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TECHNISCHE UNIVERSITÄT WIEN

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DISSERTATION

Development and Chromatographic Characterisation of Monolithic Capillary LC Columns Based on MTMS as Precursor

Ausgeführt zum Zwecke der Erlangung des akademischen Grades
eines Doktors der technischen Wissenschaften
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Kurzfassung

Ziel der Dissertation war es, monolithische Trennsäulen für die Flüssigkeitschromatographie in Kapillaren zu synthetisieren. Das verwendete synthetische Protokoll basiert auf Sol-Gel Technologie. Methyl-trimethoxysilan wird dabei als Edukt verwendet.

Als erster Schritt wurde versucht, ein Protokoll mit basischer Katalyse, das für die Synthese von Trennsäulen mit 4.6 mm Innendurchmesser entwickelt wurde, auf Kapillarformat anzuwenden. Dabei wurden in den meisten Fällen Lücken zwischen der monolithischen Trennphase und der Kapillarwand beobachtet. Als Grund wurde das Entstehen eines thermischen Gradienten von der Kapillarwand zur Mitte während der Synthese identifiziert. Durch eine schnelle Thermostatisierung der befüllten Kapillare konnte das Auftreten dieser Risse verhindert werden. Trotz Optimierung der Synthese war die Bindung der Monolithen zur Kapillarwand jedoch zu schwach, um diese Säulen erfolgreich für die Flüssigkeitschromatographie einzusetzen.

Sol-Gel Synthese basierend auf saurer Katalyse wurde in einem weiteren Teil der Arbeit eingesetzt. Es wurde der Einfluss verschiedener Parameter auf Poren- und Skelettgröße untersucht, darunter Temperatur, Konzentration des Katalysators und Zusammensetzung der Reaktionslösung. Die morphologischen Eigenschaften wurden dabei mit Rasterelektronenmikroskopie sowie der Auswertung von Stickstoffadsorptionsisothermen ermittelt. Es konnten Materialien mit Porengrößen zwischen 0,8 und 15 µm, sowie mit Skelettgrößen zwischen 0,4 und 12 µm hergestellt werden. Spezifische Oberflächen von bis zu 334 m^2/g wurden erzielt. Materialien mit Skelettgrößen von über 3 µm zeigten keine signifikante innere Oberfläche. Während die Makroporen- und Skelettgröße gut reproduziert werden konnten, war das für die spezifische Oberfläche nicht der Fall.

Chromatographische Tests wurden zur Charakterisierung der chemischen Eigenschaften der Säulenmaterialien verwendet. Der beobachtete Rückdruck der Säulen stimmte mit den erwarteten Werten, die sich aus den über die REM Messungen erhaltenen Porengrößen ergaben, überein. Die mit REM ausgewerteten Querschnittsflächen waren daher für die gesamte Säule repräsentativ. Die Selektivität zwischen hydrophoben Testanalyten variierte nur gering zwischen den Säulen. Die Methylenselektivität war dabei im Bereich von Octadecylphasen. Die Retention polyaromatischer Verbindungen war geringer als erwartet, was auf einen anderen Wechselwirkungsmechanismus schließen lässt. Säulen, die unter gleichen Bedingungen

hergestellt wurden, zeigten eine relativ hohe Varianz der Kapazitätsfaktoren, was mit der Beobachtung der wenig reproduzierbaren spezifischen Oberflächen übereinstimmt. O-Terphenyl und Triphenylen zeigten bei den meisten untersuchten Materialien keine Retention, die innere Oberfläche ist für diese Analyten aufgrund zu kleiner Mikroporen nicht zugänglich. Das Mizellen bildende Polymer Brij wurde im Weiteren als Porogen eingesetzt. Retention wurde nun für diese beiden Analyten beobachtet. Sehr breite Peaks lassen allerdings darauf schließen, dass in diesen Fällen die Mikroporendurchmesser keinen schnellen Stoffaustausch zwischen Oberfläche und mobiler Phase zulassen. Eine Bestimmung der Silanolaktivität zeigte im Vergleich zu kommerziellen Säulen mittlere Werte, ohne dass Reaktionen zur Deaktivierung durchgeführt wurden. Ein Material das basisch gealtert wurde, zeigte bei der Charakterisierung sehr breite Peaks für Naphthalin und Anthracen, die Porengröße wurde durch diesen Schritt offensichtlich verkleinert. Eine längere Alterung hat Materialien ohne jegliche Retention erzeugt – durch diesen Prozess lassen sich also Mikroporen eliminieren.

Die Ergebnisse haben gezeigt, dass es notwendig ist, Mesoporen in die Materien einzubringen - allenfalls durch das Verwenden von Templaten. Dadurch würde Größenausschluss schon für relativ kleine Moleküle eliminiert, die Diffussion in das Material und dadurch auch die chromatograpfische Auflösung würden sich deutlich verbessern. Die Synthese von effizienten Kapillarsäulen mit Umkehrphasencharakteristik wäre dann möglich. Aufgrund der hydrolytischen Stabilität der organischen Modifikation und der chemischen Homogenität des Materials wäre eine sehr gute Langzeitstabilität zu erwarten. Möglichkeiten zur Einbringung anderer Funktionalitäten sollten im Weiteren untersucht werden; MTMS basierende Materialien hätten aufgrund erhöhter pH Stabilität und kleinerer Silanolaktivität auch in diesen Fällen einen Vorteil gegenüber herkömmlichen Kieselgel basierender Materialien.

Abstract

Aim of this thesis was the development of a new synthetic protocol for the fabrication of monolithic columns in capillary format. For this purpose, methyl-trimethoxysilane was chosen as the sole precursor in a synthesis based on sol-gel technology.

In a fist step, a previously developed protocol based on basic catalysis was transferred from wide-bore format to capillary format. The occurrence of gaps to the capillary wall was a problem encountered in many cases. This was attributed to a thermal gradient from the centre of the capillary to the wall during synthesis. Adjustments in the protocol could eliminate these gaps. Adhesion between capillary wall and the monolithic material could not be improved to a point which would allow successful application for chromatography.

A second approach based on acid catalysis showed potential for the fabrication of capillary columns. The influence of reaction conditions such as temperature, reaction mixture composition and catalyst concentration on the morphology of obtained monoliths was examined. Precise control of these parameters allows to tailor the monolith´s structure on a macroscopic scale (skeleton and through pore diameters). Physical characterisation was done with scanning electron microscopy and nitrogen adsorption. Pore diameters could be varied from 0.8 to 15 µm, diameters of the xerogel network from 0.4 to 12 µm, respectively. Specific surface areas up to 334 m^2/g have been observed. Generally materials with skeleton diameters above 3 µm did not possess specific surface areas in a significant quantity. Repeatability of the macroscopic morphological characteristics was good, however it was poor regarding observed specific surface areas.

Chromatographic characterisation was successfully carried out with synthesised columns. Observed backpressure was in good agreement with values calculated form the pore diameters obtained from SEM micrographs. Hydrophobic properties of the produced columns were relatively uniform, with methylene selectivity exhibiting a value comparable to octadecyl modified stationary phases. Retention for polyaromatic compounds was less than expected, indicating the action of a second, different retention mechanism. Retention factors observed for columns synthesised under the same conditions varied which indicated a poor reproducibility for the accessible phase volume. Chromatographic characterisation carried out under different mobile phase compositions shows a dependency of the available phase volume on the content of

the organic modifier in the mobile phase. Size exclusion was observed for o-terphenyl and triphenylene in most cases. Access to the specific surface area is restricted by micropores too narrow to allow these analytes to enter. Materials which were produced with the micelle forming tenside Brij as polymeric porogen did not show size exclusion effects; very broad peaks indicate however that pores are still too small to allow a fast diffusion of the analytes within the silica skeleton. All materials showed a moderate silanol activity compared to commercial reversed phase materials without having undergone a deactivation process. Several columns were subjected to an ageing process. As a result one column showed very broad peaks for naphthalene and anthracene, indicating that pore size decreased during the ageing process. Materials which were subject to ageing for a longer time period did not show retention for any of the employed analytes which means that micropores which contain the majority of phase volume were eliminated through this process.

Further optimisation of the synthesis is needed in order to include a larger number of mesopores necessary for fast transfer kinetics into the material. The inclusion of an ageing process could eliminate unwanted micropores which exhibit unfavourable diffusional characteristics. Capillary monolithic columns with a reversed phase characteristics could be produced. The hydrolytic stability of the organic modification and the homogeneity of the material propose a long term stability of its chromatographic performance. The employment of a second precursor or surface modification could produce stationary phases with variuos of functionalities on a very inert base material.

Acknowledgements

First thanks to my supervisor Dr. Erwin Rosenberg who allowed me to work on an interesting topic in a diverse and challenging environment.

The working place consists not only of chemicals, instruments, and office hardware but fortunately also of people. It is surely better to have company for coffee or lunch. In alphabetical order thanks to Barbara, Christina, Emanuel, Eva, Evangelia, George, Lamprini, Niki, Patrick, Patricia, and Shuya.

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“There is a theory which states that if ever for any reason anyone discovers what exactly the Universe is for and why it is here it will instantly disappear and be replaced by something even more bizarre and inexplicable. There is another that states that this has already happened.”

Douglas Adams

1. Introduction

Capillary separation techniques such as micro liquid chromatography (μ-LC), nano liquid chromatography (n-LC), and capillary electro chromatography (CEC) have become increasingly important in recent years. Miniaturisation in chromatography has obvious economic advantages; moreover it is essential for many applications in the field of life sciences where only limited amounts of samples are available. Excellent compatibility of capillary separation techniques with mass spectrometers make them even more attractive for these applications.

Monolithic columns, also known as rods were a recent innovation in the field of liquid chromatography [1]. In comparison to particle based columns they offer an increased permeability to the mobile phase while providing fast mass transfer kinetics even at high flowrates. For these reasons, very rapid chromatographic separations can be carried out without compromising separation efficiency, increasing the throughput of chromatographic systems. One other major advantage lead to their breakthrough especially at capillary format. In-situ preparation of monolithic columns in capillaries provides a strong connection to the capillary wall through the establishment of chemical bonds. No frits are necessary to retain the stationary phase inside the capillary. The avoidance of sintering frits eliminates problems such as decreased column stability and uncontrolled loss of surface modification in sintered zones which negatively influences chromatographic properties or causes the formation of bubbles - a specific problem sometimes seen in CEC.

Silica based materials offer compared to polymer based phases a high mechanical stability, which is advantageous in pressure driven chromatographic applications. The modification of the micro- and mesopore structure is furthermore possible through the inclusion of an ageing step after synthesis. Another advantage silica based materials exhibit over polymer based stationary phases is that due to their rigidity they do not swell and thus do not change their morphological characteristics with the mobile phase composition. Bonds between capillary wall and monolithic phase can be established without the need to modify the capillary wall with polymerisable residues as needed for polymer based phases.

Silica based columns need to be functionalised on the surface to receive the desired selectivity for chromatographic applications. The coverage of the surface with the modification is usually less than perfect due to steric reasons. A number of special protocols were developed in order to shield the polar, silanol group containing surface of the silica base material from

interacting with the mobile phase. Problems observed in the separation of basic compounds and the susceptibility of the siloxane bond to hydrolytic attack are consequences of this. Limited stability at high pH values and a degradation of chromatographic properties due to a consistent loss of surface modification are often observed in the case of silica based stationary phases.

The use of a single alkylated precursor would include reversed phase chromatographic characteristics in the base material. A higher density of functional groups at the surface of the chromatographic materials could be achieved than with surface modification. This base material would furthermore exhibit an enhanced stability at higher pH values as alkylation would decrease the electrophility of the silica nuclei. The organic modification is introduced with a hydrolytic stable Si-C bond, which would furthermore lead to an enhanced long-term stability compared to surface modification established through siloxane bonds.

For reasons stated above, the aim of this thesis was the synthesis of capillary columns based on a single alkylated precursor, methyl-trimethoxysilane (MTMS). Sol-gel chemistry was the chosen route of synthesis for the production of materials. This technique is already frequently applied in the synthesis of particulate and monolithic columns based on bare silica supports. Parameters on how to control the macroscopic as well as the microscopic morphological characteristics had to be identified and quantified in order to enable later adjustment of the synthesis. Scanning electron microscopy and nitrogen adsorption measurements were applied in a first step to characterise synthesised materials physically. Chromatographic standard tests should in a last step be performed in order to investigate retention behaviour. Although MTMS based materials are known to exhibit hydrophobic characteristics, retention behaviour is expected to differ from standard modified octadecly reversed phase silica columns.

The theoretical part (chapter 2 of this thesis) will discuss possibilities and merits of sol-gel synthesis. Means to characterise solid materials will be discussed in chapter three of this thesis. Chapter four will give a brief introduction to advantages and applications of monolithic materials in chromatography with focus on capillary separation techniques. The results section will describe experiments on transferring an already developed protocol based on basic catalysis from wide-bore to capillary format in chapter five. Chapter six describes the optimisation of a synthetic protocol based on acid catalysis regarding macroscopic morphology. The seventh chapter in the results section summarises chromatographic characteristics of synthesised columns obtained from chromatographic standard tests.

[1] F. Svec, J. M. J. Fréchet, Anal. Chem. 64 (1992) 820.

2. Sol-gel synthesis of porous materials

2.1 Introduction

The motivation of pursuing the route of sol-gel based synthesis of porous materials is the possibility to produce materials from high purity silica alkoxide precursors under mild conditions regarding temperature, pH and solvent content. There is little limitation considering size and shape of the derived materials, as materials can be prepared as powders, spherical particles and even as monoliths. Porosity can be controlled and tailored as well, ranging from the macropore to the mesopore and micropore range. Advantages of the mild reaction conditions are for instance that biologic molecules can be incorporated in situ in the material without denaturation, which means without losing their functionality. The high purity of silica alkoxides as precursors leads to silica matrices with no or little metal impurities - to the elimination of very acidic superficial silanol groups. This improves the performance of silica alkoxide derived materials in chromatographic applications where highly acidic residual silanol groups are known to cause problems for the analysis of basic compounds. Another advantage of this route of synthesis is that surface modification can be carried out under the same conditions as for any other silica based materials, with many established protocols available for application.

Applications where materials produced by sol-gel chemistry with silica alkoxides as precursors are employed include for instance enrichment phases for solid phase extraction (SPE) and solid phase micro extraction (SPME), and solid supports for antibodies, enzymes and industrial catalysts. Sol-gel derived materials are also applied as stationary phases for liquid chromatography, from preparative down to capillary format in both, the packed particle and monolith format, and as stationary phases for capillary electro chromatography (CEC).

In the first part of this chapter the general characteristics of sol-gel chemistry will be discussed with the discussion of the difference of the two possibilities to catalyse the reaction which can be carried out under basic or acidic conditions. Aging and drying are the next steps in the route of synthesis which influence the morphology of the derived materials and will be discussed in the second part of the chapter. The final part will discuss and evaluate the possibilities to incorporate organic functionalities into the silica based materials.

2.2 The sol-gel process

General principle [1]

The synthesis, as the name indicates, involves the formation of a sol as first step, which is per definition the dispersion of a colloidal solid (with particle diameters between 10 nm and 100 nm) in a liquid. A gel is according to the generally accepted nomenclature a three dimensional, porous and continuous network. It may either be formed by aggregation of colloidal, discrete particles or by aggregation of branched, polymer like chains. The primary mechanism guiding the sol-gel transition depends, as will be discussed in the next sections, on the pH conditions prevailing during the reaction. Precursors employed for this type of reaction are typically metal alkoxides with the metal being Al, Si, Sn, Pb Zr,…, and alkoxides typically being methyl, ethyl, propyl and isopropyl. Precursors based on metals other than silica are much more reactive, requiring additives to reduce reactivity to avoid premature precipitation of metal oxides. Furthermore analytical applications utilise almost exclusively materials based on silica, therefore the following discussion will focus on reactions employing silica alkoxide precursors.

The step following gelation is the so-called ageing process. The initially formed gel is still in a state where many highly reactive free silanol groups are present at the surface of the solid. Due to the flexibility of the gel, adjacent silanol groups can undergo condensation processes, leading to further densification and curing of the gel network. This type of reaction can be promoted by exchanging the initially emerging liquid phase in the pore network with a basic aqueous solution. Hydroxy ions have the ability to break siloxane bonds, consequently dissolving silica molecules from energetically unfavourable positions in the network (strongly convex curved surface sections) which will then condensate favourably on concave shaped surface positions. This so-called Ostwald ripening leads to a total decrease of the specific surface area but enhanced stability of the network and a more defined mesopore diameter distribution.

Drying is the last step in the production of monolithic materials with the sol-gel process. Capillary forces will occur during this step which are quantitavely described with the Kelvin equation. This last step is a very critical one as here a maximum of physical stress acts upon the gel network which may exceed its stability and lead to cracks, rendering monolithic materials unusable for their purpose. One possibility to avoid this problem entirely is to employ supercritical drying. Capillary forces can not arise as in no stage of the process a gas-

liquid interface is present at the surface of the solid. Other possibilities to avoid the formation of cracks during the drying process is to minimise capillary forces, either by exchanging the pore liquid with a solvent with low surface tension, by adding surfactants with the same effect, or with hydrophobic modification of the silica surface which reduces its wettability and consequently the occurring capillary forces.

Hydrolysis and polycondensation

The first step of the sol-gel reaction is the hydrolysis of the alkoxy groups of the precursor. Parallel to the hydrolysis, polycondensation of free silanol groups either with other silanol groups or with alkoxy groups will take place as represented in equations 2.1, 2.2a and 2.2b in a simplified way:

$$Si(OR)_4 + 4\ H_2O \Rightarrow Si(OH)_4 + 4\ ROH \qquad \text{eq. 2.1}$$

$$\equiv Si\text{-}OH + HO\text{-}Si\equiv \Rightarrow \equiv Si\text{-}O\text{-}Si\equiv + H_2O \qquad \text{eq. 2.2a}$$

$$\equiv Si\text{-}OH + RO\text{-}Si\equiv \Rightarrow \equiv Si\text{-}O\text{-}Si\equiv + ROH \qquad \text{eq. 2.2b}$$

Both reactions occur simultaneously with different reaction rates, depending on the pH of the reaction mixture. The reaction rate for the hydrolysis depends on the precursor to water ratio, on pH values, as it will increase linearly with the concentration of H_3O^+ and OH^- ions, on temperature, as for instance a tenfold increase of the reaction rate was observed at an increase of temperatures from 20°C to 45.5°C. The last factor is the reactivity of the alkoxy group which will decrease with the size of the alkyl side chain.

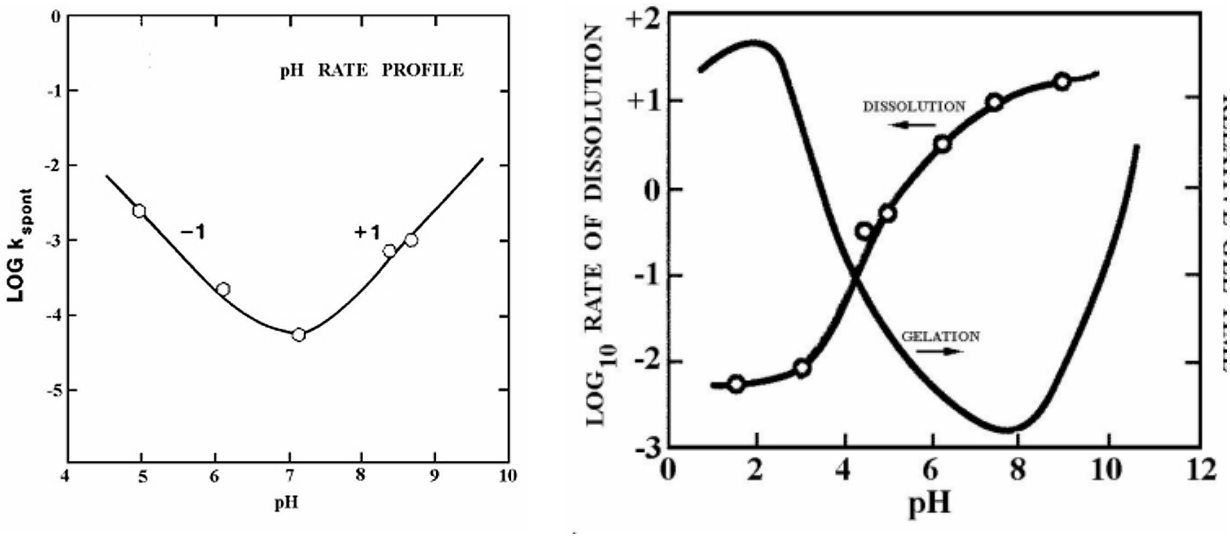

Fig. 2.1 pH dependency of hydrolysis (left), gelation time and dissolution reactions (right) [2], expressed as the logarithm of the rate constants of hydrolysis and dissolution or the relative gelation time.

As can be seen in graph 2.1 hydrolysis is a pseudo first order reaction depending on the concentration of catalytic hydroxyl or hydronium ions [2]. First products of hydrolysis are besides monomers and dimers cyclic tri- and tetramers [3]. Another reason that condensation under basic condition tends to be faster is that dissolution reactions are initiated by hydroxy ions as can be seen in figure 2.1.

This can easily be explained with the reaction mechanism. Reactive intermediate species will be formed from the protonation of either alkoxy or silanol groups. As a consequence of the protonation the silica nucleus will be more susceptible to a nucleophilic attack of either water molecules (leading to hydrolysis of alkoxy groups) or silanol groups (leading to the polycondensation reaction) with a clear SN2 type reaction mechanism [4].

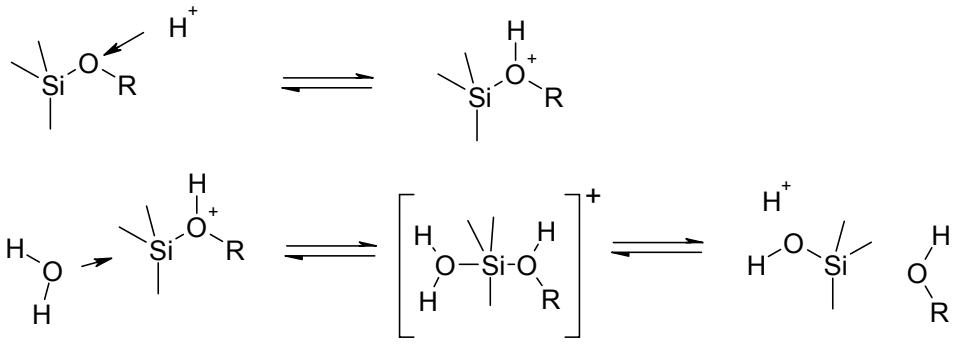

Fig. 2.2 Reaction mechanism of the hydrolysis reaction under acidic conditions.

The +I effect of alky side chains leads to a higher electron density on oxygen bonds and makes them more susceptible for protonation, which means that the reaction rate increases with the number of alkoxy groups present, oxygen from silanol groups gets for the same reason protonated at a significantly lower rate. This leads intermediately to a high concentration of free hydrolysed species in the reaction mixture. In the polycondensation reaction silanol groups are protonated prior to the condensation step, though at a much slower rate than the alkoxy groups of the precursors.

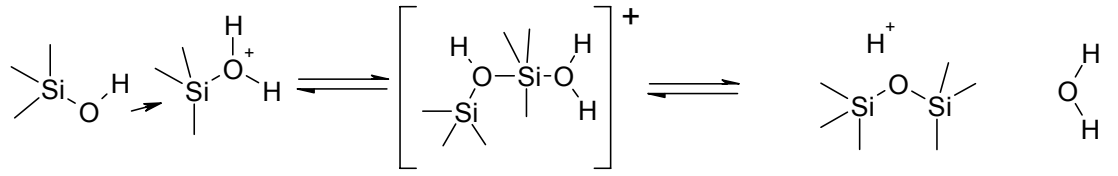

Fig. 2.3 Reaction mechanism of the polycondensation reaction under acidic conditions.

The reactivity of silanol groups for further condensation on a specific molecule decreases with the number of already siloxane bonds established. As a consequence the species formed in the first steps of the reaction will have a linear, hardly branched conformation. The growth mechanism under acidic conditions is also known as reaction limited cluster aggregation.

The mechanism for hydrolysis under basic conditions is the nucleophilic attack of hydroxyl ions on the silicon nucleus with the following elimination of the corresponding alkoholate anion.

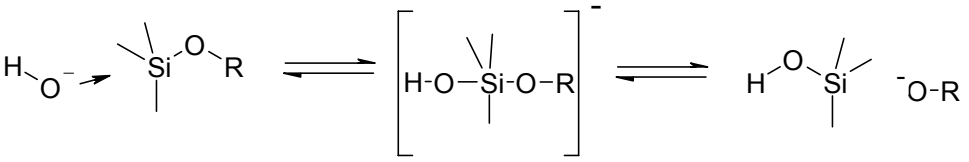

Fig. 2.4 Reaction mechanism of the hydrolysis reaction under basic conditions.

The susceptibility of the metal atom to a nucleophilic attack increases with a decreasing electron density. This means that hydrolysed species are more reactive than non hydrolysed ones, reactivity towards nucleophiles increases also with the number of established siloxane

bonds. The condensation reaction occurs in the case of basic catalysis between two silanol groups as well as between silanol and alkoxy groups.

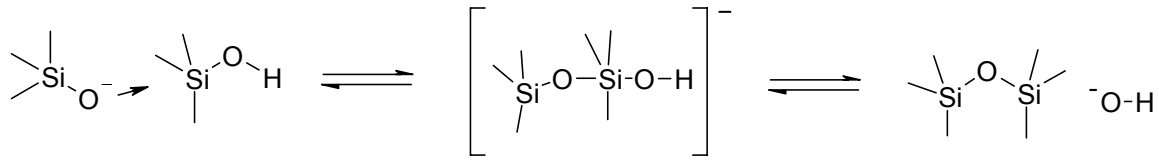

Fig. 2.5 Reaction mechanism of the polycondensation reaction under basic conditions.

Another essential characteristic under basic conditions is that the reaction rate for hydrolysis is slower than the reaction rate for condensation. This means that under basic conditions hydrolysed species will immediately undergo condensation reactions, also with not hydrolysed species. Silanol groups of intermediates with established siloxane bonds possess more acidity than those without, which means they are more likely to undergo deprotonation and therefore to form nucleophilic species themselves. This leads to a reaction mechanism called reaction limited cluster-monomer growth. Clusters are growing in a particulate form, immediately consuming dissolved silica species in the process. Another difference to acidic conditions is the fact that siloxane bonds are broken at a significant rate. Emerging particles are continuously dissolved, smaller particles at a higher rate with freed species continuously undergoing condensation reactions with existing clusters. This leads to a rather uniform distribution of the emerging spherical clusters in the reaction mixture.

Gelation

Gelation is defined as the point during the synthesis where a continuous three dimensional solid network is formed, macroscopically observable with a steep increase of the viscosity of the reaction mixture. In most cases the reaction mixture is no longer fluid enough to be removed from the reaction vessel by pouring, which is the more empirical definition of the gelation point. Once gelation has occurred, the macroscopic gel structure is more or less frozen and can only be subject to minor changes. The mechanisms guiding the phase separation until the gelation point shall be discussed in the next paragraphs as they are essential for the formation of the macroscopic structure.

As discussed in the previous paragraph, the starting point of the gelation under acidic conditions is the formation of polymer like chains which posses some side branches. Parallel to the growth of their length their miscibility in the solvent decreases. Polar silanol groups are consumed during the poly-condensation process, which caused the gel phase become more apolar. Both, the increase of the molecule size and the decrease of their polarity cause a reduced solubility in a polar medium, which is usually the case in sol-gel synthesis, as solvents typically used are low alcohols besides the aqueous catalyst. The liquid will become at a certain point saturated with the emerging polymer chains, which means that a phase separation into a solvent rich and a polysiloxane rich phase begins. This condensation induced phase separation is known as spinodal decomposition in literature.

The spinodal decomposition of the homogenous reaction mixture into a two phase system has as consequence that further condensation is accelerated because siloxane species are forced into a smaller volume. At the point of gelation of the siloxane rich phase the bicontinuous pore and skeleton structure is frozen at its current state. Further condensation can shrink the skeleton diameters, and surface area will also be lost, but densification and an enhanced stiffness of the gel structure prevent major changes to take place concerning the macroscopic structure. The key factor in the macroscopic appearance of later formed gel is therefore the relative kinetics of the polycondensation reaction and the phase separation mechanism. Gelation taking place even before the phase separation starts will lead to materials which exhibit no macroporosity at all. The later gelation occurs in relation to the progress of the phase separation, the more coarse macroporous structures will be observed for the obtained siloxane material. The other extreme case is a complete separation of the two phases with a complete loss of the desired bicontinuous structure. Phase ratios of silica precursors, aqueous catalyst and solvent have also to be adjusted for this type of reaction to obtain a bicontinuous gel; unfavourable ratios can result in solid materials with an isolated pore structure or isolated solid spheres without interstitial porosity. Any parameter influencing the separation mechanism can in return be used to influence the outcome of the synthesis. These parameters include phase ratios between precursor, solvent and aqueous catalyst, catalyst concentration, gelation temperature, solvent composition, etc. The addition of surfactants can for instance be used to alter the surface energy and tension between the two phases, polymers can adjust the viscosity of the liquid phase and therefore influence the phase separation mechanism as well. A general characteristics of this mechanism which depends on the relative kinetics of two reactions is that it is very sensitive towards a change in any of named parameters, the fabrication of a material with desired structural characteristics is often only possible within a

narrow set of parameters, which in exchange have also to be controlled rigidly to establish a reproducible synthetic protocol.

Under basic conditions the mechanism of gelation is different. The starting point of the synthesis are spherically shaped suspended particles (= sol). As discussed in the previous section, condensation under basic conditions is faster than the hydrolysis of alkoxy groups. A higher pH value means that more precursors which can be consumed by the polycondensation reaction are present in the reaction mixture, which means that more centres for particle growth emerge in the first stages of the reaction. At a certain point of the synthesis, the number and density of particles increases and so does the probability that two of them link together. Still ongoing dissolution of silica species and reprecipitation at concave shaped surface sections further solidifies the coherence between two particles. Particle aggregation takes place up to the point of gelation where a continuous network is formed. Again, parameters that influence the initial number of particles, their rate of growth and the probability that two of them actually merge will determine the macroscopic appearance of the resulting material. These are for instance the type of the alkoxy group: higher alcohols hydrolyse and undergo condensation reactions slower, resulting in bigger initial sol particles. A high pH value will lead to a higher number of particles and a faster condensation reaction, resulting in a reduced gelation time and in finer structured materials. The water to precursor ratio influences reaction rate of the hydrolysis and through dilution the probability of the cluster aggregation process taking place as shown in figure 2.6 [5].

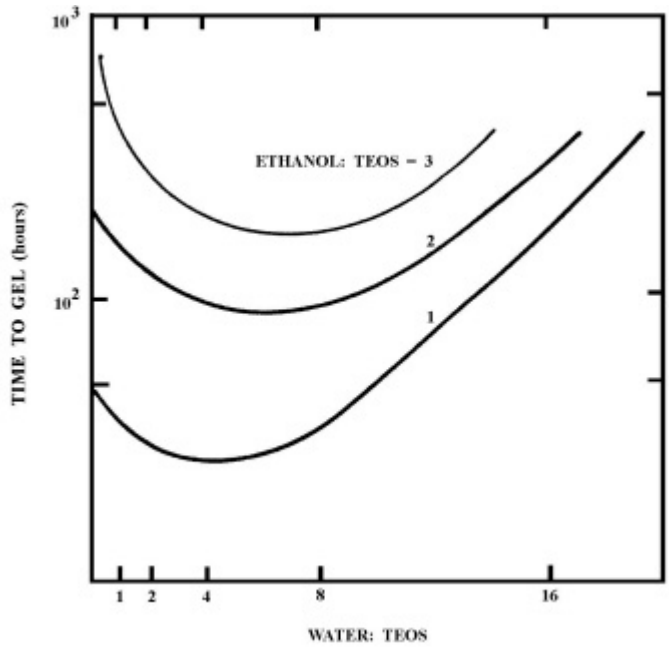

Fig. 2.6 Dependency of gelation time for tetraethoxysilane on the molaric water to precursor ratio for three different ethanol to precursor ratios. [5]

As a net result a minimum exists for gelation time, which is as seen in figure 2.6 6:1 for a ethanol:TEOS ratio of 3:1. Temperature is another parameter to be considered. Diffusion rates and reaction rates increase whereas viscosity of the solvent decreases at higher temperatures, which leads to smaller particle and pore diameters as well as to a decreased gelation time. The type of used solvent or solvent mixture influences diffusion rates of dissolved species, higher alcohols as solvents lead to an increase in gelation time and structural parameters. The type of counter ion used for the catalyst is another influential parameter, as it affects the reactivity of deprotonated species through ionic interaction. To complicate matters, the regime of turbulence acting in the reaction vessel through mixing is another parameter that will affect the gelation process. Forces acting on the sol particles and their relative velocity will determine the probability that chemical bonds are successfully established at contact (contact time) and that two attached particles are ripped apart again by the hydrodynamic forces acting.

2.3 Ageing

The time period between the formation of a gel and its drying is called ageing. During this time the gel is still immersed in liquid phase. This pore liquid can consist either of the solvent being liberated during the sol-gel process, or it can be replaced to promote particular reactions during this phase of the synthesis. Hydrolysis and condensation processes still continue during this stage, which lead to changes in the gel structure.

Polycondensation occurs as long as adjacent silanol groups are present and close enough to react. The increasing number of crosslinks leads to an enhanced mechanical stability and stiffness of the gel structure. This additionally leads to a spontaneous shrinkage of the gel and expultion of pore liquid, which is called syneresis. The initial gel structure is more or less frozen in a thermodynamically not favourable state, with areas with strongly convex curved surfaces and areas with strongly concave curved surfaces. Dissolution occurs preferably from convex curved areas whereas precipitation of dissolved silica species occurs preferably at concave curved areas, which leads to a coarsening of the gel structure, also called Ostwald ripening. As a result of this mechanism necks between particles will grow and small pores will be filled with solid material. The average pore and skeleton diameters of the gel will increase whereas the specific surface area will decrease in this stage of the reaction. Ostwald ripening is often promoted by exchanging the initial pore liquid with an aqueous solution with a rather high pH value. Although metal species can be dissolved at very low pH values as well, faster reaction rates at high pH values favour later conditions for the ageing process.

The importance of the ageing process lies in the general coarsening of the structure and the formation of additional bonds in the gel network. This leads to an increased stiffness of the gel as well as to a higher elastic modulus and modulus of rupture. This makes the chances to retain the monolithic gel without cracks after the drying step higher as emerging capillary forces can be counterbalanced by the now more strengthened gel structure. Conditions to promote aging are, as already mentioned, the exchange of the pore liquid, higher temperatures during the ageing period and the duration of the treatment. As structural changes happen in this period, a rigid control of all these parameters is essential for the repeatability of the whole synthetic process.

2.4 Drying

The last step in the fabrication of solid materials according to the sol-gel route is their drying. Disregarding supercritical drying for the following discussion, this process may be divided into three stages. In the first stage physically entrapped water evaporates and capillary forces will due to a certain elasticity of the gel network lead to its shrinkage. As the network contracts, new siloxane bridges can be formed from silanol groups now close enough to undergo condensation. When the network can resist further shrinkage due to increased packing density, the menisci will start to penetrate the pore network of the gel. Liquid is at this stage transported through an adsorbed film at the surface of the solid. In the last stage this surface film can no longer be sustained, liquid evaporates from within the pores and is transported through gas diffusion, in this last stage no structural changes occur.

If we have a look at the physical stress acting at the different stages of evaporation, cracks developing in the first stage are very unlikely. Excessive stress only appears when the rate of evaporation exceeds the rate of shrinkage of the material and its physical resistance is at the same time not high enough to sustain it, which will only happen if the gel was exposed to the drying step premature, after an insufficient period of ageing. The critical moment of the drying step is typically the first part of stage 2. Most materials possess a certain distribution of pore radii. At this point of the drying process large pores will be emptied first, whereas smaller neighbouring pores are still filled with liquid. The difference in capillary forces can be as high as 1000 bar which is enough for materials with a low strain resistance to crack.

One possibility to circumvent described problems during the drying process is to use supercritical drying. Preferably CO_2 is used in these instances as its supercritical state starts at rather mild conditions ($T > 31.3°$ C, $p > 73$ bar). The pore liquid has to be exchanged prior to the drying as water and CO_2 are not miscible, usually methanol or ethanol are used. This exchange can take a rather long time, though. The advantage is that because of a complete lack of occurring capillary forces, drying induced structural changes can entirely be avoided with supercritical drying.

An other possibility to prevent damage during the drying process is to minimise emerging capillary forces by employing surface modification techniques. As the use of these agents, for instance organically modified silica chlorides, often requires working under water free conditions, a time consuming exchange of pore liquid can not be avoided in these cases. A positive Side effect is the reduction of available surface silanol groups. Significantly less

siloxane bridges are formed in the stage where the material is shrunk due to capillary forces. When these forces disappear after drying, the material will expand to its original size, which is known as “springback” effect. This was attributed to the elimination of a meniscus during the last drying step through decreased wettability of the solid surface [6].

The last possibility is the employment of organically modified co-precursors with the general formula R-Si(OR´)$_3$ with R typically being alkyl chains in various lengths. When using several precusrors in one synthetical approach one has to pay attention to a difference in reaction rates concerning hydrolysis and condensation between precursors. To obtain materials with an organically modified surface the reaction rate of the modified precursor should be slower for both reactions which is, due to the +I effect of the alkyl chain the case for basic catalysis. Gelation processes can be tremendously influenced with the addition of a co-precursor. A new optimisation of the whole sol-gel synthesis is unavoidable in this case. One has to keep in mind when using surface modification prior to the drying step or employing a co-precursor which will lead to a surface with reduced silanol activity, that these modifications should either be reversible in nature (organics can be removed by oxidation as is discussed in the next section), or that they do not interfere with the planned application.

2.5 Thermal treatment

Even after drying many silanol groups are present at the surface of the solid material, chemical and physical stabilisation is for many applications neccessary. Chemical stabilisation aims at the elimination of adsorbed water and of surface silanol groups below a critical level to avoid later rehydroxylation. Thermal treatment and densification is aimed to remove surface area and to further strengthen the gel structure to allow the material to be used without further irreversible structural changes. In the case of pure siloxane based materials, physisorbed water can be eliminated at temperatures as low as 170 °C, chemisorbed water is eliminated irreversibly at temperatures over 400°C. Densification of the materials starts at approximately 850°C, depending on the size of the affected pores. Small pores disappear first in this process which would ultimately end in a non-porous glass, which makes it possible to eliminate unwanted microporosity by applying corresponding temperatures. The incorporation of organic rests or molecules in the siloxane gel enables the possibility to add pores in the size domain of said organics by removing them via oxidation, typically done over 400°C under an oxygen stream.

2.6 Porosity in sol-gel materials

Porosity is a key parameter to be controlled for most applications. It will determine properties such as bulk density, flow through resistance, mass transfer from pore liquid to the surface, quantity and accessibility of reactive sites.

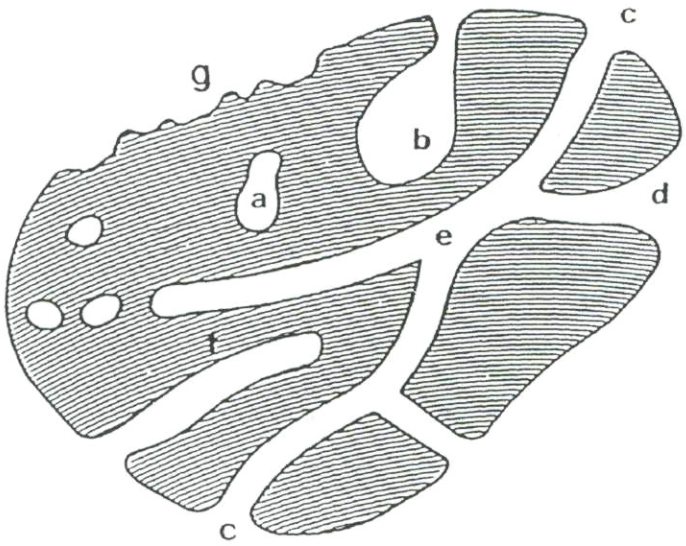

Fig. 2.7 Pore classification according to connectivity and shape [7]

One way to classify pores is according to their shape and connectivity as can be seen in figure 2.7. Closed pores from type a are not accessible to external fluids. They will influence parameters as bulk density, mechanical strength or heat conductivity. Type b-f are open pores, which means they are accessible for external fluid. Subtypes b, f, and g pores are so-called blind or dead end pores, whereas c, d, and e are referred to as through pores. Shapes can vary from funnel-shaped d, tubular shaped c and f, bottle neck shaped b, to slit shaped pores. Surface roughness as demonstrated in figure 2.7 as type g is only counted to porosity if their depth exceeds dimensions of superficial structures. The importance of pore connectivity lies first in the accessibility of the inner surface to external fluids, as almost all applications are based on reactive or adsorptive properties which are located at the surface. The second importance of pore connectivity is that it will determine the efficiency and kinetics of mass transfer from pore fluid, containing species of interest, to the surface, hosting reactive sites, through convective and diffusive mechanisms.

The second classification of pores that can be made is according to their diameter. The definition according to IUPAC is as follows: Micropores have widths smaller than 2 nm, mesopores have widths between 2 and 50 nm, and macropores have widths larger than 50 nm. As pore geometries of materials formed from processes like sol-gel synthesis tend to be highly irregular, the "size" is in most cases dependant on the analytical method it was determined with. A more detailed discussion on the importance of the various pore regimes, especially for chromatographic application, will follow later. Very simplified macropores determine the flow resistance of the material. Mesopores are very important for mass transfer via diffusion or convection. Micropores contain the main part of the specific surface area and therefore, assuming an even distribution, contain the major part of superficial sites for interaction. Their shape and size can lead to wanted or unwanted size exclusion selectivity or irreversible adsorption of chemical species. As will be shown in the following discussion, pores in all size regimes can be an inherent property originating from the sol-gel process. They can also be introduced into the system with the use of templates, and they can be tailored by already described ageing or thermal treating processes.

Macroporosity

Macropores are in the size domain above 50 nm. As dicussed in the previous part of the chapter their formation relies on the parameters chosen for the sol-gel synthesis. Under acid conditions, the phase separation mechanism accompaning the polycondensation reaction will determine pore and skeleton diameters in the macropore range. Under basic conditions the aggregation of sol particles to larger structural features is the mechanism behind the formation of macropores. Detailed discussion on the influence of conditions on the formation of macropores will be given in the results section. Beside optimising reaction conditions for the formation of macroscopic structural features, templates in the µm range can be used to form macropores. Templates utilised for this route are for instance latex particles, viruses or emulsion droplets [8,9]. These templates have then to be removed via calcination or dissolution from the silica structure; these removal procedures can pose a problem for organic modifications of the silica matrix.

Mesoporosity

Mesopores have widths in the range of 2 to 50 nm. One possible source of mesopores in the sol-gel derived materials are primary sol particles. Under acidic conditions it was found out that further growth of siloxane colloids above 2 nm size contributes little to the gel formation, instead aggregation of these colloids is after this stage the primary mechanism of network formation [10]. Under basic conditions due to continuous dissolution and reprecipitation processes, primary particles will grow in size, depending on temperatures and pH; gelation occurs due to aggregation of these. Mesoporosity is more or less a leftover of voids between the aggregated particles. Size distribution can further be manipulated in the ageing process.

Templates in the mesopore range exist for instance in the form of supramolecular structures like micelles. They are usually formed from surfactants. Alkyltriethylammonium cations were the first ones to be employed for this route of templating. Popular non-ionic surfactants are for instance Brij (polyethyleneglycol alkyl ethers) or Pluronics (polyethyleneoxide – polypropyleneoxide – polyethyleneoxide block polymers). Surfactants undergo above a critical concentration self assembly processes, resulting in an ordered mesophase. Depending on parameters such as the head diameter to tail diameter ratio and concentration of the surfactant these phases can have a hexagonal orientation of spheres, parallel tubes, bilayers or stacked bilayers. The size of said structures depends mainly on the length of the hydrophobic tail; the limit of a feasible chain length for alkyltriethyl ammonium surfactants is C_{18}, limited by a lack of solubility for surfactants with longer chains, resulting in 2-4 nm micellar structures that will later form the pores. Diameters up to 25 nm can be achieved by adding auxiliary hydrophobic molecules to the reaction mixture which will be incorporated in the micelle and lead to its swelling [11] or by using polymers as amphiphiles.

When employing sol-gel synthesis, the silica phase can only grow in the hydrophilic part of the micelles containing phase, leaving the mesostructured micelles as templates for pores. The phase diagram of the surfactant in water can usually serve as guideline for potentially achievable structures [12]. Mesoporous silica can by this route be formed in many shapes and morphologies, including fibres with axially ordered mesopores, thin films and hollow spheres [13]. Template removal is performed by calcination or extraction; preventing a collapse of formed structures makes a careful optimisation of this process necessary. Major advantage of using templates is the possibility to create ordered mesoporous structures with a narrow pore size distribution.

Microporosity

Microporosity is in the range of diameters below 2 nm. Silica materials derived via the sol-gel route are principially amorphous, micropores often result from defects in the silica structure. Again, conditions prevailing during the synthesis will influence the quantity and size distribution of micropores formed. Ageing can reduce the number of micropores through condensation of dissolevd silica species on the concave surface until a complete closure. During thermal densification which starts above 850°C micropores start to seal, larger pores at higher temperatures, which makes it possible to selectively eliminate pores below a certain diameter. Micropores with a specific diameter can be introduced into siloxane based materials through templates which can be dissolved organic molecules, metal ions with hydrate shell or complexing agents around which then the siloxane network grows. Zeolithes were the first class of silica based materials with ordered micropores. Hydrothermal reaction conditions lead here to the formation of monodisperse siloxane clusters with a defined pore structure. In contrast to this, sol-gel synthesis reaction products are in a state of a thermodynamical minimum. Another possibility to introduce the template is to utilise organically modified silica alkoxides of the type $R\text{-}Si(OR')_3$ as precursors. The organic sidegroup will be stable during the sol-gel process, but it can later be removed by calcination similar to any other organic template.

2.7 Organically modified silica

Applications of materials synthesised by the sol-gel route utilising silica alkoxides as precursors require in most cases other physical or chemical properties than what can be achieved with siloxane alone. The incorporation of organic molecules or functionalities into the inorganic network is often necessary to modify parameters such as stress resistance, ductility, superficial adsorption and retention behaviour, or enzymatic and catalytic activity. Hybrid organic-inorganic materials based on silica are known under the acronym Ormosil (=organically modified silica) or Ormocer (=organically modified ceramics) in literature. The sol-gel route of synthesis is especially suited for this type of modifications as the mild reaction conditions allow incorporated molecules or functional groups to retain their character in the whole course of synthesis, as the possibility to incorporate enzymes even in their active conformation clearly demonstrates. Possibilities for post-synthesis modification of the silica surface will not be discussed at this point of the discussion, it shall be noted that the same well developed set of silica chemistry can be used for sol-gel derived materials as for other silica based materials.

Synthetic modification of the inorganic network with functional organics can be divided into three different types [14]. The first and most simple method is to dissolve organic molecules in the reaction mixture, where these molecules do not undergo chemical reactions with inorganic molecules later forming the host matrix. After synthesis, molecules are either adsorbed at the siloxane surface or physically entrapped in the gel network. Applications for this type of incorporation include for instance dyes [15], antibodies or enzymes [16]. Even bacteria have been reported to survive the sol-gel process [17]. One main disadvantage of using adsorption or entrapment for the organic modification is that leaching cannot entirely be avoided. The control of the distribution of the organic modifier can pose another problem for this route of modification.

The second possibility for forming hybrid organic-inorganic materials is to form two interpenetrating networks. This can be done by a simultaneous co-evolution of an organic polymeric phase e.g. polymethacrylate or derivatives of it and an inorganic gel phase. For this technique monomers are added to the reaction mixture together with photoinitiators, alkoxy precursors, solvent and sol-gel catalyst. Or, as second possibility, the inorganic porous solid is soaked in a solution containing monomer, solvent and initiator. Hence the polymer network is formed in pores of an already existent inorganic solid. This technique has as field of

application for instance the synthesis of protective coatings. The flexibility and hydrophobicity of the organic polymer network blend together with the hardness of the inorganic network, leading to corrosive and abrasive resistant, and ductile coatings. This technique can also be used for the deposition of a polymer film on the surface of the solid which exhibits any desired selectivity and retention behaviour. This route to synthesise hybrid materials is therefore also used in the fabrication of solid phases applicable for extraction or chromatography. The inorganic network adds physical stiffness and resistance towards swelling whereas the polymer film adds chemical selectivity to the hybrid material.

The third possibility for forming hybrid organic-inorganic materials is to use precursors with a direct Si-C or ionic bond which is hydrolytically stable during the sol-gel process. This class of materials is also known by the registered brand name Ormocer®. These organic groups can be introduced with two different purposes, as network modifier or as network former. Network modifiers are silica molecules modified with simple organic groups that cannot undergo reactions themselves, like alkyl groups or phenyl groups. Network formers contain organic groups that can undergo linking reactions and thus contribute to the network formation process. Examples for network modifications are for instance ethylene, pyridyl or epoxy containing groups which can undergo crosslinking reactions. Precursors have the general formula $R_nSi(OR)_{4-n}$, leaving from one to three alkoxy groups for further condensation reaction with the silica network. Precursors with only one or two alkoxy groups can not form a gel network by themselves; therefore they are used as co-precursors together with tetraalkoxy or trialkoxy silica precursors. Bridged alkoxy precursors of the type $(OR)_3Si\text{-}R'\text{-}Si(OR)_3$ exist as well, where the spacer can vary in length (ethyl being very common). Mechanical properties of the sol-gel can be tuned accordingly with this set of precursors. Dialkoxy precursors as well as bridged precursors with long polymer chains as a spacer result in soft materials with a low elastic modulus, trialkoxy precursors will result in stiff materials with a high elastic modulus. Organically modified silica precursors can of course also be used to alter the surface property of the sol-gel derived material after network formation. The major advantage of chemical bonds as link between organic and inorganic components is that leaching can not occur and that the distribution of both domains is homogenous at a molecular level.

One important point when using precursor mixtures for synthesis is that organic modifications will change the course of the sol-gel process. Organic modifications with a direct Si-C bond to will generally have a +I effect on the electron density of the silica atom. The reaction rate for nucleophilic attacks will thus be significantly lower for this type of precursor. This means

that under basic conditions hydrolysis and polycondensation are slower, whereas the opposite is true for acid catalysis, where the +I effect favours the initial protonation reaction [18]. Organic side groups which can undergo reactions in the course of the sol-gel process or can stabilise certain transition states through complexation have even more influence on the sol-gel process. One example is for instance that aminopropyl modifications have an autocatalytic effect on the hydrolysis reaction because the amino group can undergo a nucleophilic attack on the silica [19]. Considering the variability of possible effects it is clear that when moving to precursor mixtures, the whole process gets complicated to a point where predictions are rather difficult to make and experimental optimisation of synthetic parameters is the only possibility to obtain materials with desired chemical and physical properties. The most simple precursor mixture consists of an organic modified precursor and a tetraalkoxy precursor. Ignoring steric effects for the moment, rates of hydrolysis are under basic conditions one order of magnitude lower for the modified precursor considering the +I effect of the Si-C bond on the silica nucleus. Because polycondensation reactions rely on the same reaction mechanism they can be expected to be slower for the organic modified precursors as well. The result is that the distribution of incorporated organics will be heterogenous in the resulting material. This difference in reactivity can be utilised specifically to create materials where the organic modified species will be found enriched in their concentration on the surface.

In the last section of this chapter the use of methyltrialkoxysilanes, or more specifically methyltrimethoxysilane (MTMS) as precursor for sol-gel synthesis shall be highlighted. Physical and chemical properties and applications of materials based on this precursor will be discussed as well in the following section. In principle the methyl derivatisation on the silica leads to materials with high hydrophobicity. Contact angles towards water of 150° were reported in literature [20]; this behaviour was attributed to a high density of outward directed methyl groups [21]. If MTMS is used as sole precursor, methyl groups responsible for adsorptive properties are distributed evenly in the material, which should eliminate a decrease of these properties through hydrolytic reactions as it is often observed for surface modification. The +I effect of the methyl group leads to a reduced sensitivity towards nucleophilic attacks, which should lead, compared to unmodified silica materials to an enhanced pH stability of the resulting methyl-silsesquioxane. The reduced order of network bonds that can be established by individual silica atoms leads to an increased flexibility of the xerogel. As consequence a general reduction of stiffness can be observed together with an

enhanced resistance towards the formation of cracks. This property makes MTMS based materials very interesting for the synthesis of monolithic structures.

Characteristics concerning synthesis are as follows. Hydrolysis and polycondensation rates are when compared to tetraalkoxy precursors faster under acidic conditions and slower under basic conditions. Regarding the phase separation mechanism guiding the formation of macroscopic features under acidic conditions, emerging intermediate condensation products exhibit a more apolar nature due to the reduced number of silanol groups and the presence of methyl groups. The driving force behind the phase separation is a higher difference in polarity between solvent and intermediates. Phase separation will therefore start at an earlier phase of the condensation. An addition of polymers to the reaction mixture to encourage phase separation can be avoided. Intermediate colloids will have a more linear structure; their arrangement during phase separation can occur with less energetically unfavourable interface area. When solid surfaces are available during the phase separation process, they can induce morphological inhomogenity because intermediates tend to adsorb on them [22]. These surface effects have to be accounted for when performing synthesis in confined spaces such as capillaries. Reactions under basic conditions are slower for MTMS than they are for tetraalkoxy precursors. It has been shown that the same mechanisms govern the resulting macropscopic morphology of the synthesised materials. MTMS / water / solvent ratios and the pH of the reaction mixture determine the size distribution of sol particles and later the gel morphology resulting from their aggregation.

Ageing is usually performed under basic conditions as here the dissolution of silica species has a higher reaction rate. MTMS as precursor leads to a reduced affinity towards nucleophiles and to a slower condensation rate, which means that higher pH values, higher temperatures or longer ageing times have to be employed to compensate this lower reactivity. Drying can be performed at ambient conditions as the hydrophobicity reduces capillary forces on one side and the increased flexibility of the network leads to an increased ductility on the other side.

2.8 References

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3. Characterisation of porous solids

3.1 Determination of morphological characteristics

Stereology

Stereology is the general term of the three dimensional interpretation of two dimensional data. For this purpose, planar cross sections are analysed from the sample, which will then be subject to microscopic techniques. Requirements that have to be fulfilled for this approach are a sufficient contrast between the pore and solid phase and that a statistically significant number of cross sections is analysed to avoid biased or misinterpretation due to macroscopic anisotropy of the material.

Laser Scanning Confocal Microscopy (LSCM)

The LSCM employs a laser beam that is expanded through the optical system of a microscope objective. This beam is further focused on a small spot of the specimen by the objective lens. An image is created from scanning the beam over a cross section of the material of interest. Backscattered or fluorescent light is captured by the same objective lens and focused on a photodetector via a beam splitter, enabling a digital capture of the image. The major advantage of this method over conventional microscopy is that light from regions of the substrate other than in the focal plane is rejected by the detector [1]. Details on the setup can be seen in fig. 3.1.

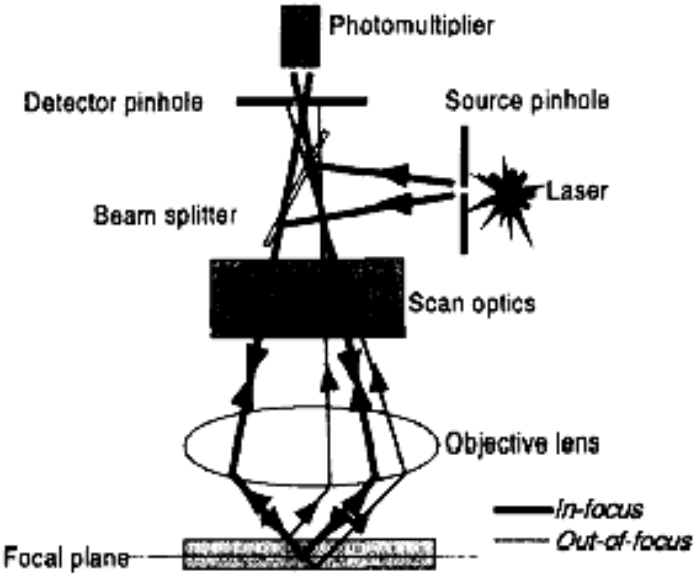

Fig. 3.1 Experimental setup for LSCM measurements [2]. As can be seen, out of focus beams are excluded from detection.

After scanning one picture, the focal plane can be slightly changed. Consequently, pictures from different focal planes can be overlaid and merged into a three dimensional plot computationally [3]. This 3D image should give a more realistic overview on pore and skeletal connectivity than other, more indirect methods which rely on idealised models for interpretation.

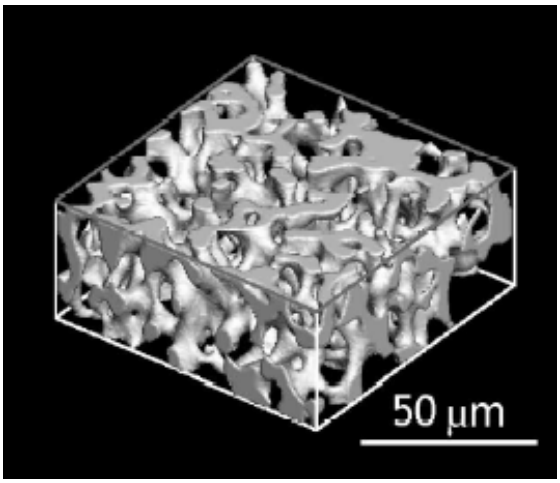

Fig. 3.2 Example of a LSCM measurement of a silica gel produced between two glass slides [4]. Pore liquid was exchanged with a fluoresceine solution prior to measurement. Bright parts represent the pore structure.

Resolutions acquired with this method are like for optical microscopy limited with half the wavelength, around 200 nm spatially and for depth, which means this method can only give information on the macropore structure. In recent developments, instrumentation has moved from cumbersome devices resembling conventional microscopes towards fibre optical devices. It shall be noted that the possibility to image fluorescence light as well makes this technique interesting to use in conjunction with fluorescent probes, as is done in many biological applications. It may be noted that although obtained 3D images might give a better impression on the appearance and homogeneity of the actual structure, the conversion of this data into practical important features like permeability is not easily possible. One requirement that has to be met is that investigated samples have to be transparent for employed incitation and emission wavelength.

Scanning Electron microscopy

Scanning electron microscopy (SEM) is used for the investigation of solid sample surfaces on a routine basis. The content of information of the sample it can provide include besides imaging also laterally resolved elemental information if used in conjunction with an x-ray detector. The principle shall be described very briefly.

An electron beam, originating from a filament, is focused on the sample with electron lenses and apertures. The beam can be focused on a spot down to 5 nm diameter. Scanning of this

beam is achieved through a pair of electromagnetic coils located in the objective part of the electron optics. To achieve a high intensity and focus of the beam one requirement is to operate under vacuum conditions.

One kind of interaction the electron beam can undergo when it hits the sample is scattering. Backscattered electrons are one possible source for imaging in electronic microscopy. Lateral resolution for this kind of interaction is limited, depending on atom number and electron beam energy, with typical values being below μm range. The electron beam can also lead to ionisation processes in the sample upon impact. Emerging secondary electrons can also be used for the generation of an image. Characteristics of secondary electron microscopy are improved lateral resolutions down to 5 nm. The decay of the excited ionic states can lead to the emission of x-rays, leading to element specific information with a depth resolution of several μm. It may be noted that the cross section of interaction with the electron beam as well as the intensity of emitted x-rays is heavily dependant on the element number; higher atomic numbers generally give more intense signals. The most common mode, scanning secondary electron microscopy (SEM) exhibits characteristics as follows: The lateral resolution is limited with ca. 5 nm. Magnifications that can be reached vary from 10x to 200.000x. The brightness of a spot in the picture is proportional to the number of secondary electrons generated. If the sample is not conductive, the sampled spot will charge up due to interaction with the electron beam, resulting in a deflection of the primary electron beam and emerging secondary electrons. Therefore gold or carbon layers are sputtered upon non-conducting samples. One has to be aware that this sputtering process and the evacuation of the sample can introduce artefacts. Environmental scanning electron microscopy (ESEM) is a new development which does not require the sample chamber to be under high vacuum conditions. Gold or carbon coating can be avoided as samples can even be in a wet state during ESEM microscopy. This reduces the chances of generating artefacts during the sample preparation step.

Transmission electron microscopy

In transmission electron microscopy (TEM), transmitted electrons are used to create pictures of the substrate which is typically done in direct imaging and not in scanning mode. Detectors can be phosphorescent screens or CCD cameras. Investigated objects are usually limited to 100-200 nm thickness, which makes an extensive sample preparation step necessary. It may be for instance necessary to embed fragile material in resins prior to generating the samples

by cutting as is done for biological samples or by grinding and etching. Conductivity is not necessary for TEM samples, the placement on a conducting net is sufficient to avoid charging effects. Scattering is the main mechanism of contrast formation, which basically relies on the product of density and thickness and therefore the average atomic number of the transmitted section. TEM is a very powerful microscopic technique, allowing resolutions down to Å level. TEM can therefore give an impression on the meso- and micropore structure of the material. Embedding the samples in resin, cutting them into thin layers and the evacuation can be sources of artefacts in TEM.

Nitrogen adsorption

One of the most commonly used techniques employed for the characterisation based on the adsorption of gas is the determination of specific surface area, micropore volume and mesoporosiy by recording gas adsorption isotherms. Nitrogen is used in the majority of applications as is allows to operate at the boiling point which leads to information gained by capillary condensation. Gas adsorption is usually a very complex phenomenon, involving mass and energy transfer; the details of the well known BET model or gas adsorption mechanisms shall not be discussed at this point. The following section will give a brief overview on the interpretation of recorded isotherms. An isotherm is in this technique usually constructed point by point, the adsorbed amount of species is plotted versus equilibrium pressure. After evacuation of the sample and cooling with liquid nitrogen to 77 K, portions of nitrogen are added which are adsorbed by the substrate; pressure is then allowed to reach an equilibrium. The desorption branch is recorded by decreasing the pressure point by point. Recorded isotherms are then analysed using the BET equation in its linear form.

$$\frac{p}{n^a\left(p^o - p\right)} = \frac{1}{n^a{}_m\, C} + \frac{\left(C-1\right)}{n^a{}_m}\frac{p}{p^o} \qquad \text{eq. 3.1}$$

In this equation n^a is the amount adsorbed at the relative pressure p/p^o, $n^a{}_m$ is the monolayer capacity and C is a constant. When plotting the term on the left side of equation 3.1 against p/p^o, C and $n^a{}_m$ can be calculated from the intercept and the slope of the resulting linear relation (linearity is usually restricted to $0.05 < p/p^o < 0.3$).

The specific surface A (BET) can be calculated from $n^a{}_m$ if the average area that is occupied by the adsorbed molecule a_m is known, L is the Avogadro constant in this equation:

$$A\ (BET) = n^{a}_{m} * L * a_{m} \qquad \text{eq. 3.2}$$

Detailed discussion on this type of evaluation can be found in [5] and [6].

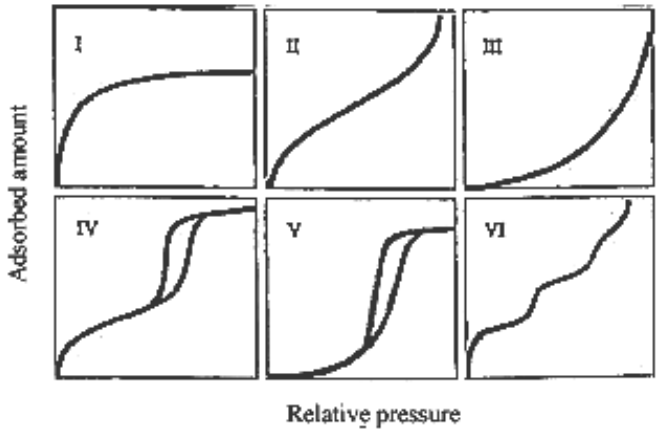

Fig. 3.3 Classification of nitrogen adsorption isotherms [7]. A description and explanation of different types of isotherms is given in the text.

Recorded isotherms can be classified into six categories as shown in figure 3.3 [7]. A steep slope of the recorded isotherms at low pressures is usually an indication of either a strong gas-substrate interaction or an indication of a rich micropore structure, the former being unlikely when employing nitrogen for adsorption. Materials with type I isotherms can therefore be classified in exhibiting microporosity only, surface area available for multilayer adsorption decreases significantly after the first adsorbed layers, which is typical for micropores. A class II isotherm indicates the presence of some micropores, a steady slope in the middle part indicates no decrease of surface area available for multilayer formation in this part, which is the case for meso- and macropores. Materials with class III isotherm lack microporosity or a strong adsorbate adsorbend interaction. Materials with class IV and V isotherms exhibit a high number of mesopores. A steep increase of adsorbed gas at pressures close to the saturation pressure is a strong indication of capillary condensation taking place. The isotherms level off when the capillaries are filled with liquid nitrogen. As capillary evaporation does occur at different pressures, a hysteresis loop can be observed for mesoporous materials. The distinction between class IV and V is that class IV also possesses a significant amount of micropores. Type VI isotherms refer to materials where adsorption occurs in a stepwise multilayer manner, representative for non-porous materials. Type I and IV are most commonly observed for sol-gel derived materials, where type IV materials exhibit also mesopores.

For the analysis of mesoporosity, that evaluation of the part in the adsorption isotherm where capillary condensation occurs is analysed. Hysteresis loops can hereby give an indication of pore shape and pore connectivity [8,9].

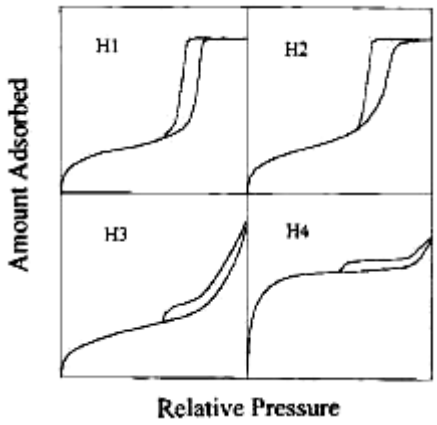

Fig. 3.4 Classification of hysteresis loops [8]. A description and explanation of different types of isotherms is given in the text.

The almost vertical slope of both hysteresis branches observed for type H1 materials is an indication of either agglomerates of uniform particles with a uniform size, but is also typical for materials with ordered, cylindrical shaped pores. In type H2 materials, the hysteresis loop is triangular shaped, the slope of adsorption is less steep than the almost vertical slope of the desorption branch. This was attributed to ink-bottle shaped pores, but can also refer to pore connectivity. Pore liquid can be trapped in pores with a diameter higher than would refer to the capillary condensation process at the current pressure by pore liquid in smaller pores connecting to the exterior of the network; this trapped liquid would appear in the desorption branch at pressures referring to smaller diameters. Type H3 isotherms do not level off at pressures close to the condensation pressure and were reported for materials consisting of aggregates of plate like structures featuring slit like pores. Type H4 loops were reported for materials with slit like pores but also for pores in the shape of spherical voids or hollow spheres with an ordered mesopore structure in the shell. In essence the shape of the hysteresis loop may be influenced by size and shape of mesopores, by their connectivity, and, if the material exhibits sufficient flexibility, by deformation processes occurring due to the resulting capillary forces.

Nonetheless, capillary condensation is used to obtain data on the size distribution of mesopores [5,6]. Mesopores are filled with adsorbate liquid at pressures lower than the

condensation pressure, depending on their size. A correlation on the pore size and the critical pressure for capillary condensation is given in the Kelvin equation.

$$\ln \frac{p^*}{p^o} = -\frac{2\gamma \nu \cos\theta}{RT\, r_m} \qquad \text{eq. 3.3}$$

The term p* refers to the critical condensation pressure, p^o to the saturation pressure of the adsorptive, γ the liquid surface tension, ν the molar volume of the condensed adsorptive, θ the contact angle between the solid and the condensed phase (taken to be zero for nitrogen, hence $\cos\theta = 1$) and r_m the mean radius of curvature of the liquid meniscus.

One thing to keep in mind is that in this equation the curvature of the liquid/vapour interface is related to the relative pressure without taking influences of the solid substrate into account. The assumption is made that the curvature only refers to pore size and shape. As multilayer adsorption occurs prior to condensation, pore radii and meniscus radii are not equal, the difference being the thickness of the adsorbed layer.

Out of the numerous mathematical approaches to determine the mesopore size distribution utilising the Kelvin equation, the most widely used approach was developed by Barrett, Joyner and Halenda [10] and is generally referred to as "BJH method". Here the Kelvin equation is applied over the whole mesopore range, adsorption is assumed to be equal on curved pore surface and flat open surface. The contact angle is set to 0°, relating to perfect wetting, so menisci curvature is only determined by pore size and shape. For calculation, the desorption process is used. Pressure is released stepwise; lost pore liquid refers to as formerly condensed and now free pore volume. Thickness of the adsorbed nitrogen layer is obtained by assuming an average molecular layer thickness. For pore size calculation a cylindrical shape of mesopores is chosen most often. Calculations are made along a whole branch of the isotherm, giving results on the pore radius, area and volume distribution.

Micropore volume is another parameter that can be determined with nitrogen adsorption measurements. No general mathematical approach exists for the mechanism of micropore filling at the moment; interpretation of the isotherm for micropores should therefore be done with care. The t-plot method and its derivatives rely on the comparison of the isotherm with the isotherm of a non-microporous material. For this purpose, the adsorbed gas volume is plotted against t, which corresponds to the thickness of the adsorbed layer. Several approaches exist to relate the relative pressure to t, the most frequently used ones are relations

developed by Harkins and Jura [11] and the model of Halsey [12]. The simplest method, of the t-plot evaluation is the so-called MP method [13].

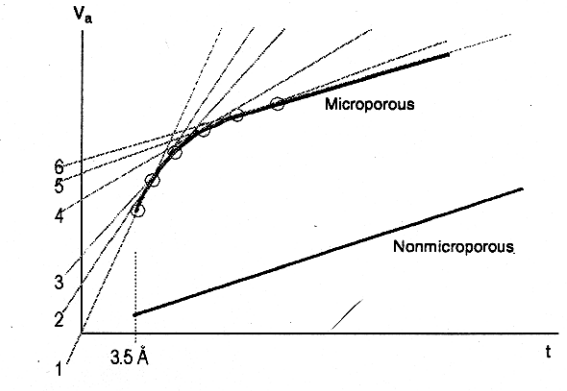

Fig. 3.5 Schematic of the evaluation of a t-plot [5]. Evaluation of this plot is described in the text.

Multilayer adsorption causes micropores to be filled, small micropores will be filled first, pores with a larger diameter later, causing the surface area available for further adsorption to decrease, which will be visible in the isotherm as reduction of the slope. As the relative pressure can be converted to thickness of the adsorbed layer, the same will be visible in the t-plot. A series of tangents is calculated from the t-plot. The tangent line for the first data point is forced through the origin, its slope V_p/t gives the total micropore surface area. The slope of the second tangent is the total micropore area of empty pores until point 2. Tangents are then subsequently laid until the slope stays constant or is increasing. The pore volume between two points can be calculated as follows:

$$V_p = \left[\frac{(S_n - S_{n+1})(t_1 + t_2)}{2}\right]K \qquad \text{eq. 3.4}$$

S_n and S_{n+1} are surface areas obtained from the slopes of the tangents n and n+1.The values of t_{n+1} and t_n refer to the thickness at the related points and K is a constant converting gas volume (STP conditions) to liquid volume and Å units to centimetres. The output of the t-plot evaluation is therefore pore volume versus pore diameter.

A short discussion on the validity of the data derived by the evaluation of adsorption isotherms shall be made at this point. The BET approach of describing isotherms has several unrealistic assumptions. It assumes for instance an equal energy distribution of the surface for

the adsorption of gas molecules, lateral interactions between adsorbed molecules in the first layer are ignored in this approach. Higher layers are assumed to exhibit characteristics of a liquid. Micropore filling is also not taken into account when evaluating the isotherm for specific surface area determination. Type I isotherms are therefore unlikely to give a valid estimation of the specific surface area with BET evaluation. For practical purposes, if C is outside the range of 20 to 200 the BET model should not be used for evaluation. Even if all requirements are met, the BET approach has 20% deviation from the true surface area, this uncertainty mainly stems from the general assumption that an adsorbed nitrogen molecule occupies 0.162 nm^2 at 77K.

BJH determination of pore size distribution has the requirement that condensing gas behaves indeed like a free liquid. In pores from 2-5 nm the question arises if this assumption is true, a smooth vapour/solid interface can not be assumed as fluid-wall interactions can become dominant [14]. Furthermore hysteresis loops rely heavily on pore connectivity, for example taking the desorption branch of H2 type isotherms for evaluation will correlate volume of large pores to a false pore size domain. Correct interpretation may therefore rely on pore morphology and connectivity data gained by other sources. As a guideline, IUPAC has published an article containing recommendations under which premises various models can be applied to evaluate recorded isotherms [8].

^{129}Xe NMR

Xe, although an inert gas, exhibits as valuable property that is utilised in this method which is its ease of polarisation. The chemical shift of an individual xenon atom can be caused by the chemical environment, by the surface curvature or by Xe-Xe interactions, the latter are pressure dependant. The chemical shift can be measured because the ^{129}Xe isotope has a magnetic spin of ½. If xenon is adsorbed on a micro- or mesoporous solid, the chemical shift resulting from the adsorption is decreasing with pore dimensions [15]. Increasing xenon concentrations will increase the chemical shift resulting from Xe-Xe interactions. If these interactions are anisotropic, which is the case if the size of Xe (4.4 Å) is negligible compared with the size of the pore, the chemical shift that can be attributed to these interactions increasing linearly with the pressure, which makes it possible to extract the shift caused by the pore surface curvature alone. Additional chemical shifts may result from the chemical nature of the surface and magnetical or electrical fields caused by the presence of charged or paramagnetic nuclei in the material [15]. If a fast exchange of Xe atoms on the surface in

relation to the NMR timescale is restricted by lowering the temperature, different signals may be observed in the spectrum. 2D NMR experiments can be utilised to observe Xe exchange on the surface, making it possible to gain data on xenon diffusion in the porous solid.

Mercury intrusion porosimetry [5,16]

Mercury intrusion porosimetry is a viable characterisation method used since more than five decades for all kind of porous solids. It can be performed in a timeframe under one hour and covers pore diameters from 3 nm to 360 μm, the only drawback are health effects of employed mercury.

The special characteristics that make mercury irreplaceable for intrusion porosimetry is that it does not wet most substrates which means that it does not enter the pore system unless it is forced with pressure. Assuming circular shaped pore entries applied pressure and pore diameter can be correlated with the Washburn equation, as given in eq. 3.5. Increasing pressure means that mercury can enter a larger fraction of the total pore volume, restricted by the minimum pore diameter it can enter.

$$d = \frac{-4\gamma\cos\theta}{p} \qquad \text{eq. 3.5}$$

The minimum pore diameter is d, applied pressure is p, γ the surface tension of mercury (485 dyne/cm at 20°C) and θ the contact angle (130°). These values are valid for most applications. Used instruments operate between 0.0034 MPa (0.5 psi) and 414 MPa (60000 psi) which corresponds to pore diameters between 316 μm and 3 nm. The resulting plot of intruded volume versus applied pressure can directly be converted into a pore volume versus pore diameter plot. Furthermore, this plot can be converted into a pore diameter versus surface area plot, with the assumption of cylindrical pores it done with the simple formula A = 4V/d. Total pore volume per weight can also be determined with mercury intrusion porosimetry, which is taken from the point of the highest applied pressure.

Concerning the interpretation of mercury intrusion plots, several effects have to be considered. All solid materials exhibit certain compressibility. Consequently pore volume obtained with mercury intrusion porosimetry will always be overestimated. Pore connectivity leads to an underestimation of pore size. Voids connected to the outside with narrow pores appear in the plot at a pore size relating to the largest connecting pore. Surface roughness can lead to a different contact angle between mercury and the solid. Narrow necks in the pore

system can lead to a rupture of the liquid mercury at extrusion, causing trapped mercury volumes which will be reflected as a not closed intrusion – extrusion hysteresis loop. Information on pore connectivity received by alternative techniques can be helpful for the interpretation of mercury intrusion plots.

3.2 Solid state NMR

NMR is an interesting technique for the determination of the electron density (= chemical shift) resulting from the chemical environment of a nucleus. Interesting nuclei for silica based stationary phases are ^{1}H, ^{13}C, ^{29}Si and if alumnium was employed during the synthesis ^{27}Al. General characteristics of solid state NMR is the strong occurrence of anisotropy effects in solid samples. They would lead to very broad and featureless spectra. However, they can be averaged and thus eliminated from the spectra by a technique known as magic angle spinning (MAS). The sample is rotated at an angle of 54.7° with respect to the magnetic field.

^{1}H NMR is a direct approach to characterise silanol groups. The chemical shift on silanol protons can be used to quantify the abundance of isolated and geminal (= on the same silica) silanol groups. Adsorbed water can lead through the formation of hydrogen bonds to peak broadening, the broad proton signal caused by adsorbed water can overlap with signals from silanol groups, further complicating quantification. Desorbing water and performing NMR experiments under dry gas is therefore recommended for ^{1}H measurements of Silanol groups. Exchange with deuterium can clarify the amount of superficial to interstitial silanol groups, later are not relevant for chromatographic applications.

A valuable method for the investigation of silica derived materials is the use of ^{29}Si NMR. The chemical shift of a silica nucleus is mainly the result from the number of oxygen bonds and the number of Si-O-Si bridges connecting to it. The classification of signals can therefore be made according to silica connectivity; Q and T are signals refering to four and three oxygen bonds, furthermore an index refers to the number of siloxane bridges – Q^3 means that this type of silica atom is connected with 4 oxygens, three of them bridging to another silica. Due to a lack of resolution a distinction if oxygen not bridging is part of a silanol group or an alkoyx group can not be made. For the same reason quantification may require a deconvolution of obtained ^{29}Si NMR spectra.

^{13}C NMR has its value when employing organic modified precursors in the course of synthesis or when conducting organic surface modifications. Success in the incorporation of organics into the solid matrix can be controlled this way, residual alkoxy groups resulting from an incomplete hydrolysis can be identified by ^{13}C NMR as well.

Multidimensional NMR can be employed to identify nuclei with proximity to each other, making it possible to clarify uncertainties regarding the conformation of solid materials. Sorption processes can be studied as well with this technique.

3.3 Calorimetric methods

Calorimetric methods have in common that they measure reaction enthalpy relating to reactions characteristic for properties of the solid substrate. These reactions can be physical and chemical adsorption from gas- or liquid phase or the dependency of melting points on pore size.

Immersion calorimetry relies on the immersion of a dry sample into a liquid. Measured enthalpy of the wetting can directly be related to the specific surface area with the help of a reference sample. Immersion of the sample into apolar liquids with a different molecular size can even give an overview on micropore accessibility. Surface polarity and the abundance of acidic or basic sites can be accessed with the use of probe liquids with different polarity, acidity, ect. [17].

Thermoporometry relies on the pore size dependant depression of the melting point of a condensed adsorbate. For this purpose, transferred thermal energy is measured as function of temperature. Pore size accessible for this kind of measurement is between 2 and 25 nm. This method can be performed on non rigid materials. Materials that swell can be studied under conditions close to the conditions they are typically applied. As an advantage, obtained values for pore size refer to the actual cavity dimensions; pore connectivity does not have an effect on the results.

Gas adsorption calorimetry performed with inert gases like argon or nitrogen relies on an enhanced adsorption enthalpy in micropores, making the determination of micropore area possible. As an alternative to chromatographic methods, calorimetry based on chemisorption processes can be used to determine the quantity of active sites present in the investigated solid.

3.4 Chromatography

Gas chromatographic methods

Other than the point by point record of the adsorption isotherm which uses nitrogen as probe, kinetic and energy related parameters of the adsorption process can give valuable information on surface chemistry properties and diffusive properties of the investigated material. Chromatographic techniques discussed in the next section can provide this kind of information.

Frontal analysis may be the most simple form of a chromatographic technique employed to observe material properties. Here the material of interest is used as stationary phase. The mobile phase consists of a carrier, either a gas or a solvent, and the probe molecule which is chosen according to the type of interaction with the solid bed one is interested in. The analyte concentration in the eluate is recorded; one will typically observe breakthrough curves for the probe molecule. At the beginning of the experiment it will be completely removed from the feed as it is adsorbed in the solid bed, whereas it will reach the initial feed concentration after the material is saturated with the probe molecule in relation to the feed concentration. Desorption can be observed by switching the feed concentration to 0 after the adsorption equilibrium is reached. For data evaluation, the classical equilibrium theory assumes the solid bed to be an ideal stationary phase – that at an equilibrium state between feed concentration and adsorbed amount exists at any point of the experiment. With this assumption, the adsorption isotherm can be constructed form the breakthrough curve or the desorption curve. Detailed information on the so-called equilibrium theory can be viewed in publications [18] and [19]. Isotherms are usually evaluated with Langmiuir type isotherm models, assuming one or a few different types of adsorption sites for a given analyte. This type of evaluation can give the number of adsorptive sites for the probe molecule in the solid substrate. The approach of frontal analysis can further be extended to feeds containing multiple analytes and therefore can be used to record competetive isotherms. Frontal analysis can therefor be a valuable tool to obtain data of acidic sites on the substrate surface, saturation levels and therefore the maximum capacity of the substrate or gain valuable information on possible competetive adsorption between species of interest. It shall be noted that in frontal analysis kinetic data contained in broadening of the curves is usually not evaluated. This technique is

used in liquid chromatography as well, analyte-solvent and substrate-solvent interactions add further complexity to data interpretation.

Reversed flow gas chromatography as technique to obtain kinetic data and the energy distribution of adsorption sites shall be mentioned at this point. It is mostly applied in the field of the characterisation of solid catalysts to obtain data on adsorption- desorption- and reaction kinetics. The principle is illustrated in figure 3.6.

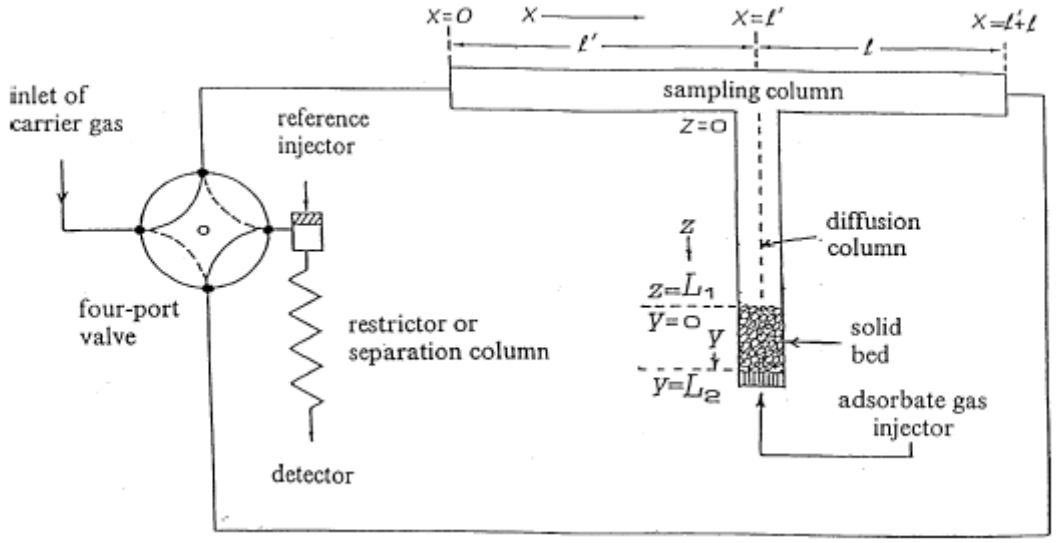

Fig. 3.6 Schematic of the reversed flow experimental setup [20]

In reversed flow experiments sample is introduced into the system at the point named adsorbate gas injector in fig. 3.3. Sampling is conducted by a column located on top of the solid bed. This means that the probe molecule is transoprted through diffusion only until this point. Without further manipulation of the gas flows, one would observe a broad peak generated by the diffusive transport of the analyte. By reversing the flow, sections of the gas present in the sampling column pass the diffusion column three times, whereas other sections pass the diffusion column only once – sample peaks are generated artificially. The base beyond these sampling peaks represents the average concentration in the sampling gas stream whereas the height of these sampling peaks contains kinetic data on such as adsorption, desorption or, if injecting reactive species, data concerning reaction rates. These sampling peaks also allow performing chromatography for separating analyte mixtures. Data evaluation is rather complicated, it shall be noted that values such as the energy distribution of adsorption sites, kinetic features of said adsorption sites as well as reaction kinetics can be

obtained through this method. Details on data evaluation are given in a comprehensive review [20].

Liquid Chromatography

Liquid chromatography is a valuable analytical tool employed in many laboratories for routine work. Its fields of application reach from the anlysis of samples, the determination of physico-chemical constants to the isolation of single compounds from a complex mixture. A principal distinction can be made between normal phase and reversed phase liquid chromatography. In normal phase chromatography a polar stationary phase (for example silica) and an apolar solvent mixture are applied with retention relying on adsorption, in reversed phase chromatography an apolar stationary phase (for example polymers or surface derivatised silica) are employed for analysis with retention relying on absorption. Reversed phase chromatography is the more widespread technique, around 75% of applications utilise RP chromatography, a majority C_{18} and C_8 modified silica [21]. Because of the importance of RP chromatography and because stationary phases synthesised in this thesis exhibit a very apolar nature, tests developed for reversed phase stationary phases will be discussed at this point. Fundamentals of chromatography, subject of the next section, are not exclusive to reversed phase applications but shall be mentioned at this point.

Fundamentals and definitions in chromatography

In chromatographic applications the porous stationary phase, which can consist either of packed particles or of a monolith, is flown through by the mobile phase. In RP chromatography a solvent mixture of water or an aqueous buffer and an organic phase is used, in most cases methanol or acetonitrile. The sample, usually a solution or extract of the analytes, is injected in a narrow rectangular pulse at the head of the stationary phase. Sample components are distributed between mobile phase and stationary phase. Transport of the analyte molecules occurs only in the mobile phase. The larger the fraction of the total dwell time is that a compound spends in the mobile phase, the faster it will elute. In turn, a compound that spends a larger fraction of dwell time in the stationary phase (which correlates often with the logKow value) and less time in the mobile phase will reach the detector at a later time. After a certain time, also known as retention time or t_r, they are eluted from the system in a peak.

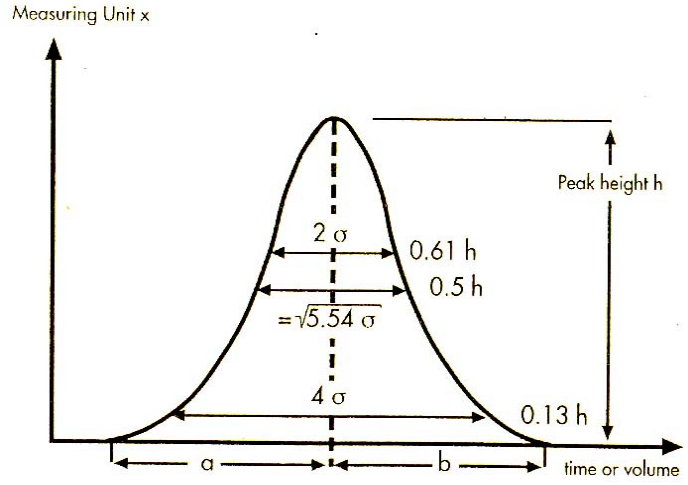

Fig. 3.4 Characteristics of a peak [21]

The retention time for a given compound spent in the chromatographic column can be described as sum of the times spent in the mobile phase (= t_m) and stationary phase (= t_s). From these parameters, a so called retention or capacity factor, characteristic for the interaction of the analyte with the stationary phase, can be calculated.

$$t_r = t_s + t_m \qquad \text{eq. 3.6}$$

$$k = (t_s - t_m)/t_m \qquad \text{eq. 3.7}$$

The time the analyte spends in the mobile phase, also known as zero or dead time, is accessible through chromatography of dead time markers which must not undergo absorptive or adsorptive interactions with the stationary phase. Chromatographic systems exhibit a certain dead volume which will contribute to t_m. This effect is marginal in wide- and narrow bore format but has to be considered for capillary columns.

The separation of two given compounds can be characterised with the selectivity α and the chromatographic resolution R_s which are calculated according to equations 3.8 and 3.9.

$$\alpha = k_2/k_1 \qquad \text{eq. 3.8}$$

$$R_S = \left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_1'}{1 + k_1'}\right)\left(\frac{\sqrt{N_1}}{4}\right) \qquad \text{eq. 3.9}$$

N_1 is the plate number of the first eluting peak in this equation. The plate number is a number relating to the efficiency of the separation. The plate theory of chromatographic separations assumes as model that a column in separated in distinct plates, which are defined that the distribution of a compound between mobile and stationary phase is in an eqilibrium state. The

higher the number of theoretical plates the better two compounds are separated and the more narrow peak widths become. Although chromatography follows a dynamic distribution mechansim, the plate number is used as measure for column efficiency. Plate numbers and plate heights can be calculated from peak widths at half height ($w_{0.5}$) and column length (L).

$$N = 5.54*(t_R/w_{0.5}) \qquad \text{eq. 3.10}$$

$$H = N/L \qquad \text{eq. 3.11}$$

Under ideal conditions, the peak shape is a symmetric Gaussian bell. Asymmetric peaks are an indication of a non ideal distribution of a compound between stationary and mobile phase (ratio of dissolved and absorbed concentration is not a constant). Peak asymmetry can for instance be observed when there are several mechanisms of retention of an analyte on a stationary phase and sites responsible for one type of interaction are saturated at one point of the separation. The most prominent case are acidic silanol groups, present in any silica based separation media, which show strong interaction with basic analytes; their relatively low concentrations can lead to a strong tailing of peaks of basic substances. Peak symmetry (= S) can be calculated according to equation 3.12, peak asymmetry (= AS) by equation 3.13 [22]. If both are calculated from the same peak and the same height, they are connected through equation 3.14.

$$S = w_{tot,5\%} / (2*w_{front,5\%}) \qquad \text{eq. 3.12}$$

$$AS = w_{rear,5\%} / w_{front,5\%} \qquad \text{eq. 3.13}$$

$$AS = 2*S - 1 \qquad \text{eq. 3.14}$$

Peak widths are usually not taken from the peak base as there can be severe problems to obtain correct values due to a correct identification of a base line. Generally taking widths from lower peak height leads to more sensitivity for peak asmmetry caused by peak tailing, peak widths are typically taken from 5% and 10% of peak height for the determination of peak symmetry or asymmetry.

Testing procedures of stationary phases

Due to the widespread application of reversed phase chromatography, a number of manufacturers are on the market of chromatographic columns with all sort of proclaimed selectivities and special features. Even materials appearing equivalent on paper, for instance C_{18} modified silica phases with equal particle diameter, may possess different

chromatographic properties. This can be caused, for instance, by a difference in specific surface area and therefore in a different phase volume present in the chromatographic material, resulting in different k values for the same analyte. Porosity in the mesopore range can lead to a difference in column efficiency, whereas micropores can lead to size exclusion phenomena. One very important feature of reversed phase media is silanol activity. Although surface modification aims at a complete coverage with the hydrophobic functionality, in practice only about 1/3 to ½ of the surface silanols can react with the organosilane reagent due to steric reasons. The number of free silanol groups is typically further reduced to about 1/3 by reaction with a small alkylsilane reagent (mostly $(CH_3)_3SiCl$), a process known as "Endcapping" (EC). For the reasons given above, the silica material still has a significant number of accessible silanol groups which can undergo sorption processes with polar functionalities of analyte molecules. Their abundance and their distribution of acidity, resulting from the silica base material on one hand and from employed surface modification technique on the other hand, is another important property of stationary phases. To enable a better intercomparison of stationary phases, several standard test procedures were established with the aim of gaining objective and comprehensive data on previously mentioned properties.

The first testing procedures aiming at C_{18} modified silica phases were developed by Tanaka and co-workers [23]. Soon afterwards, Engelhardt *et al.* published test protocols, also for surface modified silica columns [24,25]. The third widely applied test procedure was developed by Galushko and co-workers [26,27]. Waters also established a procedure intended for the classification of stationary phases [28]. Other testing procedures aiming at more defined and specific types of analytes can also be found in literature. The aim of the conducted chromatographic testing procedures was to get a general overview on the properties of synthesised columns, testing procedures for more specific applications will not be discussed here.

As there is some agreement on the mechanisms of reversed phase chromatography for alkyl modified silica materials, it is not surprising that mentioned test procedures exhibit certain similarities in their layout. Several studies have been conducted on the intercomparison of mentioned test procedures regarding hydrophobic properties, silanol activity and size and shape selectivity by Claessens [29-31], and especially for silanol activity by Rogers and Dorsey [32]. The next paragraphs will give a short comparison and evaluation of mentioned test procedures dealing with the characterisation of hydrophobic properties, silanol activity and size and shape selectivity.

Hydrophobic properties

Hydrophobic properties are for RP stationary phases the most important feature as their design aims at the retention of analytes according to hydrophobic interactions. Chromatographic standard tests utilise the retention of hydrophobic test analytes to gain data on column hydrophobicity. Test parameters of utilised standard tests are given in table 3.1.

Tab. 3.1 Test parameters for the classification of hydrophobic characteristics of RP stationary phases. Mobile phase ratios are given in v/v.

Test	Analytes	Mobile Phase	Calculated Result
Engelhardt	Toluene (T), Ethylbenzene (EB)	55 % methanol 45 % water	$\alpha=k_{EB}/k_T$
Tanaka	Butylbenzene (BB), Pentylbenzene (PB)	80 % methanol 20% water	$\alpha=k_{PB}/k_{BB}$
Waters	Anthracene (A) Benzene (B)	65 % acetonitrile 35 % water	Hydrophobicity index $\alpha=k_A/k_B$
Galushko	Toluene (T) ; Benzene (B)	60 % methanol 40 % water	Hydrophobicity: $(k_T+k_B)/2$

Differences of retention factors resulting from hydrophobic interaction between phases with the same surface modification mainly stem from differences in the phase volume of the hydrophobic phase. The phase volume is directly related to the surface area available for chromatographic separation and the packing density of organic modifications on the surface. Both, Engelhardt and Tanaka utilise aromatic compounds with an alkyl side chain for the quantification of hydrophobicity, mobile phases are adjusted to the analytes hydrophobicity. Both tests give as characteristic value the methylene selectivity, calculated from the respective retention factors of employed test analytes. Considering these abundant similarities, hydrophobicity parameters obtained from both tests correlate very strongly.

The Waters test uses two aromatic compounds as test analytes, namely anthracene and benzene. When applying this test, size exclusion phenomena in materials with narrow micropores could occur as both compounds differ considerably in size. A possible difference in π-π interactions with the stationary phase between these analytes lead to can an additional mechanism for selectivity, which is not relevant for C_8 and C_{18} columns, but may become important for phases which exhibit functional groups containing π electrons. The Galushko test for column hydrophobicity uses in comparison to previously discussed tests absolute retention factors and not selectivity as output for the result. The use of absolute retention

factors leads to a wider variation of experimentally observed values as the phase ratio has more influence on this value than on selectivity.

The comparison of these characterisation procedures performed on more than 20 stationary phases showed that parameters for hydrophobicity correlate very well which means that they rely on the same retention mechanisms [31]. Differences between columns for methods using selectivity as characteristic value can mainly be attributed to a difference in the type of surface modification. Columns with the same surface modification give virtually identical selectivity values as result. Phase ratios are therefore not accounted for when using selectivity as parameter, the main factors contributing to selectivity are rather differences in distribution coefficients between the analytes. Absolute retention factors on the other hand depend on phase ratios as well. In short, both of these values are important; absolute k values can give information on surface area and phase ratios, whereas selectivity is an important measure for surface chemistry, which can be important for the characterisation of new bonded phases.

Silanol activity

Residual silanol activity is a known feature, being both beneficial and problematic for stationary phases produced from silica materials. Silanol groups can undergo hydrogen bonding, dipole-dipole interaction and if deprotonated also ionic interactions. Silanol activity of a bonded phase depends first on the substrate as for instance silanol groups adjacent to aluminium impurities are known to exhibit strong acidity. Pretreatment and type of surface chemistry applied for modifications are the other big factors determining the abundance of silanol groups on the surface. High purity silica precursors and so-called end capping techniques were introduced successfully to minimise residual silanol activity in RP stationary phases. The problem stemming from the abundance of silanol groups is that they undergo comparatively strong interactions with usually slow desorption kinetics, especially with polar or basic compounds, results in unwanted peak tailing. For this reason a number of tests were developed with the aim to characterise silanol activity.

Tab. 3.2 Parameters of standard tests employed for silanol activity characterisation. Mobile phase ratios are given in v/v. Parameter values in the Engelhardt test refer to acceptable values for columns with good silanophilic properties.

Test	**Analytes**	**Mobile Phase**	**Reported parameter**
Engelhardt	Phenol (P), Aniline (An);	55 % methanol 45 % water	Elution order; Ratio of Asymmetry factors $AS_{An}/AS_P<1.3$
	The three isomeric toluidines (OT, MT, PT)	55 % methanol 45 % water	Selectivities $\alpha< 1.05$
	4-Ethylaniline	55 % methanol 45 % water	Asymmetry factor AS<1.30
Tanaka	Caffeine (C), Phenol (P)	30 % methanol 70% water	$\alpha_{C/P} = k_C/k_P$
	Benzylamine (BA), Phenol (P)	30 % methanol 70% water - 0.02 M Phosphate buffer pH 2.7	$\alpha_{A/P} = k_A/k_P$
	Benzylamine (BA), Phenol (P)	30 % methanol 70% water - 0.02 M Phosphate buffer pH 7.6	$\alpha_{A/P} = k_A/k_P$
Waters	N-,N-Diethyltoluamide (DETA), Anthracene (A)	Acetonitrile	SiOH-index=k_{DETA}/k_A
	Nitrobenzene	Dry n-Heptane	Alternative SiOH-index: k_{NB}
Galushko	Aniline (An), Phenol (P)	60 % methanol 40% water	silanol activity= $1+3[(k_{An}/k_P)-1]$

The test developed by Engelhardt utilises three different approaches towards the characterisation of silanol activity. The first one compares asymmetry factors of phenol and aniline and evaluates their elution order. It was reasoned that peak asymmetry can have other sources than silanol activity alone, therefore it is compared between the basic compound aniline and phenol. The value for "good" chromatographic columns showing little silanol activity should be below 1.3. Later three different toluidine isomers were used for characterisation. They possess different pK_B values between 4.4 and 5.1; differences in their polarity, size and shape are considered negligible. Selectivity factors higher than 1.05 should give an indication of ionic interactions of protonated amines with deprotonated silanol groups, separation of the toluidines is caused due to a difference in basicity.

The test developed by Tanaka is based on the assumption that there are different contributions of silanol groups to overall silanol activity. He divides these interactions into hydrogen bonding, weak acidic silanol groups and strong acidic silanol groups. For all these values, phenol is used as reference for retention based on the reversed phase mechanism only. Hydrogen bonding capacity as defined by Tanaka is the selectivity between caffeine and

phenol, hydrogen bonding is therefore argued to be a factor in the retention of caffeine. Ion exchange capacity is defined as selectivity between benzylamine, which is protonated at employed conditions, and phenol. At pH 7.6 all silanol groups are assumed to be in a deprotonated state, at pH 2.7 only strongly acidic silanol groups will be deprotonated. A higher number of deprotonated silica species should consequently result in higher retention times for benzylamine and higher values for IEC.

In both tests developed by Waters hydrophobic interactions between test analytes and stationary phases are eliminated by using highly apolar solvents, acetonitrile and dry n-heptane. In both cases non-ionic polar analytes are used; retention is caused by dipole and hydrogen bonding interactions. Specific values are retention factors, as interactions are limited to polar interactions with silanol groups, no referencing is necessary. It is strongly suggested by the author to utilise these tests prior to usage of the column in the laboratory where they get in contact with ionic agents. An employment as tool for a routine monitoring of stationary phases may be problematic with this test method.

Galushko also uses phenol and aniline for the determination of silanol activity, although at slightly different conditions than employed for the Engelhardt test. The silanol activity is calculated according to the formula presented in table 3.2. For good columns the selectivity between both analytes should be 1 according to Engelhardt, a silanol activity value according to the Galushko test should also be around 1.

Standard tests for silanol activity are still disputed amongst the scientific community. The employment of unbuffered solvents in the Engelhardt test may contribute to a lack of reproducibility, which was examined by Engelhardt and co-workers [24]; Claessens employed a modified test with a phosphate buffer at pH 7 and observed an enhanced reproducibility of results [23]. Hydrogen bonding capacity according to the Tanaka test assumes a hydrogen bonding mechanism of silanol groups with caffeine. A poor correlation with ion exchange capacities at pH 2.7 where the vast majority of silanol groups is protonated indicates that there are different mechanisms for the retention of caffeine than hydrogen bonding. IEC capacities obtained with the same test may be a good indication of the abundance of weakly and strongly acidic silanol groups; these mainly may derive from the presence of trace metals in the silica matrix and stay dissociated even at low pH values [33]. The test developed by Waters may not be feasible for monitoring; the silanol activity test developed by Galushko suffers from utilising an unbuffered solvent mixture. Concerning the ongoing discussions, employed tests and presented results should be evaluated critically in their possible shortcomings and errors.

Size and shape selectivity

Size and shape selectivity refers to the phenomenon that retention is not only influenced by chemical interactions between analyte molecule and stationary phase but also by size exclusion or shape recognition processes. Size exclusion is explained rather simple. Molecules may, depending on their relative size, be excluded from a part of the reversed phase volume located in pores too small to be accessible for the molecule. Size exclusion as retention mechanism is exploited in size exclusion chromatography; in reversed phase chromatography this effect may cause shifts in the expected retention order which is otherwise based on hydrophobicity. Size exclusion phenomena are therefore unwanted in reversed phase chromatography. In order to gain information on possible size exclusion properties of a bonded phase, a physical characterisation of pore size distribution with the recording of gas adsorption isotherms can be valuable. A second possibility to determine pore size distributions is to employ inverse size exclusion chromatography. Polymer mixtures with a known size distribution are used as test analytes. Their retention, which should be based on size exclusion alone, is used to determine pore size distributions; a detailed review on mathematical models for data evaluation was made by Gorbunov *et al.* [34]. A comparison of inverse size exclusion chromatography (ISEC) measurements with mercury intrusion porosimetry revealed a good correlation for mesopores, however artefacts in the macropore range occurred for ISEC which was attributed to effects resulting from a deformation of the spherical polymers due to an interaction with surface roughness [35].

Shape selectivity as phenomenon in reversed phase chromatography is attributed to the structure of the bonded phase and the accessibility of bonded phase located in narrow pores. Packing density also influences the retention mechanism based on the analyte shape. For the determination of this column property, both Engelhardt and Tanaka use the triphenylene – o-terphenyl pair to quantify this phenomenon. They are both of the same size, hydrophobicity and possess the same amount of π electrons. The difference is that triphenylene is a flat molecule, whereas in o-terphenyl the two ortho placed phenyl rests are twisted slightly out of plane. The only difference between both tests is that the Engelhardt test uses 79% methanol for the mobile phase compared to 80% methanol of the Tanaka test.

Another pair of test analytes was proposed by Sander and Wise [36]. Benzo[a]pyrene has a more linear structure whereas 1,2:3,4:5,6:7,8-tetrabenzonaphthalene is wider and more spherically shaped.

Tab. 3.3 Test parameters for shape selectivity standard tests

Test	analytes	Mobile Phase	Output parameter
Engelhardt	triphenylene o-terphenyl	79 % methanol 21 % water	$\alpha_{Tri/Ter}=k'_{Tri}/ k'_{Ter}$
Tanaka	triphenylene o-terphenyl	80 % methanol 20 % water	$\alpha_{Tri/Ter}=k'_{Tri}/ k'_{Ter}$
Sander/Wise	benzo[a]pyrene (BaP) 1,2:3,4:5,6:7,8-tetrabenzo-naphthalene (TBN)	85 % acetonitrile 15 % water	$\alpha_{TBN/BaP}=k'_{TBN} / k'_{BaP}$

Sander and Wise explained shape recognition with a slot model for interaction. It is argued that, depending on the density of functional groups, they form slots which can be entered more easily by smaller or more linear shaped molecules. This abundance of slots results from the derivatisation technique employed for surface modification. The terms monomeric (= monochlorosilanes) and polymeric phases (= di- and trichlorosilanes) refer to the type of silane employed for the surface modification. Polymeric phases should give a higher packing density of octadecyl chains due to siloxane bridges between organically modified silica species. Shape selectivity can be classified according to these terms as well because the shape selectivity property is different for phases where different organosilanes were employed for modification. Polymeric phases exhibit generally the highest values for shape selectivity. Critical values for the distinction of polymeric phases for the test according to Tanaka are values larger than 3. Sander and Wise classify columns with selectivity values higher than 1.7 as monomeric and smaller than 1 as polymeric. A comparison of both tests revealed a good correlation in their classification [37], it was concluded that a high carbon loading and comparatively wide pore diameters are essential to obtain materials with high shape selectivity values, ordered structures in the octadecyl phase should arise under these conditions more probably. It shall be mentioned at this point that stationary phases which exhibit functional groups that can interact with π electrons of employed test analytes can give misleading values for the classification of shape selectivity.

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4. Monolithic capillary columns in liquid chromatography

4.1 Introduction

Since its introduction, high performance liquid chromatography has become the most frequently used technique for the separation of complex mixtures in analytical chemistry, more than 70% carried out under reversed phase conditions [1]. Improvements in chromatography aim at increasing separation efficiency and reducing the time needed for each analysis. One approach to achieve these goals is to reduce the particle size of packed columns at the cost of increasing the pressure needed to pump the mobile phase through the column. Monolithic columns offer through their unique morphology the possibility to operate at higher flowrates and thus to reduce separation time without losing separation efficiency. Stationary phases based on single beds, also known as rods or monoliths, have become increasingly important as stationary phases in liquid chromatography. They are used in both the classical and capillary format as separation media, and also for electrokinetic driven capillary electrochromatography. The development of monolithic stationary phases started in the early 1970s with soft materials based on polyurethane [2]; first rigid materials were reported in the early 1990s with the fabrication of polymer based columns developed by Svec and co-workers [3]. The first inorganic monolithic materials were reported several years later [4,5]. Subsequent optimisation of the macropore and mesopore structure was performed by Tanaka and co-workers [6-11] which later resulted in the first commercial silica based monolithic columns for analytical chromatography marketed as Chromolith® by Merck. Advantageous features monolithic stationary phases exhibit over their particle based counterpart shall be highlighted in the next sections. A brief review on applications will focus on the capillary format.

4.2 Efficiency in Liquid Chromatography

One model to describe separations in liquid chromatography is the so-called plate model. It assumes a step by step process where analyte concentrations reach equilibrium between mobile and stationary phase before the mobile phase is transported to the next section. Although chromatography is a dynamic process, efficiency can be described by the number of theoretical plates achieved during one separation. The higher the plate number, the higher is the resolution of a chromatographic system and the narrower are the observed peaks. The number of theoretical plates can be determined from the chromatographic parameters retention time and peak width. Height of a theoretical plate (H) is column length (L) divided by the plate number (N).

$$N = 5.54 * \left(\frac{t_R}{W_{0.5}} \right)^2 \qquad \text{eq. 4.1}$$

$$H = L/N \qquad \text{eq. 4.2}$$

Peak broadening in chromatography depends on several factors such as diffusion, dispersion and mass transfer kinetics between mobile phase and stationary phase. The general description of dispersive terms generating peak broadening is the van Deemter equation.

$$H = H(u) = A + B/u + C * u \qquad \text{eq. 4.3}$$

H is the height of one equivalent plate, A, B and C are constants, u is the mobile phase velocity.

The A term is independent of the flow; dispersion is caused by the mobile phase being able to take different paths through the stationary phase. Heterogeneity of the pore structure and pore diameter leads to a large A term.

$$A = 2 \lambda d_p \qquad \text{eq. 4.4}$$

λ describes statistical irregularities of the macroscopic morphology and is in the range of 1-2, d_p is the particle diameter.

B/u describes dispersion through longitudinal diffusion which is independent from properties of the stationary phase; it depends on diffusive properties of the analyte in the mobile phase.

$$B = 2\,\gamma\,D_m \qquad \text{eq. 4.5}$$

γ is the so- called tortuosity factor which usually ranges from 0.6-0.8, D_m is the diffusion coefficient of the analyte in the mobile phase.

C*u describes dispersion occurring because diffusion from mobile phase to surface area and diffusion in the stationary phase cause a kinetic limitation of mass transfer between flow through pores and stationary phase. The C term can further be divided into contributions that come from diffusion of analytes in the free pore liquid in the mesopores (C_m) and into a contribution of adsorptive and desorptive mass transfer of the stationary phase (C_s).

$$C_s = \frac{qk'\,{d_f}^2}{(1+k')^2\,D_s} \qquad \text{eq. 4.6}$$

$$C_m = \frac{\omega\,{d_p}^2}{D_m} \qquad \text{eq. 4.7}$$

q is the so-called configuration factor of the adsorbed liquid film; it is 0.67 in the case of solid supports, d_f is the film thickness. D_s is the diffusion coefficient of the analyte in the stationary phase, k´ is the capacity factor. ω is a constant describing the influence of the packing morphology on radial diffusion and varies from 0.02-5.

Later adjustments to the van Deemter equation were suggested by Knox [12], who proposed the use of reduced parameters for plate height (h) and flow velocity (v). As investigations showed, the contribution of the A term also depended upon mobile phase velocity which was accounted for in the equation.

$$h = A * v^{1/3} + B/v + C * v \qquad \text{eq. 4.8}$$

$$h = H/d_p\,;\;\; v = u * d_p/D_M \qquad \text{eq. 4.9}$$

The analysis of the factors contributing to peak broadening make it clear that increasing the separation efficiency can be achieved by decreasing the size of particles in the case of packed columns. The major limitation when pursuing this route of optimisation is that backpressure also increases with smaller particle diameters. In the case of laminar flow conditions, the pressure drop can be described with the Carman-Kozeny equation [13].

$$\Delta p = \Phi * u * \eta \frac{L}{d_{pore}^2} \qquad \text{eq. 4.10}$$

Δp is the observed pressure drop, Φ the flow resistance relating to morphological characteristics of the solid bed, l is the length of the solid bed, d_{pore} is the pore diameter, η the viscosity of the mobile phase

In the case of ideally packed beds based on spherical particles, pore- and particle geometry are connected through the geometry, which means d_{pore} : d_p ratios of 0.25 to 0.4 can be achieved. There is usually no such geometrical limit on the ratio of pore- and particle diameters in the case of monolithic columns, pore and skeleton characteristics depend on pore formation mechanisms stemming from applied synthetic parameters and can –at least in theory- be controlled independently. From this point of view it is entirely possible to create stationary phases which exhibit the performance of particulate columns while possessing just a fraction of their specific backpressure.

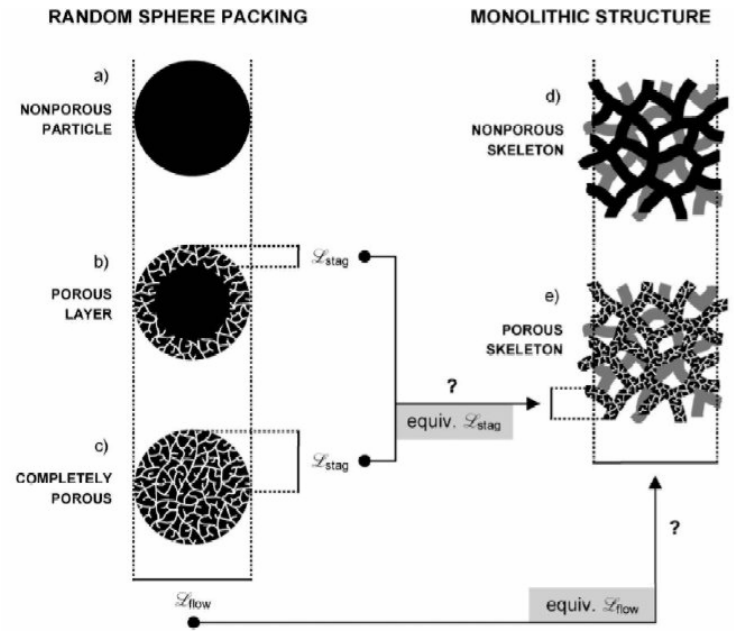

Fig. 4.1 Comparison of characteristic length scales for hydraulic permeability (L_{flow}) and hydrodynamic dispersion (L_{stag}) of particulate and monolithic stationary phases) [14].

The advantage of monolithic stationary phases can be seen in fig. 4.1. Materials c and e are equivalent according to their characteristic length describing flow resistance properties, whereas peak broadening through pore diffusion (=C_m term) can be expected to be a factor 10 or higher. Stationary phases e and b are equivalent according their characteristic length

describing flow resistance properties and mass transfer kinetics, the monolithic material has the advantage of a higher amount of specific surface area (= phase volume) per volume fraction.

The evaluation of stationary phases can usually be done with so-called van Deemter plots, where the measured plate height is plotted versus mobile phase velocity. From these plots, optimum flow conditions for separation can be derived. To gain additional means to compare speed and efficiency of a stationary phase, specific plots were introduced by Poppe [15]. These graphs plot a so-called plate time (= time in a separation needed to obtain one theoretical plate) versus the number of theoretical plates under certain pressure limits. Characteristic lines are obtained for separation media dependent on particle size, morphology and maximum pressure; it is even possible to compare pressure driven and elektrokinetic driven separation conditions. From a single graph the time required to obtain a specific number of plates at a specific pressure drop for columns of optimal length can be estimated.

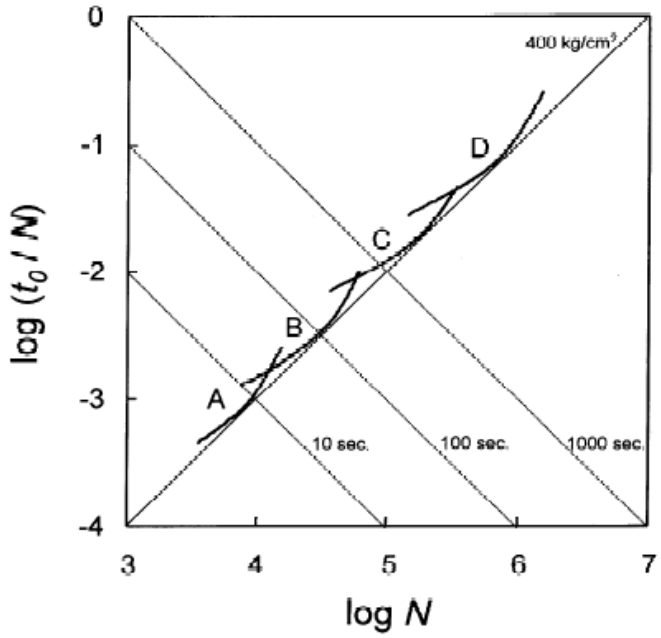

Fig. 4.2 Schematic Poppe plot of plate time, (t_0/N), vs. plate number for a particle packed column in HPLC [15]. The curves were obtained by assuming the parameters, maximum pressure: 400 bar, η = 0.001 Pa/ s, Φ = 1000, D_m = 10^9 m^2/s and Knox equation $h = v^{1/3} + 1.5/v + 0.05v$. Particle diameter: (A) 1 µm, (B) 2 µm, (C) 5 µm, (D) 10 µm. The dotted lines indicate the required t_0 values in seconds.

A comparison of the performance of monolithic columns and packed stationary phases employing Poppe plots reveal trends regarding performance limits [16]. Small particles or monolithic columns with a high permeability are preferable for performing rapid separations. When operating under the same pressure limitations and ignoring time limitations, phases with large particles give the highest numbers of theoretical plates. Regarding time limitation, highest plate numbers can again be achieved with either small particles or monolithic columns.

When comparing monolithic columns with packed stationary phases, there are several disadvantages of monolithic columns in the classic HPLC format of 4.6 to 2.1 mm ID. First of all, columns have to be produced free of voids and cracks which means that higher rates of failure have to be expected during the synthesis stage. The need of special cladding procedures of the monolithic columns proved to be another hindrance in their development. Currently Merck does the cladding by shrinking a PEEK tube onto the monolith, which on one hand poses mechanical stress to the column; on the other hand this procedure limits wall thickness and rigidity of the surrounding tube which limits its use to pressures below 200 bar.

Newest inventions from manufacturers on the LC markets were the move towards packed columns with smaller particles. Agilent presented their so-called Rapid Resolution line of columns containing 3.5 µm or 1.8 µm particles instead of the more widely used 5 µm. Separation efficiency can be sustained using smaller particles and a reduced column length while achieving reduced runtimes under the same pressure conditions. Waters presented their so-called ultra performance liquid chromatography (UPLC) systems which can operate at pressures up to 1000 bar instead of established 400 bar systems [17,18]. Together with this new generation of LC systems, 1.7 µm and 1.8 µm particle columns were launched. Shorter runtimes are achieved in these cases through shorter columns packed with smaller particles. The advantage of monolithic columns in the wide- and narrow bore format is the possibility to maintain high separation efficiency at high linear flowrates - which has compared to the use of packed columns with small particles an additional negative economic impact. Pressure limits are through the invention of UPLC 1000 bar for packed columns compared to 200 bar for the Chromolith®. The use of smaller particles allow maintaining known properties of particulate stationary phases while reducing runtimes and compared to monolithic columns a more economic use of solvents.

4.3 Monolithic columns in capillary separation techniques

Advantages of monolithic columns in capillary separation techniques

Interest in capillary separation techniques such as pressure driven µ-LC and n-LC and electrokinetic driven CEC has grown in the last couple of decades. Reasons for this shall be discussed later. At this point properties which make monolithic separation media interesting in these fields shall be presented. At first, several drawbacks of monolithic columns observed in the wide and narrow bore format do not apply any longer: Small dimensions make the occurrence of cracks in the material less probable than for the synthesis in large moulds. Cladding is no longer required as monolithic stationary phases are synthesised inside the capillary. In turn surface activation of the capillary wall has to be performed in order to ensure a high number of bonds between capillary wall and monolithic stationary phase. Under these conditions, gap formation or a flushing out of the monolithic phases at higher pressures will not occur. Fabricating packed particle phases in capillary format requires the introduction of frits into the capillary support, a process that can lead to the destruction of the capillary coating, making it more sensitive towards mechanical stress. As will be discussed in the next paragraph, frits pose severe drawbacks towards separation efficiency if electrokinetic separations are carried out [18,19].

The main procedure used in CEC to retain packed materials is by creating frits in the capillaries. The most frequent approach to introduce frits is to sinter stationary phase particles in small zones at the ends of the stationary phase. This can be done with or without the addition of water to increase reactivity of silica supports. There are several drawbacks to this approach. The polyimide coating surrounding the capillary can be damaged which decreases the mechanical stability. This procedure usually lacks reproducibility, especially concerning the permeability of sintered frits. Furthermore the surface chemistry in the sintered zone is changed through hydrolysis processes. This means on one hand that a zone with increased silanol activity and a partially destroyed stationary phase is created; the main implication is a drastic change of the ς potential and therefore of the electroosmotic flow. As a consequence of occurring differences of the electroosmotic flow across the frit, pressure differences develop up to a point where bubbles are formed which proves problematic for maintaining high resolution of the separation [20,21]. Surface modification after sintering can be performed to recover surface properties of the initial packing material; an improvement of the bubble formation problem and silanol activity has indeed been observed [22,23]. Tapering the ends

of capillaries should be mentioned as alternative method to retain the stationary phase in capillary columns.

Pressure driven vs. electrokinetic driven flow

Separation carried out in the capillary format offers the possibility to operate under two fundamentally different flow modes, namely pressure driven flow and electroosmotic flow. Flow conditions in the pressure driven mode are laminar, thus the flow velocity profiles have a parabolic shape. Electroosmotic flow profiles depend on the ratio of pore diameter to the thickness of the electrical double layer (typically 1-10 nm); at high values plug like flow profiles are obtained (pore diameters should be over 100 nm). This means that dispersion through velocity differences in the mobile phase is reduced in the case of electrokinetic separation. As a consequence, the A term is reported to be one order of magnitude lower in CEC compared to miniaturised liquid chromatography [24]. Additionally, observed C terms in van Deemter plots are also lower for CEC, which means that there is less resistance towards mass transfer through the elimination of an adsorbed solvent layer and possible electroosmotic flow through mesopores [25]. Theoretically the minimum plate height that can be achieved in pressure driven chromatography is around 2.4 d_p [26]. In CEC, observed minimum plate heights have been reported to be a fraction of the particle diameter, in theory 0.2 d_p could be possible for H_{min}. Furthermore the electroosmotic flow does not depend on the pore diameter, packed phases with particles as small as 20 nm have been reported [27], although at these dimensions efficiency is affected by double layer overlap. A comparison of stationary phases in the capillary format give principally higher plate numbers when performing CEC as compared to µ-HPLC [28,29]. However, CEC requires the stationary phase to exhibit a charged surface in order to establish a sufficient EOF. Silica based materials exhibit usually sufficient silanol groups that can provide surface charge to establish an electroosmotic flow, charged residues have to be introduced in the case of polymer based materials. Both separation techniques deliver comparable selectivity in the case of separations which rely on hydrophobic interactions. Chromatography of analytes with polar residues or analytes which exhibit electrophoretic mobility themselves can not be compared between both techniques. Capillary diameters for CEC are also limited due to Joule heating to 100 µm.

Applications of monolithic materials in capillary separation techniques

Capillary separation techniques have become increasingly important in the last decades. There are several reasons why miniaturisation in separation techniques is pursued. One economically important aspect is the reduction of solvent consumption. Molecular imprinting or immobilisation of molecules for affinity chromatography may require expensive or rare chemicals, miniaturisation has also economic advantages in these cases. More important is the reduced amount of sample needed to perform analysis. Limited sample amounts are an important issue in proteomic and genomic research, two fields which have grown vastly over recent years. Biomedical research is another field where sample amount is a limiting factor. As most of these applications require mass spectrometric detection, miniaturisation on the separation technique side is not problem as on-line coupled ionisation techniques typically exhibit flowrates in the µl/min range or lower which is perfectly compatible with µ-LC or CEC. Interfaces were developed which allow MS^n experiments when performing n-LC without compromising separation efficiency; this setup traps peaks in a 6-port valve, stops the n-LC and elutes the peaks at a reduced flow from the valve to the MS source [30]. The generation of a solvent gradient can be achieved in nl/min scale [31]. Latest developments in the course of miniaturisation are so-called "lab on a chip" approaches where liquid handling and separation are integrated in one chip; flow is typically generated with EOF. Another side effect of miniaturisation in liquid chromatography is the possibility to easily introduce a temperature gradient to the separation, realised for µ-LC [32] and chips [33].

Synthesis in capillary format can in principle be done according to the same procedures as in classic LC format. One major difference is the need to establish chemical bonds between the capillary wall and the monolithic material as synthesis is done in-situ. According to the application and required properties, monolithic materials can be produced via several routes which will be briefly described in the next paragraph; several reviews on synthesis in the capillary format are available in literature [34-35].

As mentioned in the introduction section, the first monolithic materials used for capillary separations were based on polymers. One widely used family of precursors in this field are methacrylates, introduced by Svec and co-workers [3]. Polymerisation is based on a radical mechanism which is usually initiated thermally. To form rigid materials with high porosity, crosslinkers like ethylene dimethacrylate are added to the reaction mixture. Glycidyl methacrylate is often used as co-precursor in the synthesis of monoliths. It incorporates the

reactive epoxy group into the material which can further be used to functionalise the surface of the stationary phase according to the application. The capillary wall is usually activated and derivatised prior to synthesis with metallorganic molecules containing a polymerisable function, like 3-(triethoxysilyl)-propylmethacrylate (γ-MAPS).

Polystyrene is another popular polymer based support for monolithic materials, the development of monolithic supports started in the early 1990s [39]. As crosslinker divinylbenzene is used in this case. Generally materials based on polystyrene exhibit a high stiffness, surface areas over 300 m^2/g can be achieved with novel polymerisation techniques [34]. They exhibit principally reversed-phase selectivity. Surface charge can be introduced using co-precursors like acrylic acid or by sulfonating the aromatic residues. Vinylbenzylchloride can be used to incorporate reactive residues into the material. It is possible to employ Friedel-Crafts catalysed reactions as means to derivatise the polymer.

Polyacrylamide based monolithic materials for capillary chromatography were introduced in 1996 [40]. Crosslinking is achieved with N,N'-methylenebis(acrylamide). The base material exhibits hydrophilic polarity. Again, a variety of co-precursors can be added to introduce new functionalities into the material, they have to be miscible with the usually aqueous reaction mixture, though. Advantage is that the polymerisation is usually carried out at room temperature over night. This means biomolecules can be incorporated in-situ on one hand, on the other hand the danger of temperature gradients causing inhomogenities in the materials does not exist in this case.

Silica based materials were developed by Tanaka et al. in the mid 1990s [5]. Monolithic materials are usually based on the sol-gel synthesis using tetraalkoxysilanes as main precursor; co-precursors can in principle be used to adjust properties of the material. Activation of the capillary wall is usually required in order to establish sufficient bonds to the monolithic stationary phase. The use of tri- or tetra coordinated silica results in a high rigidity of the network. Silica monoliths exhibit the best values for pressure resistance, swelling of the material due to wetting does not occur. Surface chemistry is usually adjusted after synthesis with well known agents such as functionalised chlorosilanes. 3-glycidoxypropyl-trimethoxysilane is used to functionalise the surface with epoxy groups which can for instance be used for the immobilisation of proteins. The major issue that distinguishes silica materials from polymer based ones is the presence of silanol groups, which can cause problems due to strong interactions with basic functional groups/molecules. In return they can be used to advantage as surface charge carrier for electrokinetic separations. The main advantage of

silica based monoliths is the possibility to tailor the micro- and mesopore structure through the inclusion of an ageing step after gelation - independently of the macroscopic formed structures.

This last section will give a brief overview of applications where monolithic columns are currently used in μ-LC, n-LC or CEC separation techniques. As a remark, equipment for capillary separation techniques just recently became commercially available. Many applications described in literature demonstrate the potential of synthesised capillary stationary phases. Although miniaturised LC or CEC show great potential for a variety of applications, their implementation as routine techniques still requires a broader acceptance outside the academic field. Research in biochemistry and medicine require the qualitative or quantitative determination of analytes in biological samples. Limitations of sample amount make capillary separations attractive; the complexity of samples requires high resolution and peak capacity from employed separation technique.

Protein separation is routinely performed with 2D gel electrophoresis on a polyacrylamide gel. It has several drawbacks such as a poor automatisation, problems concerning repeatability, limitations concerning the pI and molecular mass of proteins and the sensitivity of protein detection. After protein separation, they are digested and then analysed with mass spectrometric detection to obtain peptide and consequentially protein sequence coverage. Monolithic capillary columns were so far used for preconcentration, for protein separations, as support for digestive enzymes allowing on-line digestion [39] and as separation media for digested peptides. Coupling to MS detectors can be realised on-line to ESI sources or off line via targets for MALDI sources [42]. The possibility to perform rapid resolutions with monolithic columns has lead to their use as second dimension in 2D LC separations of proteins and peptides. Although potentially stationary phases with a large variety of functionalities can be used, the majority of protein and peptide analytical applications employ reversed phase capillary monoliths, either based on silica with C_{18} modification [43], on polystyrene/divenylbenzene [44] and also on methacrylates with alkyl residues [45]. Affinity chromatography for glycoproteins was performed in CEC mode by immobilising lectines in a methacrylate based column [46]. Phosphopeptides can be separated with immobilised metal affinity chromatography which was demonstrated with silica based monoliths [47]. The separation of oligonucleotides was reported to be optimal under ion pair reversed phase conditions. Polystyrene/divenylbenzene monolithic columns have successfully been applied

in this field [48]. Another problem often encountered in life science research is the need to separate enantiomers, especially important in the analysis of pharmaceuticals. The inclusion of chiral selectivity in stationary phases can be achieved in several different ways. Possibilities are the immobilisation of proteins, cyclodextrins or organic molecules exhibiting chiral centres. Chiral selective stationary phases for capillary monoliths have so far been realised for polyacrylamide, polymethacrylate, and silica based supports [49-52].

Numerous other work is published on μ-LC, n-LC and CEC; in principle any chromatographic separation can be miniaturised to capillary format. Biosciences as fields of application were highlighted because advantages of miniaturisation are most convincing; the establishment of capillary separations techniques as routine methods is in the opinion of the author most likely to happen in these applications.

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5. Implementation of MTMS based sol-gel synthesis for the fabrication of monolithic capillary monoliths

5.1 Introduction

General characteristics of basic catalysed sol-gel synthesis are given in the theory section of this thesis. A synthetic route employing MTMS / H_2O_2 with basic catalysis was used. It was first reported in literature by Gun et al [1]. Optimisation concerning the employment in chromatography was conducted in previous work for monolithic columns with 12 mm and 6 mm diameters [2]. It is characteristic for this synthetic protocol that gelation times are within 15 min after the addition of the catalyst to the reaction mixture; average times are about 5 minutes. Time for transfer to moulds which act as templates for the monolithic materials is therefore limited. Difficulties in the cladding process proved to be a major hindrance in the employment of produced materials in liquid chromatography. The cladding process as employed by Merck for the production of their Chromoloith column involves the shrinking of a PEEK tube onto the rod at high pressures and elevated temperature which lead often to the mechanical destruction of produced materials, probably because differences in mechanical stability of materials based on MTMS compared to their silica material were unaccounted for [2]. In-house cladding was performed using Teflon shrinking tubes for sealing the rods which were then glued into steel tubes. In this case the organic content of employed solvent mixtures could diffuse through the Teflon layer and start to dissolve resins used for gluing which lead to the occurrence of dead volumes at these locations [3].

For these reasons and because of the developing interest in capillary separation techniques, it was decided to switch to the capillary format. It was therefore necessary to transfer developed knowledge of the optimisation of reaction conditions in the wide bore format to the synthesis in capillary format. In this case the cladding step can be avoided, which in return means that the synthesis has to be adjusted. A lack of bonds to the capillary wall in conjunction with shrinking occurring during ageing and drying can possibly lead to the formation of gaps turning these columns useless for chromatography. For further work fused silica capillaries with 0.53 I.D. were chosen as chromatographic separations could still be carried out on standard LC systems. It should be possible to transfer successful synthesis also to capillaries with even smaller diameters as this means that there are more bonds between monolith and capillary wall per volume which can resist to the forces occurring particularly during drying.

5.2 Materials and methods

Instrumentation

A model Poly 15 VarioMag magnetic stirrer (H + P Labortechnik; Oberschleißheim, Germany) was used for mixing the reaction mixtures. A model 5890 gas chromatograph (Hewlett-Packard; Avendale, PA, USA) was used as a programmable oven for gelation under controlled conditions. Non-deactivated fused silica capillaries with 530 µm i.d. were purchased from Agilent Technologies (Palo Alto, CA, USA). Single use syringes (polypropylene) were purchased from Wagner & Munz (Vienna, Austria). Female Luer to female 10-32 adapters, 10-32 connectors for 1/16" tubes, and 1/16" O.D. 685 µm I.D. capillary sleeves were purchased from Upchurch Scientific (Oak Harbour, WA, USA).

Chemicals and buffers

Methyltrimethoxysilane (MTMS) purum, tetramethoxysilane (TMOS), methanol analytical-reagent grade, ethanol absolute, 2-propanol analytical-reagent grade, 20% hydrogen peroxide, nitric acid 69% p.a. and sodium hydroxide purum were obtained from Sigma-Aldrich (Vienna, Austria). Distilled water was produced in-house.

Column preparation

Two different synthetic procedures were employed for the synthesis of monolithic columns in capillary format. For the basic catalysed protocol typically 5 ml MTMS, 5 ml 2-propanol, 5 ml 20% hydrogen peroxide solution, and 0.1 ml 3 N NaOH catalyst were used as reaction mixture. Capillary activation was done by exposing them to 3 N NaOH for 30 min, rinsing with distilled water, flushing with air for 10 min and drying over night at 40°C. Gelation times varied between 2 and 12 min after the addition of the catalyst. The reaction mixture was filled into capillaries 3 min or less before gelation took place.

In first experiments with acid catalysed synthesis, the attachment of monoliths to the capillary wall proved sufficient, therefore capillaries were used without activation in these experiments. The reaction mixture was stirred in polypropylene vessels at room temperature for approximately 2 minutes. Reactant ratios were optimised in the course of experiments,

favourable conditions were found at following ratios: 8 ml MTMS, 4 ml methanol, and 2 ml 1 N nitric acid (molar ratios of MTMS: MeOH: H_2O: HNO_3 = 0.056: 0.99: 0.11: 0.002).

The capillaries were filled with the use of single-use syringes, sealed with parafilm and then put in vertical position into the GC oven where gelation took place over night. Gelation temperature was 40°C if not stated differently in the results section. For the ageing procedure, the pore liquid was exchanged after gelation; capillaries were sealed again and transferred into the GC oven for a given period of time. For the final drying step the capillaries were rinsed with methanol and again put into the oven. Parallel to the preparation of columns in capillary format, larger quantities of the materials were synthesised in single-use syringes with analogous post-treatment to be later used for the assessment of surface area by nitrogen adsorption measurement.

Characterisation of porosity

The microscopic morphology was observed using a JSM 6400 electron microscope instrument (JEOL; Tokyo, Japan) in the secondary electron mode. Short column sections were sputtered with gold for 2 min at 10 mA and 15 kV acceleration voltage prior to the measurements as the bulk material is not conductive. The morphology was characterised by average pore diameter and average skeleton diameter which were obtained by measuring the length of 20 of each of the respective features in the pictures obtained by SEM with the instrument specific SemAfore software.

5.3 Transfer of a synthetic protocol employing basic catalysis to the capillary format

General considerations

As discussed in the introduction section, the starting point of the thesis was a previously developed synthetic protocol employing basic catalysis which was optimised for the bulk format [2]. Several characteristics of this protocol shall be mentioned at this point. Gelation times for the large-dimension monoliths were in the low minute range. The transfer of the reaction mixture in the macroscopic format was simply done by pouring it from polyethylene vessels employed for mixing into pre-heated glass or PEEK tubes used as moulds.

In contrast to this, capillaries had to be filled using a syringe as explained in section 5.2. Capillaries were immersed with one end in the reaction mixture which was then drawn through the capillary into the syringe. A piece of parafilm was then attached to seal the lower end of the capillary which was then removed from the connector to the syringe. After sealing the other end, capillaries were transferred into the oven for gelation. Previous work in the macroscopic format showed that results depended for instance on the time delay when the reaction mixture was filled into the moulds, on the inner diameter of the mould and even on the kind of material it consisted of. These dependencies can be explained with the thermal conditions acting during the gelation process. Hydrolysis and polycondensation are both exothermal reactions, leading to an observed increase of temperature in the reaction mixture up to 60°C. Depending on the temperature of the mould, its material (and therefore its heat conductivity) and its diameter, the reaction mixture cools down more or less quickly, leading to differences between the materials and/or a radial inhomogenity within a synthesised monolith. The direct transfer of reaction conditions to capillaries which exhibit a much higher surface to volume ratio and therefore an increased heat transfer rate can be expected to give vastly different results in the structure of the monolithic material.

Another fundamental difference between both formats is the introduction of the ageing solution. To do so, monolithic materials were removed from the moulds and simply immersed in the ageing solution. Pore liquid would simply be exchanged through diffusion processes between the bulk and the surrounding solution. Exchanging the pore liquid could be performed in a much more direct and efficient manner in case of the capillary monoliths. For this purpose a syringe was filled with the ageing solution, connected to the capillary and then the solution was introduced by applying pressure to the syringe. The reduction of the time

needed for solvent exchange must be considered when transferring ageing conditions to the capillary format.

The transfer of the reaction mixture in case of the large format moulds was rather close to the point of gelation. To keep the reaction conditions as close as possible, transfer into capillaries was also aimed to be close to the point of gelation. Capillaries were pre-heated at 40°C prior to the filling process. This should minimise heat losses before and during the essential time of gelation, which would otherwise lead to differences in the morphology of the emerging xerogel. To promote the formation of bonds between the capillary wall and the gel, it was decided to activate the silica surface. This was done by exposing it to 3 N NaOH for 30 min, rinsing with distilled water, flushing with air for 10 min and drying over night at 40°C.

Variation of the precursor composition

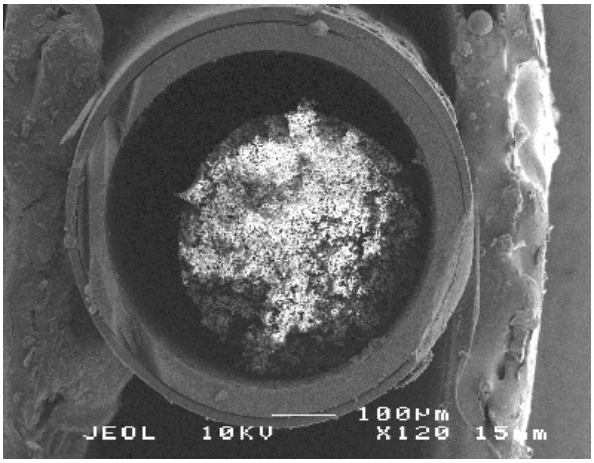
The starting point of the synthesis under basic conditions was following mixture: 5 ml MTMS as precursor, 5 ml 2-propanol or methanol, 5 ml 20% hydrogen peroxide solution and 0.1 ml 3 N sodium hydroxide as catalyst. First experiments conducted with this protocol resulted in monoliths which in most cases were washed out when trying to introduce the ageing solution. When performing drying without ageing, the monolithic materials were also only loosely attached to the capillary wall. This became evident when cutting short pieces from the columns as preparation for the SEM measurements, during which the monoliths would often slip out of the capillaries. For these reasons the initial synthetic protocol had to be adapted.

In the first set of experiments the precursor composition was varied. Tetramethoxysilane (TMOS) was added to the reaction mixture as second precursor. TMOS has under basic conditions an increased rate of hydrolysis and polycondensation (compared to MTMS) and should therefore form the backbone of the gel structure. Reaction kinetics are slower under these conditions for MTMS, this was believed to result in a methyl-silsesquioxane rich surface. Because TMOS forms silica species which can form 4 bonds instead of 3, which could increase the number of established bonds from the gel to the capillary wall. It is clear that the addition of a second precursor influences the whole course of reaction which leads to differences in the morphology. Tab. 5.1 gives employed reaction conditions for this set of experiments. Morphology was determined from pictures obtained by SEM when possible.

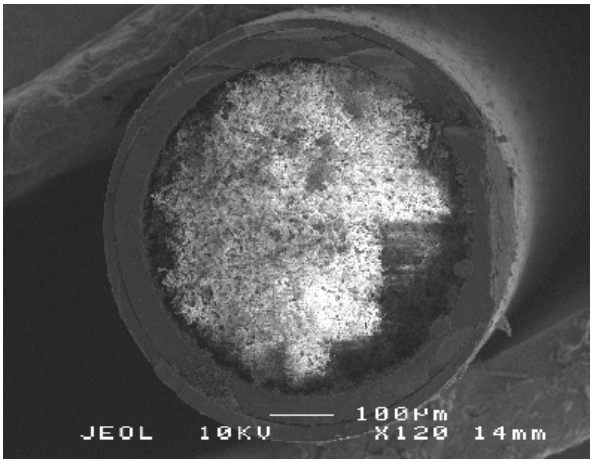
Tab. 5.1: Reaction conditions and observed morphological features for materials with various precursor compositions.

	Reaction mixture [ml]					**Transfer- and gelation time [min:sec]**		**Morphology [µm]**	
Column code	MTMS	TMOS	2-PrOH	H_2O_2	3N NaOH	t_T	t_G	skeleton	pore
12	4.75	0.25	5	5	0.1	3:00	3:20	2.53	3.77
13	4.5	0.5	5	5	0.1	2:30	3:10	<0.2 µm, strong shrinking	
14	4.25	0.75	5	5	0.1	2:30	2:50	Non porous	
15	4	1	5	5	0.1	1:30	2:10	Non porous	
16	5	0.05	5	5	0.1	2:30	3:40	Flushed out	
17	5	0.1	5	5	0.1	2:30	3:30	1.74	5.58
18	5	0.15	5	5	0.1	2:30	3:20	Very heterogenous	

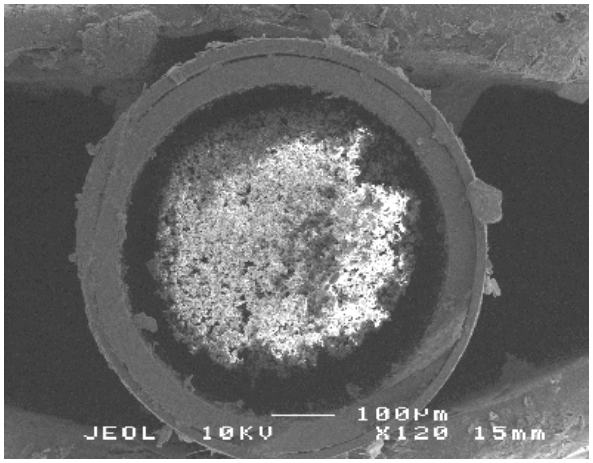
As can be seen in the results of these experiments, the precursor composition has a strong influence on the morphology of obtained monolithic materials. When substituting more than 0.25 ml of MTMS with TMOS, pore and skeleton size decrease to a point where macropores disappear entirely. These materials exhibit a glass like appearance; they are very stiff and transparent. During ageing and drying shrinking occurs; cracks are formed during these steps up to a point where the monolithic materials disintegrate into small pieces.



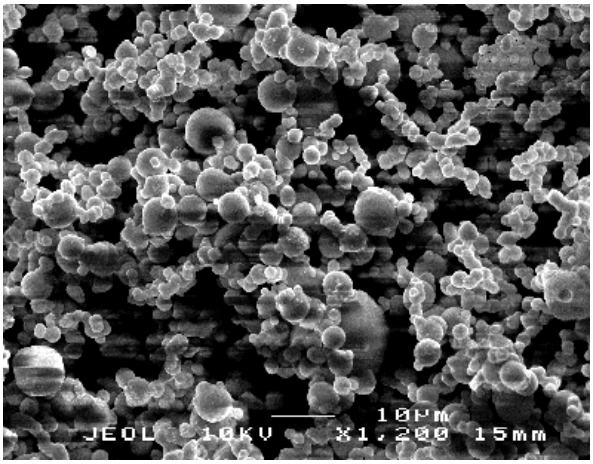
SEM micrograph of material 12



SEM micrograph of material 17



SEM micrograph of material 18



SEM micrograph of material 18, zoom in

Fig. 5.1: SEM micrographs of materials 12, 17 and 18

Experiments where the TMOS content was lower than 0.25 ml in the reaction mixture resulted in materials with a macroscopic bicontinuous structure. Material 12, which has the highest content of TMOS in the reaction mixture, was subject to significant shrinking during the drying process, which resulted in a large visible gap between the monolith and the capillary wall. This is also true for material 18, which has the second highest content of TMOS, although in this case the shrinking is less developed. As can be seen in the zoom into the monolith, the skeleton structure is very inhomogenous and consists of spherical structures with a large variability in size. The skeletal structure of materials 12 and 17 consisted of fused spheres with a narrower size distribution. The origin of these unique features of material 18 could not be identified in further experiments. The micrograph of material 17 which has the

lowest content of co-precursor does not show apparent gaps to the capillary wall. Its pore and skeleton size are in the range of feasible materials for liquid chromatography. However, the attachment to the capillary wall was too poor to employ this material for liquid chromatography.

This set of experiments showed that a co-precursor could only slightly improve the attachment to the capillary wall. An increasing content of co-precursor resulted in an increasing shrinking during ageing and drying, leading to the occurrence of gaps to the capillary wall. One explanation is that more bonds within the silica network can be established and that less methyl groups are contained within the network, which increases its density compared to pure MTMS derived materials. A higher number of silanol groups on the surface of the emerging gel can be expected as well which results in an increased wetability - capillary forces during the drying step increase as well. The macroscopic structure did not change dramatically at a low content of co-precursor with the exception of smaller observed pore sizes due to shrinking. Non-porous gels were obtained at higher contents of TMOS in the reaction mixture. This route of synthesis did not result in capillary monoliths which could be employed in chromatography.

Variation of the catalyst concentration

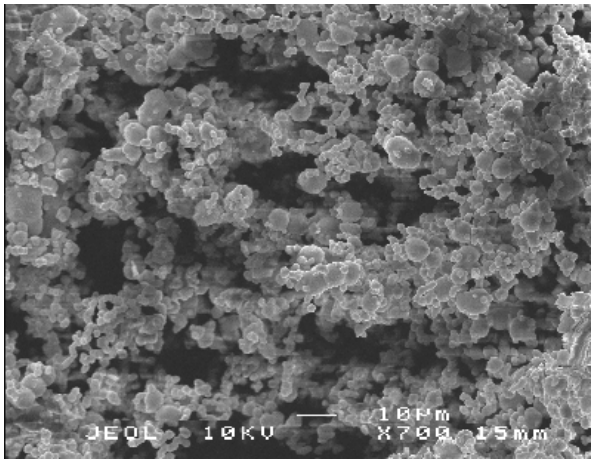
One major contribution to the lack of adhesion was believed to be caused by the temperature gradient which would cause the gelation occurring earlier at the centre of the capillary than at its wall. As ageing processes start immediately after gelation, the growing gel phase would already start to shrink, which means that expelled pore liquid would dilute the remaining reaction mixture at the capillary wall. It is clear that a temperature gradient should be avoided in the course of synthesis. The second reason for a lack of attachment of the monolithic material could be the existence of repulsive forces between the negatively charged surfaces of the gel and the silica surface of the capillary.

It was therefore decided to study the influence of the catalyst concentration on the attachment of the monolithic materials to the capillary wall. Lower catalyst concentration means that the hydrolysis and polycondensation occur at a slower rate. This means that gelation times are longer and that the final temperature of the reaction mixture is also lower, which would reduce the emerging temperature gradient. A decreasing amount of negative surface charge and therefore reduced repulsive forces between gel phase and capillary wall were also

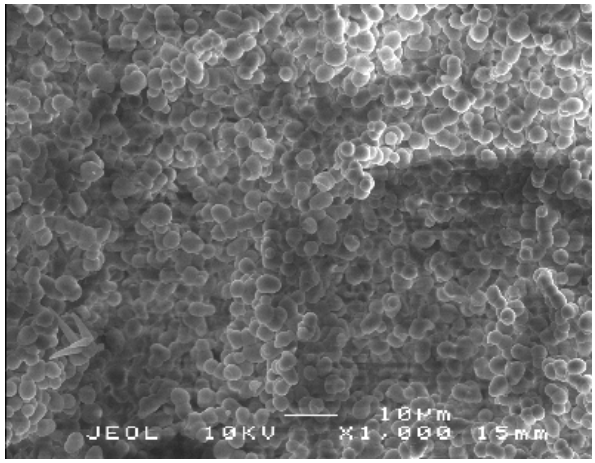
expected at lower catalyst concentrations. Detailed reaction conditions for this set of experiments are given in table 5.2.

Tab. 5.2: Reaction conditions and observed morphology from the study of the influence of catalyst concentration on the morphology of the monolithic material.

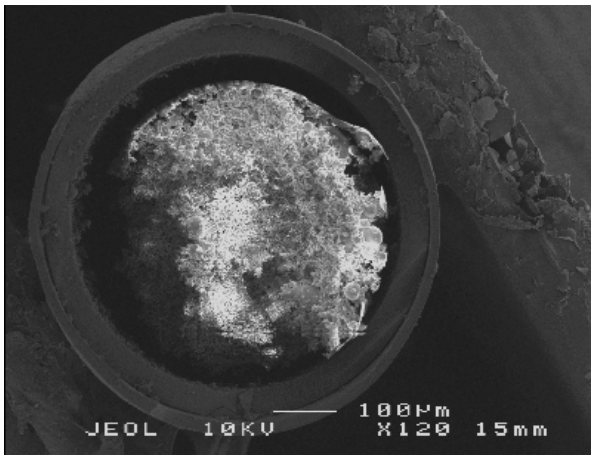
	Reaction mixture [ml]				**Transfer- and gelation time [min:sec]**		**Morphology [µm]**	
Column code	MTMS	2-PrOH	H_2O_2	3N NaOH	t_T	t_G	skeleton	pore
7	5	5	5	0.09	3:30	5:30	Flushed out	
8	5	5	5	0.08	5:00	7:00	3.14	12.33
9	5	5	5	0.07	7:00	8:25	3.09	-
10	5	5	5	0.06	7:45	9:45	Very heterogenous	
11	5	5	5	0.05	8:30	11:20	3.87	-



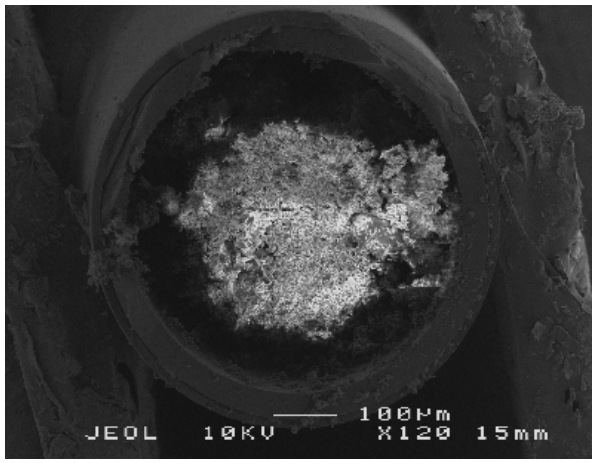
SEM micrograph of material 8



SEM micrograph of material 9



SEM micrograph of material 10



SEM micrograph of material 11

Fig. 5.2: SEM micrographs of materials 8-11

The results showed that the reduction of the catalyst content did not improve the morphology of the resulting monolithic materials. The skeleton of the monoliths consisted in all cases of connected spheres as can be seen in figure 5.2. In micrographs of materials 9 and 11 no pores could be evaluated, the softness of these materials lead to a collapse of the bicontinuous structure on the cross section during the sample preparation. In material 8 the skeleton consisted of several layers of fused spheres which had on average 3.14 µm diameter. The material also possessed visible macropores with a diameter of 12.33 µm. Both features mean that the material could be feasible for use in chromatography, however in the micrograph which showed a complete cross-section of the capillary a gap to the capillary wall was visible. Materials produced with even lower catalyst concentrations did not possess apparent

macropores as can be seen in their micrographs. They consist of more or less loosely connected spheres. Although their diameter does not change dramatically when reducing the content of the catalyst, their connectivity decreases at the same time. This is also reflected by an apparent softness these materials possess. When handling the larger batches produced in the single use syringes as moulds, xerogel material was easily rubbed off from touching. The lack of cohesion between these spheres means that in the course of ageing and drying macropores are lost due to a collapse of the very soft skeletal structure.

This set of experiments showed that catalyst concentration is a parameter which should not be changed. High concentrations of catalyst favour the formation of smaller, more strongly connected spheres which form the skeletal backbone of the monolithic material, because more nuclei for polycondensation are available. Mechanical stiffness and the formation and preservation of a macropore structure are essential if the materials should be employed for liquid chromatography. In these cases the danger of the formation of a thermal gradient to the capillary wall was higher at the employed reaction conditions which lead to poor connectivity to the capillary wall. As discussed in the next sections, other means of optimising the reaction were pursued.

Capillary pre-treatment and transfer time

As discussed in the previous part of this chapter, the change of the precursor composition and the variation of the catalyst concentration could not improve the adhesion of the monolithic material to the capillary wall. Both sets of experiments showed furthermore that only a small deviation of reaction conditions from the initial protocol resulted in materials with morphological characteristics not suitable for the application in liquid chromatography.

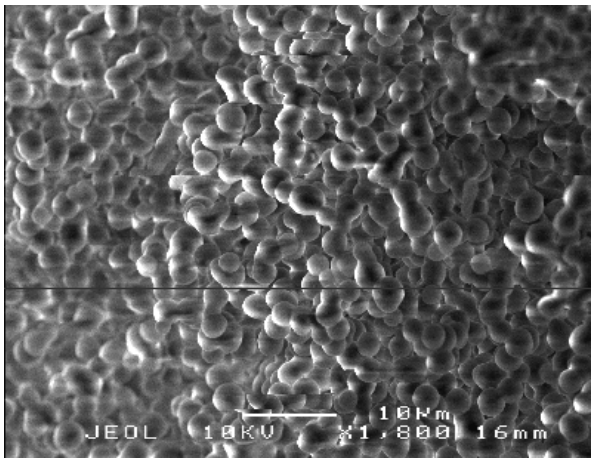
The next logical step was to investigate several possibilities to keep the synthetic protocol which resulted in materials exhibiting favourable morphological characteristics but adjust capillary activation and the transfer time of the reaction mixture into the moulds to improve the adhesion to the capillary wall and avoid shrinking processes which would lead to the formation of cracks or gaps.

Tab. 5.3: Reaction conditions and observed morphology from the study on the influence of capillary activation and transfer time

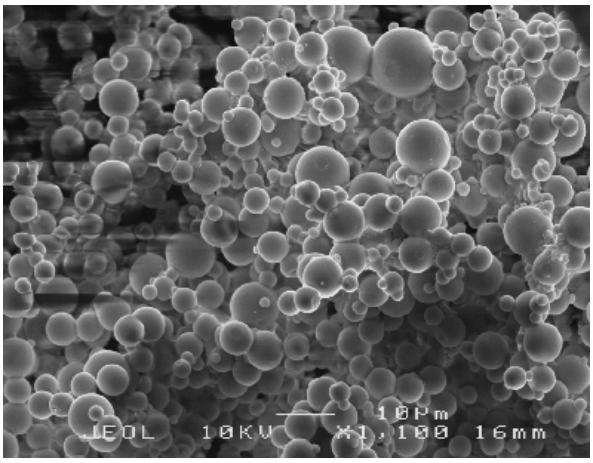
Column code	Reaction mixture [ml]				Activation	Transfer- and gelation time [min:sec]		Morphology [µm]	
	MTMS	solvent	H_2O_2	3N NaOH		t_T	t_G	skeleton	pore
27	5	2-PrOH 5	5	0.1	Yes	2:30	3:40	2.62	-
30	5	2-PrOH 5	5	0.1	No	2:30	3:40	5.10	-
29	5	MeOH 5	5	0.1	Yes	1:15	2:10	5.43	4.10
31	5	MeOH 5	5	0.1	No	1:15	2:10	Collapsed structure, huge gap	
88	5	2-PrOH 5	5	0.1	No	1:00	3:40	Flushed out	
87	5	MeOH 5	5	0.05	No	1:00	5:15	3.76	3.90
77	5	EtOH 5	5	0.1	No	1:00	2:40	3.58	5.51

In the first part of this optimisation the influence of the capillary activation on the resulting morphology was studied. For this purpose, reaction mixtures with identical composition were filled after identical transfer time into one activated and one not activated capillary.

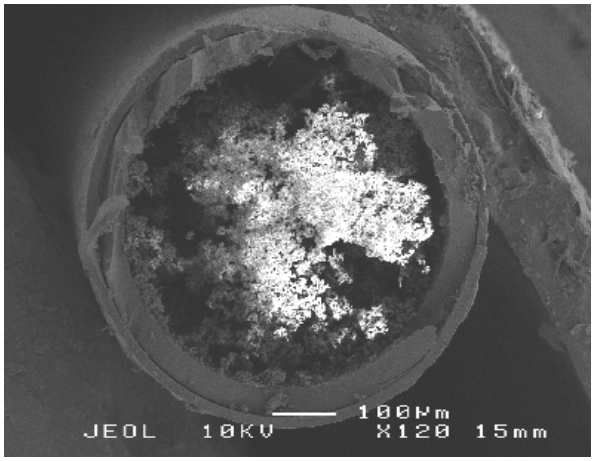
Results as shown in figure 5.3 show an influence of activation on the morphology of obtained materials, although explanations on causality could not be made; this difference is probably caused by transfer time variations. In case of materials 27 and 30, spherical structures are significantly larger for the material where the capillary was not activated prior to synthesis. Material 31 consists of spheres with a wide size distribution whereas material 29 where the capillary was activated consists of fused spheres with a narrow size distribution. Material 31 possessed not enough mechanical stability to preserve its structure after drying and cutting for the SEM measurements.



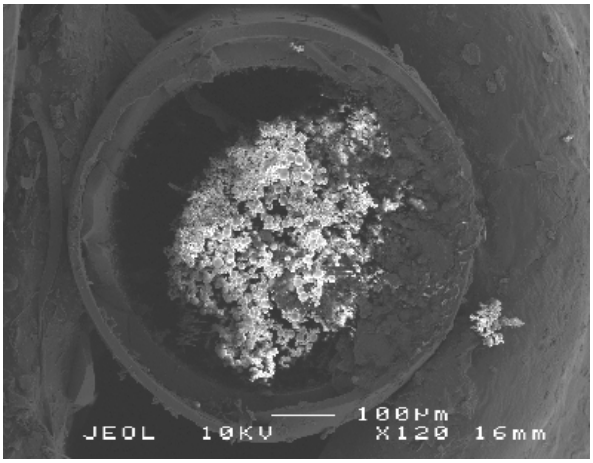
SEM micrograph of material 27



SEM micrograph of material 30

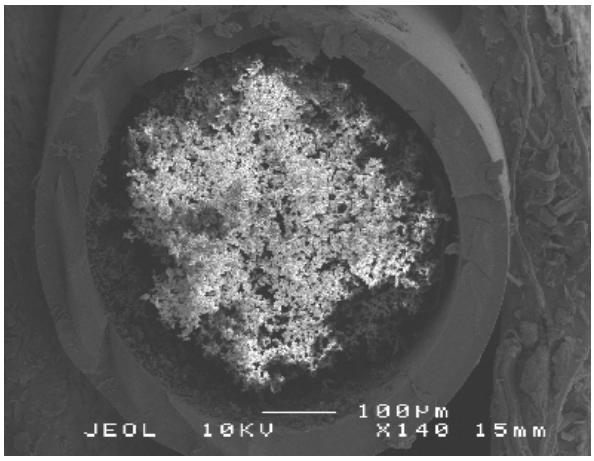


SEM micrograph of material 29

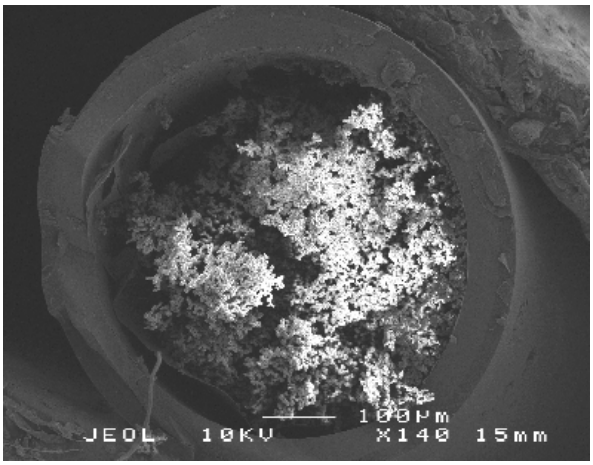


SEM micrograph of material 31

Fig. 5.3: SEM micrographs of materials with and without capillary activation



SEM micrograph of material 87



SEM micrograph of material 77

Fig. 5.4: SEM micrographs of materials where the transfer time was reduced to 1 min

In the second part of adjusting the setup for the synthesis, it was decided to reduce the transfer time after the addition of the catalyst. Reaction mixtures leading to favourable morphological characteristics typically heated very rapidly. In previous experiments the transfer was timed to be performed just before the gelation in order to minimise heat losses. Besides the apparent inability to produce materials with sufficient attachment to the capillary wall, it happened rather often that gelation occurred before or during the transfer procedure because of handling difficulties with the used setup.

A second approach to this problem was to transfer the reaction mixture 1 minute after adding the precursor which should sufficiently homogenise the solution. This would allow more buffer time for the transfer. Capillaries were immediately sealed and put into an oven at elevated temperature. Heat loss should not occur and the gel should therefore be formed simultaneously in the whole cross section of the capillaries. Two materials produced with the new approach can be seen in figure 5.5. The micrograph of material 87 shows a gap to the capillary wall as well as a gap within the material in the direction from top down. For material 77 the gap within the material is even more visible. A narrow zone with some material attached to the capillary wall is visible in the dark regions in both pictures, which means that some material was attached in both cases to the capillary wall. It can be argued that in both cases the scratching and breaking of the capillaries as preparation for the SEM measurements put enough stress to the monoliths to cause their deformation and the formation of observed gaps. The zoom into the materials showed an even size distribution of spherical structures forming the skeleton. This could be due to the reason that a temperature gradient could be avoided which leads to a more uniform gelation process. For these reasons, the following experiments were conducted with a reduced transfer time and the immediate placement of filled capillaries into the oven.

Investigations concerning the activation procedure did not produce conclusive results. Although there was a difference in the morphology between synthesis with or without activation, they were more likely to stem from variations of transfer time and therefore thermal conditions inside the capillary during the gelation. Later experiments where the heat loss could be reduced were performed in capillaries without activation. As results were suggesting a good attachment to the capillary wall, the activation step was concluded to be unnecessary and thus eliminated from the synthetic protocol. Another effect of the elimination of heat losses was that the morphology of obtained materials was closer to the results obtained for monoliths in the 6 mm I.D. format. Variations in the transfer time should have less of an

effect when performing synthesis according to the new procedure compared to previous experiments where transfer was timed just shortly before gelation.

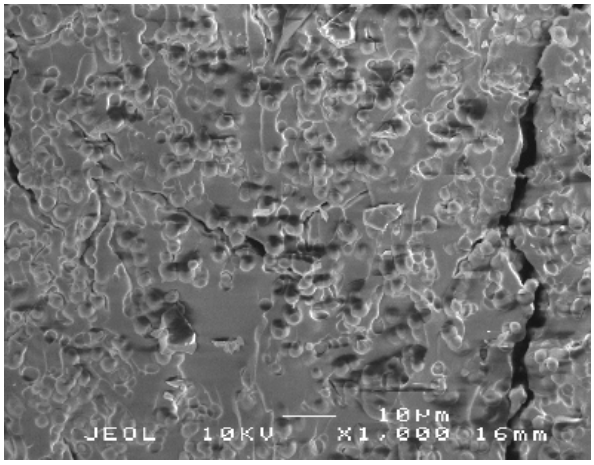
Variation of the ageing procedure

In the next series of experiments an ageing step was introduced. This step is essential to improve the mechanical stability of the monolithic materials. There are several ways to perform ageing; the most widely used one is to replace the pore liquid with a basic solution. Silica species would continuously dissolve and precipitate with the result of a general smoothening of the macroscopic structure, an increase of mesopore diameters due to a general coarsening of the structure in the nm range and the subsequent loss of micropores.

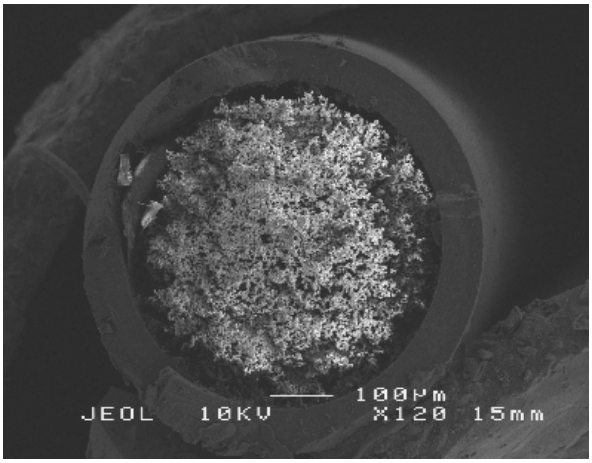
This way was ruled out as nucleophilic attacks would occur preferrably at silica nuclei without organic modification – at the capillary wall. It was therefore decided to perform ageing by introducing a precursor solution. Dissolved methyl silica species would react with available silanol groups from the gel, which should lead to a precipitation of silica species on places with negative curvature as under basic conditions.

Tab. 5.4: Reaction conditions for synthesis and ageing and observed morphological characteristics of resulting monolithic materials

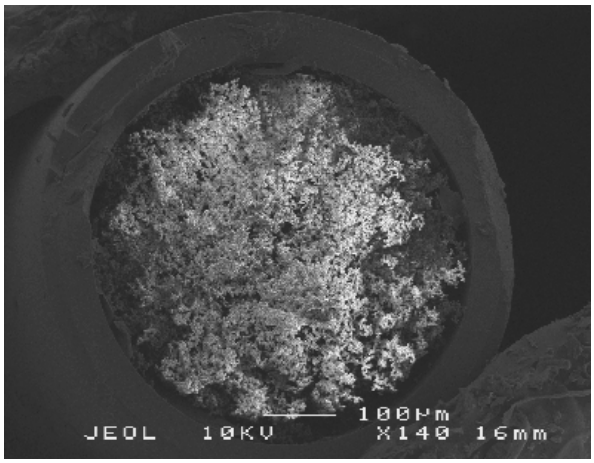
Column code	Reaction mixture [ml]				Ageing procedure	Morphology [µm]	
	MTMS	solvent	H_2O_2	3N NaOH		skeleton	pore
27b	5	2-PrOH 5	5	0.1	30 min in MTMS:0.04N HNO_3 3:1	Non-porous material	
29b	5	MeOH 5	5	0.1	30 min in MTMS:0.04N HNO_3 3:1	Non-porous material	
75	5	MeOH 5	5	0.1	a: no ageing	a: 2.38	5.30
					b: 5 min in MTMS : 2-PrOH 0.3 : 0.7	b: 3.08	3.67
76	5	2-PrOH 5	5	0.1	a: no ageing	a: 2.65	4.93
					b: 5 min	b: 2.59	5.66
					c: 15 min in MTMS : 2-PrOH 0.3 : 0.7	c: 2.66	4.75
46	5	2-PrOH 5	5	0.1	a: no ageing	a: 2.48	5.65
					b: 30 min in MTMS : 2-PrOH 0.3 : 0.7	b: 2.07	3.36



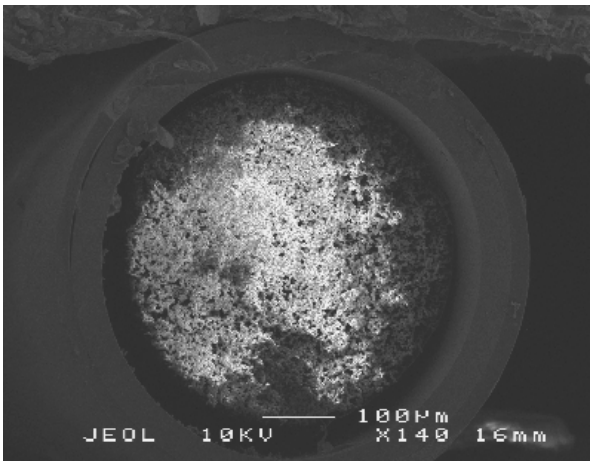
SEM micrograph of material 27b



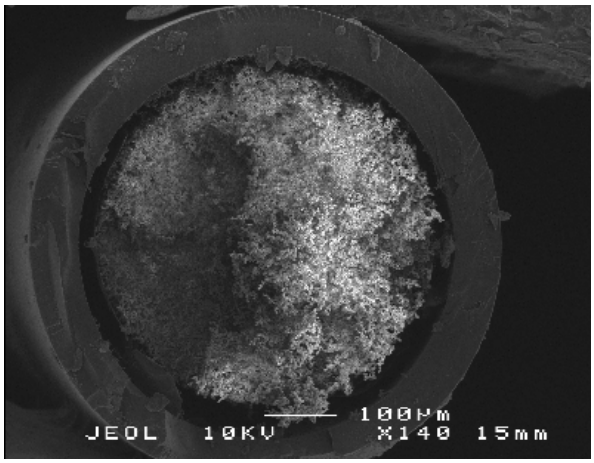
SEM micrograph of material 75a



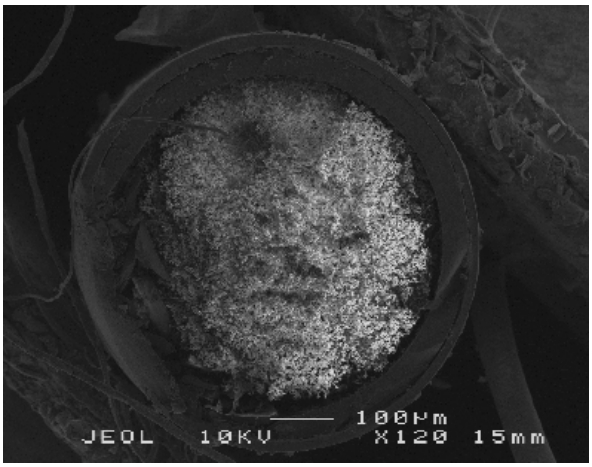
SEM micrograph of 75b



SEM micrograph of 76a



SEM micrograph of 76b



SEM micrograph of 46b

Fig. 5.5: SEM micrographs of materials where ageing was performed with MTMS containing solutions as described in table 5.4.

In the first experiments the ageing solution was produced by hydrolysing MTMS under acidic conditions. This would produce a large number of species with reactive silanol groups which could then undergo condensation reactions necessary for the ageing process. This solution was introduced for 30 min., capillaries were then rinsed with methanol and finally dried. As can be seen in the SEM micrograph of material 27b, ageing conducted with these parameters resulted in non porous material. Hydrolysed precursors would enter pores and form a non-porous gel phase under these conditions. As a result, it was decided to change the composition of the ageing solution. MTMS was diluted with 2-propanol; no catalyst or water was added. The reasoning behind this decision was that superficial silanol groups should be present at a negatively charged state after gelation, which would be enough to catalyse hydrolysis and condensation reactions. Water necessary for the hydrolysis of MTMS to species with free silanol groups should stay adsorbed at surface areas with hydrophilic properties – with a sufficient amount of silanol groups being present. No secondary gel phase should emerge under these conditions; new methyl-silsesquioxane material would precipitate only at reactive sites as it is desired for the ageing step.

A general result of this set of experiments was the observed variation of macroscopic morphology even for investigated cross sections from the same column which leads to the conclusion of a longitudinal inhomogenity, probably due to sedimentation processes. The synthetic protocol used for materials 75a and 75b had characteristic gelation times below 2:30 minutes. Monolithic materials produced with this protocol exhibited compared to other materials a higher stiffness. Ageing was performed for only 5 min with a 30% MTMS solution in 2-propanol in case of material 75b. Results as shown in table 5.4 and figure 5.6 show reduced pore diameters and increased skeleton diameters for the aged material. A gap is visible in the SEM micrograph of material 75a but not in the micrograph 75b. This means that 75b has sufficient mechanical stability to sustain the macroscopic morphology during the drying step. A second series of materials where an ageing step was included was produced with a synthetic protocol which resulted in softer base materials. In this series ageing times were varied between 5 min and 30 min. SEM micrographs show a large gap present in the picture of the material where no ageing step was included in the synthesis which gets subsequently smaller with the time the monoliths were exposed to the ageing solution. These set of experiments demonstrate that ageing can be employed to improve the mechanical stability of the materials. As aged materials resulted in capillary monoliths without gaps, these gaps are not caused by shrinking processes happening before the introduction of the ageing solution. Whether they are formed in of the drying step or during the sample preparation for

the SEM measurements could not be evaluated, either case means that the materials would not be suited to be employed in liquid chromatography.

5.4 Implementation of a synthetic protocol employing acid catalysis

Since the synthetic protocol based on basic catalysis proved to be of little success, an alternative method based on acidic catalysis of MTMS was considered for the synthesis of capillary monoliths [4]. Gelation times were reported to be one hour or more which would eliminate problems occurring in the transfer of the reaction mixture into the capillaries. Gelation temperature would be independent of the exothermal hydrolysis reaction and could completely be controlled externally with the GC oven.

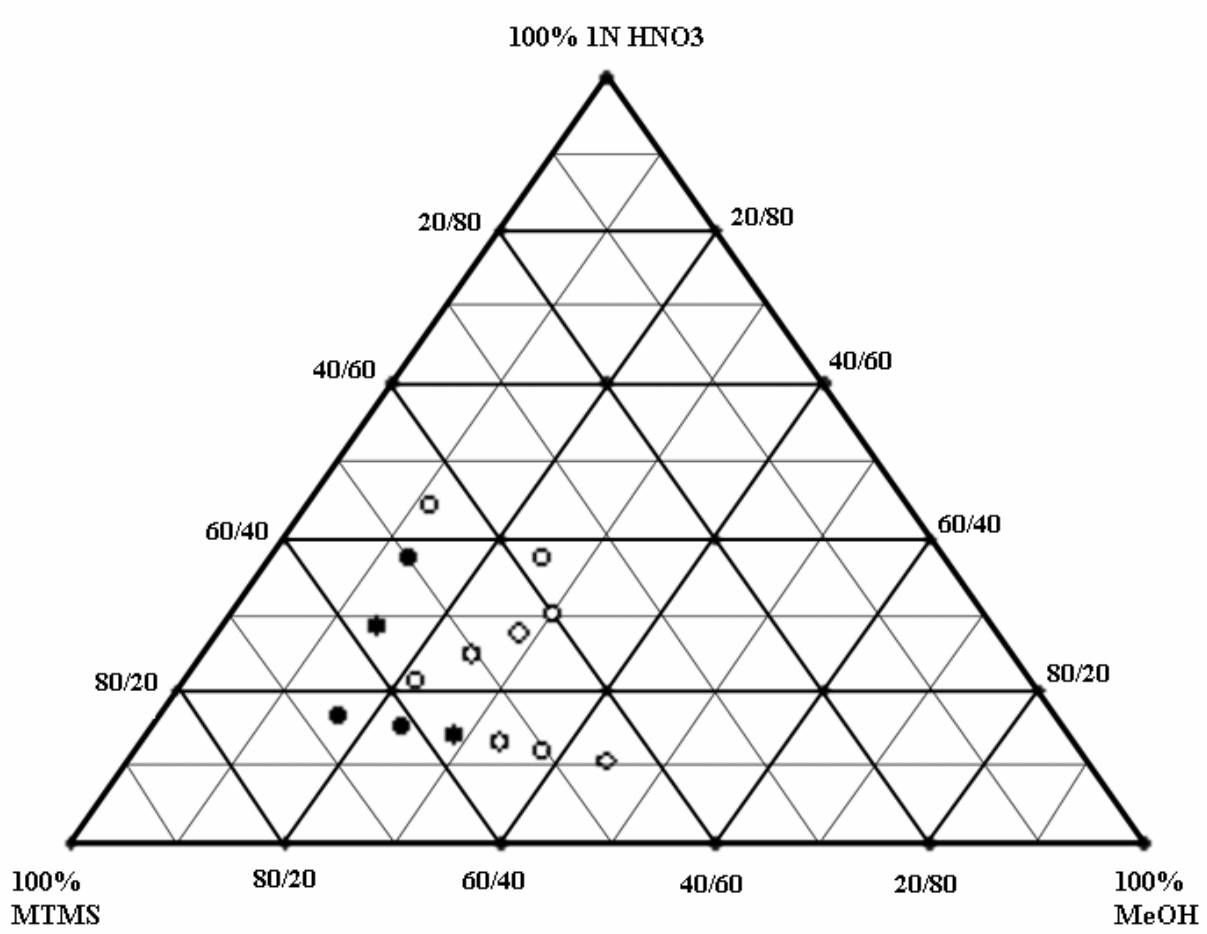


Fig. 5.6: Ternary phase diagram of the composition of the reaction mixture. Numbers are given in v/v. Full circles represent materials with continuous gel phase with porous structure; empty circles represent experiments which produced materials without these features.

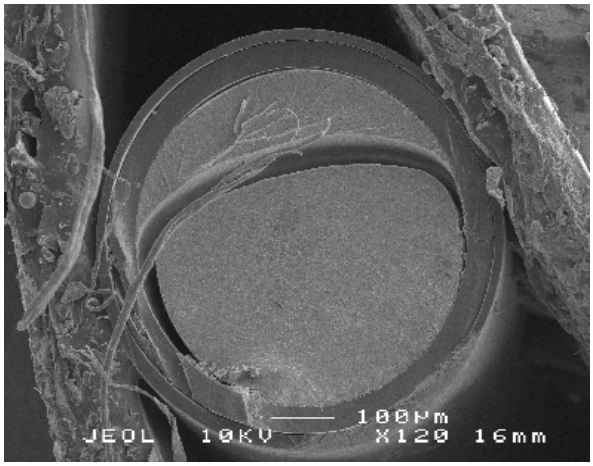
In the first step of introducing a synthetic protocol based on acid based catalysis, reaction mixtures with a variety of precursor compositions were mixed. SEM measurements were performed with each material in order to determine which mixtures produced monolithic materials with a continuous gel phase with through pores as necessary for chromatographic applications. Capillaries were stored in a horizontal position during the gelation in these experiments in order to make any inhomogenity visible caused by sedimentation processes. Results of this first set of experiments showed that materials with a desired macroscopic morphology were only obtained within a narrow region of the reaction mixture composition. Increasing the methanol content too much lead to coarse structures which were subject to sedimentation which left regions without gel phase in the capillary; increasing the content of the aqueous phase lead to similar results. When increasing the content of both phases at the same time, the region of materials with a bicontinuous pore and skeletal structures was immediately left.

Tab. 5.5: Composition of the reaction mixture for materials produced with acid catalysis

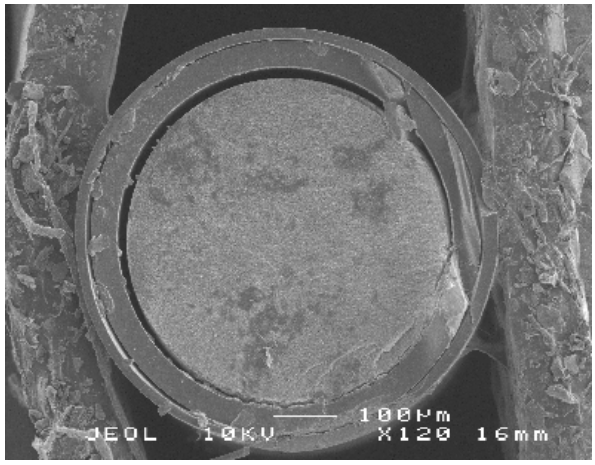
	Reaction mixture [ml]			**Morphology [µm]**	
Column code	MTMS	solvent	1N HNO_3	skeleton	pore
46	8	MeOH 2	2	0.10	0.16
47	8	MeOH 3	2	0.22	0.46
48	8	MeOH 4	2	1.36	3.13
38	8	MeOH 2	4	Non-continuous pore structure	
39	8	MeOH 2	6	Non-continuous pore structure	
57	8	2-PrOH 3	2	Non-porous	
60	8	EtOH 3	2	Non-porous	

Structures with a bicontinuous phase shall be discussed with more detail at this point. Figure 5.6 shows a series of experiments where the methanol content of the initial reaction mixture was varied between 2 and 4 ml while all other parameters were kept constant. The results show that pore and skeletal structure get coarser as precursor and aqueous phase get more diluted. This is a result of the gelation mechanism which relies on the comparative kinetics of

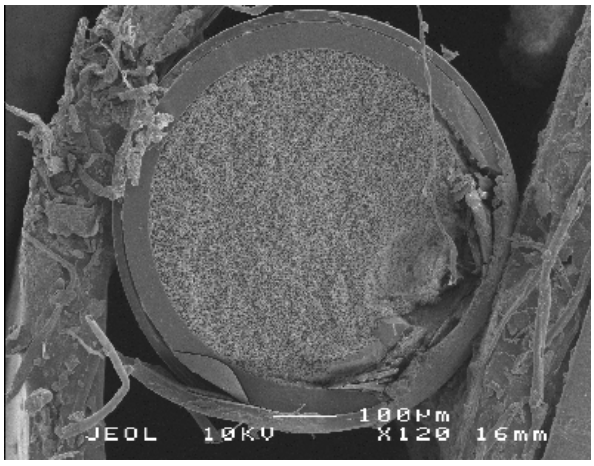
the polycondensation and phase separation mechanism as discussed in chapter 2. Shrinking caused gaps to occur for materials with pore diameters below 0.5 µm. No gaps are apparent at the SEM micrograph of material 48. It possesses an average pore diameter of 3.13 µm and a skeleton diameter of 1.36, these features are homogenous over the whole cross-section of the capillary monoliths. Adhesion to the capillary wall proved to be excellent, repeated flushing could not remove the monolith from the capillary. Further increasing the methanol content of the reaction mixture resulted in materials with a coarser structure where the premature phase separation left regions of the capillary without xerogel. This set of experiments showed that changing the methanol content had a strong effect on pore and skeleton size. The principle gel morphology of connected, smooth tubes did not change when changing the methanol content.



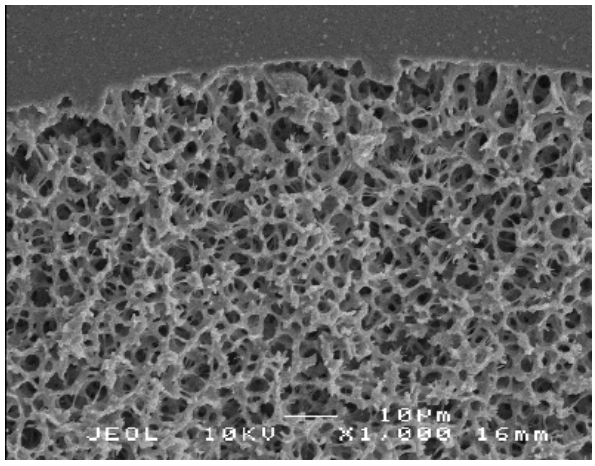
SEM micrograph of material 46



SEM micrograph of material 47



SEM micrograph of material 48

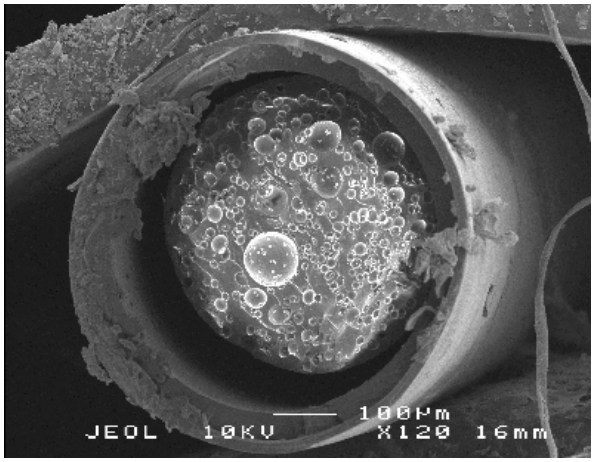


SEM micrograph of material 48, zoom in

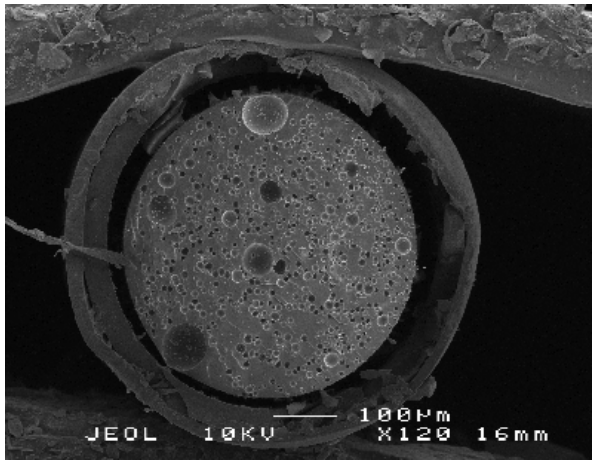
Fig. 5.7: SEM micrographs of materials 46-48. Methanol content of the reaction mixture was increased in this series as presented in table 5.5.

In the reaction mixture of materials 38 and 39 the ratio of precursor to aqueous phase was changed. As can be seen in figure 4.9, the morphology of the monolithic materials changes completely. The pore structure is not wide open as for material 48, pores appear to form enclosed spherical cavities within a non-porous methyl-silsesquioxane phase. Gaps to the capillary wall appear in both micrographs. When further increasing the water content, isolated gel spheres without porosity are formed.

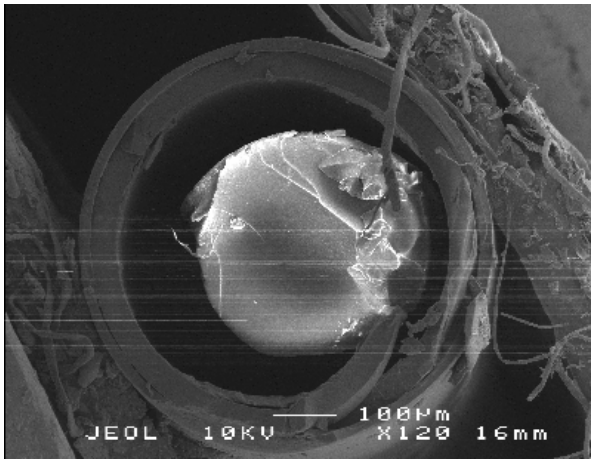
Two experiments were made where the solvent was changed to 2-propanol and ethanol. Both experiments resulted in non-porous, strongly shrinking materials not suited for chromatography.



SEM micrograph of material 38



SEM micrograph of material 39



SEM micrograph of material 57

Fig. 5.8: SEM micrographs of materials where the MTMS : aqueous phase ratio was changed as presented in table 5.5. In the protocol of material 57 the solvent was changed for methanol to 2-propanol.

First experiments conducted with the synthetic protocol based on acid catalysis showed that a small change in the composition of the reaction mixture resulted in large changes of the morphological characteristics. Diluting the mixture with solvent could change pore- and skeleton size dramatically whereas a higher amount of aqueous catalyst changed the morphology of the pore- and skeleton structure from a bicontinuous phase to isolated pores in a monolithic block. Exchanging the solvent resulted in materials with no apparent porosity at all. The reaction conditions employed for material 48 resulted in macroscopic morphological characteristics which seemed suitable for chromatography. This synthetic protocol could be used as starting point for the investigation of parameters which influence the morphology of the resulting material.

5.5 Conclusion

The transfer of the protocol using the basic hydrolysis showed, that several crucial points had to be adjusted in order to produce monolithic materials without gaps to the capillary wall, with sufficient adhesion to the capillary wall to not be flushed out at the first occasion and with sufficient repeatability in the resulting morphology. Activation of the capillaries proved to have little effect on the adhesion of the monolithic materials to the capillary wall; commercially available capillaries are already in an undeactivated and dry state after production. The initial composition of the reaction mixture could only be changed within narrow limits while still obtaining materials with favourable macroscopic morphologies. Neither the addition of TMOS as co-precursor, nor the variation of the catalyst concentration nor the solvent could improve the adhesion and the mechanical stability vitally. The crucial adjustment was to reduce the time for transferring the reaction mixture into the capillaries after adding the catalyst. The temperature at the point of gelation seems to be the most important parameter which determines the morphology; a temperature gradient will result in a gradual gelation from hot zones to cooler zones. A fast transfer into a heated oven could make the synthesis more reproducible. The morphologies were resulting from this procedure were more similar to morphologies obtained in the synthesis of the 6 mm I.D. columns. Materials with good characteristics were generally obtained in syntheses with comparatively low gelation times – with a comparatively higher temperature rise due to the exothermal reactions. Moving to protocols with even shorter gelation time was not possible as the time of mixing,

filling the capillaries and transfer into the oven could not be reduced below 1:15 min. Ageing the monolithic materials in a 30% MTMS solution could enhance the mechanical stability and the adhesion to the capillary wall. As previous work showed, further prolonging the ageing time would result in materials with insufficient specific surface area to employ them in liquid chromatography. Even the materials with the best wall adhesion were flushed out of the capillaries when it was tried to install them in a liquid chromatograph. As a summary the basic protocol could not be employed to produce monolithic capillary columns fit for chromatography.

First experiments with the acid based protocol showed a strong dependency of the resulting morphological characteristics on the composition of the reaction mixture. Diluting the solution with solvent had an influence on pore- and skeleton diameters, adding more aqueous catalyst changed the principal morphology from connected tubes to blocks with enclosed pores. Replacing methanol with other solvents produced materials which did not possess macropores. This first screening of reaction conditions resulted in one material which possessed favourable macroscopic characteristics for its employment as stationary phase. From this starting point further investigation of the acid catalysed sol-gel reaction should be made in order to quantify the dependency of the morphology on various parameters. A parallel optimisation of the micropore and mesopore structure is also needed as both features are important for the final performance of capillary monoliths as stationary phase.

5.6 References

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6. Tailoring the macroporous structure of monolithic silica-based capillary columns with potential for liquid chromatography*

6.1 Introduction

In recent years monolithic stationary phases have become increasingly important as stationary phases for high performance liquid chromatography, capillary chromatography/ electrochromatography and lab on a chip systems [1-5]. Recent reviews highlight the use of silica based monolithic stationary phases in the field of electrophoresis [6-10] as well as in the field of pressure driven liquid chromatography [11-13]. Synthesis in the capillary format is, when employing sol-gel technology as route of synthesis, typically carried out directly in the capillaries. The synthetic protocol has to be optimised to obtain materials with desired properties at all levels of feature dimensions for their application in chromatography: this is, the open pore structure in the macroscopic scale, the mesopores with suitable diameter depending on the particular range of analytes, and a microporosity large enough to offer a sufficiently large surface area to be useful for chromatography. Surface modification and post-treatment (after the synthesis of the materials) have consequently to be conducted for each monolithic column individually which can cause problems with their reproducibility [14]. A single-step synthetic protocol with an easily controllable post-treatment for the synthesis of the column material is therefore desirable. To tailor the physico-chemical properties of the product, synthetic protocols based on the sol-gel process are widely used including both tetraalkoxysilanes and organically modified silica precursors. Advances in this area of hybrid stationary phases for the application in liquid chromatorgraphy and CEC have been discussed elsewhere [15-18]. The use of organically modified silanes (ormosil) for the synthesis of sol-gel based silica materials as stationary phases offers the additional advantage of increased hydrolytic stability of the Si-C bond. This endows the column materials with practical usability in an extended pH range. The starting mixture of the synthesis typically is a mixture of a tetraalkoxysilane which is co-polymerised with the organically modified precursor (e.g., an alkyl-trialkoxysilane). A restriction of this route of synthesis is that many of the physico-chemical properties of the resulting product, like surface coverage with the organic functionality or silanol activity, cannot be optimised independently from the morphological parameters as the synthesis is carried out in one step.

A possibility to circumvent this problem is to use a single organically modified silica alkoxide as precursor. Precursors used for this route of synthesis are methyl trialkoxysilanes, either

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methyltrimethoxysilane (MTMS) [19-23] or methyltriethoxysilane (MTES) [24-26]. Monolithic stationary phases produced by the sol-gel process from MTMS have already elicited some interest: first monolithic stationary phases based on this precursor are reported to possess a chromatographic efficiency of up to 100 000 plates/m [27]. The advantage of using a single organic-inorganic hybrid precursor is that the bulk material has the same properties as the surface, which means that even when hydrolytic attack of the silica material takes place, the new surface produced is chemically similar to the previous one, and thus only a marginal deterioration of the separation performance over time can be expected. Other properties materials based on MTMS possess are enhanced stability at high pH values, which should be particularly advantageous for the separation of basic compounds, and an extremely hydrophobic surface with a contacting angle towards water of 150° [28, 29].

The work conducted uses a synthetic protocol based on MTMS as sole precursor and acid catalysis. Some characteristics of this route of synthesis shall be highlighted in the following paragraphs. The mechanism of how the particular stationary phase morphology is created in the particular synthetic protocol has been extensively investigated. It is based on phase separation, which occurs in both organic and inorganic polycondensation reactions has been extensively investigated. Phase separation, or in the case of purely acidic catalysed reactions more specifically, spinodal decomposition, starts from an initially homogeneous system. The polycondensation reaction causes this homogeneous mixture to move into a heterogeneous (two phase) region, usually fast enough to prevent a nucleation growth process. Small fluctuations in the composition induce at this point the spinodal decomposition of this homogeneous system into a two phase system. The concentration gradient between these phases will increase until equilibrium is reached. From a morphological point of view the two phases form at a certain point a bicontinuous structure, which will, as the phase separation goes on, coarsen until one phase will form isolated spheres in a matrix built by the other phase. [30, 31]

As the phase separation mechanism relies on a mass transfer of second phase components against a concentration gradient, it will come to a stop at the point where the viscosity becomes too large to allow mass transfer e.g. at the sol-gel transition. Gel morphology relies therefore on the relative kinetics of two concurrent reactions, the phase separation mechanism and the polycondensation reaction. One phase consists of siloxane polymers which after gelation will form the xerogel, the other phase is poor in silica species and will form the pore network of the resulting material. Parameters influencing one or both reactions will therefore

influence the morphology of the xerogel and pore network and can as a consequence be used to tailor the material in this domain to the needs of chromatography.

Work conducted with MTMS as precursor has shown that this system exhibits a higher tendency for spinodal decomposition than systems with tetraalkoxysilanes [32-34] or the recently investigated bridged organic modified precursors like 1,2 bis(trimethoxysilyl)ethane [35]. This is caused by its reduced compatibility with polar solvents. In synthesis with MTMS as precursor, hydrophilic polymers like polyethylene oxide (PEO) do not have to be added to induce the phase separation as is necessary for systems with tetraalkoxysilanes [31]. An observation often made with acid catalysed sol-gel synthesis is that macroscopic phase separation or the formation of cyclic or cubic structures which do no polymerise further can prevent the emergence of a macroscopic bicontinuous structure [19, 24-26, 36]. For this reason, synthesis has to be carried out at pH values near 1 to obtain a monolith with bicontinuous macroscopic morphology [34]. Specific surface areas are also rather low (normally too low to be of use for chromatographic applications) in synthetic systems which solely use MTMS as precursor. One explanation is that a maximum of three of the four valences of a silicon atom can establish bonds to other (organo-)silica tetrahedrons. This leads to a very flexible intermediate network which can arrange itself in a thermodynamically stable manner with a minimum specific surface area [37-39]. This observation is not made in systems using basic catalysed condensation which promotes a nucleation growth phase separation. An advantage for acid catalysed condensation is that the silica phase possesses an excellent wetting behaviour towards silica surfaces due to this flexibility of the intermediate state which leads to an excellent adhesion to surfaces [40-43]. A protocol using MTMS as sole precursor has been used in the following studies. Hydrolysis and polycondensation have both been catalysed with nitric acid. They have been conducted to investigate possibilities to control the macroscopic as well as the microscopic morphology of MTMS based capillary columns using a single-step synthesis.

6.2 Materials and methods

Instrumentation and material

A model Poly 15 VarioMag magnetic stirrer (H + P Labortechnik; Oberschleißheim, Germany) was used for mixing the reaction mixtures. A model 5890 gas chromatograph (Hewlett-Packard; Avendale, PA, USA) was used as a programmable oven for gelation under controlled conditions. Non-deactivated fused silica capillaries with 530 µm i.d. were purchased from Agilent Technologies (Palo Alto, CA, USA). Single use syringes (polypropylene) were purchased from Wagner & Munz (Vienna, Austria). Female Luer to female 10-32 adapters, 10-32 connectors for 1.5875 mm tubes, and 1.5875 mm O.D. 685 µm I.D. capillary sleeves were purchased from Upchurch Scientific (Oak Harbour, WA, USA).

Chemicals and buffers

Methyltrimethoxysilane (MTMS) purum, methanol analytical-reagent grade, ethanol absolute, 2-propanol analytical-reagent grade., and nitric acid 69% p.a. were obtained from Sigma-Aldrich (Vienna, Austria). Distilled water was produced in-house.

Column preparation

Fused silica capillaries were used without pre-treatment as this proved not necessary under the applied conditions. The reaction mixture was stirred in polypropylene vessels at room temperature for approximately 2 minutes. If not stated otherwise in the results section, the following volumes of chemicals were mixed: 8 ml MTMS, 4 ml methanol, and 2 ml 1 N nitric acid (molaric ratios of MTMS : MeOH : H_2O : HNO_3 = 0.056 : 0.99 : 0.11 : 0.002). The capillaries were filled with the use of single-use syringes, sealed with parafilm and then put in vertical position into the GC oven where gelation took place over night. Gelation temperature was 40°C if not stated differently in the results section. After gelation the capillaries were rinsed with methanol and then dried at 40°C. Parallel to the preparation of columns in capillary format, larger quantities of the materials were synthesised in single-use syringes with analogous post-treatment to be later used for the assessment of surface area by nitrogen adsorption measurement.

Characterisation of porosity

The microscopic morphology was observed using a JSM 6400 electron microscope instrument (JEOL; Tokyo, Japan) in the secondary electron mode. Short column sections were sputtered with gold for 2 min at 10 mA and 15 kV acceleration voltage prior to the measurements as the bulk material is not conductive. The morphology was characterised by average pore diameter and average skeleton diameter which were obtained by measuring the length of 20 of each of the respective features in the pictures obtained by SEM with the SemAfore software.

Nitrogen adsorption measurements were conducted on an ASAP 2010 instrument (Micromeritics; Norcross, GA, USA) from the large batches synthesised in the single-use syringes. The materials were vacuum dried at 60 °C for 5 hours. Nitrogen adsorption was carried out at 77 K. The adsorption isotherms were evaluated with the standard software delivered with the instrument, specific surface area was determined by the t-plot method, pore size distribution was calculated using the BJH method developed by Barrett, Joyner and Halenda.

6.3 Results and discussion

Variation of the methanol/MTMS ratio

One parameter to be investigated was the ratio of methanol added to the reaction mixture. Methanol has to be used as solvent for the synthesis because the rather apolar precursor would initially not dissolve in the aqueous phase. At the same time, the solvent acts as porogen for the formation of an appropriate macropore structure.

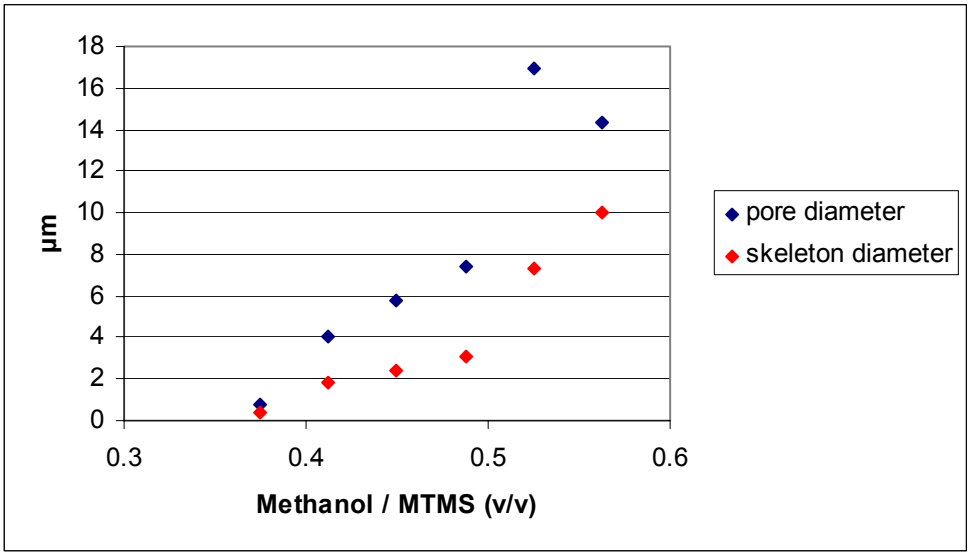
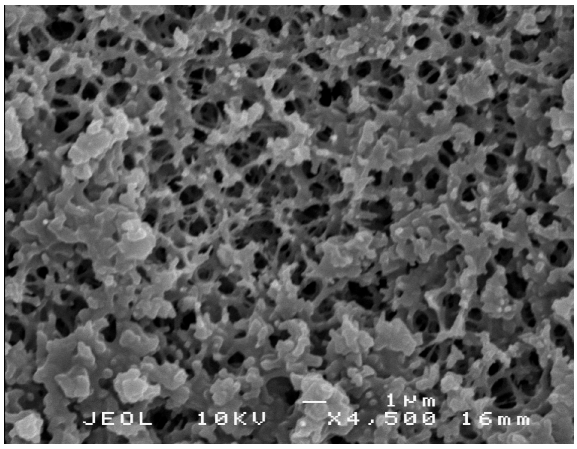


Fig. 6.1: Dependence of the average pore and skeleton diameter on the methanol/ precursor ratio. Experimental conditions are given in section 6.2

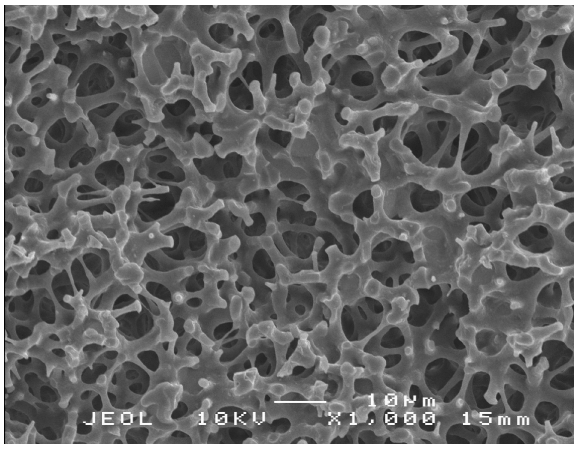
The results for average pore and skeleton diameters are presented in figure 6.1. They show the enormous importance of this parameter: Although the solvent to precursor ratio is only varied within a rather small range from 0.38 to 0.58, both the dimensions of pore and skeleton diameters vary approximately by a factor of 20.

Examples of the morphology are given in 6.2. It may be noticed that for the point with the highest fraction of methanol in the reaction mixture the pore diameter decreases compared to the material adjacent in composition. This effect is caused by macroscopic phase separation. An example is also presented in figure 6.2, leading to a densification of the phase with the siloxane skeleton. A higher fraction of methanol in the reaction mixture results in a higher dilution of hydrolysed precursor molecules and thus in a slowing-down of the

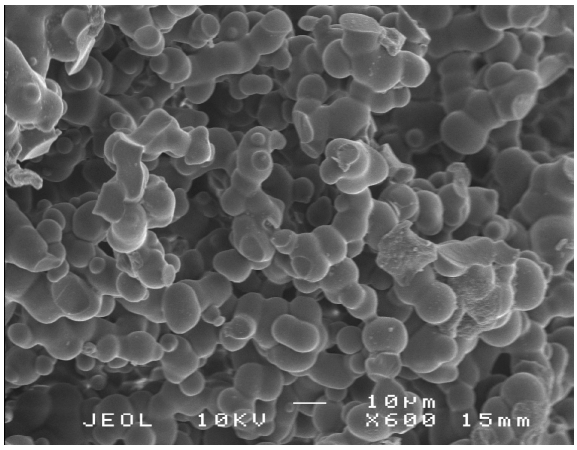
polycondensation kinetics. The phase separation mechanism is also affected by the higher dilution as the point of saturation of the solvent with oligo- and polymers is reached later. The effect on the phase separation seems to be smaller than the effect on the condensation as the increase of pore and skeleton diameters at higher contents of methanol in the initial reaction mixture indicates.



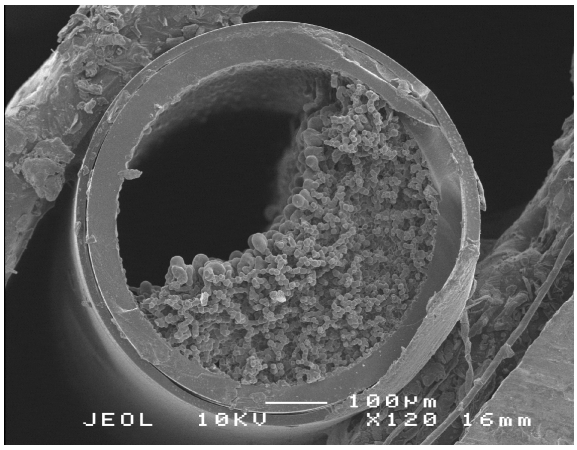
Cross section of the material with methanol / MTMS ratio (v/v) = 0.38.



Cross section of the material with 0.45 methanol / MTMS ratio (v/v) = 0.45.



Cross section of the material with methanol / MTMS ratio (v/v) = 0.56.



Cross section of the material with methanol / MTMS ratio (v/v) = 0.56. This material was synthesized in horizontal position to be able to observe macroscopic decomposition.

Fig. 6.2: SEM micrographs of materials in the series where the MTMS / MeOH ratio was varied. Skeleton diameters have been taken from cylindrical bridging structures and from the connection point of the spheroid structures, respectively.

Results from the nitrogen adsorption measurements presented in table 6.1 show that only materials with relatively small ratios of methanol to MTMS – that is, materials with comparatively small macroscopic structures – possess a considerable specific surface area. Materials with morphological characteristics above a certain size (2.4 μm skeleton diameter and 5.7 μm pore diameter in this series) do not possess a specific surface large enough to be measurable with the used instrument.

Tab. 6.1: Dependence of the specific surface area on the methanol/precursor ratio.
Experimental conditions are given in section 6.2

Methanol/ precursor ratio (v/v)	Specific surface area (m^2/g)
0.38	289
0.45	5
0.56	<LOD*

*estimated with 2 m^2/g using 200 mg of material

This observation was also made in other series of experiments. The current explanation for this observation is that the polymers formed by the polycondensation with MTMS as precursor are more flexible than those of tetraalkoxy precursors because they are less interlinked due to only three possible siloxane bridges per silica. The use of acids as catalysts leads furthermore to chainlike oligomers and polymers opposed to spherical structures when using basic catalysis. When the polycondensation continues during phase separation it is comprehensive that the flexible chainlike polymers can arrange themselves in the siloxane rich phase with a minimum of internal surface e.g. in a thermodynamically favourable conformation, whereas condensation of spheres as it happens when using basic catalysis leads inherently to pores with sizes in the range of these initial spheres. At the onset of gelation, both phases contain are composed of siloxane polymers and solvent but with different concentrations of these compounds. For the silica rich phase this means that a certain amount of solvent is still present at the beginning of the phase separation. This amount will consequently become smaller as the phase separation continues. The solvent present at the point of gelation in the siloxane rich phase acts as template for micropores in the xerogel structure. If the gelation and thus the stop of the phase separation occurs at a late stage this leads consequently to a smaller amount of accessible micropores. This explains the

observation that a smaller specific surface area is observed for materials with larger pore- and skeleton diameters.. In this series 4 m^2/g specific surface area was observed for the material with 2.4 µm average skeleton diameter and 5.7 µm pore diameter, materials with larger morphological characteristics do not possess specific surface area above the limit of detection.

Variation of the catalyst concentration

The next parameter that was studied for its influence on the macroscopic morphology of the synthesised monolithic material was the concentration of nitric acid used as catalyst. In this set of experiments the concentration of the nitric acid was varied from 0.8 to 1.2 mol/l.

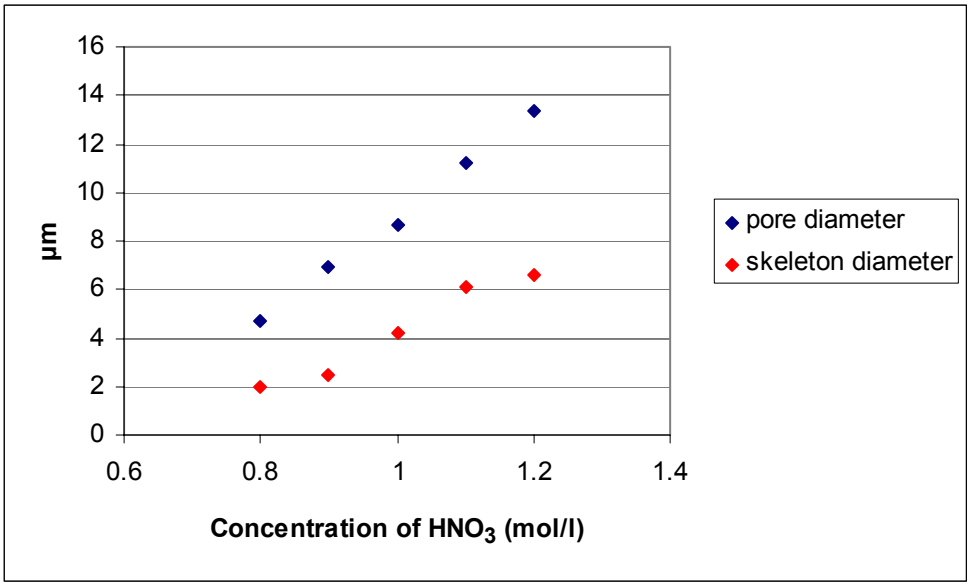


Fig. 6.3: Dependence of the average pore and skeleton diameter on the catalyst (HNO_3) concentration. Experimental conditions are given in section 6.2

Results from the evaluation of SEM pictures are presented in figure 6.3. It is seen that the medium pore and skeleton diameters of the synthesised materials increase by a factor of 3 from the material with the smallest to the material with the largest domain diameters with increasing catalyst concentration. Under the conditions used for the synthesis, the hydrolysis rate should increase with increasing concentration of catalyst. The same should be valid for the polycondensation reaction. The phase separation in the reaction mixture can be assumed to be relatively unaffected by the concentration of acid. In few instances the formation of cyclic polysiloxanes was observed at lower concentration [19, 24-26, 36]. The results

obtained here seem to indicate that indeed the phase separation starts earlier as average pore and skeleton sizes increase with the catalyst concentration. If the catalyst concentration would only affect the polycondensation reaction, both the skeleton and pore diameters should decrease in size with an increasing concentration as a faster condensation means that the two phase system would be frozen at an earlier stage of the separation.

Tab. 6.2: Dependence of the specific surface area on the catalyst (aq. HNO_3) concentration. Experimental conditions are given in section 6.2

Catalyst concentration (mol/l)	Specific surface area (m^2/g)
0.8	140
1.0	<LOD
1.2	<LOD

According to the nitrogen adsorption measurements (results shown in table 6.2) only the material produced with the lowest concentration of catalyst exhibits a considerable surface area. This is also the material with the smallest dimensions of the morphological features. The reasons for this observation are the same as discussed in previous paragraphs.

Variation of the gelation temperature

In this set of experiments the temperature at which gelation was taking place was varied between 37 and 52°C while keeping all other parameters of the synthesis constant. As the results presented in figure 6.4 show, domain dimensions become smaller at higher gelation temperatures. The average skeleton diameters vary by a factor of 7, the average pore diameters vary by a factor of 3 in the investigated range. For the material with the largest skeleton diameters macroscopic phase separation already occurs (see figure 6.2), therefore the average pore diameter is smaller than expected.

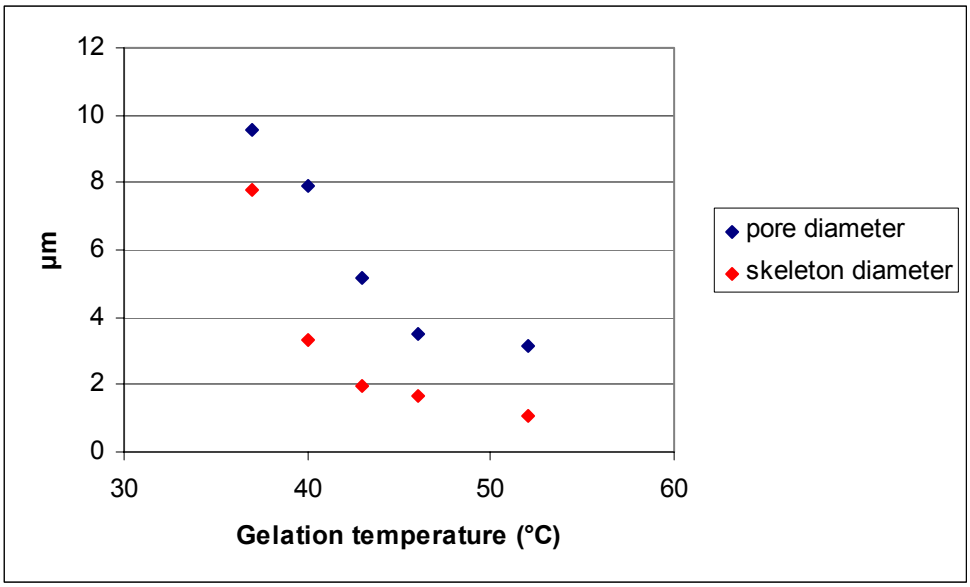


Fig. 6.4: Dependence of the average pore and skeleton diameter on the gelation temperature. Experimental conditions are given in section 6.2

Again the phase separation mechanism is influenced by the variation of synthesis parameters. One possible reason for this dependence is the relative increase of the gelation kinetics compared to phase separation kinetics. A higher reaction temperature is normally associated with a higher solubility of the emerging silica polymers in the reaction mixture, resulting in a later onset of the phase separation.

Table 6.3: Dependence of the specific surface area on the gelation temperature. Experimental conditions are given in section 2.3 and 2.4

Gelation temperature (°C)	Specific surface area (m^2/g)
40	<LOD
47	4
53	269

Nitrogen adsorption data presented in table 6.3 shows as in the previously discussed series that particularly those materials have a large specific surface area which exhibit comparatively small pore and skeleton diameters.

Substitution of methanol with ethanol

In the next series of experiments, the composition of the solvent was varied. Up to 20% (v/v) of methanol (used as co-solvent and as porogen) were substituted with ethanol. Results for the average pore- and skeleton diameter are presented in figure 6.5.

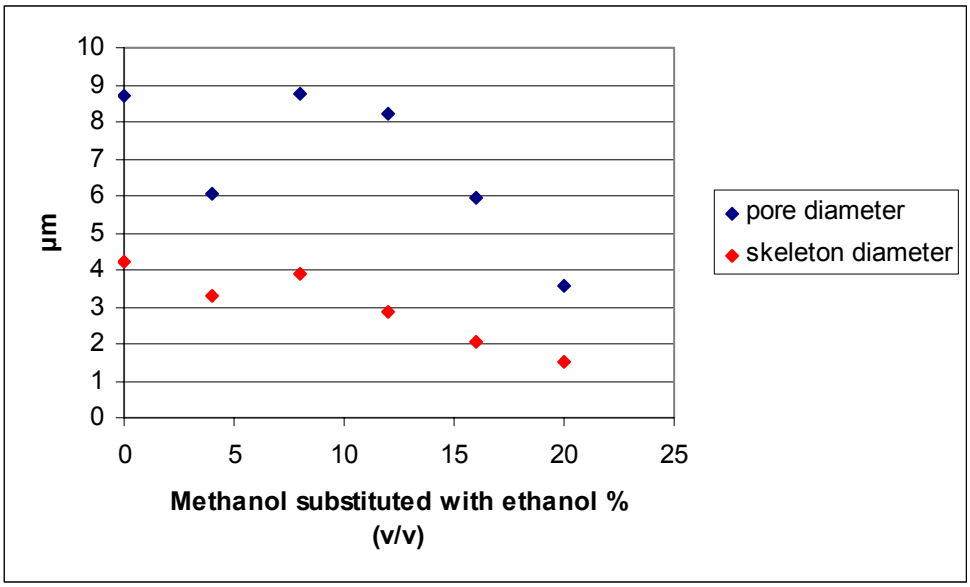


Fig. 6.5: Dependence of the average pore and skeleton diameter on the volume fraction of methanol substituted by ethanol (% v/v). Experimental conditions are given in section 6.2

The substitution of less than 8% of methanol by ethanol seems to have virtually no impact on the average pore and skeleton diameters. However, when more than 8% of methanol were substituted in the reaction mixture, the materials show a clear trend of decreasing pore and skeleton diameters with increasing amount of ethanol. Ethanol possesses a smaller dielectric constant than methanol (24.55 *versus* 32.66 at 25°C [44]), which means that the reaction mixture should be less polar with larger amounts of ethanol substituting methanol. As the solubility of the emerging oligomers should be higher in a less polar mixture, the phase separation should also be delayed in relation to the polycondensation reaction. This is in agreement with the obtained results as smaller pore and skeleton diameters mean that the two phase system is frozen by gelation at an earlier stage.

Tab. 6.4: Dependence of the specific surface area on the volume fraction of methanol substituted with ethanol (% v/v). Experimental conditions are given in section 6.2

Volume fraction of ethanol in methanol (% v/v)	Specific surface area (m^2/g)
0	<LOD
4	<LOD
12	<LOD
20	320

Nitrogen adsorption measurements are presented in table 6.4 and demonstrate that a only the monolithic material with the smallest average pore and skeleton diameter has a considerable specific surface area, a result observed throughout all previous series.

Substitution of methanol with 2-propanol

An analogous set of experiments was conducted where 2-propanol is used to substitute methanol in the reaction mixture. Since the difference in polarity between 2-propanol and methanol is larger than between ethanol and methanol (dielectric constant of isopropanol: 19.92 at 25°C [44]), the effect on the resulting morphology should also be larger.

Results of the average pore and skeleton diameter obtained from SEM measurements are presented in figure 6.6. They show a strong dependence of these parameters on the amount of methanol substituted by 2-propanol. Pore and skeleton diameter vary by a factor of 20 in these experiments. Again, the influence on the average pore and skeleton diameters can be explained by an increasing solubility of the emerging ormosil polymers in the reaction mixture due to a reduced polarity of the reaction mixture, resulting in a delay of the phase separation.

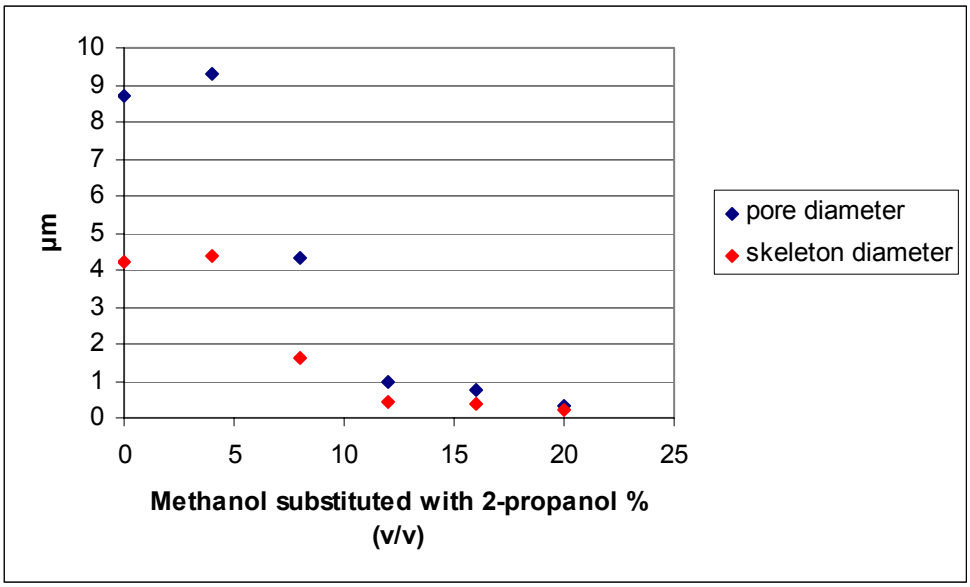


Fig. 6.6: Dependence of the average pore and skeleton diameter on the volume fraction of methanol substituted by 2-propanol (% v/v). Experimental conditions are given in section 6.2

Nitrogen adsorption measurements show already at a level of 4% substitution an increase in specific surface area from below the limit of detection to 117 m^2/g, whereas the macroscopic morphology is virtually the same as for the material without substitution.

Tab. 6.5: Dependence of the specific surface area on the volume fraction of methanol substituted with 2-propanol (% v/v). Experimental conditions are given in section 2.3 and 2.4

Volume fraction of 2-propanol in methanol (% v/v)	Specific surface area (m^2/g)
0	<LOD
4	117
12	334
20	321

The considerable surface area resulting already at very small amounts of 2-propanol added to the reaction mixture indicates that an additional effect becomes important when using 2-propanol instead of ethanol. It seems that 2-propanol acts as a kind of solubility promotor in this case, lowering the surface energy between the two phases and therefore enabling a larger

specific surface to be formed. An effect of the dielectric constant of the solvent composition on the specific surface area was reported for the synthesis of magnesia and zirkonia xerogels using sol-gel synthesis with alkoxide precursors [45].

Repeatability of the synthetic protocol

To study the repeatability of the synthesis five materials with identical synthetic parameters have been produced. The protocol applied was with 8% of methanol substituted with 2-propanol. As the results presented in table 6.6 show, average pore and skeleton diameters of all materials are well within standard deviation values of each other.

Tab. 6.6: Results of the repeatability study. Experimental parameters are given in section 6.2 and 6.3. Standard deviation relies to 15 random length measurements of the according parameter in obtained SEM pictures. Materials 3-5 have been produced in one single batch whereas materials 1 and 2 have been produced in individual batches.

	Average skeleton diameter (μm)	Average pore diameter (μm)
Material 1	1.64 ± 0.62	4.34 ± 1.32
Material 2	1.60 ± 0.65	4.36 ± 1.33
Material 3	1.73 ± 0.47	4.98 ± 1.71
Material 4	2.06 ± 0.69	4.48 ± 1.11
Material 5	1.68 ± 0.45	4.86 ± 1.68

Chromatographic testing

First chromatographic tests have been conducted to demonstrate the feasibility of produced capillary columns for their use in chromatography. One column was installed in a Hewlett-Packard 1090 HPLC system with a UV/VIS detection system. The used capillary column had approx. 180 mm length and an inner diameter of 0.53 mm. The stationary phase had 1.7 µm average skeleton diameter and 4.9 µm average pore diameter. The separation was carried out under isocratic conditions with 70% acetonitrile in water at a flow of 20 µl / min (corresponds to 71 mm / min linear flow). It may be noted that the installed detector flowcell has a volume of 8 µl and therefore contributes to a large extent to the observed peakwidths. The column exhibited a backpressure of 6 bar under these conditions and 14 bar with 50 µl / min (177.5 mm / min). The results as shown in figure 6.7 demonstrate that the used column shows indeed retention for the investigated analytes. The overall performance of the column is rather poor under these conditions as can be seen in the poor separation of the chosen alkylbenzenes. It may be noted that the polycyclic aromatic compounds are overall retained less than the alkylbenzenes. Their retention time is also very close. This behaviour is interpreted to be caused by size exclusion phenomena occurring for these substances, micropores might not be accessible for these compounds. Current synthetic work aims at producing materials with larger pore diameters in the micropore range. The low specific backpressure observed in these experiments leaves the possibility to operate under higher flow rates and to use columns with smaller pore and skeleton diameters.

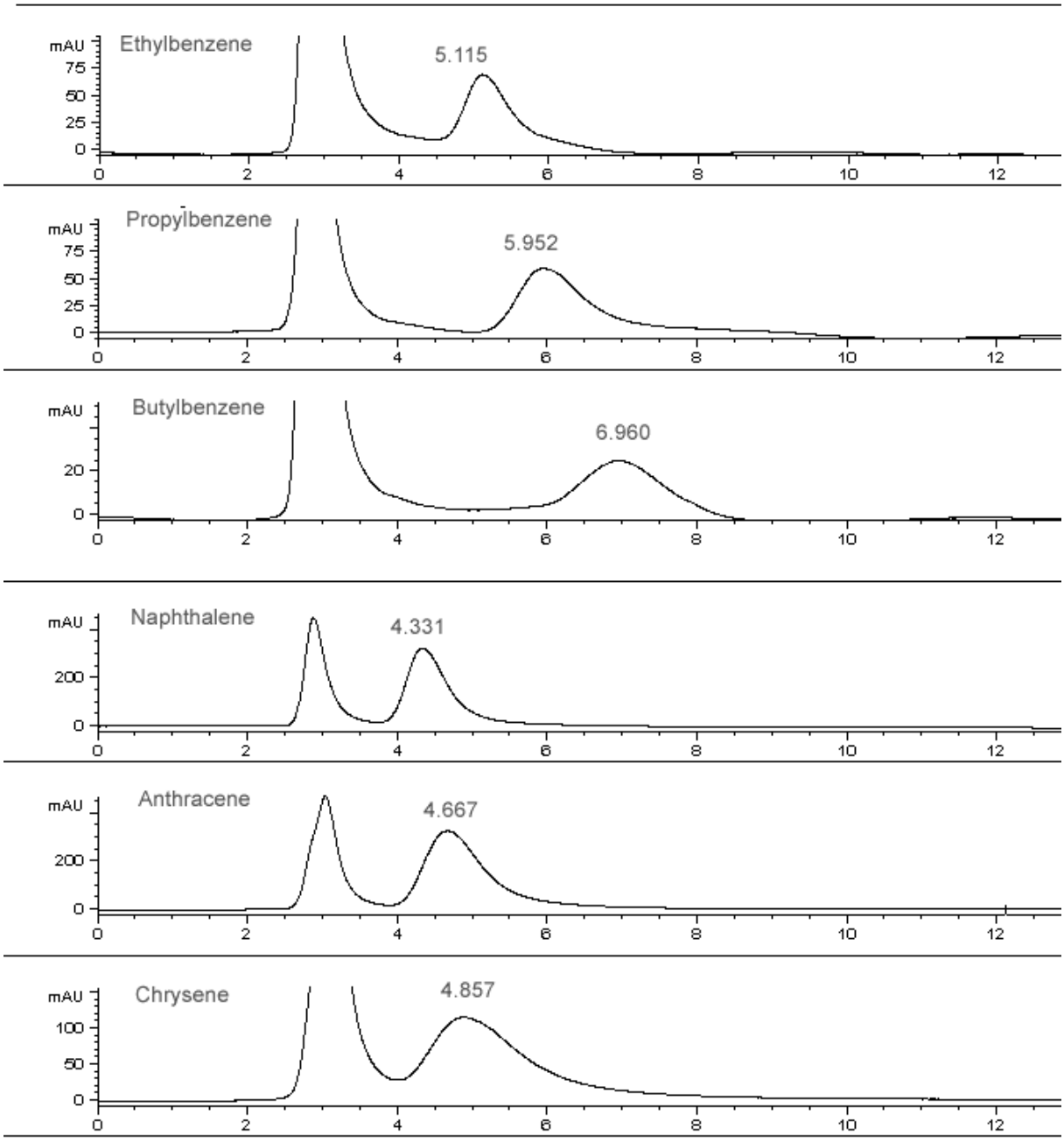


Fig. 6.7: Single substance chromatograms with deadtime marker from top to bottom: ethylbenzene, propylbenzene, butylbenzene, naphthalene, anthracene and chrysene, the injected amount were approx. 30 ng per substance. For these experiments a Hewlett-Packard 1090 HPLC with UV/VIS detection was used. The separation was carried out under isocratic conditions with 70% acetonitrile in water at a flow of 20 µl / min (corresponds to 71 mm / min linear flow). Alkylbenzenes and naphthalene were detected at 210 nm, anthracene at 254 nm and chrysene at 270 nm wavelength.

6.4 Conclusion

The sol-gel method was employed for the production of monolithic materials directly in capillaries with MTMS as sole precursor, which may find use as stationary phases for chromatography. The mechanism leading to the formation of the macroscopic morphology is polymerisation-induced phase separation. The influence of various experimental parameters on the morphology of the products, characterised by the average pore- and skeleton diameter in the macropore range and the specific surface area has been studied. Experimental results indicate that already small variations of any of the investigated parameters influence the macroscopic morphology of the resulting materials, which means that all of them are suited and can be employed to tailor the macroscopic morphological features for chromatography. Specific surface areas useful for chromatographic applications were observed for materials with comparatively small pore- and skeleton diameters (depending on the series 2 - 2.5 μm skeleton diameter and 4.5 – 5.7 μm pore diameter respectively). An exception to this behaviour occurred in the series where 2-propanol was used to substitute methanol. This indicates that 2-propanol can be used as porogen for micropores. Further experiments will be conducted in order to test the feasibility of other chemicals as porogens in the micropore range. The creation of mesopores (which at the current time are practically unavailable in the materials described here) and their tailoring to the desired dimensions will be the next step on the way to have complete control over the morphology of capillary monoliths derived from MTMS in all domains.

6.5 References

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7. Chromatographic characterisation of synthesised MTMS based columns

7.1 Introduction

Chromatographic testing is an important step for the characterisation and quality control of produced columns. To obtain direct evidence on hydrophobic properties, silanophilic properties, shape selectivity, size exclusion effect, and column efficiency, several testing protocols have been developed as discussed in chapter 3 of this thesis. Preliminary experiments on MTMS based materials have shown principally hydrophobic characteristics, however special retentive properties can be expected from these columns. The surface exhibits a high density of methyl groups, which can not be achieved by surface modification techniques; retention itself should be caused by adsorption on the surface opposed to distribution in phases based on longer alkyl chains. Little is known about the silanol activity of MTMS based columns. No surface modification or ageing was employed to minimise the occurrence of silanol groups Methyl groups can probably not sterically hinder interaction with adjacent silanol groups. As retention is probably based on adsorption, shape selectivity could occur due to an adsorption based mechanism, whereas retention for commercial octadecyl products follows the partition mechanism for small analytes.

7.2 Materials and methods

HPLC testing parameters

Synthesised capillary columns had an I.D. of 0.53 mm and were installed with the help of a capillary sleeve with an I.D. of 0.685 mm in a standard HP 1090 system. For detection an in-built UV-DAD was used with a standard flow cell of 1.7 µl volume. All measurements were performed under isocratic conditions; the volumetric flow was varied between 15 µl/min and 50 µl/min. Injected amounts of analytes were in each case around 20 ng per substance. This range of concentration was chosen to not overload the capilalry columns; detection was due to excessive peak broadening not possible for peaks with retention factors over 30. Details on the synthetic procedures can be seen in the materials and methods section of chapter 5 and 6.

Chromatographically tested columns

Tab. 7.1: Reaction conditions and morphological characteristics of monolithic materials employed for chromatographic tests in this chapter.

	Reaction mixture [ml]				**Gelation Temperature [°C]**	**Morphology [µm]**	
	MTMS	MeOH	Additive	1N HNO_3		skeleton	pore
188B	8	3.6	0.4 2-PrPH	2	40	2.06	4.48
189	8	3.4	0.6 2-PrOH	2	40	1.01	2.85
191	8	4	-	2	52	2.16	5.06
192	8	4.5	-	2	40	6.75	14.42
194	8	4	-	2	49	2.22	5.39
195A	8	5	-	2	40	8.57	11.88
195B	8	5	-	2	40	n.d.	n.d.
196A	8	4	-	2	49	5.30	8.47
198	8	4	0.1 Brij	2	49	1.86	5.30
201	8	4	0.1 Brij	2	49	1.83	5.38
203	8	4	-	2	49 Ageing in 1 N NH_4OH at RT for 48h	2.15	5.82

Evaluation of chromatographic data

Peaks were evaluated by integration using the ChemStation software. For the determination of retention factors, the system dead time (= t_{sys}) was determined by installing a short PEEK capillary instead of the column. The injection of thiourea as deadtime marker with the installed column gave the column dead time (= t_0). So t_0-t_{sys} represents the time a volume fraction needs to pass the column. The deadvolume of the HPLC system (determined with 25 µl) can be neglected in comparison to the column deadvolume in wide and narrow bore format, however it has to be considered for separations with capillary columns (typical volumes were around 35 µl). Retention of a solute was calculated as time difference between solute and deadtime marker t_R-t_0 (system deadtime affects both and is eliminated).

Retention factors were therefore calculated according to equation 7.1

$$k' = (t_R - t_0)/(t_0\text{-}t_{sys}). \qquad \text{eq. 7.1}$$

7.3 Physical characteristics

Test columns employed were optically controlled for channelling effects after installing them into the chromatograph; capillaries are transparent enough to follow from outside how the solvent front wets the dried stationary phase. Channelling was never observed, the liquid front showed longitudinal dispersion of maximal 2 mm at column lengths of about 150 mm. Several physical characteristics could be determined from chromatographic data. Flow-through pore volume (= V_{pore}) was calculated from column dead time (=t_0-t_{sys}) and volumetric flowrate (=v) (eq. 7.1). Capillary volume (= V_{cap}) was calculated assuming a homogenous cylindrical shape (eq. 7.2); porosity was defined as ratio between these values (eq. 7.3). Linear flow rates (= u) were calculated by dividing column length by column deadtime according to equation 7.4

$$V_{pore} = (t_0\text{-}t_{sys})*v \qquad \text{eq. 7.2}$$

$$V_{cap} = r_{cap}^2\pi*L \qquad \text{eq. 7.3}$$

$$\text{Porosity} = V_{pore}/V_{cap} \qquad \text{eq. 7.4}$$

$$u = L/(t_0\text{-}t_{sys}) \qquad \text{eq. 7.5}$$

To correlate pore diameters determined from SEM micrographs with flow through properties, pressure drop was determined at different flow rates. For this purpose, 100% methanol was used as mobile phase at different flow rates at 40°C; pressure drop caused by monolithic columns was determined by subtracting observed pressure values for the empty system from observed values with installed columns. Accuracy of the measuring of the actual pressure was limited by the 1090 instrument with 1 bar. Linear flow was calculated according to Eq. 7.5. Pressure drop was normalised to 100 mm columns for better comparison between the columns.

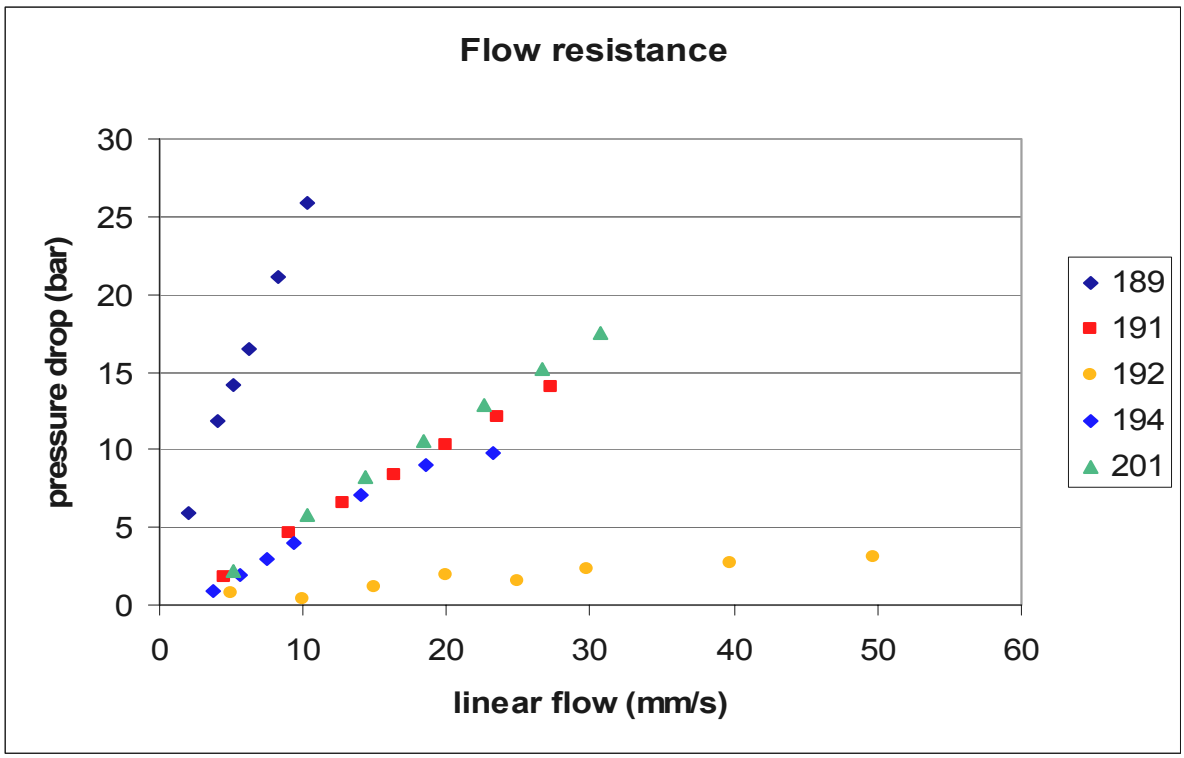


Fig. 7.1: Observed pressure drop of monolithic columns versus linear flow rate (normalised to 100 mm length of a 0.53 mm I.D. column). Series numbers refer to the column code.

An outlier is present at the highest employed flowrate for material 194, low observed backpressures in the range of the accuracy of the instrument result in a high relative deviation for material 192. Reynolds numbers were determined for all experiments according to eq. 7.6, measured average pore radii were used as characteristic length. Values for viscosity and density of methanol at 40°C were obtained from www.efunda.com; $\eta = 4.5\ 10^{-4}$ Pa*s, $\rho = 774$

kg/m^3. As observed Reynolds numbers were below 1000, laminar flow conditions were present in all cases. Pore diameters were calculated according to the Darcy-Weisbach equation which described the pressure drop in capillaries as shown in eq. 7.7. and eq. 7.8. It was used instead of the Carman-Kozeny equation which describes the pressure drop in solid beds, because porosity values determined by chromatography and the assumption of perfectly tubular capillaries seemed not reliable enough. The term u/Δp was obtained for each column from linear regression of the plot as presented in figure 7.1.

$$\mathrm{Re} = \frac{2\rho * u * r}{\eta} \qquad \text{eq. 7.6}$$

$$\Delta p = \frac{64}{\mathrm{Re}} * \frac{l}{d} * \frac{\rho * u^2}{2} \qquad \text{eq. 7.7}$$

$$d = \sqrt{\frac{u}{\Delta p} * 32 * l * \eta} \qquad \text{eq. 7.8}$$

Re ..... Reynolds number
η ..... viscosity
ρ ..... density
l ..... column length
d = 2r .. pore diameter and radius
u ..... linear flow velocity
Δp ..... pressure drop

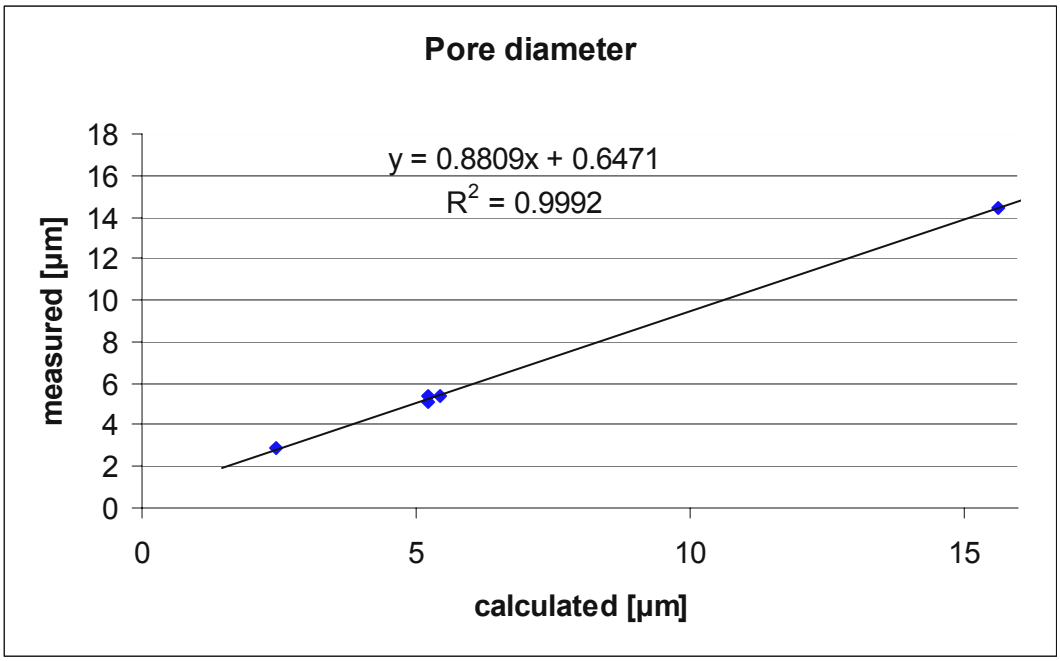


Fig. 7.2: Correlation of pore diameters calculated from backpressure experiments and average pore diameters determined from SEM micrographs

As can be seen in figure 7.2, calculated values for pore diameter and measured values of the average pore diameter show a good correlation. It can therefore be assumed that morphology values obtained from the evaluation of cross sections of the capillary column are representative for the whole material. The slope between measured and calculated values is different from 1, mainly because the equation can not be completely applied to solid beds. Tab. 7.2 summarises results for this set of experiments.

Tab. 7.2: Physical properties of materials subject to flow resistance investigations

Material	Porosity	Skeleton diameter µm	Pore diameter (SEM) µm	Pore diameter (calculated) µm
189	72.0%	1.01	2.85	2.46
191	81.6%	2.16	5.06	5.21
192	74.6%	6.75	14.42	15.62
194	79.6%	2.22	5.39	5.43
201	72.3%	1.83	5.40	5.21

7.4 Hydrophobic properties

Hydrophobic interaction is the main source of retention in reversed phase liquid chromatography. It is therefore essential to assess information on this property for synthesised columns. As already stated previously, MTMS based materials exhibit a high hydrophobicity due to the high methyl group density on the surface of the material. The retention mechanism can be expected to rely on adsorption; retention mechanisms for columns with alkyl modification are still under discussion [1-5].

For a comparison of retention mechanisms, properties of octadecyl modified columns shall be discussed at this point. Principally two different types of surface modification are employed which result in different retention characteristics. Monomeric surface modification will lead to a comparatively low density of functional groups, which means that analytes can enter the octadecyl phase with little hindrance as is reflected in shape selectivity factors close to 1 for these phases. The model of solution and partition of the analyte molecules into the alkyl phase will therefore be the main mechanism for retention in the case of monomeric stationary phases.

Polymeric phases have a higher density of functional groups; larger molecules will have problems to penetrate the hydrophobic phase. In the case of larger molecules, the mechanism of retention will be closer to adsorption on a hydrophobic surface.

Concerning MTMS based phases which exhibit superficial methyl groups as separation phase, adsorption can be expected as dominant retention mechanism.

As investigations showed, solvent molecules are also adsorbed on the hydrophobic surface of reversed phase columns, leading to the dynamic formation of a hydrophobic phase on top [6-9]. It is clear that an adsorbed liquid phase will also cause retention by the formation of dynamic distribution equilibrium of an analyte between mobile phase and a more hydrophobic adsorbed liquid phase. Retention is therefore a summation of several stages of absorption and adsorption. Possible mechanisms for the retention of hydrophobic compounds on the methyl rich surface of methyl-silsesquioxane are therefore the solution in an adsorbed solvent phase with adsorption of analyte molecules on the methyl groups of the stationary phase.

Comparison of retention factors

As discussed earlier, retention factors rely basically on two characteristics. The first important property is the relative distribution of the analyte between the stationary phase and the mobile phase. The higher it is on the side of the stationary phase, the longer is the retention time of the analyte. A second factor is the phase volume of the stationary phase. If two stationary phases exhibit the same chemical properties, the main contribution to differences in retention is the difference in accessible surface area. Therefore, retention factors of hydrophobic molecules should reflect a difference in phase volume of stationary phases. The test analytes should not be subject to a size exclusion mechanism as they exhibit roughly the same size.

Preliminary experiments showed retention factors over 30 when conducting experiments according to conditions as employed in chromatographic tests according to Engelhardt; due to sensitivity it was often not possible to detect the resulting peaks. For these reasons it was decided to employ conditions as used in the Tanaka test with a methanol content of 80%.

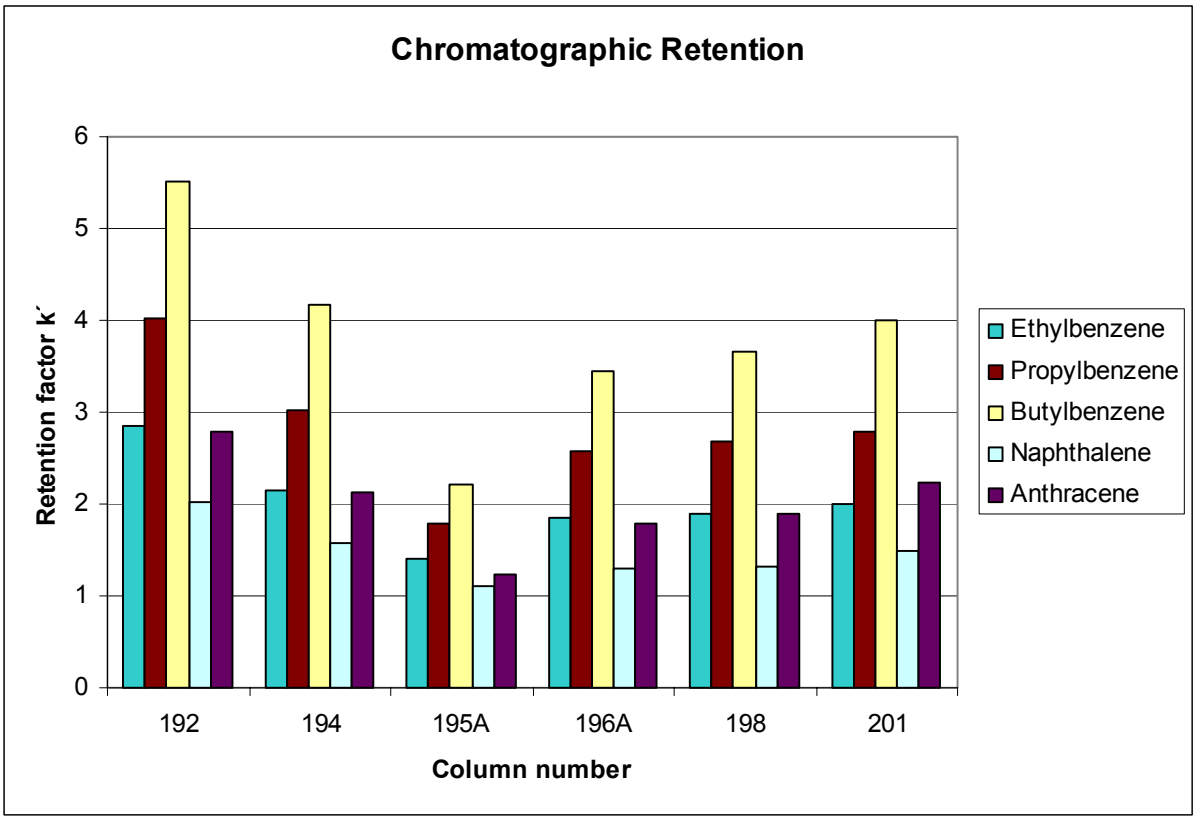


Fig. 7.3: Comparison of retention factors. Employed conditions were as follows: solvent: 80% methanol / 20% water, unbuffered; flow: 20 µl/min volumetric flow. Numbers refer to the coding of employed columns

As mentioned previously, results for retention factors will give differences in the phase volume of the stationary phase which reflects accessible surface area. Employed columns for this set of experiments exhibit significant differences in the macroscopic morphology. Results can be seen in figure 7.3. Materials 194, 198 and 201 are rather similar in their macroscopic morphology. They possess average pore diameters between 5.4 and 5.5 µm, their skeleton size is around 2 µm each. 198 and 201 were produced under identical conditions. Results for the retention factor differ between 5% and 15% depending on the analyte for these columns. Columns 192, 195A and 196A possess completely different morphological characteristics. In their synthesis, gelation occurred at a rather late stage of the phase separation process, leading to coarsened, rounder structures of the silica skeleton. Specific surface area was in all cases below the limit of detection for materials with these characteristics, leading to the conclusion that open micropores did not exist within these materials. Results for retention factors however indicate that column 192, which shows the highest retention for hydrophobic test compounds of all investigated materials, possesses the highest amount of accessible specific surface area. 195A and 196A were produced under identical conditions. Retention factors vary from 15% to 50%, this difference is increasing with molecule size. This size dependent selectivity could be the result of size exclusion selectivity. Results for retention factors demonstrate that an accessible surface area is present in all employed columns. Differences in retention are present between columns which were fabricated under identical conditions. This is in accordance with the results for specific surface area obtained during the optimisation of the synthetic parameters. Although capillaries are dried at controlled temperatures, differences in the permeability of the cross section of the monolithic capillary result in different drying rates. It seems that the preservation of micropores, where most of the specific surface area is present, depends heavily on the conditions during the drying process.

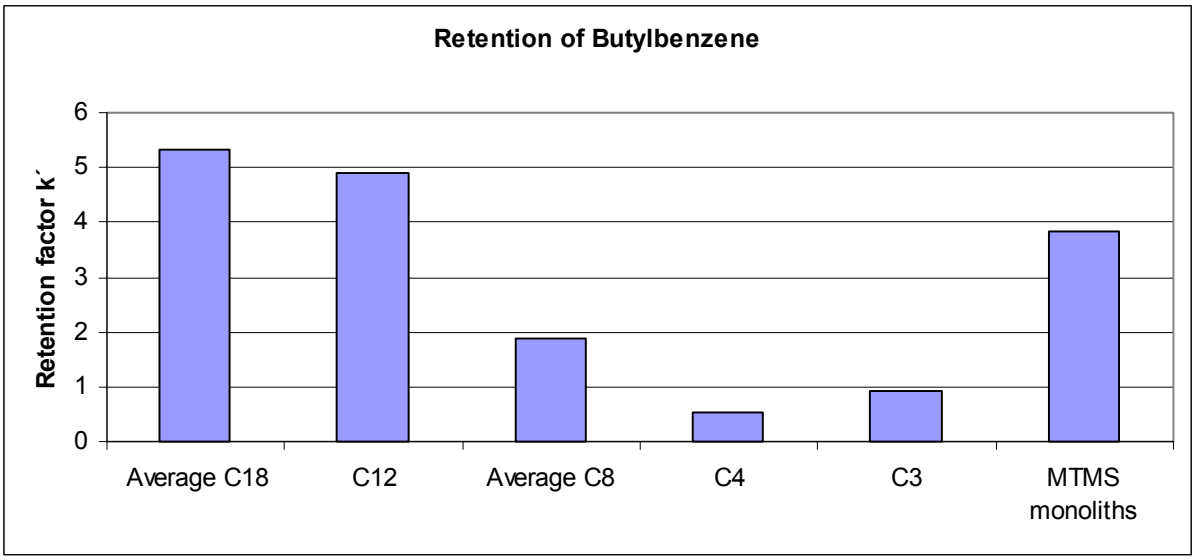


Fig. 7.4: Comparison of average retention factors of butylbenzene with data extracted from literature [10]. Conditions of the experimental data for MTMS monoliths were as follows: solvent: 80% methanol / 20% water unbuffered; flow: 20 µl/min volumetric flow.

A comparison of retention factors of butylbenzene was made with values obtained from a database of commercially available reversed phase columns [10]. The columns to which our MTMS based capillary columns were compared exhibit stationary phases consisting of alkyl chains with different length. As can be seen in the table, retention factors generally decrease with the chain length of the alkyl modification. Neglecting at the moment a difference in specific surface area, and assuming an equal density of functional groups, it is clear that as the chain length decreases the total volume of the reversed phase also decreases. This trend is not true for MTMS columns produced according to our route of synthesis. The application of a hybrid organic-inorganic precursor group leads to a much higher density of functional groups on the surface than what can be achieved with surface modification techniques. Therefore observed average retention factors are higher than observed for the average of commercial C_8 columns. Another factor which has to be considered is that a low density of functional groups combined with small chain lengths will lead to a different surface polarity, as can be seen in the comparison of selectivity factors in the next section.

Comparison of selectivity

The simplest model for retention is the so-called partition model. Depending on their affinity to the stationary phase, a distribution equilibrium between mobile and stationary phase is achieved. Depending on the fraction retained by the stationary phase, analyte molecules are transported on average at a decreased rate through the column. So retention would rely on the distribution coefficient and on the phase volume. Selectivity is defined as ratio between the retention of two analytes. Applying the partition model of retention, it is independent of the phase ratio and relies therefore only on the ratio of distribution coefficients. Selectivity factors are therefore an indication of chemical properties of the retentive phase. Values of the octanol - water distribution coefficient can be used to extrapolate the retention order in the case of alkyl modified columns as in both cases hydrophobic interactions occur with alkyl chains.

Tab. 7.3: Experimental logKow values of employed hydrophobic test compounds obtained from: Syracuse research corporation http://www.syrres.com/esc/physdemo.htm

	ethylbenzene	propylbenzene	butylbenzene	naphthalene	anthracene
logKow	3.15	3.69	4.38	3.30	4.45

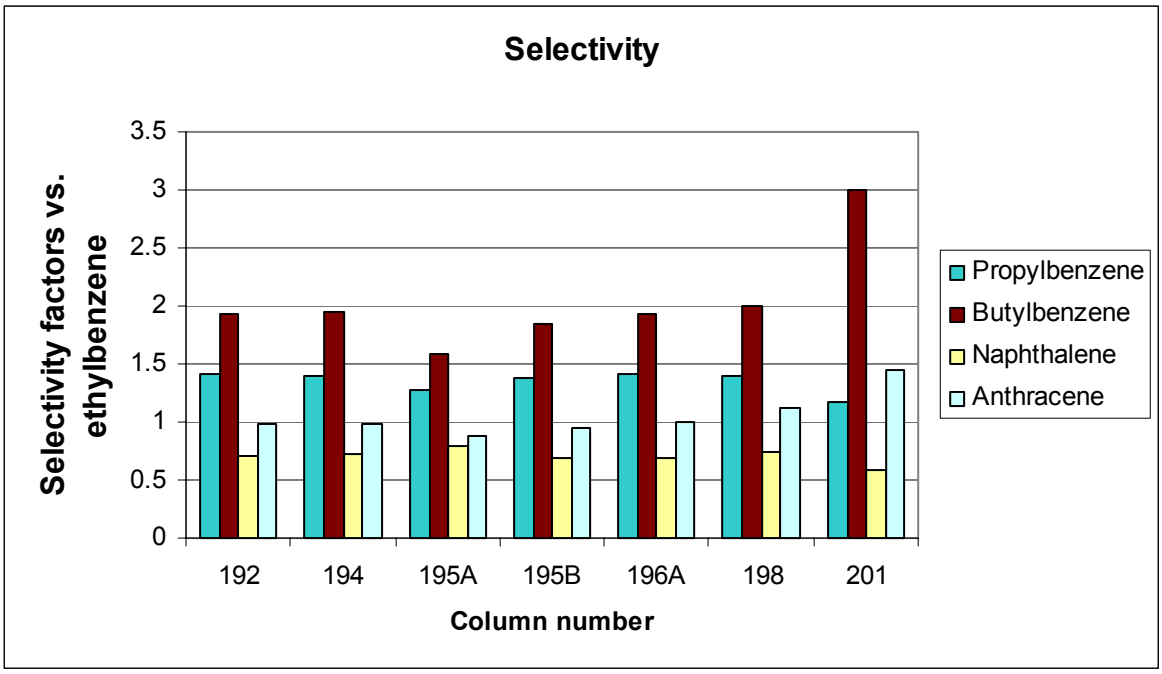


Fig. 7.5: Comparison of selectivity factors versus ethylbenzene. Employed conditions were as follows: solvent: 80% methanol / 20% water unbuffered; flow: 20 µl/min volumetric flow. Numbers refer to the column coding.

To obtain data on the difference in surface chemistry between tested columns, selectivity factors of test analytes versus ethylbenzene were determined as presented in figure 7.5. Selectivity values of propyl- and butylbenzene deviate less than 5% between all columns except 195A, which means that surface properties are very similar for these columns. Regarding 195A, selectivity for propylbenzene differs by 10% and for butylbenzene by 20% which can be seen as significant (standard deviation of k´values was determined with 0.03, which would be 1.5% for butylbenzene). The composition of the surface should be almost identical for all columns, so the difference in selectivity can be attributed to a selectivity which is caused by size exclusion.

When comparing the logKow of the test analytes, naphthalene should have a similar polarity as ethylbenzene, whereas anthracene should be comparable to butylbenzene. As results for the selectivity of naphthalene show, its retention factors are only around 0.7 compared to ethylbenzene. Anthracene which is according to its pKow value more apolar than butylbenzene is only retained in the range of ethylbenzene. This was observed for all tested columns. It seems that the methyl rich surface exhibits a selective retention mechanism for alkyl chains. This retention mechanism is different to the absorption based mechanism

existent in the case of octanol - water distribution or in the case of stationary phases with long alkyl chains as surface modification. As current explanation, superficial methyl groups cause a structured surface with pocket like voids which exhibit an enhanced affinity towards alkyl chains whereas poylaromatic compounds cannot undergo this kind of interaction because of their size and the rigidity of their skeleton. Size exclusion from micropores can be excluded as cause for this selectivity as columns no. 201 and 198, which also show retention for triphenylene and o-terphenyl, exhibit the same selectivity of naphthalene and anthracene versus ethylbenzene as other tested columns, which did not retain these test analytes.

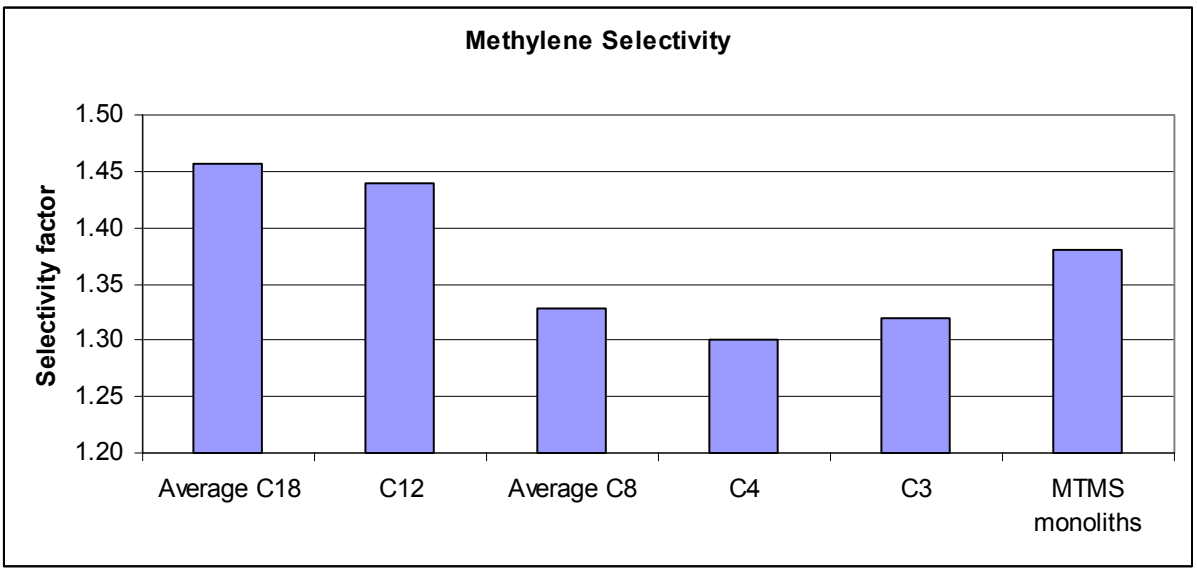


Fig. 7.6: Comparison of average methylene selectivity values with data from literature [10]. Conditions of the experimental data for MTMS monoliths were as follows: solvent: 80% methanol / 20% water unbuffered; flow: 20 µl/min volumetric flow.

The comparison of methylene selectivity between commercially available stationary phases with different alkyl modification as presented in figure 7.6 shows a general decrease with chain length. As this factor does not depend on phase volume, these columns possess different retentive characteristics. Long alkyl chains can shield the more polar silica backbone more effectively from the mobile phase, but already at octyl modification methylene selectivity decreases significantly. Stationary phases with propyl- and butyl modification exhibit nearly the same selectivity factors as octyl modified columns. The synthesised monolithic columns show a methylene selectivity of around 1.38 which is well above the average observed for C_8 columns. As explained before, MTMS based columns show a special

retention behaviour towards alkyl chains which explains enhanced retention factors and enhanced methylene selectivity compared to stationary phases with short alkyl modification.

7.5 Silanol activity

General importance of silanol groups in chromatography

In reversed phase chromatography, the interaction of analyte molecules with silanol groups is mainly viewed as unwanted. Especially basic compounds undergo a strong interaction. This second type of interaction shows a poor desorption kinetics which leads to distinctive tailing of basic compounds. One primary aim of surface modification is to shield these residual silanol groups from interaction with the analytes. Monomeric modification utilises silane species with three hydrophobic groups, one of them being the actual stationary phase. The two other residues shield the unreacted silanol groups in the direct environment so that after modification they form a closed layer above the silica surface which sterically hinders analytes to interact with it. Polymeric modification aims at creating a dense reversed phase by utilising monofunctional silica species; cross condensation between those species should result in a closed alkyl phase. The MTMS based materials presented in this thesis did not undergo reactions aimed to minimise the number of free silanol groups. Although there is a high density of methyl groups on the surface they may not sterically hinder analyte molecules from interacting with silanol groups present.

For the purpose of characterising the silanol activity of reversed phase materials, a number of tests were developed. All employed tests utilise the selectivity between basic compounds and phenol. The selectivity value should be representative for the interaction with silanol groups either in a protonated or, when working under neutral or basic conditions, deprotonated state.

Tab. 7.4: Experimental logKow values and dissociation constants of employed polar test compounds obtained from: http://www.syrres.com/esc/physdemo.htm

	Phenol	Caffeine	Benzylamine	Aniline	4-ethylaniline	4-isopropylaniline
logKow	1.46	-0.07	1.09	0.90	1.96	2.49
pKd	9.99	10.4	9.33	4.63	5.0	4.85

Silanol activity

One of the parameters chosen to characterise the activity of silanol groups was the silanol activity according to the Galushko test. The parameter is calculated according to eq. 7.9.

$$\text{silanol activity} = 1 + 3[\ k_{Aniline}/k_{Phenol} - 1) \qquad \text{eq. 7.9}$$

Chromatographic conditions were as follows:

30% methanol / 70% 20 mmol/l phosphate buffer pH 7.6 at 40°C

Chromatographic columns exhibiting a low amount of silanol activity should elute aniline before phenol, so good values for this parameter are lower than 1.

Hydrogen bonding capacity

Hydrogen bonding capacity was performed according to the standard test developed by Tanaka. It is defined as the selectivity between caffeine and phenol. Test parameters were as follows.

30% methanol / 70% 20 mmol/l phosphate buffer pH 2.7 at 40°C

The proposed mechanism is that an additional retention occurs by the formation of hydrogen bonds between silanol groups and caffeine if silanol groups are abundant. When comparing the experimental octanol water distribution coefficients, phenol should be retained significantly more through hydrophobic interaction. Under employed chromatographic conditions caffeine can be expected to elute almost in the dead volume.

Ion exchange capacity

Ion exchange capacity is in the Tanaka test defined as selectivity between benzylamine and phenol at a given pH value. It is conducted with the aim to identify silanol groups which exhibit a moderate acidity with pKa values higher than 2.7 and silanol groups with values below that. These groups can either come from geminal silanol groups, which are usually not present in surface modified silica, or groups where for instance alumina is connected to the silica in question through an oxygen bridge. Tests are performed under following conditions:

30% methanol / 70% 20 mmol/l phosphate buffer pH 2.7 and pH 7.6 at 40°C

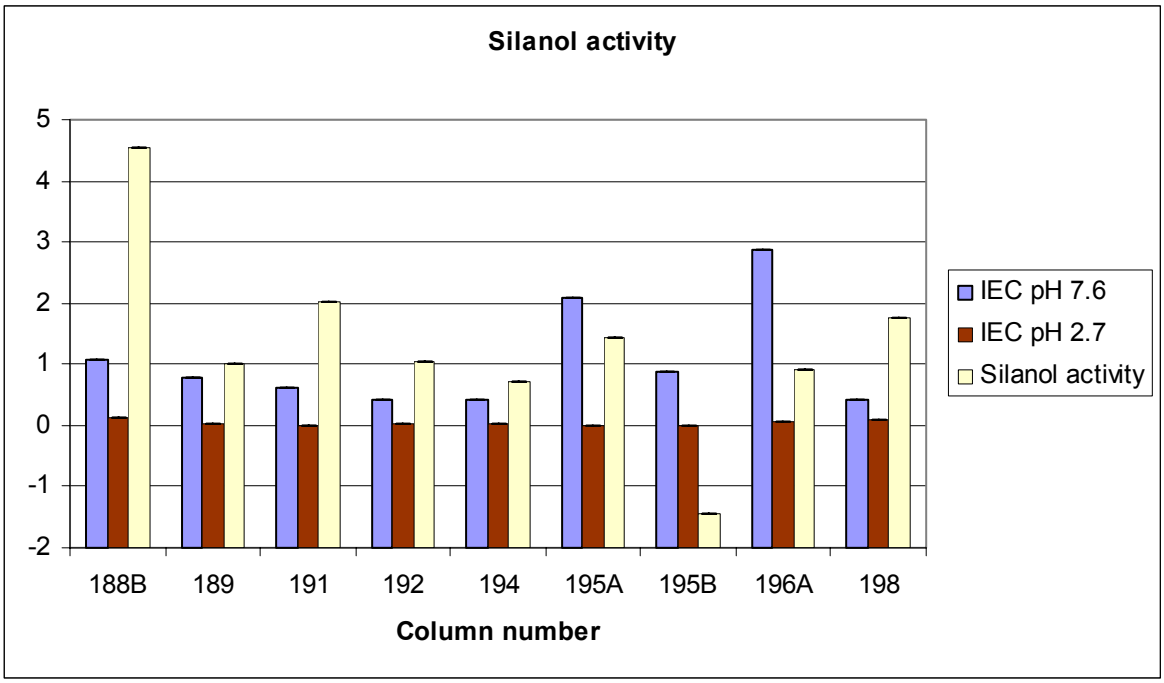


Fig. 7.7: Comparison of silanol activity and ion exchange capacities (IEC) at a pH of 2.7 and 7.6. Calculations and experimental conditions are described in the text. Numbers represent the column coding.

Results for the ion exchange capacity at a pH of 2.7 are that almost no silanol groups are dissociated at this pH. This was expected as highly acidic silanol groups are mainly a result of metal impurities in the silica material, which increase due to a –I effect the acidity of silanol functions of adjacent silica nuclei. As every silica nucleus has a methyl modification which has a direct +I effect, pKa values of present silanol groups can be expected to be lower than for silanol groups of silica nuclei with four oxygen bonds. Ion exchange capacity at a pH of

7.6 and silanol activity recorded at the same conditions do not correlate well. The difference is that benzylamine was employed as basic compound for the former parameter whereas aniline was employed for the determination of the later parameter. The comparison of the pKa values shows that aniline is a weaker base than benzylamine. The computation of the silanol activity value makes it more sensitive towards a shift in selectivity between employed test analytes. As can be seen in the results for these parameters, a correlation between them can not be made. Silanol activity is highest for column 188B, whereas ion exchange capacity has its highest value for column 196A. Materials 189, 192 and 194 show similarities concerning these parameters although they have been produced under different conditions. 195A and 195B exhibit clear differences in their silanophilic properties although they were produced in one batch. The results obtained make it clear that silanol activity depends not only on the experimental parameters during the synthesis. Investigations made on the repeatability of the synthesis showed that although macroscopic properties could be reproduced, values for specific surface area determined by parameters out of control of the proposed synthetic route. The current explanation is that the rate of drying varies due to differences in the permeability of the column cross section which will result in different specific surface area and silanol activity properties even for materials synthesised under the same conditions. Results for hydrogen bonding capacity are shown in the next section.

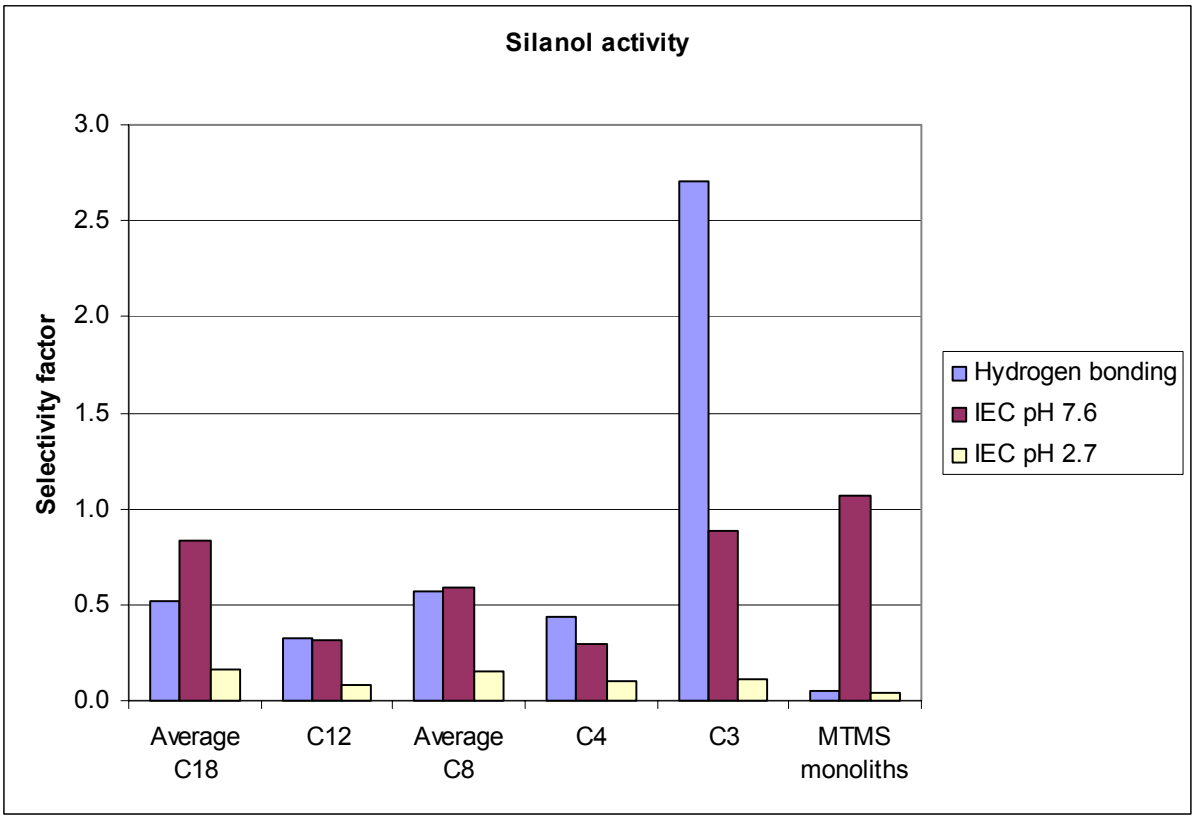


Fig. 7.8: Comparison of observed hydrogen bonding capacity and ion exchange capacity at pH 2.7 and 7.6 with average values for alkyl based reversed phase columns with data from literature [10].

The comparison of silanophilic properties with commercially available columns with different surface modification shows that values for hydrogen bonding capacity, which is defined as selectivity between caffeine and phenol at a pH of 2.7, are close to 0 for all tested MTMS based columns. Retention for caffeine should be significantly lower than for phenol if hydrophobic interaction is the main source of retention. The situation is different for commercially available columns. Values around 0.5 for most of them indicate a polar interaction of caffeine with the stationary phases. The investigated C_3 column shows that under employed conditions caffeine is retained by a factor of 2.7 more than phenol, showing that polar interaction exceeds apolar interaction in this case. Ion exchange capacities at a pH of 7.6, representing the sum of all accessible silanol groups, vary between commercial columns depending on the quality of the modification process. An interesting point is the low ion exchange capacity for the C_3 column, which means that this phase exhibits a polar character without many accessible silanol groups present. Ion exchange capacity values at a pH of 7.6 are on average higher for the investigated MTMS based columns. It shall be noted

that values as high as 4 were observed for commercial C_{18} columns. There were MTMS based columns which exhibited values around 0.4, which could be classified as good. An optimisation of the ageing and drying process should be able to produce columns with a very low abundance of silanol activity. Ion exchange capacities at pH values of 2.7 or below are an indication of metal impurities. MTMS based columns show on average lower numbers than commercial columns, which is a result of the use of a high purity precursor.

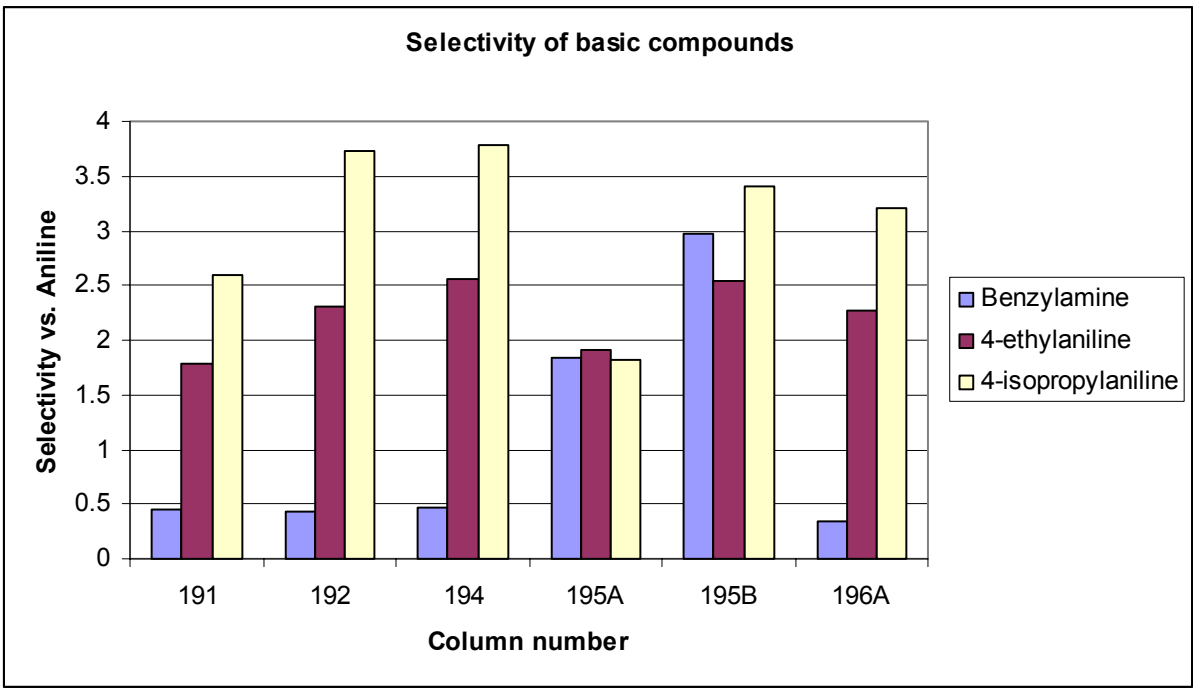


Fig. 7.9: Selectivity of basic compounds versus aniline. Chromatographic conditions were as follows: 30% methanol / 70% 20 mmol/l phosphate buffer pH 7.6; 40°C.

As last step in the evaluation of chromatographic properties for basic test solutes, selectivity factors of several compounds versus aniline were calculated. Benzylamine possesses a similar polarity as aniline but is a stronger base as can be seen in table 7.4. Differences in interaction should therefore be caused by their different basicity. Results show selectivity values are similar for all investigated columns except for 195A and 196A, which would indicate that they possess a difference in their silanol activity; the same observation was made for the selectivity versus phenol which gives the values for IEC. Selectivity of alkylated aniline species versus aniline should reflect differences in their hydrophobic properties, differences in basicity are very small between these species. As more or less uniform hydrophobic properties were observed between all columns, this should be reflected in these selectivity

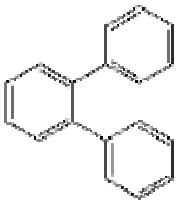
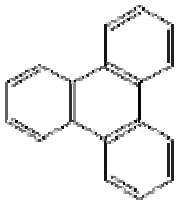
factors as well. Values for ethylaniline show a deviation of around 15%, which is marginally higher than observed for hydrophobic properties. For the selectivity values of isopropylaniline, however, a clear outlier was observed in the case of column 195A. It is retained less than ethylaniline although it exhibits clearly higher hydrophobicity. This specific column showed also a reduced selectivity for anthracene in the investigation of hydrophobic properties as demonstrated in section 7.4.

7.6 Shape selectivity and size exclusion effects

Shape selectivity

The test analytes employed for the determination of shape selectivity are o-terphenyl and triphenylene. Their logKow values which reflect their hydrophobicity are, as shown in table 7.5 very similar. Observed differences in retention should, as they do not possess any polar groups, reflect retention mechanisms which are caused by their different morphology.

Tab. 7.5: Experimental logKow values of o-terphenyl and triphenylene obtained from: http://www.syrres.com/esc/physdemo.htm

	o-Terphenyl	Triphenylene
logKow	5.52	5.49
		

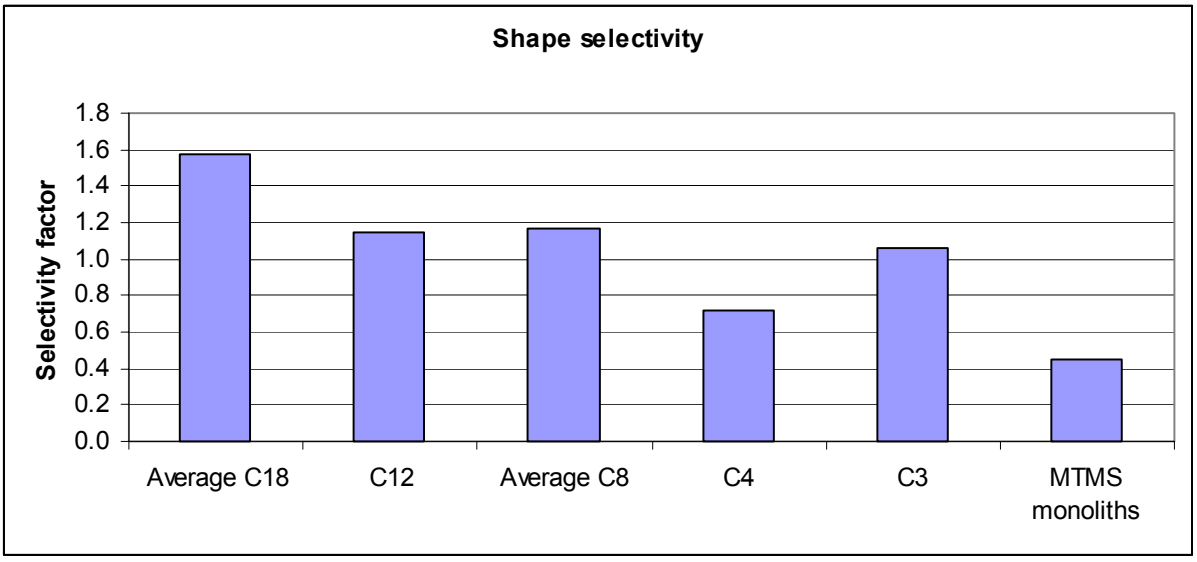


Fig. 7.10: Comparison of shape selectivity factors of different commercial alkyl reversed phase columns [10] with columns 198 and 201. Conditions of the experimental data for MTMS monoliths were as follows: solvent: 80% methanol / 20% distilled water, unbuffered; flow: 20 µl/min volumetric flow.

The mechanism of selective interaction for the flat shaped triphenylene is known for polymeric stationary phases. Here the high density of alkyl chains which form liquid crystal like structures allow only the flat shaped molecule to enter the phase whereas o-trephenyl which is not flat can not penetrate it. The highest observed values for this parameter are in the case of octadecyl phases around 4, on average the shape selectivity is close to 1.6. This property decreases generally with the alkyl chain length. Values below 1 were only observed for commercial products in the case of the C_4 modified stationary phase. The investigated MTMS based columns did in most cases not show retention for either test analyte. This indicated that they were excluded from the surface area because of their size. Reason for this was probably that the main part of surface area was only accessible through micropores with a too small diameter to be entered by these analytes. After this observation, the micelle forming Brij was added to the reaction mixture which should leave mesopores after extraction.

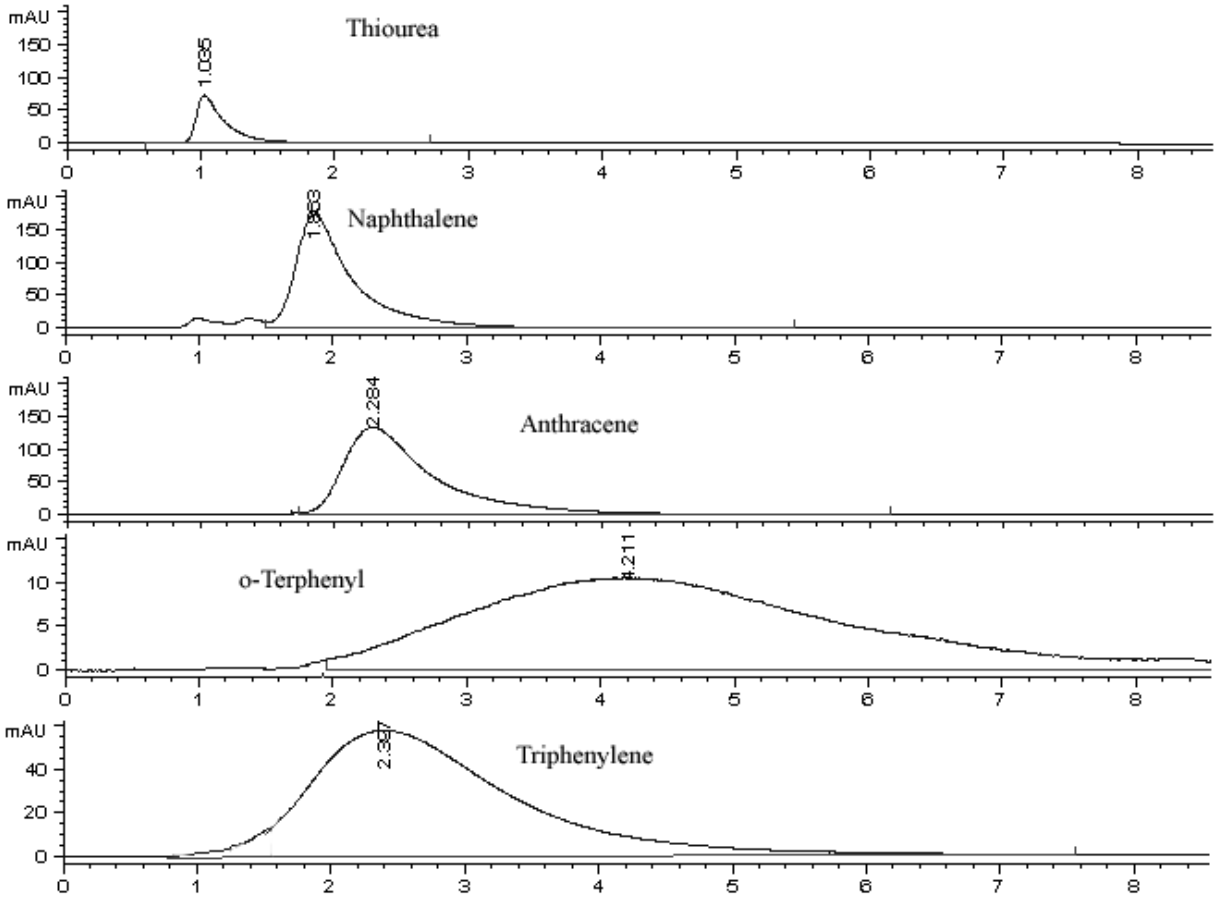


Fig. 7.11: Chromatograms of thiourea and aromatic compounds for column 201. Conditions were as follows: solvent: 80% methanol / 20% water unbuffered; flow: 50µl/min volumetric flow.

The investigation of columns produced with this addition showed both retention factors of 5.7 for o-terphenyl and 2.4 for triphenylene, respectively. Shape selectivity is therefore around 0.45, which is a rather low value. The interaction between the curved o-terphenyl with the stationary phase is therefore better than the interaction of the flat triphenylene molecule. Peak widths observed for both compounds are very broad with 2.5 and 1.5 min at retention times of 4.2 and 2.4 min, as can be seen in figure 7.11. This is believed to be caused by limiting diffusional characteristics stemming from comparatively small micropore diameters.

Observed size exclusion phenomena

For the purpose of investigating size exclusion phenomena and pore size dependence on the mobile phase composition more deeply, hydrophobic analytes were tested at different contents of methanol in the mobile phase. As previous results indicated that micropore diameters are in the range of the molecular size of employed test solutes, effects on retention should be visible.

Tab. 7.6 gives the results on selectivity of hydrophobic test compounds versus ethylbenzene recorded under the same mobile phase compositions. As can be seen, there is some deviation in the selectivity values between the columns. Apart from some outliers, observed differences are within 20%. This indicates that the chemical properties regarding hydrophobic interaction reflected by this selectivity factor do deviate within this range. The surface of the tested columns should be chemically almost identical, additional selectivity could come from differences in micropore diameters causing size exclusion in some cases. Results do not indicate the presence of this mechanism for selectivity.

Tab. 7.6: Selectivity values of hydrophobic test solutes vs. ethylbenzene recorded at different mobile phase compositions

Selectivity versus Ethylbenzene				
	192	194	196A	201
85% Methanol				
Propylbenzene	1.32	1.32	1.31	1.29
Butylbenzene	1.70	1.67	1.65	1.78
Naphthalene	n.d.	n.d.	n.d.	0.81
Anthracene	0.96	0.68	0.91	1.01
80% Methanol				
Propylbenzene	1.41	1.40	1.38	1.39
Butylbenzene	1.94	1.95	1.85	2.00
Naphthalene	0.71	0.73	0.70	0.74
Anthracene	0.98	0.99	0.96	1.12
75% Methanol				
Propylbenzene	1.56	1.38	1.26	1.51
Butylbenzene	2.40	1.83	1.64	2.22
Naphthalene	0.56	0.58	0.51	0.74
Anthracene	0.83	0.79	0.77	1.14
70% Methanol				
Propylbenzene	1.69	1.63	1.56	1.70
Butylbenzene	2.74	2.54	2.52	2.98
Naphthalene	0.69	0.66	0.69	0.73
Anthracene	1.25	1.19	1.15	1.43

The surface properties of the employed columns are rather similar as shown in table 7.6. Therefore the coefficients between analyte concentration in the mobile phase and the concentration of adsorbed species should be similar. The ratio of k´ values for one analyte should according to the partition model give then the ratio of the accessible surface areas between two columns.

Tab. 7.7: Ratios of k´ values versus material 201 to demonstrate differences in phase ratios.

Capacity factor ratios versus column 201				
	192	194	196A	201
85% Methanol				
Mean value	1.73	1.67	1.13	1
Std. dev.	0.06	0.35	0.06	-
80% Methanol				
Mean value	1.37	1.04	0.88	1
Std. dev.	0.08	0.05	0.06	-
75% Methanol				
Ethylbenzene	1.61	1.06	1.42	1
Propylbenzene	1.66	0.97	1.18	1
Butylbenzene	1.73	0.87	1.05	1
Naphthalene	1.21	0.83	0.99	1
Anthracene	1.18	0.74	0.96	1
70% Methanol				
Mean value	1.06	0.73	0.64	1
Std. dev.	0.06	0.06	0.05	-

Ratios of k´ values of hydrophobic compounds were calculated for values obtained under different mobile phase compositions. As ratios within one mobile phase compositions varied little with the exception of 75% methanol content, mean values and standard deviation values are shown in table 7.7 in the other cases. The high value for deviation in case of column 194 was caused by outliers for naphthalene and anthracene. Results show that the ratios of the phase volume compared to material 201 change with the mobile phase composition. Values obtained at 85% methanol content are for all investigated materials a factor of at least 1.5 smaller compared to values obtained at 70% methanol content. This seemingly significant difference suggests that the access or interaction with the surface area is somehow depending on the mobile phase composition. Whether some pores become inaccessible because they shrink at higher mobile phase polarity or mass transfer is limited for other reasons remains speculation at this point.

Influence of ageing on chromatographic properties

As last part in the investigation of size exclusion effects, one material was subjected to ageing under basic conditions for 24 h at 40°C. It is also known that dissolution and precipitation of silica species occur under basic conditions, which consequently decreases micropore diameters until they are completely filled with silica material.

The chromatographic results presented in figure 7.12 show that distinctive peak tailing occurs at a methanol content of 80% for naphthalene and anthracene. This indicates limited transfer kinetics due to decreased micropore diameters. Another material which was subject to 96 hours of ageing under the same conditions did not show retention for employed test analytes at all, which means that micropores can be eliminated by this procedure.

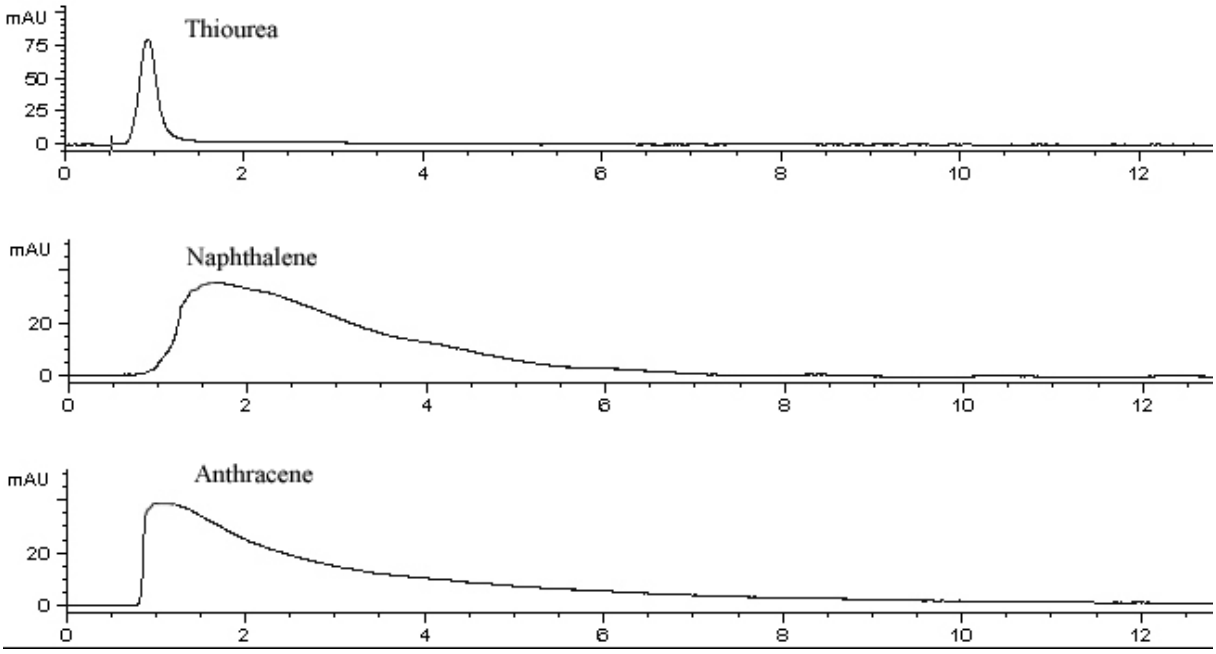


Fig. 7.12: Chromatograms of thiourea, naphthalene and anthracene of material 203. Employed conditions were as follows: solvent: 80% methanol / 20% water, unbuffered; flow: 50 µl/min volumetric flow.

7.7 Conclusion

Capillary columns synthesised according to the proposed protocol could be employed for chromatographic standard tests as proposed by Tanaka *et al*. The determination of flow through properties showed that morphological characteristics obtained from the evaluation of SEM micrographs correlate very well with values obtained from the specific flow resistance. It can therefore be concluded that the determination of skeleton and pore diameters from the SEM micrographs is a suitable way for the evaluation and that investigated cross sections were representative for the whole column.

Absolute retention factors of hydrophobic test solutes indicate the presence of an accessible specific surface area even for materials where no significant area was observed in nitrogen adsorption measurements. Retention factors deviated between columns produced under the same conditions. Conditions prevailing during the drying process could probably not be controlled precisely enough to guarantee repeatable results. As most specific surface area is located in micropores, it is sensitive towards ageing processes and pore closure during drying.

Selectivity values for hydrophobic test analytes were nearly identical for all investigated columns. As surface polarity can be assumed to be identical, size exclusion would be the only mechanism to cause a different selectivity as was observed in one case. Naphthalene and anthracene were in comparison to employed alkylbenzenes retained less than expected according to their log Kow. The reason for this is an adsorption based retention mechanism; the methyl rich surface exhibits probably voids which show adsorptive properties towards alkyl chains but to a lesser degree towards aromatic groups.

The results for ion exchange capacity confirmed that the number of silanol groups with a pKa lower than 2.7 is negligible, which results from using high purity precursors for synthesis. The value recorded at a pH of 7.6 indicates in comparison to commercial columns moderate silanol activity. Values varied by a factor of 5 between the synthesised columns; some materials showed very low activity without being subject of deactiviation procedures. An optimisation of ageing and drying is therefore likely to produce materials with excellent properties regarding silanol activity. Values obtained for hydrogen bonding capacity were very low. This indicates that hydrophobic interaction is dominant under employed conditions. Size exclusion occurring for the employed test analyte caffeine can not be ruled out at this point, though.

Shape selectivity could only be determined for one material where a porogen was added to the reaction mixture; other materials did not show retention for o-terphenyl or triphenylene. The explanation is that micropore diameters are too small to be entered by the test solutes in these cases. Results for two materials where ageing under basic conditions was conducted showed that micropores can be eliminated with this procedure.

The largest fraction of the specific surface area is –for the proposed materials- only present in micropores which cause unwanted size exclusion effects and limit kinetics for interaction of solutes with the surface. Further templating in the mesopore range should be pursued as means to introduce mesopores into the material. Ageing could then be employed to eliminate micropores which exhibit unfavourable transfer kinetics; remaining mesopores should provide sufficient specific surface area for retention. Silanol activity can be expected to be very low after optimising said ageing procedure. Drying should have less influence on accessible surface area as micropore closure would not occur after stabilising the surface with ageing.

7.8 References

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8. Conclusions and perspectives

The transfer of a synthetic protocol based on basic catalysis showed that several crucial points had to be adjusted in order to avoid gaps to the capillary wall. Activation of the capillary wall proved to be of little influence on the adhesion of the monolithic material to the capillary wall. The composition of the reaction mixture could only be adjusted within narrow limits as the macroscopic morphology of the obtained xerogel has to be within certain limits in order to be feasible for liquid chromatography. The main hindrance in the establishment of sufficient bonds between monolithic material and capillary wall was identified as the temperature gradient that forms inside the capillary. Gelation would start at the centre of the capillary where temperatures would be highest. The emerging gel would start to shrink due to ageing processes; the reaction liquid at the border to the capillary wall would be enriched with solvent. Gaps could be eliminated by filling the reaction mixture at an early stage of the reaction into the capillary with an immediate transfer into a heated compartment to prevent the formation of a temperature gradient. Nonetheless, adhesion to the capillary wall proved to be insufficient to employ these columns for chromatographic applications. Ageing was performed by exchanging the pore liquid of the xerogel with a 30% MTMS solution. Mechanical stability and adhesion could be enhanced with this procedure; however columns were still flushed out of the capillary when flushing the stationary phases with the mobile phase. Further extending the ageing procedure was not feasible as it would result in a loss of a significant amount of internal specific surface area. For further experiments a synthetic protocol based on acid catalysis was pursued.

The reaction under acid catalysed conditions relies on the competitive kinetics of a decomposition of the reaction mixture into two phases and the gelation process. A first screening was made to determine boundaries for the composition of the precursor mixture within which a desired bicontinuous pore- and skeleton structure was observed. These first experiments showed that connectivity between monolithic material and capillary wall was sufficient to avoid the occurrence of gaps to the capillary wall. The influence of various parameters on morphological characteristics prevailing during the synthesis was investigated in a next step.

The precursor composition could only be varied within 10% to deliver bicontinuous structures, observed macroscopic characteristics varied by a factor of 5 or more within this series. Gelation temperature had less effect on morphological characteristics; higher

temperatures were accompanied with smaller observed pore diameters and skeleton dimensions. Changing temperatures from 40 to 52°C resulted in a decrease of a factor of 3. Higher catalyst concentrations resulted in larger pore- and skeleton diameters, the variation of the concentration from 0.8 M to 1.2 M resulted in an increase of characteristics by a factor of 3. Using mixtures of MeOH with variable fractions of EtOH and 2-PrOH instead of pure MeOH as solvent resulted in a decrease of pore- and skeleton size. As example, 20% substitution with 2-propanol resulted in an observed skeleton size of only 0.2 µm. Specific surface area could generally be observed for materials exhibiting skeleton diameters of 2 µm and less. The evaluation of nitrogen adsorption measurements showed in these cases the abundance of micropores, mesopores were never observed. Results of nitrogen adsorption measurements showed furthermore a poor repeatability whereas the macroscopic morphology could be reproduced very well. These sets of experiments showed that several possibilities exist to control the macroscopic morphology over a wide range.

Fabricated monolithic columns were then employed in chromatographic characterisation. The evaluation of backpressure revealed a good correlation with pore diameters determined from the SEM micrographs, which confirms that the chosen approach for the assessment of macroscopic characteristics is principally viable. Standard tests were then carried out to gain information on chromatographic characteristics. The investigation of hydrophobic properties showed that methylene selectivity was in the range of other reversed phase materials. Polyaromatic compounds were retained less than expected, indicating a difference in the retention mechanism compared to octadecyl modified columns. Investigated columns showed very similar selectivity factors. Surprisingly, some chromatographic columns exhibited retention for the test analytes, although the BET measurements indicated only very low specific surfaces for these materials. Retention factors were not reproducible for columns synthesised under the same conditions.

Results of silanophilic properties indicated that only a small population of silanol groups can undergo interaction with the employed test solutes; ageing intends to minimise their occurrence and could potentially lead to very inert stationary phases with regard to silanol activity. Shape selectivity investigation confirmed that specific surface area is mainly present in very small micropores; most materials did not retain the test analytes used in the shape selectivity test. The addition of a micelle forming polymer as porogen resulted in a material that showed retention for test solutes used to determine shape selectivity. However, very broad peaks indicated slow transfer kinetics- pore diameters are probably still too small to allow a fast diffusion within the stationary phase. The same observation was made for

naphthalene and anthracene in the case of a material which was subjected to ageing, which confirms that pore diameters can be reduced through ageing. Increasing the time of ageing resulted in a material that showed virtually no retention. Micropores can obviously be eliminated through this process.

The vast majority of the specific surface area is present in micropores. This causes a poor reproducibility of observed area and retention factors because micropores seem to be sensitive towards ageing processes occurring after synthesis and during the drying stage. They possess furthermore unfavourable diffusional kinetics causing peak broadening. Size exclusion effects even for small molecules were observed. The next step in the synthesis has to be the creation of mesopores through the use of a suitable porogen. Micropores were demonstrated to be eliminated through ageing – a higher reproducibility for retentive properties should be the possible. Although optimised synthesis should produce chromatographic materials with reversed phase characteristics ready to use in applications, possibilities to include different functionalities should be investigated in a next step. One possibility could be to modify the silanol groups still present, although chromatographic tests have shown their low abundance. The inclusion of a co-precursor like glycidoxypropyl-trialkoxysilanes is another interesting possibility to generate reactive groups which can in a further step be modified with the desired functionality. The main merit of methyl-silsesquioxane based materials compared to siloxane based would be their enhanced inertness of the base material as well as an enhanced stability at high pH values and better long term stability.

9. Appendix

9.1 Abbreviations

A	anthracene
ACN	acetonitrile
An	aniline
BA	benzylamine
BB	Butylbenzene
BET	Brunauer / Emmett / Teller
BJH	Barrett / Joyner / Halenda
C	caffeine
CEC	capillary electrochromatography
CP-MAS-NMR	cross polarization - magic angle spinning - NMR
DAD	diode array detector
EB	ethylbenzene
EOF	electroosmotic flow
ESI	electrospray ionisation
GC	gas chromatography
HETP	height equivalent to a theoretical plate
HPLC	high performance liquid chromatography
I.D.	inner diameter
IEC	ion exchange capacity
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LSCM	laser scanning confocal microscopy
MALDI	matrix assisted laser desorption-ionisation
MT, OT, PT	meta-, ortho-, para-toluidine
MPTMS	methacryloxypropyltrimethoxysilane
MTES	methyltriethoxysilane
MTMS	methyltrimethoxysilane
NMR	nuclear magnetic resonance
n-LC	nano liquid chromatography

ORMOCER	Organically Modified Ceramics
ORMOSIL	Organically Modified Silica
P	phenol
PAH	polycyclic aromatic hydrocarbons
PB	propylbenzene
PEEK	poly(ethyletherketone)
PEO	polyethylene oxide
RP	reversed phase
SEM	scanning electron microscopy
SPE	solid phase extraction
SPME	solid phase micro extraction
T	temperature; toluene
TEM	transmission electron microscopy
TMOS	tetramethylorthosilicate (Tetramethoxysilane)
UPLC	ultra performance liquid chromatography
γ-MAPS	3-(triethoxysilyl)-propylmethacrylate
µ-LC, µ-LC	micro liquid chromatography

9.2 Symbols

A	term of the van-Deemter equation
AS	peak asymmetry factor
a_m	average area occupied by an adsorbed molecule
B	term of the van-Deemter equation
C	BET constant; term of the van-Deemter equation
C_s	contribution of the stationary phase to term C
C_m	contribution of the mobile phase to term C
d_f	film thickness
D	reduced dispersivity
D_L	longitudinal dispersion coefficient
D_m	diffusion coefficient in the mobile phase
D_s	diffusion coefficient in the stationary phase
D_{stag}	effective diffusion coefficient in the stagnant zone
d_p	particle diameter; macropore diameter

d_{pore}	macropore diameter
F	Darcy-Weißbach friction factor
h	reduced theoretical plate height
H	theoretical plate height
h_{min}	minimum plate height
K	permeability
k, k´	retention factor
L	Avogadro constant (chapter 2); column length (chapter 4 and 5)
L_{flow}	characteristic length for hydraulic permeability
L_{stag}	characteristic length for hydrodynamic dispersion
N	number of theoretical plates
n^a	adsorbed amount
n^a_m	monolayer capacity
p	pressure
p^o	saturation pressure
p*	critical condensation pressure
Q^n	silicon atom bonded to four oxygen atoms; n = number of siloxane bonds
q	configuration factor
q_i	factor that describes particle shape
Re	Reynolds number
r_m	mean radius of curvature of the liquid meniscus
r_p	pore radius
S	surface area; peak symmetry factor
T^n	silicon atom bonded to three oxygen atoms; n = number of siloxane bonds
t_g	gelation time
t_0	retention time of a compound that is not retained
t_r	retention time
t_{sys}	system dead time
u	mobile phase velocity
V	pore volume
V_a	adsorbed volume
α	selectivity factor

γ	surface tension, tortuosity factor
η	dynamic viscosity of the mobile phase
Φ	specific flow resistance
θ	contact angle
λ	constant describing statistic irregularities of the packing
ν	molar volume of the condensed adsorptive; reduced mobile phase velocity
ρ	density
υ	kinematic viscosity of the mobile phase
ψ_i	factor that describes the size distribution of particles
ω	constant describing influence of packing on radial diffusion

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5th May 2003	Diploma exam; the study of Analytical Chemistry (graduation with distinction)
July 2002 - May 2003	Diploma thesis in the research group "Organic Trace Analysis" under supervision of Ao. Prof. Dr. Egon Erwin Rosenberg.
March 1998 - May 2003	Study of Technical Chemistry on the University of Technology, Vienna. With focus on analytical chemistry
July 1997 - March 1998	Military service
6th June 1997	Graduation with main focus on biology and chemistry
September 1989 – June 1997	Attendance of high school Oberschützen, 8 years education in English, 4 years in French; focus on education in natural sciences
September 1985 - June 1989	Primary school, Oberwart

Work experience

October 2003 – October 2007	Employment as scientific assistant, TU Vienna
July 2003 – October 2003	Employment as project assistant at the working group for organic trace analysis
2004 ,2005 ,2007	Counsellor for Technical Chemistry at the exhibition for study and education (BeSt), Vienna
July and August 2000	Baxter Vienna, Industriestrasse; Summerjob at the serum protein precipitation facility
July 1999	Beiersdorf Vienna: Summerjob in the storehouse
Juli 1994	Retirement home Oberwart: Summerjob in the kitchen

Publications and presentations

27th-30th March 2007	S. Laschober, E. Rosenberg: *Synthesis and Characterisation of Monolithic Capillary Columns using Sol-Gel Synthesis based on Methyl-trimethoxysilane*, poster presented at "Anakon 2007", Jena, Germany
2007	S. Laschober, E. Rosenberg, M. Sulyok: *Tailoring the macroporous structure of monolithic silica-based capillary columns with potential for liquid chromatography*, published in J. Chrom. A 1144 (2007) 55.
28th-31st May 2006	S. Laschober: *Towards Silica based capillary columns as stationary phases for LC*, oral presentation at "Monolith Summer School 2006", Portoroz, Slovenia.
20th April 2006	S. Laschober, E. Rosenberg: *Silica Based Capillary Monoliths. New Stationary Phases for Liquid Chromatography*, poster, oral presentation, and publication at "Junior Scientist Conference 2006", TU Vienna, Austria.
31st March 2006	S. Laschober: *Mikrowellen unterstützte Probenvorbereitung in der Spurenanalytik,* oral presentation at "Seminar moderne Analytik", TU Vienna, Austria.
15th April 2005	S: Laschober, E. Rosenberg, M. Sulyok: *Towards Monolithic Silica Based Capillary Columns as Stationary Phases for Liquid Chromatography*, oral presentation and publication at "Seminar for Young Scientists working in the Area of Analytical Chemistry", CEEAM Gdansk, Poland.
11th April 2003	S. Laschober: *Untersuchung von Silica basierten Sol-Gel Materialien für die chromatographische Probenvorbereitung,* oral presentation at "Seminar moderne Analytik", TU Vienna, Austria.
15th-19th June 2003	S: Laschober, E. Rosenberg, M. Sulyok: *Sorption Studies on Sol-Gel Derived Silica-Based Materials to Assess their Potential for Solid Phase Extraction,* poster presented at HPLC, Nice, France.
1st-2nd May 2003	M. Sulyok, E. Rosenberg, S. Laschober: *Characterisation of Sol-Gel Derived Monolithic Silica Designed for Chromatographic Application*, poster presented at Austrian Tunesian Meeting, Tunis, Tunisia.
2nd-5th April 2003	M. Sulyok, E. Rosenberg, S. Laschober: *Charakterisierung von Sol-Gel basierenden Silica Mololithen in Hinblick auf chromatographische Anwendungen*. poster presented at "Anakon 2003", Konstanz, Ger.