

Doctoral (PhD) Dissertation

**SYNTHESIS OF SILICA-GELATIN HYBRID
AEROGELS AND THEIR APPLICATION AS
DRUG DELIVERY SYSTEMS**

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Dr. Lázár István

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“Stay hungry. Stay foolish.”

/Steve Jobs/

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Glossary, Acronyms and Abbreviations

TMOS	tetramethoxysilane
HMDS	1,1,1-trimethyl-N-(trimethylsilyl)silanamine
C ₁₆ -TMOS	hexadecyltrimethoxysilane
Ph-TMOS	trimethoxyphenylsilane
IBU	(RS)-2-(4-isobutylphenyl)propanoic acid, ibuprofen
KET	2-(3-benzoylphenyl)propanoic acid, ketoprofen
TRF	2-acetyloxy-4-(trifluoromethyl)benzoic acid, triflusal
HTB	2-hydroxy-4-(trifluoromethyl)benzoic acid
PBS	phosphate buffer saline: pH = 7,4; 0,14 mol L ⁻¹ NaCl; 2,70 mmol L ⁻¹ KCl; 0,01 mol L ⁻¹ PO ₄ ³⁻
sc.	supercritical
wt. %	weight percent
V/V %	volume percent
PGSE	pulsed gradient spin echo
SEM	scanning electron microscopy
DDS	drug delivery system

1. Introduction

Modern pharmaceutical industry faces certain challenges related to the administration of biologically active agents. In order to reach safer and more effective drug administration, new types of carriers have to be developed. Aerogel is the name of a group of materials with extraordinary structural and physico-chemical properties. From the early 1990's aerogels are considered to have a huge potential in life sciences applications. They represent such features, like enormous specific surface area, open mesoporous structure, very low density, and easily tunable composition and surface characteristics that can be advantageously applied in drug delivery. This trend can be clearly seen if one summarizes the number of research papers and reviews published in the last two decades.

First, the most thoroughly characterized inorganic aerogels were used as proof-of-concepts. Researchers could lay down the fundamental connections between structure and application by studying the change in their adsorption and release properties with composition and functionalization. These early prototypes were promising candidates, yet had some disadvantages which prevented their widespread use. Some of these were overcome by using bio-based organic aerogels. The biodegradable backbone opened new ways of applications. Nowadays the attention of the scientific community is directed towards hybrid and composite materials which unite the favorable features of both inorganic and organic matrices thus represent endless possibilities in material design and applications.

The studies related to aerogels showed high adsorption capacities and the possibility of both fast and retarded drug release. However, the mechanism of drug dissolution and the context between structure and delivery behavior was never fully described in any system. The main reasons for this can be the lack of appropriate probing techniques or the lack of appropriate approaches. Nevertheless, it is evident, that the infinite

number of matrix compositions require some degree of prediction, for which a deep understanding on the relationship between structure and behavior is indispensable.

In this thesis, we present a new type of hybrid drug delivery system synthesized using a newly developed method. Commonly used structural investigation techniques were combined with NMR based characterization methods in order to gain a better understanding how these complex matrices work. The effect of composition on the release behavior is highlighted and possible release mechanisms are presented.

2. Research objectives

The main objective of this research was the synthesis and characterization of such hybrid aerogel structures, where the hybrid 3D backbones are formed with the combination of inorganic (silica) and protein (gelatin) matrices. The aim of the synthesis was to combine the biodegradability of gelatin with the beneficial structural and mechanical properties of silica aerogels (higher surface area, easier manufacturing and lower shrinkage) to produce fully biocompatible and also partially biodegradable matrices. With the application of two surface modification strategies, our aim was to study the effect of functionalization on the structures and adsorption properties of the products. Co-gelation and silylation was used to prepare hexadecyl-, phenyl- and trimethylsilyl functionalized hybrid matrices. The structural changes were studied with different thermoanalytical techniques, electron microscopy and N₂ porosimetry.

The second aim was to examine the feasibility of the application of the hybrids in life sciences as drug delivery systems. Therefore, the matrices were impregnated with 3 model drugs of low water solubility (triflusal, ibuprofen and ketoprofen). Overall loadings were determined with RP-HPLC methods. Preliminary drug release tests were also carried out using the batch method combined with RP-HPLC.

Another goal was to understand the mechanistic background of the difference between the delivery properties of the two structurally similar porous materials, pure silica and silica-gelatin aerogels. By simultaneously investigating the pore structure, surface properties and hydration behavior of silica-gelatin aerogel, factors altering the loading and release characteristics can be explored. The structural investigation was complemented with a detailed study on the release kinetics of the model drugs from the silica-gelatin matrix.

Finally, we wanted to understand the main differences in drug delivery from hybrid aerogels with different gelatin contents. By summarizing the results obtained during the research program we wanted to present a

comprehensive overview on the possible applications of these newly synthesized biodegradable hybrid aerogels in drug delivery.

3. Literature overview

3.1. Properties of aerogels

Aerogels are dry, solid materials with extraordinary properties, such as high specific surface area, open mesoporous structure, extremely low density and very low thermal and electrical conductivity. They can be produced using various types of precursors, thus their composition and surface characteristics can be easily tuned.[1,2] Aerogels were first synthesized in the late 1920's. Samuel S. Kistler introduced these new solid materials to the scientific community in 1931.[3]

As their composition can be very distinct, their features also vary in a large scale. Due to this huge variety in their attributes, it is hard to give an overall picture on their properties. In this chapter, they are presented through the example of silica aerogels.

Silica aerogels represent the most studied and commercialized types of aerogels. Their specific surface area is between 500-1200 m² g⁻¹ associated with high porosity (80-99.8%) and very low density (ca. 3×10⁻³ g cm⁻³). They have very low thermal conductivity (ca. 5×10⁻³ W K⁻¹ m⁻¹), refraction index (ca. 1.05) and dielectric constant (1.0-2.0). They can be easily surface functionalized, and prepared in different shapes, such as monoliths, granules and powders. Owing to these characteristics, aerogels have very good and tunable adsorption properties.[1,2,4–6]

3.2. Aerogel preparation

The synthetic route to produce the dry aerogels has three stages independently of the composition of the aerogel backbone. Every step has a significant influence on the properties of the final products. First, a gel structure is formed with different kinds of gelation strategies. For silica aerogels, a sol-gel process is used to obtain the alcogel or hydrogel depending on the dispersion medium. This is followed by the aging, when

the solid structure is further strengthened to avoid shrinkage during further processing. Also, all the co-products of the synthesis and all impurities are removed in this step. Typically, the mother liquid inside the pores are changed for pure organic solvents via a multiple-step solvent change during aging. The last step is the drying, where all the liquid is removed to obtain the three dimensional solid structure of aerogels. [7,8]

The solid, 3D backbone of the gel is built up from primal globules. These globules form during the hydrolysis and the following polycondensation of various types of alkoxy silane precursors. The gelation time is rather slow, thus catalysis (acidic, basic or two-step) is required to reach optimal gelation time. Depending on the type of the applied catalyst the reaction mechanism is completely different. Using acids, the hydrolysis rate will be faster, resulting a polymeric gel with linear, polymer-like globules. Under basic conditions, the condensation will be faster leading to the formation of colloidal gels with nanoparticle like globules. Moreover, the structure of the alkoxy silane precursors, the silane–water ratio and the organic solvent used during the synthesis also have their own effect on the gel structure.[7,8]

During aging, the still ongoing hydrolysis and condensation of unreacted silanes strengthen the structure of the alcogel. This step is crucial to avoid any unwanted shrinkage during drying, and to obtain better mechanical properties. The water and alcohol produced during the reactions need to be removed from the porous skeleton, thus a multiple-step solvent exchange is carried out. [7,8]

To get dry aerogels the liquid needs to be removed from the pores without damaging the solid structure. For this purpose 3 techniques can be used.[2,7] During freeze-drying, the frozen solvent is removed via sublimation at low pressure. The freezing of the liquid results in the collapse of the pristine gel structure caused by the crystal formation. This leads to powder like final products called cryogels.[9] Drying on ambient pressure can cause significant shrinkage due to the capillary stresses,

which occur when gas-liquid menisci appear in the small pores. Many new techniques were developed to reduce the shrinkage and the possibility of structural damage during drying. One strategy to minimize capillary stresses is to use functionalized silane reagents, silylation, and low surface tension organic solvents.[10,11] The strength of the gel skeleton can be increased with further aging. Another very promising possibility to enhance the flexibility of the gel is to utilize the so called spring back effect.[12–14] This gives the possibility to produce aerogels with ambient pressure drying. Without these treatments the gel structure cracks due to shrinkage resulting dense, microporous materials, called xerogels.[7]

The most often used method to produce aerogels is the supercritical drying. During this process the liquid-gas interface ceases to exist as the liquid in the gel structure reaches its critical pressure and temperature. Without the interface, no capillary stress occurs, thus the shrinkage becomes negligible. The drying can be performed with supercritical organic solvents (hot method) or with supercritical CO₂ (cold method).[15,16] The latter is the most common medium used in sc. drying owing to the low critical point and the easy access of CO₂. Supercritical drying can produce large, crack-free monoliths.[17] Although, there are a few disadvantages of the technique. These are e.g. the need for special high pressure equipment and expertise, and slowness of the process. The technique of rapid supercritical drying also exist. This combines the synthesis, aging and drying in a single step. Here, the high pressure reactor is the mold itself where the gelation occurs, which is followed by a short aging period and drying. It is usually a temperature and pressure assisted process, in which higher temperature and pressure is applied compared to the normal gelation and aging techniques.[18,19]

Aerogel frameworks can be easily and readily modified during or after the synthesis. There are numerous feasible alternatives, and we demonstrate only the most common and widely applied techniques here. Various functional groups can be introduced during the aging by co-gelation of

different silanes. Another possibility is to use highly reactive agents during aging, which can functionalize the unreacted silanols (Si-OH) of the backbone. Using sc. methanol as the drying medium, or methoxylation or vapor treatment after drying converts the surface silanols to Si-OCH_3 groups and thus produces hydrophobic aerogels. [20–23]

3.3. Aerogel applications

Due to their extraordinary properties their applications are very divers. However, the need for specialized equipment and expertise, and the high cost of production hinder their everyday usage. Nowadays, there are many initiatives to develop cheap and reproducible manufacturing techniques, but these are still 5-10 years far from large scale industrial implementation.[8,24,25]

Silica aerogels are one of the best thermal insulation materials owing to their low thermal conductivity (around $0.015 \text{ W K}^{-1} \text{ m}^{-1}$). They are used for building insulation, however the best-known insulation applications of aerogels are related to the aerospace industry. NASA and ESA applied them in several missions (PATHFINDER MARS, EURECA etc.).[26]

Their optical properties allow them to be excellent candidates for Cherenkov radiation detectors. For this purpose low density aerogels with low refraction indexes are needed. Their 1.1 dielectric constant makes them ideal for integrated circuits, however with modification they can be good electric materials as well.[27–29]

The high specific surface area combined with the open mesoporous structure and the tunable surface properties ensure excellent and flexible adsorption properties. They can be sorbents of gases, liquids and a wide variety of volatile compounds. They have huge potential in environmental applications that include oil-spill clean up, removal of toxic organic compounds and heavy metal ions from waste water, and adsorption of high industrial value metal ions.[30,30–35] Different pharmaceutical

agents, enzymes or even cells can be trapped inside the porous framework. These preparations can be utilized in the pharmaceutical industry as drug delivery systems, biosensors or matrices for tissue engineering.[36,37]

3.4. Drug delivery systems

The modern pharmaceutical industry has to face certain challenges such as safer and controlled drug administration, the reduction of side effects and the stabilization of sensitive active agents. To overcome these challenges new types of drug delivery systems need to be developed. In the early 1990's Schwertfeger et al. and Berg et al. patented the use of inorganic, organic, and biodegradable organic aerogels in pharmacy.[38,39] Numerous projects aiming to develop aerogel based drug delivery systems started from their ideas. In the early 2000's more patents followed the early concepts, and many research groups started to work in this topic. Today, aerogel based drug delivery system development is one of the key driving factor of aerogel research.[36,37]

3.4.1. Loading of aerogels with active ingredients

There are several possibilities to load aerogels with active pharmaceutical ingredients. The first method is the addition of the drug to the initial sol. In this way the molecule will be trapped in the 3D structure. It is a flexible and easy way to impregnate the gel, however there is the possibility of dissolution during aging and drying. It is well applicable for xerogel impregnation, where no additional aging and sc. drying is needed. Covalent linking of the agent to one of the starting materials can be a feasible alternative to overcome this disadvantage. In this way the forming gel structure will contain the drug from the first step of the aerogel synthesis. With a suitable link, it is also possible to attain controlled drug release initiated by pH change, enzyme activity etc. The adsorption of the drug from the aging organic solution can be implemented, however dissolution during drying needs to be carefully

considered. Adsorption from liquid phase can be carried out after sc. drying, but the collapse of the aerogel structure is inevitable after the second drying.[36]

The most promising and widely used technique is the supercritical impregnation, mentioned above. With this technique high loadings can be achieved without damaging the aerogel skeleton. Nevertheless, this method requires the same high pressure equipment increasing the cost. Also, the drug also has to be soluble in sc. CO₂.

To enable the use of low adsorption capacity aerogels as drug delivery systems, a new technique called adsorptive precipitation or adsorptive crystallization was developed. The aerogels are impregnated with a supercritical solution during the process, and by changing the properties of the solution (temperature, pressure) small particles form inside the pores. The formation of small particles ensures high loadings, since the drug is not only adsorbed on the inner surface of the aerogel, but inside the pores as well. The porous structure also prevents the aggregation of these nanoparticles that otherwise tend to stick together without pore walls between them. In order to reproducibly realize adsorptive precipitation, not only the experimental conditions (temperature, pressure, concentration etc.) need to be controlled, but the surface properties of the matrix has to be optimal as well. Drugs can be precipitated in amorphous or crystalline form. Weak interactions between the active agent and the matrix lead to the formation of nanocrystals, while strong interactions result an amorphous phase.[40–42]

3.4.2. Silica based aerogels

Because silica aerogels represent the most intensively studied aerogels, they were the first to be implemented in pharmaceutical applications. Schwertfeger et al. loaded ambient pressure dried aerogel like materials with different pharmaceutical agents (methylprednisolone and Hoe277) and studied their release behavior. They discovered that depending on the surface characteristics of the matrix (hydrophilic or hydrophobic) the

release can be either rapid or retarded. Aerogel containing capsules, lotions and suppositories were also prepared. The proof-of-concept was confirmed, however, these aerogel-like materials had lower porosity, lower surface area and higher density than the aerogels obtained by supercritical drying.[39] Impregnation from solution is also not a beneficial technique, because the gel structure collapses during the second drying step after the impregnation.

Smirnova et al. used supercritical media to load aerogels. They studied the adsorption isotherms, the achievable loadings, the crystallinity of the entrapped drugs, and the release rates as well. The applied drugs displayed Langmuir isotherms, which indicates that after a certain drug concentration the loading cannot be further increased with this technique. Very high loadings were achieved with miconazole and ibuprofen, $0.6 \text{ g}_{\text{drug}} \text{ g}_{\text{aerogel}}^{-1}$ and $0.7 \text{ g}_{\text{drug}} \text{ g}_{\text{aerogel}}^{-1}$, respectively. It was shown with X-ray diffraction that the drugs adsorbed as either mono- or multilayers. Suttiruengwong et al. showed that with increasing surface area and mesopore volume higher loadings can be achieved. The main factor determining the loading are the solubility of the drug in sc. media, and the affinity of the drug to the aerogel surface. The latter depends on the aerogel properties such as the aforementioned surface area and mesopore volume, and moreover the density, pore size distribution and surface functionality.[40,41,43,44]

As it was previously shown the matrix properties have a significant role in drug release rate. Hydrophilic aerogels are readily hydrated by the release media (different aqueous solutions) thus the release rate is faster, than for hydrophobic aerogels which are non-hydrated. Drug release from hydrophilic materials are usually faster than the dissolution of the microcrystalline drug. The factors driving this effect are the following. The adsorbed drug is distributed on a large surface, the hydrophilic structure collapses rapidly in contact with water and the drug is either in molecular form or in an amorphous phase, from which dissolution does not require any additional energy to collapse the crystal lattice.

The solubility of the drug is also increased, because the re-crystallization of the drug can be initiated only through dissolution.[43–46]

Alnaief et al. published that with different functionalization strategies it is possible to synthesize amino functionalized aerogels with high surface area ($800 - 1000 \text{ m}^2 \text{ g}^{-1}$) and improved drug adsorption capacity toward ketoprofen. They also showed that the surface modification had no negative effect on the rapid drug release.[23]

Murillo-Cremaes et al. proved that silica based aerogels are able to stabilize the moisture sensitive antithrombotic drug (triflusal) and enhance the bioavailability of poorly water soluble pharmaceutical agents.[47]

3.4.3. Biodegradable aerogels

In spite of amorphous silica (thus silica aerogels) are considered harmless to human body, the lack of biodegradability may cause unwanted side effects and serious illnesses in the case of certain delivery routes (e.g. nasal delivery). This fact directed the attention of the scientists to biodegradable biological materials such as proteins and polysaccharides. From 2005, the trend in aerogel based drug delivery system development shifted towards this area. Biodegradable aerogels with various shapes and compositions were synthesized and thoroughly studied. As Zhao et al. showed, more than 55% (up to 100) articles were published in this topic after 2012, which points out the interest in the topic.[37]

Polysaccharides and proteins have low toxicity, high biocompatibility and biodegradability. They are common additives in food and drug formulations, and easy to access. In recent publications the synthesis and characterization of aerogels derived from starch, cellulose, chitosan, alginate, whey protein, gelatin and other sources were reported.[37]

Their synthesis follows the previously presented general procedure with a change in the gelation, i.e. the 3D alcogel structure is not the product of hydrolysis and polycondensation reactions. The hydrogel is formed via cross-linking reactions that can be initiated via chemical reactions

(chemical cross-linking) or with the change of pH or temperature (physical cross-linking). In general, these types of aerogels have different porosities than inorganic ones because of the presence of more macropores. The density is higher and the specific surface area is lower. They also have a huge variety of surface functional groups, which can be beneficial in drug delivery.

Starch based aerogels were extensively studied. Mehling et al. prepared potato and corn starch aerogels with $70 - 100 \text{ m}^2 \text{ g}^{-1}$ specific surface area. They used two methods to load the gels. Supercritical adsorption was used for ibuprofen, while liquid phase adsorption was used for paracetamol. In spite of the low surface area, they could achieve considerably high loadings (10 – 20 wt.% of ibuprofen). Due to the high density of surface functional groups, the specific drug loadings were much higher than that for silica aerogels. Both accelerated and retarded drug release is feasible depending on the structure.[48] García-González et al. continued this work and synthesized monolithic and microspherical aerogels using pea and corn starch. They studied both the gelation conditions and the ketoprofen adsorption from sc. medium. They were able to raise the specific surface area to $250 \text{ m}^2 \text{ g}^{-1}$ and achieve high loading for ketoprofen (up to 16 wt.% in amorphous form). They showed that ketoprofen release is a two-step process, and more sustained than from the microcrystalline form. The release followed the Korsmeyer-Peppas model, which is governed by the diffusion of the drug and the erosion of the matrix.[49–51]

Alginate is a polysaccharide copolymer built up from β -D-mannuronic acid and α -L-guluronic acid, and can form a gel via cross-linking by di- or multivalent metal ions.[52] Mehling et al. presented the preparation and impregnation of Na-alginate aerogels. They showed, that the drug dissolution is fast from this matrix owing to the good wetting properties and the larger pores which can enhance the transport of the active agent.[48] Veronovski et al. showed that the properties of the gel can be tuned by changing the initial alginate concentration. The higher the

alginate concentration is, the denser and more compact gel structure formed. This also shifts the drug release to a more sustained profile. Multi-layered alginate aerogel beads were also prepared and impregnated with nicotinic acid before cross-linking in aqueous solution. Results show that the loading can be increased significantly with the addition of more layers, and with this retarded drug release is feasible.[53,54]

Haimer and coworkers investigated the application of bacterial cellulose aerogels as drug delivery systems. They impregnated the gels with dexphantenol and L-ascorbic acid. Loadings are dependent only on the solute concentration in the loading solution, and the release profile is independent from the amount of entrapped drug, but highly dependent on the aerogel layer thickness. With the use of the effective diffusion coefficient of the drug, they were able to predict the release kinetics.[55,56]

Novel whey protein aerogels were prepared from protein hydrogels via sc. drying. Betz et al. found that the structure of the 3D backbone, the adsorption capacities and the drug release profiles are better than those of cryogels.[57] Silk fibroin aerogels were synthesized and loaded with ibuprofen by Marin et.al. They reported a 21 wt.% loading and a Fickian diffusion governed slower drug release compared to the free microcrystalline drug.[58]

3.4.4. Aerogel hybrids and composites

As the previous examples showed, inorganic and organic aerogels possess a great potential in pharmaceutical applications. Both types have their own advantageous properties, like the high specific surface area of the silica based materials, or the biodegradability and high surface functional group density of bio-based materials. With the combination of these new, novel materials the possibilities are almost unlimited. As Smirnova and Gurikov pointed out, purely empirical methods cannot be used for large scale investigations, because the combination of the possible precursors are too high. They proposed the use of molecular level modelling based

on the understanding of basic correlations. As this area is still in its early years fewer papers were published than those for non-hybrid or non-composite aerogels.[59]

Kanamori et al. presented versatile synthesis possibilities to gain siloxane based aerogel composites and hybrids with enhanced physico-chemical properties, that can be utilized in life science applications.[60,61]

Chitosan-silica hybrid aerogels were prepared by Ayers et al. They showed, that the overall shrinkage of the wet gel during preparation depends on the chitosan-silica ratio. The porosity and specific surface area of the hybrid matrices were ca. 30 times higher than those of chitosan-L-glutamic acid aerogels. Biocompatibility tests showed low cytotoxicity and high hemolysis. Based on these data diverse applications in drug delivery and waste water treatment were proposed.[62]

Smirnova and her colleagues introduced a new technique to prepare alginate based hybrid aerogels, that can be used for multiple purposes. Alginate based hybrid materials were synthesized by carbon dioxide induced gelation. The second component in these composites was either lignin, starch, pectin, carrageenan, cellulose, gelatin or other biopolymers, polymers or filler materials. They highlighted potential applications such as thermal insulation, mucosal drug delivery and tissue engineering. [63]

The preparation of silica-gelatin hybrids for biomedical use is mentioned in several papers, however no real hybrid aerogels were produced so far. Nonetheless, hybrid hydrogels, composite materials for applications in life sciences are well known.

As Santoro and coworkers demonstrated there is a huge variety of gelatin based composites. These have strengths which can be utilized, however they also bears some weaknesses, that need to be further improved. Gelatin-ceramics systems have high compressive strengths and they are osteoconductive, but slowly biodegradable. Gelatin-biopolymer composites are biomimetic, but their isoelectric points are not tunable, while gelatin- synthetic polymer matrices have highly tunable properties.

The lack of inherent cell-recognition sites also considered a setback. Some possible applications (wound dressing, drug delivery, bone cements) were also shown. [64]

A mesoporous silica-gelatin sandwich scaffold for bone tissue engineering was developed and its bioactivity was extensively tested. During the biological tests the scaffold promoted alkaline phosphatase activity and the viability of human osteoblast-like cells increased. These results make the scaffold an ideal candidate for bone replacement.[65] Sachithanadam et al. improved the strength of gelatin-silica aerogel composites by adding SDS into the structure.[66,67]

Gelatin can also be used as a template to reach bimodal porosity in mesoporous silica structures, that have enhanced lysozyme adsorption capacities. Xu et al. used gelatin as a matrix metalloproteinase degradable coating for silica nanoparticles to achieve microenvironment-triggered release for cancer treatment. A new foaming/freezing technique was used to synthesize a highly macroporous gelatin-silica scaffold which had higher porosity than the pure gelatin matrix and showed high in vitro biocompatibility.[68] Silica-gelatin hybrid coatings were also prepared.[69] Nevertheless, true gelatin-silica hybrid aerogels were never synthesized and characterized before.

3.5. Aerogel characterization

3.5.1. N₂ adsorption/desorption porosimetry

Gas sorption porosimetry is the most commonly used technique to characterize open-pore structures. The method is based on the interaction of the probing gas and the solid surface of the porous material. This interaction can be the mono- or multi-layer adsorption of the gas on the inner surface of the pores and liquid-solid interaction when the gas condenses inside the pores. The most often used gas is N₂, however other gasses, like CO₂ also can be used. It is important to note that the measurable pore size range depends on the characteristics of the probe

gas. The adsorption and desorption branches of the isotherms are recorded by monitoring the adsorbed amount of gas while changing the relative gas pressure between vacuum and standard atmospheric pressure and vice versa at 77.3 K temperature. The adsorbate uptake is measured by volumetric or gravimetric methods or with monitoring the concentration of the probing gas in a mixture with an inert gas. Modern instruments use the volumetric approach to register the isotherms. Both the sample preparation (grinding, degassing etc.) and the instrument parameters (e.g. equilibration time) have a huge effect on the experimental isotherms.[70]

Standard isotherms are classified by the IUPAC (Fig. 1.). Type IV and V isotherms are characteristic for aerogels where capillary condensation takes.[71] The adsorption and desorption branches form a hysteresis loop. Certain regions of the isotherms such as the shape and the position of the loop, the shape of the curves at low relative pressures along with the slope of the plateau in the middle regions can reveal fundamental information on the material structure. Numerous methods can be utilized to quantify these features.

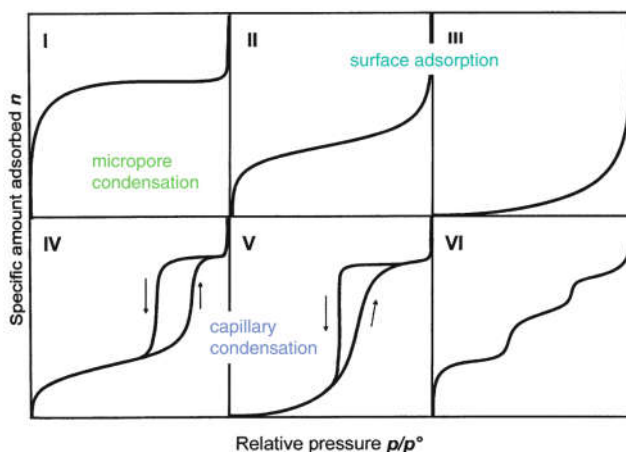


Figure 1. Standard adsorption-desorption isotherms as classified by the IUPAC. Taken from Ref. [70].

Total pore volume (V_t), which includes the micro- ($d < 2$ nm), meso- ($2 \text{ nm} < d < 50$ nm) and macropores ($50 \text{ nm} < d$) is determined around 0.99 relative pressure, based on the assumption that the total amount of the probing gas condense.

$$V_t = V_{ads}c \quad (1)$$

Where V_{ads} is the amount of adsorbed gas in $\text{cm}^3 \text{ g}^{-1}$ at standard temperature and pressure and c is a gas dependent constant ($c = 0.001547 \text{ cm}^3 \text{ cm}^{-3}$ for N_2). The bulk density of the material can also be calculated from Eq. 1., if there are no macropores in the material and the shapes and sizes of the pores are uniform.[70]

Specific surface area can be calculated using numerous models. The most commonly used one is the BET method (Brunauer-Emmett-Teller). The mesopore surface area can be determined by using the BET equation.[72]

$$\frac{1}{W \left(\left(\frac{p_0}{P} \right) - 1 \right)} = \frac{1}{W_m C} + \frac{C - 1}{W_m C} \left(\frac{P}{p_0} \right) \quad (2)$$

Where W is the weight of the gas adsorbed at the relative pressure P/p_0 and W_m is the weight of the adsorbate constituting a monolayer of surface coverage. The BET C constant (C) is related to the strength of adsorbent-adsorbate interaction and can be used to characterize the surface hydrophobicity.

The BET equation is usually represented by a linear plot of $1/(W(p_0/P)-1)$ vs. P/p_0 . The linearity is the best in the range between 0.05 and 0.35 relative pressure. W_m can be calculated from the slope and the intercept.

$$s = \frac{C - 1}{W_m C} \quad (3)$$

$$i = \frac{1}{W_m C} \quad (4)$$

$$W_m = \frac{1}{s+i} \quad (5)$$

The total surface area (S_t) can be calculated using Eq. 6.

$$S_t = \frac{W_m N A_{cs}}{M} \quad (6)$$

Where N is Avogadro's number and M is the molecular weight of the adsorbate. A_{cs} is the molecular cross-section area of the adsorbate. The specific surface area can be calculated from the total surface area and the weight of the sample.[72]

Mesopore size distribution usually calculated from the desorption branch of the isotherm, however in some special cases the adsorption branch can also be used. Mesopore size is usually calculated assuming cylindrical pores using the Kelvin equation:

$$r_K = \frac{-2\gamma V_m}{RT \ln\left(\frac{P}{p_0}\right)} \quad (7)$$

In which γ is the surface tension of nitrogen at its boiling point, V_m is the molar volume of liquid nitrogen, R is the gas constant, T is the boiling point of nitrogen, P/p_0 is the relative pressure and r_K is the Kelvin pore radius. Equation 8 takes the following form for nitrogen:

$$r_K = \frac{4.15}{\log\left(\frac{P}{p_0}\right)} \quad (8)$$

However, the Kelvin radius is not equal to the pore radius, because an adsorbed layer with t thickness will be always present.

$$r_p = r_K + t \quad (9)$$

Mesopore size distribution in the conventionally available softwares are calculated with BJH method (Barrett, Joyner, Halenda).[73]

When the relative pressure is close to 1, it is assumed that all the pores are filled with liquid. All pores with radii (r_p) have a physically adsorbed layer of nitrogen with a thickness of t . An inner capillary with radius (r_K) is inside each pore from which the evaporation takes place when the

relative pressure is lowered. The relation between the pore volume (V_p) and the Kelvin volume (V_K) of the inner capillary can be expressed as:

$$V_p = \frac{V_K r_p^2}{r_K^2} \quad (10)$$

When the relative pressure is lowered from (P_1/p_0) to (P_2/p_0) a given volume of the adsorbed liquid (V_l) will desorb from the surface. Not only the largest pore are emptied by this, but the thickness of the inner layer decreases as well (Δt_1). The average change in thickness will be $\Delta t_1/2$. The pore volume of the largest pore will be:

$$V_{p1} = V_1 \left(\frac{r_{p1}}{r_{K1} + \frac{\Delta t_1}{2}} \right)^2 \quad (11)$$

The same phenomena occurs as the relative pressure is lowered again. Based on the previous deduction the pore volume of the second largest pore (V_{p2}) will be:

$$V_{p2} = \left(\frac{r_{p2}}{r_{K2} + \frac{\Delta t_2}{2}} \right)^2 (V_2 - V_{\Delta t_2}) \quad (12)$$

$$V_{\Delta t_2} = \Delta t_2 A c_1 \quad (13)$$

$A c_1$ is the area exposed by the previously emptied pores. This can be generalized to present any step of the desorption. Pore volumes at various relative pressures can be calculated by the combination of Eq. 12 and the generalized form of Eq. 13.

$$V_{pn} = \left(\frac{r_{pn}}{r_{Kn} + \frac{\Delta t_n}{2}} \right)^2 \left(\Delta V_n - \Delta t_n \sum_{j=1}^{n-1} A c_j \right) \quad (14)$$

$A c$ for any pore size varies with the relative pressure, thus needs to be evaluated.

A_p , the area of each pore, is constant and can be calculated from the pore volume. In the case of cylindrical pores:

$$A_p = \frac{2V_p}{r_p} \quad (15)$$

The pore areas can be summed, thus A_p is known for every step of the desorption. The BJH method can calculate $\sum A c_j$ from A_p .

$$c = \frac{\bar{r}_c}{\bar{r}_p} = \frac{\bar{r}_p - t_{\bar{r}}}{\bar{r}_p} \quad (16)$$

Where $\bar{r}_p$ and $\bar{r}_c$ is the average pore and capillary radius, while $t_{\bar{r}}$ is the thickness of the adsorbed layer at the average radius. With Eq. 16 the exact expression of Eq. 14 is feasible.[73]

It has to be noted that the BJH method assumes cylindrical pores, while NMR cryoporometry evaluation uses either cylindrical, spherical or slit geometry. Thus, the interpretation of the results of the different methods needs to be done with extreme caution.[74,75]

3.5.2. NMR techniques

NMR spectroscopy is a powerful tool to gain structural information of molecules, study the geometrical arrangement of the constituent elements in compounds and to determine reaction mechanisms. With the application of some special techniques it is possible to study the porosity of solid matrices, the wetting mechanism of their backbones and the structural changes during their interaction with liquids.

Diffusiometry

In diffusiometry the self-diffusion of different probing liquids are studied. The self-diffusion coefficient of a molecular species depends on its effective molecular weight, size and shape, thus diffusion can be applied to map intermolecular interactions or detect any restriction caused by physical barriers (e.g. pore walls).[76,77] At small observation times the molecules move freely, thus the self-diffusion coefficient is equal to that

of the bulk liquid. However, with increasing observation time the probability that the solvent molecules contact the pore walls will increase. For long observation times the molecule hits the pore walls again and again, thus the effective path and the observed self-diffusion coefficient decrease. After a certain point the solvent molecule wanders the whole available distance inside a pore as shown in Fig. 2.[78] From this point on the observed self-diffusion coefficient does not decrease any more. NMR diffusimetry can provide a huge amount of information on the interaction of molecules with other molecules or with their environment. The number of publication related to the investigation of polymers, zeolites and other porous systems increased significantly with the development of high-resolution gradient enhanced NMR spectroscopy demonstrating, that it is indeed a powerful tool to characterize solid matrices.

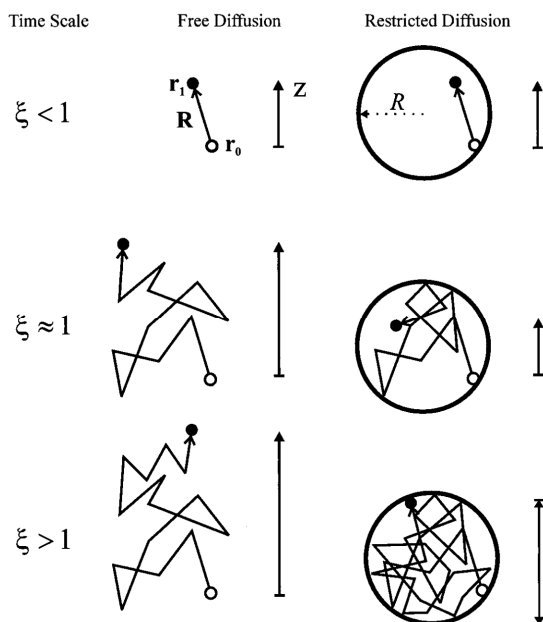


Figure 2. Demonstration of the random diffusion at various measurement time-scales. Taken from Ref. [78].

The PGSE technique is commonly used in NMR diffusimetry. It is based on spin-echo sequences (Fig. 3).[79]

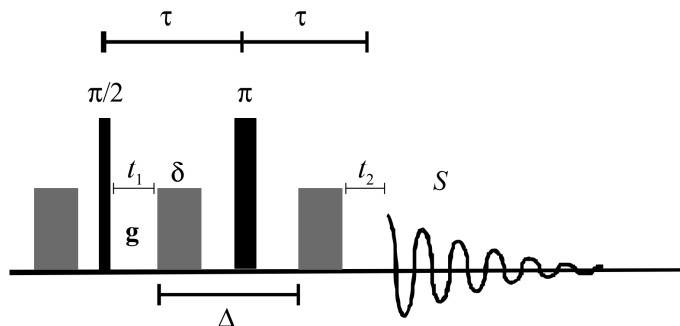


Figure 3. An illustrative PGSE sequence based on a Hahn-spin echo. $\pi/2$ and π are 90° and 180° rf. pulses each of them followed by a gradient pulse with a length of δ and a magnitude of g separated by Δ . The pulses are applied in every τ period. A pre-pulse gradient (BIPLD) is usually used before the 90° rf. pulse. The second half of the echo is used as the NMR signal (S). Taken from ref. [79].

In the sequence, two gradient pulses with a length of δ and a magnitude of g separated by Δ is applied in every τ period. The second half of the echo is used as the NMR signal (S). The echo attenuation is defined by $E(g) = S(g)/S(g=0)$. This sequence can be used to determine the diffusion coefficients, as it lets the spin relaxation effects to normalize out. A pre-pulse gradient (BIPLD) is usually used before the $\pi/2$ rf. pulse (90°) to reduce eddy currents and gradient mismatches.

The diffusion data can be evaluated according to the well-known expression given in Eq. 17:

$$I = I_0 \left\{ -D_{obs} \gamma^2 \left(\Delta - \frac{\delta}{3} \right) \delta^2 g^2 \right\} \quad (17)$$

During a normal experiment the pulsed gradient strength (G) is increased with square-equidistant steps and the echo intensity (I) is measured. The coefficient $D_{obs} \gamma^2$ can be calculated for each experiment by fitting the exponential curve of Eq. 17 to the measured echo intensity (I) as a function of g^2 , and using the known parameters. The real diffusion

coefficient (D) can be deduced after calibration, which is based on measuring the diffusion of D_2O in water by the same method.[77]

Cryoporometry

In NMR cryoporometry the basis of pore shape and size determination is the detection of the shift of the phase transition temperature caused by confinement in pores. The shift in melting and freezing point is caused by the fact that the energy cost to create new solid-liquid interfaces is not zero. For bulk materials this energy cost is negligible, but for liquids inside small pores it is quite significant. The physico-chemical background of the method is given by the modified Gibbs-Thomson equations, which describe the melting and freezing point depressions of liquids in confined spaces.

$$\Delta T_m = T_m - T_0 = -\frac{n_m K_c}{r_p} \quad (18)$$

$$\Delta T_f = T_f - T_0 = -\frac{n_f K_c}{r_p} \quad (19)$$

In Eq. 18, ΔT_m is the melting point depression expressed as a difference between the phase transition temperature of the bulk (T_0) and the confined liquid (T_m). K_c is the cryoporometry constant, n_m is the geometry factor describing melting and r_p stands for the average pore radius. In Eq. 19, the symbols denote the corresponding parameters for freezing. The values of ΔT_m and ΔT_f , and n_m and n_f are different, because melting and freezing of liquids in confined spaces show a hysteresis in general. This is not a kinetic phenomenon, but a thermodynamic hysteresis. The mechanism of freezing and melting is different, thus at a given temperature the two states are different based on the starting conditions. In NMR cryoporometry, the amount of liquid is quantified at different temperatures during melting- freezing cycles. The liquid signal intensity is plotted as a function of temperature and the melting and freezing points

of the confined liquid are determined from the inflection of the two hysteresis curves.[75,80,81]

From the ratio of freezing and melting temperature shifts it is possible to determine the shape of the pores as it is presented in Table 1 and Figure 4.

Table 1. Freezing and melting temperature shifts of confined liquids for different pore geometries. Taken from ref [75].

Pore shape	$ \Delta T_f $	$ \Delta T_m $
Sphere	$3K_c r^{-1}$	$2K_c r^{-1}$
Cylinder	$2K_c r^{-1}$	$K_c r^{-1}$
Slit	$K_c r^{-1}$	0

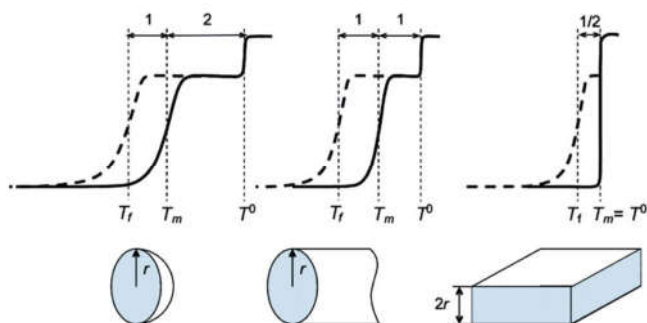


Figure 4. Illustration of the shape of freezing-melting hysteresis curves in NMR cryoporometry for different pore shapes. Taken from ref. [75].

Relaxometry

It was shown that proton spin-spin (T_2) relaxation times of liquids are different depending on the position of molecules inside pores. Near to the pore walls the relaxation time is decreased caused by the interaction with the solid matrix, while inside the pores the relaxation time approaches that of the bulk liquid. If the liquids in this regions do not mix with each other in the NMR relaxation time-scale multiple relaxation times are measured. Every region will have their own characteristic decay-time. However, if the mixing is rapid an average value will be obtained. The value of the

averaged T_2 time can be calculated based on the fast-exchange theory using Eq. 20:

$$\frac{1}{T_2} = \frac{V_s}{V} \frac{1}{T_{2s}} + \frac{V_b}{V} \frac{1}{T_{2b}} \quad (20)$$

Where T_{2s} and T_{2b} are the surface-liquid and bulk liquid relaxation times, respectively. V_b and V_s are the volume of the bulk and surface liquids, and $V=V_s+V_b$.

To determine T_2 relaxation time a CPMG (Carr-Purcell-Meiboom-Gill) sequence is used. After a 90° rf. pulse, a second 180° pulse is applied. The relaxation is studied by increasing the delay time between the pulses. The method will result a curve with exponential decay. If the experimental curve can be fitted with two exponentials, it is regarded as the sum of two curves with 2 relaxation times: T_{21} (shorter relaxation time) and T_{22} (longer relaxation time).

$$I = y_0 + A_1 e^{-\frac{x}{T_{21}}} + A_2 e^{-\frac{x}{T_{22}}} \quad (21)$$

Where I is the intensity of the signal, A_1 and A_2 are the amplitudes of T_{21} and T_{22} and x is the delay time.[82,83]

3.6. Drug release experiments

The United States Pharmacopeia (USP), along with the European Pharmacopeia (EP) and the Japanese Pharmacopeia (JP), describes four dissolution apparatuses. These are the following: USP 1 basket, USP 2 paddle, USP 3 reciprocating cylinder and USP 4 flow-through cell. The most frequently used one is USP dissolution apparatus 2. The vessels are submerged in 37.0 ± 0.5 °C temperature water bath and have their design and operation cause unique hydrodynamics, which significantly affect the drug dissolution. For every type of dosage forms (immediate-, extended-, delayed-release) there is a well-defined protocol which has to be applied.[84] In spite of these methods being applied in the whole pharmaceutical industry, the sampling frequency sometimes does not

fulfill the need during drug delivery system development. For example, if the initial burst release is fast for the method to keep pace, the information gathered to study the release mechanism is insufficient. This setback often observed in the case of aerogel based drug delivery systems.

A possible alternative can be on-line detection, but the dead volume of the equipment and the attached tubes can also alter the results. Thus, only the direct detection inside the vessel could be the ultimate technique to use. The drug forms disintegrate during dissolution and a suspension forms. The measurement of the analyte concentration is not evident in this slurry (e.g. because of light scattering).

If the active agent has significantly different and sufficiently large UV-Vis absorbance compared to the background, UV-Vis spectrophotometry is an ideal technique to use recording drug release curves. It should be noted, that the aerogel suspension scatters the detection light of the spectrophotometer. However, the apparent absorbance of the suspended aerogel quantitatively adds to the absorbance of the dissolved substances and do not change the molar absorbances of these analytes, as shown previously. The apparent absorbance of the aerogel can be taken into correction by subtracting it from each recorded spectrum using the “dual-wavelength method” developed by Liu and Zhu. [85,86] First, the spectrum of the aerogel needs to be measured under the applied conditions. Second, a reference wavelength range needs to be chosen where the dissolved analytes have zero absorbances. The spectrum of the aerogel suspension is reconstructed from the reference absorbances by using the known apparent molar absorbances of the suspension. Finally, the spectrum of the suspension is subtracted from the recorded UV-Vis spectrum in the whole wavelength range. This calculation needs to be done in every experiment for each recorded spectrum.

The corrected, time-resolved UV-Vis spectral series correspond exclusively to the dissolved analytes (as shown in Fig. 5), thus, providing the drug release kinetic curve. This curves can be recorded with high time

resolution. The release curves will be more reproducible and will contain significantly more data points than the commercial methods. This allows the use of high precision curve analysis.[87]

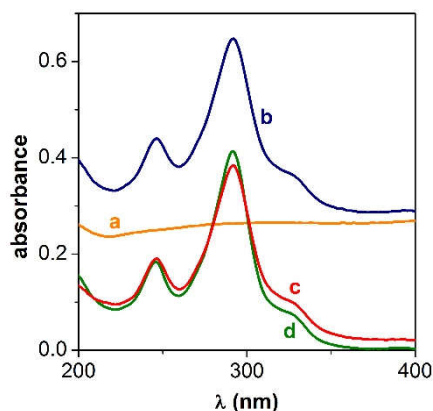


Figure 5. Demonstration of the “dual-wavelength method” for baseline correction in UV-Vis measurements of suspensions. a) spectrum of well stirred aerogel suspension, b) spectrum of aerogel suspension immediately after the addition of methylene blue solution, c) the difference of spectra a) and b), d) spectrum of pure methylene blue solution. Taken from ref. [87].

3.7. Mathematical modeling of drug release

Understanding drug dissolution mechanism from various dosage forms can be a serious help in dosage form design, production and drug delivery system development. If the dosage is well characterized and every variables that can lead to the alteration of drug release are well-known not only development time, but production costs can be saved.

For a well-characterized DDS, the evaluation of drug release curves and the deduction of key features are feasible by using different types of mathematical models. After Higuchi published the first equation to describe drug release from ointments, numerous models were proposed.[88] These can be categorized as empirical, semi empirical and mechanistic realistic models. The first group is not based on any physical or chemical phenomena, thus does not convey information about drug release mechanism and cannot be used for any predictions. However, they

can be used to compare different systems studied under the same conditions. Mechanistic models are based on phenomena like diffusion, dissolution, erosion, swelling etc. System-specific parameters can be determined by these, thus more knowledge can be gained on the release mechanism. The quantitative prediction of formulation effects is feasible by the application of mechanistic models. Moreover the system parameters can be easily calculated to reach a predetermined release profile.[89]

Depending on various factors (drug type, preparation technique, conditions during release etc.) several phenomena can control the drug dissolution. For silica-gelatin aerogels one or more of the following phenomena can be considered as key effects to determine the rate of drug release: a) wetting of the aerogel surface and water penetration into the matrix (hydrophilic vs. hydrophobic matrices); b) matrix disintegration caused by the wetting and the shear forces during the release experiment; c) interaction of the dissolution medium with the aerogel backbone which can lead to hydration, swelling, hydrogel formation, and other structural changes; d) drug dissolution and possible hindrance in dissolution due to solubility; e) drug interaction with the pore walls; f) inhibited drug diffusion through the hydrogel.[89]

Mechanistic realistic models use equations to describe real phenomena. To solve these equations initial and boundary conditions has to be predetermined, and either analytical or numeric solutions can be used to solve them based on their complexity. These theories can be based on Fick's law of diffusion, polymer swelling, polymer and drug dissolution and erosion.

As Siepmann et al. pointed out empirical and semi-empirical models should not be used if the release mechanism or the effects of formulation is needed to be predicted. Yet, they can be very effective for the comparison of drug dissolution profiles. In such a way, by combining

them with structural information it is possible to determine certain mechanistic aspects of drug release.[89]

Aerogels are considered eroding delivery systems, meaning that the kinetics of release is primarily governed by the rate of matrix erosion and only secondarily by the limited diffusion of drug molecules from the pores.[44] The Hopfenberg model is a semi-empirical model which can be advantageously used to describe the kinetics of drug release from eroding matrices, where a zero order surface detachment is the limiting step of release:[90]

$$\frac{M_t}{M_{max}} = 1 - (1 - k_{obs}t)^n \quad (22)$$

Here M_t and M_{max} are the cumulative absolute amounts of drug released at time t and infinite time, respectively. The observed rate constant is k_{obs} , which depends on the initial concentration of drug in the system and on the physical dimensions of the carrier. Parameter n is a “shape factor” representing the geometry of the carrier: spherical ($n = 3$), cylindrical ($n = 2$) or slab ($n = 1$).[90]

Gelatin forms hydrogel in aqueous media, thus using it as a matrix component may lead to diffusion controlled drug release. A versatile empirical model to describe the diffusion controlled release of drugs is the Peppas model (Eq. 23). The model was proposed in 1985 to simplify the analysis of release from non-swellable polymeric matrices of varying geometry. It was first applied to thin films.[91] Later Ritger and Peppas showed that the model can be applied for other geometries as well.[92]

$$\frac{M_t}{M_{max}} = k_{obs}t^n \quad (23)$$

Here M_t and M_{max} are the cumulative absolute amounts of drug released at time t and infinite time, as defined previously. The observed rate constant is k_{obs} , which incorporates the structural and geometric characteristics of the delivery system. Parameter n is the “release exponent” and its value

can be indicative of the mechanism of drug release. As Ritger et. al. showed the limiting values of the exponent change with the geometry of the release vehicle and it also depends on the particle size distribution of the delivery system. For spherical particles $0.43 < n < 0.85$ indicates anomalous transport, n below this range marks Fickian diffusion and above this suggest Case II transport. Anomalous transport refers to overlapping of various phenomena and can be the combination of drug diffusion and polymer swelling.[92]

4. Experimental methods

4.1. Materials

Tetramethoxysilane (TMOS) was used as the silica precursor and it was obtained from Fluka. Gelatin was used as a second component to produce hybrid aerogels. Food grade gelatin was purchased from Dr. Oetker due to the high purity and the constant quality of the product. Methanol and acetone were the solvents for aerogel synthesis and ammonium carbonate ((NH₄)₂CO₃) was added as catalyst. All of these were purchased from Fluka. The surface modifying agents – hexadecyltrimethoxysilane (C₁₆-TMOS), trimethoxyphenylsilane (Ph-TMOS) and 1,1,1-trimethyl-N-(trimethylsilyl)silamine (HMDS)–, were supplied by Sigma–Aldrich. Ibuprofen (IBU) [(RS)-2-(4-isobutylphenyl)propanoic acid] and ketoprofen (KET) [2-(3-benzoylphenyl)propanoic acid] were purchased from Sigma Aldrich, while triflusal (TRF) [2-acetyloxy-4-(trifluoromethyl) benzoic acid] and its main metabolite 2-hydroxy-4-(trifluoromethyl) benzoic acid (HTB) were kindly donated by Uriach S.A. The molecular structures of the surface modifying agents and the model drugs are shown in Figure 6.

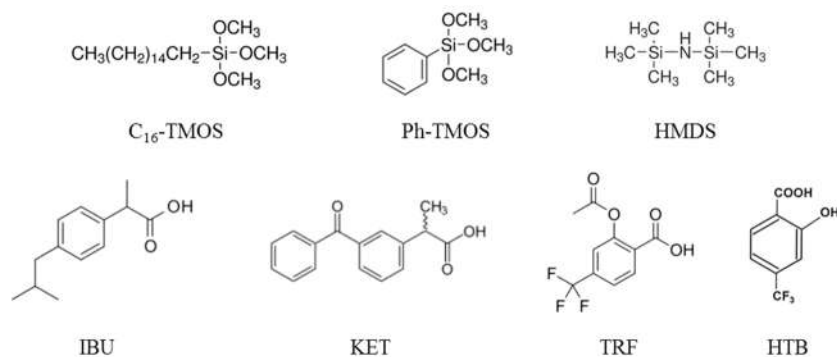


Figure 6. Molecular structure of modifying agents (top) and used drugs (bottom).

Supercritical CO₂ was produced from 99.95% pure gas (Carbueros Metalicos SA). All aqueous solutions were prepared with Milli-Q water

(Millipore). Formic acid (99% w/w, Merck) and methanol (HPLC grade, Panreac) were used for the preparation of the mobile phase for the chromatographic method. Other chemicals (HCl, NaOH, NaH_2PO_4 , cyclohexane and hexane) were ACS reagent grade (Sigma-Aldrich).

4.2. Aerogel synthesis and loading

Silica aerogel (Sil) was prepared by a sol-gel procedure described by Lázár et. al.[93] Two solutions, TMOS dissolved in methanol and $(\text{NH}_4)_2\text{CO}_3$ dissolved in distilled water were mixed in a beaker. The sol was poured into plastic molds, where gelation took place in 5-10 minutes. The gel was kept in the mold for 24 hours, then it was placed into a perforated aluminum container and a multiple step solvent exchange protocol was carried out. First, the sample was soaked in methanol for 24 h. Next, methanol was replaced by acetone in four 24 h soaking steps, and acetone was replaced 2 more times after 24 h soaking. The acetone was replaced 3 more times using a continuous-mode solvent regeneration and extraction unit to remove any trace amounts of water from the porous system. Finally, acetone was extracted with liquid CO_2 at 5.4 MPa (room temperature), then the gel was dried with supercritical CO_2 at 14 MPa and 80 °C.

Hybrid alcogels were prepared by a modified sol-gel procedure using different organic/inorganic precursor ratios. The exact amount of reagents are given in Table 2. In a typical procedure, a given quantity of solid gelatin was first dissolved in hot water, then the solution was cooled to near room temperature before adding the corresponding amount of $(\text{NH}_4)_2\text{CO}_3$. A viscous solution was obtained. TMOS dissolved in methanol was added to this solution under vigorous stirring. In selected experiments, either Ph-TMOS or C_{16} -TMOS functionalizing agent was added at this point in a fraction of ca. 10, 20 and 30 V/V% with respect to TMOS. After the formation of the sol, the same protocol was followed as in the case of silica aerogel production. For post-silanized samples, the HDMS reagent was added after the first soaking step with methanol, and

the alcogel/HDMS system was additionally aged for 48 h. Monolithic pieces of dry aerogels were obtained in all cases, which were further micronized mechanically to obtain a powder.

Table 2. Composition of the precursor mixture for each synthesized aerogel. All data is expressed in grams (g).

Sample	TMOS	MeOH	H ₂ O	(NH ₄) ₂ CO ₃	Gelatin	Ph-TMOS	C ₁₆ -TMOS	HMDS
Sil	3.10	5.55	20	0.07	-	-	-	-
H3	3.10	5.55	20	0.07	0.10	-	-	-
H10	3.10	5.55	20	0.07	0.30	-	-	-
H20	3.10	5.55	20	0.07	0.60	-	-	-
H30	3.10	5.55	20	0.07	1.00	-	-	-
H3_Ph10	2.79	13.5	10	0.10	0.10	0.32	-	-
H3_Ph20	2.48	13.5	10	0.10	0.10	0.64	-	-
H3_Ph30	2.17	13.5	10	0.10	0.10	0.96	-	-
H3_C₁₆10	2.79	13.5	10	0.10	0.10	-	0.27	-
H3_C₁₆20	2.48	13.5	10	0.10	0.10	-	0.53	-
H3_C₁₆30	3.10	13.5	10	0.10	0.10	-	0.80	-
H3_Sil	3.10	5.55	20	0.07	0.10	-	-	2.31
H10_Sil	3.10	5.55	20	0.07	0.30	-	-	2.31
H20_Sil	3.10	5.55	20	0.07	0.60	-	-	2.31
H30_Sil	3.10	5.55	20	0.07	1.00	-	-	2.31

The drug loading process (impregnation¹) in sc. CO₂ was performed in a high pressure equipment. Experiments were carried out in the batch mode. In a typical run, the 100 mL autoclave was charged with powdered aerogel wrapped in 0.45 µm pore size filter paper. An excess of drug (IBU, KET or TRF) was placed to the bottom of the reactor, enough to saturate the sc. CO₂. This experimental design ensured the physical separation of the aerogel and the solid drug in the autoclave. The reactor was then filled with liquid CO₂ and heated up to 45 °C. The pressure was increased to 12 MPa for the highly soluble TRF and to 20 MPa for IBU

¹ As an extension of the IUPAC definition we consider the saturation of aerogels with sc. solution and the removal of the solvent as well impregnation. We use this terminology during the dissertation and the thesis as well. *IUPAC – Gold Book* Version 2.3.3 2014.02.24.

and KET. The autoclave was magnetically stirred at 100 rpm for a period of 6 h, after which the reactor was depressurized and the residue of loaded aerogel was recovered. The depressurization rate was $0.2 - 0.3 \text{ MPa min}^{-1}$ to avoid drug crystallization.[47]

4.3. Aerogel characterization

Scanning electron microscopy (SEM) images were recorded on a Hitachi S-4300 instrument (Hitachi Ltd., Tokyo, Japan). Aerogel shards, freshly split from the monoliths were immobilized with superglue and Wood's metal and covered by 5 – 6 atomic layer thick sputtered gold conductive layers. Typically, 10 – 30 kV accelerating voltage was used.

The thermal degradation of hybrid aerogels and the quantification of the surface modifiers were assessed by thermogravimetric analysis (TGA) using a PerkinElmer 7 TGA instrument. The amount of loaded drug was also measured by this method. Thermal transitions were studied by differential scanning calorimetry (DSC) with a Mettler Toledo 822e/400 instrument. Both kinds of measurements were carried out under N_2 atmosphere at a $10 \text{ }^\circ\text{C min}^{-1}$ heating rate.

The crystallinity of the loaded drugs was analyzed by X-ray powder diffraction (XRD) using a Rigaku Rotaflex RU-200 B spectrometer.

The specific surface area, the pore size distribution and the pore volume of the pristine and the impregnated aerogels were measured by low-temperature N_2 adsorption-desorption porosimetry in 2 different instruments (Quantachrome Nova 2000e and ASAP 2000 Micromeritics Inc.). All samples were degassed at $80 \text{ }^\circ\text{C}$ for 24 h before the measurements. Specific surface area and pore size distribution were calculated using the multipoint BET and BJH methods, respectively. The results acquired by the 2 instruments deviate only by ca. 5 – 7 %. In this thesis the data measured by the Quantachrome instrument is shown only.

The FT-IR spectra of the silica-gelatin aerogel, crystalline IBU, KET and TRF together with the drug loaded aerogels were recorded using a

PerkinElmer Spectrum One spectrophotometer after pelleting the samples into KBr.

The particle size distribution of ground silica-gelatin aerogel particles suspended in either water or in hexane was measured in a laser diffraction particle size analyzer (Malvern Mastersizer 2000). Angular scattering intensity data were evaluated by the instrument controlling software using the Mie theory of light scattering.[94] A representative refraction index of 1.006 was used in the calculations for the aerogels. Particle size is reported in this work as volume equivalent sphere diameter.

4.4. Light-scattering during drug release experiments

The release of IBU and KET from silica-gelatin aerogel was followed on-line by UV-Vis spectrophotometry. Milled and sieved ($< 125\ \mu\text{M}$) loaded silica-gelatin aerogel samples were used. The suspension of the loaded aerogels was stirred in a spectrophotometric cuvette under temperature control. Full UV-Vis spectra (220 – 800 nm) were recorded at 1 s time intervals. Neither IBU, nor KET absorb above 400 nm, thus the apparent absorbance at this spectral range is the direct consequence of the light scattering of the dispersed aerogel particles. Changing particle size distribution changes the intensity of light scattering, and the change in the intensity of light scattering changes the measured apparent absorbance. Thus by taking the measured absorbance versus time, the temporal change in the particle size distribution of the suspension can be followed. It should be noted that the method is only qualitative in the applied experimental setup.

4.5. NMR measurements

For NMR measurements, dry (as prepared), ground silica-gelatin aerogel was introduced into glass or Teflon NMR tubes and wetted with either Millipore water, hexane (puriss), or with cyclohexane (puriss). The mass of the aerogel and the liquid added was carefully measured in each experiment. The samples were degassed by sonication.

^1H -NMR spin echo and PGSE (Pulse Field Gradient Stimulated Echo) experiments were performed with a Bruker Avance II 400 NMR spectrometer using standard pulse programs provided with the spectrometer. MestreNova 8.1 software was used for FID post processing.

4.5.1. Diffusiometry

Silica-gelatin aerogel suspensions in water and in hexane were studied. The self-diffusion coefficients of water and hexane were measured by standard PGSE protocols.[77] The self-diffusion of the liquids were determined under different pore-filling conditions. First, having the pores of the aerogel only partially filled with one of the liquids, and later having the pores totally filled with either water or hexane with additional bulk liquid present in the suspension. A stimulated spin echo pulse sequence was employed using bipolar gradient pulses to decrease eddy currents (BIPILED) at 298 ± 0.2 K. Typical parameters for diffusion experiments were: diffusion time (Δ) from 16 to 150 ms and length of gradient pulse (δ) from 1.6 to 4 ms. The diffusion data were evaluated according to Eq. 1. The pulsed gradient strength (g) was increased with 64 square-equidistant steps from 0 to approximately 50 Gauss cm^{-1} . Coefficient $D_{\text{obs}}\chi^2$ was calculated for each experiment by fitting the exponential curve of Eq. 1 to the measured echo intensity (I) as a function of G^2 , and using the known parameters. The real diffusion coefficient (D) was calculated after calibration, which was based on measuring the diffusion of D_2O in water by this method.

4.5.2. Cryoporometry

The pore size distribution of suspended silica-gelatin aerogel micronized particles was studied by using 2 different probe liquids, either water or cyclohexane. The aerogel slurries were cooled to -15 °C in a Teflon NMR tube. The probe head was also cooled to this temperature before measurements using a built-in regulator for stabilizing and regulating the temperature with air flow through a Bruker BSCU-05 cooling unit. The

temperature was calibrated using glycol and methanol.[95] After temperature equilibration, the ^1H NMR spectrum of the sample was recorded by a spin echo sequence. The typical echo time was 1.5 ms, the length of the 90° pulse was $10.2\ \mu\text{s}$. Temperature ranges of $-14 - 4\ ^\circ\text{C}$ and $-14 - 11\ ^\circ\text{C}$ were studied for water and cyclohexane, respectively. The samples were melted by elevating the temperature to $4\ ^\circ\text{C}$ or to $11\ ^\circ\text{C}$ in $0.2\ ^\circ\text{C}$ steps by using an automated program. From $4\ ^\circ\text{C}$ (or 11°C), the samples were cooled to $-14\ ^\circ\text{C}$ in $0.2\ ^\circ\text{C}$ steps and freezing was monitored. In most freezing experiments, the samples were not completely melted before a freezing cycle was started in order to avoid overcooling. The melting and freezing point depressions of liquids in confined spaces and the melting-freezing hysteresis are given by the modified Gibbs–Thomson equations (Eqs. 18 and 19).[75,80,81]

4.5.3. Relaxometry

T_1 relaxation times of the dispersing liquid in aerogel suspensions were measured by inversion recovery. The delay between the 180° and 90° power pulses was changed from 0.001 to 5 or 15 s for water and hexane, respectively, in 14 – 24 steps. For T_2 measurement, the CPMG sequence was used. The echo time was set to 0.5 ms and the number of echoes increased from 2 to 50 or to 100. T_1 and T_2 relaxation times were estimated from non-linear exponential fitting of the measured values by the MestreNova and Origin softwares. The standard deviations of the estimated parameters were also determined in the fitting procedure.

4.6. Drug loading measurements and release experiments

4.6.1. HPLC measurements

The liquid chromatograph equipment consisted of an Agilent 1100 series instrument furnished with a G1311 quaternary pump, a G1379A degasser, a G1392A autosampler and a G1315B diode array spectrophotometric detector. Instrument control and data acquisition were carried out with the Agilent Chemstation software. The analytical column was a Synergy

Hydro-RP C18 (150 mm × 4.6 mm inner diameter, particle size 4 µm) from Phenomenex. Analytes were eluted isocratically using 0.1 v/v% formic acid aqueous solution and methanol. The percentage of methanol in the eluent was 80 v/v% for TRF/HTB and KET, and 90 v/v% for IBU. The mobile phase flow rate was 1 mL min⁻¹ and the injection volume was 10 µL. TRF/HTB, KET and IBU were detected at 280, 254 and 220 nm, respectively. External standard method and calibration curves with 7 points were used to measure the quantity of dissolved drugs during the determination of the loading efficiency and in dissolution experiments.[96]

The amount of drug loaded into a given aerogel was determined after soaking the loaded sample (~5 – 10 mg) in 10 mL of methanol and filtering the solution through a PTFE membrane filter (pore size = 0.25 µm). In the case of TRF/HTB, both the concentrations of the drug and the metabolite were quantified. Data presented in this work correspond to the sum of these values expressed as the total triflusal content (TRF(T)).

HPLC was also used to determine the kinetics of the dissolution of pristine drugs and the release of drugs from loaded aerogel samples. Drug release experiments were carried out in either 10 mM HCl (pH 2.0) or 50 mM PBS (pH 7.4) aqueous solutions, simulating gastric and intestinal pH-conditions, respectively. In these experiments, a weighted sample (~5 – 7 mg) was added to 25 mL preheated (37 °C) buffer solution. The stirring rate was 60 rpm and the temperature was 37 ± 0.5 °C. For each measurement, 250 µL solution was recovered, and 10 µL were injected into the column. All experiments were performed in triplicates that allowed the establishment of variability ranges.

4.6.2. Spectrophotometric method

The release of IBU and KET from silica-gelatin aerogel is fast both in HCl and PBS. In order to follow the fast release of the drugs with sufficiently high time-resolution, a new experimental method was

developed. Dry, loaded aerogel was milled, and sieved to uniform size ($<125\ \mu\text{m}$) in order to avoid any size-related effects. A portion of the loaded aerogel powder was weighted with 0.01 mg precision into a carefully dried spectrophotometric cuvette. The cuvette was placed into the cell-holder of a custom built fiber optic UV–Vis spectrophotometer equipped with a fast CCD detector (Avantes) and thermostated at $37.0 \pm 0.1\ ^\circ\text{C}$. [97] On-line detection was started, and 3.0 mL pre-heated release medium (pH 2.0 HCl solution or pH 7.4 PBS) was injected into the cuvette. During this process and under the whole release experiment the suspension was stirred by a $2 \times 8\ \text{mm}$ PTFE coated magnetic stir-bar at 300 rpm. The detector was typically operated with 30 ms integration time and 20 subsequent spectra were averaged. Absorbance change was followed in the 200 – 800 nm wavelength range in 1.0 nm steps for at least 1000 s with a minimum time resolution of 1.0 s. As the drug was released from the aerogel, the characteristic absorbance signal of either IBU or KET was detected in the suspension.

As we mentioned before, the aerogel suspension scatters the detection light of the spectrophotometer. This light scattering was taken into correction by subtracting it from each recorded spectrum using the “dual-wavelength method” developed by Liu and Zhu, detailed in Section 3.6. [85,86]

The release experiments were conducted in the concentration range between 0.20 and 0.57 mg mL^{-1} loaded aerogel in the dissolution medium. This translates to final concentrations of 0.048 – 0.14 mg mL^{-1} and 0.028 – 0.080 mg mL^{-1} of IBU and KET, respectively.

5. Results and discussion

5.1. Aerogel composition

Four different hybrid aerogels were synthesized by using gelatin and silica precursors with weight ratios between 3 and 30 wt.% for gelatin. In particular, ratios of 3, 10, 20 and 30 wt.% were used to synthesize samples referred to as H3, H10, H20 and H30, respectively. The mass of the gelatin incorporated into these hybrid aerogels after processing was estimated by TGA from the weight loss in the range 300 – 500 °C (Fig. 7). In this temperature interval, some condensation of vicinal –OH groups could also take place in the inorganic silica phase. The weight loss due to the loss of water molecules expelled by the inorganic phase is below 1 wt.%, and was considered negligible.[47] The TGA estimated weight percentages of gelatin in the hybrid aerogels are given in Table 3. The gelatin content varies from ca. 3 to 24 wt.% for samples H3 to H30.

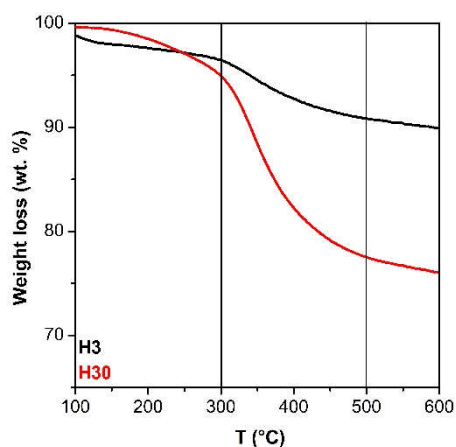
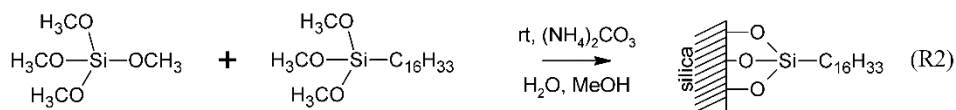
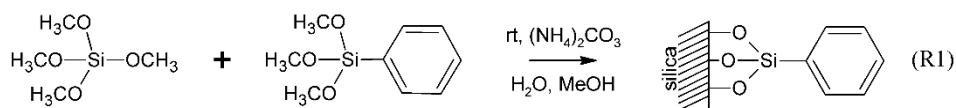


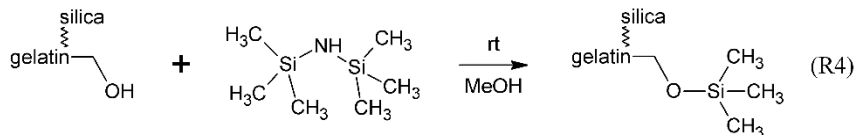
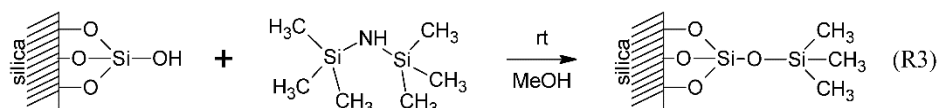
Figure 7. Representative TGA profiles of silica-gelatin hybrid aerogels with 3wt.% (H3, black) and 24 wt.% (H30, red) gelatin content. Gelatin content was calculated from the weight loss between 300 – 500 °C (marked with vertical lines).

The silica-gelatin aerogels are hydrophilic porous materials. To obtain products with different characteristics and possibilities of applications, the

hybrid aerogels were further modified by the functionalization of the backbones, i.e. the surfaces of the pores. Two different functionalization approaches were evaluated, applied either during the sol-gel process or after gelation. In the first method, aerogels with low gelatin content (H3), were modified during the sol-gel reaction by the co-condensation of TMOS and either Ph-TMOS (sample H3_Ph) or C₁₆-TMOS (sample H3_C₁₆). The ratio of TMOS and the functionalizing agent was 90:10, 80:20 or 70:30 V/V% (Table 2). In the case of both additives, a hydrophobic group incorporates into the silica matrix via a hydrolytically stable $\equiv\text{Si}-\text{C}$ covalent bond following reaction equations R1 and R2.[98,99]



In the second hydrophobization strategy, the hybrid gel was post-functionalized during aging, by adding HMDS, a difunctional trimethylsilyl silane. HMDS is highly reactive towards the surface silanols of the silica backbone and the hydroxyls of the gelatin backbone, giving ammonia as a side product.[100] The hydrogens in the surface silanol and hydroxyl groups are replaced by the hydrolytically stable $-\text{Si}-(\text{CH}_3)_3$ group.[101] Following this procedure, hybrid aerogels with different silica-gelatin ratios were derivatized by adding an excess of HMDS. Silylation took place following reaction equations R3 and R4.



TGA was used to estimate the final composition of all functionalized aerogels. Gelatin decomposition was previously established to occur between 300 and 500 °C (Fig. 7). The degradation temperatures of the modifiers depend on the structure of the grafted molecules. Fig. 8 shows the TGA profiles corresponding to H3 aerogels treated with each of the studied modifiers. For Ph-TMOS and HMDS treated aerogels, the hydrolytically stable $\equiv\text{Si}-\text{C}$ covalent bond in the $\equiv\text{Si}-\text{Ph}$ and $\equiv\text{Si}-(\text{CH}_3)_3$ layers remain intact up to 550 °C. As a consequence, the weight loss occurred in two uneven stages for these samples: the first one corresponds to gelatin degradation (300 – 500 °C) and the second results from the Si-C cleavage in the 550 – 700 °C temperature interval. From the TGA weight loss in the second temperature interval, it could be estimated that aerogel samples were modified with ca. 4 – 9 and 5 – 6 wt. % of $-\text{Ph}$ and $-(\text{CH}_3)_3$ organic moieties, respectively (Table 3). In contrast, in the aerogel modified with $-\text{C}_{16}\text{H}_{33}$ (H3_C16 samples) the grafted hydrocarbon chain starts to decompose at 250 – 300 °C by the cleavage of C–C bonds and continue up to 700 °C where the Si–C bonds break. Therefore, the weight losses corresponding to the degradation of gelatin and the modifier overlap. Assuming a gelatin weight loss of ca. 4 wt.% in the H3 type samples, the estimated amount of C₁₆ modifier incorporated to the sample is 2 – 6 wt.% (Table 3).

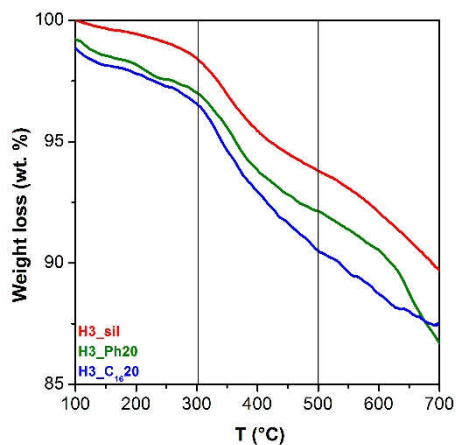


Figure 8. Representative TGA profiles of silica-gelatin hybrid aerogels with 3wt.% functionalized with silyl (red), phenyl (green) and hexadecyl (blue) moieties. Gelatin degradation takes place between 300 – 500 °C (marked with vertical lines).

The 3 modifiers have remarkably different molar masses (M_w): 77, 225 and 45 g mol⁻¹ for $-C_6H_5$ (Ph), $-C_{16}H_{33}$ (C₁₆) and $-(CH_3)_3$ (HMDS), respectively. This results in different surface densities even at similar weight percentages of the incorporated functional groups. Molecular surface densities ($S\rho$ [molecule nm⁻²]) were calculated to facilitate comparison by using the BET specific surface values of the raw matrices (a_s [nm² g⁻¹], shown in Table 3) and the measured amount of modifiers (d_s [gmodifier g_{aerogel}⁻¹]). Surface grafting density was calculated using the following formula:

$$\frac{d_s \times 6 \times 10^{23}}{M_w \times a_s} = S\rho \quad (24)$$

Relatively high density values of 1.2 – 1.7 molecule nm⁻² were estimated for the silanized products, and 0.3 – 1.0 molecule nm⁻² for $-Ph$ treated aerogels. On the contrary, aerogels functionalized with the long carbon chain $-C_{16}$ had surface grafting density values of only 0.1 – 0.2 molecule nm⁻².

Table 3. Composition and textural properties of the hydrophilic (Sil, H3, H10, H20, H30) and the surface modified aerogel samples. (* tentatively assigned)

Sample	Gelatin [wt.%]	(CH ₃) ₃ [wt.%]	Ph [wt.%]	C ₁₆ [wt.%]	Sp [molecule nm ⁻²]	a _s [m ² g ⁻¹]	C
Sil	-	-	-	-	-	863	93
H3	3.7	-	-	-	-	644	105
H10	11	-	-	-	-	627	102
H20	18	-	-	-	-	415	87
H30	24	-	-	-	-	285	81
H3_sil	3.6	6.0	-	-	1.26	636	21
H10_sil	11 *	6.0*	-	-	1.34	596	23
H20_sil	19	5.0	-	-	1.31	509	27
H30_sil	24	6.5	-	-	1.71	504	24
H3_Ph10	5.0	-	4.2	-	0.38	860	54
H3_Ph20	3.8	-	7.6	-	0.82	724	56
H3_Ph30	4.0	-	9.2	-	0.91	790	56
H3_C ₁₆ 10	4.0*	-	-	2.1	0.09	638	86
H3_C ₁₆ 20	4.0*	-	-	4.0	0.14	736	75
H3_C ₁₆ 30	4.0*	-	-	5.8	0.22	711	73

5.2. Microstructure and textural properties of the aerogels

SEM images of silica and H3 silica-gelatin aerogels in 25k× magnification are shown in Fig. 9. Both aerogel backbones are built from spherical blocks. The determination of the diameters of the globules is difficult, because they overlap in the image. A rough estimation gives a globule size between 75 and 100 nm for both aerogels. The majority of pores are in the mesopore range, although some macropores can also be seen in the images. No significant morphological differences are revealed between the two aerogels. Only the microstructure typical of silica aerogel can be observed in the images. No separate polymer phase is visible in any portion of the sample, thus indicating that the two phases are intimately mixed at this scale. The measured percentages of gelatin in the hybrid aerogels (Table 3) were similar to the added amounts (Table 2), suggesting the full incorporation of this phase into the silica primary particles.

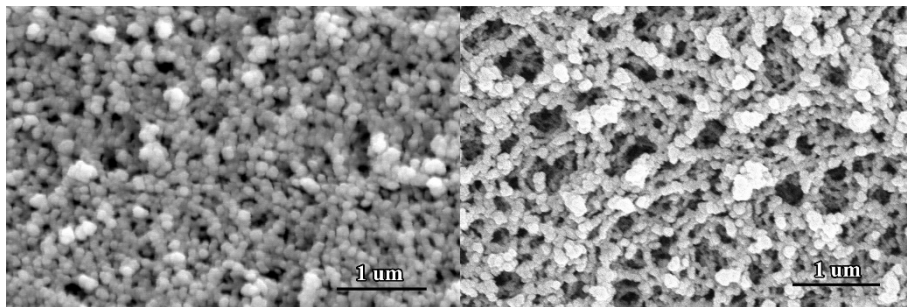


Figure 9. SEM images of silica aerogel (Sil, left) and silica-gelatin hybrid aerogel (H3, right).

The N_2 adsorption and desorption isotherms of the dry silica-gelatin aerogels are shown in Fig. 10. Type IV hysteresis curves were observed for all samples, that are characteristic for mesoporous materials. The steep rise of the isotherms in the vicinity of relative pressure = 1 indicates the presence of macropores. The specific surface area of silica aerogel is $863 \text{ m}^2 \text{ g}^{-1}$ and that of the unmodified hybrids decreases from $644 \text{ m}^2 \text{ g}^{-1}$ to $285 \text{ m}^2 \text{ g}^{-1}$ depending on the gelatin content (Table 3). The C constants are around 100 suggesting highly hydrophilic surfaces in every case. The total pore volume of silica and silica-gelatin aerogels are in the range of 5.00 and $7.00 \text{ cm}^3 \text{ g}^{-1}$, respectively. Micropore volumes of both types of aerogels were near zero as determined from the BET “t-plots”. The pore size distributions for the materials are calculated by the BJH method from the desorption curves are shown in Fig. 10. The shape of the pore size distribution curves clearly show that the contribution of both the smaller ($d = 5 - 10 \text{ nm}$) and the larger ($d = 50 - 100 \text{ nm}$) pores increase with increasing gelatin content. The incorporation of gelatin induces the overall decrease of the number of pores, which is expressed in a decrease of the specific surface area.

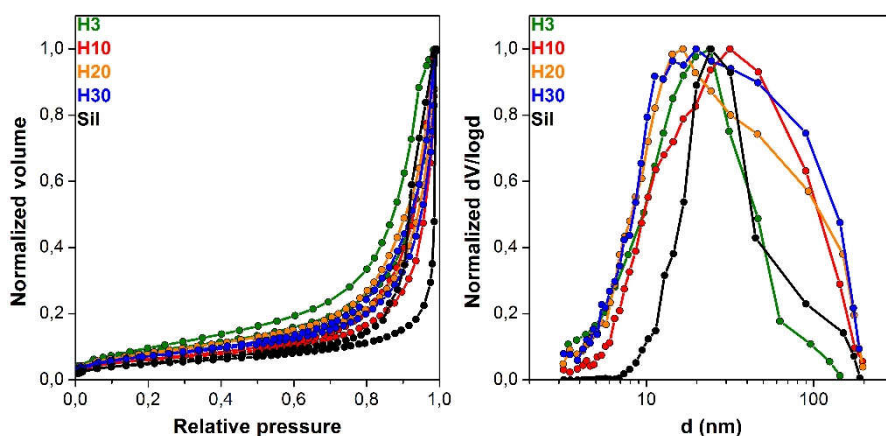


Figure 10. Nitrogen adsorption-desorption isotherms (left) and pore size distribution curves (right) of pure silica aerogel and silica-gelatin hybrid aerogels. The values of the Y-axis are normalized. The distributions were calculated from the corresponding desorption curves by the BJH method.

Functionalization of the aerogel surfaces by co-condensation or silane derivatization affect the specific surface area in different ways (Table 3). Silylation and phenyl modification prevent the gelatin-induced decrease of the surface area to some degree. The C_{16} modification results in a slight increase of the surface area. A possible explanation for the positive effect of the modifiers on the surface area can be that the hydrophobized matrices shrink only to a lower extent during aging and drying compared to the hydrophilic gels.

In N_2 adsorption-desorption measurements, the intensity of the interaction of the adsorbate with the surface of the adsorbent can be assessed from the C constant of the BET equation.[102] Although, the rigorous interpretation of the C parameter and its use for the determination of surface energies have fundamental limitations, a simplistic qualitative approach gives an idea of the hydrophobic/hydrophilic character of the adsorbent. Namely, the lower the C constant value, the lower the hydrophilicity is. Pure silica aerogels have values of C of ca. 100. As it can be observed in Table 3, both normal silica and hybrid aerogels prepared in this work have C values in the order of 90 – 100,

independently of the gelatin content. This indicates that the matrices are highly hydrophilic. The C values for the phenyl modified aerogels and the silylated samples are reduced to ca. 50 and to ca. 25, respectively, pointing towards a decrease of the aerogel surface polarity. This is the result of the presence of the hydrophobic functional groups. In contrast, C values measured for the $-C_{16}$ modified aerogel were similar to the corresponding pristine hybrid matrix (H3), likely reflecting the low surface grafting density of this modifier ($0.2 \text{ molecule nm}^{-2}$). This is probably not enough to significantly modify the thermodynamic properties of the aerogel surface.

5.3. Characterization of impregnated aerogels

The hybrid aerogels were impregnated with IBU, KET or TRF by using sc. CO_2 as a solvent. The high hydrophobicity of these drugs makes them soluble in sc. CO_2 at $35 - 45 \text{ }^\circ\text{C}$ and pressures above 90 bar. The solubility of ibuprofen in sc. CO_2 was found to be 4×10^{-3} mole fraction at 20 MPa and $35 \text{ }^\circ\text{C}$. [103] Under similar conditions, the solubility of ketoprofen is lower, 8×10^{-5} mole fraction due to its higher molar mass. [104] For triflusal, the presence of the hydrophobic trifluoromethyl group has a pronounced effect on the interaction of TRF molecules with sc. CO_2 enhancing the solubility to 3×10^{-2} mole fraction at $35 \text{ }^\circ\text{C}$ and 20 MPa. [105]

The efficiency of the impregnation process was studied by FT-IR spectroscopy. The comparison of the infrared spectra of the pure drugs, the unloaded and the loaded hybrid aerogels clearly show the successful impregnation of the matrix (Fig. 11). The characteristic broad bands of the silica based matrix can be observed at 3400 cm^{-1} , 1650 cm^{-1} , 1100 cm^{-1} , 960 cm^{-1} and 800 cm^{-1} . These can be assigned to the stretching of silanol groups and molecular water linked to the surface silanols with H-bonds, adsorbed water, the asymmetric stretching vibration of Si–O–Si, the deformation of Si–O–H and the symmetric vibration of Si–O–Si, respectively. [106]

The characteristic bands of the drugs can be detected in superposition to those of the matrix. The new bands in the matrix impregnated with ibuprofen between 3090 and 2850 cm^{-1} are related to the C–H stretching of methyl groups and the aromatic ring. The band originating from the C=O stretching of carboxylic group can be seen at 1710 cm^{-1} . The C=C ring vibration causes a lower intensity band at 1515 cm^{-1} . [107] As the chemical structure of ibuprofen and ketoprofen are similar, the same specific transitions can be detected in the spectrum of the hybrid aerogel impregnated with ketoprofen as described above. [108] However, the lower loadings in the case of ketoprofen result lower intensity bands. Addition to the aromatic C–H, C=O and O–H (of carboxylic group) stretchings characteristic for triflusal, the asymmetric C–F stretching at 1335 cm^{-1} is present in the IR spectra of the hybrid aerogels loaded with triflusal.

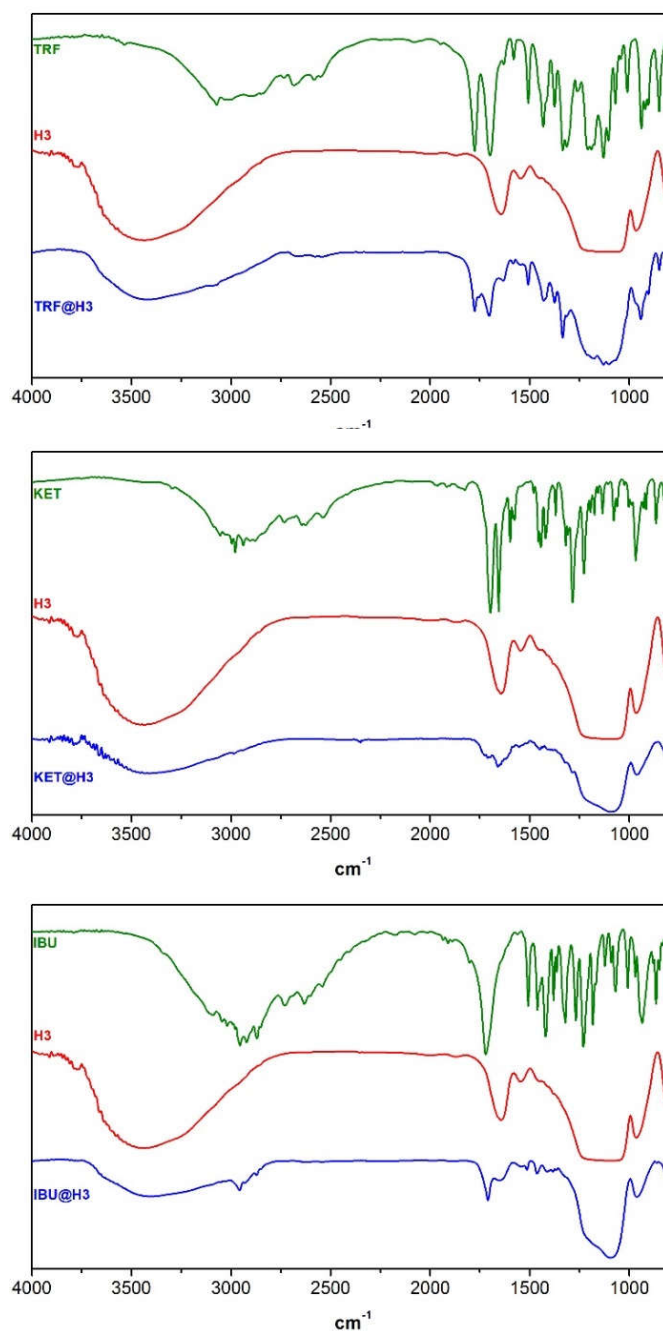


Figure 11. FT-IR spectra of microcrystalline drugs, pristine hybrid aerogels and loaded hybrid aerogels.

The estimation of drug loading values was performed by TGA. Illustrative TGA profiles corresponding to aerogel H3 loaded with one of the 3 studied drugs (IBU@H3, KET@H3 and TRF(T)@H3), are shown in Fig. 12. Loadings were approximated from the weight loss in the 150 –550 °C interval, after subtracting the weight loss of gelatin (ca. 4 wt.%). High loading values were found in the order of 19, 13 and 27 wt.% for IBU@H3, KET@H3 and TRF(T)@H3, respectively. In comparison, pure silica aerogels can have loadings of ca. 40 wt.% ibuprofen and ca. 25 wt.% ketoprofen. The successful impregnation is due to the high specific surface area of the matrices and the effectiveness of the adsorptive precipitation technique. In order to confirm the loading percentages, dissolution experiments combined with HPLC analysis were conducted with three independent replicates. Data given in Table 4 correspond to HPLC measurements. It should be noted that the loadings measured by TGA and HPLC were in good agreement in the case of all samples. The highest loading values were obtained for TRF (25 – 29 wt.%), followed by IBU (19 – 24 wt.%) and KET (11 – 15 wt.%).

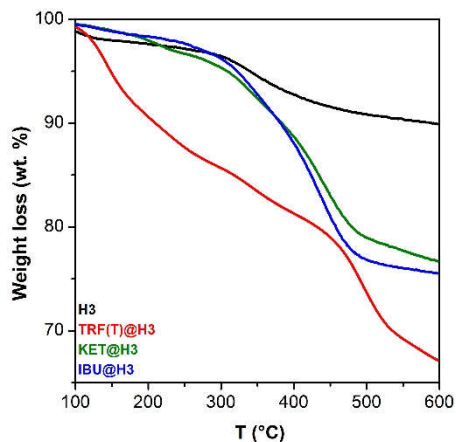


Figure 12. TGA profiles of different hybrid H3 aerogel samples loaded with one of the 3 studied drugs.

XRD was used to assess the crystallinity of the impregnated drugs. Spectra were recorded in the 2θ range of $5 - 35^\circ$ (see examples in Fig. 13). The three studied drugs can be readily identified in this range by their most intense peaks. All the impregnated drugs showed only a broad band centered at ca. 22° , which is typical for amorphous silica. Diffraction peaks corresponding to the crystalline forms of TRF, IBU or KET were not detected in the XRD patterns of the impregnated products indicating the absence of crystalline drugs inside the aerogel pores after supercritical impregnation.

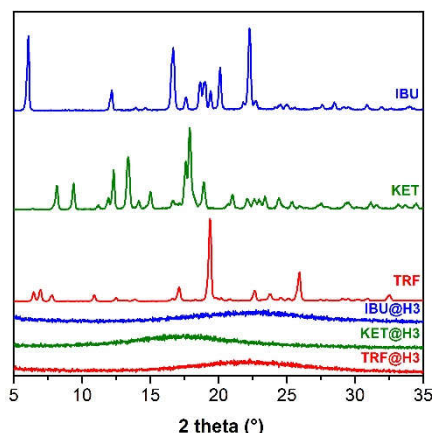


Figure 13. XRD patterns of different hybrid H3 aerogel samples loaded with one of the 3 studied drugs.

Complementarily, DSC analysis was used to further confirm the absence of drug crystals in the impregnated products. Up to 600°C no thermal transition takes place in the studied pristine and loaded matrices (Fig. 14). The pure drugs melt in the $80 - 120^\circ\text{C}$ temperature interval exhibiting sharp endothermic peaks in the DSC patterns. Hence, XRD and DSC results suggest that the drugs are most likely dispersed inside the matrices at amorphous form, rather than present in crystalline form.[105,109] In fact, in order to generate crystalline particles inside of the pores a sudden change in solubility, e.g. created by a pressure drop would be necessary.

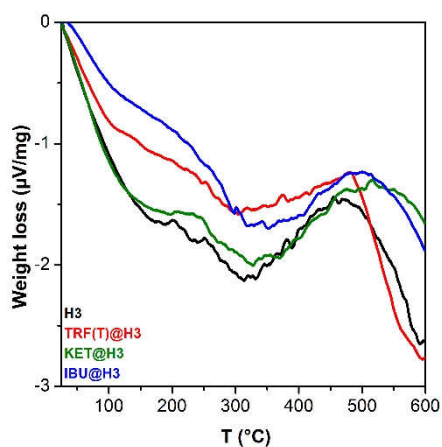


Figure 14. DSC profiles of hybrid H3 aerogel samples loaded with one the 3 studied drugs.

The pristine hybrid aerogels are highly hydrophilic, having a high number of surface -OH and -NH_2 groups. These reactive surface moieties form hydrogen bonds with the carboxylic acid groups of the studied drug molecules. This can stabilize the drugs against thermal degradation, since TGA analysis showed that they were stable up to 300 °C and higher (Fig. 12). Drug impregnation modifies the textural properties of the samples, resulting in a reduction of the specific surface area and pore volume to 400 – 450 m² g⁻¹ and 1.5 – 1.6 cm³ g⁻¹, respectively. A simple explanation for this is that the adsorbed drugs occupy considerable space inside the pores. The C values of the impregnated matrices decrease by ca. 50% compared to those of the pristine aerogels (Table 3), indicating the hydrophobization of the surface as a consequence of the impregnation by the drugs.

The influence of aerogel functionalization on drug loading was studied by HPLC (Table 4). Two main factors determine the overall loading of a given drug delivery system. The first one is the specific surface area of the carrier matrix which determines the number of the active adsorption sites of the system. The second one is the affinity of the drug molecules to the surface of the matrix. It was shown in Chapter 5.2. that the specific surface area of the hybrid aerogels decrease with increasing gelatin

content, meaning the reduction of the number of the adsorption sites. This significantly alters the overall loadings. The area specific loadings, that reflect the affinity of the drug molecules to the surface, increase with increasing gelatin content from 2.5 $\mu\text{mol m}^{-2}$ to 5.8 $\mu\text{mol m}^{-2}$, from 2.4 $\mu\text{mol m}^{-2}$ to 4.0 $\mu\text{mol m}^{-2}$ and from 1.0 $\mu\text{mol m}^{-2}$ to 1.7 $\mu\text{mol m}^{-2}$ for TRF, IBU and KET, respectively. Gelatin has numerous $-\text{OH}$ and $-\text{NH}_2$ groups which can serve as potential hydrogen bonding sites, and thus can facilitate the adsorption of the drug molecules. However, the decreasing surface area compensates for the favorable change in the surface properties. Overall, the gelatin content does not have any significant influence on drugs uptake in the case of the studied non-modified hybrid aerogels. Both surface modification techniques prevent the reduction of the surface area with increasing gelatin content, hence the trends in specific loadings change. Silylation has a positive effect on TRF and KET uptake, both increase from 18 wt.% to 26 wt.% and from 7.0 wt.% to 8.4 wt.%, respectively. The phenyl modification has a negative effect on both the KET and the IBU loadings, while the C_{16} modification reduces the adsorbed amount of TRF.

Table 4. Drug loading values of hybrid silica-gelatin aerogels. (* tentatively assigned)

Sample	Gelatin (wt.%)	(CH_3) ₃ (wt.%)	Triflusal (wt.%)	Ibuprofen (wt.%)	Ketoprofen (wt.%)	Triflusal ($\mu\text{mol m}^{-2}$)	Ibuprofen ($\mu\text{mol m}^{-2}$)	Ketoprofen ($\mu\text{mol m}^{-2}$)
H3	3.7	-	29	24	14	2.56	2.38	0.99
H10	11	-	25	23	14	2.14	2.31	1.02
H20	18	-	29	22	15	3.97	3.29	1.67
H30	24	-	29	19	11	5.78	3.99	1.71
H3_sil	3.6	6.1	18	15	7.0	1.39	1.35	0.47
H10_sil	11*	6.0*	22	14	7.7	1.91	1.32	0.55
H20_sil	19	5.0	25	16	9.1	2.64	1.81	0.77
H30_sil	24	6.5	26	15	8.4	2.81	1.70	0.72
Ph (wt.%)								
H3_Ph10	5.0	4.2	27	23	18	1.73	1.68	1.00
H3_Ph20	3.8	7.6	29	19	13	2.27	1.57	0.81
H3_Ph30	4.0	9.2	27	18	9.1	1.89	1.35	0.50
C₁₆ (wt.%)								
H3_C ₁₆ 10	4.0*	2.1	24	27	11	1.99	2.81	0.76
H3_C ₁₆ 20	4.0*	4.0	21	25	7.2	1.46	2.20	0.41
H3_C ₁₆ 30	4.0*	5.8	19	25	11	1.33	2.27	0.68

5.4. Drug dissolution and release monitored by HPLC

Drug dissolution profiles of pristine drugs and loaded aerogel systems were monitored in vitro using an HPLC method. Assays were carried out in two dissolution media, biorelevant and widely applied to similar studies, with a pH of either 7.4 (intestinal pH) or 2.0 (gastric pH). A preliminary dissolution test was carried out for each pristine drug under similar pH conditions (Fig. 15).

Crystalline drugs rapidly dissolve in the first few minutes, either totally at basic pH or up to saturation values at acidic pH. This is the common behavior expected for the studied acidic drugs, with pK_a values of 4.40, 4.76 and 4.15 for IBU, KET and TRF, respectively.[110] The measurements gave an estimation of drug solubility under the studied experimental conditions. At pH 2 and 37 ± 0.5 °C, the solubilities are in the order of 0.06, 0.26 and 0.69 mg mL^{-1} for IBU, KET and TRF(T), respectively. At pH 7.4 and 37 ± 0.5 °C, the solubilities of the three compounds are higher than 0.7 mg mL^{-1} , which was the highest value assayed. The observed increase in solubility at pH 7.4, particularly evident for IBU and KET (Fig. 15), can be related to an enhanced driving force for the forward reaction between the weakly acidic drugs and the basic species in the medium. The behavior of TRF is more complex, since aside from solubility issues, this molecule is unstable at neutral and alkaline pH and undergoes a progressive decomposition to HTB.[96,111] The metabolite HTB is also highly active as a platelet aggregation inhibitor, hence, its degradation does not represent a remarkable shortcoming from the pharmaceutical point of view. However, analytically this decomposition makes the evaluation of the behavior of the drug more difficult. The total TRF amount was calculated by adding the amount of HTB and TRF.

Drug release profiles were studied for samples involving either a hydrophilic (H3) or hydrophobic (H3_sil) matrix. Drug release profiles

from both the hydrophilic H3 and the hydrophobic H3_sil matrices are shown in Fig. 15.

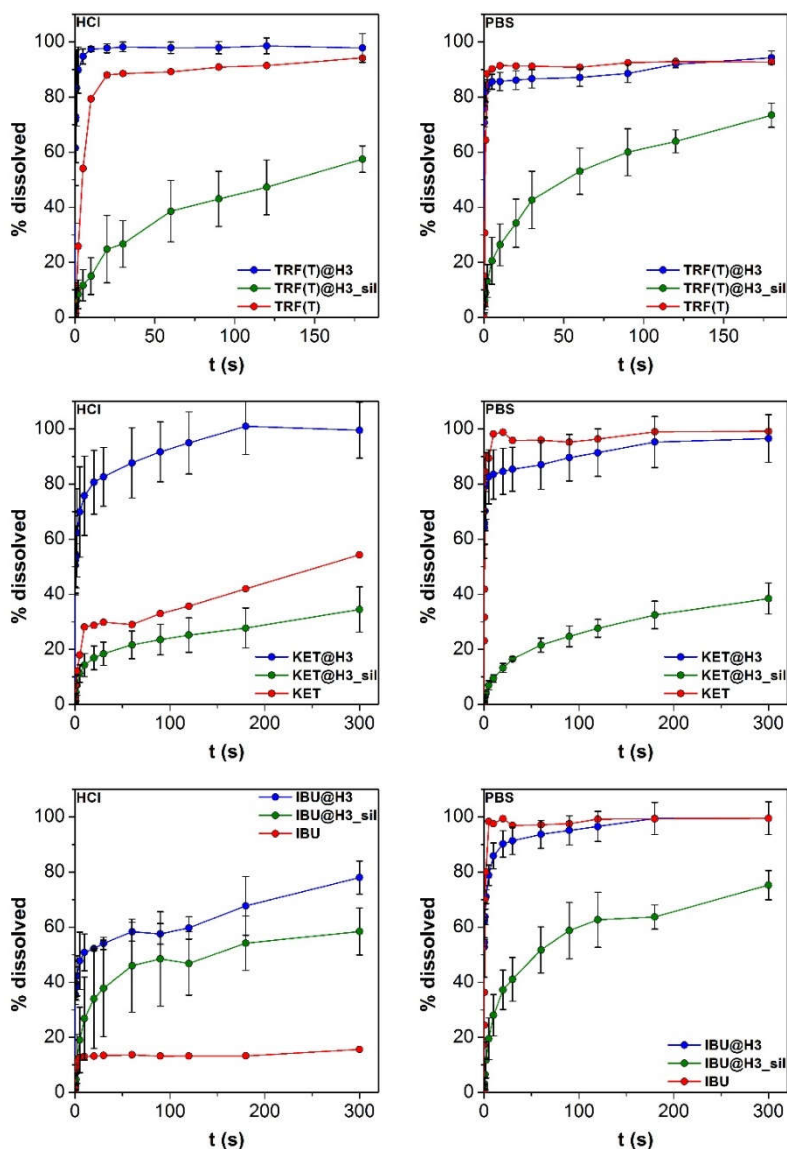


Figure 15. Drug release from microcrystalline drug (red), drug@H3 (blue) and drug@H3_sil (green) systems for: triflusal (top), ibuprofen (middle) and ketoprofen (bottom) at pH 2.0 (HCl) and pH 7.4 (PBS), 37.0 ± 0.5 °C, 60 rpm.

At pH 7.4, the dissolution of the 3 active agents occurred almost instantaneously. On the contrary, semiretarded release profiles were recorded at pH 2.0. For TRF(T) at pH 2.0, the initial burst provided an immediate release of 40% of the loaded drug, reaching a value of drug dissolution of 90% after 5 h (Fig. 15 top). For KET at pH 2.0, the initial burst release attained a value as high as 80% and after 5 h the drug was completely released to the acidic medium (Fig. 15 middle). For IBU at pH 2.0, a release of ca. 50% was observed in a first burst stage, followed by a slow delivery step giving ca. 70% of drug released after 5 h (Fig. 15 bottom). It is worth mentioning that the dissolution rate for the 3 studied drugs from the H3 matrix is faster in basic medium than at pH 2.0. The observed semiretarded release behavior under acidic conditions cannot be related to drug solubility, because sink conditions were applied. The key factor is the change in the specific interactions established between the matrix and each drug as a function of pH. The studied analytes are quite nonpolar (the estimated $\log P$ values for IBU, KET and TRF are 3.7, 2.8 and 2.3, respectively) and are mainly protonated (neutral) at pH 2.0, while at pH 7.4 the deprotonated (anionic) species of all the drugs are present as the major species. The protonated species at pH 2.0 interact more strongly with the hydroxylated matrix surface, probably through hydrogen bonds, thus, retarding drug release.

The role of modifiers in the silica aerogel, including gelatin in the bulk and hydrophobic agents on the surface, is analyzed by comparison with published data for pure silica aerogels having surface areas of 600 – 800 m² g⁻¹ and drug loadings of 10 – 20 wt.%. For the IBU@silica aerogel system, release data is published at neutral pH showing a delivery of 80% of the drug after 30 min.[48] For the IBU@H3 system studied in this work, the release of 80% was attained after 10 min in neutral pH and it was retarded to more than 300 min at acid pH. For the KET@silica aerogel system, it was shown that 60 min are necessary to release 80% of the drug at acid pH, similar to the value found in this work for the KET@H3 system.[44] For the TRF@silica aerogel system, previous

studies showed that 80% of the drug was released after 20 min in both acidic and basic pH.[47] However, for the TRF@H3 samples, periods of 60 and >300 min are necessary to reach a delivery of 80% at neutral and acidic pH, respectively.

The release behavior of the hydrophobic drugs dispersed in modified hydrophobic aerogels was assessed in the matrix with the lowest gelatin content treated with HDMS (H3_sil sample). For the 3 studied drugs, a prolonged release was observed for the impregnated drug@H3_sil samples when compared to the release profiles of similar drug@H3 aerogels (Fig. 15). This behavior was observed at both of the studied pH values. The fast release for the drug@H3 samples at pH 7.4 turned into a two-step release profile in similar drug@H3_sil samples, involving an initial burst of 40, 15 and 20% release for IBU, KET and TRF(T), followed by a slow delivery reaching values of 75, 35 and 80% release for IBU, KET and TRF(T) after 5 h (Fig. 15). At pH 2 initial burst release – like at pH 7.4–, takes place for drug@H3_sil samples, although a slightly slower drug release was measured at the acidic pH. At pH 2, values of only 55, 30 and 65% release occur for IBU, KET and TRF(T) after 5 h (Fig. 15). The appearance of the initial burst can be explained with the fact, that during the impregnation process not only the inner pores of the matrix are loaded with the drug but the outer surface of the particles is also impregnated. During the release test the release media can easily and rapidly dissolve this ratio of the drugs causing the burst stage of the dissolution profiles, which is followed by the prolonged release controlled by the poor wettability of the matrix.

5.5. Aerogel behavior in release media

5.5.1. Particle size distribution in suspension

The rate of drug release from a delivery matrix is highly dependent on the size distribution of the carrier particles dispersed in the release medium. Silica aerogel particles dispersed in deionized water (or in aqueous

buffers) change in time and the size distribution stabilizes about 3 h after dispersing.

In order to compare the size distribution of particles in silica (Sil) and silica-gelatin aerogel (H3) suspensions, laser diffraction measurements were performed. The size distributions of both aerogels change after dispersing, which interfere with the measurements. Thus, in the first set of experiments only stable suspensions were studied. The calculated, nearly symmetrical log-normal particle size distributions of the dispersed aerogels are shown in Fig. 16. Silica-gelatin aerogel particles are smaller ($d = 10 - 30 \mu\text{m}$) and more uniform in size, while pure silica aerogel particles are larger ($d = 20 - 50 \mu\text{m}$) and have a wider distribution. In this sense, laser diffraction data do not indicate that incorporating gelatin into the backbone would significantly alter the suspension phase properties of the aerogel.

The particle size distribution of the silica-gelatin aerogel H3 was also studied in hexane–EtOH (9:1). The calculated distribution (Fig. 16) displays a uniform, ca. $5 \mu\text{m}$ shift towards higher particle sizes compared to measurements performed in water. This effect can be attributed to the smaller solvate spheres of the particles in hexane.

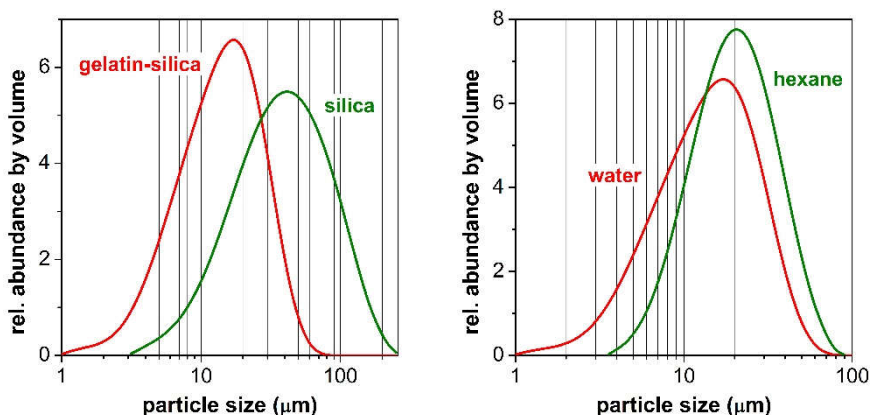


Figure 16. Particle size distribution of silica and silica-gelatin aerogels dispersed in aqueous phosphate buffer (left). Particle size distribution of silica-gelatin aerogel dispersed in aqueous phosphate buffer (red) and dispersed in hexane (green) (right). The distributions were calculated from laser diffraction measurements. Stable, at least 3 h old suspensions were studied.

In order to directly correlate drug release kinetics with the temporal change in particle size, the change in size distribution should be followed on the timescale of the release.[112] It was previously shown, that the complete release of IBU and KET from silica and silica-gelatin aerogels takes only a few minutes.[43–45,112] Unfortunately, meaningful time-resolved laser diffraction measurements could not be carried out at this timescale, thus the direct measurement of particle size-distribution during release was not possible. On the other hand, the change of the intensity of scattered light, which also gives indirect information on particle size, was followed with on-line spectrophotometry during drug release experiments (Fig. 17).

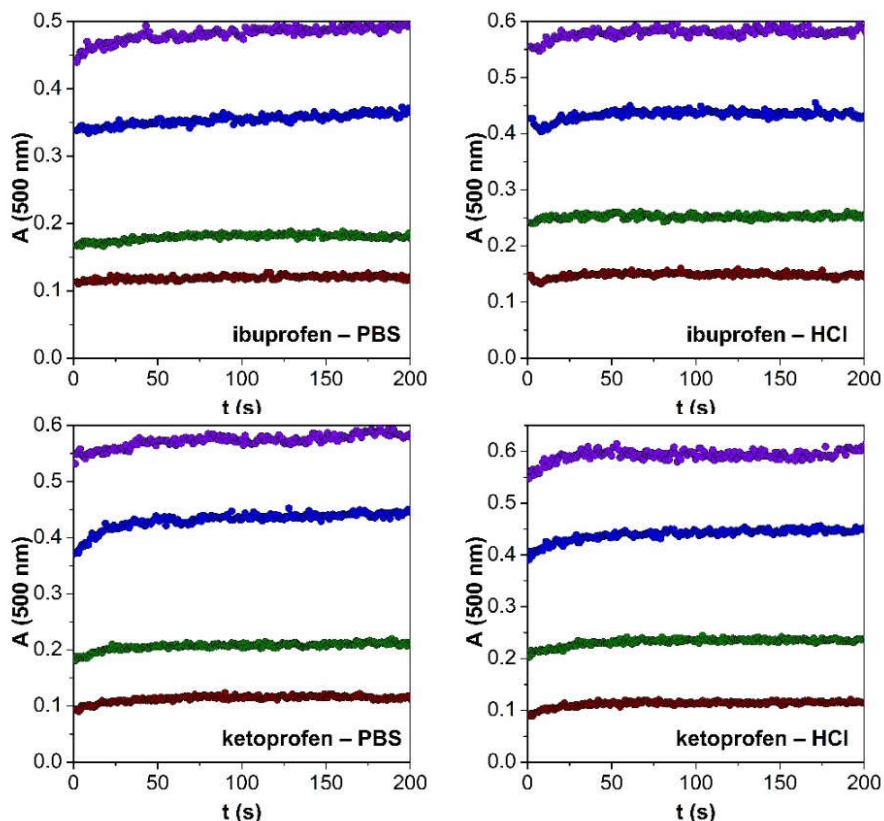


Figure 17. Light scattering measured as the apparent absorbance at 500 nm during drug release experiments. The dissolution of ibuprofen (top) and ketoprofen (bottom) from loaded silica-gelatin aerogel in pH 2.0 HCl and in 10 mM PBS (pH 7.4). The mass of the loaded aerogel changed in the cell from 0.56 mg to 1.72 mg, while the volume of the release medium was constant. Parallel experiments with different masses of aerogel are shown in each panel. Volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring.

5.5.2. NMR studies

Cryoporometry

The pore size distribution of silica aerogel (Sil) dispersed in water was measured by Kalmár et al. using NMR cryoporometry. The ratio of the measured melting and freezing point depressions was almost 2 : 3, which indicates the presence of spherical pores. The reconstructed pore size distribution curves are in good agreement with those obtained using N_2

porosimetry. The general conclusion drawn from cryoporometric data is, that dispersed silica aerogel particles retain the pore structure of the dry (as prepared) aerogel following the disintegration of the monolith in water. These observations were completed with the study of particle size distribution in aerogel suspensions (Chapter 5.5.1). During stirring and agitation, the shear forces cause the fragmentation of the large aerogel particles until a suspension with a well-defined particle size distribution is reached, which is confirmed by the laser diffraction particle sizing results. However, the pore structure remains intact. This phenomenon was independently investigated by small-angle neutron scattering (SANS), and it was shown that the introduction of water into the pores does not affect the structure in the mesoporous range.[113]

The pore structure of silica-gelatin aerogel H3 was probed by two different liquids using NMR cryoporometry. The first liquid was cyclohexane, a nonpolar liquid in which neither gelatin nor silica swell. The melting and freezing curves of H3 silica-gelatin aerogel dispersed in cyclohexane is shown in Fig. 18. The position of the melting-freezing hysteresis indicates the presence of mainly spherical pores in the suspension.[75] The reconstructed pore size distribution is shown in Fig. 18.

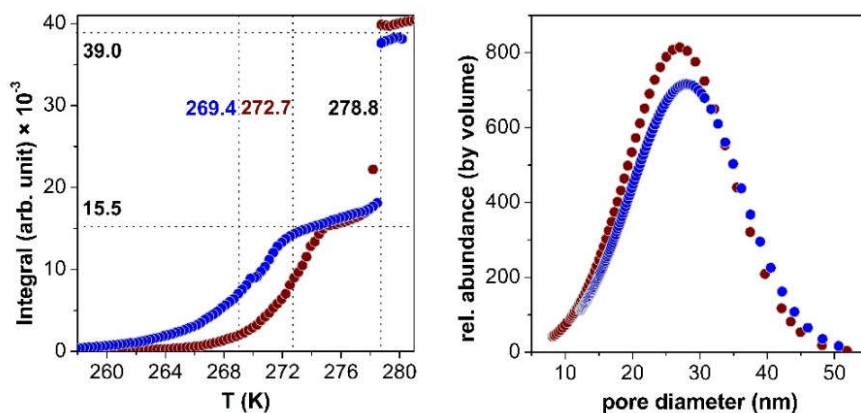


Figure 18. NMR cryoporometry. Left: Melting (red) and freezing (blue) curves of silica-gelatin aerogel H3 dispersed in cyclohexane. The positions of the inflection points and the heights of the plateaus are given in the plot. Right: Pore size distribution of dispersed silica-gelatin aerogel H3, reconstructed from the left figure assuming spherical pores (red: from the melting curve, blue: from the freezing curve).

The comparison of the distribution curves in Figs. 10 and 18 indicates that the pore structure of silica-gelatin aerogel is not deformed significantly when the material is dispersed in cyclohexane. This observation is in-line with the expected weak interaction between the hydrophilic silica-gelatin skeleton and cyclohexane.

The second liquid used for probing H3 silica-gelatin aerogel was water. A suspension of well-defined size distribution was studied (Fig. 16). The cryoporometric melting and freezing curves of the aqueous aerogel suspension show hysteresis, but do not have a well-defined plateau before the melting of bulk water at 273 K (Fig. 19). Cryoporometric curves of this type are characteristic for hydrogels, where no solid-walled pores exist and the apparent pore size distribution is extremely wide.[114] When the silica-gelatin aerogel is wetted with water, stirring and agitation cause the fragmentation of the large aerogel particles until dispersed particles of well-defined sizes are produced. Additionally, the silica-gelatin aerogel shows no observable swelling (or shrinking) in water. Still, cryoporometry indicates that its backbone strongly interacts with

water. It is rational to assume that the gelatin content in the backbone strongly binds water, and the well-hydrated protein chains deform the whole polymer backbone during their conformational change. The resulting structure resembles a hydrogel in the sense that the well-defined pore structure of the dry aerogel ceases to exist.

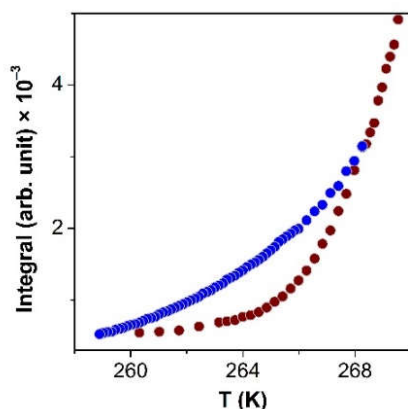


Figure 19. NMR cryoporometry. Melting (red) and freezing (blue) curves of silica-gelatin aerogel dispersed in water. The absence of inflection points and plateaus on the curves indicates the dense hydrogel like structure of the dispersed aerogel particles with no well-defined pore size distribution.

To further study the effect of gelatin content on the structure of the hybrid aerogel matrices cryoporometric measurements were carried out on hybrid aerogels with higher (11 wt.%, 18 wt.% and 24 wt.%) gelatin content (samples H10, H20 and H30) in cyclohexane. In spite of the fact that cyclohexane do not form a solvate sphere with gelatin, the results interestingly show that the hysteresis curve vanishes with increasing gelatin content. A hysteresis curve and a well-defined plateau can only be seen for H3 and H10 hybrid aerogels (Fig. 20). The Gibbs-Thomson equations (Eqs. 18–19) demonstrate, that the melting and freezing point differences are inversely proportional to the pore size, thus larger pores cause smaller shifts in melting and freezing points compared to the bulk phase. The pore size distribution curves reconstructed from the freezing curves clearly show a shift towards larger pores, which is in good

agreement with the pore size distribution curves calculated from N₂ porosimetry data (Fig. 21).

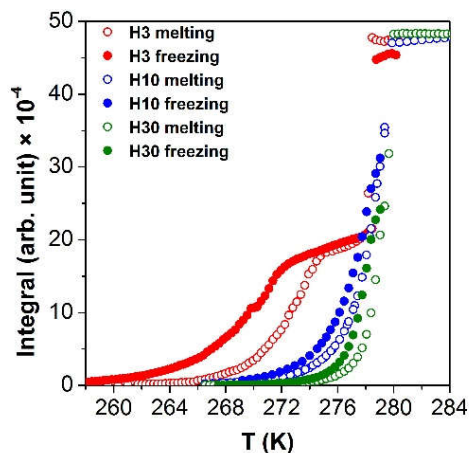


Figure 20. NMR cryoporometry: Melting and freezing curves of silica-gelatin aerogels with different gelatin content dispersed in cyclohexane.

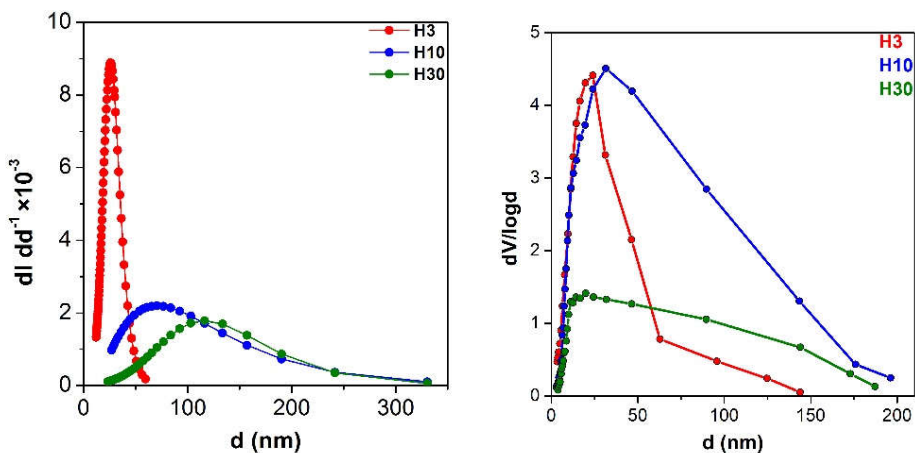


Figure 21. Right: Pore size distributions of dispersed silica-gelatin aerogels reconstructed from the freezing curves of NMR cryoporometry assuming spherical pores. Left: Pore size distribution of dry silica-gelatin aerogels calculated from the desorption branches of the N₂ isotherms using the BJH method.

Diffusiometry

The self-diffusion of hexane and water was measured in the suspensions of silica-gelatin aerogel H3 by the PGSE NMR method. First, the aerogel was dispersed in ca. twice the amount of hexane necessary to completely fill the pores of the material. Diffusion experiments revealed that the self-diffusion of hexane is restricted in this case. The intensity decay in this diffusion experiment is clearly single exponential in a wide observation time range from 4 to 120 ms. The value of the observed self-diffusion coefficient is $D_{obs} = (3.30 \pm 0.09) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, while that is $4.15 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for bulk hexane.[115] The explanation for the single diffusion domain is related to the fact that hexane molecules in the bulk diffuse freely, but molecules inside the pores are restricted in movement. The two groups of molecules would normally give two diffusion domains, unless they exchange rapidly in the timescale of the diffusion experiment. Rapid exchange between bulk hexane and hexane inside the pores indicates that the structure of the dispersed silica-gelatin aerogel is open and the pores are highly interconnected. Small molecules can enter and exit the pore network without restriction in the millisecond timescale. This is not surprising, since cryoporometry showed that dispersion in hexane does not deform the open pore structure of the aerogel.

In the second experiment the silica-gelatin aerogel H3 was dispersed in ca. twice the amount of water necessary to completely wet and fill the pores. PGSE experiments revealed that the self-diffusion of water is restricted in this aqueous suspension. Surprisingly, we detected only one diffusion domain in the whole timescale of the diffusion experiments (4 – 120 ms). The observed self-diffusion coefficient is $D_{obs} = (1.5 \pm 0.1) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, while this is $2.3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for bulk water.[116] By applying the same reasoning as in the case of hexane, the single diffusion domain indicates that hydrated and dispersed aerogel particles are still highly permeable for water. Cryoporometry revealed that silica-gelatin aerogel particles develop a hydrogel like character in water. Now, the results of diffusiometry complete this information by suggesting

that in spite of the high extent of hydration and deformation of the aerogel, the pore structure remains interconnected and highly permeable.

In order to gain more information on the interaction of H3 silica-gelatin aerogel with water, additional PGSE experiments were performed with wet aerogel samples, containing only ca. 70% of the amount of water necessary to completely fill the pores of the aerogel. In this case, the value of the observed self-diffusion coefficient decreases by increasing observation time below 40 ms. At longer observation times two separate diffusion domains (i.e. double exponential decays) were observed. The dependence of the effective diffusion coefficients on observation time is shown in Fig. 22. We propose, that the slower diffusion domain is characteristic for water molecules in the close hydration sphere of the silica-gelatin polymer backbone. Water molecules outside the hydration sphere, diffusing in the pores, belong to the faster diffusion domain. At short observation times the apparent diffusion rate of water molecules are the same in the two environments. At diffusion times longer than 40 ms, the motion of water molecules become restricted in the hydration sphere, because they cannot leave for the other domain. It manifests in the decrease of the effective diffusion coefficient. Those water molecules which are in the pores do not have this kind of restriction and keep their apparent diffusion rate versus observation time.[117,118]

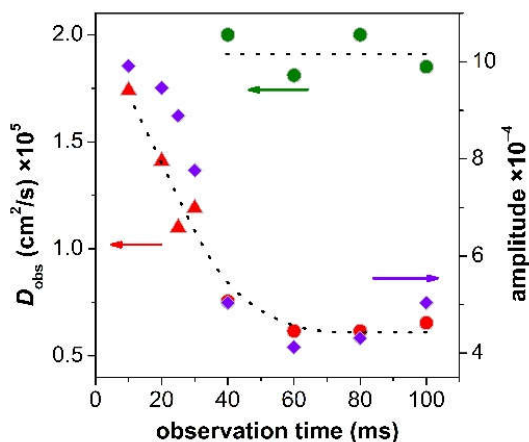


Figure 22. NMR diffusimetry. The self-diffusion coefficient (D_{obs}) of water measured in a wet silica-gelatin aerogel sample. Approximately 70% of the total pore volume of the aerogel is filled with water. Two diffusion domains were detected at observation times ≥ 40 ms. The diffusion coefficient of the faster domain is given with green, and that of the slower domain is given with red. The amplitude characterizing the slower domain is also given with violet. The measured D_{obs} of the slower domain is increasing by decreasing observation time. Dashed lines are only for guiding the eye.

The size of the restricting space can be approximated by using the methodology of Cho et al., as D_{obs} is inversely proportional to the observation time in this region.[118] By using the Einstein equation, we found that water molecules in the hydration sphere can travel about 26 μm freely, which is the approximate size of a dispersed aerogel particle. Evidently, when the silica-gelatin aerogel particles are only wet and no bulk water is present, water molecules in the close hydration sphere of the polymer backbone are restricted from moving from one particle to another. This is another piece of evidence for the strong hydration of silica-gelatin aerogel.

In order to investigate the effect of gelatin on the hydration of the hybrid aerogels additional PGSE experiments were carried out on hybrids with higher gelatin content (H10 and H30 containing 11 wt.% and 24 wt.% gelatin, respectively). Above 40 ms observation time, where the apparent diffusion coefficients are independent of the observation time, the

dependence of the self-diffusion coefficients on water content was studied (Fig. 23). For lower gelatin content (3 – 11 wt.%) and lower water content two diffusion domain were detected. As we previously showed, the slower diffusion domain is characteristic for water molecules in the close hydration sphere, while the faster domain belongs to the water molecules moving freely inside the pores. With higher water content the slower domain vanishes, because the amount of the liquid inside the pore becomes more and more dominant. Interestingly, for the gelatin content of 24 wt.% only one slow diffusion domain can be seen at low water content, which indicates, that the gelatin backbone forms a very strong and large hydration sphere. As the amount of water increases D_{obs} tends toward the diffusion coefficient of bulk water (Fig. 23). These results suggest that with increasing gelatin content the hybrid aerogels form denser hydrogel like structures in water which can significantly alter the drug release properties of the matrix.

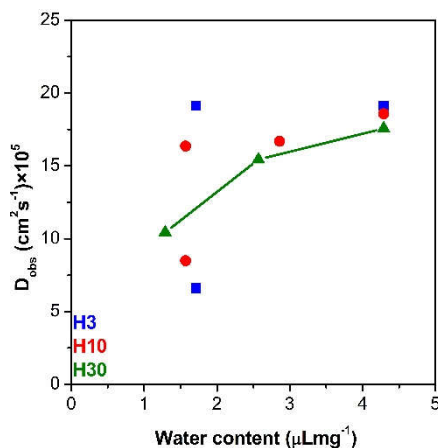


Figure 23. Dependence of the self-diffusion coefficient (D_{obs}) of water on water content in suspensions of silica-gelatin aerogel samples of different gelatin content. Observation times ≥ 40 ms.

Relaxometry

The T_1 and T_2 relaxation times of the dispersing liquids were measured in suspensions of H3 hybrid aerogel made with hexane or with water. Two

pairs of experiments were performed. The relaxation times were measured when the pores of the aerogel were partially filled with either water or hexane, and also when the pores were completely filled and bulk liquid was present in the suspension. The relaxation times together with the standard deviations of the estimated parameters are shown in Table 5.

Table 5. NMR relaxation times of liquids used for dispersing silica-gelatin aerogel with and without of bulk phase present in the suspensions. Two T_2 relaxation processes were observed in each sample. The errors are the standard deviations of the estimated parameters determined in non-linear fitting processes.

Solvent	Saturation of pores with liquid	T_1 (s)	T_{2a} (s)	T_{2b} (s)
water	ca. 70%	1.84 ± 0.02	$(3.2 \pm 0.1) \times 10^{-3}$	$(9.9 \pm 0.3) \times 10^{-3}$
	100%, bulk liquid present	0.91 ± 0.01	$(3.9 \pm 0.8) \times 10^{-3}$	$(1.8 \pm 0.1) \times 10^{-2}$
hexane	ca. 70%	2.97 ± 0.09	$(4.7 \pm 0.2) \times 10^{-3}$	$(2.1 \pm 0.1) \times 10^{-2}$
	100%, bulk liquid present	3.88 ± 0.05	$(1.0 \pm 0.2) \times 10^{-2}$	$(1.2 \pm 0.1) \times 10^{-1}$

In both liquids, two different T_2 relaxation times were observed independently from the amount of liquid used for dispersion. The smaller T_2 can be attributed to solvent molecules in close interaction with the surface of the aerogel (adsorbed), while the higher values can be attributed to the average relaxation of molecules inside the pores and in the bulk. By increasing the amount of the dispersing liquids, the T_2 relaxation times increase, as the relative amount of solvent molecules in close interaction with the surface decrease. According to the cryoporometry experiments the interaction of silica-gelatin is much stronger with water, than with hexane, resulting in faster transverse relaxation (T_2) due to the longer rotation correlation time (c) of water molecules in the hydrated matrix.

While T_2 relaxation time decreases monotonously with increasing rotation correlation time, T_1 relaxation time shows a minimum curve.[119] Water molecules in the strongly hydrated aqueous sample are in a higher rotation correlation time range, where T_1 decreases with c . By increasing

the amount of water in the sample and introducing some bulk liquid, the movement of water molecules become less hindered. The rotation correlation time of water molecules decreases and T_1 decreases, as seen in Table 5. In the case of hexane, the aerogel is not well-solvated and the solvent molecules can rotate faster. Thus, hexane molecules are in a smaller c region where the T_1 relaxation time is inversely proportional with c . This results in increasing T_1 with increasing amount of the dispersing liquid (Table 5).

5.6. Drug release mechanism for hybrid aerogel with 3 wt.% gelatin

The mechanism of release of IBU and KET from loaded silica and silica-gelatin aerogels have not been previously compared. In spite of the obvious similarities of the 2 delivery matrices the mechanism of drug release can be governed by different phenomena in these systems, as discussed in Chapter 3.7. The release of KET from silica aerogel always starts with a rapid (burst) release, in which 20 – 40% of the total loading dissolves in 5 min, while complete release is reached in a subsequent slower step in 50 – 100 min.[23,36,41,44,45] Interestingly, the complete dissolution of both KET and IBU from silica-gelatin aerogel H3 are almost ten-times faster processes, complete in only a few hundred seconds.

In order to gain sufficient kinetic data on the rapid release of IBU and KET from H3 silica-gelatin aerogel, a special, on-line spectrophotometric technique was developed to follow the phenomenon. The background absorbance caused by the light scattering of the aerogel suspension was taken into consideration using the dual-wavelength method (Fig. 24). The basics of this technique were discussed in Chapter 3.6. [85–87,97]

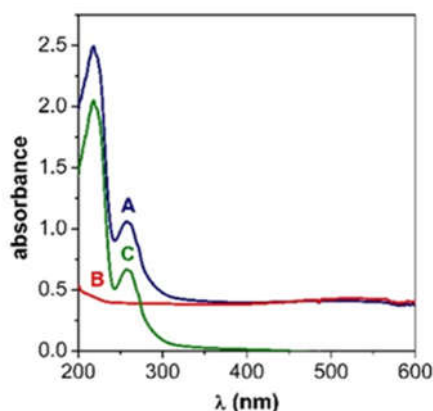


Figure 24. Demonstration of the “dual-wavelength method” in the case of ibuprofen release from H3 hybrid aerogel in pH 2.0. HCl. A) spectrum of well stirred loaded aerogel suspension at the 200th second of the release test, B) reconstructed spectrum of the aerogel suspension (background), C) the difference of spectrum A) and B).

Owing to the 1.0 s time resolution of the method, high quality kinetic curves could be recorded even in the case of the rapid silica-gelatin delivery systems. It is also important to note that in the current study, milled and sieved aerogel particles were used to avoid any size related effects during dissolution. The release profiles measured with loaded H3 hybrid aerogel samples in pH 2.0 HCl and in pH 7.4 PBS media are shown in Fig. 25. These high quality profiles can be analyzed quantitatively, and the intimate mechanism of release can be elucidated with model fitting, and compared to the structural properties of the delivery matrix.

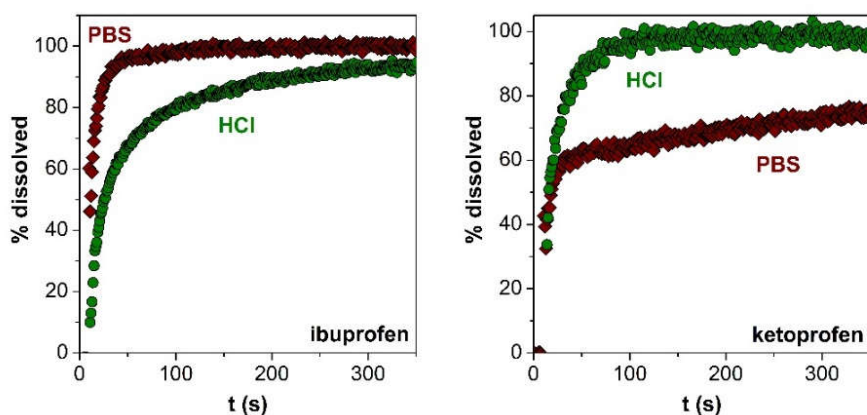


Figure 25. Drug release experiments. Dissolution of ibuprofen (left) and ketoprofen (right) from loaded silica-gelatin aerogel in pH 2.0 HCl (green) and in pH 7.4 PBS (red). Mass of loaded aerogel: 1.50 mg, volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring.

Therefore, the results presented here are considered richer in information and more reliable than those measured by the batch technique, as discussed in Chapter 5.4. The release kinetics was re-evaluated using the new, more comprehensive experimental data set in order to gain a deeper understanding on the mechanism of drug dissolution and on the various factors governing it.

The release of IBU is complete in 100 s in PBS. In one set of parallel experiments the amount of loaded aerogel in the dissolution cell was modified keeping the volume of PBS constant. In these runs, the absolute amount of dissolved IBU increases with increasing amount of loaded aerogel. However, the relative amount of dissolved IBU at a given time, i.e. the release profile, does not change with changing aerogel concentration, as seen in Fig. 26. This observation makes two facts clear: first, the solubility of IBU does not limit its release, and second, the relative rate of dissolution is invariant to both the concentration of loaded aerogel and the concentration of dissolved IBU.

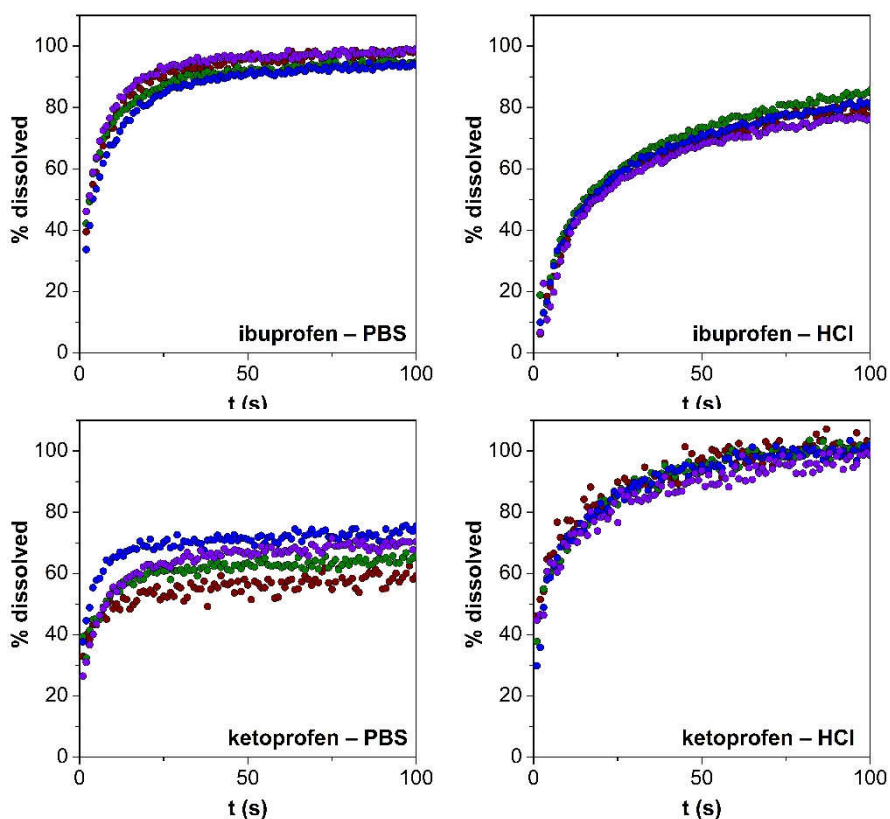


Figure 26. Drug release experiments. The dissolution of ibuprofen (top) and ketoprofen (bottom) from loaded silica-gelatin aerogel in pH 2.0 HCl and in pH 7.4 PBS. The mass of the loaded aerogel changed in the cell from 0.56 mg to 1.72 mg, while the volume of the release medium was constant. Parallel experiments with different masses of aerogel are shown in each panel. Independently from the concentration of the loaded aerogel or the absolute amount of dissolved drugs the corresponding release profiles overlap. Volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring.

Aerogels are considered eroding delivery systems, meaning that the kinetics of release is primarily governed by the rate of matrix erosion and only secondarily by the limited diffusion of drug molecules from the pores.[36,89,112] Nonlinear fitting of the release curves shown in Fig. 25 was performed accordingly. The best results were obtained by using the Hopfenberg model (Eq. 22) valid for spherical ($n = 3$) degrading particles, as shown in Fig. 27. The Hopfenberg model is a semi-empirical model

which can be advantageously used to describe the kinetics of drug release from eroding matrices.[89,120] Additionally, the first 60% of the release curves were fitted to a widely used empirical release model (Peppas, Eq. 23), but none of these models are able describe the release of IBU in PBS from silica-gelatin aerogel, as shown in Fig. 27. Thus, it is evident, that the rate of drug release is controlled by matrix specific physico-chemical processes, rather than general transport processes. Possible factors that might control the release kinetics are the specific interaction of the drug and the carrier, and the erosion, deformation and hydration of the silica-gelatin backbone.

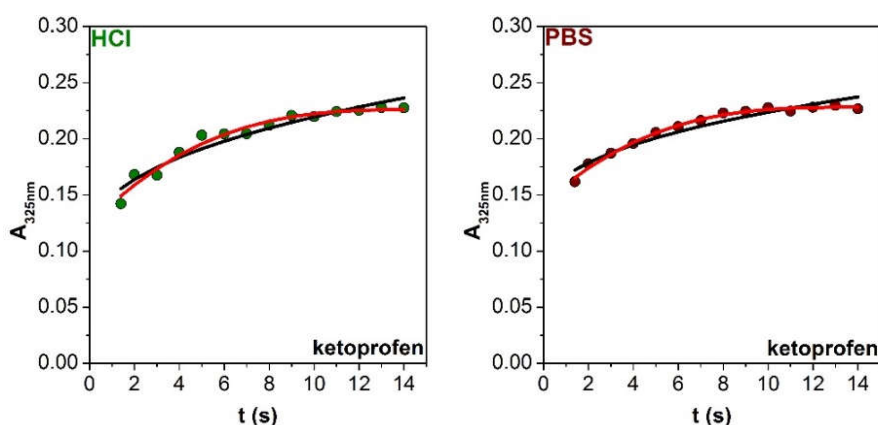


Figure 27. Representative ketoprofen release curves from H3 silica-gelatin hybrid aerogels in pH = 2.0 HCl (left) and pH = 7.4 PBS (right) solutions. The experimental curves (points) were fitted with Peppas (black) and Hopfenberg (red) models.

The kinetics of drug release is affected by the strength (i.e., the energy) of the specific interaction between the drug molecules and the pore walls.[121,122] If the interaction is strong, the effective diffusion of the drug molecules are slowed. In this case, the release kinetics is at least partially controlled by the aforementioned specific interaction.[121,122] In Section 5.3, it was shown that the affinity of IBU is somewhat higher towards the surface of silica-gelatin than towards pure silica. Thus, a stronger surface interaction is expected between IBU and silica-gelatin,

than between IBU and silica. In spite of the stronger interaction, the release rate is much faster from the H3 silica-gelatin matrix. A feasible explanation for this phenomenon is that the release rate is not interaction controlled, and the rate determining processes are probably the degradation, deformation and hydration of the aerogel matrix.

The next logical step to understand the mechanism of drug release was the investigation of the kinetics of the erosion of the silica-gelatin aerogel matrix in water. Unfortunately, as mentioned in Section 5.5.1, we could not use time-resolved laser diffraction to follow the degradation of the dispersed aerogel during drug release. However, we could follow the light scattering of the suspension on-line by UV–Vis spectrophotometry at wavelengths where dissolved IBU does not absorb. As it was shown in Chapter 5.5.1 (Fig. 17), the light scattering of the suspension is practically constant in the timescale of release, meaning that the size distribution of the dispersed aerogel particles does not change. This indicates, that the observed limited erosion of the silica-gelatin particles can control only the initial, burst phase of the release kinetics.[36,41,43,122] Evidently, the later phase of the release kinetics is controlled by other processes, most probably by the deformation and the hydration of the pore structure.

To summarize all the experimental results and theoretical considerations, it seems reasonable that the key factor of forcing the rapid desorption and dissolution of IBU from the silica-gelatin aerogel is the hydration of the matrix. Furthermore, the changing, opening pore structure can also be advantageous in facilitating the rapid release of the model drug. Another important conclusion is that the only major difference between the silica and silica-gelatin delivery systems is the strong hydration of the latter, which property radically changes the rate of drug release.

On the longer time-scale, however, the hydration of the silica-gelatin aerogel matrix is expected to hinder the diffusion of drug molecules, because the effective cross-section of the pores decrease. This scenario can be observed in the case of the release of KET in PBS (Fig. 25). The

release of KET is significantly slower than the release of IBU in PBS. In the case of KET, there is enough time for the silica-gelatin matrix to reach complete hydration and develop a hydrogel like structure, which results in retarded release after 100 s.

Furthermore, the relative rate of drug dissolution is invariant to the IBU concentration in HCl (Fig. 26). The release curves in Fig. 25 were fitted to the Hopfenberg model, and additionally to empirical release models. Fitting was again unsuccessful, indicating that the mechanistic considerations discussed in the case of the PBS medium are valid here as well.

It is interesting to note that IBU is released much faster in PBS, than in HCl. The reason can be the stronger interaction between the protonated drug and the pore wall in HCl. The formation of hydrogen bonds between gelatin and IBU is also possible, leading to the conclusion that the mechanism of drug release can be interaction controlled in HCl.[121,122]

The shape of the release profiles of KET are markedly different in PBS and in HCl (Fig. 25). The initial rate is slightly lower in HCl, but the retarded release characteristic in PBS is not present in HCl. A possible kinetic explanation is as follows. The shape of the release curve is determined by the relative rate of desorption of KET and the formation of the deformed hydrogel structure. When hydrogel formation is much slower than desorption, no retarding effect can be seen. Most probably, hydrogel formation is much faster in PBS than in HCl, thus the relative rate of desorption significantly increases in HCl. This effect can compensate for the decrease of the absolute rate of desorption due to the protonation of KET in HCl.

5.7. Drug release mechanism for hybrid aerogels with high gelatin content

In Section 5.2, it was shown, that silica-gelatin hybrid aerogels containing higher wt.% of gelatin form a hydrogel like structure in water which can

alter the drug release from the matrix. To gain a better understanding on the influence of gelatin content additional drug release experiments were carried out for H10, H20 and H30 hybrid aerogels impregnated with IBU and KET (Fig. 28). No significant deviation was detected in the release behavior of H10 hybrid compared to H3. This indicates, that 11 wt.% gelatin incorporated in the hybrid backbone has the same effect on the release as 3 wt.%, thus the conclusions summarized before can be applied for this matrix as well. However, as Fig. 28 shows 18 wt.% and 24 wt.% gelatin alter the release curves significantly. The initial stage of release becomes slower and the overall shapes of the curves reveal a prolonged release profile. As the diffusimetric and relaxometric measurements already showed, a dense hydrogel structure forms in the case of high gelatin content aerogels during release. The diffusion through this hydrogel is slower compared to an eroding system (like hybrid aerogel H3). To confirm our assumption, the release curves were fitted to the some widely used release models. Unlike for aerogel H3, the Hopfenberg model was not applicable to describe the release process. In contrast, fitting with the Peppas model gave good results for high gelatin content gels (Fig. 29). As it was mentioned in Chapter 3.7, the Peppas model is a versatile empirical model to describe controlled release systems, where the release rate is dependent on the diffusion of the drug molecules through a polymer matrix.

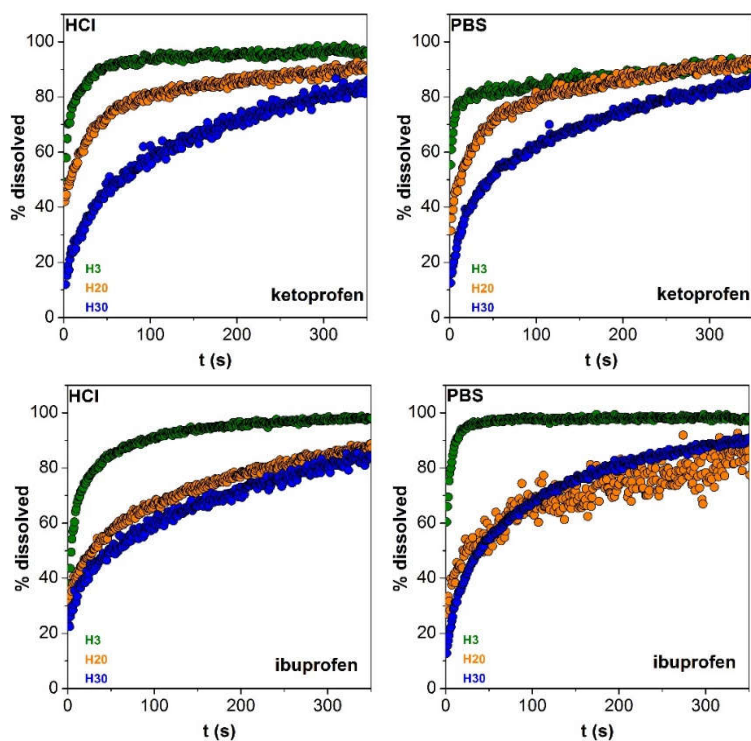


Figure 28. Drug release experiments. The dissolution of ketoprofen (top) and ibuprofen (bottom) from silica-gelatin hybrid aerogels containing 3-24 wt.% gelatin in pH 2.0 HCl and in pH 7.4 PBS. Mass of loaded aerogel: 1.50 mg, volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring.

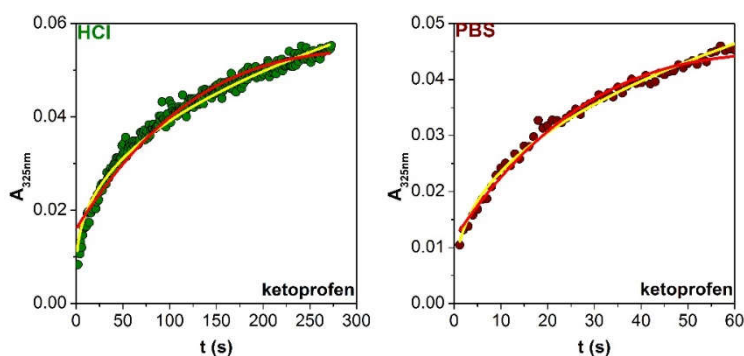


Figure 29. Representative ketoprofen release curves from H30 silica-gelatin hybrid aerogels in pH = 2.0 HCl (left) and pH = 7.4 PBS (right) solutions. Data points were fitted with Peppas (yellow) and Hopfenberg (red) models.

Summarizing the previous results, we can propose the following model for drug release from hybrid silica-gelatin aerogels. Depending on the gelatin content the matrices show two completely distinct release behaviors. For low gelatin content (3 – 11 wt.%), an erosion controlled process is present, where the hydration and the deformation of the matrix both control the drug release. The initial aerogel particles disintegrate to smaller particles due to shear forces and water penetration, and the adsorbed non-crystalline drug gets in contact with the release medium through the open pores. The strong interaction between the water molecules and the pore walls remove the adsorbed drug which leads to its very fast release. This can be slightly altered with the protonation of the drug molecules in acidic media, where the protonated species form stronger H bonds with the polar surface groups. However, the fast release does not change significantly. Water hydrates the gelatin, but in the H3 and H10 gels the gelatin content is too low to give a dense hydrogel like structure, thus the pores remain open and permeable for the drug molecules. On the other hand, higher gelatin content (18 – 24 wt.%) alters the release process by forming a dense hydrogel. In this case the disintegration of the particles also happens, but now the gelatin content is high enough to form a hydrogel in the presence of water. Finally, drug diffusion through this hydrogel structure becomes the controlling parameter, which limits the release rate.

6. Summary

During my research we were able to develop a new synthetic method to produce silica-gelatin hybrid aerogels for the first time. With two modification strategies we introduced three functional groups (phenyl-, hexadecyl- and trimethyl silyl-) on the surface of the hybrid matrices. With the thorough characterization of the hybrid porous materials we determined the composition of the aerogels and also studied the effect of composition and surface modification on their porosity. Impregnating them with three low water soluble drugs (triflusal, ibuprofen and ketoprofen) in supercritical carbon dioxide we proved the feasibility of pharmaceutical application. Both the initial and the functionalized matrices adsorbed high amounts of pharmaceutical agents (up to 29wt.% triflusal, up to 27 wt.% ibuprofen and up to 18 wt.% ketoprofen) in amorphous form. Compared to silica aerogels the hybrid aerogels showed higher specific loadings which is tunable with aerogel composition. The porous matrices were also able to improve the thermal stability of the adsorbed drugs. The preliminary batch release tests confirmed that both enhanced and retarded drug release is achievable with the use of hydrophilic and hydrophobic matrices, respectively.

We developed a new on-line spectrophotometric method with high time resolution to gain dissolution curves richer in information and with better reproducibility. These are feasible to be used for high precision curve analysis. We proved that the technique is capable to register information rich release curves, can be used with extremely low sample sizes (0.3 – 2.0 mg per test) and has good reproducibility.

To gain a better understanding of the 10 times faster release from hybrid aerogels compared to normal silica aerogels, we used NMR diffusimetry, cryoporometry and relaxometry to study the behavior of the mesoporous structure in different liquids. Silica-gelatin aerogels disintegrate to smaller particles than normal silica gels. For low gelatin content, the drug dissolution is governed by the erosion of the matrix and

the hydration of the aerogel backbone, which leads to the removal of the active compound from the pore walls. The latter effect accelerates the dissolution of the drug. Drug release from low gelatin content aerogels can be modeled using the Hopfenberg model, which also verifies the presumed delivery mechanism. NMR diffusometry showed that with increasing gelatin content the hydrogel-like characteristics of the hydrated aerogels become more and more pronounced, which leads to the change of drug release. While drug dissolution from aerogels with 3 – 11 wt.% gelatin is controlled by erosion, hydrogel formation in the case of 18 – 24 wt.% gelatin will cause a hindered drug transport from the carrier towards the bulk phase, resulting in a more retarded drug release.

The results presented in this thesis demonstrate, that with changing the composition and modifying the surface of mesoporous hybrid matrices, it is possible to fine tune the drug adsorption and release of biodegradable drug delivery systems. The gained data and the newly developed investigation methods can be utilized in the future to develop new and advanced controlled release drug delivery systems.

7. Összefoglalás

Doktori munkám során kidolgoztunk egy új szintézisutat különböző zselatintartalmú szilika-zselatin hibrid aerogélek előállítására. Két eltérő funkcionizálási technika felhasználásával három funkciós csoportot (fenil-, hexadecil- és trimetil-szilil-) vittünk fel a gélváz felszínére. Különböző vizsgálati módszerekkel meghatároztuk az aerogélek összetételét és a felületi módosítás hatását a kialakuló aerogélek szerkezetére.

Három, vízben rosszul oldódó hatóanyag (triflusal, ibuprofén, ketoprofén) szuperkritikus szén-dioxidból történő adszorpciójával demonstráltuk, hogy a szilika-zselatin hibrid aerogélek alkalmasak lehetnek gyógyszerhordozóként történő felhasználásra. Mind a normál, mind a funkcionizált hibrid mátrix képes volt nagy mennyiségű hatóanyag felvételére (29 tömeg % triflusal, 27 tömeg % ibuprofén és 18 tömeg % ketoprofén maximális felvett hatóanyag). Zselatin mint társ-vázalkotó alkalmazásával sikeresen növeltük meg a hatóanyag affinitását a vázhoz, amelyet az egységnyi felületre vonatkoztatott felvett hatóanyag mennyisége jelez. A mezopórusos váz emellett képes volt a felvett hatóanyag termikus stabilitásának növelésére. Az előzetes batch kioldódás vizsgálatok bizonyították, hogy hidofil, illetve hidrofób mátrix alkalmazásával rendre azonnali, illetve lassú hatóanyag-eladás érhető el.

A gyors hatóanyag-leadás vizsgálata érdekében kifejlesztettünk egy új, gyors, on-line kioldódás vizsgáló módszert. Bizonyítottuk, hogy a módszer alkalmas kis mintamennyiség esetén is olyan görbék reprodukálható regisztrálására, amelyeken nagyhatékonyságú görbealak analízis végezhető. Ezzel sokkal alkalmasabbak a hatóanyag-leadás mechanizmusának vizsgálatára, mint a konvencionálisan alkalmazott technikák.

NMR krioporozimetriával, diffuziometriával és relaximetriával vizsgáltuk a hibrid gélváz viselkedését különböző folyadékokban, annak érdekében, hogy jobban megértsük, hogy 3 tömeg% zselatin tartalmazó aerogélek

esetén miért jelentkezik rendkívül gyors hatóanyag-leadás. Az eredmények azt mutatták, hogy a szilika-zselatin hibrid vízben kisebb részecskékre esik szét, mint a normál szilika aerogél. Megmutattuk, hogy kis zselatin tartalmú aerogélek esetén a hatóanyag-leadást a mátrix degradációja, valamint hidratációja vezérli. A hibrid gélvázról a víz az erős hidratáció miatt leszorítja az adszorbeálódott hatóanyagot, amely a normál szilika aerogélek esetén tapasztaltnál tízszer gyorsabb leadáshoz vezet.

Az NMR diffuziometriás eredmények arra engednek következtetni, hogy a zselatintartalom növelésével az aerogél váz vízben egyre inkább hidrogélként viselkedik, amely kihatással lehet a hatóanyag-leadásra is. Ezt a jelenséget erősítik meg a kioldódás görbék is. Kis zselatintartalomhoz viszonyítva, ahol a leadást az erózió irányítja, magasabb (18 – 24 tömeg%) zselatintartalom esetén a kioldódást a hatóanyag molekulák hidrogélen keresztül történő gátolt diffúziója irányítja.

A kapott információkkal az eddigieknél mélyebb betekintést nyertünk a felhasználás során lejátszódó folyamatokba, ezzel is segítve a jövőbeni fejlesztéseket.

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