previously reported columns, the same isocratic analysis has been performed as the one used to analyze the commercial columns in chapter 5 (see figure 5.17 on page 108). This allowed for an ideal comparison between this column and the earlier produced MCM-41 based C18 column. This MCM-41 based C18 column showed a retention factor of 33 for toluene, which was double that of a Luna C-18(2) column (the best retaining column of the tested commercial columns). Current PMO column under investigation has a retention factor on toluene of 44 (see figure 7.6D), which is more than three times that of the Luna C18(2) column.



Figure 7.6: Analysis of a mixture containing uracil (1), methyl-4-hydroxybenzoate (2), ethyl-4-hydroxybenzoate (3), propyl-4hydroxybenzoate (4) and butyl-4-hydroxybenzoate (5) (A); (C) Analysis of a mixture containing uracil (1), 1-phenyl-1-ethanone (2), 1-phenyl-1-butanone (3), 1-phenyl-1-pentanone (4), 1-phenyl-1-hexanone (5), 1-phenyl-1-heptanone (6), 1-phenyl-1-octanone (7), 1-phenyl-1-decanone (8) and 1-phenyl-1-dodecanone (9) (B); Analysis of a mixture of uracil (1), benzene (2), naphtalene (3), 7,12-dimethylbenz[a]anthracene (4), fenantrene (5), fluoranthene (6) (C); Analysis of a mixture of uracil (1), caffeine (2), phenol (3), 1-phenyl-1-ethanone (4), propyl-4-hydroxybenzoate (5), benzene (6) and toluene (D).

The improvement in retention factor can be attributed to the larger carbon content of the material. The unmodified particles have a carbon content of 24 μ mol/m² and the C18 modified particles have a carbon content of 31.5 μ mol/m² measured by elemental analysis. This means the carbon loading representing the stationary

phase is 7.5 μ mol/m² which means there are 0.4 μ mol/m² of C-18 chains on the surface. This is the same as the MCM-41 based C18 column but much less than the Luna C18(2) column (3 μ mol/m²).

The different analyses in figure 7.6 show that the particle size distribution is sufficiently low so that acceptable columns could be packed for the evaluation of the chromatographic properties of this prototype material. This is reflected in the van Deemter curve of figure 7.7, where an optimal velocity can be determined at 0.02 mL/min and a plate height of 14 μ m. This is nearly as low as the lowest plate height reported in literature on experimental particles, which was 10 μ m on 1.7 μ m particles.¹¹⁹ However this is not yet close to the optimal plate heights reached by commercial columns (between 2 and 5 μ m). This can be attributed to a broader particle size distribution than observed for the commercial columns.



Figure 7.7: The H versus u plot of 1-phenyl-1-ethanone under isocratic conditions $(40/60 \text{ acetonitrile/H}_2\text{O})$ on the rPMO-SD3 material.

7.3.3.3 Analysis of polar compounds.

To test the prototype reversed phase column for its ability to separate highly polar compounds, a mixture of atrazine derivatives and analogues has been injected on the column. Figure 7.8 shows this in comparison with a contemporary state-of-theart column (Kinetex, Phenomenex). The most difficult compounds to retain and therefore separate are obviously the most polar compounds and are either eluted with the dead time marker or just thereafter. When zoomed in at the first four compounds it is clear that the new ringPMO column can separate them whereby the commercial column cannot.



Figure 7.8: Analysis of a mixture containing NaI (1), desethyl-2-hydroxy atrazine (2), desethyl desisopropyl atrazine (3), desisopropyl atrazine (4), aldicarb (5), cyanazine (6), simazine (7), prometryne (8), atrazine (9) and propazine (10).

7.3.3.4 Column stability tests.

Chromatographic stability

To test the column stability a sequential flush analysis series has been performed on the column. The column was first flushed 24 times with a pH 10.5 gradient (step 1) and then tested at this pH with diphenhydramine (step 2). Figure 7.9 shows an overall low efficiency with an average plate number of 500 but remains more or less constant through the 13 runs. The retention of this component appears to be slightly increasing with the number of cycles.



Figure 7.9: Left: the plate numbers for diphenhydramine, phenol, acetophenon and propylparaben as a function of the run number; right: the retention factors for diphenhydramine, phenol, 1-phenyl-1-ethanone and propyl-4-hyrdoxybenzoate as a function of the run number.

Step 3 consisted of a neutral flush to equilibrate the column for the next analysis and to prevent salt formation with the next flush step at low pH. After this a

neutral pH test was performed (step 4) with a mixture of uracil, caffeine, phenol, 1-phenyl-1-ethanone, propyl-4-hydroxybenzoate, benzene and toluene. Figure 7.9 only shows the retention and the plate number measured for three relevant analytes, namely phenol, acetophenon and propylparaben. The variation on the plate number of these compounds is larger than compared to those of the basic compound. The variation is such that it is not to suspect there is a correlation between the amount of runs and the rendered plates. Note that the retention factor for these components remains constant during the 13 runs. This implies a hydrolytically stable stationary phase.

During step 5 the column was flushed 24 times with a gradient at pH 2.0 to test the stability of the anchored C18 chain. The retention factor for the various compounds remains constant which implies that the C18 is anchored well to the surface.

From these results it can be concluded that the stationary phase is stable against these very harsh flushing conditions. It can withstand both the high and the low pH treatments. The structural porosity and a wall thickness of no more than a nanometer appear not to reduce the stability of the material. On the contrary, the hybrid nature of the material even improves the stability of the material since it is now at least as stable as the commercial column which is marketed for its extreme pH stability (Gemini, Phenomenex). Nevertheless thirteen cycles is not enough to clearly assess the columns stability.

Particle stability analysis

From the SEM micrographs in figure 7.10 it is immediately clear that the particles are stable towards packing and the very harsh flushing conditions used during the extensive test of this prototype column.



Figure 7.10: SEM micrographs for rPMO-SD3 before C18 grafting (A), after C18 grafting (B) and after 1500 injections (C).

Figure 7.11 depicts the isotherms of the packed material before packing and after 1500 injections. From these it is clear that the pore structure is not affected by the harsh separation conditions and the packing procedure.



Figure 7.11: $\rm N_2$ adsorption isotherms for rPMO-SD3 before, before grafting and after 1500 injections.

7.4 Conclusions

This study reports the first successful spray drying procedure for PMO materials. The 1,3,5-tris[diethoxysila]cyclohexane precursor was used to produce spherical PMO particles of 2.3 µm in size to be applied as an ultra stable packing material in high performance liquid chromatography. Based on previous experience these materials were optimized, functionalized with C18 and packed into a steel HPLC column.

The column was extensively tested to obtain information on the retention, the column efficiency, the column stability and to obtain an idea of the improved applicability range of the new prototype material. Previously it was reported that the improved retention was primarily due to the ordered and uniform porosity of the system. This is again the case but the higher carbon content of the seed material improved the retention further to three times that of the state-of-the-art columns.

This has been illustrated by performing the same separation earlier reported for an ideal comparison with previous columns and commercial columns. Additionally a mixture of highly polar pesticides have been analyzed by the new column and by a contemporary state-of-the-art column (Kinetex, Phenomenex) which confirms the previous results.

Finally the column was subjected to a harsh flushing procedure to test the bed stability and the stability of the C18 chain. After thirteen cycles the column was

be made in less steps than the commercial column.

8

Superior Retention with Ordered Mesoporosity: Re-enabeling WCOT Capillary Electrochromatography

8.1 Introduction

As mentioned in chapter 2, ultimate column performance, in terms of plates, is best achieved with the open tubular column. However, in order to be able to obtain comparable column performance in HPLC in comparison to what is possible with GC, the column internal diameter should be much reduced. Because the diffusion coefficient in the liquid phase is about a 1000 times lower than in the gas phase column diameters between 1 and 5 μ m internal diameter (I.D.) are considered the most promising for LC (in comparison to 50 - 250 μ m in GC) as this should allow to obtain several hundred thousands of plates for a column of a few meters length, while still allowing detection. Hence, the main limitation to this development is the much reduced amount of sample which can be injected on the column and the detrimental phase ratio which reduces the achievable retention.

Since more available stationary phase and therefore more retention can be obtained when using mesoporous materials they pose for interesting candidates to synthesize wall coated open tubular columns for liquid chromatography (WCOT-LC) techniques and also columns for the electrodriven variant: capillary electrochromatography (CEC).

Ordered mesoporous materials can generally be subdivided into two major pore structures, either 2D or 3D pore structures. For coated layers where the pores have to be accessible by the analytes in the mixtures it is therefore best to choose a 3D pore structure. Consequently SBA-16 layers were synthesized on the inner wall of 50 µm capillaries and evaluated in the capillary electrochromatograhy mode. CEC was thereby selected over micro-LC, because of the greater accessibility and instrumental simplicity of the technique. A schematic drawing of the capillary and of the CEC system is shown in figure 8.1.



Figure 8.1: Illustration of ordered mesoporous WCOT columns and how their installation on a commercial capillary electrodriven system.

8.2 Experimental

The sols were first optimized on silicon and glass substrates before the synthesis was attempted inside narrow capillaries, as this much facilitated characterization of the formed layers.

8.2.1 Optimization process of the mesoporous films

First Pluronic F127 was dissolved in ethanol and HCl and left stirring for 2 hours. A second solution with TEOS, ethanol, HCl and H₂O was mixed at room temperature for one hour and then refluxed at 80°C for an additional hour. All ratios are provided in tables 8.1 and 8.2 Subsequently these two solutions were mixed together and refluxed for an additional hour at 80°C. The SBA-sol compositions were based on an article published by Wang et al.²⁰⁴ The sols were coated both on silicon and

on glass substrates after which they were stabilized at 120°C for 12 hours. Hereafter, the samples were calcined at different temperatures (see table 8.5) with a heating rate of 2.95°C per minute and then kept at this temperature for an additional two hours.

8.2.1.1 Surfactant study

To study the influence of the amount of surfactant on the porosity and the viscosity of the samples, 3 different sol compositions were prepared and tested (see table 8.1). The three samples were dip coated on silicon wafers and analyzed with an optical microscope to test for film irregularities. The best films in terms of uniformity and smoothness were then dip coated at different speeds and analyzed with ellipsometry to obtain information on the porosity and on the film thickness.

Sample	TEOS	F127	H_2O	EtOH	HCl
TL-SBA16-1	1	0.0035	2.8	28	0.0003
TL-SBA16-2	1	0.0070	2.8	28	0.0003
TL-SBA16-3	1	0.0140	2.8	28	0.0003

Table 8.1: The sol composition in molar ratios.

8.2.1.2 Calcination experiments

For this study the composition of TL-SBA16-2 was used and calcined under air from room temperature to either 350°C, 450°C or 550°C at a heating rate of 2.95°C per minute and a dwelling time of 2 hours at the maximum temperature. The samples were dip coated at 120 mm/min and the rest of the sol underwent exactly the same procedure in a petri dish to obtain a powder with similar properties as the thin films. Both the film and powders were characterized with XRD, SEM and N₂-adsorption.

8.2.2 From substrate thin layer to coated capillary

The capillaries were flushed with sol TL-SBA16-2 for various lengths of time. The flushing pressure and the length of the column was varied (see table 8.2). Figure 8.2 depicts the set up used to coat the fused silica capillaries, the sol is thereby blown through the column by applying a N_2 pressure which depended on the column length.

To assess the effect of the ordered mesoporous layer on the separation properties of open tubular columns CC2 and CC4 two columns without this coating were fitted with C18 chains (CC0 and CC1).

After coating, the columns were dried under a N₂-flow for 12 hours and stabilized in an oven without gas flow for another 12 hours at 120°C after which they were calcined under N₂-flow at 350°C. The set up to perform the calcination comprised of a GC oven coupled to a N₂-gas source. The GC oven was heated up to 350°C at a heating rate of 2.95°C per minute with a N₂ pressure of 2.5 bar. The maximum temperature the polyimide coating of the fused silica capillaries can with stand is 400°C therefore the calcination temperature was deliberately kept below that value. As the previous calcination study indicated that most porous layers are formed at 350°C, this set up provided the suitable conditions to perform this column manufacturing process.



Figure 8.2: The coating set up for fused silica capillaries.

After calcination, the columns were amendable for coupling of the C18 groups to the enlarged surface of the coating. The column was therefore reattached to the coating set up and this time the sol reservoir was filled with an octadecyl dimethylchlorosilane solution in toluene. The time of reaction was 12 hours at room temperature to ensure optimal coating of the silica surface. To benchmark the performance of the WCOT columns, a non coated fused silica capillary was also treated with the C18 chain solution to obtain information on the retaining influence of the porous coating.

Table 8.2: Conditions used for the manufacturing of the various capillaries which were tested.

Capillary	Total column length	Time	ID	Coating pressure
	(cm)	(\min)	(μm)	(bar)
CC0	60	/	50	/
CC1	200	/ 50 /		
CC2	60	5	50	8
CC3	200	2880	50	100

8.2.3 Chromatographic testing

Due to the convenience of use of a capillary electrophoresis system to evaluate the capillaries and because of the low peak dispersion associated to the use of this type of instrumentation, the choice was made to evaluate their potential in the electrodriven mode rather than the pressure driven micro-LC mode.

Two different CE equipment set ups were used to analyze these columns. Columns CC0 to CC2 were evaluated with a P/ACETM system MDQ CE/UV from Beckman. The background electrolytes (BGE) consisted of a mixture of acetonitrile with a MES (2-(N-morpholino)ethanesulphonic acid) buffer at pH 6 (50 mM) with a v/v composition of 20/80 ACN/MES. A mixture of parabenes containing uracil (250 µg/mL), methyl-4-hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate (100 µg/mL) each dissolved in 20/80 acetonitrile/MES was thereby injected electrokinetically at 5 kV for 1 s. Detection was performed at 214 nm.

Columns CC1 and CC3 were evaluated on an Agilent 7100 CE with a UV DAD detector. The used buffer thereby consisted of a mixture of acetonitrile with a MES (2-(N-morpholino)ethanesulphonic acid) buffer at pH 6 (50 mM) with a v/vcomposition of 20/80 ACN/MES. The injection was performed at 60 mbar for 5 seconds and the EOF was generated by a 30 kV voltage without external pressure. Two mixtures were injected, the first mixture was a mixture of thiourea (250 $\mu g/mL$) and naphthalene (50 $\mu g/mL$) each dissolved in 20/80 acetonitrile/MES; the second was a mixture of parabenes containing uracil (250 μ g/mL), methyl-4hydroxybenzoate, ethyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate (100 μ g/mL) each dissolved in 20/80 acetonitrile/MES). The third mixture comprised of a set of phenones consisting of thiourea (200 μ g/mL), 1-phenyl-propanone, 1-phenyl-butanone, 1-phenyl-hexanone, 1-phenyl-heptanone, 1-phenyl-decanone and 1-phenyl-dodecanone (100 $\mu g/mL$). They were injected at 50 mbar for 5 seconds and the EOF was generated by a 30 kV voltage without external pressure. One set of phenones was dissolved in a 50/50 mM MES pH 7 buffer/acetonitrile and a second set was dissolved in a 60/40 mixture of the same MES buffer with acetonitrile.

8.2.4 Characterization

Scanning electron microscopy (SEM) was performed on a FEI Quanta 200 FEG microscope with 4 nm resolution operating at 30 kV.

Nitrogen adsorption experiments were done at 77 K with a Belsorp-mini II gas analyzer. The different samples were dried overnight under vacuum at 120°C to remove adsorbed water.

XRD measurements were performed on an ARL X'TRA Diffractometer from Thermo (radiation CuK α generated by 45kV and 44mA, with slits of 2, 4, 1.5 and 0.2 for the divergence, scatter, receiving scatter and receiving slit respectively, at 0.25° 2θ min⁻¹).

Ellipsometric measurements were performed on a J.A. Woolem Co. Inc. ellipsometer with a FC5300 75W Xe-lamp.

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Thermo Gravimetric Analysis (TGA) was performed on an SDT 2960 from TA Instruments.

8.3 Results and Discussion

8.3.1 Characterization of the thin layer on silicon substrates

8.3.1.1 Surfactant study

Microscopic analysis of the three samples (see table 8.1) shows that sample one and two both have a smooth surface but that sample three exhibits local irregularities (figure 8.3). Ellipsometric measurements were not possible on these sample because of these deformations in the film, leading to an excessive high background signal in the spectra.



Figure 8.3: Optic microscopy figures of samples 1 (a), 2 (b) and 3 (c) at a 25 times magnification.

These observation could be related to the viscosity of the sol, which is 3.0 cP and 3.9 cP for sol 1 and 2 respectively (water has a viscosity of 1 cP). Higher surfactant concentrations therefore most probably lead to more viscous sols and more viscous sols lead to thicker films. Secondly, the film thickness depends on the speed of dip coating (see table 8.3).

Table 8.3: The thickness of the samples as a function of the viscosity and the dip coat speed.

Sample	Viscosity (cP)	120 mm/min	130 mm/min	150 mm/min
TL-SBA16-1	3.0	130 nm	135 nm	140 nm
TL-SBA16-2	3.9	220 nm	225 nm	230 nm

The viscosity and the dip coat speed also influence the porosity of the material. This is depicted in table 8.4 where it can be seen that the porosity follows an inverse

relation with the dip coating speed and a direct relationship with the viscosity. This means that with more surfactant in the initial sols more porosity is obtained. This is also in accordance with the results obtained in chapter 4. More surfactant means more templating molecules in the sol which in turn leads to increased porosity.

Table 8.4: The porosity of the samples as a function of the viscosity and the dip coat speed.

Sample	Viscosity (cP)	120 mm/min	130 mm/min	150 mm/min
TL-SBA16-1	3.0	21%	20%	18%
TL-SBA16-2	3.9	34%	30%	28%

The relation between the dip coat speed and the lower porosity is less obvious. A possible explanation could be that as thicker films are formed, more solvent has to evaporate which slows down the formation of micelles in the coated sol. Therefore polycondensation of the silica precursor molecules has already started before it has the chance to co-assemble with the formed micelles. Note that the atmospheric drying conditions were kept constant for all the samples.

8.3.1.2 Calcination study

All the films exhibited clear smooth films when analyzed with optical microscopy, therefore it could be concluded that the films are homogeneous with very few impurities present.

Evaluation of the films

Table 8.5 summarizes the ellipsometric analysis of the thin films. Both the film thickness and the porosity decrease as a function of the temperature. A general shrinkage of the layer is to be expected as a function of the temperature because of induced condensation of bridged silanol groups in the silica structure.

Since the amount of SBA material remains the same with decreasing film thickness as the temperature goes up, the only explanation for the drop in porosity is an increase in density. The material shrinks which means that the pores in the coating will also shrink or collapse, this is reflected in the isotherms of the powders in figure 8.6.

Temperature (°C)	Thickness (nm)	Porosity (%)
350	220	38
450	206	35
550	190	33

Table 8.5: The samples porosity as a function of the calcination temperature.

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Figure 8.4: Low angle XRD measurements of the silica thin films as a function of the calcination temperature.

Low angle XRD measurements were performed directly on the films to determine whether an ordered mesoporous pore system is present. As can be seen from figure 8.4 the intensity of the diffracted signal is not very intense. This can be attributed to the fact that it is a very thin layer that has been measured. The evolution in the diffractrograms was clear, as an increase in order as a function of the calcination temperature was observed. The reason for this can be two-fold, either the pore structure is not completely formed at the lower temperature requiering elevated temperatures to obtain ordered pores. The second reason can be that there is still surfactant present in the pores of the system and because of the low amount of material this further reduces the intensity of any diffraction peak. There is however a hint of a peak visible in the first diffractogram at $0.9^{\circ}\theta$ which becomes more and more visible as a function of the calcination temperature and could correspond to the (110) plane. By the third diffractogram the secondary diffraction peaks become more visible at $1.35^{\circ}\theta$ and $1.55^{\circ}\theta$ which could correspond to the (200) and the (211) diffraction plane which together with the (110) plane could be an indication that the Im3m pore structure has been formed. To elucidate this further, TGA measurements of the powders were performed to test for the presence of surfactant in the materials.

Evaluation of the powders



Figure 8.5: TGA measurements as a function of the calcination temperature.

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Thermo-gravimetrical measurements indicate that when the materials are calcined at 350°C there is still surfactant present in the porous material (see figure 8.5). From these measurements a presence of 3w% of surfactant was calculated. This is a low value corresponds with the low values of remaining surfactant measured after extraction of the surfactant with acetone.³⁴ XRD measurements on ethene bridged ordered mesoporous materials revealed that 3 clear diffraction peaks could be discerned. This could indicate that, if there is order present in the samples, they should be visible in the XRD patterns. However they are not, either indicating the structure is still forming during calcination or for this small amount of SBA-16, 3w% of remaining surfactant is enough to reduce the contrast of the diffraction peaks. If no repetitive structure is formed a disordered highly mesoporous material could be formed. However, for the application at hand we believe that an ordered pore system is not imperative, contrary to a uniform pore size, as long as it has a lot of connections to the open inside of the capillary.



Figure 8.6: N_2 -sorption measurements as a function of the calcination temperature (A). BJH pore size distributions for the adsorption (B) and the desorption (C) isotherm.

The N_2 -sorption measurements of these powders, taken from the petri dishes, indicate that as the temperature of calcination increases, the silica material looses pore volume and surface area (see figure 8.6 and table 8.6). Since the pore size remains constant, this reduction in pore surface and pore volume could be attributed to a collapse of the pores.

These measurements are in accordance with the porosity measurements on the thin films (see table 8.5), indicating there is a correlation between the powders and the thin films when they are treated under the same conditions to form ordered mesoporous materials.

Temperature	$SA_{(BET)}$	V_p	$d_{p(BJH,ads)}$	$d_{p(BJH,des)}$
$(^{\circ}C)$	(m^2/g)	$(cm^{\hat{3}}/g)$	(nm)	(nm)
350	523	0.35	3.9	3.5
450	397	0.30	4.0	3.5
550	356	0.27	4.1	3.5

Table 8.6: The samples pore properties as a function of the calcination temperature.

8.3.2 Characterization of the capillary

The capillaries manufactured in table 8.2 were analyzed by scanning electron microscopy. Since the capillary is made up of fused silica and the final coating is also silica, characterizing the capillaries was a challenging task. The only characterization which could successfully be performed was a SEM analysis of the inside of the capillary. However, since the coating time of CC2 was only 5 minutes and the applied pressure during coating only 8 bar no apparent layer can be observed. The pore sizes were also too small to be distinguished by SEM. Figure 8.7 shows the difference between a non coated and a coated capillary, on the coated capillary a hint of a coating is visible. The coating has a rather rough surface texture, which increases the interaction with the mobile phase and solutes but is inherently hard to reproduce. In order to be able to characterize the pore system TEM measurements could be performed to visualize the pore structure but due to the extensive sample preparation time involved, this has not yet been done, it will be addressed in the near future.



Figure 8.7: SEM pictures from the inside of CC0 and CC2.

However the SEM micrographs for CC3 depict a clear coating, the thickness of this coating was an average thickness of 600 nm. The reason for this increased thickness can be found in the longer reaction time and the use of a much higher pressure to percolate the reactive solution through the column. It can only be expected

that this will have an improved influence on the retention of analytes on these wall coated open tubular capillaries.



Figure 8.8: SEM micrographs of CC3 indicating a thick layer of 600 nm of mesoporous material deposited on the inner wall (marked red) of the capillary.

8.3.3 Chromatographic evaluation

8.3.3.1 Benchmarking the coated capillaries

To clearly ascertain the retentive effect of the coated porous layer in an early stage of this research both a column with and without the layer were evaluated under the same conditions. Figure 8.9 shows the separation of 4 parabens and thiourea on CC0. On this uncoated column all the compounds eluted within one minute, indicating little to no retention. When this is compared with the same separation on CC2, improved retention becomes visible. The same compounds are eluted over a time frame of 7.5 minutes under the same conditions. The resulting current varied between 20 and 25 μ A indicating an EOF is generated. Preliminary proof is evidenced in the separation shown in figure 8.9. Although poor efficiency was observed on CC2, this initial test clearly illustrated the potential of the use of coated mesoporous walls in OT-CEC.

The peaks could not be identified because after 3 separations the analysis was no longer reproducible, hence no additional injections could be done on this column.



Figure 8.9: The chromatograms of CC0 (A) and CC2 (C). Thiourea and 4 parabens were separated on a ACN/MES 20/80 mobil phase with a 30 kV applied voltage and an elektrokinetic injection at 5 kV for 1 second. The detection was performed by UV at 254nm.

8.3.3.2 The influence of coating thickness on the retention

However, when the open tubular column with the much ticker layer was evaluated, improved results were obtained. During the following analysis a 70 cm column was used in the Agilent 7100 system, whereby detection is performed at 4.5 cm from the capillary end, which means that the actual chromatographic length is 66.5 cm. Figure 8.10 shows a separation of thiourea as the dead volume marker and naphthalene as a retained compound. The plate number for naphthalene was thereby 36000 plates corresponding to a plate height 1.85 µm and a reduced plate height of 0.037 µm. The amount of injected analyte on the column was estimated 390 pmol. Without apparent overloading of the column as can be ascertained by the good peak symmetry whereas the loading capacity of wall coated open tubular

capillaries is usually much less $(50 - 100 \text{ fmol})^{109}$.



Figure 8.10: Analysis of a mixture of thiourea (1) and naphthalene (2) on CC3. Injection at 60 mbar for 5s; mobile phase: 20/80 ACN/pH6 50 mM MES; measurement at 30 kV; flow rate: 0.28 mm/s.

In order to compare the results obtained by the separation of a mixture of thiourea and naphthalene, presented by figure 8.10 and table 8.7 with literature¹⁰¹ an independent factor has been introduced, k'/h. This way both the effect on the retention and the column efficiency are analyzed at once, irrespective of the column properties and the separation conditions. As can be seen from table 8.7 the number of plates for the same compound is much lower for our home made column in comparison with what is provided by Guo et al., (36000 plates vs 140000 plates). However, the lower the retention the sharper peaks are and the higher N will be for that compound. To account for column properties, the reduced plate height, h, has been calculated, this was 37% higher for CC3. If the retention of both columns are compared then CC3 has a 7 times higher retention for naphthalene than the column reported in literature. To account for both parameters at once, k'/h was proposed as an independent indicator. So a higher k'/h ratio is better since then the retention is maximized while the reduced plate height is minimized. CC3 has a k'/h value of 1.34 whereas the column in literature exhibited a k'/h of 0.25 on the same component. This improvement could be attributed to the ordered porous layer that increases the retention. Note that the column in literature had an internal diameter of only 13 μ m, indicating that the huge improvement on efficiency is useless, unless enough retention can be generated on the respective columns.

Table 8.7: The plate numbers, retention factors, plate height, and reduced plate height of the analysis of a mixture of thiourea and naphthalene on CC3 compared with data in literature.

Naphthalene	Ν	k'	Н	h	k'/h	
			(μm)			
CC3	36000	0.50	18.5	0.37	1.34	
$Literature^{101}$	140000	0.069	3.57	0.27	0.25	
CC3: mobile phase: 50mM MES pH6/ACN 80/20, 30kV, flow rate: 0.28 mm/s						

Figure 8.11 shows the analysis of a paraben mixture on CC1 and CC3. Both columns have been evaluated under exactly the same conditions and with the same column length. As can be seen from the chromatograms the retention of CC3 is much higher than on the CC1, the capillary without the mesoporous layer. The rentention factor for butyl-4-hydroxybenzoate on CC3 is 3.16 whereas k' for the same compound on CC1 is 0.038, this is 83 times lower.



Figure 8.11: Analysis of a mixture of thiourea (1), methyl-4-hydroxybenzoate (2), ethyl-4-hydroxybenzoate (3), propyl-4-hydroxybenzoate (4) and butyl-4-hydroxybenzoate (5) on CC3. Injection at 50 mbar for 5s; mobile phase: 20/80 ACN/pH6 50 mM MES; measurement at 30 kV; flow rate CC1: 0.74 mm/s and CC3: 0.45 mm/s.

The figures of merit of the separation on CC3 are given in table 8.8. This confirms what is visible on the chromatogram, namely satisfactory baseline separation of all peaks in the electrochromatogram. Note that all solutes are neutral under the conditions of analysis and that the electro osmotic flow was 0.45 mm/s. Although the velocity of the mobile phase was still clearly sub-optimal a peak capacity of 28.6 could be obtained.

Table 8.8: The plate numbers, retention factors, separation factors and the resolution of the analysis of a paraben mixture on CC3.

Compound	t_r	Ν	k'	α	R_S	N_{eff}	Н	h
	min						μm	
1	24.2	110384					6.0	0.12
2	32.7	31093	0.35			2129	21.4	0.43
3	40.1	16284	0.66	1.87	5.89	2579	40.8	0.82
4	58.5	8976	1.42	2.15	7.44	3094	74.1	1.48
5	100.6	6012	3.16	2.23	8.11	3471	191.6	2.21

mobile phase: 50mM MES pH6/ACN 80/20, 30kV, 0.45 mm/s

Figure 8.12 shows the analysis of a phenone mixture under two different mobile phase compositions on the same capillary. The resolution of the chromatographic evaluation is shown in table 8.10. Further more the EOF velocity is 0.29 mm/s for the 50/50 composition, for the analysis with 40% acetonitrile the EOF velocity is 0.33 mm/s. The peak capacity n for the 50/50 composition was calculated to be 16.6 and for the 60/40 composition n was 24.8.

This also demonstrates the known high dependency between the EOF velocity and the BGE composition. The obtained results clearly demonstrate the importance of the use of more elevated EOFs, which can be achieved by means of higher field strengths (larger applied voltage or shorter capillaries) or by the presence of a larger amount of free silanol functions on the silica wall.

Compound	t_r	Ν	k'	α	R_S	N_{eff}	Η	h
	(min)						(μm)	
1	34.1	51499					12.9	0.26
2	37.2	30250	0.09			214	22.0	0.44
3	39.0	27127	0.14	1.56	1.86	427	24.5	0.49
4	46.1	22271	0.35	2.46	5.76	1512	29.9	0.60
5	52.6	18087	0.54	1.54	4.15	2240	36.8	0.74
6	62.3	14431	0.83	1.52	4.68	2959	46.1	0.92
7	98.5	8029	1.89	2.28	8.24	3434	82.8	1.66

Table 8.9: The plate numbers, retention factors, separation factors and the resolution of the analysis of a phenone mixture on CC3.

mobile phase: 50 mM MES pH7/MeCN 60/40, 30 kV, 0.33 mm/s

Interesting is also the extremely low reduced plate heights which are obtained. This suggests a promising future for the technology once it has been adopted to effective coating of more narrow capillaries.

Compound	t_r	Ν	k'	α	R_S	N_{eff}	Η	h	
	(\min)						(μm)		
1	37.9	41191					16.1	0.32	
2	39.4	36311	0.04			50	18.3	0.37	
3	40.1	37140	0.06	1.49	0.86	110	17.9	0.36	
4	42.5	38931	0.12	2.07	2.73	443	17.1	0.34	
5	44.2	36282	0.17	1.39	1.91	736	18.3	0.37	
6	46.5	31216	0.23	1.36	2.18	1065	21.3	0.43	
7	53.4	23917	0.41	1.80	4.99	2012	27.8	0.56	
8	65.3	13280	0.72	1.76	5.22	2329	50.1	1.00	
mobile phase, 50mM MES pH7/MeCN 50/50, 20 kV, 0.20 mm/s									

Table 8.10: The plate numbers, retention factors, separation factors and the resolution of the analysis of a phenone mixture on CC3.

mobile phase: 50 mM MES pH7/MeCN 50/50, 30 kV, 0.29 mm/s



Figure 8.12: Analysis of a mixture of thiourea (1), 1-phenyl-propanone (2), 1-phenyl-butanone (3), 1-phenyl-hexanone (4), 1-phenyl-heptanone (5), 1-phenyl-octanone (6), 1-phenyl-decanone (7) 1-phenyl-dodecanone (8) on CC3. Injection at 50 mbar for 5s; mobile phase: 50 mM MES buffer pH 7/ACN; measurement at 30 kV; flow rate: 60/40 = 0.33 mm/s and 50/50 = 0.29 mm/s.

8.4 Conclusion

In this chapter wall coated open tubular capillaries for capillary electrochromatography were effectively developed and investigated. It seems that the loading capacity of these WCOT columns is high enough to accommodate all of these separations without the issue of over loading. This, however, has to be quantified in the future to assess the loading capacity of these columns. Due to the low phase ratio an low rate of diffusion in the liquid phase a coated capillary is easily overloaded and usually cannot generate enough retention for an efficient separation of the reaction mixture. In an attempt to solve these problems a coating, supposedly with ordered mesopores, of 600 nm has been deposited to the inner lining of a fused silica capillary. The data in chapter 5 demonstrates to some extend that an ordered mesoporous system increases the retention of analytes on the column.

The chromatographic evaluation of the wall coated open tubular columns showed that the improved retention ordered mesoporous materials generated can be used to make separations possible on open tubular columns without having to resort to ultra narrow capillaries although in this way the expected ultra high efficiencies could not yet be achieved. Additionally the loading capacity is higher than thus far reported in literature.¹⁰⁹

However, additional evaluation is needed in terms of column to column reproducibility and coating uniformity. Also, the work has to be translated to the manufacturing of more narrow capillaries. This chapter lays the foundation for a potentially important field of research, with much envisageable applications in the future.
9

General Conclusions and Outlook

The main aim of this research was to investigate the effect of ordered mesoporosity on chromatographic separations. The research was split up in two major parts, one dealing with particle synthesis for packed columns and one dealing with the deposition of a thick mesoporous layer (around $0.5 \ \mu m$) on the inner lining of fused silica capillaries.

9.1 Particle design

9.1.1 Proof of concept

MCM-41 materials were the first materials ever to be spray dried and most of the research on spray dried ordered mesoporous particles has been performed on MCM-41 type materials. Therefore these materials are perfectly fit to be used in a proof of concept study. The added value of this contribution to the scientific community is a systematic analysis of the synthesis procedure to retrieve the factors influencing both the pore properties and the morphology of the materials. This combined with a thorough chromatographic evaluation are the foundations upon which the subsequent research is performed.

Particle design

The main factors which appeared to influence the spherical morphology of the final material are the surfactant concentration, the water fraction and the gas flow. Two of these factors influence the surface tension of the spray dried solution, namely the surfactant concentration and the water fraction. It has been confirmed and found logical that the higher the surface tension, the easier it is to form spherical

particles. A surfactant influences the surface tension negatively and water influences this positively. The gas flow on the other hand only has an influence on the particle size distribution. Higher gas flows mean higher atomization forces, which in turn means that the particle size distribution will shift to lower particle sizes.

When the production was upscaled and thus larger volumes of solution were made it became clear a third factor played a significant role in the synthesis. The surface tension of the solutions tend to drop as a function of the reaction time. This could be attributed to an extended oligomerisation of the silica precursor. Due to formation of large silica oligomers, the surface tension of the solution dropped, leading to collapsed spheres.

The pore properties are directly proportional to the surfactant concentration. This means a trade off between the particle morphology and the pore properties has to be made.

Once the ideal composition was found, the solution was spray dried on different spray driers to test the robustness of the synthesis. This enabled upscaling the entire production procedure. This was necessary, firstly to form large enough quantities to be able to pack a column, secondly to be able to partly classify the material.

Chromatographic evaluation

To put the particles to the test they were fitted with C18 chains and end-capped to allow them to be used for reversed phase chromatography. After this the materials were slurry packed at 900 bar and then chromatographically evaluated. It became immediately clear that this type of materials exhibited exceptional retention. Even with a lower carbon loading than conventional columns the retention was doubled when compared to the best retaining silica column on the market. The improved retention was attributed to the higher surface area resulting from the uniform porosity of the materials. This in turn, together with better accessible C18 groups, leads to the superior retention.

The next steps in the further development of these materials were to increase the pore size and to improve the particle stability.

9.1.2 Improving the stability

With the basic factors elucidated in the previous section, the next step is to impose the findings on different materials. The hydrothermal stability of silica materials is directly dependent on the stability of the siloxane bridge in the system. When water can attack these bridges and reduce them to two silanol groups the entire structure will eventually collapse. This in turn will lead to a collapse of the packed bed, completely destroying the column. The proposed solution is to replace the siloxane bridges with carbon bridges. This way a so called periodic mesoporous organosilica or PMO material has been spray dried. To do this a ring precursor molecule was used as organosilica source which infers that half of the siloxane bridges are replaced by carbon bridges. After the ideal sol composition was found the materials were spray dried and packed in a column for evaluation.

The column showed an external porosity of 45%, indicating that the particle size distribution was not yet optimal to ensure an ideally packed column. This means that an additional classification will be necessary in the future to improve the packed bed. Additionally, the maximum pressure at which the column was packed was 900 bar, which in fact is too low for 2.5 µm particles. For this particle size a pressure of at least 1500 bar should be used, but due to equipment limitations this was not possible.

In terms of retention, this column triples the retention of the best commercial column. Since the pore structure of this material is exactly the same as the MCM-41 type column in the previous section this means that the added retention can be attributed to the increased carbon content of the material. So not only will it improve the stability of the material it also increases retention significantly.

In order to test for applicability of the huge retention of this column, a mixture of highly polar pesticides was easily separated on this column. Moreover the column was compared to a kinetex column, which could not separate the most polar compounds.

The last test performed on this column was a stability stress test, normally used to stress Gemini columns. These commercial columns consist of normal silica particles but with an organosilane layer grafted to the surface of the material. This is done in order to improve the stability of the material. The home made column resisted perfectly the harsh conditions of the stress test and in doing so it proves to be as stable as a Gemini column, without having to add an extra layer of organosilanes. This reduces the production time of such an ultra stable column and might in the future allow for new methods to be developed. For example organic modifier/water gradients might be replaced by water/temperature gradients. This way separations become more environmentally friendly and more economical.

Parallel to this, the spray drying of larger pore systems has been investigated. This proved to be a big challenge, as the next section explains.

9.1.3 Increasing the pore size

Although good progress has been achieved in the synthesis of spray dried SBA-15 materials, the morphology of the materials is still an issue. The achieved surface areas and pore properties exceeded previously reported spray dried SBA materials. In terms of obtaining a good spherical morphology a lot of work still has to be performed, both on the feed solution as on the spray drying set up itself. The assembly process of an SBA type material cannot be done by a fast EISA process, the reaction is just to slow for this. While CTAB forms cylindrical micelles within

seconds, this is not the case for the, in comparison, gigantic Pluronic surfactants. The fastest way they assemble into cylindrical micelles is within 30 minutes. The problem which arises next is a possible precipitation of the assembled surfactant/ silica structures. They gain mass very quickly so ideally the assembly process will have to be stopped right before it is spray dried, or it has to be performed by an online mixing system. Once precipitates are formed the particle morphology cannot be controlled anymore. If stabilized nano-sols of mesoporous material can be formed then these would be ideal to be spray dried, because then the formation of micrometer sized particles are easily controlled morphologically. It would be just a case of surface tension as was the case in the previous two sections.

The same restrictions are present for SBA-16 but with one small difference, it is easier to form spherical particles with the larger Pluronic F127 surfactant. This surfactant preferentially forms spherical micelles, which are smaller and thus faster to assemble into a cubical array of micelles. But as with SBA-15 materials the precipitation rate has to be controlled to be able to ascertain complete control over the morphology of the final particles.

Nevertheless it would be very interesting to just be able to spray dry both types of materials, irrespective of the particle morphology because then there would be a starting point to spray dry large pore PMO materials. Secondly it would facilitate and speed up the production proces of these types of materials.

9.2 The design of thick mesoporous layers

During the previous sections it has been proven that an ordered mesoporous system with a high surface area strongly improves the retention mechanism in packed columns. During this section this principle is exploited to generate the retention necessary to perform chromatography in the liquid phase on wall coated open tubular capillaries, which is still monopolized by gas chromatography.

9.2.1 Open tubular capillaries for liquid chromatography

During this part of the research an ordered mesoporous SBA-16 coating has been deposited onto the inner wall of a fused silica capillary and subsequently been functionalized by a C-18 chain. This column has then been analyzed with CEC to test its retentive properties. The thickness of the coating was 600 nm with an estimated porosity of 38%. Obtained plates on 1-phenyl-propanone were 61000 plates per meter and a k factor of 3.2 on butyl-4-hydroxybenzoate could be reached. This means that proof has been delivered that for a 50 micrometer capillary it is possible to make a wall coated open tubular column that can be used for the standard analysis of mixtures.

The column was compared with a non-coated capillary for the separation of four parabens. A 83 times higher retention was observed for the WCOT capillary.

However, to unambiguously identify the porous layer on the surface to be the reason for this improved retention on WCOT columns extensive research still has to be performed. First off all characterizing the layer will be the biggest challenge. It is not possible to see the pores with HR-SEM and the sample preparation of a capillary for TEM is very time consuming. Currently these two techniques seem to be the only ones to be able to determine the layer properties.

9.3 Outlook

Ordered mesoporous materials have real potential for high performance liquid chromatography and for capillary electro chromatography. The high retention they infer can really broaden the application range of common C18 columns for reversed phase chromatography. Additionally the materials can be fitted with organic groups which are anchored into the structure itself, generating superiorly stable packing materials.

However the materials are not yet uniform enough to be competitive with the state of the art, currently on the market. During the course of this research the main problem that was encountered for spherical particles was the particle size distribution. This has to become much more narrow to acquire superior plates and resolutions.

The materials that were evaluated chromatographically during this research were from the MCM-family meaning that the pore sizes did not exceed the 4.5 nm barrier. To broaden their scope of application materials with larger pores (the SBA-family) should be developed. This way a broad range of materials with different pore sizes can be developed to be used in size exclusion chromatography. So future research should comprise the development of large pore ordered mesoporous spheres with a uniform particle size distribution.

The coated capillaries need to be benchmarked further in such a way that there is clear proof of the fact that the porous layer is responsible for the improved retention on these columns. Additionally column to column reproducibility should be investigated and different capillary diameters should be tried.

Once the particle stability is improved and the particles exhibit uniform particle sizes, these materials have the ability to change the routine HPLC analysis methods. New more economical and environmentally friendly methods will be developed, changing the way separations are performed today.

10

Nederlandse Samenvatting

10.1 Inleiding

Deze doctoraatsthesis behandelt de synthese en toepassing van geordende mesoporeuze materialen voor vloeistof chromatografie. Deze klasse van materialen werd voor het eerst ontdekt in 1992 gedurende een onderzoek om de poriegrootte van zeolieten te vergroten. Het algemeen procédé voor de synthese van deze materialen maakt gebruikt van een surfactant als porogen. Een (organo)silica bron wordt gepolycondenseerd rondom een micellaire structuur die gevormd wordt door het surfactant. Na afloop wordt het surfactant er ofwel uitgebrand ofwel uitgewassen en blijft een poreus systeem achter met poriën van uniforme grootte. Behalve deze uniforme poriegrootte hebben deze materialen ook een grote specifieke oppervlakte en een groot porie volume.

Door hun eigenschappen kunnen deze materialen potentiële voordelen opleveren voor verschillende toepassingen zoals katalyse, milieutechnologie, gezondheidszorg, micro-elektronica en uiteraard ook chromatografie. Het overgrote deel van de publicaties met betrekking tot deze klasse van materialen vermeldt telkens de mogelijkheden maar er zijn er maar weinig die effectief de mogelijke toepassingen onderzoeken.

Dit onderzoek legt de focus op hun toepassing binnen de chromatografie en vertrekt vanuit dat standpunt om nieuwe geordende mesoporeuze materialen te ontwikkelen die een antwoord bieden op specifieke problemen binnen de vloeistofchromatografie. Zo zijn er bijvoorbeeld de vaak problematische instabiliteit van silica materialen in basisch milieu en de lage retentie van open tubulaire capillaire kolommen. Vanuit deze problemen werd vertrokken om nieuwe materialen te ontwikkelen die de tekortkomingen van de huidige materialen kunnen teniet doen.

10.2 Het silanolgetal van de bekendste geordende mesoporeuze (organo)silicas: de totale versus de bereikbare silanolen

Het silanolgetal (uitgedrukt als het aantal silanol groepen per nm^2) wordt al onderzocht sinds de jaren 50. Aangezien alle functionele groepen die gebruik worden in de chromatografie om tussen verschillende kolommen te differentiëren, verankerd worden via de silanolgroep aan het oppervlak van silica materialen is het gemakkelijk in te zien dat een goede kennis van het aantal silanolgroepen essentieel is binnen de chromatografische wereld. Bovendien leiden overblijvende groepen tot interacties met de meer basische analyten in een te analyseren mengsel. Voor standaard silica materialen zijn er voldoende databases voorradig die silanolgetallen oplijsten, maar voor de klasse van de geordende mesoporeuze materialen werd nog geen eenduidig resultaat gevonden. Daarom werden tijdens dit onderzoek drie chromatografisch belangrijke functionele groepen (een trimethylsilyl groep, een octyl dimethylsilyl groep en een octadecyl dimethylsilyl groep) vastgezet op dit type materialen en vergeleken met het totale aantal silanolgroepen in het materiaal. De geteste materialen waren SBA-15, SBA-16, MCM-41 (hydrothermaal), MCM-48, MCM-41 (gesproeidroogd), etheen PMO, ethaan PMO, benzeen PMO en Nucleosil als vergelijkingsmateriaal.

Materiaal	$SA_{(BET)}$	Vn	d_n	an	h
	(m^2/g)	(cm^3/g)	(nm)	(nm)	(nm)
SBA-15	656	0.7	6.9	10.7	4.2
SBA-16	738	0.6	6.2	14.6	6.4
MCM-41HT	1144	0.6	2.9	3.8	1.1
MCM-48	1293	0.8	2.8	7.3	1.0
MCM-41SD	719	0.4	2.9	3.6	1.2
EtheenPMO	923	1.0	6.2	9.0	3.1
Nucleosil	88	0.4	~ 30	/	/

Tabel 10.1: De porie eigenschappen van de materialen.

10.2.1 Bepaling van alle silanol groepen in de structuur

Met behulp van in-situ infrarood spectroscopie werden alle OH-groepen (kolom 1 in tabel 10.2) in het materiaal bepaald, dit betekent zowel de oppervlakte OH-groepen als de silanolgroepen die zich in de wand bevinden. In tweede instantie werden via CP MAS H¹ ²⁹Si NMR de ratios aan silicium atomen bepaald met 1, 2 of zonder OH-groepen. Dit gaf informatie over de condensatiegraad van de gemaakte materialen.

Kolom 2 en 3 geven de respectievelijke percentages aan enkelvoudige en geminale silanol groepen in het materiaal. Kolommen 4 en 5 geven respectievelijk de totale ratio aan silanol groepen en zijn inverse, de condensatiegraad van het materiaal. Van deze kolommen kan afgeleid worden dat SBA materialen een hogere condensatiegraad bezitten dan MCM materialen, met de gesproeidroogde MCM als uitzondering. Een verklaring hiervoor kan de wanddikte zijn; MCM materialen hebben per hoeveelheid silica meer oppervlakte waardoor er bijgevolg meer oppervlakte OH's zijn. Ethaan PMO's zijn dan weer een ander verhaal; door de structuur van de precursor molecule ligt de condensatiegraad hier een stuk lager dan voor pure silica materialen.

	1	2	3	4	5
	(IR)	SiO_3OH	$SiO_2(OH)$	2 Totale	Condensatie-
		(%)	(%)	SiOH ratio	graad
Materiaal	α_{OH}	$Q^3/$	$Q^2/$	$(Q^2 + Q^3)/$	$Q^4/$
	$(\#/nm^2)$	$(Q^2 + Q^3)$	$(Q^2 + Q^3)$	$(Q^2 + Q^3 + Q^4)$	$(Q^2 + Q^3 + Q^4)$
SBA-15	3.2	88	12	0.25	0.75
SBA-16	6.8	96	4	0.35	0.65
MCM-41	1.9	94	6	0.40	0.60
MCM-48	2.4	94	6	0.42	0.58
MCM-41SD	2.7	97	3	0.26	0.74
	(IR)	SiO_2OH	$SiO_1(OH)$	$_2$ Totale	Condensatie-
		(%)	(%)	SiOH ratio	graad
Materiaal	α_{OH}	$T^{2}/$	$T^1/$	$(T^1 + T^2)/$	$T^3/$
	$(\#/nm^2)$	$(T^1 + T^2)$	$(T^1 + T^2)$	$(T^1 + T^2 + T^3)$	$(T^1 + T^2 + T^3)$
EtheenPMO	11.0	95	5	0.55	0.45

Tabel 10.2: Het silanol getal en de Q^2 , Q^3 en Q^4 groepen in het materiaal.

10.2.2 Bepaling van de maximale loading van de functionele groepen

De beladingsgraad van de functionele groepen op de verschillende materialen werd met elementaire analyse bepaald. De resultaten worden weergegeven in tabel 10.3. Aangezien de reactietijd voor de 3 verschillende functionele groepen gelijk werd gehouden kan besloten worden dat de belading daalt met de ketenlengte. Voor de hydrothermaal gesynthetiseerde materialen (MCM, SBA en Nucleosil) stijgt de belading per oppervlakte eenheid met de poriediameter. Maar voor het C18 silaan gaat deze logische tendens niet meer op aangezien MCM-41 een hogere belading heeft dan SBA-15. De reden hiervoor moet gezocht worden in de lange en bochtige poriestructuur van SBA-15. Deze maakt het moeilijk voor grotere functionele groepen om zich over de gehele porie te verspreiden. Een bijkomende observatie kan gemaakt worden wanneer SBA-15 vergeleken wordt met SBA-16 en MCM-41 met MCM-48 (dus hexagonaal vs kubisch), de belading voor de kubische systemen ligt systematisch hoger dan voor de hexagonale. De kubische systemen zijn toegankelijker door hun 3D porie systeem waardoor deze hogere belading kan verklaard worden. Dit betekent dat de functionering van oppervlakte OH groepen heel sterk diffusie gereguleerd is.

Materiaal	d_p	C3		C	8	C18	
	nm	mmol/g	$\#/nm^2$	mmol/g	$\#/nm^2$	$\mathrm{mmol/g}$	$\#/nm^2$
SBA-15	6.9	1.8	1.7	0.7	0.7	0.1	0.1
SBA-16	6.2	2.3	1.9	0.8	0.7	0.5	0.4
MCM-41	2.9	2.2	1.2	0.9	0.5	0.4	0.2
MCM-48	2.8	2.5	1.2	1.2	0.6	0.5	0.3
MCM-41SD	2.9	1.9	1.6	1.0	0.8	0.3	0.3
EtheenPMO	6.2	1.1	0.7	0.8	0.5	0.4	0.2
Nucleosil	~ 30	0.4	2.8	0.5	3.3	0.3	1.9

Tabel 10.3: De beladingscapaciteit van de drie chromatografische groepen op de verschillende geordende mesoporeuze materialen.

10.2.3 Enkele theoretische beschouwingen

Via het modelleren van de bereikbare oppervlakte OH groepen op silica kon berekend worden dat de maximale belading 2,5 groepen per nm^2 is voor een oppervlakte met enkelvoudige silanol groepen. Indien er geminale silanol groepen aanwezig zijn stijgt de maximale belading naar 4,5 groepen per nm^2 .

	1	2	3	4	5	6	7
			α_{OH}	h	d_p		
	totale	enkele	geminale opp		interne		
Materiaal			$(\#/nm^2)$	(nm)	(nm)		
SBA-15	3.2	2.8	0.4	1.7	1.5	4.2	6.9
SBA-16	6.8	6.5	0.3	1.9	4.9	6.4	6.2
MCM-41	1.9	1.8	0.1	1.2	0.7	1.1	2.9
MCM-48	2.4	2.3	0.1	1.2	1.2	1.0	2.8
MCM-41SD	2.7	2.6	0.1	1.6	1.1	1.2	2.9
EtheenPMO	11.0	9.3	1.7	0.8	8.5	3.1	6.2

Tabel 10.4: Het silanol getal, de hoeveelheid enkelvoudige, geminale, oppervlakte en interne silanol groepen per oppervlakte eenheid.

Kolom 4 geeft het aantal OH groepen dat beschikbaar was voor het C3 silaan. Aangezien deze waarden nergens de maximale belading van 2,5 groepen per nm^2 overschrijden, kan beschouwd worden dat alle oppervlakte OH groepen meegenomen werden door het C3 silaan. Verder kan geobserveerd worden dat het aantal beschikbare oppervlakte OH groepen voor SBA-15, SBA-16 en MCM-41(SD) groter is dan voor MCM-41 en MCM-48. Dit kan verklaard worden door de synthese procedure, de eerste groep werd in zuur milieu gemaakt terwijl de laatste groep in basisch milieu gemaakt werd. Dit kan verklaard worden door het verschil in interactie tussen de silica species en het surfactant. Etheen PMO's vormen weerom de uitzondering; door het toevoegen van deze organische groep aan de structuur wordt het aantal oppervlakte OH groepen sterk gereduceerd.

10.3 De productie van sferische MCM-41 materialen via sproeidrogen: een verdubbeling van de retentie factor van HPLC kolommen

MCM-41 werd als *proof-of-concept* materiaal gebruikt om het effect van geordende mesoporeuze materialen te analyseren op een chromatografische scheiding. Een statistische studie werd opgestart om de verschillende invloeden te onderzoeken van de synthese parameters op zowel de deeltjes morfologie als de porie-eigenschappen. Hierbij was het doel om een zo goed mogelijke uniforme sferische morfologie te bekomen samen met een zo hoog mogelijk oppervlak. Het ontwikkelde materiaal werd vervolgens gefunctionaliseerd met een C18 groep en gepakt in een HPLC kolom om uitgebreid geëvalueerd te worden.

10.3.1 Het controleren van de porie eigenschappen

De grootste invloed op de porie eigenschappen van het gesproeidroogde materiaal kan toegeschreven worden aan de hoeveelheid surfactant in het reactiemengsel. Hoe hoger de hoeveelheid surfactant hoe hoger de oppervlakte van het materiaal, waar een maximum kon opgemeten worden bij een silaan/surfactant verhouding van 1/0.24. Verder bleek ook dat wanneer de hoeveelheid water werd verhoogd zodat de surfactant concentratie lager dan de kritische micel concentratie lag, er nog altijd een geordend mesoporeus materiaal gemaakt werd. Dit betekent dat de vorming van een micellaire structuur samen met de *co-assembly* van de silica-surfactant structuur minstens even snel verloopt als het sproeidroogproces dat niet meer dan 6 seconden duurt.

10.3.2 Het controleren van de deeltjes morfologie

De invloed van de gas-flow

De gas-flow heeft vooral een invloed op de deeltjesgrootte van het gesproeidroogde materiaal. Hoe hoger de gas-flow, hoe groter de vernevelingskracht en dus hoe kleiner de druppels. Bijgevolg zullen de sferische deeltjes ook kleiner zijn.

De invloed van de hoeveelheid CTAB

Hoe meer CTAB wordt toegevoegd aan het reactiemengsel, hoe slechter de morfologie van de materialen wordt. De deeltjesgrootteverdeling schuift bovendien ook op van een brede naar een meer uniforme verdeling met een verlaging van de hoeveelheid surfactant.

De invloed van de hoeveelheid water

De hoeveelheid water heeft een positieve invloed op de morfologie van de deeltjes, hoe meer water aan het reactiemengsel wordt toegevoegd hoe beter de morfologie. Dit kan toegewezen worden aan de oppervlakte spanning van het reactiemengsel. Om een mooie ronde druppel te vormen in het sproeidroogproces speelt de oppervlaktespanning een heel belangrijke rol. Hoe hoger de oppervlaktespanning, hoe beter de druppels de vervormingskrachten die werkzaam zijn in de sproeidroger kunnen weerstaan. Dit is tevens de reden waarom een verhoging van de hoeveelheid surfactant een negatieve invloed heeft op de morfologie van de materialen.

10.3.3 Op zoek naar de balans tussen een ideale porositeit en perfecte uniforme sferen

Aangezien de hoeveelheid surfactant een positieve invloed heeft op de porie-eigenschappen maar een negatieve invloed op de sferische morfologie van de gesproeidroogde materialen zal een compromis gezocht moeten worden waarbij beide materiaal eigenschappen gemaximaliseerd worden.

Hierbij zijn de water-ratio en de CTAB-ratio de twee componenten die ten opzichte van elkaar zullen moeten afgewogen worden om tot een goed resultaat te komen. Figuur 10.1 en figuur 10.2 tonen de variatie van de oppervlakte en de morfologie in functie van de CTAB- en de water-ratio.



Figuur 10.1: De morfologie en de specifieke oppervlakte in functie van de CTAB/ TEOS ratio.

Deze laatste figuur laat zien dat hoge water hoeveelheden ervoor zorgen dat er deeltjes kunnen gemaakt worden die zowel een goede morfologie als een goede poriestructuur bezitten. Het enige nadeel bestaat erin dat de opbrengst bij hoge hoeveelheden water sterkt verlaagt en er bijgevolg snel naar grotere toestellen moet overgeschakeld worden. Nadat er voldoende materiaal gemaakt werd zijn de MCM-41 sferen gefunctionaliseerd met C18 ketens (8 m%, met een oppervlakte van 958 m^2/g komt dit neer op 0.2 groepen per nm^2) en gepakt in een kolom (5 cm, 2.1 mm I.D.) om vervolgens uitgebreid geëvalueerd te worden.



Figuur 10.2: De morfologie en de specifieke oppervlakte in functie van de $H_2O/TEOS$ ratio.

10.3.4 De evaluatie van de kolom met behulp van HPLC

De stabiliteit van de pakking

Figuur 10.3 laat duidelijk zien dat de pakking zeker 300 scheidingen stabiel blijft, zowel in termen van deeltjes morfologie als in termen van porositeit.

Figuur 5.16 op pagina 107 laat zien dat de kolom met gemak verschillende mengsels kan scheiden aan verschillende snelheden. Bovendien laat chromatogram d zien dat zelfs de grote polyaromatische koolwaterstoffen vlot door het porie systeem van het materiaal kunnen. Verder kunnen we ook afleiden dat van de theoretische 13000 platen die kunnen bereikt worden met deze kolom er slechts 5000 behaald werden. De reden hiervoor kan worden gevonden in het feit dat de deeltjesgrootteverdeling nog steeds te breed was voor een perfecte pakking van de kolom.

Het grootste voordeel van dit materiaal moet niet gezien worden in een verbeterde efficiëntie maar eerder in een sterk verbeterde retentie. Figuur 10.4 toont de prototype MCM-41 kolom in vergelijking met 3 commerciële kolommen, een Luna C18(2), een Zorbax SB C18 en een X-bridge kolom. Deze figuur toont mooi aan hoe de dezelfde analyse op de vier kolommen werd uitgevoerd en het verschil in retentiefactor duidelijk naar voor komt. De geordend poreuze kolom heeft een k' factor die 2 keer hoger ligt dan deze van de beste geteste commerciële kolom, terwijl de belading slechts een vijfde besloeg (zie tabel 10.4). De reden voor dit verschil in retentie kan gevonden worden in de geordende porositeit en de grotere oppervlakte. De toegankelijkheid van de binnenkant van de deeltjes, en bijgevolg de stationaire



fase, wordt op deze manier sterk vergroot.

Figuur 10.3: Deeltjes en porie stabiliteit in functie van de pakkings condities. SEM vlnr: na grafting en na pakken en 300 chromatografische runs.

Kolom	k'	$SA_{(BET)}$	C belading	Groepen	k/groep
		$(m^2/g)^2$	(m w%)	per nm^2	
mesoporous	33	957	8	0.2	0.17
Luna	14.5	400	17.5	1.1	0.03
X-bridge	8.6	185	18	2.3	0.02
Zorbax	12.2	180	10	1.3	0.05

Tabel 10.5: Een uitgebreide retentie studie van de de geteste kolommen.



Figuur 10.4: (A,B) Analyze van een mengsel dat uracil (1), caffeine (2), fenol (3), 1-fenyl-1-ethanon (4), propyl-4-hydroxybenzoaat (5), benzeen (6), and tolueen (7) op de X-bridge (A), de Zorbax SB C18 (B), de Luna C18 (2) (C) en de MCM-41 (D) kolom.

10.4 Hybride silica partikels met een geordende porositeit: chromatografische pakkingen met sterk verhoogde stabiliteit

Silica materialen zijn gevoelig voor hydrolyse ter hoogte van de siloxaanbruggen in de structuur, vooral in een sterk basisch milieu vindt deze hydrolyse plaats. Maar ook in water bij verhoogde temperatuur gaan de siloxaan bruggen wegreageren met vorming van twee silanol groepen. De huidige oplossingen voorzien een pacificering van het oppervlak met trimethylsilyl groepen of met een volledige organosilaan laag. Dit vertraagt de hydrolyse van het silica materiaal maar het laat evenwel nog niet toe om de materialen lang bij hoge pH of hoge temperatuur te gebruiken. Daarom werd voor dit onderzoek gekozen voor een organosilaan dat het aantal siloxaanbruggen in de deeltjes halveert door ze te vervangen door koolstofbruggen. De synthese van de bewuste precursor wordt weergegeven door het onderstaande reactiemechanisme.



Figuur 10.5: De vorming van de ring precursor met R1 als een ethoxy groep.

10.4.1 De ring-PMO sproeidrogen



Figuur 10.6: N_2 -sorptie isothermen voor de startsamenstelling (rPMO-SD1) en de twee optimalisaties (rPMO-SD2 en rPMO-SD3).

Hier werd vertrokken van de eerste rapportering van de hydrothermale synthese van ring PMO materialen.⁴⁰ Deze samenstelling werd verder geoptimaliseerd zodat een materiaal met een specifieke oppervlakte van 903 m^2/g en een sferische morfologie werd bekomen (zie figuren 10.6 en 10.7). De grootste invloed op de porie-eigenschappen werd veroorzaakt door een verdubbeling van de hoeveelheid precursormolecule. De morfologie werd voornamelijk beïnvloed door de hoeveelheid water in de oplossing, zoals eerder in sectie 10.3 aangetoond.



Figuur 10.7: SEM foto's voor de startsamenstelling (rPMO-SD1 (A)) en de twee optimalisaties (rPMO-SD2 (B) en rPMO-SD3 (C)).

Nadat een voldoende grote hoeveelheid materiaal geproduceerd was, werden de materialen gefunctionaliseerd met C18 (0.22 groepen/ nm^2), gepakt in een kolom (5 cm, 2.1 mm I.D.) en chromatografisch geëvalueeerd.

10.4.2 Kolom en materiaal evaluatie

De zuiverheid van de materialen

Via elementaire analyse werd ontdekt dat de geproduceerde materialen naast koolstof, silicium, waterstof en zuurstof ook nog enkele metaal inclusies bevatten. Deze metalen zijn afkomstig van de sproeikop van de sproeidroger want de oplossingen bevatten voor het sproeidrogen geen metalen. Dit betekent dat de materialen een behandeling zullen moeten ondergaan zodat er geen metaalinclusies en andere ionen in de finale deeltjes meer zitten. Dit wordt voornamelijk bekomen met verdunde HF oplossingen.

De pakkingsdichtheid

Via de total pore blocking method werd de densiteit van de pakking bepaald. Dit is een maat voor de uniformiteit van de deeltjes, de minimale externe porositeit van een kolom kan berekend worden vanuit de hexagonaal dichtste bolstapeling (35%). Met deze kolom wordt een externe porositeit bekomen van 45% (zie figuur 10.8) en dit is 5 à 7% hoger dan wat er standaard bereikt wordt bij commerciële kolommen.

Standaard kolom evaluatie

Wanneer dezelfde analyse als voor de 3 commerciële kolommen uit sectie 10.3 wordt toegepast dan blijkt dat de huidige kolom een nog grotere retentie oplevert. Deze verbetering in retentie (3 keer hoger dan een Luna C18 (2)) kan toegewezen worden aan de hogere specifieke oppervlakte, net zoals het geval is bij de op MCM-41

gebaseerde C18 kolom. De verdere verbetering ten opzichte van deze laatste kolom kan toegewezen worden aan de grotere koolstof belading die het gevolg is van de ring precursor die als startsilaan werd gebruikt. Figuur 10.9 toont het van Deemter plot voor 1-fenyl-1-ethanon, hierop kan een plaathoogte van 14 µm afgelezen worden. Dit is vergelijkbaar met wat er in de literatuur werd bekomen, maar dan op 1.7 µm deeltjes, de deeltjesgrootte in de ring PMO kolom bedroeg 2.3 µm. Dit is evenwel nog niet de plaat hoogte die bereikt wordt voor commerciële kolommen (tussen 2 en 5 µm), de reden hiervoor kan gevonden worden in het feit dat de deeltjesgrootteverdeling nog niet nauw genoeg is.



Figuur 10.8: De externe kolom porositeit.

De analyse van polaire componenten

De sterk verbeterde retentie kan toegepast worden voor de scheiding van polaire componenten. De gemaakte prototype kolom werd vergeleken met een *state-ofthe-art* kolom voor de scheiding van enkele polaire pesticiden. Hierbij werd snel duidelijk dat de nieuwe kolom de meest polaire analieten kon scheiden terwijl dit niet het geval was bij de commerciële kolom.

De stabiliteit van de kolom en zijn pakking

De laatste test die werd uitgevoerd was een stabiliteitstest. Hierbij werd de kolom onderworpen aan een reeks van spoelingen en analyses afwisselend bij hoge, neutrale en lage pH. In het totaal zijn er 1500 analyses uitgevoerd op de kolom en figuur 10.10 laat duidelijk zien dat de kolom deze test goed doorstaan heeft. Bovendien

zijn er geen fysieke verschillen te zien tussen de verschillende deeltjes voor en na de test, bovendien bleef de poriestructuur van het materiaal perfect behouden.



Figuur 10.9: Het H versus u plot vah 1-fenyl-1-ethanon onder isocratische condities $(40/60 \text{ acetonitril/H}_2\text{O})$ op het rPMO-SD3 materiaal.



Figuur 10.10: Links: het aantal platen voor diphenhydramine, fenol, acetofenon and propylparabeen in functie van het aantal runs; rechts: de retentie factoren voor diphenhydramine, fenol, 1-fenyl-1-ethanon and propyl-4-hyrdoxybenzoaat in functie van het aantal runs.

10.5 Het vergroten van de porie diameter van gesproeidroogde silica sferen

Om de poriegrootte van de materialen te vergroten kan gebruik gemaakt worden van grotere surfactanten. In dit deel van het onderzoek werd gebruik gemaakt van twee verschillende Pluronic triblok co-polymeren, namelijk P123 dat voornamelijk hexagonale systemen vormt en F127 dat voornamelijk kubische systemen vormt. Maar in vergelijking met CTAB wordt de beoogde micelstructuur niet binnen enkele seconden gevormd. De Pluronic surfactanten zijn daarvoor veel te omvangrijk waardoor dit proces samen met de de polycondensatie van de silica precursoren minstens een half uur duurt. Hierdoor worden grote co-assemblages van silica en surfactant moleculen gevormd die op het moment dat ze te groot worden zullen neerslaan in de oplossing. Om de uiteindelijke morfologie van de deeltjes te kunnen controleren moet dit ten allen tijde vermeden worden.

10.5.1 Het hexagonale SBA-15 systeem

Gedurende de ontwikkeling van een gesproeidroogd SBA-15 systeem werd duidelijk dat de oplossing niet langer dan 1.5 uur mag roeren, dit om te verhinderen dat er een neerslag in de oplossing ontstaat. Bovendien moet de oplossing tijdens de voorbereiding op 90°C gehouden worden om te verzekeren dat een SBA-15 systeem ontstaat met voldoende oppervlakte. Om de morfologie te kunnen controleren werd een tweede surfactant (CTAB) toegevoegd aan het reactie mengsel. Dit had een positieve invloed op de oppervlakte van het eindmateriaal waarbij 742 m^2/g werd bereikt. Door het toevoegen van 1-butanol aan dit reactiemengsel kon een meer sferische morfologie bereikt worden (zie figuur 10.11). De porie diameter van materiaal B bedroeg 7.1 nm en is ongeveer het dubbele van de MCM materialen. Dit materiaal biedt de beste opties om een pakkingsmateriaal voor chromatografie te ontwikkelen.



Figuur 10.11: De morfologie van SBA-15 gesproeidroogd met een co-surfactant (A: 742 m^2/g) en met een co-surfactant en butanol als cosolvent (B: 436 m^2/g).

10.5.2 Het kubische SBA-16 systeem

Tijdens de ontwikkeling van dit poriesysteem bleek dat het mogelijk was om hiermee cilindrische poriën te maken. Dit bleek hoofdzakelijk een functie te zijn van de reactietijd voor het sproeidrogen, zoals af te leiden is uit de N₂-isothermen in figuur 10.12. Bovendien bleek het gemakkelijker te zijn om een sferische morfologie te bekomen met dit surfactant. De reden hiervoor zou kunnen zijn dat er preferentieel sferische micellen gevormd worden met dit surfactant.



Figuur 10.12: De $\rm N_2\text{-}isothermen van gesproeidroogde SBA-16 in functie van de reactie tijd.$

Dit onderzoek staat duidelijk nog in zijn kinderschoenen, er moet nog heel wat optimalisatie gebeuren van de synthese om deze materialen in grote hoeveelheden te kunnen produceren via sproeidrogen. Dit zou het onderwerp moeten uitmaken van toekomstig onderzoek.

10.6 De superieure retentie van geordende mesoporeuze materialen brengt *Wall Coated Open Tubular CEC* terug naar de voorgrond

Net zoals bij gas chromatografie kan de beste kolom performantie bereikt worden door gebruik te maken van *Open Tubular* kolommen, dit zijn kolommen zonder

pakking maar met een wand die voldoende retentie heeft om een scheiding te bekomen. Doordat de diffusiecoëfficient in de vloeistoffase 1000 keer lager ligt dan in de gasfase, moet de kolomdiameter terug gebracht worden tot 1 a 5 µm interne diameter. Maar de meest limiterende factor is de lage faseratio en de lage hoeveelheid analiet dat kan geïnjecteerd worden zonder de kolom te overladen. De verhoogde retentie van geordende mesoporeuze materialen kan de oplossing blijken voor deze problemen, waardoor WCOTLC kan doorbreken in de routine analyse. Omwille van het gemak in kolom en detector koppeling werd voor deze kolommen gekozen voor een elektrogedreven variant op capillaire chromatografie, namelijk capillaire elektrochromatografie of CEC.

Gedurende dit onderzoek werd een SBA-16 (kubisch porie systeem) off-column geoptimaliseerd om in een laatste fase als dikke coating op de binnenzijde van de capillairen te leggen. Op deze manier kan de gemeten porositeit enigszins gekoppeld worden aan de porositeit op de binnenwand van het capillair. Deze laag is anders niet eenvoudig te analyseren.

10.6.1 Karakterisatie van de gecoate laag op *off-column* substraten

De laageigenschappen werden onderzocht voor verschillende parameters, waaronder de coating snelheid, de calcinatie temperatuur en de viscositeit van de coating oplossing. Hierbij werd duidelijk dat de laagdikte stijgt met de viscositeit en de coating snelheid. De porositeit daarentegen daalt met de coating snelheid maar stijgt met de viscositeit. Aangezien de viscositeit van de oplossing een gevolg is van het toevoegen van meer surfactant leidt dit ook tot een hogere porositeit (net als in sectie 10.3). De relatie tussen de coating snelheid en de lagere porositeit is minder logisch. Een mogelijke verklaring kan zijn dat als er met de hogere snelheid een dikkere laag wordt gevormd, de vorming van micellen in de coating vertraagd wordt waardoor de porositeit verlaagt.

De invloed van de calcinatie temperatuur op de porie eigenschappen werd ook onderzocht. Hierbij blijken de porositeit en de laagdikte beide naar beneden te gaan met een stijging van de calcinatie temperatuur. Dit kan verklaard worden door het feit dat de hogere temperatuur ervoor zorgt dat meer silanolgroepen omgezet worden in siloxaanbruggen waardoor de volledige laag een hogere densiteit krijgt.

10.6.2 Karakterisatie van het capillair

Eens de lagen geoptimaliseerd waren, werd overgestapt naar capillairen. Hierbij werd de silica oplossing doorheen een fused silica capillair gestuurd met behulp van een N_2 -flow. De reactie tijd werd gevarieerd van 5 minuten tot 48 uur. De dikste laag werd bekomen bij een reactie tijd van 48 uur. De analyse van de laag kan voorlopig enkel en alleen via SEM metingen. Figuur 10.13 toont dat er een 600 nm dikke silica laag afgezet werd op de binnenzijde van een 50 µm fused silica capillair.



Figuur 10.13: SEM foto's van een gecoat capillair na 12 uur coaten.

Tabel 10.6: De plaat getallen, retentie factoren, scheidings factoren en de resolutie van de analyze van een fenonen mengsel op CC3, mobiele fase: 50mM MES pH7/MeCN 50/50.

Component	t_r	Ν	k	α	R_S	N_{eff}	Н	h
	min						μm	
1	34.1	43135					15.4	0.31
2	37.2	40395	0.09			281	16.5	0.33
3	39.0	39461	0.14	1.58	2.29	623	16.9	0.34
4	46.1	35865	0.35	2.45	7.29	2430	18.5	0.37
5	52.6	31225	0.54	1.54	5.46	3863	21.3	0.42
6	62.3	24314	0.83	1.52	6.07	4982	27.4	0.55
7	99.1	17685	1.91	2.30	12.3	7608	37.6	0.75

10.6.3 Chromatografische evaluatie van de gecoate capillairen

Hierbij werd een gefunctionaliseerde kolom met een laagdikte van 600 nm en een lengte van 70 cm (66.5 cm chromatografische lengte) vergeleken met eenzelfde nietgecoate kolom. Hierbij werd onmiddellijk duidelijk dat onder dezelfde condities de niet-gecoate kolom (CC1) vrijwel geen scheiding van het mengsel kon bewerkstelligen terwijl de gecoate kolom (CC3) dat wel kon (zie figuur 10.14). Bij verdere analyse van de kolom bleek dat de electro osmotische flow (EOF) vooral afhankelijk is van de samenstelling van de mobiele fase. Wanneer de mobiele fase veranderd wordt van 50% buffer naar 60% buffer dan gaat de EOF van 1.95 cm/min naar 1.99 cm/min en wanneer de hoeveelheid buffer verder verhoogd wordt naar 80% stijgt de EOF verder naar 2.75 cm/min. Dit is logisch daar de EOF vooral bepaald wordt door de hoeveelheid ionen die zich in de mobiele fase bevinden. Tabel 10.5 geeft de resolutie voor de scheiding van een mengsel van fenonen met 50% buffer. Hieruit blijkt dat de plaathoogte voor de scheiding, afhankelijk van de geanalyseerde component tussen 15 en 38 μ m ligt.



Figuur 10.14: Analyse van een mengsel van thiourea (1), methyl-4hydroxybenzoaat (2), ethyl-4-hydroxybenzoaat (3), propyl-4-hydroxybenzoaat (4) en butyl-4-hydroxybenzoaat (5) op CC1 (niet gecoate kolom) en CC3 (gecoate kolom). Injectie bij 50 mbar voor 5s; mobiel fase: 50 mM MES buffer pH 7/ACN 80/20; meting bij 30 kV; flow rate: 0.45 mm/s.

De chromatografische evaluaties laten zien dat de verbeterde retentie, inherent aan de grote specifieke oppervlakte van deze geordende mesoporeuze materialen, kan gebruikt worden om open tubulaire vloeistof chromatografie weer naar de voorgrond te brengen. In de toekomst zal de kolom-tot-kolom reproduceerbaarheid uitgebreid getest moeten worden en bovendien zal moeten nagegaan worden indien dezelfde verbetering ook kan gerealiseerd worden zonder een surfactant aan de oplossing toe te voegen. Dan pas zal onomwonden bewezen zijn dat deze materialen echt noodzakelijk zijn om deze tak van de chromatografie rendabel te maken.

10.7 Conclusie en Toekomstperspectieven

Geordende mesoporeuze materialen hebben in eerste instantie een grote invloed op de retentie van analyten in een kolom. Dit is in hoofdzaak te wijten aan de verhoogde specifieke oppervlakte van de materialen. Maar de deeltjesgrootteverdeling van de huidige materialen is onvoldoende nauw om het volledige potentieel van deze materialen in kaart te brengen. Bijgevolg is dit het belangrijkste punt waaraan gewerkt dient te worden in de toekomst. Zonder de uniforme deeltjes kunnen geen goede kolommen gepakt worden waardoor deze materialen een curiositeit zullen blijven als stationaire fase binnen de chromatografie.

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Conferences

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Ordered Mesoporous (Organo)Silica Particles as Packing Material in RPLC: Increasing Retention and Improving Particle Stability M. Ide, F. Lynen, P. Sandra and P. Van Der Voort, HPLC36, 2011, June 19-23, Budapest, Hungary.

Presentations

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Hierachical and Bimodal Ethenylene PMO Materials

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Ordered mesoporous (organo)silica particles as packing material in RPLC: increasing retention and improving particle stability

M. Ide, F. Lynen, P. Sandra and P. Van Der Voort, HTC12, 2012, February 1-3, Bruges, Belgium.

Appendix

Ellipsometry²⁰⁵

Ellipsometry measures a difference in polarisation when light is reflected by a measured sample. The change in polarisation is depicted by its amplitude ratio (Ψ) and faseratio (Δ). The measured response depends on the optical properties and the thickness of the sample. The technique is primarily used to determine the layer thickness and the optical constants. Nevertheless it can also be used to characterize the composition, crystallinity, surface roughness and much more material properties relative to a change in optic response.

Experimental values can be transformed to physical parameters such as layer thickness, diffraction index, roughness and optical focal point with a sample specific mathematical model.



Figure 10.15: The ellipsometric set up.

During the course of this study a J.A. Woolem Co. Inc. ellipsometer with an FC5300 75W Xe-lamp.

N_2 -adsorption^{103,206}

To characterize of the surface of solid porous materials, N_2 -adsorption measurements are most often used. The technique makes use of the physisorption and condensation of N_2 -gas on the surface of the porous solid. It is a non-destructive analysis technique due to the fact that physisorption does not change the surface chemistry. Also at higher temperatures the surface is free from physisorbed gasses. A second property, inherent to physisorption, is the possibility to form multilayers of gasses.



Figure 10.16: A N₂-isotherm of an ordered mesoporous material.

Figure 10.16 shows the N₂-isotherm of an ordered mesoporous material. The figure is divided in 4 different areas. In the first area, Langmuir area, a monolayer of gas is adsorbed to the surface. In the second area multilayers of gas are formed with a more or less direct relation between the amount of gas adsorbed and the relative pressure. From this area the specific surface area of the material is calculated with the BET (Brunauer, Emmet and Teller) theory¹⁸⁸. In the third area a capillary condensation of gas takes place in the pores. Here the pore size distribution is calculated with the BJH (Barrett, Joyner and Halenda) calculations²⁰⁷. The sharper the jump in adsorbed gas the more narrow the pore size distribution will be. The higher the pressure at which the capillary condensation takes place the larger the pores of the system are. In the fourth and final area the pores of the material are completely filled, so here the total pore volume is calculated. However, if further adsorption occurs here, it is due to inter particle porosity.

Upon desorption a so-called hysteresis loop is observed in the third area. The form of this loop can give a lot of information on the type of pores the material comprises.

Solid-State NMR²⁰⁸

Solid-state NMR spectroscopy is a variation on nuclear magnetic resonance spectroscopy, characterized by the presence of anisotropic interactions. The spin of the atom core interacts with the magnetic field of the NMR equipment. The proximity of two atoms, bonded or not, can give rise to interactions between the two nuclei. In the solid state, the atoms have little to no mobility. Here aniostropic interactions have a substantial influence on the behavior of a system of nuclear spins, whereas in the liquid-state the Brownian motion leads to an average of these interactions. There are several of those interactions of which *chemical shift anisotropy* and *dipolar coupling* are the most common.

These interactions modify the nuclear spin energy levels of all the sites in a molecule. They often contribute to line-broadening in NMR spectra. In solids these cannot be avoided, so here high resolutions are established using magic angle spinning (MAS) (see figure 10.17). The residual line width after spinning at the magic angle (ca .54.74, where $\cos^2\theta_m = 1/3$) at a rate of 5-15 kHz is in the order of 0.5-2 ppm. Other interactions, such as second order quadrupolar interactions, account for more line broadening.

Other interactions that play a role in solid-state NMR are chemical shielding, Jcoupling, dipolar coupling and the previously mentioned quadrupolar interaction. Chemical shielding is a local property of each nucleus and is dependent on the external magnetic field B, because this field induces an electric field in the molecular orbitals of the material. These currents have their own magnetic field. Under sufficiently fast MAS this directionally dependent factor is removed and the isotropic chemical shift is left. This shift is unique for the nucleus and its surroundings (both chemical and spatial) and is used to characterize the materials. A different shift infers a different atomic surrounding of the nucleus under observation.

J-coupling describes the interaction of nuclear spins through chemical bonds. Dipolar coupling originates from the dipole moment of one nucleus interacting with another. It depends on the spin of the species, the internuclear distance and the orientation of the vector connecting the two nuclear spins (visualized in figure 10.17). Quadrupolar interactions originate from the non spherical charge distribution of nuclei with a spin greater than 1.5. This is the largest interaction in NMR and is the predominant cause of peak broadening in solid-state NMR. An actual measurement starts with an RF pulse that starts with cross-polarization (CP), which is common in solid-state NMR (see figure 10.17). The CP is used to enhance the signal of nuclei with a low gyromagnetic ratio, by a magnetization transfer from nuclei with a high gyromagnetic ratio. To establish this transfer, the pulse applied on the two channels must fulfill the Hartmann-Hahn condition. Under MAS, it defines a relation between the voltage through the RF coil and the rate of sample rotation. To increase the resolution nuclear spin interactions are decoupled. However, these broad lines can still contain a lot of information on the sample. For instance dipolar coupling is distant dependent, so it may be used to calculate interatomic distances in labeled molecules.



Figure 10.17: Left: An illustration of MAS in the external magnetic field B under the angle θ . Middle: An illustration of the dipolar coupling between two nuclei I_1 and I_2 . Right: CP (cross-polarization) pulse sequence.