Doctoral Thesis

Retention, diffusion and release of flavor molecules from porous silica sol-gel-made particles

Author(s): Veith, Susanne Regine
Publication Date: 2004
Permanent Link: https://doi.org/10.3929/ethz-a-004715255

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RETENTION, DIFFUSION AND RELEASE OF FLAVOR MOLECULES FROM POROUS SILICA SOL-GEL-MADE PARTICLES

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of

DOCTOR OF SCIENCES

presented by

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Zurich, 2004
Acknowledgements

This PhD project was carried out in the Particle Technology Laboratory at the ETH Zurich in cooperation with the Nestlé Product Centers Kemptthal and Konolfingen and with the Nestlé Research Center Lausanne. I would like to thank Nestlé for supporting and sponsoring this work and Dr. Klaus Zimmermann and Dr. Konrad Lerch for making this cooperation possible.

A special thankyou goes to Matthias Perren, who continuously followed this project with great interest, for his productive scientific and personal discussions and suggestions. I very much enjoyed working together with the encapsulation group at Kemptthal. Thanks a lot to Clara Ong and Dr. Susann Neiser for their help with the GC work. I am especially thankful to Dr. Eric Hughes for his cooperation and enthusiasm in diffusion NMR, for many scientific discussions and last but not least for the combined working/snowboarding weekends.

I would like to express my deep gratitude to Prof. Dr. Sotiris E. Pratsinis, who supervised this PhD project, for his continuous encouragement and valuable advice throughout this work. I am also very grateful to my co-examiner Prof. Gary Reineccius for his useful advice and interest and for serving on my PhD committee. I would like to thank Prof. Magnus Nyden for his fruitful discussions and his scientific input in the diffusion NMR part.

This work would not have been possible without the contributions and help of my colleagues at the ETH. I very much enjoyed the inspiring atmosphere and many stimulating discussions within the group. A special thank you to Oliver Wilhelm, Martin Heine, Dr. Lutz Maedler, Dr. Karsten Wegner, Dr. Roger Müller, Rainer Jossen, Reto Strobel, Dr. Hendrik Kammler and Dr. Jinsoo Kim.

I would also like to thank the workshop at the Institute of Process Engineering under the supervision of Dr. Doerfler for their technical assistance, Markus Huber for his help with chemical problems and Sascha Jovanovic and Benni Cadonau for their aid to quickly solve computer problems. I greatly acknowledge the administrative support of our secretaries Ileana Eugster and Particia Horn, especially also in keeping me "female company" within the group.
Many thanks to the students Sven Richard, Tobias Rothenfluh, Yannick Loosli, Marc Stipsicz and Tobias Weber, who contributed to this work in their semester projects or as Hilfsassistenten.

I am also very grateful to Gilles Vuataz, Britta Folmer, Dieter Welti and to France Berruex for their experimental support at the CRN in Lausanne.

Finally, I would like to express my deepest gratitude to my family, especially to my parents, who have been supportive and encouraging throughout these years. And last but not least to Kai, who always managed to make me enjoy the life beneath science, for his endless trips to Zurich and his understanding moral support.
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Zusammenfassung


Das Einleitungskapitel gibt zunächst einen Überblick über die verschiedenen Einkapselungstechniken mit dem Schwerpunkt auf dem Sol-Gel Verfahren. Die Motivation Sol-Gel Partikel für die Aromeneinkapselung zu untersuchen, wird erläutert.

zeigen, dass Aromenmoleküle selbst bei hohen Temperaturen recht effizient zurückgehalten werden.


Im Gegensatz zu den Wasserdiffusionsstudien gestaltet sich die Messung von organischen Molekülen in porösen Partikeln mittels PFG-NMR wesentlich schwieriger, da eine signifikante Linienverbreiterung des NMR-Signals aufgrund von Suszeptibilitätsunterschieden zwischen den einzelnen Phasen auftritt. Im Anhang A wird deshalb gezeigt, dass NMR Spektren mit hoher Auflösung durch gleichzeitige Rotation der Probe um den sogenannten „magischen Winkel“ („MAS“, magic angle spinning) erhalten werden und dass die Rotationsbewegungen der Probe die so gemessenen Diffusionsergebnisse nicht beeinflussen. Es wird ein Rotationsfrequenzfenster ermittelt, in dem die Messung von Diffusionskoeffizienten sinnvoll erscheint. Moderne interne Feldgradienten spielen dabei eine untergeordnete Rolle, obwohl die angelegten Feldgradienten vergleichbar klein sind.

Schlussendlich werden im letzten Kapitel die mikroskopischen Porendiffusionskoeffizienten von Aromenmolekülen in Sol-Gel Siliziumdioxidpartikeln mittels
Seite Leer / Blank leaf
Summary

The scope of this thesis is to investigate the encapsulation and release performance of organic molecules in silica sol-gel-made particles for applications in the food and pharmaceutical industry. The first part focuses on the influence of particle morphology and chemical nature of the organic compound on the retention performance. The second part is dedicated to the observation of water and organic compound diffusion in these matrices by pulsed field gradient nuclear magnetic resonance (PFG-NMR). These experimental pore diffusion coefficients are also compared with model predictions that are based on particle morphology and the relation of the size of the diffusing molecules to the pore dimensions. The macroscopic release from those porous particles can be calculated by an integration of Fick’s second law with the knowledge of the pore diffusion coefficient from the model or from PFG-NMR measurements. These predictions are compared to measured release kinetics.

The introduction chapter gives an overview over various encapsulation techniques with the focus on sol-gel encapsulation. It also describes the motivation of studying sol-gel materials for the encapsulation of flavor molecules.

The following chapter investigates the retention performance and stability of an entrapped model flavor, decanoic acid, in silica matrices made by hydrolysis and condensation of tetraethyl orthosilicate (TEOS). The morphology, specific surface area, porosity and pore size distribution of the particle matrix is controlled by the hydrolysis ratio of the sol-gel preparation method. During drying, the weakly cross-linked polymers in a slowly hydrolyzing silica gel deform and wrap around flavor molecules creating a denser gel structure. There the flavor molecules are entrapped more efficiently than in fast hydrolyzing matrices that result in more porous particles. The influence on decanoic acid retention by residual precursor was investigated by thermogravimetric analysis and a similar surface structure was found in all blank samples. Therefore changes in retention performance are mainly attributed to differences in particle morphology. Kinetic annealing studies show that flavor molecules are entrapped quite efficiently even at high temperatures.

The retention performance of flavor molecules from different chemical classes (e.g. alcohols, esters, aldehydes and terpenes) was studied in the third chapter of this thesis. Since particle morphology, porosity and pore size distribution can be controlled by the sol-gel preparation method, the influence of the nanoconfinement in the microporous matrix on
flavor retention is studied as well as the effect of initial flavor load. As the porosity is decreased, flavor molecules are entrapped more efficiently in the silica particles. The retention performance decreased in the order of: alcohol > aldehyde > ester > terpene consistent with the retention behavior of organic matrices. In contrast to these matrices, open sol-gel-made silica particles show an increased retention with increasing flavor load while denser silica matrices show a maximum with increasing load.

The fourth chapter of this thesis deals with the measurement of the restricted diffusion coefficient of water molecules through porous silica sol-gel-made particles by pulsed field gradient (PFG) NMR as a function of loading in order to develop a model for self-diffusion at full pore filling. This model describes the pore or intraparticle diffusion coefficient as a function of particle porosity, tortuosity and the steric hindrance applied on the molecules by the pore space. The particle morphology is characterized by nitrogen adsorption and an appropriate tortuosity model is chosen in comparison with literature data. To characterize the material, NMR relaxation and diffusion studies at different degrees of pore filling were carried out in relation to the silica/water adsorption isotherm.

In contrast to the water diffusion studies pulsed field gradient NMR diffusion measurements of entrapped organic molecules are difficult to obtain under high spectral resolution due to broad resonances arising from magnetic susceptibility differences between the different phases. It is shown in appendix A that high resolution spectra can be obtained by utilizing magic angle spinning and that the measured diffusion coefficients are not influenced by the spinning. There is a reasonably sized spinning frequency window where diffusion coefficients can be measured. Moderate internal field gradients present in the materials of interest do not affect the experiment even though the applied gradients that the probe is capable of are small.

Finally, the restricted diffusion coefficients of flavor molecules entrapped in sol-gel-made silica particles are measured in-situ by pulsed field gradient magic angle spinning (PFG-MAS) NMR in the last chapter of the thesis, while the release kinetics are calculated by the Crank equation. Their macroscopic release profiles are obtained directly by UV-VIS spectroscopy. Measured and calculated release profiles agree within experimental error. Furthermore, the pore diffusion coefficients obtained by PFG-MAS-NMR are in agreement to those obtained by model calculations indicating its potential for characterization and screening of encapsulation formulations.
## Notation

### Nomenclature and Units:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Space between restricting barriers</td>
<td>[m]</td>
</tr>
<tr>
<td>$a_w$</td>
<td>Water activity</td>
<td>[-]</td>
</tr>
<tr>
<td>$b$</td>
<td>Pore spacing</td>
<td>[m]</td>
</tr>
<tr>
<td>$c$</td>
<td>Concentration of solute in the pore</td>
<td>[g/cm$^3$]</td>
</tr>
<tr>
<td>$c_0$</td>
<td>Starting concentration at $t = 0$</td>
<td>[g/cm$^3$]</td>
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<tr>
<td>$c_e$</td>
<td>Equilibrium concentration at particle surface</td>
<td>[g/cm$^3$]</td>
</tr>
<tr>
<td>$d$</td>
<td>Particle diameter</td>
<td>[m]</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient</td>
<td>[m$^2$/s]</td>
</tr>
<tr>
<td>$D_o$</td>
<td>Molecular free self diffusion coefficient</td>
<td>[m$^2$/s]</td>
</tr>
<tr>
<td>$D_{av}$</td>
<td>Average diffusion coefficient</td>
<td>[m$^2$/s]</td>
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<tr>
<td>$D_{eff}$</td>
<td>Effective diffusion coefficient</td>
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</tr>
<tr>
<td>$D(t)$</td>
<td>Time dependent self diffusion coefficient</td>
<td>[m$^2$/s]</td>
</tr>
<tr>
<td>$f$</td>
<td>Gradient strength (PGSTEBP)</td>
<td>[T/m]</td>
</tr>
<tr>
<td>$F(\lambda)$</td>
<td>Correlation function for steric hindrance</td>
<td>[-]</td>
</tr>
<tr>
<td>$g$</td>
<td>Gradient strength</td>
<td>[T/m] or [G/cm]</td>
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<tr>
<td>$g_i$</td>
<td>Internal gradient</td>
<td>[T/m] or [G/cm]</td>
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<tr>
<td>$H$</td>
<td>Hydrolysis ratio (H = [H$_2$O/Precursor])</td>
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<tr>
<td>$I$</td>
<td>Intensity of the spin echo</td>
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<td>Relaxation weighted intensity</td>
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<td>$k$</td>
<td>$k = \gamma^2 \cdot \delta^2 \cdot g^2 \cdot t_d$</td>
<td>[s/m$^2$]</td>
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<td>$k_B$</td>
<td>Boltzmann constant</td>
<td>[J/K]</td>
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<tr>
<td>$K_D$</td>
<td>Drag coefficient</td>
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<td>$K_m$</td>
<td>Michaelis-Menten Constant</td>
<td>[mM]</td>
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<td>$K_p$</td>
<td>Partition coefficient between pores and bulk</td>
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<td>$l_D$</td>
<td>Diffusion length</td>
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<td>Description</td>
<td>Unit</td>
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<td>--------</td>
<td>-----------------------------------------------------------------------------------------------</td>
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<td>$m_D$</td>
<td>Decanoic acid mass measured by GC</td>
<td>[mg]</td>
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<tr>
<td>$m_{D0}$</td>
<td>Decanoic acid input mass</td>
<td>[mg]</td>
</tr>
<tr>
<td>$m_E$</td>
<td>Mass of powder extracted</td>
<td>[mg]</td>
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<tr>
<td>$m_i$</td>
<td>Mass of compound i in extraction sample (GC-analysis)</td>
<td>[mg]</td>
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<td>$m_{i0}$</td>
<td>Theoretical flavor input mass of compound i</td>
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<td>$m_{OH}$</td>
<td>Mass loss by thermal dehydroxylation</td>
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<td>$m_{So}$</td>
<td>Silica input mass in sol</td>
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<tr>
<td>$m_{SE}$</td>
<td>Mass of dry silica (extraction sample)</td>
<td>[mg]</td>
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<tr>
<td>$m_w$</td>
<td>Water mass</td>
<td>[mg]</td>
</tr>
<tr>
<td>$M_t$</td>
<td>Amount released at time t</td>
<td>[min]</td>
</tr>
<tr>
<td>$M_\infty$</td>
<td>Total amount released at infinite time</td>
<td>[min]</td>
</tr>
<tr>
<td>$n$</td>
<td>Integer ($n = 1 \ldots \infty$)</td>
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<tr>
<td>$p$</td>
<td>Empirical parameter (Archie's law)</td>
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<tr>
<td>$p_i$</td>
<td>Fraction of the molecules in domain i</td>
<td>[-]</td>
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<td>$p/p_0$</td>
<td>Relative pressure (adsorption isotherm)</td>
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<tr>
<td>$p'$</td>
<td>Empirical parameter (Archie's law)</td>
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</tr>
<tr>
<td>$q$</td>
<td>Reciprocal wave vector ($q = \gamma \cdot g \cdot \delta / 2\pi$)</td>
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</tr>
<tr>
<td>$r$</td>
<td>Radius</td>
<td>[m]</td>
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<tr>
<td>$r_m$</td>
<td>Radius of molecule/solute</td>
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</tr>
<tr>
<td>$r_p$</td>
<td>Pore radius</td>
<td>[m]</td>
</tr>
<tr>
<td>$R$</td>
<td>Particle radius</td>
<td>[m]</td>
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<tr>
<td>$R^2$</td>
<td>Regression coefficient</td>
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<tr>
<td>$R_D$</td>
<td>Retention of decanoic acid</td>
<td>[%]</td>
</tr>
<tr>
<td>$R_t$</td>
<td>Retention of flavor component i</td>
<td>[%]</td>
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<tr>
<td>$RI$</td>
<td>Retention index</td>
<td>[-]</td>
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<tr>
<td>$S_p$</td>
<td>Pore surface area</td>
<td>[m$^2$]</td>
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<tr>
<td>(V)</td>
<td>Volume of water in the pores</td>
<td>[m³/g]</td>
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<tr>
<td>(V_A)</td>
<td>Adsorbed gas volume (plateau value)</td>
<td>[cm³/g STP]</td>
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<tr>
<td>(V_0)</td>
<td>Total pore volume</td>
<td>[cm³/g]</td>
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<tr>
<td>(V_p)</td>
<td>Pore volume</td>
<td>[m³]</td>
</tr>
<tr>
<td>(V_v)</td>
<td>Void volume</td>
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<tr>
<td>(x)</td>
<td>Displacement</td>
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<td>(x_0)</td>
<td>Initial mass fraction</td>
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<tr>
<td>(x_{i0})</td>
<td>Initial mass fraction of component (i)</td>
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</tr>
<tr>
<td>(x_{\text{init}})</td>
<td>Total initial mass fraction</td>
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**Greek Letters:**

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<td>(\gamma)</td>
<td>Gyromagnetic constant</td>
<td>([1/(T \cdot s)])</td>
</tr>
<tr>
<td>(\delta)</td>
<td>Length of gradient pulse</td>
<td>[s]</td>
</tr>
<tr>
<td>(\Delta)</td>
<td>Time between gradient pulses (observation time)</td>
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<tr>
<td>(\Delta \delta)</td>
<td>Chemical shift</td>
<td>[ppm]</td>
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<td>(\Delta \nu)</td>
<td>Line width at half height of the Fourier transformed signal</td>
<td>[Hz]</td>
</tr>
<tr>
<td>(\Delta m)</td>
<td>Total mass loss of flavor-laden sample by TGA</td>
<td>[mg]</td>
</tr>
<tr>
<td>(\varepsilon_b)</td>
<td>Void fraction between particles</td>
<td>[-]</td>
</tr>
<tr>
<td>(\varepsilon_p)</td>
<td>Particle porosity</td>
<td>[-]</td>
</tr>
<tr>
<td>(\theta)</td>
<td>Temperature</td>
<td>[K]</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Ratio of molecular radius to pore radius</td>
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<tr>
<td>(\lambda_{\text{surf}})</td>
<td>Surface thickness parameter</td>
<td>[m]</td>
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<td>(\mu)</td>
<td>Solvent dynamic viscosity</td>
<td>[kg/(m s)]</td>
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<td>(\rho_p)</td>
<td>Particle density</td>
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<td>(\tau)</td>
<td>Time between r.f. pulses</td>
<td>[s]</td>
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<td>(\tau_b)</td>
<td>Bed tortuosity</td>
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<td>(\tau_p)</td>
<td>Pore tortuosity</td>
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1. Encapsulation Techniques: A General Overview

Abstract

Encapsulation is a relatively new technology that is used for protection, stabilization and controlled release of pharmaceuticals, enzymes and food ingredients, for example flavors and nutraceuticals. This introduction provides a general overview of different encapsulation techniques with the focus on sol-gel encapsulation. The current state of research in the field of sol-gel encapsulation is summarized for microorganisms, enzymes, pharmaceuticals and other organic molecules such as flavors.

1.1. Physical encapsulation methods

1.1.1. Spray drying

Spray drying is the oldest and most commonly used encapsulation method in the food industry (Giunchedi and Conte, 1995). This process is economical and flexible and delivers dry particles of good quality (Risch, 1995). Although most often spray drying is considered as a dehydration process, it can be used as an encapsulation process entrapping “active materials” within a protective matrix formed from a polymer (Shahidi and Han, 1993). In spray drying processes an aqueous solution or emulsion of the core material and the carrier is atomized by a nozzle or a spinning wheel into the hot drying air. The quality of the emulsion is decisive, since the creation of a finer emulsion increases flavor retention (Risch, 1995). Hot air flowing either in a co-current (Figure 1.1.) or counter-current direction contacts the atomized droplets, evaporates the water and produces dried particles. Co-current drying conditions lead to a more gentle drying of the product and are therefore the preferred configuration. Dried particles and air are separated by a cyclone or a filter. Several factors influence the product quality including the nature of the carrier and the active component, the in-feed viscosity and concentration, the drying temperature and the emulsion droplet size besides atomization conditions and the direction of the drying air. Research has shown, that the in-feed concentration seems to be the major factor influencing flavor retention. Increasing
the solids contents seems to benefit flavor retention (Reineccius and Coulter, 1969). One main disadvantage of this process however is, that it produces a very fine powder, which in some cases needs further processing by agglomeration or compaction.

![Diagram of spray drying](image)

**Fig. 1.1: Spray drying**

### 1.1.2. Spray chilling and spray cooling

Spray chilling or spray cooling are similar to spray drying regarding process steps, however, the core material is dispersed in a liquefied lipid carrier. Consequently this technique is attractive for water-soluble materials like minerals, vitamins and enzymes. The core and wall mixture are atomized in either cool or chilled air, which causes the wall to solidify around the core. The two methods differ only in the melting point of the wall material (spray chilling: 32-42 °C; spray cooling: 45-122 °C). With the ability to select the melting point of the wall, these methods can be used for controlled release (Risch, 1995).
1.1.3. Spray granulation

Spray granulation is, in analogy to spray drying and spray cooling, a matrix encapsulation process, where the active ingredient is integrated within the carrier matrix. In most of the cases the granulation is started with seed particles from spray drying at the beginning of the granulation while the fines are recycled from the filter during the course of the process. The particles are suspended by means of a fluidized bed. The circulation of the hot gas flow and the particles within the fluidized bed is realized by different opening sizes of the dispersion plate. The granulation liquid is dispersed by an atomization nozzle located either at the top or at the bottom of the fluidized bed. The particles grow in thin layers as the droplets are deposited on the particle surface and dried in the hot fluidizing air. The desired particle size fraction can be removed by means of an integrated classifier (Uhlemann and Mörl, 2000).

1.1.4. Spray coating or fluidized bed coating

Spray coating is a core-shell encapsulation process in contrast to the matrix encapsulation obtained by spray drying and spray granulation. The experimental set-up, however, is similar to the one of spray granulation. The main difference is, that the coating liquid is different from the core material. The latter is often a product of spray drying and is suspended in an upward moving air flow that can be heated or cooled. This upward moving air stream is generated by the dispersion plate. The holes in the center of the plate are bigger than at the outside creating an upward flow in the center of the plate and a downward flow at the outside. The coating material is either dissolved in a liquid or in the molten state. The coating liquid is dispersed by an atomization nozzle located either above or within the fluidized bed (Wurster process). During coating a thin layer is deposited around the fluidized particles and in some cases an integrated classifier removes the desired particle size fraction from the fluidized bed (Uhlemann and Mörl, 2000). Several parameters influence the quality of the coating like fluidizing air flow and temperature, atomization conditions and substrate and coating characteristics like attrition and film-forming ability (DeZarn, 1995).
1.1.5. Extrusion

Encapsulation by extrusion involves dispersion of the core material in a molten carbohydrate carrier matrix. This mixture is then forced through a die into a dehydrating bath which hardens the material. The most common liquid for the hardening process is isopropyl alcohol. The extruded filaments are broken into pieces, separated from the liquid bath and dried. The advantage of this process is that extrusion provides a true encapsulation process, since all core material is surrounded by the carrier matrix and residual surface oil is removed in the dehydration step. This absence of surface oil and the complete encapsulation result in a good shelf life stability (Shahidi and Han, 1993).

1.1.6. Freeze drying

Freeze drying is the desired encapsulation process for heat sensitive products like pharmaceuticals, flavors and enzymes. However, production costs are comparably high due to long dehydration times in the batch mode (Shahidi and Han, 1993). The encapsulation matrix is cooled down until all the water is frozen. As the pressure is reduced below the sublimation pressure, the frozen water is directly sublimed. Retention of flavor molecules was shown to be increased with decreasing molecular weight of the carbohydrate and increasing solids content (Flink and Karel, 1970).

1.1.7. Centrifugal extrusion

The centrifugal extrusion process is a liquid coextrusion process utilizing nozzles consisting of two concentric orifices located on the outer circumference of a rotating cylinder. The liquid core material is pumped through the inner orifice and the shell material through the outer one. The forming filaments consist of core material surrounded by a shell, which break into smaller capsules. Parameters to control capsule size and production rate are: nozzle size, rotational speed, feed rates and fill. As the capsules leave the nozzle, they are in the liquid state and have to be hardened before further processing. Depending on the shell material, the
capsules can be collected by a liquid reaction bath by simple cooling, solvent evaporation or coacervation (Schlameus, 1995).

1.1.8. Rapid expansion of supercritical solutions (RESS)

The RESS-process is a technology that produces small particles (< 0.1-225 µm) with a narrow particle size distribution and it offers interesting applications especially for hydrophobic and heat-sensitive organic compounds. The extraordinary solvating properties of supercritical fluids for non-volatile compounds are used at high pressures. The rapid expansion of a supercritical solution through a nozzle leads to a large cooling rate, resulting in high supersaturation with homogeneous nucleation and particle growth. An increasing number of newly developed pharmaceutical substances are poorly soluble in both aqueous and organic media. Thus, the application of oral or injectable drugs is often limited by its low bioavailability. An alternative and promising method to improve the bioavailability of pharmaceutical agents is the production of nanoscale particles by the rapid expansion of supercritical solutions (RESS). However, the disadvantage of this process is, that the low solubility of non-volatile solutes in supercritical solutions limits the output of RESS products and until now typical values are less than a kilogram per day. Due to its highly sophisticated and expensive set-up, this process is only interesting for expensive products with low production rates like proteins and pharmaceuticals (Turk, 1999).

Microencapsulation of proteins using this process can be realized by the rapid expansion of a supercritical solution with a nonsolvent (RESS-N). There a polymer is suspended in a first step in the cosolvent (polar organic solvent such as ethanol) and the polymer and the protein are suspended in supercritical CO₂. As the two suspensions are mixed together, the polymer is dissolved and the protein is still insoluble in the mixture of supercritical CO₂ and the polar organic solvent. During RESS, the polymer will precipitate and coat the protein microparticles. The advantage of the process is that neither toxic surfactants nor undesirable organic solvents are used. The disadvantages, however, are the same as in the RESS process (Mishima et al., 2000).
1.2. Chemical encapsulation methods

1.2.1. Interfacial polycondensation

Interfacial polymerization involves a reaction of two different monomers by condensation polymerization at the interface of two immiscible phases. The resulting polymer film will be a polyester, polyurea, polyurethane or a polycarbonate. Microcapsules obtained by interfacial polymerization range from 1-200 µm. Usually two reactive monomers are employed, one dissolved in the aqueous disperse phase containing a solution or a dispersion of the core material, and the other dissolved after the emulsification step in the nonaqueous continuous phase. Since most polymers used in interfacial polymerization are not food grade, this technique is investigated for its application in the field of microencapsulation of pharmaceuticals, enzymes and cosmetics. Despite the considerable interest in medical applications, very few applications have been exploited commercially. This arises from toxicity problems associated with unreacted monomers of the resulting polymer, excessive drug degradation caused by reaction with the monomer, high permeability of the formed coating especially for low-weight core material, fragility of the microcapsules and the lack of biodegradability. Polyamide encapsulation has been widely investigated in the field of enzyme encapsulation, since enzymes are high-molecular weight substances with low diffusion coefficients. But these substances tend to undergo chemical reactions resulting in inactivation of the enzyme. Despite the addition of protection substances, Chang (1971) reported that often more than 50% of the enzyme activity is lost upon encapsulation. Further product formulation is done by the removal of the residual moisture and organic solvent by spray-, vacuum or freeze drying. However, during this step the initially spherical particles may become irregular and clump together.
1.3. Physicochemical encapsulation methods

1.3.1. Phase separation or coacervation

Due to the small particle size (1-2000 μm) of coacervation products and the lack of toxicity of the coating materials this technique is widely applied in the pharmaceutical and cosmetic field. Since this process is carried out in a moderate temperature level, it has been studied in the field of immobilization of enzymes and other biological molecules. Coacervation is a very effective, but expensive process especially because of the costs of recovery of the microcapsules. After the coacervation step itself, subsequent washing, hardening, filtration and drying steps e.g. spray or freeze drying are necessary, that make this process only attractive for high-price products. Furthermore, the controllability of the particle size is very poor and the microcapsules tend to stick together to form agglomerates, particularly during subsequent hardening and drying stages (Deasy, 1984).

The scheme of the process of coacervation is shown in Figure 1.2. In coacervation a colloidal dispersion can be caused to separate into colloid-rich and colloid poor regions by careful control of the temperature, pH, addition of electrolyte (compression of the double-layer), addition of a second polymer or supplement of a non-solvent. The coacervate or colloid region form as droplets, that make the system opaque and sediment to form a separate lower layer unless prevented by stirring. The deposition of the coating material is aided by a reduction in total interfacial energy of the system. In this step the core droplets act as nuclei for the deposition of the coating. Coacervation can be distinguished into two types: simple and complex coacervation. In simple coacervation phase separation is induced by a temperature, a pH shift or by the addition of an electrolyte thus decreasing the solubility of the system. Complex coacervation occurs with the mutual neutralization of two oppositely charged polymers an in aqueous solution. (e.g. gelatin and gum acaica; alginate and chitosan; chitosan and polyphosphates). Gelatin and acaica are a most frequently used system. Since acaica contains only free carboxylic groups, it always carries a negative charge except at very low pH-values. Pig-skin gelatin, which is produced at a high isoelectric point (8.0 to 8.5) carries a positive net charge and leads to neutralization upon mixing, which is accompanied by loss of bound water by the polymers. Concerning the applicability for biological systems Hsu and Chu (1992) have reported that it is more suitable for cell viability to have a core
consisting of an anionic polymer e.g. carrageenan, carboxymethyl-cellulose, alginatedextran sulfate or a mixture of these two polymers. The cationic counterpart may be poly-L-lysine (PLL), polyethyleneimine (PEI), chitosan, DEAE-dextran or polyvinylamine (Wang et al., 1992).

1.3.2. Inclusion complexation

Cyclodextrins are chemically and physically stable molecules formed by the enzymatic modification of starch. They have the ability to form complexes with a variety of organic substances and include compounds that fit dimensionally within their cavity. Due to the hydrophobic nature of this cavity, molecules of suitable size, shape and hydrophobicity are able to interact noncovalently with the cyclodextrins to form stable complexes (Shahidi and Han, 1993). Several kind of forces such as van der Waals forces, hydrophobic interaction and dipole-dipole interaction are involved in the binding of guest molecules with the cavity of the cyclodextrin (Hedges et al., 1995). These forces are sufficiently strong to form a stable complex, but are not so strong that the guest molecule can not be released from the complex. β-cyclodextrin is the most readily available cyclodextrin and most work is therefore done using β-cyclodextrin as a complexing agent.
1.3.3. Liposome entrapment

A liposome (or lipid vesicle) is defined as a structured compound of lipid bilayers that enclose a number of aqueous or liquid compartments. The liposome may be composed of a single bilayer or hundreds of bilayers depending on its composition and means of manufacturing (Reineccius, 1995). Liposomes are prepared from phospholipids such as egg yolk or soybean lecithin. They form spontaneously, when the phospholipids are properly placed in an aqueous environment. Therefore, the problems in the manufacture of liposomes relate to the formation of the desired size and structure of the vesicles and their efficient loading. Virtually any substance, regardless of solubility, electrical charge and molecular size can be incorporated into liposomes, provided that the substance does not interfere with liposome formation (Gregoriadis et al., 1993). Water soluble materials will be incorporated in the aqueous phase of liposomes, whereas lipid-soluble materials will be incorporated into the lipid phase.

![Molecular organization in liposome entrapment](image)

Fig. 1.3: Molecular organization in liposome entrapment (left); electron micrograph of a liposome (right) (Shahidi and Han, 1993).

1.3.4. Multiple emulsions (solvent evaporation process)

The solvent evaporation process is a technique mostly applied in the encapsulation of pharmaceuticals. There are two different systems: either a w/o/w-emulsion for the entrapment of water soluble species or an o/w/o-emulsion for oil-soluble core materials. In the case of a w/o/w system an initial w/o emulsion is formed, that is in turn emulsified in an aqueous
solution. The resulting microcapsules are in the form of a triple wall structure. The choice of the hydrophilic polymer is much wider including e.g. gum acacia, sodium alginate, Eudragit, gelatin, PVP, polyethylene glycol, dextran or polyacrylic acid derivatives as Duquemin (1987) demonstrated. Whereas the choice of the lipophilic polymer (e.g. ethyl cellulose) is limited. In order to form the w/o emulsion, the aqueous solution or dispersion of the drug is emulsified into a hydrophobic polymer, that is dissolved in a water-immiscible solvent (e.g. methylene dichloride, ethyl acetate) having a boiling point below the one of water. The initial w/o emulsion is again emulsified into an aqueous solution of a hydrophilic colloid to get a w/o/w emulsion. Upon raising the temperature, the organic solvent will evaporate causing phase separation of the hydrophobic polymer as a coating around the inner aqueous droplets. The dispersion will be spray dried and the microcapsules will be incorporated in the matrix. The different stages of the preparation of multiple emulsion systems are explained in Figure 1.4.

**Fig. 1.4: Stages in the formation of microcapsules from multiple emulsions**
1.3.5. Sol-gel encapsulation

The sol-gel process is in principle a phase separation process resulting in a ceramic material by preparation of a sol, gelation of the sol and removal of the solvent. This process has the unique feature of producing inorganic glasses at lower temperatures than is possible using conventional melting techniques. Depending on the preparation procedure dense oxide particles or polymeric clusters will be obtained. The sol (colloidal suspension of solid particles in a liquid) may be produced from inorganic and organic precursors (e.g. nitrates or alkoxides). It generally involves the use of metal alkoxides M(OR)n (where M can be Si, Al, Ti and R represents an organic group), which undergo hydrolysis and condensation reactions to form a gel. Because water and alkoxy silanes are immiscible, a mutual solvent such as alcohol is normally used as a homogenizing agent. However, gels can be prepared from silicon-water mixtures without added solvent, since alcohol produced in the hydrolysis reaction is sufficient to homogenize the initially phase separated system (Brinker and Scherer, 1990). The formation of silica glass, for example, can be realized by hydrolysis of Si(OCH₃)₄ (TMOS or tetramethyl orthosilicate) followed by condensation reactions to yield a polymeric oxo-bridged SiO₂ network. In the first hydrolysis step, the Si-OCH₃ bonds are converted to Si-OH, which condense together to form the oxo-bridged Si-O-Si structure like it is shown in the following reactions:

**Hydrolysis:**

\[
\begin{align*}
\text{Si(OR)}_4 + \text{H}_2\text{O} & \rightarrow \text{HO-Si(OR)}_3 + \text{ROH} \\
\text{Si(OR)}_4 + 4 \text{H}_2\text{O} & \rightarrow \text{Si(OH)}_4 + 4 \text{ROH}
\end{align*}
\]

**Condensation:**

\[
\begin{align*}
(\text{OR})_3\text{Si-OH} + \text{HO-Si(OR)}_3 & \rightarrow (\text{OR})_3\text{Si-O-Si(OR)}_3 + \text{H}_2\text{O} \\
(\text{OR})_3\text{Si-OR} + \text{HO-Si(OR)}_3 & \rightarrow (\text{OR})_3\text{Si-O-Si(OR)}_3 + \text{ROH}
\end{align*}
\]
First these reactions take place at localized regions, where silica polymerizes in stages to nuclei of silica, which then lead to the formation of sol particles of the size of 2-3 nm (a suspension containing these colloidal particles is called sol). As polycondensation continues in the case of an acid hydrolysis the degree of cross-linking between the particles (5-10 nm or smaller) increases. The resulting viscous material solidifies and leads to the formation of a porous gel. In this step the solution temperature is a major factor determining gelation time. Increasing the gelation temperature leads to a more rapid gelation (Brennan et al., 1999).

In the case of an alkaline hydrolysis (in the absence of salt) a particulate sol is obtained. The ionization of the polymer species is much higher, so that the monomer polymerizes and decreases in concentration very rapidly. However, no aggregation or chain formation occurs due to the highly charged surfaces that repel each other provided that the salt concentration is low. If salt is present during an alkaline hydrolysis the compression of the double layer leads to the aggregation of the dense particles as can be seen in Figure 1.5.

The gel derived from an acid hydrolysis consists of a two-phase system comprising the inorganic solid and the trapped solvent phase. During the subsequent aging process the formation of new Si-O-Si bonds due to further polycondensation reactions results in gel-shrinkage expelling solvent from the pores. Air dried gels are called xerogels, where the evaporation of the pore liquid results in shrinkages of approximately 1/8 of the initial wet gel volume due to pore collapse. At this point gel to glass conversion occurs.

![Diagram](image_url)

Fig. 1.5: Difference in acid and alkaline catalyzed hydrolysis in the generation of silica sol-gel (Brinker and Scherer, 1990).
1.3.5.1. Aspects of biocompatibility and toxicity

Biocompatibility is defined as the ability of biomaterial to perform with an appropriate host response in a specific application. The goal is to produce protective materials, which can be smoothly integrated into living systems instead of fighting them. In view that silica is present in natural water and foods, it is not surprising, that it is considered harmless in industrially manufactured food matrices and drinks. It is therefore considered to be a safe additive in foods and pharmaceuticals. At least in small amounts, the ingestion of amorphous silica appears to be completely harmless since it dissolves to give only monomer and finally will be excreted in this form (Iler, 1979).

The idea to encapsulate microorganisms in silica can be directly deduced from nature itself. Silica is distributed in many living organisms and is supposed to have even played a role in the existence of life. It was found that silica was adsorbed and incorporated by several biological species like mammalian cells, amphibians, plants, viruses, bacteria, fungi, sponges and algae. The diatome, one group of algae, even impregnated its walls with silica and deposit it as an external supportive structure. The major mechanism of the precipitation of silica on earth is considered to be of a biochemical nature. Benefits arising from the incorporation are attributed to strengthening of the structure of the biological species. Also is has been reported by several authors, that silica incorporated in plants increases the resistance to fungus diseases (Iler, 1979). Therefore silica structures seem to be highly compatible with biological materials, which in some cases may even form skeleton silica structures around them as a self-support.

An excellent biocompatibility of silica sol-gels has also been shown in the entrapment of Langerhans cells and the successful transplantation into diabetic mice. These islets have proved to be viable and to release insulin for several weeks without adverse reaction (Pope, 1995a; Pope, 1995b). However, for the application in food and pharmaceutical science the influence and biocompatibility of possible residual precursor has not yet been addressed, although sol-gel entrapment in silica monoliths has been suggested and even patented for the above applications (Böttcher and Slowik, 1998; Böttcher et al., 1999a; Böttcher et al., 1999b).
1.3.5.2. Encapsulation of biological molecules

Combining novel materials with biological science is currently one of the most innovative topics of research. Recent modified sol-gel techniques enable the encapsulation of bioactive components within an inorganic layer. The resulting glasses allow the transport of small molecules into and out of the glasses at reasonable rates but retain the protein molecules within their pores (Zink et al., 1994). General studies to date indicate, that depending on the precursors and protocols used, proteins with molecular weights of 8000-15000 Da can be irreversibly encapsulated in sol-gel glasses (Gill and Ballesteros, 2000). Enzymes, catalytic antibodies, DNA, RNA, antigens, live bacterial, fungal, plant and animal cells have been entrapped in silica, metal-oxide, organo-siloxane and hybrid sol-gel polymers (Table 1.1). The living ceramics are easy to separate and can be used over and over again.

Conventional immobilization techniques such as covalent attachment result in chemical modifications of the proteins. In contrast, sol-gel immobilization is characterized by physical entrapment without any chemical modification and conformational changes (Edminston et al., 1994; Lan et al., 1999). Studies carried out by Margolis and Harley (1961) investigating the denaturation of proteins adsorbed on silica surfaces showed, that denaturation was found to increase with increasing size of the colloidal particles that were added to the system. It was observed that when the individual silica particles are very small, the molecular segments of the protein could be attached to different particles without the segment being stretched. This study shows the potential benefit of applying nanostructured systems to the field of encapsulation. It is in agreement with observations that these bioactive ceramics retain their conformational, chemical and physical properties, displaying activities approaching or in some cases even exceeding those in free solution (Akbarian et al., 1997; Dave et al., 1996; Dave et al., 1997).

Kinetic studies show, that enzymes entrapped in sol-gel particles perform the same enzymatic reactions as in free solution and that, in many cases, enhanced activities have been observed (Gill and Ballesteros, 2000). Bio-doped sol-gels function in gaseous, liquid (aqueous, aqueous-organic, low-water organic), solid-liquid, subcritical and supercritical environments. The conversion of carbon dioxide to methanol by an enzymatically coupled reduction of three different dehydrogenases revealed that the yield of methanol production is substantially increased (up to 14 fold) in the case of sol-gel encapsulated enzymes when
compared to those in free solution. This feature is attributed to the nanoconfinement of the protein (Obert and Dave, 1999). Reetz and coworkers achieved up to 88 times higher enzyme activities of entrapped lipase in a hydrophobic inorganic-organic hybrid material derived from TEOS and organically modified silanes (ormosils) compared to the non-immobilized species (Reetz et al., 1996b; Reetz, 1997). Those enzymes catalyze the hydrolysis of esters with formation of carboxylic acids and alcohols. Both Reetz and Brennan et al. (1999) showed, that lipophilic biomolecules, such as lipases, are stabilized by organically doped hydrophobic sol-gel materials, whereas they did not remain functional in polar matrices derived solely from TEOS. Avnir and coworkers have proven that even multienzymatic reactions can be performed in a single sol-gel matrix (Avnir et al., 1994). However, due to their matrix structure and the resulting diffusion of both substrate and product it has been shown frequently, that the reaction kinetics is slowed down in the silica matrix compared to the reaction of the enzyme in free solution (Zink et al., 1994; Dave et al., 1995). Encapsulated enzymes showed Michaelis-Menten kinetics, but the values of the Michaelis-Menten constant $K_m$ of the encapsulated enzymes were higher than those of the enzymes in solution indicating the presence of partitioning and diffusional effects in the pores of the matrix (Dave et al., 1995; Bhatia and Brinker, 2000).

Furthermore, an increased stability and robustness against thermal and chemical denaturation of the protein upon sol-gel entrapment was observed in numerous studies (Avnir et al., 1994).

Sol-gels doped with antibodies were shown to retain their ability to bind free antigens from aqueous solution so that even immunoassays could be carried out in a sol-gel matrix (Turniansky et al., 1996; Livage et al., 1997).

In conclusion, these novel bioactive layers offer interesting new applications for e.g. biocompatible coatings on implants and medical products (Hench, 1998), the preparation of biosensors (Dave et al., 1994) and biocatalysts (Reetz et al., 1996a; Obert and Dave, 1999).

1.3.5.3. Encapsulation of organic molecules and controlled release effects

The ability to entrap organic molecules into oxide matrices using the sol-gel technique also offers new and interesting perspectives for obtaining particles with controlled release effects. Such systems could be used for different therapeutic and antibacterial depot systems, where
the metal oxide is only an inert carrier for the diffusible compound (Böttcher, 2000). Only a small number of studies have been carried out in this field, yet. For example the incorporation of pharmaceuticals like nifedipin (Böttcher and Slowik, 1998) and the release of perfumed essences and oils from hybrid SiO₂ lyogels, xerogels and films for cosmetic and even food application (Böttcher et al., 1999a; Böttcher et al., 1999b; Caturan et al., 1997) have been studied so far. From this it was suggested that the sol-gel matrix is applicable as a reservoir system for the encapsulation of flavors and fragrances for the long-term aromatization of food, cosmetics or textile fibers. Although Bolton and Reineccius (1992) showed when plating flavors on various carriers, that amorphous silica was a more effective matrix compared to conventional organic flavor carriers both regarding flavor retention and oxidative protection, not much research has been carried out in this field. This work is therefore dedicated to elucidate the retention performance and mechanisms of sol-gel-made silica matrices and to provide an understanding of the diffusion and the release kinetics of the entrapped compound.

1.4. Discussion

In comparison to the other conventional encapsulation techniques, sol-gel encapsulation is a relatively new process. It has been widely studied in the field of enzyme encapsulation and is, up-to date, the most efficient encapsulation method for enzymes due to its nanoparticulate nature. These findings in combination with enhanced shelf-life properties of amorphous silicas were the motivation for studying the retention and release properties of these materials in more detail for organic flavor molecules in this work.
Table 1.1: Examples for sol-gel encapsulation.

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<td>Coupled enzyme reaction</td>
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<tr>
<td>Oxalate oxidase</td>
<td>Prevention of cracking and elimination of swelling of the hydrogel</td>
<td>Yamanaka et al. (1996)</td>
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<td>G-6-P dehydrogenase</td>
<td>Kinetic studies (Michaelis-Menten)</td>
<td></td>
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<tr>
<td>Peroxidase</td>
<td>Selective intake and release of proteins in matrices with designed pore structure</td>
<td>Rao and Dave (1998)</td>
</tr>
<tr>
<td>Oxalate oxidase</td>
<td>Study of electrostatic effects</td>
<td>Shen and Kostic (1997)</td>
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<tr>
<td>Peroxidase</td>
<td>Largest entrapped enzyme: 100 Å</td>
<td>Lan et al. (1996)</td>
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<tr>
<td>Coupled enzyme reaction</td>
<td>Crystallinity of Fe oxide cores remained</td>
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<td>Ferritin</td>
<td>Gelation time studies and activity studies of trypsin/acetylated trypsin as a function of pH</td>
<td>Braun et al. (1992)</td>
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<td>Trypsin</td>
<td>Review: Heme proteins, Thermal denaturation, pH studies (gel/solution), Self specific pore design</td>
<td>Lan et al. (1999)</td>
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<td>Cholinesterase</td>
<td>Spectroscopic characterization of protein conformation</td>
<td>Edminston et al. (1994)</td>
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<td>Bovine serum albumin (BSA)</td>
<td>Spectroscopic characterization of protein conformation</td>
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<td>Horse heart myoglobin</td>
<td>Diffusivity studies in hydro-and xerogels</td>
<td>Tang and Dave (1998)</td>
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<tr>
<td>Bovine serum albumin (BSA)</td>
<td>Spectroscopy studies in solution, aged and dried gels</td>
<td>Zink et al. (1994)</td>
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<td>Alkaline phosphatase</td>
<td>Mesoporous matrix via glucose templating, Increased diffusion and activity</td>
<td>Wei et al. (2000)</td>
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<td>Carbonmonoxy-myoglobin</td>
<td>Denaturation studies (unfolding/refolding)</td>
<td>Samuni et al. (2000)</td>
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| **Lipase** | **Enhanced activity in hybrid hydrophobic sol-gel material (up to 88 times higher)** | **Organic catalyst** | Reetz et al. (1996a)  
Reetz et al. (1996b)  
Reetz (1997) |
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<td><strong>Activity studies: Effects of dopants (PEG/PVA)</strong></td>
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<td>Keeling-Tucker et al. (2000)</td>
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<tr>
<td><strong>Tryptophan</strong></td>
<td><strong>Kinetic studies</strong></td>
<td><strong>Mobility studies</strong></td>
<td>Zheng et al. (1997)</td>
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</table>

**Antibodies/Antigens**

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<tr>
<th><strong>Monoclonal anti-atrazine antibodies</strong></th>
<th><strong>ELISA of pesticides (atrazine)</strong></th>
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<th>Bronshtein et al. (1997)</th>
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<tr>
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<td><strong>ELISA of pesticides (atrazine)</strong></td>
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<td>Turniansky et al. (1996)</td>
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| **Anti-trinitrotoluene antibodies** | **Immunassays**  
**Stability studies** | | Lan et al. (2000) |
| **Antigen: cell parasites** | **Preservation of cellular organization**  
**Immunoenzymatic reaction: ELISA** | | Livage et al. (1996) |
| **Echinoccosis antigens** | **Immunoenzymatic reaction: ELISA** | | Livage et al. (1997) |

**Other Microorganisms**

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<tr>
<th><strong>Yeast</strong></th>
<th><strong>Accumulation of metal ions</strong></th>
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<th>Al-Saraj et al. (1999)</th>
</tr>
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| **Sulfate reducing bacteria** | **Maintained viability**  
**Cell division and growth in gel in the presence of nutrient** | | Bartlett and Woolfrey (2000) |
| **Aspergillus terreus** (fungus) | **Elucidation of biosynthetic pathway** | | Bressler and Braun (1996) |
| **Escherichia coli** | **Preservation of cellular organisation**  
**Michaelis-Menten kinetics (decreased activity in xerogels)** | | Fennough et al. (2000) |
| **E. coli bacteria** | **Gel characterization as a function of PEG concentration, aging time, temperature of prehydrolyzed sol and hydrolysis ratio** | | Conroy et al. (2000) |
| **Pseudomonas** | **Atrazine degradation**  
**Loss of activity** | | Kauffmann and Mandelbaum (1996) |
| • Pseudomonas ADP | • Atrazine degradation  
| | • Addition of nutrient partially restored activity  
| | Rietti-Shati et al. (1996)  
| • Plant cells | • Immobilization due to adsorption on gel  
| | • Maintenance of viability  
| | Campostrini et al. (1996)  
| • Microbial cells | • Phenol biodegradation  
| | • PCB biodegradation  
| | • No cell growth in silica  
| | Branyik et al. (1998)  
| • Yeast Cells | • Heavy metal bioabsorbent  
| | Kuncova et al. (1998)  
| • Pancreatic islet | • Successful in vitro and in vivo tests  
| | Pope (1995a)  
| • Saccharomyces cerevisiae | • Bioactivity studies  
| | Pope (1995b)  
| Other |  
| • Organic liquids (flavours) | • Release studies  
| | Böttcher et al. (1999b)  
| • Biocides | • Food/cosmetic preservation (packaging material)  
| | Böttcher et al. (1999a)  
| • Nifedipine | • Drug delivery / controlled release  
| | Böttcher and Slowik (1998)  
| • Bioactive gel glasses  
| (SiO₂-CaO-P₂O₅) | • Apatite formation  
| | • Bone growth  
| | • Osteoproduction  
| | • Implant  
| | Wheeler and Greenspan (1998)  
| • Steroids | • Impregnation of sol-gel samples with steroids  
| | • Diffusion studies (model and experiment)  
| | Sieminska et al. (1997)  
| • Liposomes | • Enhancement of sensitivity to metal ions  
| | Yamanaka et al. (1997)  

1.5. References


2. Encapsulation and Retention of Decanoic Acid in Sol-Gel-Made Silicas

Abstract

Porous sol-gel-made silica particles are investigated as encapsulation matrices for controlled release of substances in food and pharmaceutical applications. Here the retention performance of an entrapped model flavor, decanoic acid, inside silica matrices made by hydrolysis of tetraethyl orthosilicate (TEOS) is studied. The retention of decanoic acid is measured by gas chromatography and thermogravimetric analysis. The morphology, specific surface area, porosity and pore size distribution of the particle matrix is controlled by the sol-gel preparation method. During drying, the weakly cross-linked polymers in a slowly hydrolyzing silica gel deform and wrap around flavor molecules creating a denser gel structure. There the flavor molecules are entrapped more efficiently than in fast hydrolyzing matrices that result in more porous particles. Kinetic annealing studies show that flavor molecules are entrapped quite efficiently even at high temperatures.

2.1. Introduction

Sol-gel-made materials can be used as encapsulation matrices for different chemical (Böttcher et al. 1999a) and even biological species (Avnir et al. 1994). These materials have applications as biocompatible coatings on implants and medical products (Hench and West 1990), biosensors (Dave et al. 1994), biocatalysts (Reetz et al. 1996), coatings for controlled release of biocides (Böttcher et al. 1999b), pharmaceuticals and vitamins. Of particular interest here are matrices for encapsulation of nutraceuticals, flavors and fragrances for the aromatization of food, cosmetics, packaging materials or textile fibers (Böttcher et al. 1999a). Silica matrix characteristics like porosity and pore size distribution affecting compound retention and controlled release can be selected in the manufacturing process (Brinker et al. 1982). Furthermore, Bolton and Reineccius (1992) showed an improved retention and oxidative protection of flavor molecules using silica matrices in comparison to conventional organic ones.
Böttcher and Slowik (1998) embedded the coronary therapeuticum nifedipin in porous sol-gel-made matrices and showed that its release in water can be influenced either by particle size or partial substitution of the precursor TEOS with methyl-triethoxysilane or copolymerisation with polyethylene glycol. Release of the preservative benzoic acid from thin silica films was enhanced by coentrainment with penetration agents like polyethylene glycol 10000 and sorbitol (Böttcher et al. 1997). Retinol, a cosmetic ingredient, was entrapped in sol-gel-made silica particles using an oil/water/oil (o/w/o) multiple emulsion by Lee et al. (2001) who studied encapsulation efficiency and release kinetics. Caturan et al. (1997) investigated the kinetic release of perfumed essences (e.g. menthol) in alkyl-modified silicon alkoxides by thermogravimetric analysis and discrepancies in release kinetics were mainly attributed to differences in matrix porosity and chemical interactions of the organic molecules with the matrix.

Typically in sol-gel encapsulation silica nanoparticles surround the flavor molecules during gel formation. In principle, the sol-gel process can be considered as a phase separation resulting in a ceramic material by sol-reactions, sol-gelation and, finally, removal of the solvent. Depending on preparation, dense oxide particles or polymeric clusters will be obtained (Hench and West 1990). The sol (colloidal suspension of solid particles in a liquid) may be produced from inorganic and organic precursors (e.g. nitrates or alkoxides). Generally metal alkoxides M(OR)n (where M can be Si, Al, Ti and R is an organic group) undergo hydrolysis and condensation reactions to form a gel. As water and alkoxy silanes are immiscible, a mutual solvent such as alcohol is normally used as a homogenizing agent (Brinker and Scherer 1990). In acid hydrolysis, silica forms initially by precursor hydrolysis followed by polycondensation reactions to yield a polymeric oxo-bridged SiO2 network (Hench and West 1990). Condensation and hydrolysis take place at localized regions where silica polymerizes in stages to nuclei of silica, which then lead to the formation of sol particles of 2-3 nm in diameter. At this stage flavor molecules can be added to the sol. As polycondensation continues, the degree of cross-linking between particles (5-10 nm or smaller) increases and flavor molecules will be embedded in the arising network structure. The resulting viscous material solidifies and leads to the formation of a porous gel. Increasing the gelation temperature enhances gel formation. During the subsequent aging process, the formation of new Si-O-Si bonds by further polycondensation reactions results in gel-shrinkage expelling solvent from the pores (Dunn et al. 1998). Sols made with a lower hydrolysis ratio exhibit shorter gelation times (Brinker and Scherer 1990).
The mechanisms of flavor retention during plating and adsorption on silica surfaces occur by physical and/or chemical adsorption (Iler 1979, Ringwald and Pemberton 2000). In physical adsorption, the formation of hydrogen bonds between the electronegative atoms of the adsorbed molecule and the hydrogen atoms of the silica silanol groups is primarily responsible for flavor adsorption. Compounds with oxygen electron donors, such as acids, ketones, ethers, alcohols and esters can therefore hydrogen-bond with surface hydroxyl groups (Bolton and Reineccius 1992). In chemical adsorption, a stronger bonding between the molecules and the surface is obtained by possible reaction between silanol and e.g. functional OH-groups of the flavor molecules (Hair 1967). Further mechanisms are adsorption at the surface of an adsorbed water film and dissolution into the adsorbed water (Ringwald and Pemberton 2000). During sol-gel encapsulation, flavor molecules are embedded into the silica matrix whose characteristics are determined by the hydrolysis ratio (molar ratio of water to TEOS), hydrolysis time and drying.

The focus of previous studies was on the release behavior rather than on the retention performance of sol-gel-made silica matrices which is the topic of this study. As a model flavor substance, decanoic acid (DA) was chosen. Blank and DA-laden silica sol-gel-made matrices with different pore size distributions and porosities were prepared by hydrolysis of TEOS and characterized by nitrogen adsorption and thermogravimetric analysis. The decanoic acid retention in these matrices was determined by extraction and subsequent GC-analysis and was related to matrix characteristics.

2.2. Experimental

2.2.1. Materials and preparation

Six standard protocols (Sol A-F) were used for flavor encapsulation (Table 2.1). Typically TEOS (Sigma Aldrich, Germany, 98%), absolute ethanol (Baker, Switzerland), deionized H₂O (18 MΩ·cm at 25°C) and 0.06M HCl (prepared from a 1M stock solution, Merck KGaA, Germany) were mixed under room temperature in the molar ratios given in Table 2.1. Hydrolysis was carried out for 48 hours. In order to induce gelation, the sol was neutralized with either a 0.09 M or 0.9 M NaOH-solution (prepared from a 1M stock solution, Merck KGaA, Germany).
Table 2.1: Sol-gel composition and powder characterization for DA encapsulation. The particle morphology of blank and corresponding DA-laden samples (after removal of DA) is characterized by nitrogen adsorption and the decanoic acid retention (initial load: 10 wt%) is determined by GC analysis. The blank samples are microporous (Type I isotherm), whereas the DA-laden particles possess micro- and mesopores (Type IV isotherm) due to DA entrapment.

<table>
<thead>
<tr>
<th></th>
<th>Sol A</th>
<th>Sol B</th>
<th>Sol C</th>
<th>Sol D</th>
<th>Sol E</th>
<th>Sol F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blank</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEOS, mol%</td>
<td>8.7</td>
<td>8.7</td>
<td>2.4</td>
<td>2.4</td>
<td>1.7</td>
<td>1.7</td>
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<tr>
<td>Ethanol, mol%</td>
<td>47.4</td>
<td>47.4</td>
<td>37.3</td>
<td>37.4</td>
<td>34.8</td>
<td>34.8</td>
</tr>
<tr>
<td>HCl, mol%</td>
<td>0.047</td>
<td>0.047</td>
<td>0.065</td>
<td>0.065</td>
<td>0.065</td>
<td>0.065</td>
</tr>
<tr>
<td>H₂O, mol%</td>
<td>43.9</td>
<td>43.9</td>
<td>60.3</td>
<td>60.3</td>
<td>63.4</td>
<td>63.4</td>
</tr>
<tr>
<td>NaOH, Molarity</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>H = [H₂O]/[TEOS]</td>
<td>5.1</td>
<td>5.1</td>
<td>25.3</td>
<td>25.3</td>
<td>37.9</td>
<td>37.9</td>
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<tr>
<td>Pore Volume, cm³/g</td>
<td>0.08</td>
<td>0.1</td>
<td>0.13</td>
<td>0.21</td>
<td>0.28</td>
<td>0.33</td>
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<tr>
<td>Porosity, %</td>
<td>15.4</td>
<td>18.5</td>
<td>22.4</td>
<td>31.5</td>
<td>38.2</td>
<td>42.1</td>
</tr>
<tr>
<td>SSA *, m²/g</td>
<td>156.2</td>
<td>188.1</td>
<td>283.2</td>
<td>403.7</td>
<td>541.8</td>
<td>602.6</td>
</tr>
<tr>
<td><strong>DA-laden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retention, %</td>
<td>75.38</td>
<td>60.35</td>
<td>62.65</td>
<td>52.22</td>
<td>32.09</td>
<td>33.14</td>
</tr>
<tr>
<td>SSA ***, m²/g</td>
<td>319.0</td>
<td>253.6</td>
<td>111.4</td>
<td>118.3</td>
<td>58.4</td>
<td>42.8</td>
</tr>
<tr>
<td>Porosity, %</td>
<td>37.4</td>
<td>49.2</td>
<td>34.7</td>
<td>44.1</td>
<td>43.1</td>
<td>52.8</td>
</tr>
</tbody>
</table>

* Powders are microporous.

** The SSA's and the porosities of the DA-laden samples may be affected by residual carbon that may not be fully removed from the sample upon heat treatment especially at low hydrolysis ratios.
TEOS was chosen as a precursor since ethanol is a non-toxic byproduct. At pH=6 decanoic acid (Sigma Aldrich, Germany, 99%) was added to the sol. For the blank sol A the gelation time was about 5 minutes, whereas sol F gelled within approximately 15 minutes. In the presence of DA, the gelation time decreased by about 50%. The gel was then dried in a vacuum oven at 40°C and 750 mbar for 24h (Böttcher et al. 1999b).

The initial mass fraction $x_0$ of DA for the flavor-loaded particles in the sol-gel matrix was determined by:

$$x_0 = \frac{m_{D0}}{m_{D0} + m_{S0}}$$

where $m_{D0}$ is the DA input mass and the amount of dry silica, $m_{S0}$, was calculated assuming complete hydrolysis of TEOS. Sol-gels A-F were prepared with an initial mass fraction of 10 wt% decanoic acid.

### 2.2.2. Characterization and instrumentation

The N$_2$-sorption isotherms of the powdered samples were obtained on a Micromeritics ASAP 2010 surface area and pore size analyzer (Micromeritics, Nocross, GA) at -196 °C (liquid nitrogen). All samples were degassed at 200°C and 0.003 mm Hg prior to the measurement. The surface and pore characteristics were calculated using Micromeritics software. To avoid any influence by helium adsorption on the nitrogen adsorption isotherm, the free space was measured in a separate experiment (Webb and Orr 1997). The specific surface area was determined by the multipoint BET method using adsorption data in the pressure range $(p/p_0)$ from 0.05 to 0.25. In the case of a type I isotherm, the pore volume and particle porosity can be calculated from the amount of gas adsorbed in the plateau region (Sing 1982). Assuming the ideal gas law with a liquid N$_2$ density of 0.808 g/cm$^3$, the pore volume $V_0$ is (Nair et al. 1997):

$$V_0 = 1.547 \cdot 10^{-3} \cdot V_A$$

where $V_A$ is the adsorbed gas volume in [cm$^3$/g STP]. With a silica density $\rho_p$ of 2.2 g/cm$^3$ (Iler 1979), the particle porosity $\varepsilon_p$ (Vacassy et al. 2000) is:
The morphology of the silica sol gel samples was examined using scanning electron microscopy (SEM, Model S-900, Hitachi Co., Ltd., Tokyo, Japan).

Thermogravimetric analysis was carried out in a thermobalance (TGA/SDTA 851°, LF/1100°C, Mettler Toledo AG). Three different temperature programs have been applied in this study: in the first, the silica powders (blank and flavor-loaded) were initially heated in pure oxygen flow (180 ml/min) from 30 to 1000 °C at 20 °C/min and then the powders were kept at 1000 °C for 15 min.. In the second, the samples were subjected to a constant temperature of 100 and 200 °C for about six hours in a nitrogen flow of 50 ml/min. In the third, the powders were first heated up to 120 °C at 10 °C/min in an oxygen flow of 180 ml/min, followed by an isothermal step at 120 °C for 30 min. to remove adsorbed water followed by heating at 20 °C/min up to 1000 °C where it was kept for 10 min.. The CO₂-concentration of the outlet gas from the TGA was measured with a Metrosoft aq-5000 CO₂ sensor (Metrosonics, Inc., Rochester, N.Y.). To guarantee a minimum CO₂ level in the TGA sample chamber at the beginning of the experiment, the measuring cell was flushed with oxygen (180 ml/min) at 30 °C for 30 min. to achieve a constant baseline (less than 30 ppm CO₂).

The flavor load of the samples was determined with a gas-chromatograph (GC, Varian Chemstation 6890, Darmstadt, Germany) equipped with a polar DB-FFAP column (J&W 123-3232, JW Scientific, Folsom, USA) and a flame ionisation detector (FID) at 250 °C. Helium (35 ml/min) was used as make-up gas. The injection technique was on column (40 °C) with a sample volume of 0.001 ml. The split injection mode was used with a ratio of 10:1. Initially the column was at 40 °C for 2 min. followed by an increase of 4 °C/min up to 180 °C and then by another increase of 10 °C/min to the final 240°C, where it was held for 10 min..

DA retention was determined by extraction and subsequent GC analysis. Since the silica matrix is not soluble, DA has to diffuse out of it. Depending on the initial flavor load, 0.2-0.5 g of sol-gel powder was taken for extraction (mₑ). As extraction media, 4 ml of deionized water and 20 ml of dichloromethane (DCM) were used with decanol as internal standard having a final concentration of about 0.1 mg/ml. The samples were extracted in 25 ml Pyrex bottles for 12 hours, though the extraction was already complete after two hours. In a subsequent step, the organic phase was collected and the residual water was removed with sodium sulfate before GC analysis. The calibration was carried out with known
concentrations of the pure DA (0.05-1.59 mg/ml) in DCM with an internal standard of a similar concentration. Calibration curves were linear for DA and its concentration was calculated according to the corresponding GC areas. The retention index (RI) of decanoic acid was measured to be 2346.3 based on alkane standard solutions and was comparable to a literature value for a polar FFAP column of 2265 (Baser et al. 2000). Each flavor-loaded sample A-F was prepared twice as well as the corresponding blank silica ones. Three extractions from each flavor loaded sample A-F were carried out and the extract was subjected to GC analysis four times. The same stock solution of internal standard was used throughout the study. A calibration curve consisting of at least five points was made before each run.

2.2.3. Encapsulation of decanoic acid in sol-gel-made particles

The sol-gel powder that was subjected to GC analysis \( m_E \) contains mainly dry silica \( m_{SE} \), water \( m_w \) and DA \( m_D \):

\[
m_E = m_{SE} + m_w + m_D \tag{2.4}
\]

The retention \( R_D \) of DA in the matrix is calculated according to:

\[
R_D = \frac{m_D / m_{SE}}{m_D / m_S0} \tag{2.5}
\]

where \( m_D \) is the mass of decanoic acid in the extraction sample determined by GC analysis and, \( m_{SE} \), the mass of the dry silica subjected to extraction which is determined by thermogravimetric analysis (TGA) of the DA-laden sample. The weight loss detected gravimetrically, \( \Delta m \), consists of the mass loss of water, \( m_w \), the mass loss of DA, \( m_D \), and of the thermal dehydroxylation, \( m_{OH} \), of the silica surface:

\[
\Delta m = m_D + m_w + m_{OH} \tag{2.6}
\]

During dehydroxylation, a condensation reaction between two surface silanol groups (\( \equiv Si-OH \)) results in one siloxane group (\( \equiv Si-O-Si\equiv \)). Fully hydroxylated silica contains 4.6
OH/nm² (Taylor et al. 1965). According to Vasant et al. (1995) this value can be considered as a physical constant regardless of the silica type and/or structure, e.g. pore size distribution or specific surface area. Thermal dehydroxylation leads to one residual OH/nm² at 800 °C and to 0.4 OH/nm² at 1000 °C (Curthoys et al. 1974). From the specific surface area SSA (Table 2.1) of the sol-gel-made powder and its change in OH-density during TGA, the contribution of thermal dehydroxylation, $m_{OH}$, to the total mass loss, $\Delta m$, can be quantified (Mueller et al. 2003). Knowing the mass of DA, $m_p$, from GC analysis, the mass of water in the sample can be determined by eq. 2.6. Hence, the mass of the dry silica, $m_{SE}$, can be obtained by eq. 2.4.

2.3. Results

2.3.1. Characterization of the blank silica sol-gel particles

Figure 2.1 represents a typical SEM micrograph of a blank silica powder made by an acid hydrolysis of TEOS according to the sol-gel protocol A (Table 2.1). In the polycondensation reactions a network structure is formed consisting of single nano-sized silica particles, in which flavor molecules can be entrapped.

Fig. 2.1: SEM micrograph of the blank sol-gel-made silica A (Table 2.1).
Figure 2.2 shows the nitrogen adsorption isotherms of the six blank sol-gel-made SiO$_2$ powders A-F.

All isotherms exhibit a Type I behavior, which is characteristic for a microporous solid with pore diameters less than 2 nm (Sing 1982). If a Type I isotherm exhibits a nearly constant adsorption at high relative pressure, the micropore volume and the porosity can be calculated according to eq. 2.2 and 2.3 (Table 2.1).
Figures 2.3 and 2.4 show cumulative (for all samples) and frequency (for samples B, D and F) pore size distributions using the Horvath-Kawazoe model (Horvath and Kawazoe 1983; Elferink et al. 1996). An increasing hydrolysis ratio (from A to F) increases the pore volume consistent with Lenza and Vasconcelos (2000) and Elferink et al. (1996). The latter report a comparable pore size distribution for a silica sol-gel with a hydrolysis ratio of 6.4.

**Fig. 2.3:** Cumulative specific pore volume of the blank silica powders calculated according to the Horvath-Kawazoe method (Horvath and Kawazoe 1983) (compare Table 2.1). The pore volume increased with increased hydrolysis ratio from A to F.
Fig. 2.4: Pore size frequency distribution calculated according to the Horvath-Kawazoe method (Horvath and Kawazoe 1983) for the samples B, D and F (Neutralisation: 0.09 M NaOH) specified in Table 2.1.

Differences in chemical structure of the silica surface groups may influence flavor retention by chemical and/or physical adsorption of the flavor molecules. Thermogravimetric analysis of the blank powders in combination with CO₂ detection in the outlet gas stream was used to characterize the powders regarding residual precursor and purity. Figure 2.5 shows the TGA analysis in oxygen atmosphere of the present blank silica samples A and F and silica powders R5 (made at a low hydrolysis ratio: H=5 containing products of incomplete reaction) and pure R1000 (made at a high hydrolysis ratio: H=1000) from Mueller et al. (2003). The TGA weight loss of the other blank samples (B-E) lays in between the samples A and F. Since the weight loss below 120 °C is attributed mainly to the desorption of water, the weight was normalized to 100% at the end of the isothermal step at 120 °C. The weight loss above 120 °C is ascribed to surface dehydroxylation and possible loss of residual precursor.
Fig. 2.5: Thermogravimetric analysis of the sol-gel samples A and F of this study and R5 and R1000 from Mueller et al. (2003). No large difference in weight loss above 120 °C can be seen in the samples A and F indicating a similar surface structure. The weight loss is comparable to the sol-gel-made pure silica powder R1000. Furthermore, CO₂ analysis of the off-gases of the sol-gel-made samples A-F did not show any degeneration of possible residual precursor. The TGA weight loss of the samples B-E lays in between those of A and F.

Silicas A-F do not reveal any significant differences in weight loss above 120 °C and are comparable to the weight loss of the sol-gel R1000 sample that did not show any impurities or volatile compounds when analyzing the TGA off-gases with a mass spectrometer or a CO₂ sensor (Mueller et al. 2003). Likewise no CO₂ was detected in the TGA off-gases from the blank sol-gel samples A-F indicating their purity. In contrast to the samples A and B (hydrolysis ratio: H=5.1), the sol-gel powder R5 from Mueller et al. (2003) exhibits a higher weight loss by degeneration of incomplete reaction products that were also detected by mass spectroscopy. Present samples A-F do not show this as they were hydrolyzed for 48 hours in contrast to the one hour hydrolysis time in the synthesis of R5 and R1000 by Mueller et al. (2003). Brinker and Scherer (1990) observed, that not only the hydrolysis ratio, but also the hydrolysis time is an important parameter affecting the constitution of the sol. Sols made at a
high hydrolysis ratio undergo fast hydrolysis, so one hour might not have been enough to complete the reaction for their R5 sol-gel sample (Song and Pratsinis 2000).

2.3.2. Flavor retention

Thermogravimetric analysis in combination with CO₂ measurements in the outlet gas of the TGA are presented in Figure 2.6 for the DA-laden sol-gel-made powder A.

The total weight loss and the corresponding sample temperature are shown. The first reduction in mass (below 120 °C) is mainly attributed to water loss, whereas the steep decline at about 263 °C (boiling point of decanoic acid) is ascribed to the loss of decanoic acid and the partial conversion to CO₂, since the decomposition of decanoic acid also results in CO and smaller hydrocarbon molecules. Below 263 °C decanoic acid seems to remain in the silica
matrix. The marginal decline in mass at high temperatures (> 600 °C) can be attributed to silica dehydroxylation.

Blank silica samples (without DA) show a total mass loss of about 13-20% (Figure 2.5) when heating them up to 1000 °C by TGA. Figure 2.7 shows kinetic annealing data of DA-laden powders A and D in a nitrogen flow at constant temperatures of 100 and 200 °C for about six hours. Annealing at 100 °C does not remove the water completely in the DA containing samples (total mass loss: 9.2 % for sample D and 7.9 % for sample A at 100 °C). The higher weight loss of the more porous sample D indicates, that DA is more stable in the denser sample A. Annealing at 200 °C does not reveal differences in weight loss between the samples A and D indicating that powder structure may not matter at these high temperatures.

![Graph showing mass loss over time](image)

**Fig. 2.7:** Mass loss measured by TGA-annealing of decanoic acid (DA)-laden powders A and D (Table 2.1) at 100 and 200 °C in a nitrogen flow (50 ml/min). At 100 °C decanoic acid (DA) is retained better (less mass loss) in the denser sample A. At 200 °C the matrix structure has little effect on DA loss.
Decanoic acid retention determined from powder extraction followed by GC analysis is shown in Table 2.1 and Figure 2.8. The GC reproducibility is within 4.5%, while the maximum error between single extraction samples is smaller than 13%. The corresponding error bars are shown in Figure 2.8. Powders E and F reveal the lowest retention, whereas the recovery in sample A is twice as high. Comparing these results with the corresponding blank powder porosities shows that a decrease in particle porosity enhances decanoic acid retention. Flavor losses in sol-gel encapsulation seem to take place mainly during the drying process. Hence a denser matrix seems to prevent diffusional losses especially during drying. About 25% of decanoic acid is lost in the denser sample A, whereas 70% of the initial flavor is lost during drying in the more porous sample F.

Fig. 2.8 Decanoic acid retention (GC-analysis) in various silica powders (Table 2.1) as a function of porosity of the corresponding blank powders. Flavor retention is increased with decreasing porosity indicating that a denser matrix seems to prevent diffusional losses especially during drying. Furthermore, the polymers in a slowly hydrolyzed sol-gel are still able to deform during drying entrapping the flavor molecule more efficiently compared to coarser gels made by fast hydrolysis of TEOS.
To study the pore morphology of the different silica powders containing decanoic acid, these samples were degassed at 300 °C for 16 hours. In Figure 2.9 the nitrogen adsorption isotherms of the sol-samples A and F after DA removal (filled symbols) are compared to the ones of the corresponding blank samples (open symbols).

![Graph showing adsorption isotherms](image)

*Fig. 2.9: Adsorption isotherms of blank (open symbols) sol-gel powders A and F and of the corresponding ones loaded with 10 wt% decanoic acid (DA) (filled symbols) after DA removal by heating for 16h at 300 °C. In contrast to the blank adsorption isotherms, the DA-laden powders exhibit a type IV isotherm after DA removal. The hysteresis loop in the DA-laden samples indicates mesopores in addition to micropores at p/p₀ < 0.2. Porosity and average pore size are increased upon entrapment. Sample A with a higher retention capacity (Figure 2.7) shows a bigger porosity and pore volume increase compared to sample F. Hence flavor molecules act as a template for silica forming around them by condensation reactions.*

In contrast to the nitrogen adsorption isotherms of the blank samples, the DA-laden samples exhibit a type IV isotherm after DA removal. The hysteresis loop in the DA-laden samples is an indication of the presence of mesopores in addition to the micropores at low relative pressures (Sing 1982). Furthermore, the pore volume and porosity are increased significantly upon DA entrapment in comparison to the corresponding blank samples. Sample F has a
porosity of 52.8% (corresponding blank sample: 42.1%) and sample A 37.4% (corresponding blank sample: 15.4%). This porosity increase upon entrapment of sample A is significantly bigger than in sample F for its increased retention capacity (Figure 2.8). The average BJH desorption diameter (Song and Pratsinis 2000) of sample F is about 5.6 nm and the one of sample A is around 3.0 nm. The increased porosity and average pore size of sample F result in higher diffusional losses leading to a lower retention performance compared to sample A (Figure 2.8). Hence, the DA molecules seem to act as a template, where silica is forming around during condensation reactions. This is consistent with sol-gel enzyme encapsulation (Dave et al. 1996), where the protein structure does not seem to be influenced by the particle formation. The enhanced stability of enzymes in such materials is attributed to the fact that the enzyme designs self-specific pores in the silica network according to its size and shape requirements (Lan et al. 1996).

2.4. Discussion

2.4.1. Particle morphology

The structures obtained from slowly and rapidly hydrolyzed gels respond differently to the removal of solvent during drying, since condensation reactions become even more important in this step. Upon removal of the solvent, both the individual polymer units comprising the gel and the gel structure itself may shrink. This permits additional condensation reactions within and between polymers. Faster drying rates generally result in denser consolidated structures (Delange et al. 1995). Powders E and F were prepared at the highest hydrolysis ratios (Table 2.1). The hydrolysis reaction is therefore rapid and the resulting polymers are reported to be larger and highly cross-linked. Upon impingement, the silica polymers will not deform as readily to fill the voids nor will they shrink as much by solvent removal from within the pores. Consequently the gel dries to a more or less random packed array of identifiable particles with large voids in between (Brinker et al. 1982). This is in agreement with the increased pore volume and porosity (Table 2.1, Figures 2.3 and 2.9) at higher hydrolysis ratios. However, in slowly hydrolyzed, acid catalyzed gels prepared at low hydrolysis ratios strong surface forces are generated by the removal of the solvent between the polymers (Brinker et al. 1982). As the weakly cross-linked polymers impinge on one
another, they deform readily and form a dense gel structure in agreement with the porosity and pore volumes in Table 2.2 and in Figures 2.3 and 2.9. Therefore conditions of slow hydrolysis result in fine gel features that dry to a high bulk density (low porosity) while fast hydrolysis develops coarser gels, that dry to a low bulk density (higher porosity) (Brinker et al. 1982).

2.4.2. Influence of the particle morphology on decanoic acid retention and stability

The major mechanism of flavor retention on silica is physical adsorption primarily by hydrogen bonding between the hydrogen atoms of the silanol groups and electronegative atoms or molecules with free π electrons (Iler 1979; Bolton and Reineccius 1992). However, the investigations in this study show, that an additional mechanism may take place incorporating decanoic acid in microporous silica sol-gel-made particles, which is the nanoconfinement of the silica network structure employing a capillary pressure on the flavor molecule thus increasing the total retention performance of the matrix as the porosity is decreased (Figure 2.8).

During the drying process the weakly cross linked polymers in a slow-hydrolyzing sol-gel are reported to still deform around the flavor molecule creating a dense gel structure (Brinker et al. 1982), where the flavor is entrapped more efficiently in contrast to the fast-hydrolyzing types, where the structure is more coarse and open (Figures 2.8 and 2.9). TGA analysis in combination with CO₂ detection in the TGA outlet gases of all sol-gel silica powders shows no big difference in weight loss and thus surface structure, that might have influenced flavor retention.

Furthermore, treating the flavor loaded powders at 100 and even 200 °C indicates that DA remains quite stable in the silica matrix, since the total weight loss is well below the water loss of a blank silica sample heated up to 1000 °C in the non isothermal TGA protocol. This agrees well with the observations made by Bolton and Reineccius (1992) that a flavor would not deteriorate or be lost as quickly when plated on amorphous silica as with traditional organic food carriers.
2.5. Conclusions

Decanoic acid retention in porous silica sol-gel-made matrices can be influenced by their particle characteristics that are controlled by the hydrolysis ratio during preparation. As the porosity is decreased, the flavor molecules experience an additional nanoconfinement within the porous network structure resulting in an enhanced retention by capillary forces and a decreased diffusional loss during drying. Furthermore, the polymers in a slowly hydrolyzed sol-gel are still able to deform during drying entrapping the flavor molecule more efficiently compared to coarser gels made by fast hydrolysis of TEOS. Possible influence on decanoic acid retention by residual precursor was investigated by TGA-analysis and a similar surface structure was found in all blank samples. Analysis of the TGA off-gases by a CO2 sensor showed that the powders are reasonably pure indicating that the particle structure is a major factor influencing retention. Decanoic acid remains quite stable in the sol-gel-made silica matrix, even treating the decanoic acid loaded powders at modest temperatures (100-200 °C) for a prolonged time. Therefore these inert materials seem to be attractive carrier systems for the food and pharmaceutical industry so that retention performance and release kinetics can be closely controlled.

2.6. References


3. Flavor Retention in Sol-Gel-Made Silica Particles

Abstract

The retention performance of flavor molecules from different chemical classes (e.g. alcohols, esters, aldehydes and terpenes) is investigated in silica particle matrices made by hydrolysis of tetraethyl orthosilicate (TEOS). Since particle morphology, porosity and pore size distribution can be controlled by the sol-gel preparation method, the influence of the nanoconfinement in the microporous matrix on flavor retention is studied as well as the effect of initial flavor load of the particles. As the porosity is decreased, flavor molecules are entrapped more efficiently in the silica particles. The retention performance decreased in the order of: alcohol > aldehyde ≥ ester > terpene consistent with the retention behavior of conventional organic matrices. In contrast to these matrices, open sol-gel-made silica particles show an increased retention with increasing flavor load while denser silica matrices show a maximum retention with increasing load.

3.1. Introduction

Porous sol-gel-made particles are used as encapsulation matrices for controlled release in the food and pharmaceutical industry. Of special interest in this study are matrices for encapsulation of nutraceuticals, flavors and fragrances for the aromatization of food, cosmetics, packaging materials or textile fibers (Böttcher et al., 1999). Sol-gel-made silica particles differ significantly from organic carriers due to their inorganic, inert and thus biocompatible nature (Iler, 1979). The incorporation of organic molecules is a typical matrix encapsulation process, where the silica nanoparticles surround the molecules during the formation of the gel (Brinker and Scherer, 1990).

Enhanced retention and oxidative protection of flavor compounds was observed in plating experiments by Bolton and Reineccius (1992) when using silica rather than conventional organic flavor encapsulation matrices. Although the exact protection mechanism of silica is uncertain, it is believed to be a result of hydrogen bonding between the flavor molecules and silica, physical protection from oxygen within the active sites and uptake of
singlet oxygen by silica. Furthermore, Zeller et al. (1999) highlighted the potential of microporous materials like porous carbohydrates as effective adsorbent flavor carriers. As a consequence, microporous sol-gel-made silicas are investigated here more closely as encapsulation matrices for various flavor compounds.

A basic introduction into traditional encapsulation techniques for flavor molecules is given by Risch and Reineccius (1995) and by Shahidi and Han (1993). In general, two concepts of flavor retention are discussed: selective diffusion in glassy matrices (Menting et al., 1970; Thijssen, 1971) and entrapment in the so-called microregions (Flink and Karel, 1970). In conventional carrier systems like maltodextrin-based materials, the glass transition of the material affects flavor retention. The objective is to entrap the flavor in a glassy matrix that inhibits its molecular mobility. Once in the glassy state, amorphous carriers exhibit very low permeation rates and the amount of released flavor depends upon the chemical composition of the matrix, the pore and particle size, the size of the flavor reservoir pockets (microregions) entrapped within the particles and the wall thickness around these areas. Smaller water molecules will be able to diffusive selectively, whereas the bigger, organic molecules, that are more volatile than water, are retained preferentially. In the concept of entrapment within microregions, the flavor molecules are associated with the carrier matrix and each other by hydrogen bonding to form complexes within these microregions (Whorton, 1995). There, the influence of physicochemical properties of volatiles and carbohydrate carrier is a decisive factor for flavor retention (Goubet et al., 1998). The nature of functional groups, molecular weight, polarity and volatility of the flavor compound are key parameters in determining retention performance. Generally, the retention of high molecular weight molecules is favored. Molecular weight and size are linked and smaller molecules diffuse more easily through the matrix during drying compared to bigger molecules. Retention seems to decrease in the order of: alcohols > ketones ≈ esters > acids for carbohydrates (Goubet et al., 1998), which was also confirmed by Boutboul et al. (2002) for different types of starch carriers. However, exceptions to this indicate that other physicochemical parameters besides chemical functionality are relevant e.g. polar molecules are retained less than non-polar ones and compounds with a higher relative volatility are lost preferentially. Overall, the retention of volatiles depends therefore on their steric hindrance, polarity, functionality and relative volatility aside from the nature of the carrier matrix (Arvisenet et al., 2002). Reineccius and Coulter (1969) investigated the influence of spray-drying operating conditions on retention performance and the in-feed flavor concentration was identified as one of the major parameters. An increase in flavor load resulted in a decreased retention performance.
Therefore these physicochemical parameters and the influence of flavor load are also investigated here to study the retention performance of silica carriers in comparison to conventional organic matrices.

Typically, a flavor consists of several chemical compounds that result in the desired smell and taste. Upon entrapment, however, this flavor composition may change by differences in retention of the single compounds. This change in flavor composition has to be accounted for prior to designing an appropriate encapsulation matrix. Therefore, this study focuses on the retention performance of typical flavor molecules like alcohols, aldehydes, esters and terpenes to unravel the mechanisms of entrapment in sol-gel-made silica particles and to compare their performance with conventional carriers. As a consequence, the retention of a homologous series within these chemical classes was investigated. Furthermore, the silica particle characteristics were controlled by the sol-gel preparation method (Brinker et al., 1982). The effect of flavor molecule nano-confinement on retention performance was studied as well as the effect of initial flavor load.

### 3.2. Experimental

#### 3.2.1. Materials and synthesis

Four standard protocols (Sol A-D) were used in this study for flavor encapsulation (Table 2.1) and the sols were prepared as described in paragraph 2.2.1. At pH=6 the flavor mixture was added to the sol and after neutralization gelation took place. The obtained gel was dried in a vacuum oven at 40 °C and 750 mbar for 24h. Blank and flavor loaded particles were prepared accordingly.

Table 3.1 shows physicochemical properties of the investigated flavors. The single flavor compounds were received from Fluka (Switzerland). For each chemical class (esters, aldehydes, alcohols and terpenes) an artificial mixture with equal proportions of each component for every homologous series (e.g. alcohol) was prepared. For example when alcohol was used as a flavor it contained 1/3 by weight of butanol, octanol and decanol. The initial mass fraction $x_{io}$ for each flavor component $i$ in the sol-gel matrix was determined by:
\[ x_{i0} = \frac{m_{i0}}{\sum_{i} m_{i0} + m_{S0}} \]  

(3.1)

where \( m_{i0} \) is the input mass of each flavor component and \( m_{S0} \) the dry amount of silica, that was calculated assuming complete hydrolysis of the precursor. The total initial flavor mass fraction \( x_{\text{tot}} \) is defined accordingly:

\[ x_{\text{tot}} = \frac{\sum_{i} m_{i0}}{\sum_{i} m_{i0} + m_{S0}} \]  

(3.2)

Flavor-laden silicas A-D were prepared with total initial mass fractions \( x_{\text{tot}} \) ranging from 5 to 50 wt%. The hydrophobic terpenes were scarcely soluble in the sol and an emulsion was obtained for initial mass fractions exceeding 5 wt%. So the maximum initial load was just 10 wt%.

**Table 3.1: Physicochemical properties of the flavor molecules.**

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Flavor Component</th>
<th>Molar Weight g/mol</th>
<th>Boiling Point °C</th>
<th>Oil/Water Partition Coeff. ( K_{ow} ), -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Butanol</td>
<td>74.12</td>
<td>117.7</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Octanol</td>
<td>130.23</td>
<td>195.1</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>Decanol</td>
<td>158.29</td>
<td>231.1</td>
<td>4.57</td>
</tr>
<tr>
<td>Ester</td>
<td>Ethyl butanoate</td>
<td>116.16</td>
<td>121.5</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>Ethyl hexanoate</td>
<td>144.22</td>
<td>167.0</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td>Ethyl octanoate</td>
<td>172.27</td>
<td>208.5</td>
<td>3.81</td>
</tr>
<tr>
<td></td>
<td>Ethyl decanoate</td>
<td>200.32</td>
<td>241.5</td>
<td>4.79</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>Hexanal</td>
<td>100.16</td>
<td>131.0</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>Octanal</td>
<td>128.22</td>
<td>171.0</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>Decanal</td>
<td>156.27</td>
<td>208.5</td>
<td>3.78</td>
</tr>
<tr>
<td>Terpene</td>
<td>Myrcene</td>
<td>136.23</td>
<td>167.1</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Limonene</td>
<td>136.23</td>
<td>176.0</td>
<td>4.57</td>
</tr>
<tr>
<td></td>
<td>Linalool</td>
<td>154.25</td>
<td>197.0</td>
<td>2.97</td>
</tr>
</tbody>
</table>
3.2.2. Characterization and instrumentation

The N$_2$-sorption isotherms of the powdered samples were obtained on a Micromeritics ASAP 2010 surface area and pore size analyser (Micromeritics, Nocross, GA) at -196°C (liquid nitrogen) as described in paragraph 2.2.2.

Thermogravimetric analysis was carried out in a thermobalance (TGA/SDTA 851e, LF/1100°C, Mettler Toledo AG). The silica powders (blank and flavor-laden) were initially heated in a pure oxygen flow (180 ml/min) from 30 to 1000 °C at a rate of 20 °C/min. In a second isothermal step the powders were kept at 1000 °C for 15 min (Mueller et al., 2003).

The flavor load of the samples was determined with a gas-chromatograph (GC, Varian Chemstation 6890, Darmstadt, Germany) equipped with a polar DB-FFAP column (J&W 123-3232, JW Scientific, Folsom, USA) and a flame ionisation detector (FID) at 250 °C. The GC parameters and extraction procedure is described in paragraph 2.2.2. The calibration curves, consisting of at least five points, were linear for all flavor components shown in Table 3.1. The concentrations of each component were calculated according to the corresponding GC areas taking concentration and area of the internal standard into account. Each flavor-laden sample A-D was prepared twice as well as the corresponding blanks. Three extractions from each flavor-laden sample were carried out and the extract was subjected to GC analysis four times. The same stock solution of internal standard was used throughout the study.

3.2.3. Encapsulation of flavor

The total mass of the sol-gel powder that is subjected to GC analysis ($m_E$) consists of:

$$m_E = m_{SE} + m_w + \sum_i m_i$$

(3.3)

where $m_i$ is the mass of the flavor component in the extraction sample determined by GC analysis, $m_w$ the mass of water and $m_{SE}$ the mass of dry silica subjected to extraction which is determined by thermogravimetric analysis (TGA) of the flavor-laden sample. The flavor retention of each component $R_i$ in the matrix is:
\[ R_i = \frac{m_i / m_{SE}}{m_{r0} / m_{s0}} \]  

(3.4)

where the subscript 0 stands for the initial conditions. The weight loss of the flavor-laden sample, \( \Delta m \), detected gravimetrically is given by:

\[ \Delta m = \sum_i m_i + m_w + m_{OH} \]  

(3.5)

where \( m_{OH} \) is the mass loss attributed to thermal dehydroxylation of the silica surface. From the specific surface area SSA (Table 2.1) of the sol-gel-made powder and its change in OH-density during TGA, the contribution of thermal dehydroxylation, \( m_{OH} \), to the total mass loss, \( \Delta m \), can be quantified (Mueller et al. 2003). Knowing the mass of all flavor compounds, \( m_i \), from GC analysis, the mass of water in the sample can be determined by eq. 3.5. Hence, the mass of the dry silica, \( m_{SE} \), can be obtained by eq. 3.3.

3.3. Results

Table 2.1 shows the pore volume, porosity and the SSA measured by nitrogen adsorption of the four blank sol-gel-made SiO₂ powders A-D. All isotherms exhibit Type I behavior, which is characteristic of microporous solids, where the average pore size does not exceed 2 nm (Webb and Orr, 1997). Samples C and D, made at a high hydrolysis ratio (\( H = 25 \)), exhibit a higher specific pore volume and therefore a higher porosity compared to A and B, made at \( H = 5 \) (Table 2.1) consistent with Lenza and Vasconcelos (2000) and Elferink et al. (1996). The porosity increase is 45% (A, C) and 70% (B, D).
3.3.1. Flavor retention and influence of particle morphology

3.3.1.1. Alcohol flavors

A flavor mixture containing butanol, octanol and decanol in equal proportions was encapsulated in the silicas A-D (Table 2.1) with total initial flavor loads ranging from 5 to 50 wt%. Figure 3.1 shows the average alcohol retention as a function of blank silica porosity for four different initial loads.

Fig. 3.1: Average alcohol retention in silicas A-D (Table 2.1) as a function of the blank porosity for total initial loads of 5, 10, 20 and 50 wt%. The average alcohol retention increases with increasing alcohol load but it decreases with increasing particle porosity. However, at the lowest porosity (15%), retention is hardly affected by load.

The average retention increased with increasing initial load and decreases with increasing porosity for all silicas. This is in contrast to conventional carbohydrate matrices, where the flavor retention generally decreases as the load increases (Reineccius and Coulter, 1969). Especially for lower initial loads the retention is significantly increased with decreasing porosity as alcohol adsorption to the silica surface becomes more pronounced by an increased...
nanoconfinement in the pore space. For an initial load of 5 wt% the retention is enhanced about five-fold by halving the porosity. This trend, however, diminishes at higher loadings indicating that the pore space could be too confined. Figure 3.2 shows the nitrogen adsorption isotherm of the densest blank sample A and the corresponding flavor loaded sample after removal of the alcohol. This sample was initially loaded with 50 wt% alcohol that was removed by annealing for 16 hours at 300 °C. It can be seen, that the pore volume increased drastically upon alcohol entrapment. During encapsulation, flavor molecules seem to act as a template, over which silica polymer chains are forming. The total accessible pore space for encapsulation is therefore 0.70 cm³/g (eq. 2.2) corresponding to a porosity of 62%. However, an initial alcohol load of 50 wt% would occupy about 1 cm³/g assuming 100% retention. Hence, the available pore space is not sufficient to incorporate the total amount of flavor and therefore the retention is not affected by load anymore at lower porosities.

Fig. 3.2: Nitrogen adsorption isotherms of the densest blank sample (circles, sample A in Table 2.1) and the corresponding one (squares) after removal of the alcohol by annealing for 16 hours at 300 °C. The latter sample was initially loaded with 50 wt% alcohol. The pore volume is increased drastically upon alcohol entrapment.
To compare the retention behavior of each alcohol component, Figure 3.3 shows their retention as a function of the blank silica porosity for an initial load of 20 wt% (open symbols). Smaller molecules like butanol are retained rather poorly, whereas the bigger molecules, octanol and decanol, are retained far better. The increase in retention with reduced powder porosity can be seen for both butanol and octanol while decanol deviates from this trend at low porosities. Possible reasons could be either that bigger molecules are less well entrapped since they are squeezed out more easily during the gel shrinking process or that they are no longer fully extracted from the denser particles. At lower initial loadings (5 wt%; filled symbols) this behavior is not that pronounced, since for all alcohols an increase in retention with decreased particle porosity can be observed. In general, a decreased particle porosity and an increased flavor load lead to an enhanced alcohol retention. The retention pattern at a total initial load of 50 wt% (not shown) is similar to that at 20 wt%. The maximum alcohol content of the particles was up to 37 wt% (sample A, initial load 50 wt%) corresponding to an average retention of about 50 wt%.

**Fig. 3.3: Alcohol component retention in silicas A-D (Table 2.1) as a function of the blank porosity for a total initial load of 20 wt% (open symbols) and 5 wt% (filled symbols). Smaller molecules like butanol are retained rather poorly in contrast to bigger ones. An increased retention with reduced porosity is seen for all alcohols for an initial load of 5 wt%. At an initial load of 20 wt% this trend is seen for the smaller molecules butanol and octanol. However, decanol shows a decreased retention in the densest sample at high loadings.**
3.3.1.2. Ester flavors

In the ester series a flavor mixture containing ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate (Table 2.1 and 3.1) in equal weight proportions was entrapped in the sol-gel-made silica samples A-D for total initial flavor loadings of 10, 20 and 50 wt%. Figure 3.4 shows that the average ester retention increases with decreasing blank silica porosity.

![Graph showing ester retention vs silica porosity](image)

Fig. 3.4: Average ester retention in silicas A-D (Table 2.1) as a function of blank porosity for total initial flavor loadings of 10, 20 and 50 wt%. An increase in retention performance is observed with a decreased porosity. A drop in the total retention is seen at high loadings in the densest sample.

Again a drop in the total retention is observed at high loadings in the densest sample. Figure 3.5 shows the retention of each ester component at an initial load of 20 wt%. Here again, the general trend is observed that, except for ethyl decanoate in the densest sample, bigger molecules with lower volatility (Table 3.1) are retained preferentially and that the denser pore structures increase drastically the retention performance. This is also seen in samples with total initial loads of 10 and 50 wt% (not shown). However, small molecules like ethyl butanoate could only be recovered to a small extent in the densest sample. The maximum ester load of 20 wt% was reached in the densest sample with an initial load of 50 wt%.
Fig. 3.5: Ester component retention in silicas A-D (Table 2.1) with a total initial flavor load of 20 wt%. A decrease in particle porosity increases the retention performance for all esters. Bigger molecules with a lower volatility are retained preferentially.

3.3.1.3. Aldehyde flavors

Aldehydes (hexanal, octanal and decanal) were entrapped in a flavor mixture of equal proportions in sol-gel-made silicas A-D for initial loadings of 5, 10 and 20 wt%. In sols with a higher aldehyde content the flavor was not soluble anymore. Figure 3.6 also shows that the average aldehyde retention increases with decreasing blank porosity, especially in the sample with an initial load of 5 wt%. Figure 3.7 shows the retention behavior of each aldehyde component at an initial load of 20 wt%, which is similar to samples with an initial load of 5 and 10 wt%. For all three components a decreased blank porosity results in an increased retention. Large molecules of low volatility (Table 3.1) are retained better than smaller ones as the former stay preferentially in the matrix during drying. Furthermore, bigger molecules possess lower diffusion coefficients that retard diffusional losses during drying. A maximum aldehyde load of about 5 wt% was reached in the densest sample with an initial load of 20 wt%.
Fig. 3.6: Average aldehyde retention in silicas A-D (Table 2.1) as a function of the blank porosity for total initial loadings of 5, 10 and 20 wt%. A decrease in porosity results in an enhanced retention.

Fig. 3.7: Aldehyde component retention in silicas A-D (Table 2.1) as a function of the blank porosity for a total initial load of 20 wt%. An increase in retention was observed for all aldehydes with a decreased particle porosity. Bigger aldehyde molecules with lower volatility are retained preferentially.
3.3.1.4. Terpene flavors

Terpenes (myrcene (methylene-7-methyl-1,6-octadiene), limonene (1-methyl-4-(1-methylethenyl)cyclohexene) and linalool (3,7-dimethyl-1,6-octadien-3-ol)) were entrapped in the sol-gel-made silicas A-D (Table 2.1) of various blank porosities for total initial flavor loadings of 5 and 10 wt%. Terpenes are hydrophobic molecules, especially myrcene and limonene, that do not possess any polar groups. Hence an emulsion was obtained in the case of an initial flavor load of 10 wt%. In Figure 3.8 the average retention of the terpenes in sol-gel silicas is shown as a function of the blank porosity.

![Figure 3.8: Average terpene retention in silicas A-D (Table 2.1) as a function of the blank particle porosity for total initial flavor loadings of 5 and 10 wt%. An increase in retention can be observed with decreasing particle porosity. However, in the case of terpene entrainment an emulsion was obtained for initial loads exceeding 5 wt%.](image)

For all initial loads the average retention can be increased with decreasing porosity. However, the average retention is only slightly increased with increasing flavor load in highly porous samples, whereas a decline in retention with increasing initial load can be noticed in the denser samples. But one should not forget, that an emulsion was already obtained in the case...
of an initial flavor load of 10 wt%. The emulsion droplets are probably less well retained than single dissolved molecules especially in low porosity samples. In Figure 3.9 the retention of the single terpene compounds is presented. Myrcene and limonene as hydrophobic molecules are nearly not retained in contrast to linalool, that possesses a hydroxyl group, which can bind to the hydrophilic silica surface. In the case of linalool, a decreased particle porosity increased its retention in agreement with the other chemical series.

Fig. 3.9: Terpene component retention in silicas A-D (Table 2.1) as a function of the blank particle porosity for a total initial load of 5 wt%. The hydrophobic molecules myrcene and limonene are barely retained in contrast to linalool, which possesses a hydroxyl group, that can hydrogen-bond to the hydrophilic silica surface. A decreasing particle porosity enhances linalool retention.
3.4. Discussion

3.4.1. Influence of particle morphology

The above results show that flavor retention in porous silica sol-gel carriers can be influenced by the blank powder porosity and hence by the hydrolysis ratio during their preparation (Table 2.1). As the porosity is decreased, the flavor molecules are believed to experience an additional nanoconfinement within the microporous network structure resulting in an enhanced retention by capillary forces and a decreased diffusional loss during drying. Furthermore, the silica polymers in slowly hydrolyzing sol-gels (e.g. A and B) are still able to deform during drying and entrap the flavor molecules more efficiently compared to more grainy, fast-hydrolyzing sols like samples C and D made at high hydrolysis ratios (Brinker et al., 1982). Therefore in the microporous particles A and B an additional retention mechanism is expected to take place. This is the nanoconfinement of the silica network applying a capillary pressure on the flavor molecules that increases the retention performance of the matrix as porosity is decreased. This was also observed by Smirnova et al. (2003), who entrapped organic, pharmaceutical molecules in aerogels. The importance of the microstructure to improve flavor retention is furthermore discussed by Zeller et al. (1999), Seuvre et al. (2000) and Boutboul et al. (2002). The presence of a functional group, however, that can either form a chemical or physical bond with silica, seems to be a prerequisite for flavor retention. The terpenes myrcene and limonene are nearly not retained due to their hydrophobic nature since they are not able to form any bond with the hydrophilic silica surface. Boutboul et al. (2002) investigated the influence and nature of different organic types of carrier materials (e.g. starches and maltodextrin). These hydrophilic materials show the same retention pattern as inorganic silicas regardless of the type of starch used. Molecules with an increased polarity and thus an increased tendency to form hydrogen bonds with the starch matrix are retained preferentially.
3.4.2. Influence of chemical nature

The influence of the chemical class of the entrapped component is explored by comparing the sol-gel-made powders with the highest and lowest porosity. In Figure 3.10 a comparison of the retention performance of the different chemical classes in the most porous sample D is shown at an initial total load of 10 wt%. The retention of the homologous series is presented as a function of the molecular weight of the single components. It can be clearly seen that alcohols are retained best for molecules with equal molar weight. Alcohols are able to chemically react with the silica surface (Hair, 1967). In this chemisorption or condensation reaction the two hydroxyl-groups react under separation of water to form a stable bond. Furthermore, hydrogen bonding between the hydroxyl-groups can lead to a physisorption of the molecules on the silica surface (Maier, 1972; Boutboul et al., 2002).

Fig. 3.10: Flavor retention in the most porous powder (sample D) as a function of the molecular weight of the employed species (Table 3.1) for a total initial load of 10 wt%. Increasing molecular weight enhances retention nearly linearly indicating the influence of adsorption in the samples. Alcohol molecules (circles) are retained best, whereas the hydrophobic terpenes (diamonds) are retained rather poorly.
Esters and aldehydes both possess a carboxyl- or carbonyl- group, respectively, which are quite similar in nature. They are both able to form hydrogen bonds with the silica surface, and hence their retention performance is quite similar. However, aldehydes are also able to form hemiacetals with the silica surface, which may explain their slightly improved retention performance in comparison to the esters. The terpenes myrcene and limonene (same molecular weight) are non-polar molecules that cannot form any bond to the silica surface resulting in a rather poor retention. Linalool (MW=154 g/mol), however, possesses a hydroxyl group that can both chemically and physically adsorb to the silica surface resulting in a better retention than the other terpenes.

For alcohols, esters and aldehydes the retention performance is nearly linear with molecular weight. This is a general indication of physical adsorption between the molecules due to van der Waals forces, where every additional CH₂ group contributes to the energy of adsorption (Atkins, 1998).

![Graph showing flavor retention in the densest powder (sample A) as a function of the molecular weight of the employed species (Table 3.1) for a total initial load of 10 wt%. In comparison to Figure 3.10, the retention of all components is higher in the denser sample A than in sample D. However, a drop in retention performance of bigger molecules can be seen for dense powders.](image-url)
In Figure 3.11 the retention of the components of each chemical class is shown as a function of molecular weight for a total initial load of 10 wt% in the most dense silica matrix (sample A). The retention in the denser matrix (Figure 3.11) is better than in the more porous samples (Figure 3.10) for all species that can either chemically and/or physically adsorb to the silica surface. Furthermore, referring to the concept of encapsulation in microregions (Risch and Reineccius, 1995) where molecules adsorb on the carrier surface and/or form associations among themselves, these regions are believed to be much more stable in a confined pore space. The influence of nanoconfinement can be nicely seen in the terpene series, where the retention performance of the non-polar myrcene and limonene cannot be improved by a denser pore structure in contrast to the one of linalool, that increased from 4 to 50 wt%.

A reduction in retention, however, is observed for the largest alcohol and ester molecules in the densest silica matrix. This can be either an indication that larger molecules are less well retained or that they cannot be fully extracted.

Conventional carbohydrate food carriers also offer hydroxyl groups to form hydrogen bonds with flavor molecules. A review about the physicochemical characteristics of both flavor and carrier and their influence on retention (Goubet et al., 1998) shows some interesting parallels with the sol-gel-made materials. There it is stated that the functionality of the flavor molecules affects the retention performance. Moreover, the retention increases with molecular weight and decreases with relative volatility of the flavor compound (Rosenberg et al., 1990; Voilley, 1995) in agreement with this study. This behavior can be explained by the influence of the molecular weight on flavor diffusion through the matrix during drying. Diffusional losses decrease with increasing molecular weight. As a consequence, bigger flavor molecules will not be lost as readily compared to smaller molecules. However, a molecular size limit seems to exist for the sol-gel materials to be still able to release the flavor.

In general, comparing retention performances from Rosenberg et al. (1990), Voilley (1995) and Bangs and Reineccius (1981) in carbohydrates as a function of the chemical nature of the flavor, alcohols were retained best. The retention seemed to decrease in the following order: alcohols > esters > aldehydes. Also Boutboul (2002) found a decrease in retention in the order: alcohol > aldehyde > ester > terpene investigating different starch carriers in agreement with the present results.

Rosenberg et al. (1990) and Voilley (1995) found that the retention in a homologous series decreases with increasing polarity. In general the oil-water partition coefficients (Table 3.1) bear information about the polarity of the molecule. However in the examples discussed
here, the influence of polarity and molecular weight cannot be isolated. But in accordance with this study, Rosenberg et al. (1990) observed that the more polar ethyl butanoate was less well retained compared to ethyl hexanoate in the ester series. Polar volatiles are more soluble in water and thus are believed to diffuse through the porous matrix influenced also by the drag imposed by the water molecules.

The lack of any hydrophobic interactions of the flavor molecules with the hydrophilic silica matrix seems to be responsible for the poor retention of the non-polar terpenes myrcene and limonene. An introduction of a polar group (linalool) increases the retention performance. Alternatively, it would be possible to incorporate hydrophobic molecules in the sol-gel matrix (Caturan et al., 1997; Reetz, 1997), which might lead to an increased retention performance of hydrophobic flavor molecules due to van der Waals forces. However the use of these functionalized particles is limited to the aromatization of packaging materials since the resulting ormosils (organic modified silicates) are no longer food-grade.

In contrast to carbohydrate matrices, where generally an increased flavor load leads to a decreased retention (Reineccius and Coulter, 1969), sol-gel matrices show a different behavior. Particles with a more open porosity tend to show an increase in retention with an increased total load. Denser matrices however reveal either an optimum or a reduced retention with increasing load.

3.5. Conclusions

Retention of flavor compounds is a complex phenomenon in which several factors play a role. For sol-gel-made silicas the concept of entrapment in microregions seems to be prevail.

The flavor retention in porous sol-gel-made silica carriers can be influenced by the morphology of the carrier and hence by its preparation. As the porosity is decreased, the flavor molecule is believed to experience an additional nanoconfinement within the microporous network structure resulting in enhanced retention by capillary forces and decreased diffusional loss during drying. Molecules that possess a hydrophilic group are brought in close contact to the silica surface, where they can chemically and/or physically adsorb more efficiently compared to a more open pore morphology. The silica polymers in a slow hydrolyzed sol-gel material are still able to deform during drying, entrapping the flavor molecule more efficiently compared to the more rigid, fast-hydrolyzing systems.
Similar to conventional carbohydrate food carriers, the retention of the volatiles depends on their steric hindrance (or molecular weight), their polarity and especially on their chemical nature. In general, alcohols are retained better than aldehydes and esters. Flavor molecules with a functional group that are able to associate either chemically and/or physically with the hydrophobic silica surface are retained more efficiently in contrast to the hydrophobic terpenes limonene and myrcene. As the molecular size increases, however, a drop in retention performance, especially in the densest particles, is observed that may be attributed to either a decrease of retention performance of bigger molecules or to an incomplete extraction from the pore space.

3.6. References


4. Restricted Diffusion in Silica Particles Measured by Pulsed Field Gradient NMR

Abstract

The restricted diffusion coefficient of water through porous silica is measured by pulsed field gradient (PFG) NMR as a function of loading in order to develop a model for self-diffusion at full pore filling in sol-gel-made porous silica particles. This model describes the pore or intraparticle diffusion coefficient as a function of particle porosity, tortuosity and the steric hindrance applied on the molecules by the pore space. The particle morphology is characterized by nitrogen adsorption and an appropriate tortuosity model is chosen in comparison with literature data. To characterize the material, NMR relaxation and diffusion studies at different degrees of pore filling were carried out in relation to the silica/water adsorption isotherm.

4.1. Introduction

Porous sol-gel particles can act as an encapsulation matrix for different biological and chemical species. Applications for the latter can be found as controlled release systems in the pharmaceutical and food industry (Böttcher and Slowik, 1998) or as biocatalysts (Reetz et al., 1996) and biosensors (Dave et al., 1994). The kinetic response, in the case of biocatalysts depends on the substrate diffusion through the porous matrix. Furthermore, the release kinetics of entrapped chemical molecules is governed by their diffusion through the porous sol-gel particles. The particle morphology of such sol-gel-made materials can be controlled by their method of preparation, and hence their release characteristics (Brinker et al., 1982). Therefore a knowledge of the pore diffusion coefficient is crucial to understand the transport of molecules within these porous systems. In this study this diffusion coefficient is determined in two ways. It is measured by pulsed field gradient NMR and calculated by accounting for particle morphology, the size of the diffusing molecules and the pore space.

A detailed introduction to the theory of pulsed field gradient NMR and restricted diffusion can be found in Price (1997; 1998). Pulsed field gradient NMR is a powerful

\(^{a)}\) A part of this chapter was accepted for publication in the *J. Colloid Interface Sci.*
technique for studying diffusion and characterizing material structure (Hagslätt et al., 2003) as in porous rocks (Hürlimann et al., 1994), microemulsions (Söderman and Nyden, 1999) and cellulose fibers (Topgaard and Söderman, 2002). The NMR experiments used in this study are mainly based on diffusion and relaxation studies by Seland et al. (2000, 2001) and D'Orazio et al. (1989, 1990a). Seland et al. (2000) performed basic PFG-NMR studies of restricted diffusion of water in order to show how to correctly deal with the presence of large internal field gradients within the samples. Based on these investigations the authors proceeded to measure restricted diffusion in more complex porous particles (Seland et al., 2001). D'Orazio and co-workers have used self-diffusion and spin-relaxation NMR of deionized water in porous glass at different degrees of filling in order to characterize the pore morphology. Both longitudinal and transverse relaxation revealed a linear behavior with respect to the degree of fluid filling down to monolayer coverage. This was attributed to both a homogeneous pore space and an equal distribution of the water. The pore diffusion coefficient decreased as the pore filling ratio was reduced, following a relationship derived from a combination of the Stokes-Einstein equation and Archie's law. At submonolayer conditions a change in this trend was observed, which was attributed to the crossover from bulk to surface transport of the molecules. There the diffusion coefficient decreased more rapidly as the filling was reduced (Bhattacharja et al., 1989; D'Orazio et al., 1990b). The majority of other previous studies varying the degree of filling focused on the proton relaxation of water adsorbed on porous silica as a function of the relative humidity or pore filling. Early transverse relaxation studies of water in porous silica studies revealed the coexistence of two adsorbing phases of water above a bilayer coverage. The spin-spin ($T_2$) relaxation seemed to consist of a contribution from the strongly bound protons close to the surface and the weakly bound water, which shows an increase in $T_2$ relaxation as the coverage is increased by an enhanced molecular motion (Zimmermann et al., 1956). The relaxation behavior of water in silica membranes with different pore sizes at different coverages was also investigated by Almagor and Belfort (1978). They proposed a three-state model where the motional restriction of the water decreases with distance from the solid surface until the properties of bulk water are obtained at a distance exceeding two monolayers (about 5 Å) from the surface. They also observed that a decrease in pore size increased the fraction of spins restricted in motion. Hills (1994) measured proton relaxation time distributions for suspensions of compact silica particles at varying silica/water ratios and discussed the existence of different regions of water fillings down to the surface diffusion at low water contents.
Based on these previous studies the distribution of water and the homogeneity of the pore space as prerequisites for the pore diffusion experiments were investigated in this work by nuclear magnetic relaxation, varying the degree of pore saturation. Silica particles with different degrees of filling were prepared by equilibration with saturated salt solutions. Their pore size distribution was characterized by nitrogen adsorption. The focus, however, of this study was to establish a model to describe restricted diffusion in porous sol-gel silica particles, which was validated by the pore diffusion coefficient obtained by pulsed field gradient NMR at complete pore saturation. In this model the pore or intraparticle diffusion coefficient is defined as a function of particle porosity, tortuosity and the steric hindrance applied on the molecules with respect to the pore dimensions (Limbach and Wei, 1990). Several empirical expressions currently exist describing particle tortuosity as a function of porosity. To decide on the most appropriate tortuosity model for porous silica sol-gel particles experimental pore diffusion coefficients by Kunetz and Hench (1992; 1998) of chromium ions through silica sol-gel slabs at different pore morphologies were used. A comparison between experimental data and model calculations for the restricted pore diffusion coefficient in silica sol-gel slabs revealed an appropriate tortuosity model. This equation was hence applied to calculate pore diffusion within the sol-gel particles in this study. The validity of the model was furthermore demonstrated by comparison to the experimental pore diffusion coefficient obtained by D'Orazio et al. (1990b).

4.2. Theory

4.2.1. Pulsed field gradient NMR

Nuclear magnetic resonance can be used to observe the displacement $x$ of molecules undergoing Brownian motion. The relationship for the molecular mean square displacement and the self-diffusion coefficient $D_0$ is given by the Einstein-equation:

$$x^2 = 6D_0t$$  \hspace{1cm} (4.1)

where $t$ is the diffusion time.

With NMR, the diffusion coefficient is obtained by labeling the position of the molecules at the start of the experiment through the use of a field gradient. After a certain
period of time, during which the molecules will have moved to a different random position due to self-diffusion, the positions of the molecules are labeled again by a second gradient. The final signal that is observed will be a function of the diffusion coefficient $D_0$, the gradient strength $g$ and duration $\delta$, and the observation time $\Delta$. A typical pulse sequence, that was first introduced by Stejskal and Tanner (1965), is given in Figure 4.1a. For unrestricted self-diffusion, where the random motion of the molecules is assumed to follow Gaussian behavior, the signal attenuation $I/I_0$ has the functional form given in Figure 4.1a (Stejskal and Tanner, 1965).

**a) PGSE:**

\[
\frac{I}{I_0} = \exp\left(-\left(\gamma \delta g\right)^2 \cdot D \cdot t_d\right) \quad \text{with} \quad t_d = \Delta - \frac{\delta}{3}
\]

**b) PGSTEBP:**

\[
\frac{I}{I_0} = \exp\left(-\left(\gamma \delta g\right)^2 \cdot \left(1 - \frac{1}{2} \frac{t_d - \frac{1}{2} \tau}{t_d}\right)^2 \right) D t_d \quad \text{with} \quad t_d = \Delta + \frac{3}{2} \tau - \frac{\delta}{6}
\]

Fig. 4.1: Pulse sequences for pulsed field gradient NMR experiments and corresponding echo attenuations (Kärger et al., 1988; Seland et al., 2001). a) Pulsed field gradient spin echo sequence (Stejskal and Tanner, 1965); b) 13-interval bipolar pulsed field gradient stimulated echo sequence (PGSTEBP) (Cotts et al., 1989; Sorland et al., 1997) (Appendix C).
4.2.2. Restricted diffusion

For heterogeneous systems such as fluids in porous media or for molecules diffusing between compact spheres, the displacement of the diffusing species depends on the interactions with the porous matrix and may be restricted by pore walls. Therefore the mean-square displacement is usually reduced from that for free molecular diffusion obtained by the Stokes-Einstein equation (Cussler, 1997):

\[ D_0 = \frac{k_B \cdot \theta}{6\pi \cdot \mu \cdot r_m} \]  \hspace{1cm} (4.2)

In obstructed diffusion, restrictions in the structure are reached as the diffusion time increases and the apparent diffusion rate is decreased. Hence, the longer the molecules diffuse the more restricting barriers will be encountered. As a result, the measured diffusion coefficient \( D(t) \) becomes time-dependent and information about the nature of the restricting boundaries can be derived (Helmer et al., 1995; Seland et al., 2001). The long-time behavior of \( D(t) \) therefore provides an indirect measure of the macroscopic structure. If the molecules diffuse between compact particles, the effective restricted interparticle diffusion coefficient is (Latour et al., 1995):

\[ \lim_{t \to \infty} \frac{D(t)}{D_0} = \frac{D_{\text{eff}}}{D_0} = \frac{\varepsilon_b}{\tau_b} \]  \hspace{1cm} (4.3)

where \( \varepsilon_b \) is the particle bed porosity and \( \tau_b \) the tortuosity that the molecules experience diffusing through the bed of particles. In this case, the spacing between the barriers, \( a \), can be calculated according to Graton and Fraser (1935):

\[ a = \frac{V_p}{S} = \frac{1 - \varepsilon_b}{6(1 - \varepsilon_b)} d \]  \hspace{1cm} (4.4)

If the molecules diffuse inside porous particles, the intraparticle or pore diffusion coefficient can be obtained according to Ek et al. (1995), Latour et al. (1995) and Seland et al. (2001):
In the case of restricted diffusion, different regions can be distinguished depending on the size relations of the single barriers $a$, duration $\delta$ of the gradient pulse and the interval $\Delta$ between the gradient pulses (Wang et al., 1995). Generally in heterogeneous systems, the average propagator is not Gaussian and, hence, the spin echo attenuation deviates from monoexponential behavior (Stallmach et al., 2001). Only in two cases, the so-called "free-diffusion" and the "rapid-diffusion" regimes, the determination of the true self-diffusion coefficient is straightforward (Vasenkov, 2001). In the "free-diffusion" regime ($\Delta D << a^2$ and $\delta D << a^2$), when the diffusional distance is relatively small compared to the barrier separation, only a small fraction of molecules will be influenced by the barriers. Then the diffusive motion of the molecules leads to a Gaussian distribution of the spin phases, if $qa << 1$ with $q = \sqrt{g} \delta / 2\pi$. In the "rapid diffusion" regime, where $\Delta D >> a^2$ and $\delta D >> a^2$, all molecules of the ensemble will be influenced by the restricting boundaries. Although the displacements in this case are not Gaussian due to the influence of the barriers, random diffusion causes the phase behavior of the spins to be Gaussian during the gradient pulse if $qa << 1$. In the "restricted diffusion" regime, where $\Delta D >> a^2$ and $\delta D << a^2$, a Gaussian distribution of the spins is obtained if $qa << 1$ (Wang et al., 1995). However, if $\delta D \approx a^2$ it is difficult to extract the true diffusion coefficient and it is only possible if the geometry of the system is simple and well defined (Seland et al., 2001).

4.2.3. Diffusion domains

In a pulsed field gradient (PFG) experiment the echo decay of the molecules diffusing in the sample under observation can be described by the equations in Figure 4.1. In the systems studied here, various diffusion domains may occur having different diffusion coefficients (e.g. diffusion of water between and within porous particles). In the case of slow exchange between the two domains, a multi-exponential echo decay in the following form will be observed with each domain possessing its own diffusion coefficient $D_i$ (Kärger et al., 1973; Kärger and Pfeifer, 1987):
The fraction of molecules in each domain is expressed as $p_i$. A diffusion time dependence of $p_i$ can be an indication for an exchange process (Valiullin et al., 1997). In the above definitions, the effects of differences in relaxation times are not considered (Seland et al., 2001). If relaxation effects play a role, the fraction $p_i$ is a function of the different time intervals used in the pulse sequence.

When the exchange between the different domains is fast, an average diffusion coefficient $D_{av}$ will be obtained:

$$D_{av} = \sum_{i=1}^{n} p_i \cdot D_i$$  \hspace{1cm} (4.7)

and the echo attenuation is monoexponential (Valiullin et al., 1997):

$$\ln \frac{I}{I_0} = -4\pi^2 q^2 D_{av} t_d$$  \hspace{1cm} (4.8)

Between these two limits (eq. 4.6 and 4.8) different degrees of exchange may occur between various domains within the observation time.

**4.2.4. Restricted diffusion at different degrees of pore filling**

Studies about restricted diffusion at different degrees of pore filling show that the intraparticle diffusion coefficient $D_{\text{intra}}$ is reduced with a decreased filling ratio $(V/V_0)$ of the pores (Kärger et al., 1983). D'Orazio et al. (1990a) derived the following equation from the correlation between conductivity and particle porosity $\varepsilon_p$, known as Archie's law (Archie, 1942), and the relationship between self-diffusion of fluid molecules and conductivity according to the Einstein equation (Cussler, 1997):

$$\frac{D_{\text{intra}}}{D_0} = \varepsilon_p^{\rho' - 1} \left( \frac{V}{V_0} \right)^{\rho' - 1}$$  \hspace{1cm} (4.9)
The parameters $p$ and $p'$ generally range between 1.5 and 3 depending on the sample (D'Orazio et al., 1990b).

### 4.2.5. Calculation of restricted diffusion at complete pore filling

The unrestricted molecular diffusion coefficient $D_0$ in an ideally diluted system is a constant and can be calculated according to the Stokes-Einstein-equation (eq. 4.2). The fluid transport through a network of fluid-filled pores is described by Fick's first law (Cussler, 1997), where the effective pore diffusion coefficient is smaller than the diffusivity in a straight cylindrical pore as a result of the random orientation of the pores leading to a longer diffusion path. In general, the effective restricted diffusion coefficient of the solute in the porous medium, $D_{\text{intr}}$, is related to the bulk diffusion coefficient $D_0$ by eq. 4.5 (Perry and Green, 1998). The tortuosity $\tau_p$ can be obtained from pore structure, pore size and shape distributions (Carniglia, 1986), but typically correlations have been used by Mackie and Meares (1955):

$$\tau_p = \frac{(2 - \varepsilon_p)^2}{\varepsilon_p}$$

(4.10)

or by Wakao and Smith (1962):

$$\tau_p = \frac{1}{\varepsilon_p}$$

(4.11)

and by Suzuki and Smith (1972):

$$\tau_p = \varepsilon_p + 1.5(1 - \varepsilon_p)$$

(4.12)

Though the predictive value of these equations is rather limited, the common trend of tortuosity increase with the reduction of porosity is reflected by all of them. For adsorbent materials, experimentally determined tortuosities generally range from 2 to 6 (Perry and Green, 1998). In practice, the tortuosity factor is about the same for one type of material (Satterfield, 1970; Kärger and Ruthven, 1992).

When the radius of the solute molecule is comparable to the pore radius, significant steric hindrance and hydrodynamic interactions with the pore wall might occur. This
phenomenon is addressed as "restricted diffusion or steric hindrance" and becomes more pronounced, when the ratio of molecular to pore radius \( \lambda = \frac{r_m}{r_p} \) exceeds 0.1. The solute transport is then retarded by the viscous drag of the solvent, which is a function of the pore walls and the partitioning between the pores and the bulk solution. An additional restrictive factor \( F(\lambda) \) is therefore introduced to eq. 4.5 to account for steric hindrance:

\[
\frac{D_{\text{inter}}}{D_0} = \frac{\varepsilon_p}{\tau_p} \cdot \frac{K_D}{K_p} = \frac{\varepsilon_p}{\tau_p} \cdot F(\lambda)
\]

where \( K_p \) and \( K_D \) are the partition and enhanced drag coefficients, respectively (Baltus and Anderson, 1983). The \( K_p \) is defined as the equilibrium ratio of solute concentration within the interstitial space of a porous network to that in a bulk solution. The enhanced drag coefficient \( K_D \) is a measure of additional hydrodynamic resistance to the diffusion of a molecule in a porous material as compared to that in bulk solution (Limbach and Wei, 1990). Typically the ratio of these two coefficients, the steric hindrance factor \( F(\lambda) \), is defined by Brenner and Gaydos (1977) for \( \lambda < 0.1 \):

\[
F(\lambda) = \left[ 1 + \frac{9}{8} \lambda \cdot \ln \lambda - 1.54 \lambda \right]
\]

and by Kärger and Ruthven (1992) for \( 0.1 < \lambda < 0.5 \):

\[
F(\lambda) = \left( 1 - 1.83\lambda + 4.18\lambda^2 \right) \cdot \exp(-6.52\lambda)
\]

or by Mavrovouniotis and Brenner (1988) for \( \lambda \to 1 \):

\[
F(\lambda) = 0.98\lambda \left( \frac{1-\lambda}{\lambda} \right)^{3/2}
\]
4.3. Experimental

4.3.1. Materials and sample preparation

Tetraethoxysilane or tetraethyl orthosilicate (TEOS, Sigma Aldrich, 98%) and absolute ethanol (Baker, Switzerland) were used for the sol-gel preparation of the solid matrix (Brinker and Scherer, 1990). A 0.06 M HCl solution was prepared with deionized water from a 1M stock solution (Merck KGaA, Germany). TEOS, ethanol, HCl and H2O in the molar ratio 2.39 : 37.24 : 0.07 : 60.30 were mixed in a beaker at room temperature. Hydrolysis was carried out for 48 hours in a closed beaker. The sol was then neutralized with a 0.09 M NaOH solution, that was prepared from a 1M stock solution (Merck KGaA, Germany). The resulting gel was dried in a vacuum oven at 40°C and 750 mbar for 24h. The sol-gel powder obtained was ground with mortar and pestle and sieved with a vibration sieve (Retsch, Haan, Germany).

The 20-90 \( \mu \text{m} \) powder fraction was used for the NMR diffusion experiments. After vacuum drying, the water content of the sample was determined by a thermobalance (Seiko Instruments SII, TG/DTA 220, SSC/5200) applying a heating rate of 2 °C /min up to 120 °C in a nitrogen flow of 200 ml/min. The corresponding water activity of the starting sample at laboratory conditions was 0.55. Sol-gel samples at different water activities were prepared by equilibration with saturated salt solutions (Table 4.1) in dessicators until the weight of the sample was constant (Wexler and Hasegawa, 1954). The saturated salt solutions generated different partial water vapor pressures in the dessicators thus leading to different water contents in the sol-gel samples. The final water content of the sol-gel-made silica particles was also measured by a thermobalance. The sample with a water activity of 0.73 was conditioned with a saturated NaCl solution and the adsorption was stopped before equilibration was reached.
Table 4.1: Water activities and filling ratios $V/V_0$ of silica matrices at equilibrium with the saturated salt solutions and the one stored with NaCl without reaching equilibrium state. Also parameters for the initial matrix under ambient conditions and at complete pore saturation are shown.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water Acticity $a_w$</th>
<th>Filling Ratio $V/V_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$COOK</td>
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</tbody>
</table>

4.3.2. Determination of the particle morphology

The N$_2$-sorption isotherms of the sol-gel powder were conducted on a Micromeritics ASAP 2010 surface area and pore size analyser (Micromeritics, Nocross, GA) at -196°C (liquid nitrogen). The sample was degassed at 200 °C down to 3 µm Hg prior to the measurement. From the total pore volume, the particle porosity $\varepsilon_p$ is (Fukuda et al., 1989; Vacassy et al., 2000):

$$\varepsilon_p = \frac{V_0}{V_0 + 1/\rho_p}$$

(4.17)

where $\rho_p$ is the solid density of silica: 2.2 g/cm$^3$ (Iler, 1979). The specific surface area was determined by the multipoint BET method using adsorption data in the pressure range $(p/p_0)$ from 0.05 to 0.25. The desorption isotherm was used to determine the pore size distribution.
using the Barrett, Joyner and Halender (BJH) method for a cylindrical pore geometry (Song and Pratsinis, 2000).

4.3.3. NMR sequences and data analysis

The NMR experiments were performed on a widebore NMR spectrometer (Bruker Spectrospin, 9.4 Tesla) using a Bruker Diff 25 probe with a homebuilt coil to improve gradient homogeneity (Prof. Magnus Nyden, Chalmers University, Sweden). The field gradients were calibrated with a 1 vol% H₂O in D₂O solution containing 1 wt% CuSO₄ to 1.9x10⁻⁹ m²/s at 298 K (Mills, 1973). The temperature was calibrated with ethylene glycol (Amann et al., 1982)(Appendix B).

In porous systems magnetic field gradients may affect the measurement of the diffusion coefficient by pulsed field gradient NMR. These gradients result from differences in magnetic susceptibility between the different regions (Hürlimann, 1998). According to Seland et al. (2000) the size of these internal gradients in a system of particles surrounded by a liquid is:

\[ g_i = \frac{2\pi Av}{\gamma d} \]  

(4.18)

where \( d \) is the mean diameter of the particles and \( Av \) the line width at half-height of the Fourier transformed signal (Drain, 1962). Thus an estimation of the internal gradient can be obtained by readily measurable quantities. The 13-interval bipolar pulsed field gradient stimulated echo (PGSTEBP) sequence (Figure 4.1b) (Cotts et al., 1989; Sorland et al., 1997) is often applied in heterogeneous systems to minimize associated internal gradients that may be a significant source of error (Seland et al., 2001). For the diffusion experiments in this study the PGSTEBP sequence (Figure 4.1b) with equal gradients was applied, since the internal gradient was not negligible especially at longer observation times. Both bipolar pulse sequences with symmetric and asymmetric bipolar gradient pulses were tested on the sol-gel matrices and the experimental results were identical. With line widths at half-height of 400-1 100 Hz (depending on the degree of filling) and an average particle diameter of 55 μm, internal gradients between 17 G/cm and 47 G/cm were calculated according to eq. 4.18. The maximum gradient strength was varied from around 600 G/cm for an observation time of \( \Delta = 10 \) ms down to about 90 G/cm for \( \Delta = 400 \) ms. The gradient pulse duration \( \delta \) was 1 ms and a
sufficient recycle delay (> 5T₁) was taken to be 3.5s as determined by T₁ relaxation measurements. All diffusion studies were carried out at 25°C.

The measurements of the longitudinal relaxation time T₁ were performed using the inversion recovery sequence (Farrar and Becker, 1971). In the case of the transverse relaxation T₂ the Carr-Purcell-Meiboom-Gill (CPMG) technique (Carr and Purcell, 1954; Meiboom and Gill, 1958) was used applying a spacing between the rf-pulses of 50 μs. Provided that molecules in a single isolated pore with volume Vₚ and surface area Sₚ diffuse sufficiently fast so that they experience all the parts of the pore volume during the experimental time scale, Tₑ, one can write (Brownstein and Tarr, 1977):

\[
\frac{1}{T_i} = \frac{1}{T_{IB}} + \frac{\lambda_{surf} S_p}{T_{IS} V_p}
\]  

which is appropriate for the fast diffusion regime considering magnetic relaxation in the bulk T_{IB} and the surface T_{IS}. Tᵢ is the relaxation time, that stands for longitudinal or spin-lattice relaxation (i=1) and transverse or spin-spin relaxation (i=2), respectively. The surface thickness parameter λ_{surf} can be taken as the thickness of a molecular monolayer.

Error analysis for the fitting procedures (Levenberg-Marquadt algorithm) according to eq. 4.6 were performed by Monte-Carlo simulations of the experimental noise to check the reliability and the stability of the results (Alper and Gelb, 1990; Schönhoff and Söderman, 1997)(Appendix K). In the first step a biexponential fit on the input data set is performed. From this fit the level of noise is calculated by the standard deviation of the experimental data. Afterwards a loop of n fits adding artificial noise on the experimental data set is carried out resulting in distributions of the parameters Dᵢ and pᵢ (Topgaard and Söderman, 2002). From these distributions the standard deviations shown in the figures were determined. An error analysis on a diffusion experiment of 1vol% H₂O in D₂O containing 1 wt% CuSO₄ (PGSTEBP sequence) revealed an error of about 1.8% (monoexponential fit).
4.4. Results and discussion

4.4.1. Particle morphology of the sol-gel-made silica particles

The nitrogen adsorption isotherm and the corresponding pore size distribution are shown in Figure 4.2 a, b. The nitrogen adsorption/desorption isotherm is of type IV with its hysteresis loop associated with capillary condensation in the mesopores (Webb and Orr, 1997) and the limiting uptake at high $p/p_0$. The initial part of the isotherm is associated with monomultilayer adsorption (Sing, 1982). The specific surface area of the sol-gel-made powder is 215.5 m$^2$/g. The average BJH pore diameter obtained from the desorption branch of the isotherm is 4.3 nm, which is a typical value for porous silica (Brinker and Scherer, 1990; D'Orazio et al., 1990b).

![Graph](image_url)

**Fig. 4.2a:** Nitrogen adsorption/desorption isotherm of the silica sol-gel sample. The isotherm shows a hysteresis loop, which is typical for a type IV isotherm of a mesoporous solid.
4.4.2. Water adsorption/desorption on sol-gel-made silica particles

Figure 4.3 shows the adsorption and desorption kinetics of water on silica sol-gel particles (about 50 mg dry weight). It can be seen that the silica particles respond quickly to changes in the surrounding atmosphere. Therefore great care was taken to quickly fill the samples into a NMR tube that was sealed with parafilm (Pechiney Plastic Packaging, Menasha, USA). The sample weight did not change before and after NMR measurement.

In Figure 4.4 the corresponding water adsorption isotherm of the sol-gel-made silica sample calculated from the experimental points according to the BET theory is shown (Webb and Orr, 1997). Since this isotherm was created from both desorption experiments (for samples with a final water activity smaller than the initial 0.55) and from adsorption experiments (for samples with a final water activity above 0.55) the typical hysteresis loop for a type IV isotherm is not seen. However, nitrogen adsorption data indicate a mesoporous
solid, where, according to Sing (1982), the initial part of the isotherm is associated with mono-multilayer adsorption. Samples with a water activity smaller than 0.6 are therefore in the region of mono-multilayer coverage. Whereas capillary condensation takes place in the mesopores above a water activity of 0.6, indicated by the steep increase of the silica water content with increasing water activity (Webb and Orr, 1997).

Fig. 4.3: Adsorption and desorption kinetics of water in silica sol-gel particles (dry weight: 50 mg). The silica samples were equilibrated by storing them in dessicators with saturated salt solutions.

$^1$H single pulse experiments were carried out with the samples before the diffusion experiments. The peak areas were determined by the spectrometer software (X-WIN-NMR) and normalized on the dry sample weight. The normalized areas of the individual samples bear information about the adsorbed quantity of water and should be proportional to the water content. In Figure 4.4 it is shown that the water content determined by peak integration fits with the BET-isotherm and confirms that the equilibrated sol-gel samples were stable and consistent with the determination of the water content by thermogravimetric analysis. Furthermore, it shows that all water present in the sample is detected by NMR.
Fig. 4.4: Adsorption isotherm of water on sol-gel silica at 21°C. The experimental points were determined by equilibrating the sol-gel samples with saturated salt solutions and the water content was measured by thermogravimetric analysis. The BET adsorption isotherm was calculated according to the BET theory. The normalized areas of the $^1$H single pulse experiments follow the BET adsorption isotherm.

4.4.3. Validation

In order to validate the measurements of restricted diffusion, the diffusion of water and butanol in monodisperse polystyrene particles was measured here and compared to Seland et al. (2000). In Figure 4.5 the normalized time dependent self diffusion coefficient of water between monodisperse polystyrene beads (particle diameter: 100 μm, Duke Scientific) obtained in this study is shown. Experiments were carried out using a PGSTE-BP-sequence at 25°C. For short diffusion times ($t_d \to 0$) the measured apparent diffusion coefficient $D(t)$ is nearly equal to the free diffusion coefficient $D_0$ of water (self diffusion coefficient of water at 25°C: $2.3 \times 10^{-9}$ m$^2$/s (Mills, 1973), since the molecules diffuse only a short distance and only a
few molecules will feel the surrounding particle surfaces. As the diffusion time increases, more and more molecules will encounter these restrictions. Therefore the plateau value in Figure 4.5 corresponds to the restricted interparticle diffusion coefficient (eq. 4.3) and contains information about the bed packing.

Fig. 4.5: Normalized time dependent self-diffusion coefficient of water in water and butanol in D$_2$O between monodisperse polystyrene beads (particle diameter: 100 µm). At long observation times a plateau value is reached corresponding to the restricted interparticle diffusion coefficient, which carries information about the bed packing. The echo decay of the different peaks results in the same normalized diffusion coefficients regardless of whether butanol or water diffuses around the polystyrene beads. Errors estimates obtained from a Monte-Carlo fit to the data set are smaller than 2%.

A normalized diffusion coefficient $D/D_0 = 0.68$ is a typical value for monodisperse beads of this size (Seland et al., 2000). According to Mitra et al. (1992) the surface to volume ratio ($S_p/V_p$) was determined to $S_p/V_p = 73657$ m$^{-1}$ from the short time diffusion data resulting in a porosity of 0.45, which is identical to the one found by Seland et al. (2000) for the same
system (Appendix D). To prove that the molecules only probe the interparticle space and that this plateau value is independent of the diffusing molecular species, the diffusion of butanol (1 wt% in D$_2$O) between the monodisperse polystyrene beads was measured as well. In Figure 4.5 the restricted diffusion coefficients are shown using all different resonances of butanol (chemical shifts were measured with respect to tetramethylsilane (TMS)) and compared to the water peak. The normalized restricted diffusion coefficients obtained from each butanol echo-decay are identical to those of water. At short diffusion times the self-diffusion coefficient of butanol, $D_0$ (at 15°C: 7.7 $\times$ 10$^{-10}$ m$^2$/s (Satterfield, 1970)), in D$_2$O at infinite dilution is obtained (Latour et al., 1995). In all cases a biexponential fit to the data was unsuccessful leading to overfitting and negative values for the diffusion coefficients. From this it may concluded that there exists only one diffusion domain between the particles. Diffraction effects were not observed in this study, because the maximum observation time (up to 1 s) was too short to see diffraction in a 100 µm polystyrene sample (Callaghan et al., 1991) (Appendix E).

4.4.4. Relaxation at different degrees of pore filling

Generally relaxation behavior is dominated by interactions involving the probing molecules at the liquid-solid interface. Typically the presence of paramagnetic impurities and/or physisorption are considered to be key aspects of this phenomenon (D'Orazio et al., 1990b). If water molecules exchange with protons of the silica surface their relaxation behavior will be influenced to a large extent by this exchange. However, if exchange is negligible, the $T_2$ relaxation time represents the local motions of the water molecules, diffusion rates and the degree of magnetic susceptibility differences between the silica/water/air surfaces.

The $T_1$ and $T_2$ relaxation measurements were conducted with different water activities using the NMR-sequences illustrated in the experimental section to describe the homogeneity of water adsorption on the silica particle surface. In Figure 4.6 the relaxation times are shown as a function of the filling ratio ($V/V_0$). The total available pore volume $V_0$ was taken from the nitrogen adsorption isotherm. A summary of the samples with the corresponding filling ratios is shown in Table 4.1. It is known that the nuclear magnetic relaxation rates are enhanced near solid interfaces ($T_{1,h}^{-1} << T_{1,s}^{-1}$)(Halperin et al., 1989) and, therefore, the corresponding relaxation times $T_1$ are decreased. Consequently, both nuclear relaxation times $T_1$ shown in Figure 4.6 are decreased as the pore filling ratio is reduced. At high degrees of filling nuclear
magnetic relaxation is increased by an enhanced molecular motion of the molecules due to an increased distance to the solid surface (Zimmermann et al., 1956).

In all cases the echo attenuation is exactly monoexponential in time, which was also observed by Bhattacharja et al. (1989). This supports the theory of fast exchange between bulk and surface water in the pore space (D’Orazio et al., 1989). Furthermore, it indicates a high homogeneity of the pore structure which confirms the monomodal pore size distribution obtained by nitrogen adsorption measurement (Figure 4.2b). The linear dependence at all filling ratios shows not only the homogeneity of the pore space, but also an equal distribution of water at different degrees of filling. The latter is a prerequisite for the diffusion experiments.

Fig. 4.6: $T_1$, $T_2$-relaxation times as a function of the water filling ratio in the pores. $V_0$ is the total pore volume, which is obtained from the nitrogen adsorption isotherm. The linear fits confirm the fast exchange theory and show the homogeneity of the pore space and the water distribution.
4.4.5. **Restricted diffusion at different degrees of pore filling**

The restricted diffusion of water in the silica matrix with fully wetted pores \((V/V_0 = 100\%)\) was investigated as a function of the observation time (Figure 4.7a). A biexponential decay of the echo attenuation was obtained indicating a two domain diffusion that can be described by eq. 4.6. Since the exchange between these two diffusion domains is very slow, a single restricted diffusion coefficient for each domain will be observed. These two domains are attributed to the diffusion within the pores of the particles and the interparticle space.

![Graph showing the restricted diffusion of water in a fully wetted packing of porous sol-gel-made silica particles.](image)

**Fig. 4.7a:** Restricted diffusion of water in a fully wetted packing of porous sol-gel-made silica particles. At low observation times \((t_d \rightarrow 0)\) not all the molecules feel the restrictions from the silica matrix. As the observation time is increased more and more molecules will encounter the barriers and a plateau value is reached for the interparticle diffusion coefficient (triangles) at long observation times \((t_d > 0.3s)\), which carries information about the bed packing. The region of rapid diffusion prevails in the intraparticle diffusion domain (circles), since even at low observation times all molecules experience the restricting boundaries (eq. 4.5). The error bars result from the Monte-Carlo simulation.
The interparticle diffusion shows the typical behavior of a "restricted diffusion" regime. At low observation times \( t_d < 0.01\text{s} \) not all the molecules feel the restrictions. As the observation time is increased more and more molecules will encounter barriers and a plateau value is reached \( (t_d > 0.3\text{s}, \frac{D}{D_0} = 0.58) \) providing information about the bed packing (eq. 4.3). The plateau value of the interparticle diffusion coefficient (Figure 4.7a) reached at long observation times is almost equal to the one obtained in Figure 4.5 for water and butanol diffusion between monodisperse polystyrene beads. However, Figure 4.7a does not show the pronounced decrease at short observation times compared to Figure 4.5, since the silica particle size distribution (range 20-90 \( \mu m \)) is broader leading to a more dense packing of the bed. The average silica particle size is 55 \( \mu m \) resulting in a smaller interparticle void (eq. 4.4) compared with the monodisperse polystyrene particles with a diameter of 100 \( \mu m \). Therefore, the water molecules between the sol-gel particles experience the restricting boundaries at even lower diffusion times. The increased restriction in the case of the polydisperse sol-gel-made particles is also reflected in the plateau value reached at long observation times in both cases. For the polystyrene beads (Figure 4.5) the stationary plateau is obtained at a normalized diffusion coefficient around 0.68, whereas the one between the sol-gel-made particles is reached at around 0.58 due to both denser packing and smaller particle sizes.

The regime of rapid diffusion holds in the intraparticle diffusion domain throughout the whole observation time, since even at low observation times all molecules experience the restricting boundaries (eq. 4.5). The average pore diameter is about 4.3 nm (Figure 4.2b) and the conditions for the rapid diffusion regime are met \( (\Delta D >> a^2 = 1.8 \times 10^{-17} \text{ and } \beta D = 1.7 \times 10^{-12} >> a^2 \text{ for all } \Delta = 0.08 - 0.4\times) \). Consequently a plateau value for the restricted self-diffusion coefficient is reached even at short observation times \( t_d \) (Figure 4.7a) and an average pore diffusion coefficient of around \( 4.84 \times 10^{-10} \text{ m}^2/\text{s} \) is obtained. A comparison of this value with model calculations and a published value by D'Orazio et al. (1990b) measuring water diffusion in water saturated silica glass (average pore diameter of 3.5 nm) is made later on. The errors were calculated by a Monte-Carlo simulation of the data fit. The contribution of each diffusion coefficient \( p_i \), expressed in area percentage of each signal (eq. 4.6) shows that the majority of molecules (80%) diffuse in the interparticle voids (Figure 4.7b). The fraction of both signals \( p_i \) did not vary significantly with the observation time indicating that exchange of liquid between the two diffusion domains is not significant (Valiullin et al., 1997).
Fig. 4.7b: Restricted diffusion of water in fully wetted porous sol-gel particles. A biexponential echo decay is obtained corresponding to inter- and intraparticle diffusion (eq. 4.6). According to eq. 4.6 the proportion of molecules in each of the two domains is shown. About 80% of the molecules are in the interparticle space, whereas 20% of the molecules are within the pores. Errors resulting from the Monte-Carlo simulation are below 8%.

Figure 4.8 shows the intraparticle diffusion coefficients at different degrees of pore filling (29.3-100%) as a function of observation time obtained from a biexponential fit to the echo decay (eq. 4.6). It can be seen that for all different pore fillings the rapid diffusion regime is present throughout all observation times ($\Delta D \gg a^2 = 1.8 \times 10^{-17}$ and $\delta D = 1.7 \times 10^{-12} \gg a^2$ for all $\Delta = 0.08 - 0.4s$). Again a plateau value for the restricted self-diffusion coefficient is obtained even at small observation times ($t_d \to 0$).
Fig. 4.8: Intraparticle diffusion coefficient as a function of the observation time at various degrees of pore filling. The region of rapid diffusion is prevailing in the intraparticle diffusion domain, since even at low observation times all molecules experience the restricting boundaries (eq. 4.5). The error bars result from the Monte-Carlo simulation.

However, large error estimates are encountered at lower observation times ($t_d < 0.15\text{s}$) and constant proportions $p_i$ in each domain are only achieved for observations times bigger than $0.15\text{s}$ (Figure 4.9). A time dependency of $p_i$ might be due to either exchange between the two domains (Valiullin et al., 1997) or to differences in relaxation times in the two domains at very short observation times (Seland et al., 2001). Since the water molecules stay preferentially in the pores of the sol-gel particles by capillary forces, the proportion $p_i$ in the intraparticle space is increased with decreasing water content as shown for four samples in Figure 4.9.
Fig. 4.9: Proportion of molecules diffusing in the intraparticle domain as a function of the observation time. As the degree of filling is reduced the proportion of molecules in the pore space seems to be increased by capillary forces.

The diffusion coefficient of the remaining water molecules in the interparticle space is mainly dependent on the packing of the particles (eq. 4.3) and shows the same behavior as in Figure 4.7a for all filling ratios at observation times $t_d > 0.15s$.

To compare the quality of the biexponential fits, the echo decays at different degrees of pore filling are shown in Figure 4.10 as a function of $k (k = (\gamma \cdot \delta \cdot g)^2 \cdot t_d)$ at $t_d = 0.197s$ for three representative data sets. The lines represent biexponential fits to the data sets and demonstrate that the echo decay is correctly described. Since the $T_1$ and the $T_2$-relaxation times of the samples equilibrated at water activities of 0.228 and 0.432 are so small that most of the magnetization is already vanished before the echo acquisition, no diffusion experiments were carried out with these samples. As the degree of filling is decreased the corresponding echo decay is reduced and the quality of the fit drops since $T_1$ and $T_2$ relaxation times become smaller with a decreased pore filling (Figure 4.6).
Fig. 4.10: Echo decay curves at three different degrees of pore filling at an observation time $t_d = 0.197s$ as a function of $k = (\gamma \cdot \delta \cdot g)^2 \cdot t_d$. The lines show the biexponential fit to the data set. As the degree of filling is reduced the echo decay is reduced and the quality of the fit is decreased slightly.

Figure 4.11 shows the intraparticle diffusion coefficient as a function of the pore filling at $t_d = 0.15 - 0.4s$. It can be seen that the pore diffusion coefficient decreases significantly with a reduced filling ratio, which is in accordance to Kärger et al. (1983) and the model of D'Orazio et al. (1990b) (eq. 4.9). The fit to the data in Figure 4.11 (eq. 4.9) was made for $p = p' = 3$. The reason for this reduction in the intraparticle diffusion coefficient, $D_{\text{intra}}$, with decreasing pore filling might be twofold. On the one hand, smaller pores are filled preferentially due to their stronger capillary forces and so the diffusion within these pores is restricted by a greater extent than that in larger pores. On the other hand, the ratio of surface adsorbed water to free pore water is increased if the pores are not fully filled, thus leading to a smaller diffusion coefficient compared with that for increasing pore filling. The samples in the capillary branch of the adsorption isotherm, where necks are formed in the pores, seem to
reveal more or less similar diffusion coefficients and major changes appear in the region of mono-multilayer coverage ($V/V_0 < 0.6$). This is consistent with D'Orazio et al. (1990b), who reported that below a filling ratio of 0.3 a steep decline in the diffusion coefficient was noticed which is attributed to surface diffusion in the submonolayer region.

![Graph](image)

**Fig. 4.11:** Intraparticle diffusion coefficient obtained by a biexponential fit as a function of the pore filling ratio. The pore diffusion of water increases with an increased pore filling consistent with D'Orazio et al. (1990b). The line represents the fit according to eq. 4.9 for $p=p'=3$. At complete pore saturation the calculated restricted diffusion coefficients according to the model (eq. 4.13) are shown. These are in quite good agreement with the experimentally determined $D_{\text{intra}}$ and fall well within the error estimates calculated by the Monte-Carlo simulation.
4.4.6. Calculation of the restricted diffusion coefficient at complete pore filling

The proposed model calculates the restricted diffusion coefficient at complete pore saturation based on the knowledge of the dimension of a diffusing water molecule with respect to the pore space, the particle porosity and the tortuosity of the pores. However, since the determination of the tortuosity is not straightforward, it is often introduced as a characteristic of the material (Kärger and Ruthven, 1992). Tortuosities cannot be measured directly, but they can be determined indirectly from diffusion and release measurements (Ek et al., 1995). In this study the experimental restricted diffusion coefficients measured by Kunetz and Hench (1992, 1998) were used to determine an appropriate description of the tortuosity in silica sol-gel particles, taking eq. 4.10 to 4.12. There the authors investigated chromium diffusion through silica sol-gel slabs of different porosities and pore sizes. From the release curve of chromium ions diffusing through slabs with varying morphology they determined an effective pore diffusion coefficient. Since porosity, average pore diameter of the different sol-gel slabs, free diffusion coefficient of the chromium ion $D_0$ and its molecular dimension are known, Kunetz and Hench's experimental restricted diffusion coefficients can be compared with a calculation according to eq. 4.13 (Kunetz et al., 1992; Kunetz and Hench, 1998). The results are shown in Figure 4.12. It can be seen that the tortuosity model according to Suzuki and Smith (1972) seems to be adequate to describe restricted diffusion through porous particles and therefore it is used to calculate the restricted diffusion of water here.

From the total pore volume determined by nitrogen adsorption ($V_0 = 0.374 \text{ cm}^3/\text{g}$) the particle porosity $\varepsilon_p$ was calculated according to eq. 4.17 to be 45.14. The tortuosity of the sample is 1.27 (Suzuki and Smith, 1972). The radius of a water molecule is 1.9 Å for a water density of 997 kg/m$^3$ at 25 °C. With an average pore diameter of about 4.3 nm, the relation of molecular to pore diameter $\lambda$ gives 0.09. Since $\lambda$ is close to 0.1, the restricted diffusion coefficient is calculated applying both eq. 4.14 and 4.15. For these two cases the restricted intraparticle self-diffusion coefficients of water, calculated according to eq. 4.13, are shown in Table 4.2 and Figure 4.11.
Experimental Diffusion Coefficient, [m²/s]

Fig. 4.12: Diffusion of chromium ions through porous silica sol-gel slabs. Comparison of the effective diffusion coefficient determined experimentally by Kunetz et al. (1992) and by the model given in this paper. The tortuosity defined by Suzuki and Smith (1972) best describes the experimental data.

Both of them are comparable to the experimentally determined diffusion coefficient and fall well within the error estimates from the Monte-Carlo simulation. The same calculations were applied to reproduce the restricted self-diffusion coefficient found by D'Orazio et al. (1990b) at complete pore filling in porous silica glass with an average pore diameter of 3.5 nm and a porosity of 35%. In a PFG-NMR experiment the intraparticle diffusion coefficient was determined as $D_{\text{intra}} = 3.25 \times 10^{-10}$ m²/s. In both cases the model according to Kärger and Ruthven (1992) underpredicts the experimental diffusion coefficients, whereas the steric hindrance by Brenner and Gaydos (1977) leads to bigger diffusion coefficients due to its derivation for smaller $\lambda$-values. All parameters for the calculation of the restricted diffusion coefficient in this study and the comparison with D'Orazio et al. (1990b) are summarized in Table 4.2.
Table 4.2: Restricted pore diffusion coefficients are shown from pulsed field gradient experiments obtained from the silica sol-gel used in this study and from D'Orazio et al. (D'Orazio et al., 1990b). A comparison with the calculated pore diffusion coefficients is drawn.

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<td>Exp. Pore Diffusion Coeff. $D_{\text{intra}}$, [m$^2$/s]</td>
<td>4.84\times10^{-10}</td>
<td>3.25\times10^{-10}</td>
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</table>

4.5. Conclusions

Restricted diffusion in silica sol-gel particles was investigated at different degrees of filling as a function of the observation time in order to verify a model to calculate the restricted diffusion coefficient at complete pore filling. Studies of transversal and longitudinal relaxation as a function of the degree of pore filling reveal a linear behavior showing that both the pore space and the water within the pores are equally distributed. The homogeneous distribution of the water in the pore space is a prerequisite for the measurements of the diffusion coefficients at various degrees of filling.

Pulsed field gradient studies result in a biexponential echo decay for water diffusion between and within the porous particles. The intraparticle diffusion coefficient is significantly
reduced as the pore filling decreases and follows the relationship between diffusion and degree of filling derived from a combination of the Einstein-equation and Archie's law.

The proposed model describes the restricted diffusion of water in porous sol-gel derived particles at complete pore filling quite well both for the sample in this study and in comparison with literature. This model may therefore be used as a basis to study mass transfer processes in sol-gel derived materials like biocatalysts and encapsulation matrices.

4.6. References


5. Restricted Diffusion and Release of Flavor Molecules from Porous Silica Sol-Gel-Made Particles

Abstract

The macroscopic release profile of flavors from sol-gel-made particles is measured directly by UV-VIS spectroscopy and their microscopic restricted pore diffusion coefficient is obtained in-situ by pulsed field gradient (PFG) magic angle spinning (MAS) nuclear magnetic resonance (NMR) while the release kinetics are calculated by the Crank equation. Measured and calculated release profiles agree within experimental error. Furthermore, restricted pore diffusion coefficients obtained by model calculations are in agreement with those measured by PFG-MAS-NMR indicating its potential for characterization and screening of encapsulation formulations.

5.1. Introduction

Sol-gel-made silicas can be applied as encapsulation matrices for organic (Böttcher et al., 1999) and biological molecules (Dave et al., 1994) with applications in the food and pharmaceutical industry. The release kinetics of organic or flavor molecules entrapped in these particles is governed by the diffusion of the active molecule through the matrix structure (Böttcher and Slowik, 1998). Knowledge of the molecular mobility through porous particles is therefore crucial to predict release kinetics (Kortesuo et al., 2000). Diffusion is important in food and pharmaceutical applications since it is the dominant mechanism in controlled release from encapsulation matrices (Crank, 1975; Cussler, 1997). Restricted diffusion occurs in heterogeneous systems, when molecular Brownian motion is significantly hindered by pore walls. Then pore diameters are in the same order with the diffusing molecules so significant steric hindrance takes place (Anderson and Quinn, 1974; Deen, 1987; Limbach and Wei, 1990; Lee et al., 1991). Typically release kinetics are measured directly and an effective diffusion coefficient is determined from a release profile (Siepmann et al., 1998; Siepmann et al., 1999). There the effective drug diffusivity is obtained from a cumulative release profile using theoretical models under certain assumptions (Gao and Fagerness, 1995). In this study
PFG-NMR is used to measure the microscopic mobility of the flavor molecules in silica particles, which is then correlated to the macroscopic release kinetics. Pulsed field gradient NMR is a powerful technique to study diffusion in drug delivery systems (Stockman and Dalvit, 2002; Moniot and Kuchel, 2003), and to characterize material structures (Hagslätt et al., 2003) as in porous rocks (Hürlimann et al., 1994), microemulsions (Söderman and Nyden, 1999) and cellulose fibers (Topgaard and Söderman, 2002). However, PFG-NMR experiments in sol-gel-made particles suffer from the poor spectral resolution arising from magnetic susceptibility differences between the phases. This problem was overcome successfully in diffusion studies of pharmaceutical molecules in liposomes and liquid-crystalline phases (Pampel et al., 2002; Gaede and Gawrisch, 2003). There a combination of PFG-NMR and MAS-NMR techniques was applied to measure diffusion coefficients under high resolution conditions. Generally, MAS techniques have become a standard for obtaining high resolution spectra in solids (Andrew et al., 1958).

PFG-NMR and NMR imaging techniques are widely used to characterize encapsulation matrices and the mobility of the entrapped component since this technique benefits from its non-destructive and non-invasive nature. NMR imaging has been primarily applied in medical diagnostics, but it has been also used to visualize solvent penetration in polymers and pharmaceutical excipients. Ek et al. (1995a) were able to predict drug release from porous cellulose beads by determining the pore tortuosity using PFG-NMR. These techniques have been also applied to gain insight into water transport in cellulose fibers and beads (Ek et al., 1994; Ek et al., 1995b) and to characterize material structures (Topgaard and Söderman, 2002a; Topgaard and Söderman, 2002b). Wohlgemuth and Mayer (2003) showed the applicability of PFG-NMR to different nanocapsule encapsulation systems to study the exchange rate of benzene tracer molecules. Qualitative and quantitative characterization of drug delivery systems regarding penetration kinetics (Harding et al., 2000), drug distribution and diffusion were carried out by Fyfe and Blazek-Welsh (2000) and by Hyde et al. (1995) applying NMR imaging. This technique also provides information about temporal changes in liquid distribution within a sample, that enables to monitor water transport and distribution during drying (Harding et al., 2001) and water penetration into swelling amylose-starch tablets (Baille et al., 2002; Malveau et al., 2002). Drug and water diffusivity in multicomponent HPMC gels was studied by Gao and Fagerness (1995) and by Rajabi-Siahboomi et al. (1994; 1996). Hyde et al. (1995) observed a strong drug-polymer interaction in similar NMR imaging studies, which affected the polymer chain configuration and mobility and therefore retarded the buffer transport into the polymer matrix in contrast to the placebo.
There are only a few studies so far, that tried to relate the microscopic pore diffusion coefficients obtained by PFG-NMR to macroscopic release kinetics, which is also a topic of the current work. Kwak et al. (2003) compared micro- and macroscale diffusion of phosphate in hydrogels. The macroscopic diffusion coefficient was obtained by NMR imaging from release experiments, whereas the microscopic self-diffusion coefficient was determined by PFG-NMR. It appeared, that the macroscopic diffusion values are systematically lower than the ones obtained by PFG-NMR. However, they differed by a factor of less than 1.2, which suggests, that PFG-NMR estimates well the molecular transport in porous gel structures. Furthermore, Duval et al. (1999) studied water self-diffusion in lamponite gels. Diffusion coefficients extracted from the concentration profiles measured by NMR imaging are in reasonably good agreement to the ones measured by PFG-NMR.

In this study a method is presented to directly determine the macroscopic flavor release kinetics from porous particles using measured microscopic pore diffusion coefficients and particle dimensions. This intraparticle or pore diffusion coefficient is measured by PFG-MAS-NMR and the average particle radius is obtained by Frauenhofer laser-diffraction spectroscopy. The flavor release kinetics from sol-gel-made silica particles is then calculated by Fick’s second law and compared to measured release profiles obtained by UV-VIS spectroscopy. The pore diffusion coefficient of the organic compound is calculated accounting for steric hindrance and compared to the one obtained by PFG-MAS-NMR.
5.2. Theory

5.2.1. Calculation of the pore diffusion coefficients

In a thermodynamically ideal system the free diffusion coefficient $D_0$ of a solute in a solvent is a constant and can be calculated according to the Stokes-Einstein-equation (Cussler, 1997):

$$D_0 = \frac{k_B \cdot \theta}{6\pi \cdot \mu \cdot r_m} \tag{5.1}$$

In case of diffusion of a solute in a porous material, where the size of the solute is relatively small compared to the pore size, the effective pore or intraparticle diffusion coefficient of the solute $D_{\text{intra}}$ in the porous medium is related to the bulk diffusion coefficient $D_0$ by the following relation:

$$\frac{D_{\text{intra}}}{D_0} = \frac{\varepsilon_p}{\tau_p} \tag{5.2}$$

where $\varepsilon_p$ is the particle porosity and $\tau_p$ the tortuosity of the porous particle. The tortuosity is described by an empirical expression by Suzuki and Smith (1972), which was validated for sol-gel-made materials in paragraph 4.4.6:

$$\tau_p = \varepsilon_p + 1.5(1 - \varepsilon_p) \tag{5.3}$$

When the ratio of the solute molecule radius to that of the pore ($\lambda = r_m / r_p$) exceeds 0.1, steric hindrance and hydrodynamic interactions with the pore wall occur. Then the effective diffusion coefficient in the pores $D_{\text{intra}}$ is decreased observably since the solute transport is retarded by the viscous drag of the solvent which is affected by the pore walls. Therefore a restrictive factor $F(\lambda)$ is added to eq. 5.2 accounting for steric hindrance (Limbach and Wei, 1990):

$$\frac{D_{\text{intra}}}{D_0} = \frac{\varepsilon_p}{\tau_p} \cdot F(\lambda) \tag{5.4}$$
This steric hindrance factor \( F(\lambda) \) is for \( \lambda < 0.1 \) (Brenner and Gaydos, 1977):

\[
F(\lambda) = \left(1 + \frac{9}{8} \lambda \cdot \ln \lambda - 1.54\lambda \right)
\]

(5.5)

and for \( 0.1 < \lambda < 0.5 \) (Kärger and Ruthven, 1992):

\[
F(\lambda) = (1 - 1.83\lambda + 4.18\lambda^2) \cdot \exp(-6.52\lambda)
\]

(5.6)

5.2.2. Release from spherical particles

Flavor release from porous particles is determined by molecular diffusion through an isotropic porous medium using Fick's second law in radial coordinates (Crank, 1975):

\[
\frac{\partial c}{\partial t} = D_{\text{mpra}} \left( \frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right)
\]

(5.7)

The initial and boundary conditions for desorption to an infinite bath from a spherical particle are:

\[
t = 0, \ 0 < r < R \quad \quad c(t, r) = c_0
\]

\[
t > 0, \ r = R \quad \quad c(t, R) = c_e
\]

\[
\text{all } t \quad \quad \left( \frac{\partial c}{\partial r} \right)_{r=0} = 0
\]

where \( c_0 \) is the starting concentration at \( t=0 \), which is assumed to be uniform throughout the particle and \( c_e \) is the surface equilibrium concentration, which typically has a rather low value. Eq. 5.7 can be solved subject to these boundary conditions to obtain an analytical solution for the release kinetics from a spherical particle with diameter \( d \) (Crank, 1975):

\[
\frac{M_t}{M_\infty} = \frac{c - c_0}{c_e - c_0} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( -\frac{4D_{\text{mpra}} n^2 \pi^2 t}{d^2} \right)
\]

(5.8)
where $M_t$ is the amount released at time $t$ and $M_\infty$ is the total amount released after infinite time. The intraparticle or pore diffusion coefficient $D_{\text{intra}}$ is assumed to be independent of the solute concentration. NMR imaging studies indicate that liquid penetration proceeds much faster than the rate of release indicating that penetration is not the rate-limiting step in the release process (Ashraf et al., 1989). Therefore the kinetics of liquid penetration have been neglected here. Appendix I contains the Fortran program to calculate the release kinetics.

5.3. Experimental

5.3.1. Materials and sample preparation

The flavor-laden sol-gel-made silica particles were prepared according to paragraph 2.2.1. During neutralization model flavor molecules: ethyl butanoate (EB), ethyl hexanoate (EH), ethyl octanoate (EO) and ethyl decanoate (ED) (Fluka, Switzerland) were added to the sol A (Table 2.1) before the onset of gelation. All flavors were entrapped with an initial concentration of 50 wt%, except of ED that was entrapped 20 wt%, for a complete conversion of TEOS to silica. The obtained gel was dried in a vacuum oven at 40°C and 750 mbar for 24 h. The resulting powder was ground with mortar and pestle and sieved with a vibration sieve (Retsch, Haan, Germany) to obtain the size fractions for NMR ($d = 20-90 \ \mu \text{m}$) and release ($d = 510-710, 710-1000 \ \mu \text{m}$) experiments. The amount of remaining flavor after drying was determined by extraction of the entrapped compound from the silica matrix and subsequent GC-analysis as described in paragraph 2.2.2.

5.3.2. Determination of particle morphology and size

The particle morphology was measured by N$_2$-sorption isotherms according to paragraph 4.3.2. The average size of the silica particle fraction used in the release experiments was determined by Frauenhofer laser-diffraction spectroscopy on a Sympatec-Helos (Germany) in a sucell system with a cell width of 2 mm (Appendix F).
5.3.3. NMR sequences and data analysis

The PFG-NMR experiments to determine the free diffusion coefficient of the single flavor molecules (1 wt%) in per-deuterated ethanol (Fluka, Switzerland) were performed on a widebore Bruker Avance DSX 400 MHz NMR spectrometer (\(^{1}\text{H} \text{Larmor frequency: 400.13 MHz}\)) using a Bruker Diff 25 probe with a homebuilt coil to improve gradient homogeneity (Prof. Magnus Nyden, Chalmers University, Sweden). The field gradients were calibrated with a 1 vol % H\(_{2}\)O in D\(_{2}\)O (Fluka, Switzerland) solution containing 1 wt% CuSO\(_{4}\) to 1.9-10\(^{-9}\) m\(^{2}\)/s at 298 K (Mills, 1973). The temperature was calibrated with ethylene glycol (Amann et al., 1982). A pulsed field gradient stimulated echo sequence (PGSTE) was applied (Callaghan, 1991).

All PFG-MAS-NMR experiments of the flavor-laden silica powders (d = 20-90 μm) in per-deuterated ethanol were performed on a Bruker Avance DRX 600 MHz spectrometer (\(^{1}\text{H} \text{Larmor frequency: 600.13 MHz}\)) using a 4 mm HR-MAS probe with z-gradient. The maximum gradient strength was a nominal 50 G/cm. The silica particles were packed into a 4 mm rotor. The sample size in the rotor was constrained to a spherical shape with a diameter of 2.7 mm by spacers. Pulsed field gradient stimulated echo (PGSTE) sequences (Callaghan, 1991) were used with sine shaped gradient pulses and the sample spinning was controlled to within 1 Hz using the Bruker controller. The spinning frequency was 2.5 kHz. Experiments were carried out at 25°C controlled by the spectrometer. Self-diffusion coefficients were obtained by fitting the experimental echo decay to the following equation (Price and Kuchel, 1991) using the Levenberg-Marquadt algorithm accounting for flavor diffusion in the intra- and interparticle domain (biexponential echo decay) (Kärger et al., 1973):

\[
\frac{I}{I_0} = \sum_{i} p_i \cdot \exp \left( - (\gamma_0 g)^2 \frac{(4 \cdot t_d - \delta)^2}{\pi^2} D_i \right) \tag{5.9}
\]

where \(I\) is the measured echo intensity, \(I_0\) is the relaxation weighted intensity, \(\gamma\) is the gyromagnetic constant, \(g\) is the field gradient strength, \(\delta\) is the duration of the field gradient pulse, \(t_d = \Delta\) is the effective diffusion time, \(D\) is the self-diffusion coefficient and \(p\) is the fraction of the molecules in each domain \(i\). The fitting procedures to eq. 5.9 were performed according to paragraph 4.3.3. and a Monte-Carlo statistical error analysis (Appendix K) was
carried out to obtain estimates of the standard deviations of the diffusion parameters $D_i$ and $p_i$, respectively (Alper and Gelb, 1990).

### 5.3.4. Release kinetics

The release experiments were conducted in absolute ethanol at 25°C. A size fraction ($d = 510–710$ or $710–1000$ µm) of the silica particles (about 0.2 g) containing various flavors was suspended in 20 ml of dissolution liquid and was stirred at 200 rpm by a magnetic stirrer. During the experiment, the released flavor was monitored by a UV-VIS-NIR spectrophotometer (Varian, Cary 500 Scan, Switzerland) at a wavelength of 210 nm in a Suprasil-flow cell (175.000-QS, Hellma, Germany) (Siepmann et al., 1999). The liquid was circulated by a HPLC pump (Model 541, Waters Division of Millipore, MA, USA) at a flow rate of 8 ml/min. To avoid the influence of dust particles, the liquid was sieved by a filter (Waters Division of Millipore, MA, USA) before entering the measuring cell. All experiments were conducted in triplicates.

### 5.4. Results and discussion

#### 5.4.1. Flavor diffusion and particle characterization

Table 5.1 shows the free diffusion coefficients $D_0$ of the single flavors at the zero concentration limit (1 wt% in ethanol) measured by PFG-NMR at 25°C and averaged over the different chemical shifts of the compound with respect to tetramethylsilane (TMS). It can be seen, that the diffusion coefficient decreases with increasing molecular weight from EB to ED (Baille et al., 2003; Kwak et al., 2003). The errors represent two times the standard deviation divided by the average diffusion coefficient. The average hydrodynamic molecular radii $r_m$ are calculated according to eq. 5.1 with a dynamic viscosity of ethanol at 25°C of 1.055 mPas (Kuchling, 1989). For a comparison with literature data the diffusion coefficient of butanol in D$_2$O was determined at 25°C to $7.92 \times 10^{-10}$ m$^2$/s (error: 2.46%) in the same set-up which is consistent with the literature value of $7.7 \times 10^{-10}$ m$^2$/s (Cussler, 1997).
Table 5.1 shows also the porosity, pore volume and average pore radius of the different flavor-laden samples after flavor removal by heating at 300 °C for 16h. Figure 5.1 presents the nitrogen adsorption isotherms of the EB-, EH-, EO- and ED-laden silica samples after flavor removal by heat treatment (16h at 300 °C). The adsorption isotherm of the corresponding blank silica (without any flavor) is also shown for comparison. The pore volume is significantly increased upon flavor entrapment. The more flavor is retained (Table 5.1), the higher the pore volume or porosity increase since the silica chains form around the flavor molecules during polycondensation reactions and the flavor molecules act as a kind of template where the chains are wrapping around (paragraph 2.3.2).

![Graph showing nitrogen adsorption isotherms](image)

**Fig. 5.1: Nitrogen adsorption isotherms of EB, EH, EO and ED silica particles after removal of the organic compound by heat treatment (16h at 300 °C). The corresponding adsorption isotherm of the blank sample is presented as well in comparison. The pore volume is increased significantly upon entrapment.**
Table 5.1: Free diffusion coefficients $D_0$ (PFG-NMR) and the corresponding molecular hydrodynamic radii $r_m$ of the single flavor compounds in D-ethanol are shown at 25°C along with the pore morphology and diffusion coefficients of the flavor molecules entrapped in silica. The pore morphology of the single flavor-laden silica particles is characterized after removal of the organic compound by heat treatment (16h at 300 °C) by nitrogen adsorption. The flavor load is determined by extraction of the flavor and subsequent GC analysis. The average, $\bar{D}_{\text{intra}}$, minimum, $D_{\text{intra,min}}$, and maximum, $D_{\text{intra,max}}$, pore diffusion coefficients of the single flavor molecules entrapped in silica particles in D-ethanol are measured by PFG-MAS-NMR. A comparison is drawn with the calculated pore diffusion coefficients, $D_{\text{intra,c}}$, and the diffusion coefficient, $D_{\text{intra,R}}$, obtained by a fit of eq. 5.8 to the measured release kinetics ($R^2$: regression coefficient).

<table>
<thead>
<tr>
<th></th>
<th>EB</th>
<th>EH</th>
<th>EO</th>
<th>ED</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_0$, m$^2$/s</td>
<td>1.09·10^{-9}</td>
<td>9.58·10^{-10}</td>
<td>8.41·10^{-10}</td>
<td>7.59·10^{-10}</td>
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<td>$D_0$ error, %</td>
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<td>1.13</td>
<td>1.23</td>
<td>2.18</td>
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<tr>
<td>$r_m$, Å</td>
<td>1.90</td>
<td>2.16</td>
<td>2.46</td>
<td>2.73</td>
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<tr>
<td>$\varepsilon_p$, %</td>
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<td>51.13</td>
<td>69.95</td>
<td>37.4</td>
</tr>
<tr>
<td>SSA, m$^2$/g</td>
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<td>537.9</td>
<td>888.9</td>
<td>602.2</td>
</tr>
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<td>$V_0$, cm$^3$/g</td>
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<td>0.48</td>
<td>0.74</td>
<td>0.27</td>
</tr>
<tr>
<td>$r_p$, nm</td>
<td>1.36</td>
<td>1.55</td>
<td>1.47</td>
<td>1.22</td>
</tr>
<tr>
<td>Load, wt%</td>
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<td>13.39</td>
<td>36.12</td>
<td>13.06</td>
</tr>
<tr>
<td>$\tau_p$, -</td>
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<td>1.24</td>
<td>1.19</td>
<td>1.31</td>
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<tr>
<td>$\lambda$, -</td>
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<td>0.14</td>
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<td>0.22</td>
</tr>
<tr>
<td>$F(\lambda)$, -</td>
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<td>0.33</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>$D_{\text{intra,c}}$, m$^2$/s</td>
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<td>1.31·10^{-10}</td>
<td>1.17·10^{-10}</td>
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</tr>
<tr>
<td>$D_{\text{intra,R}}$, m$^2$/s</td>
<td>5.57·10^{-11}</td>
<td>1.39·10^{-10}</td>
<td>1.73·10^{-10}</td>
<td>3.14·10^{-11}</td>
</tr>
<tr>
<td>$R^2$, -</td>
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<td>0.89</td>
<td>0.87</td>
<td>0.95</td>
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<tr>
<td>$\bar{D}_{\text{intra}}$, m$^2$/s (NMR)</td>
<td>1.23·10^{-10}</td>
<td>1.32·10^{-10}</td>
<td>1.67·10^{-10}</td>
<td>3.44·10^{-11}</td>
</tr>
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<td>$D_{\text{intra,min}}$, m$^2$/s (NMR)</td>
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<td>$D_{\text{intra,max}}$, m$^2$/s (NMR)</td>
<td>1.81·10^{-10}</td>
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<tr>
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<td>2.20</td>
<td>0.94</td>
<td>0.97</td>
<td>1.09</td>
</tr>
</tbody>
</table>
5.4.2. Flavor diffusion through sol-gel-made silica particles

Figure 5.2 shows the $^1$H spectra of ethyl decanoate entrapped in silica particles (20-90 μm) that are fully wetted with per-deuterated ethanol under static (a) and MAS (b) conditions.

Under static conditions, the signal is broadened by the presence of internal gradients and the resonances of the different chemical groups cannot be distinguished. Therefore the spectrum cannot be resolved for peaks arising from ethyl decanoate and the solvent. Under magic angle spinning (MAS) conditions, the spectral resolution is significantly increased since
susceptibility broadenings are removed by MAS (Vanderhart et al., 1981). The individual resonances arising from the flavor molecule can be resolved and the echo decay of the single resonances can be measured with a PFG-MAS experiment.

Figure 5.3 shows the ED diffusion coefficients of the above sample under MAS conditions as a function of time that were obtained by a biexponential fit to the experimental data set according to eq. 5.9 at the 4.1 ppm resonance (Figure 5.2).

![Graph showing diffusion coefficients](image_url)

Fig. 5.3: Diffusion of ethyl decanoate entrapped in silica fully wetted with D-ethanol. The biexponential echo decay according to eq. 5.9 describes flavor diffusion between the particles (squares) and within the particle pores (triangle). The error bars result from a Monte-Carlo simulation to the experimental data set.

The interparticle diffusion coefficient (squares) corresponds to the ED diffusion in D-ethanol between the particles, whereas the intraparticle or pore diffusion coefficient corresponds to ED diffusion through the particle pores (triangles) (paragraph 4.4.5). The error bars result from a Monte-Carlo simulation to the experimental data set. The interparticle diffusion coefficient follows the characteristic shape of a restricted diffusion regime (Vasenkov, 2001) and the stationary plateau that provides information about the packing density of the particles.
is already reached at \( t_d > 0.1 \) s. The pore diffusion coefficient is constant with time and exhibits the shape of a rapid diffusion regime (Vasenkov, 2001) since all ethyl decanoate molecules are restricted even at low observation times in the narrow particle pores (compare paragraph 4.4.5). This diffusion pattern is also seen for the remaining resonances of ethyl decanoate and for the other flavor molecules entrapped in the silica particles.

Table 5.1 shows also the pore or intraparticle diffusion coefficients measured by PFG-MAS-NMR of the four flavor molecules entrapped in the silica particles: the average, \( D_{\text{intra}} \), minimum, \( D_{\text{intra,min}} \), and maximum, \( D_{\text{intra,max}} \), diffusion coefficients are averaged over all resonances of the component (minimum and maximum pore diffusion coefficients result from the Monte-Carlo analysis). It also presents the calculated intraparticle diffusion coefficients, \( D_{\text{intra,c}} \), according to eq. 5.3, 5.4 and 5.6. Calculated and measured average pore diffusion coefficients are in good agreement for all the four compounds (error < 16.6%). Steric hindrance becomes more pronounced as the molecular weight and size of the diffusing molecules increases. This can be seen in an increase in \( \lambda \) and a decrease in the steric hindrance factor \( F(\lambda) \) from EB to ED in Table 5.1. EO and ED are the samples with the highest and lowest porosity and, hence, EO possess the highest and ED the lowest average pore diffusion measured by PFG-NMR since the differences in porosity seem to play a more dominant role compared to the effect of steric hindrance. This is also reflected by the calculated diffusion coefficients. The present pore diffusion coefficients are comparable with literature values of drug molecules in polymer-plasticizer systems \((8.5\times10^{-11} - 5.6\times10^{-10} \text{ m}^2/\text{s})\) (Siepmann et al., 1999) and in dialysis membranes (around \(4.2\times10^{-11} \text{ m}^2/\text{s}\)) (Nardviriyakul et al., 1997).

### 5.4.3. Flavor release from spherical particles

In the following flavour diffusion and release will be discussed comparing the densest (ED-laden) and the most porous (EO-laden) samples (Table 5.1). Figure 5.4 shows the measured release kinetics of EO (dotted line) and ED (solid and dashed line) from silica in ethanol (UV-VIS) of particle size fraction \( d = 710 - 1000 \mu \text{m} \). The amount released at time \( t \), \( M_t \), is normalized to the total amount released, \( M_w \) (e.g. when the amount of released flavor did not change anymore). The ED release profile is much flatter than that of the more porous EO-
laden sample. After 10 min all EO is extracted from the porous particle. ED revealed a lower diffusion coefficient both in the PFG-MAS-NMR measurements and according to the concept of steric hindrance (Table 5.1) in agreement with the macroscopically determined release profiles. Figure 5.4 also compares the release kinetics of two different particle size fractions \((d = 510-710 \text{ and } 710-1000 \mu\text{m})\) of the ED-laden sample. The release from the smaller size fraction (solid line) is faster than from the bigger one (broken line) according to eq. 5.8 (Crank, 1975).

Fig. 5.4: Measured release kinetics (UV-VIS) of ethyl octanoate (EO) and ethyl decanoate (ED) in ethanol from the silica particles with a size fraction of \(d = 510-710\) and 710-1000 \(\mu\text{m}\). Ethyl octanoate is released much faster from the more porous particles than ethyl decanoate, which is entrapped in the denser silica matrix. The release of ED from the smaller particle size fraction is, according to eq. 5.8, much faster than from the bigger one.
The effective pore diffusion coefficients, $D_{intra,R}$, are obtained by matching the measured release profiles to eq. 5.8 (Gao and Fagerness, 1995; Siepmann et al., 1999). They are shown in Table 5.1 along with the regression coefficients, $R^2$, obtained in the interval $0 < M_t / M_\infty < 0.98$. The Fortran code can be found in appendix J. The pore diffusion coefficients calculated, $D_{intra,c}$, and measured either by PFG-MAS-NMR, $\overline{D}_{intra}$, and from the release kinetics, $D_{intra,R}$, are in fairly good agreement. Only the pore diffusion coefficient of EB determined from release kinetics seems to be slightly lower than the other ones. A possible reason could be an inhomogeneous initial flavor distribution especially at low loadings (Table 5.1) that is not accounted for by the model (eq. 5.8). Flavor molecules are entrapped in sol-gel-made materials in microregions (Whorton, 1995). There the molecules are associated so that the microscopic pore diffusion coefficient measured by PFG-MAS-NMR might be enhanced even though conventional release measurements on a macroscopic scale indicate that diffusion has been retarded. This retardation on a macroscopic level might arise from obstruction effects bigger than the PFG-NMR length scales (several micrometers) (Kwak et al., 2003). These microregions are detected also by nitrogen adsorption, which explains the good agreement of $D_{intra,c}$ and $\overline{D}_{intra}$ in contrast to $D_{intra,R}$ calculated from the release kinetics.

Figure 5.5a shows the release kinetics of EO in ethanol from the silica particles of $d = 710$-1000 $\mu$m. The average particle diameter of the applied particle fraction was determined by laser diffraction to be 820 $\mu$m. The measured release kinetics are compared to the calculated ones according to eq. 5.8 using the average pore diffusion coefficient determined by PFG-MAS-NMR ($1.67 \times 10^{-10}$ m$^2$/s; Table 5.1). The measured release profile is in agreement with the calculations and falls within the error interval ($\pm 27.9\%$) determined by the Monte-Carlo simulation for the PFG-MAS-NMR experiments.

The average measured PFG-MAS-NMR pore diffusion coefficient of ED ($3.44 \times 10^{-11}$ m$^2$/s) is nearly an order of magnitude smaller than that of EO (Table 5.1) and the release profile is flatter (Figure 5.4). Again, the calculated release profiles describe quite well the measured ED release kinetics (Figure 5.5b). The confidence interval for the PFG-MAS-NMR pore diffusion coefficient given by the Monte-Carlo simulation is $\pm 20.8\%$. The measured release profiles of the EB- and EH-laden samples shown in appendix G are also in agreement with the model calculations with Monte-Carlo confidence intervals of $\pm 48\%$ (EB) and $\pm 43.6\%$ (EH). The predicted release kinetics, however, underpredict the measured ones in all four cases at short times ($t < 2$ min) (Figure 5.5 a,b).
Fig. 5.5: Measured release kinetics (UV-VIS) of a) ethyl octanoate (EO) and b) ethyl decanoate (ED) in ethanol from the silica particles with a size fraction of 710-1000 μm. The average particle diameter was determined by laser-diffraction to be 820 μm. The measured release kinetics are compared to the calculated ones according to eq. 5.8 taking the pore diffusion coefficients from the PFG-MAS-NMR experiment (Table 5.1). The measured release profiles are in agreement with the calculations and fall within the error interval determined by the Monte-Carlo simulation.
Deviations at these early times can be attributed to the higher relative noise level at low concentrations (Fyfe and Blazek-Welsh, 2000), to initial adsorption of the flavor compound on the tubes connecting the measuring and the release cell and to the influence of ethanol penetration at the beginning of the release experiment which are not accounted for by the model. In the case of ED, the measured release is slower compared to the theory at higher times (t > 20 min). This might arise from sorption effects of ED molecules as retention is increased in the homologous ester series with increasing molecular weight (paragraph 3.3.1.2). The influence of particle size on the release kinetics is shown in Appendix H for ED.

Kwak et al. (2003) compared the microscopic phosphate ion pore diffusion coefficient by PFG-NMR in dextran gels to the corresponding macroscopic diffusion coefficient calculated from concentration profiles (that were obtained by NMR imaging) during a release experiment. The ratio of microscopic to macroscopic diffusion coefficient was around 1.2, which is comparable to $\bar{D}_{\text{micro}} / D_{\text{macro}}$ obtained here for EH, EO and ED (Table 5.1). Microscopic and macroscopic diffusion probe different diffusional phenomena by their different time and spatial scales (Kwak et al., 2003). Macroscopic diffusion coefficients are always slower than microscopic ones (Kwak et al., 2003) as with EB here, suggesting that the structures of dextran gels and sol-gels lead to obstruction effects at length scales larger than the PFG-NMR ones. The presence of microregions that are not regularly distributed and not interconnected at low flavor concentrations in the silica particles may obstruct flavor diffusion at larger length scales and thus leading to lower macroscopic diffusion coefficients as in Kwak et al. (2003).

A further comparison of macroscopic and microscopic diffusion with literature studies is done through characteristic release times. Table 5.2 summarizes the characteristic release times $t_{50}$ and $t_{90}$ for 50 and 90% release, respectively, of all the compounds from the silica particles of diameter 710-1000 μm. The characteristic times from the UV-VIS measurements ($t_m$) are compared to the ones extracted from the release profiles calculated with the PFG-MAS-NMR pore diffusion coefficients ($t_{NMR}$) and with the pore diffusion coefficients calculated according to the concept of steric hindrance ($t_c$). The measured characteristic times $t_m$ are close to $t_c$ and $t_{NMR}$ and within the time interval predicted by the PFG-MAS-NMR results for EH, EO and ED. However, the measured release kinetics of EB are rather at the slow diffusion limit and the release kinetics of ED seem to be retarded at long time scales (Figure 5.5b) as already mentioned before. NMR to measured characteristic times, $t_{50,NMR}$ /
$t_{50,m}$ range from 0.45 - 1.21 (Table 5.2) in correspondence to Ek et al. (1995a), who predicted the release kinetics of different pharmaceutical molecules from cellulose beads with the knowledge of the PFG-NMR pore diffusion coefficient after determination of the particle tortuosity by PFG-NMR. In their study, NMR to measured characteristic release times $t_{50}$ and $t_{90}$ ranged between 1.09 and 2.11.

Table 5.2: Comparison of measured $t_m$ (particle size fraction: $d = 710 - 1000 \mu m$) and predicted characteristic release times $t_{50}$ and $t_{90}$ describing 50% and 90% release of the compound, respectively. In the case of the PFG-MAS-NMR experiments release times $t_{NMR}$ for the average pore diffusion coefficients are given (the characteristic times for the minimum and maximum diffusion coefficients are shown in parenthesis). Furthermore, the characteristic times $t_c$ are given for the calculated pore diffusion coefficients.

<table>
<thead>
<tr>
<th></th>
<th>EB</th>
<th>EH</th>
<th>EO</th>
<th>ED</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{50,m}$, min</td>
<td>1.70</td>
<td>0.72</td>
<td>0.66</td>
<td>2.15</td>
</tr>
<tr>
<td>$t_{50,c}$, min</td>
<td>1.82</td>
<td>0.65</td>
<td>0.73</td>
<td>2.13</td>
</tr>
<tr>
<td>$t_{50,NMR}$, min</td>
<td>0.86 (0.58-1.65)</td>
<td>0.65 (0.45-1.13)</td>
<td>0.51 (0.39-0.71)</td>
<td>2.49 (2.06-3.15)</td>
</tr>
<tr>
<td>$\bar{t}<em>{50, NMR}$ / $t</em>{50,m}$, -</td>
<td>0.45</td>
<td>0.90</td>
<td>0.77</td>
<td>1.16</td>
</tr>
<tr>
<td>$\bar{t}<em>{50, NMR}$ / $t</em>{50,c}$, -</td>
<td>0.52</td>
<td>1.00</td>
<td>0.70</td>
<td>1.17</td>
</tr>
<tr>
<td>$t_{90,m}$, min</td>
<td>9.90</td>
<td>3.45</td>
<td>2.53</td>
<td>18.39</td>
</tr>
<tr>
<td>$t_{90,c}$, min</td>
<td>9.85</td>
<td>3.83</td>
<td>4.38</td>
<td>12.78</td>
</tr>
<tr>
<td>$\bar{t}_{90, NMR}$, min</td>
<td>5.15 (3.5-9.81)</td>
<td>3.82 (2.67-6.71)</td>
<td>3.06 (2.39-4.25)</td>
<td>14.9 (12.35-17.85)</td>
</tr>
<tr>
<td>$\bar{t}<em>{90, NMR}$ / $t</em>{90,m}$, -</td>
<td>0.52</td>
<td>1.11</td>
<td>1.21</td>
<td>0.81</td>
</tr>
<tr>
<td>$\bar{t}<em>{90, NMR}$ / $t</em>{90,c}$, -</td>
<td>0.52</td>
<td>1.00</td>
<td>0.70</td>
<td>1.12</td>
</tr>
</tbody>
</table>
5.5. Conclusions

Microscopic and macroscopic diffusion of model flavor molecules in silica particles was investigated by PFG-MAS-NMR and classic UV-VIS release kinetics and compared to theoretical models. The PFG-MAS-NMR pore diffusion coefficient can be predicted within a confidence interval < ±48% (Monte-Carlo simulation). The average PFG-MAS-NMR pore diffusion coefficients are in good agreement with calculations according to the concept of steric hindrance (error < 16.6%). The effective diffusion coefficients from the measured release profiles deviate less than 8.7% (EH, EO, ED) and 54% (EB) from the average diffusion coefficient measured by PFG-MAS-NMR. It can be therefore concluded that pore diffusion coefficients determined by PFG-MAS-NMR reflect quite well the microscopic mobility of the flavor molecules in the porous particle matrix and can be related to macroscopic release kinetics obtained by UV-VIS spectroscopy.

PFG-MAS-NMR may be therefore used to measure diffusion and to provide useful insight into the mechanisms of release. It may be applied in the formulation development process of encapsulation matrices to e.g. assist in the prediction of the release kinetics if those can not be measured directly.

5.6. References


6. Research recommendations

This thesis shows, that sol-gel-made silica particles can act as encapsulation matrices for organic molecules for applications in the food and pharmaceutical industry. The influence of particle morphology and chemical nature of the molecules on the retention performance has been investigated in this work. These inert materials show many advantages for the use as encapsulation matrices (Böttcher and Slowik, 1998; Böttcher et al., 1999b), biocatalysts (Reetz, 1997), packaging materials (Böttcher et al., 1999a), implants and other applications like e.g. human consumption as food additives. However, the influence of possible residual precursor and possible related health risks demand careful toxicological studies. So far implantations of sol-gel-made materials containing pancreatic islet cells (Pope, 1997; Pope, 1998) in diabetic mice for the treatment of diabetes mellitus indicate, that these materials are not toxic to the human body and in vivo efficacy was demonstrated recording their blood sugar level. Kortesuo et al. (2000) investigated controlled drug delivery of sol-gel silica in vitro and in vivo. A sustained drug release over a period of 42 days was observed. In a histopathological study silica did not show any tissue irritation at the site of implantation and in other organs like liver, kidneys etc. Also current research on bone regeneration (Pereira and Hench, 1996) shows the biocompatibility of these materials with the human body.

The second part of this thesis is dedicated to the measurement of the restricted diffusion coefficients by pulsed field gradient NMR. These pore diffusion coefficients provide information about the microscopic diffusibility and have been related successfully to the macroscopically measured release kinetics of flavor molecules entrapped in sol-gel-made particles. These observations make this technique an attractive tool to investigate retarded diffusion in glassy matrices (Delassus, 1994) and to study the influence of water activity on the mobility of the entrapped compounds (Whorton, 1995) in conventional food matrices. Furthermore, PFG-NMR might be used to verify models that describe diffusion in different matrices like e.g. polymers (Hyde et al., 1995) or other pharmaceutical excipients (Gao and Fagerness, 1995). PFG-NMR can therefore be applied as an alternative technique to measure diffusion and describe release kinetics to gain a better understanding of the underlying diffusion mechanisms and can be used to characterize and screen different encapsulation formulations in the development process (Stockman and Dalvit, 2002).
6.1. References


Appendix A. Measuring Restricted Diffusion in Heterogeneous Media under Magic Angle Sample Spinning

Abstract

Pulsed field gradient NMR diffusion measurements of adsorbed molecules on porous heterogeneous media are difficult to obtain under high spectral resolution as broad resonances arise from magnetic susceptibility differences between the different phases. High-resolution spectra can be obtained by utilizing magic angle spinning and the measured diffusion coefficients are not influenced by the spinning. There is a reasonably sized spinning frequency window where diffusion coefficients can be measured. Moderate internal field gradients present in the materials of interest do not affect the measurements even though the applied gradients that the probe is capable of are small.

A.1. Introduction

Pulsed field gradient nuclear magnetic resonance (PFG-NMR) has become one of the most common methods for determining self-diffusion parameters of liquids in porous media (Cotts et al., 1989; Ek et al., 1995; Sorland et al., 1997; Sorland et al., 1999; Seland et al., 2001;). In porous media, the standard NMR techniques suffer mainly from two main problems: the first is poor resolution due to susceptibility differences between liquid and solid interfaces (Figure 5.2). The second is the presence of artefacts in the experiment arising from the presence of varying internal magnetic gradients that lead to the measurement of incorrect diffusion coefficients. A number of new pulse sequences have been put forward which attempt to compensate for the interference between internal magnetic field gradients and an applied field gradient (Cotts et al., 1989; Ek et al., 1995; Sorland et al., 1997; Sorland et al., 1999; Seland et al., 2001), but still the problem of broad resonances in the final spectrum remains. It is difficult, therefore, to perform diffusion experiments on samples containing a mixture of molecules in the presence of heterogeneous media due to severe spectral overlap; for example, predicting release kinetics using diffusion studies of organic flavor molecules in porous particles.
Magic angle sample spinning (Andrew et al., 1958; Lowe, 1959) is a standard method in solid-state NMR used to obtain high resolution spectra in solid and semi-solid materials by spinning the sample rapidly at an angle of 54.7° relative to the static magnetic field. Recently, commercial magic angle spinning probes equipped with a gradient coil that deliver modest magnetic field gradients (50 G/cm) have become available (Maas et al., 1996). The gradient coil produces a linear magnetic field gradient along the direction of the spinning axis so that interference with sample spinning is avoided. Such probes have been used to investigate water and guest molecules in model membranes (Pampel et al., 2002a; Gaede and Gawrisch, 2003) and lyotropic systems (Pampel et al., 2002b). In model porous media the spinning side bands of the liquid signal under slow magic angle spinning have been shown to contain information relating to the diffusion of the liquid and the pore geometry (Leu et al., 2000; Liu et al., 2001; Sen et al., 2003).

In this chapter the possibility of obtaining effective diffusion coefficients of water and flavor molecules in porous media by PFG-NMR under high-resolution conditions is addressed. In order to obtain a high-resolution spectrum the diffusion experiment is performed while the sample undergoes magic angle sample spinning (MAS). Results are presented from experiments that have been performed on model porous systems and on silica sol-gel-made porous particles impregnated with a single model flavor compound. The effect of the spinning speed on the measured diffusion coefficient is investigated along with temperature effects. The influence of internal magnetic field gradients on the measured diffusion coefficients is investigated using different model porous materials and pulse sequences.

A.2. Experimental

All magic angle sample spinning diffusion experiments were performed on a Bruker Avance DRX 600 MHz NMR spectrometer (¹H Larmor frequency 600.13 MHz) using a 4-mm HR-MAS probe with Z-gradient. The maximum gradient strength was a nominal 50 G/cm. Pulsed field gradient stimulated echo (PGSTE) (Tanner, 1970) sequences and pulsed field gradient echo sequences with bipolar gradients (PGSTEBP) (Cotts et al., 1989) were used with sine shaped gradient pulses. Sample spinning was controlled to within ±1 Hz using the Bruker controller. Experiments were performed at temperatures of 25 °C (controlled by the
spectrometer) and 22 °C (uncontrolled). The controlled temperature experiments were kept to within ±0.1 °C of the set temperature and the unregulated temperature experiments were within ±0.2 °C (room air-conditioning). The effect of spinning on the temperature of the sample in the probe was measured using the chemical shift of ethylene glycol (Amann et al., 1982). Polystyrene beads of 100 μm diameter (Duke Scientific, Palo Alto, Ca) were packed into a 4-mm rotor. The sample size in the rotor was constrained to a spherical shape with a diameter of 2.7 mm by spacers.

Distilled water was added to the samples to fill up the void space between the beads. Sol-gel-made particles (sieve size 20-90 μm) containing the flavor (ethyl octanoate, Fluka, Switzerland) were prepared as described in paragraph 5.3.1 and packed into a rotor with per-deuterated ethanol (Glasser, Switzerland) as the solvent. The gradient field of the probe was calibrated using distilled water. Effective self-diffusion coefficients were obtained by fitting the experimental data points to the following equation (Price and Kuchel, 1991) using the Levenberg-Marquadt algorithm (Appendix K):

\[
I = I_0 \cdot \exp\left(-\left(\frac{\gamma^2 g^2 \delta^2}{\pi^2} \right) \frac{4t_d - \delta}{D}\right)
\]

where \( I \) is the measured echo intensity, \( I_0 \) is the relaxation weighted intensity, \( \gamma \) is the gyromagnetic constant, \( g \) is field gradient strength, \( \delta \) is the duration of the field gradient pulse, \( t_d = \Delta \) is effective diffusion time and \( D \) is the self-diffusion coefficient. A program written in the Python programming language was used for the fitting (paragraph 4.3.3) and a Monte-Carlo statistical error analysis (Alper and Gelb, 1990) was performed to estimate the standard deviation in the values of the diffusion parameters obtained.

A.3. Results and discussion

A.3.1. Comparison of static and spinning flavor sol-gel materials

Sol-gel-made silica particles are of interest as they are able to encapsulate molecules of various sizes (Böttcher et al., 1999) (paragraph 1, 2, 3). Their pore structure can be modified during their manufacture (Brinker and Scherer, 1990). Small molecules which are entrapped in the matrix during the preparation process will be expected to have different retention and
release characteristics depending on the pore structure and porosity. Characterizing the self-diffusion coefficient of the entrapped molecules will help to rationalize the pore morphology of the matrix relative to the release kinetics. However, size, shape and chemical constitution of these materials, create internal magnetic field gradients when placed in a superconducting magnet (Drain, 1962). This effect arises from the magnetic susceptibility differences between the solvent and the solid particles such that the NMR signal is dramatically broadened. This can be seen in Figure A.1a where a static proton spectrum of ethyl octanoate in a 20-90 μm silica sol-gel material fully wetted with per-deuterated ethanol is shown.

Fig. A.1: Comparison between non-spinning (a) and spinning (b) proton spectra of ethyl octanoate on sol-gel nanoparticles. Data taken with a 4-mm MAS triple resonance probe on a 400 MHz Bruker DSX Avance spectrometer (Spinning frequency: 2.5 kHz). Spectral resolution is increased significantly upon spinning.
The NMR signals are so severely broadened that there is little hope of distinguishing individual resonances from different chemical groups or resolving the residual proton spectrum of the solvent from the flavor molecule peaks. Figure A.1b shows the same sample under magic angle sample spinning conditions. This time a high resolution spectrum is obtained, individual resonances of the flavor molecule can be assigned and the residual proton resonances of the solvent and the flavor molecule are resolved. It is clear from the results in Figure A.1b that it would be very desirable to perform pulse field gradient experiments under such conditions to determine self-diffusion coefficients. The question arises, however, if the diffusion coefficients obtained under magic angle spinning will be true values uncorrupted from the effects of spinning and the small gradient strengths available with the magic angle spinning probe.

A.3.2. Stability of the system

In order to test the quality of the gradient system of the probe and whether the two techniques (PFG-NMR under MAS conditions) can be combined successfully the diffusion coefficient of water under magic angle spinning was first measured. The water was confined to a small spherical sample space and a simple PGSTE diffusion experiment was acquired under MAS conditions. The temperature of the sample was set to 25 °C and the spinning frequency was 1.5 kHz. The response of the echo decay should follow eq. A.1, as the diffusion coefficient measured should be equal to the free diffusion coefficient of water. Figure A.2a shows that the experimental data fit well eq. A.1 as the decay in the log plot is linear over two orders of magnitude. From this result one can judge that the magnetic field gradient is linear over the sample volume and as the electric current is incremented through the gradient coil system the applied gradient strength increases linearly in response. Both a linear gradient across the sample and linearity in gradient response are essential prerequisites for obtaining accurate diffusion coefficients (Price, 1997; Price, 1998).

In Figure A.2b the echo response under the same experimental conditions is shown for water surrounding mono-dispersed polystyrene spheres of 100 μm diameter. The measured diffusion coefficient of the liquid depends solely on the packing of the particles and reaches a constant at long observation times. The susceptibility broadening induced by the polystyrene particles is moderate (Seland et al., 2000) and its effect will be addressed later. If the packing
of the particles is sufficiently random, a single exponential decay is expected for the experiment that would result in a single diffusion coefficient. Figure A.2b shows that the data can be well described with a single exponential and the linearity of the log plot is comparable to the pure water system (Figure A.2a). After calibration of the gradients using the water sample, the effective diffusion coefficient for the water surrounding the polystyrene beads was $2.11 \times 10^{-9} \text{ m}^2/\text{s}$ for an observation time $\Delta = 20 \text{ ms}$ and a gradient pulse-width $\delta = 4 \text{ ms}$. This value is slightly less than the free self-diffusion coefficient of water ($2.3 \times 10^{-9} \text{ m}^2/\text{s}$) showing that the polystyrene beads are restricting the diffusion of the water at the employed conditions.

**Fig. A.2:** Linearity of PGSTE diffusion experiment under magic angle sample spinning conditions (Spinning frequency: 1.5 kHz). (a) Water only. (b) Water surrounding polystyrene beads.
A.3.3. Effective diffusion coefficient at long observation times

For a randomly packed bed of monodisperse spheres the effective self-diffusion coefficient at long observation times, $D_{\text{inter}}$, reaches a plateau, which is governed by the tortuosity and the porosity of the packing (eq. 4.5).

$$
\lim_{t \to \infty} \frac{D(t)}{D_0} = \frac{D_{\text{inter}}}{D_0} = \frac{\varepsilon_b}{\tau_b}
$$

The ratio of effective interparticle self-diffusion coefficient at long observation times $D_{\text{inter}}$ to the pure liquid free self-diffusion coefficient, $D_0$, is a characteristic of the sphere packing and size and should be independent of the solvent type (Ek et al., 1994). For monodisperse randomly packed beads of diameter 100 μm the ratio $D_{\text{inter}}/D_0 \approx 0.64$ (Figure 4.5)(Latour et al., 1993).

Figure A.3 shows the effective self-diffusion coefficient of water in the presence of 100 μm polystyrene beads as a function of observation time $\Delta$ for spinning (1.5 kHz)(filled symbols) and static (open symbols) conditions and between the simple stimulated-echo (PGSTE, circles) diffusion experiment and the bipolar stimulated-echo (PGSTEBP, triangles) experiment at 22 °C. The temperature was not regulated as it was difficult to keep the sample static when heating air was passed over it. In the simple stimulated-echo experiments (PGSTE) the results from the spinning and non-spinning regimes are quite different: the final diffusion ratios $D_{\text{inter}}/D_0$ were 0.66 and 0.5, respectively. If these results are taken in isolation then one might conclude that the packing of the polystyrene spheres changed upon spinning or the sample temperature had increased by spinning when compared to static results. In the two PGSTEBP experiments, however, the final diffusion ratios coincide with one another, within experimental error, and are equal to the value obtained in the spinning PGSTE echo sequence. It would appear that the temperature is nearly the same under these low spinning speed conditions and that the packing of the beads is unaltered.

The bipolar sequence was designed to compensate for interference of internal magnetic field gradients arising from susceptibility differences within the sample and the applied external gradients used in the diffusion experiment. The size of the internal gradients $g$, can be estimated from the line-width at half height $\Delta v$ of the water resonance and knowledge of the sphere diameter $d$ (Holz et al., 2000; Seland et al., 2000).
Fig. A.3: Comparison of different pulse sequences for the self-diffusion of water between 100 μm polystyrene beads under spinning and static conditions. (a) spinning PGSTE. (b) static PGSTE. (c) spinning PGSTEBP. (d) static PGSTEBP. $D_0$ for water was set to $2.11 \times 10^{-9}$ m$^2$/s at 295 K. The spinning frequency was 1.5 kHz.

\[
g_i = \frac{2\pi \Delta \nu}{\gamma d} \quad \text{(A.3)}
\]

The line-width of the water resonance when the sample was not spinning was 200 Hz, which gives an estimation for the internal gradient of 6 G/cm. Although this value would be considered negligible under normal static experimental pulsed field gradient experiments, where the maximum gradient strength can be around 500 G/cm, under the current conditions it cannot be ignored. This is especially the case at long observation times where the maximum gradient strength has to be reduced to keep the echo decay constant as a function of observation time. In the experiments shown in Figure A.3 the maximum gradient strength at long observations times is around 15 G/cm, only just over twice that of the estimated internal gradient in the static case. Magic angle spinning averages the broadening from isotropic susceptibility effects (Vanderhart et al., 1981) and, therefore, the simple PGSTE experiment works when the sample is spinning.
A.3.4. Effect of spinning speed on the diffusion coefficient

All diffusion measurements were performed on a 600 MHz spectrometer and therefore, the minimum spinning speed required for an effective 10 ppm spinning sideband free spectrum would be 6 kHz. One can imagine that the spinning frequency will have an effect on the measured diffusion coefficient in terms of spinning out the water and heating the sample. In order to observe the effect of the spinning speed on the measured effective diffusion coefficients the diffusion coefficient of the water surrounding the polystyrene beads was measured as a function of increasing and then decreasing spinning speed. The diffusion conditions for the experiment were chosen ($\Delta = 300$ ms) such that the diffusion plateau was reached. The measured diffusion coefficient of the water should be reduced relative to the unrestricted value and any effects of the spinning should, therefore, become apparent. The results are shown in Figure A.4a. The spinning frequency covered is from 1.0 to 5.5 kHz. As the spinning frequency was increased from a starting value of 1 kHz the observed diffusion coefficient remained constant until the spinning frequency reached a value of 4.0 kHz. After 4.0 kHz the observed diffusion coefficient became dependent upon the spinning speed of the sample. At 5.5 kHz the diffusion coefficient was close to that for the free diffusion coefficient value for water. The spinning frequency was then systematically reduced back down to 1 kHz. The measured diffusion coefficient of the water followed, within experimental error, the same path observed during the first half of the experiment. The experiment shows no evidence of hysteresis and a usable maximum spinning frequency of 4.0 kHz that equates to a spectral width at 600 MHz of 6.67 ppm.

In Figure A.4b the temperature of the rotor as a function of spinning speed is shown using the chemical difference observed with ethylene glycol. The temperature of the rotor follows the same trend as the diffusion experiments. The temperature of the rotor stays nearly constant up until about 3 kHz and then it starts to increase rapidly. The temperature increase over the spinning frequency covered is about 2.3 °C. The corresponding temperature dependence of the self-diffusion coefficient of water is $0.05 \cdot 10^{-9} \text{ m}^2/\text{s}$ (Holz et al., 2000). The expected increase due to the temperature is too small to account for the change observed in the diffusion coefficient. It maybe that above 4 kHz the packing of the sample changes and that the void space between the particles becomes non-uniform leading to large void spaces which would in turn lead to a higher effective self-diffusion coefficient for the water.
Fig. A.4: The effect of sample spinning frequency on the measured diffusion coefficient of water between polystyrene beads (a). Measured temperature of sample containing ethylene glycol as a function of spinning frequency (b). The expected increase in the diffusion coefficient due to temperature (b) is too small to account for the change in diffusion coefficient shown in (a).
A.3.5. Flavor results

Finally, self-diffusion experiments under magic angle sample spinning conditions were performed on a porous sol-gel matrix that was impregnated with ethyl octanoate during its synthesis. This material is mesoporous with an average pore size of about 2.4 nm. Unlike the polystyrene spheres, where there is only one pore space between the particles, with this sample there are two: the interparticle pore space between the particles and the intraparticle pore space of the mesopores (Figure 5.3). If the exchange between the two spaces of the flavor molecules is slow compared to the diffusion experiment observation time one would, therefore, expect to obtain two effective diffusion coefficients when the material is wetted with the appropriate solvent (eq. 5.9). This was found to be the case for this study. The echo decay curve of the diffusion experiment is best described by a two exponential curve. The results are shown in Figure A.5 as a function of observation time $t_d = \Delta$. The effective diffusion coefficient for the flavor molecules between the particles is around $6.1 \cdot 10^{-10}$ m²/s at long observation times and within the mesopores around $1.67 \cdot 10^{-10}$ m²/s. Unlike the polystyrene beads the diffusion values have reached a plateau by the first data point at 100 ms for both the intra- and interparticle effective diffusion coefficients. This is because the average particle size of the sol-gel material is around 55 μm, half the diameter of the polystyrene beads, and their polydisperse nature (20-90 μm) that enables a denser packing (paragraph 4.4.5). The amounts of each component in the two diffusion regimes remains constant at a 60:40 ratio, showing that there is little exchange of flavor molecules between the two diffusion regimes over the observation time of the diffusion experiment. From the static spectrum the line broadening from susceptibility effects is around 350 Hz which would give internal magnetic field gradients of around 16 to 17 G/cm, low enough for their effects to be compensated for by magic angle spinning.
Fig. A.5: Effective diffusion coefficients of ethyl octanoate present in sol-gel matrix particles when fully wetted with per-deuterated ethanol. The bi-exponential echo decay according to eq. 5.9 describes flavour diffusion between the particles (squares) and within the particle pores (triangles). The error bars result from a Monte-Carlo simulation to the experimental data set.

A.4. Conclusions

It has been shown that one can successfully perform pulsed field gradient NMR experiments under magic angle sample spinning on porous media to obtain diffusion coefficients of liquids in porous particles. It has been demonstrated that there is a reasonable spinning frequency window where diffusion coefficients are independent of the spinning frequency. The effect of inhomogeneous internal gradients on the diffusion experiment arising from the porous material are removed by the spinning of the sample and only simple pulse sequences are needed to obtain correct diffusion coefficients.
A.5. References


Appendix B. Calibration of the Field Gradients

The field gradients were calibrated with a 1 vol% H₂O in D₂O solution containing 1 Mass% CuSO₄ and the diffusion coefficient was set to 1.9·10⁻⁹ m²/s at 298 K (Mills, 1973). The temperature calibration was done with an ethylene glycol sample, where the shift Δδ between the -CH₂ and the -OH peak was taken to calculate the actual temperature of the sample according to (Amann et al., 1982).

\[ \theta[K] = 466.5 - 102.00 \times \Delta\delta \]  
(B.1)

Afterwards the literature value of the diffusion coefficient of butanol (chemical shifts: 0.79 and 3.5 ppm) in water of 7.7·10⁻¹⁰ m²/s (Cussler, 1997) was confirmed by a measurement of butanol diffusion in D₂O with an error of about 1%.

B. References


Appendix C. Comparison of the Pulse Sequences

PGSE (Figure 4.1a):
In a PFG-NMR experiment the diffusion coefficient $D$ can be obtained from the ratio of the spin echo amplitude $I$ normalized to the relaxation weighted intensity $I_0$ by the equation given by Stejskal and Tanner (Stejskal, 1965):

$$\frac{I}{I_0} = \exp\left(-\left(\gamma g \delta\right)^2 D \frac{\Delta - \delta}{3}\right)$$  \hspace{1cm} (C.1)

where $\gamma$ is the gyromagnetic ratio, $g$ is the applied magnetic field gradient, $\delta$ is the effective length of the applied gradient pulses, $D$ is the self-diffusion coefficient and $t_d$ is the effective observation or diffusion time.

The actual diffusion time $t_d$ of the molecules is defined as:

$$t_d = \Delta - \frac{\delta}{3}$$  \hspace{1cm} (C.2)

PGSTE:
Alternative pulse sequences like the pulsed field gradient stimulated echo sequence (PGSTE) may allow the use of considerably larger time durations $\Delta$ between the two gradient pulses and hence the observation of smaller diffusion coefficients (Kärger et al., 1988). Another advantage over the steady gradient technique is, that $\Delta$ can be varied independently from $\tau$, thus avoiding uncertainties regarding effects of changing the time $\tau$ (Cooper et al., 1974). The echo attenuation for the PGSTE sequence can be written in the following form:

$$\frac{I}{I_0} = \exp\left(-\left(\gamma g \delta\right)^2 D \left(\Delta + \tau - \frac{\delta}{3}\right)\right)$$  \hspace{1cm} (C.3)

where the diffusion time $t_d$ is given by:

$$t_d = \Delta + \tau - \frac{\delta}{3}$$  \hspace{1cm} (C.4)
PGSTEBP (Figure 4.1b):
The 13-interval bipolar PFG stimulated echo sequence (PGSTEBP) is often applied in heterogeneous systems in order to minimize internal gradients. These internal gradients imposed by the heterogeneity of the system may be a significant source of error (Sorland et al., 1997; Seland et al., 2001). They are due to differences in magnetic susceptibility between the different areas in a heterogeneous sample (Hürlimann, 1998). The coupling between the applied and the internal gradients may result in erroneous diffusion coefficients. However, recent publications have shown that at long observation times such a suppression is difficult to obtain, even if a bipolar sequence is used (Seland et al., 2000). The echo attenuation of the PGSTEBP-sequence is described as:

\[ \frac{I}{I_0} = \exp \left( -\gamma \delta \gamma \right) \left( g - \frac{1}{2} (g - f) \left( \frac{\Delta + \tau - \delta}{6} \right) \right)^2 \left( \Delta + \frac{3}{2} \tau + \frac{\delta}{6} \right) \] (C.5)

with the diffusion time \( t_d \):

\[ t_d = \Delta + \frac{3}{2} \tau - \frac{\delta}{6} \] (C.6)

In the absence of restricting barriers all the different pulse sequences (PGSE, PGSTE, PGSTEBP) should show a mono-exponential decay measuring the self-diffusion coefficient of 1 wt% H₂O in D₂O. The sample used for the calibration was subjected to the different sequences and the same diffusion coefficients were obtained using the following sequence parameters.
Table C.1: Sequence Parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Gradient Strength $g$ / [G/cm]</td>
<td>100</td>
</tr>
<tr>
<td>Gradient pulse duration $\delta$ / [ms]</td>
<td>0.75</td>
</tr>
<tr>
<td>Recycle delay / [ms]</td>
<td>1082</td>
</tr>
<tr>
<td>Number of steps / [-]</td>
<td>64</td>
</tr>
<tr>
<td>Scans</td>
<td>192</td>
</tr>
</tbody>
</table>

Figure C.2 shows a typical echo decay (monoexponential decay) obtained from the three sequences varying the gradient strength. The diffusion coefficient was calculated according to the eq. C.1, C.3 and C.5 using a Levenberg-Marquadt algorithm (python programming language) or the accompanying software from Brucker. It can be seen, that all the three sequences result in the same echo decay and hence in the same free diffusion coefficient of 1 wt% $\text{H}_2\text{O}$ in $\text{D}_2\text{O}$ (Mills, 1973).

![Graph](image-url)

*Fig. C.2: Echo decay in a typical spin echo experiment using the PGSE, the PGSTE and the PGSTEBP sequence.*
C. References


Appendix D. Determination of the Bed Porosity

The diffusion of water was measured in monodisperse polystyrene beads (d = 100 μm) by PFG-NMR (paragraph 4.4.3). For short diffusion times the measured apparent diffusion coefficient $D(t)$ is equal to the free diffusion coefficient $D_0$ in bulk solution, since the molecules diffuse only a short distance and only a few molecules will encounter restrictions. In this regime, $D(t)$ reflects only the local properties of the pore medium. Latour et al. (1993) showed that the short-time behavior of the diffusion coefficient is independent of the microscopic details of the restricting geometry and the surface relaxation and depends only on the surface-to-volume ratio $S_p / V_v$ of the pore space. $S_p$ is the surface area of the spherical beads and $V_v$ the pore or void volume between the beads. The time dependence in this region can be expressed as (Mitra et al., 1992; Latour et al., 1993; Helmer et al., 1995):

$$ \frac{D(t)}{D_0} = 1 - \frac{4}{9\sqrt{\pi}} \cdot \frac{S_p}{V_v} \cdot \sqrt{Dt} \quad (D.1) $$

The physical origin of this equation derives from the fact, that at short times only molecules in the surface layer, whose thickness is equal to the diffusion length $l_D = \sqrt{Dt}$ are sensitive to the restrictions of the pore walls. The fraction of the molecules affected by restrictions at short times is given by $S_p / V_v \cdot \sqrt{Dt}$. These studies dispel the common assumption that the effects of restrictions are negligible when the diffusion length $l_D$ is much less than the characteristic size of the restricting structures (e.g. average radius of the interstitial spaces of a porous medium) in the system.

Figure D.1 shows the $\sqrt{t}$ dependency described in eq. D.1 for the diffusion of water between monodisperse 100 μm polystyrene beads (Seland et al., 2000). From the slope of the curve at low observation times (-0.03 1/s$^{0.5}$) the surface-to-volume ratio $S_p / V_v$ of the pore space was determined and the mean void diameter $a$ was calculated to be around 12.7 μm, which corresponds to a packing porosity of about 45% using eq. 4.4 and D.1.
Fig. D.1: $\sqrt{t}$ dependency of the normalized diffusion coefficient in the system of 100 μm polystyrene beads (slope: $-0.03 \text{ ms}^{-0.5}$) (Seland et al., 2000).

D. References


Appendix E. Diffraction Effects

The diffusion of water was measured in monodisperse polystyrene beads (d = 100 μm) by PFG-NMR (paragraph 4.4.3). The polystyrene beads were expected to be close-packed, but no diffraction effects were observed in this study in the signal attenuation curves as a function of gradient strength and observation time as the latter was too short. According to Latour et al. (1993) the surface-to-volume ratio \( S_p/V_v \) was determined to \( S_p/V_v = 73657 \text{ m}^{-1} \) from the short time diffusion data resulting in a porosity of 0.45, which is identical to the one found by Seland et al. (2000) for the same system. According to Callaghan et al. (1992) diffraction effects are best observed, when the spins have enough time to diffuse between pores. The sharpest coherence peak is seen at an observation time:

\[
\Delta = \frac{b^2}{2 \cdot D_{\text{eff}}}
\]  

measuring \( D_{\text{eff}} \) in the low \( q \) limit (\( b \): pore spacing). Callaghan et al. (1991) studied the diffusion of water in monodisperse polystyrene beads (\( d=16 \mu\text{m} \)) and packing porosity of 0.44. From the low \( q \) data, a \( D_{\text{eff}} = 2.0 \cdot 10^{-9} \text{ m}^2/\text{s} \) was measured. Since the pore spacing \( b \) is roughly equal to the particle diameter \( d \) (Callaghan et al., 1991), diffraction effects can be seen at observation times bigger than 70 ms (Figure 2 in Callaghan et al. (1991)). Eq. E.1 results in an optimum observation time of about 64 ms. According to eq. E.1 diffraction effects would be observable above 3.2 s in this study, which was not reached at a maximum observation time of 1s (\( D_{\text{eff}} = 0.67 \cdot 2.3 \cdot 10^{-9} \text{ m}^2/\text{s} \) (Figure 4.5); \( b = d = 100 \mu\text{m} \)).

E. References


Appendix F. Determination of the Particle Size

The particle size distribution of the silica particle fraction used in the release experiments in chapter 5 was determined by Frauenhofer laser-diffraction spectroscopy on a Sympatec-Helos (Germany) in a scell system with a cell width of 2 mm. Figures F.1 a,b show the particle size distributions of the ethyl octanoate (EO) and the ethyl decanoate (ED) sample determined for the particle size fraction of 710 – 1000 μm.

Fig. F.1a: Particle size distribution (EO) determined by laser diffraction for the particle size fraction of 710 – 1000 μm.
Fig. F.1b: Particle size distribution (ED) determined by laser diffraction for the particle size fraction of 710 – 1000 μm.
Appendix G. Release from Flavor-Laden Silica Particles

The release of ethyl butanole (EB) and ethyl hexanoate (EH) from sol-gel-made silica particles was measured by UV-VIS spectroscopy for a particle size fraction of 710 – 1000 μm. The average diffusion coefficient measured by PFG-NMR was $1.23 \times 10^{-10}$ (EB) and $1.32 \times 10^{-10}$ m$^2$/s (EH) (paragraph 5.4.1).
Fig. G.1: Measured release kinetics (UV-VIS) of a) ethyl butanoate (EB) and b) ethyl hexanoate (EH) in ethanol from the silica particles with a size fraction of 710-1000 μm. The average particle diameter was determined by laser-diffraction to be 910 (EB) and 810 (EH) μm. The measured release kinetics are compared to the calculated ones according to eq. 5.8 taking the pore diffusion coefficients from the PFG-MAS-NMR experiment (Table 5.1). The measured release profiles are in agreement with the calculations and fall within the error intervals determined by the Monte-Carlo simulation.
Appendix H. Influence of Particle Size on the Release Kinetics

Figure H.1 demonstrates the influence of particle size on the release kinetics in the case of ethyl decanoate. The amount released is calculated according to eq. 5.8 with the average PFG-MAS-NMR diffusion coefficient \( 3.44 \times 10^{-11} \text{ m}^2/\text{s} \) and the boundary diameters (710, 1000 \( \mu \text{m} \)) of the particle size fraction used in the release experiment. It can be seen, that the measured release curve (average diameter: 820 \( \mu \text{m} \) determined by laser-diffraction) lays within the given particle size interval.

![Figure H.1: Measured release kinetics (UV-VIS) of ethyl decanoate (ED) in ethanol from the silica particles with a size fraction of 710-1000 \( \mu \text{m} \). The average particle diameter was determined by laser-diffraction to be 820 \( \mu \text{m} \). The measured release kinetics are compared to the calculated ones according to eq. 5.8 with the average PFG-MAS-NMR pore diffusion coefficient \( 3.44 \times 10^{-11} \text{ m}^2/\text{s} \) varying the particle diameters.](image-url)
Appendix I. Fortran Code: Release Kinetics

! Diffusion through a sphere
! Crank "The mathematics of diffusion" page 98 (a=0)

program sphere
IMPLICIT double precision (a-h, o-z)

! b: Radius,
! D: Diffusionskoeffizient

real b, pi, t, D
parameter (pi=3.1415)
real n
real M1,M2,M3, Mmal(10000), M
open(Unit=62, file='output.dat', status='new')

b=378d-6; !(m) Radius der Kugel
D=3.17d-11 !Diff. koeff (m2/s)

print *,D
print *,b
Write(62,*) D
Write(62,*) b

M1 =6/(pi**2*b**2)
Mmal(0)=0; !t / (s)
do t=0,5000
    do n=1,10000
        M2=((b*cos(n*pi))/n)**2
        M3=EXP(-D*n**2*pi**2*t/b**2)
        Mmal(n)=Mmal(n-1)+M2*M3
    end do
    M=1-M1*Mmal(10000)
    print *, t, M
    Write(62,*) t, M
end do
close (Unit=62)
end program sphere
Seite Leer / Blank leaf
Appendix J. Fortran Code: Regression Analysis

! Diffusion through a sphere
! Crank ("The Mathematics of Diffusion") page 98 (a=0)
! b: Radius,
! D: Diffusionskoeffizient
! $R^2 = Rtwo$: Regression Coefficient

program sphere

integer*4 MAX_data
parameter (MAX_data = 15000)
character*30 file_input, file_output
integer*4 n_tot, lun, i, i_tot
integer*4 k, k_max
real*8 b, t, deltats
real*8 D, D_min, D_max, delta_D
real*8 n, PI, tmax
real*8 M1, M2, M3, Mmal
real*8 SSE, SST1, SST2, SST, Rtwo
real*8 t_exp(1:MAX_data), M_exp(1:MAX_data), M_cal(1:MAX_data)
! M_mal(0:MAX_count)

! parameters ED R410
!-----------------------------------------------

b = 410d-6  ! (m) Radius of sphere
D_min = 2.72d-11  ! Diff. coeff (m²/s) smallest value
D_max = 4.15d-11  ! Diff. coeff (m²/s) largest value
delta_D = 0.01d-11  ! Diff. coeff (m²/s) step size
file_input = 'ED R410.txt'  ! Experimental data (UV-VIS)
file_output = 'result ED R410.txt'  ! Results for $R^2$ and SSE (def. see Excel: trend line)
number_data = 8000  ! # of data in the experimental source file
t_max = 62.89583588d0  ! Statistics up to this time: (x min)

! constants
!-----------------------------------------------
n_tot = 50  ! Inner loop for integration
PI = 3.141592653589793d0
print*, t_max, 'min'
print*, file_input
print*, file_output

$ t_{max} = t_{max} * 60.0d0 $  ! (tmax in seconds)
! read data from experimental file
! t_exp are the experimental times [s]
!
!---------------------------------------------------------------
  lun = 10
  open (lun, file=file_input)
  do i=1,number_data
     read(lun,*) t_exp(i), M_exp(i)
     t_exp(i)=t_exp(i)*60.0d0 !result in seconds
  end do
  close(lun)
!

! loop for different diffusion coefficients
!
!---------------------------------------------------------------
  lun = 11
  open(Unit=lun, file=file_output, status="unknown")
  k_max = int((D_max-D_min)/delta_D+0.999)
  do k=0,k_max
     D = D_min + dfloat(k) * delta_D
     ! do one calculation: Release Kinetics
     !---------------------------------------------------------------
     ! print *,'Diffusion coefficient: ',D
     ! print *,'radius: ',b
     !Write(lun,*) D
     !Write(lun,*) b
     !initialize values
     M1 = 6.0d0/(pi**2*b**2)
     t = 0
     i_tot = number_data-1
     !print *, t, M_cal(1), M_exp(1)
     SSE = 0.0d0
     SST1= 0.0d0
     SST2= 0.0d0
     !start loop for one D
     do i=2,i_tot
        delta_t = t_exp(i) - t !_exp(i-1)
        t = t + delta_t
        Mmal = 0
        do n=1,n_tot
           ...
\[ M_2 = ((b * \cos(n \pi)) / n)^2 \]
\[ M_3 = \exp(-D * n^2 * \pi^2 * t / b^2) \]
\[ M_{mal} = M_{mal} + M_2 * M_3 \]

end do

\[ M_{cal}(i) = 1.0d0 - M_1 * M_{mal} \]

! print *, t, M_{cal}(i), M_{exp(i)}

\[ SSE = SSE + (M_{cal}(i) - M_{exp(i)})^2 \]
\[ SST1 = SST1 + M_{cal}(i) * M_{cal}(i) \]
\[ SST2 = SST2 + M_{cal}(i) \]

! write(lun,*), t, M

if (t .GT. t_{max}) exit
end do

\[ SST = SST1 - SST2 * SST2 / dfloat(i) \]
\[ Rtwo = 1.0d0 - SSE / SST \]

print 10,k,'D = ',D,'R2 = ',Rtwo,'SSE = ',SSE
write (lun,10) k,'D = ',D,'R2 = ',Rtwo,'SSE = ',SSE

end do

close (unit=lun)

10 format(i4,A10,e13.5,A10,f13.9,A10,e16.8)
end program sphere
Appendix K. Python Code and Example Calculation: NMR Data Analysis and Monte-Carlo Simulation

The measured echo decay of ethyl decanoate (peak at 1.28 ppm; $\Delta = 100$ ms) in sol-gel-made silica particles (paragraph 5) is fitted by a biexponential decay function according to eq. 5.9. The pulsed field gradient stimulated echo (PGSTE) sequence with sine shaped gradient pulses (PGSTEsine.py) was used in this example calculation.

Figure K.1 shows the biexponential fit (fitroutines.py) to the measured echo decay and the corresponding errors for $D_i$ and $p_i$, resulting from the Monte Carlo analysis ($n = 1000$). Figure K.2 a,b present the frequency distributions of the intraparticle diffusion coefficient ($D_1$) and the fraction of the molecules in the pore space ($p_2$) obtained from the Monte-Carlo analysis.

$$D_1 = 1.92 \times 10^{-10} \pm 1.87 \times 10^{-12} \text{ m}^2/\text{s}$$
$$p_1 = 0.69 \pm 0.006$$
$$D_2 = 1.95 \times 10^{-11} \pm 8.07 \times 10^{-13} \text{ m}^2/\text{s}$$
$$p_2 = 0.31 \pm 0.006$$

Fig. K.1: Biexponential fit to the echo decay of ethyl decanoate (peak at 1.28 ppm; $\Delta = 100$ ms) entrapped in silica. The intra- and interparticle diffusion coefficients and the proportion of the molecules in each of the diffusion domains are given along with the errors resulting from the Monte-Carlo simulation.
Fig. K.2a: Frequency distribution of the intraparticle diffusion coefficient ($D_2$) obtained from the Monte-Carlo analysis.

Fig. K.2b: Frequency distribution of the molecules in the intraparticle diffusion domain ($p_2$) obtained from the Monte-Carlo analysis.
```python
import Numeric
import MLab
import RandomArray
import scipy
from scipy import gplt
import math
import string
import fpformat
import copy
from os.path import *
from string import *
from sys import *
import os
import readbruker2dc

class FitRoutine:
    """A base class for defining fit functions and optimization""

    def __init__( self, argv ):
        self.parse_command_line( argv )
        self.firsttime = 1
        pass

    def parse_command_line( self, argv ):
        """Parses command line to set up parameters""
        if len(argv) != 22:
            print "\n\nCommand line not correct\n\n"
            exit(-1)
        else:
            self.directory = argv[1]
            self.expno = argv[2]
            self.function = argv[3]
            self.numpts = int(argv[4])
            self.start_pts = int(argv[5])
            self.start_data = int(argv[5])
            self.enddata = int(argv[6])
            self.numcomponents = int(argv[7])
            self.num_opt_components = int(argv[8])
```

self.num_fit_components = int(argv[8])

self.diff_vals[0]=float(argv[9])
self.diff_vals[1]=float(argv[10])
self.fraction_vals[0]=float(argv[12])
self.fraction_vals[1]=float(argv[13])
self.fraction_vals[2]=float(argv[14])

self.do_MonteCarlo = argv[15]
self.monteCarlo_iterations=int(argv[16])
self.data_origin = argv[17]
self.intensity_area = argv[17]

self.list_of_expts = argv[18]
self.grad_incr = float(argv[19])
self.peak_pos = int(argv[20])-1
self.grad_start = float(argv[21])

def plot_data(self):
    """Plots final fit in x y format"""
    gplt.figure()
    plot1=gplt.current()
    gplt.plot( self.x_axis[self.start_data:self.end_data],
               self.y_axis[self.start_data:self.end_data], " wp ",
               self.calculated_data[self.start_data:self.end_data], " w l" )
    gplt.xtitle( self.xtitle )
    gplt.ytitle( self.ytitle )
    gplt.title( self.graph_title )
    gplt.legend( "hide" )

def plot_data_straightline(self):
    """Plots final fit in x log y format to produce straight line"""
    gplt.figure()
    plot1=gplt.current()

    max_val = max(self.calculated_data)
    self.calculated_data1 = self.calculated_data/max_val
    self.calculated_data1 = Numeric.log10(self.calculated_data1)

    max_val = max(self.y_axis)
    self.y_axis1 = self.y_axis/max_val
    self.y_axis1 = Numeric.log10(self.y_axis1)

    gplt.plot( self.x_axis_squared[self.start_data:self.end_data],
               self.y_axis[self.start_data:self.end_data], " wp ",
               self.x_axis_squared[self.start_data:self.end_data],
               self.calculated_data[self.start_data:self.end_data], " w l" )
gplt.yaxis([min(self.y_axis)-0.05*min(self.y_axis), 1.1])
gplt.logy()
gplt.xticks(self.xtitle)
gplt.yticks(self.ytitle)

gplt.title(self.graph_title)
gplt.legend("hide")

def save_data(self):
    
    f = open( "results.txt", "w" )

    for i in range(len(self.x_axis)):
        line = str(self.x_axis[i]) + " "
        line += str(self.x_axis_squared[i]) + " "
        line += str(self.y_axis[i]) + " "
        line += str(self.calculated_data[i]) + "n"

        f.write(line)

    f.close()

def generic_function( self, x,fvec=None, iflag=0, m=0, n=0 ):
    """Template for diffusion fitting functions"""
    if fvec is None:
        fvec = Numeric.zeros( self.end_data -self.start_data, Numeric.Float )

    for e in range( len(x)):
        self.parameters[e] = x[e]

    for i in range( self.numpts ) :
        self.calculated_data[i] = self.virtual_func( 0, i )
    for j in range( 1, self.num_components):
        for i in range( self.numpts ):
            self.calculated_data[i] += self.virtual_func( j, i )

    for i in range( self.start_data, self.end_data ):
        self.diff_data[i] = self.y_axis[i] - self.calculated_data[i]
    for i in range( self.start_data, self.end_data ):
        fvec[i-self.start_data] = self.diff_data[i] * self.diff_data[i]

    scipy.gplt.plot( self.x_axis, self.calculated_data, self.x_axis, self.y_axis, "w p 1" )
    return fvec

def area( self ): 
""" returns true if Area integration used, false if not """
if self.intensity_area == "Area":
    return( True )
else:
    return( False )

def intensity( self):
    """ returns true if Intensity is used, false if not """
if self.intensity_area != "Height":
    return( False )
else:
    return( True )

def run_optimization( self):
    """Sets up optimization, reads in data, does Monte Carlo error analysis"
    for self.expno in self.list_of_expts:
        self.read_acq_parameters()
        self.read_expt_data()
        self.setup_pulsesequence_params()
        self.parameters = []
        for i in range(len( self.diff_vals )):
            self.parameters.append( self.fraction_vals[i] )
            self.parameters.append( self.diff_vals[i] )
        x = []
        for j in range(self.num_fit_components):
            x.append(self.parameters[2*j])
            x.append(self.parameters[2*j+1])

        ## Do optimization
        fvec = self.generic_function( x, m=self.num_fit_components, n=self.end_data-
self.start_data)
        z= scipy.optimize.minpack.leastsq( self.generic_function, x )
        print z[0]

        ## Do Monte Carlo error analysis
        if self.do_MonteCarlo == 1:
            self.monte_carlo_error_analysis( x, z, self.monteCarlo_iterations)
            self.xtitle = "Gradient Strength^2 [ Gauss^2/cm^2 ]"
            self.ytitle = "log(I/I_0)"
            self.graph_title = "Test of two exponential Decay"
            self.set_title( z[0] )
            self.plot_data_straightline()
            self.xtitle = "Gradient Strength [ Gauss/cm ]"
            self.ytitle = "Signal"
            self.plot_data()
            self.save_data()
            for i in range(self.num_fit_components*2):
                self.create_histogram(self.results[i])
else:
    pass

def read_acq_parameters( self):
    """Read in acquisition parameters from Bruker files"""
    file_acqu = self.directory + "/" + self.expno + "/acqu"
    if os.path.exists( file_acqu ) :
        print " Opening File: " + file_acqu + "\n"
    else:
        print " Cannot find file: " + file_acqu + "\n"
        exit(1)

    f = open( file_acqu, 'r' )

    while 1:
        line = f.readline()
        if line == "":
            break
        if string.find( line, "##D=" ) >= 0:
            self.Dvalues = string.split( f.readline() )
            while( len(self.Dvalues) < 32 ) :
                self.Dvalues += string.split( f.readline() )
        if string.find( line, "##P=" ) >= 0:
            self.Pvalues = string.split( f.readline() )
            while( len(self.Pvalues) < 32 ) :
                self.Pvalues += string.split( f.readline() )

    def read_expt_data( self):
        """Read in spectra and parameters to do with peak analysis"""

        self.brukerparams = readbruker2dc.Bruker2DdataFile( self.directory, self.expno, "1" )
        self.brukerparams.read_acq_parameters()
        self.brukerparams.read_proc_parameters()
        self.brukerparams.read_proc2_parameters()
        self.brukerparams.read_spec()
        self.brukerparams.read_peak_positions()
        self.brukerparams.read_gradient_list()

        self.xaxis = Numeric.zeros( self.brukerparams.num_spectra(), 'd' )
        self.yaxis = Numeric.zeros( self.brukerparams.num_spectra(), 'd' )
        self.calculated_data = Numeric.zeros( self.brukerparams.num_spectra(), 'd' )
        self.diff_data = Numeric.zeros( self.brukerparams.num_spectra(), 'd' )
        self.diffusion_data = Numeric.zeros((len(self.brukerparams.peak_pos["points"])), Numeric.Float)
## Check to see if data is taken from expt or text input file

```python
if self.dataorigin == "Area":
    self.input_file = "false"
elif self.dataorigin == "Height":
    self.input_file = "false"
else:
    self.input_file = "t1t2.dx"
```

```python
scipy.gplt.plot( self.brukerparams.return_spectrum_rr(0) )
```

## keypress = raw_input( "press Enter to continue" )

```python
if self.input_file == "false":
    for i in range(self.brukerparams.num_spectra()):
        spec = self.brukerparams.return_spectrum_rr(i)
        self.x_axis[i] = i*self.grad_incr

    if self.area():
        print "self.area()"
        self.brukerparams.read_area_values()
        self.brukerparams.calc_area_values_in_points()

        for i in range(self.brukerparams.num_spectra()):
            spec = self.brukerparams.return_spectrum_rr(i)
            self.x_axis[i] = i*self.grad_incr + self.grad_start

        j=0
        for area in self.brukerparams.area_pos["points "]:
            sum = 0.0
            for k in range( area[0],area[1] ):
                sum += float(spec[k]) / 10000.0
            self.diffusion_data[j][i] = sum
            j += 1

    else:
        for i in range(self.brukerparams.num_spectra()):
            spec = self.brukerparams.return_spectrum_rr(i)
            self.x_axis[i] = i*self.grad_incr + self.grad_start

        j=0
        for peak in self.brukerparams.peak_pos["points "]:
            self.diffusion_data[j][i] = max(spec[peak-10:peak+10])
            j += 1
```

```python
for i in range(self.brukerparams.num_spectra()):
    self.y_axis[i] = self.diffusion_data[self.peak_pos][i]
```

else:
## Read data from input file created by prxy command in bruker xwinmr program if processed by xwinmr 3.5 ##

```python
file_datapts = self.directory + "\"" + self.expno + "/\"pdata/1\"" + self.input_file
if exists( file_datapts ):
    file_buff = []

f = open( file_datapts, 'r' )
while 1:
    line = f.readline()
    if line == ":
        break
    file_buff.append( line )
f.close()
data_start = -1
for i in range( len(file_buff)):
    words = string.split( file_buff[i] )
    if len(words) > 0:
        if words[0] == ":XYPOINTS=":
            data_start = i+1
    data_matrix = []
    exp_data = {}
    for i in range( data_start, data_start+self.numpts):
        datarow = split( file_buff[i] )
        data_matrix.append( datarow )
        i = 0
        for xypair in data_matrix:
            self.x_axis[i] = string.atof(xypair[0][:-1])
            self.y_axis[i] = string.atof(xypair[1])
            i += 1
        else:
            print "************* Could not find file"
            exit()

maxehy = copy.deepcopy(self.y_axis[0])
for i in range(len(self.y_axis)):
    self.y_axis[i] = self.y_axis[i]/maxehy

self.y_axis_orig = copy.deepcopy(self.y_axis)
self.x_axis_squared = self.x_axis * self.x_axis

def set_initial_guess( self, x ):
    self.x_initial = x
    self.x = x
```
def monte_carlo_error_analysis( self, init_guess, z, num_iterations=500, num_bins=20):
    
    """Estimate errors in final parameters using Monte Carlo Approach"""
    self.num_iterations = num_iterations
    self.num_bins = num_bins
    num_params = len(z[0])
    self.results = Numeric.zeros((num_params,self.num_iterations),'d')

    for i in range(num_params):
        self.results[i][0] = z[0][i]

    std_res = MLab.std( self.diff_data )
    RandomArray.seed()

    for i in range(1,self.num_iterations):
        for j in range(len(self.y_axis)):
            self.y_axis[j] = RandomArray.normal( self.y_axis_orig[j], std_res )
            self.set_initial_guess( init_guess )
            z = scipy.optimize.minpack.leastsq( self.generic_function, init_guess )
            for k in range(num_params):
                self.results[k][i] = z[0][k]

    self.final_mean = Numeric.zeros( num_params, 'd' )
    self.final_std = Numeric.zeros( num_params, 'd' )

    for k in range( num_params ):
        self.final_mean[k] = MLab.mean( self.results[k] )
        self.final_std[k] = MLab.std( self.results[k] )

    for i in range( len(self.y_axis)):
        self.y_axis[i] = self.y_axis_orig[i]

    self.set_initial_guess( self.final_mean )

    ## z = self.fit_data(self.final_mean)

def create_histogram(self, results1):
    num_bins = 50
    results=Numeric.sort(results1)
    max_val = max(results)
    min_val = min(results)

    width = max(results)-min(results)
    bin_size = width/num_bins

    histogram = {}

    freq = 0
    i = 1
for val in results:
    if val <= bin_size*i+min_val:
        freq += 1
    else:
        histogram[bin_size*i+min_val]=freq
        freq = 1
        i += 1

xl = histogram.keys()
yl = []
xl.sort()
for key in xl:
    yl.append( histogram[key] )

gplt.figure()
plot_h=gplt.current()
gplt.figure(plot_h)
gplt.plot(xl,yl, "w")

f = open( "histograms.txt", "a" )
for i in range(len(xl)):
    f.write( str(xl[i]) + " " + str(yl[i]) + "n" )
f.write( "n"
f.close()
import fitroutines
import math
import fpformat
import string

class Fit_pgste_sine( fitroutines.FitRoutine):
    """Fitting class for PGSTE sequence using SINE shaped gradients""

    def __init__( self, argv ):
        self.diff.vals = [0.0,0.0,0.0]
        self.fraction.vals = [0.0,0.0,0.0]
        fitroutines.FitRoutine.__init__(self, argv )

    def set_title( self, z ):
        """Creates title for plotting""
        self.graph_title = ""
        self.graph_title = string.replace( self.directory, "\", "/" ) + " " + self.expno + "\n"

        for i in range(self.num_fit_components):
            self.graph_title += "D = " + fpformat.sci(z[2*i+1],2) + " +/- " +
            fpformat.sci(self.final_std[2*i+1],2) + " mA2/s "
            self.graph_title += " A = " + fpformat.sci(z[2*i],2) + " +/- " +
            fpformat.sci(self.final_std[2*i],2) + "\n"

    def setup_pulsesequence_params(self):
        """Calculate delta and big elta from pulse sequence parameters""
        p1 = float(self.Pvalues[1])*1.0e-6
        p30 = float(self.Pvalues[30])*1.0e-6
        p19 = float(self.Pvalues[19])*1.0e-6

        d16 = float(self.Dvalues[16])
        d20 = float(self.Dvalues[20])

        self.gamma = 4.257e3

        Delta1 = d20 - 2.0 * p1 - p30 - d16 - p19 - d16
        tau = p1 + p30 + d16
        Delta = p1 + p19 + d16 + Delta1
self.LD = p30
self.BD = tau + Delta

def virtual_func(self, i, j):
    return( self.parameters[2*i]*math.exp(-self.parameters[2*i+1]*math.pow(2.0*math.pi*self.gamma*self.LD*self.x_axis[j],2))*((4.0*self.BD-self.LD)/(math.pi*2.0*10000.0)))
Curriculum Vitae

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11/2000 – 03/2004 Research Assistant at the Institute of Process Engineering of the Swiss Federal Institute of Technology Zurich, Particle Technology Laboratory (Prof. Dr. S.E. Pratsinis)

05/1998 – 10/2000 Process Research Engineer at Bayer Technical Development Center in Leverkusen, Germany

01/1998 - 03/1998 Research Assistant at the Institute for Mechanical Process Engineering at the University of Karlsruhe, Germany

10/1991 - 12/1997 Master in Chemical Engineering at the Technical University (TH) in Karlsruhe, Germany

04/1995 - 08/1995 Semester Project at the University College London (UCL), United Kingdom

08/1982 - 06/1991 High School, Karlsruhe, Germany

09/1978 – 06/1982 Primary School, Karlsruhe, Germany
Publications:


S. R. Veith, E. Hughes, G. Vuataz, S. E. Pratsinis, “Restricted Diffusion in Silica Particles measured by Pulsed-Field Gradient NMR”, accepted for publication in the *J. Colloid Interface Sci.*


Presentations:


S.R. Veith, E. Hughes, G. Vuataz, M. Perren and S. E. Pratsinis, “Restricted Water and Aroma Diffusion through Silica Sol-Gel Made Particles Measured by Pulsed-Field Gradient NMR”, Presentation at the AIChE Annual Meeting, 17-21th November 2003, San Francisco, California, USA

S.R. Veith, M. Perren and S. E. Pratsinis, “Flavor Retention in Sol-Gel Made Silica Particles”, Presentation at the AIChE Annual Meeting, 17-21th November 2003, San Francisco, California, USA
S.R. Veith, E. Hughes, M. Perren and S. E. Pratsinis, “Aroma Retention, Diffusion and Release from Silica Sol-Gel Made Particles”, Presentation at the Institute of Mechanical and Process Engineering at the ETH Zurich, 11th November 2003, Switzerland

S.R. Veith, E. Hughes, M. Perren and S. E. Pratsinis, “Restricted Diffusion and Aroma Release from Silica Sol-Gel Particles”, Presentation at Nestlé Central Research, 27th October 2003, Lausanne, Switzerland

S. R. Veith, E. Hughes, G. Vuataz, S. E. Pratsinis, “Restricted Water Diffusion Through Silica Sol-Gel Made Particles Measured by PFG-NMR”, Poster Presentation at the DECHHEMA/GVC Conference, 16-18th September 2003, Mannheim, Germany

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S.R. Veith, “Retention and Diffusion of Aroma Molecules in Silica Sol-Gel Particles”, Presentation at Nestlé Central Research, 3th June 2002, Lausanne, Switzerland


S.R. Veith, J. Hinderer, D. Gehrmann, "Improved Retention of Volatile Components", GVC-Conference on Drying Technology/Food Process Engineering, 15-17th March 2000, Würzburg, Germany