

Theses of Doctoral (PhD) Dissertation

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

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Debrecen, 2018

Glossary, Acronyms and Abbreviations:

TMOS	tetramethoxysilane
C16–TMOS	hexadecyltrimethoxysilane
Ph–TMOS	trimethoxyphenylsilane
HMDS	1,1,1-trimethyl-N-(trimethylsilyl)silanamine
TRF	2-acetyloxy-4-(trifluoromethyl)benzoic acid, triflusal
IBU	(RS)-2-(4-isobutylphenyl)propanoic acid, ibuprofen
KET	2-(3-benzoylphenyl)propanoic acid, ketoprofen
PBS	phosphate-buffered saline: $\text{pH} = 7,4$; $0,14 \text{ mol L}^{-1} \text{ NaCl}$; $2,70 \text{ mmol L}^{-1} \text{ KCl}$; $0,01 \text{ mol L}^{-1} \text{ PO}_4^{3-}$
wt.%	weight percent
v/v%	volume percent
PGSE	pulsed gradient spin echo
sc.	supercritical
SEM	scanning electron microscopy

INTRODUCTION AND RESEARCH OBJECTIVES

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.¹

First, the most thoroughly characterized inorganic aerogels, silica 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 bio-based 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 reason 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.

The main objective of this research was the synthesis and characterization of such hybrid aerogel structures, where the hybrid 3D backbones incorporate both 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 (high surface area, easy

¹ Z. Ulker, C. Erkey *J. Controlled Release*, 177 (2014), 51–63.

manufacturing and low shrinkage) of silica aerogels 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 structural 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.

We also wanted to study the feasibility of the application of these hybrids in drug delivery. 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 carried out using the batch method combined with RP-HPLC.

Another goal was to understand the mechanistic background of the striking difference between the delivery properties of 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 cause of the significant difference in release kinetics from hybrid aerogels with different gelatin content. 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.

EXPERIMENTAL METHODS

Synthesis of silica and silica-gelatin aerogels

Silica aerogel (Sil) was prepared by a conventional sol-gel method catalyzed by ammonium carbonate.² Two solutions, TMOS dissolved in methanol and (NH₄)₂CO₃ 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 wet gel was dried with supercritical (sc.) CO₂ at 14 MPa and 80 °C. Hybrid alcogels (H3 – H30) were prepared by a modified sol-gel procedure using different organic/inorganic precursor ratios. Aqueous gelatin solutions (35 – 40 °C) were used as the sources of the second components of the hybrid gel skeletons. During the gelation a hybrid silica-gelatin matrices were formed. After the formation of the sol, the same protocol was followed as in the case of silica aerogel production.

Impregnation of silica-gelatin hybrid aerogels

The drug loading process (impregnation³) 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 and KET. The autoclave was magnetically stirred at 100 rpm for a period of 6 h, after which the reactor was depressurized and the loaded aerogel was recovered. The depressurization rate was 0.2 – 0.3 MPa min⁻¹ to avoid drug crystallization.

² I. Lázár, I. Fábián, *Gels*. 2 (2016) 26.

³ 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.

Aerogel characterization

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. Scanning electron microscopic (SEM) images were recorded on a Hitachi S-4300 instrument (Hitachi Ltd., Tokyo, Japan). The specific surface area, the pore size distribution and the pore volume of the pristine and the impregnated aerogels were measured by low-temperature N₂ adsorption-desorption porosimetry in 2 different instruments (Quantachrome Nova 2000e and ASAP 2000 Micromeritics Inc.). 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 crystallinity of the loaded drugs was analyzed by X-ray powder diffraction (XRD) using a Rigaku Rotaflex RU-200 B spectrometer and by differential scanning calorimetry (DSC) with a Mettler Toledo 822e/400 instrument.

Determination of aerogel loadings

The amount of drug loaded into a given aerogel was determined after soaking the loaded sample (~5 – 10 mg) in 10 mL of methanol by RP-HPLC. The liquid chromatograph 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.

Release experiments

HPLC was also used to monitor 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 μ L solution was recovered, and 10 μ L was injected into the column. All experiments were performed in triplicates that allowed the establishment of variability ranges.

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 a uniform size (<125 μ 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 ± 0.1 °C.⁴ 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 2×8 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. 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.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 J. Kalmár et. al. *RSC Adv.*, 5 (2015), 107237–107246.

⁵ H. Liu, J.Y. Zhu, X.S. Chai *Langmuir*, 27 (2011), 272-278.; Q.Q. Wang, J.Y. Zhu, C.G. Hunt, H.Y. Zhan *Biotechnol. Bioeng.*, 109 (2012), 1965-1975.

For NMR diffusometry, a method developed by Cohen et al. was used.⁶ The self-diffusion of the probe 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 (BILED) 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} .

$$I = I_0 \exp \left\{ -D_{\text{obs}} \gamma^2 \left(\Delta - \frac{\delta}{3} \right) \delta^2 G^2 \right\} \quad (1)$$

Coefficient $D_{\text{obs}} \gamma^2$ 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.

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.⁷ 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.

$$\Delta T_m = T_m - T_0 = -\frac{n_m K_c}{r_p} \quad (2a)$$

$$\Delta T_f = T_f - T_0 = -\frac{n_f K_c}{r_p} \quad (2b)$$

In Eq. 2a, 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 cryoporometric constant, n_m is the geometric factor describing melting and r_p stands for the average pore radius. In Eq. 2b, the symbols denote the corresponding parameters for freezing.

⁶ Y. Cohen, L. Avram, L. Frish *Angew. Chem. Int. Ed.*, 44 (2005), 520–554.

⁷ O.V. Petrov, I. Furó *Prog. Nucl. Magn. Reson. Spectrosc.*, 54 (2009), 97–122.

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.

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 $-15\text{ }^{\circ}\text{C}$ 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. 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 $10.2\text{ }\mu\text{s}$. Temperature ranges of $-14 - 4\text{ }^{\circ}\text{C}$ and $-14 - 11\text{ }^{\circ}\text{C}$ were studied for water and cyclohexane, respectively. The samples were melted by elevating the temperature to $4\text{ }^{\circ}\text{C}$ or to $11\text{ }^{\circ}\text{C}$ in $0.2\text{ }^{\circ}\text{C}$ steps by using an automated program. From $4\text{ }^{\circ}\text{C}$ (or 11°C), the samples were cooled to $-14\text{ }^{\circ}\text{C}$ in $0.2\text{ }^{\circ}\text{C}$ 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.

NEW SCIENTIFIC RESULTS

1. Silica-gelatin hybrid aerogels were synthesized, functionalized and characterized for the first time.

1.1 A sol-gel method was developed for the synthesis of silica-gelatin hybrid aerogel monoliths.

With the use of aqueous gelatin solution as the source of the second component of the hybrid gel structure, a sol forms in which the gelation of silane and gelatin takes place simultaneously. Homogeneous hybrid gels can be produced by this method. Furthermore, the later stages of the routine synthetic route used for aerogel production are not needed to be modified.

SEM images of silica and H3 silica-gelatin aerogels in 25k $\times$ magnification are shown in Fig. 1. 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.

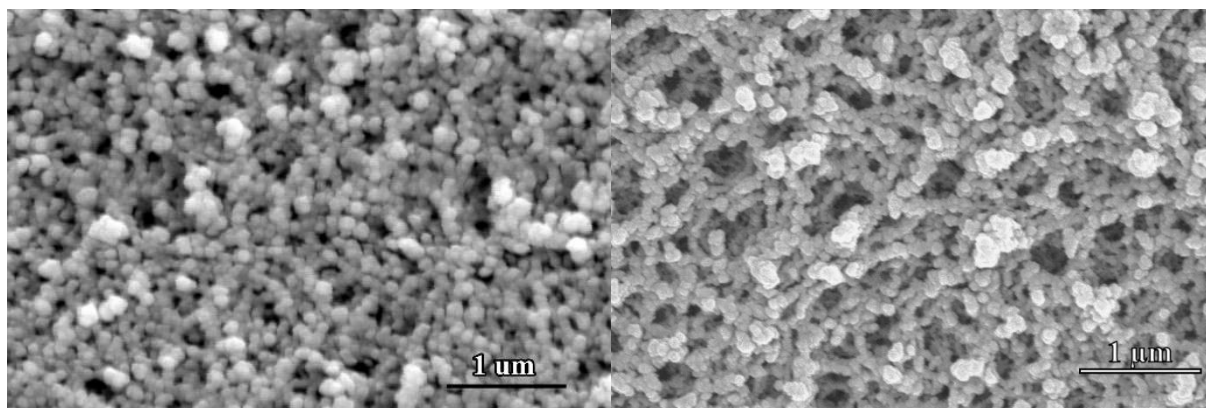
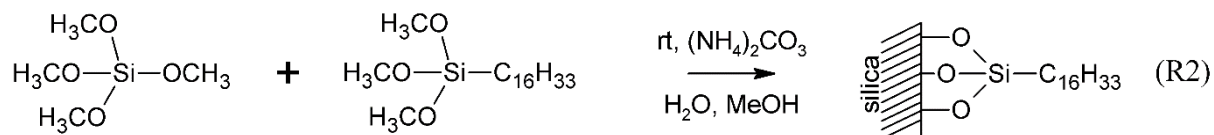
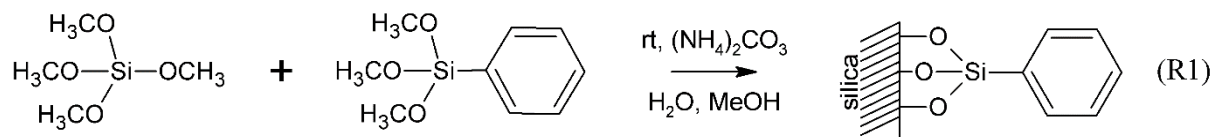


Figure 1. SEM images of silica aerogel (Sil, left) and silica-gelatin hybrid aerogel (H3, right)

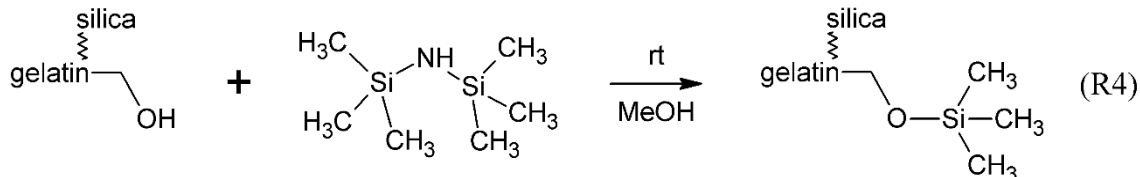
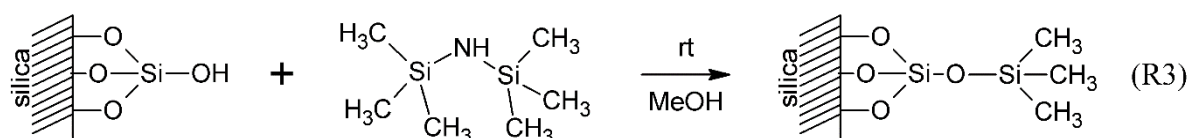
1.2 Two different functionalization approaches were used to produce aerogels with different, hydrophobic surface groups.

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). A certain amount of TMOS was replaced with the functionalizing agents to produce a sol with different silane ratios. 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.



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. Following this procedure, hybrid aerogels with different silica-gelatin ratios were derivatized by adding HMDS in excess. Silylation took place following reaction equations R3 and R4.



1.3 The composition of the hybrid aerogels were determined. We showed, that the hybrid aerogels have open mesoporous structure with high specific surface area. It was revealed that the composition has a significant influence on the structural and surface properties of the aerogels.

The mass of gelatin incorporated into the hybrid aerogels was estimated by TGA from the weight loss in the range 300 – 500 °C. The gelatin content of the samples varies between 3.6 and 24 wt.% for H3 – H30 aerogels, respectively (Table 1). As the result of surface functionalization the hybrid structures contain 4.2 – 9.2; 2.1 – 5.8 and 5 – 6.5 wt.% of phenyl-, hexadecyl- and trimethylsilyl moieties, respectively. The aerogels have high specific surface area (a_s), which changes with the composition (shown in Table 1.). Due to the surface modifications, the C constants of the samples decrease, which indicates a change in hydrophilicity.

Table 1. Composition and textural properties of hydrophilic (Sil, H3, H10, H20, H30) and surface modified aerogel samples. (* tentatively assigned)

Sample	Gelatin (wt. %)	(CH ₃) ₃ (wt. %)	Ph (wt. %)	C ₁₆ (wt. %)	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	-	-	636	21
H10_sil	11 *	6.0*	-	-	596	23
H20_sil	19	5.0	-	-	509	27
H30_sil	24	6.5	-	-	504	24
H3_Ph10	5.0	-	4.2	-	860	54
H3_Ph20	3.8	-	7.6	-	724	56
H3_Ph30	4.0	-	9.2	-	790	56
H3_C ₁₆ 10	4.0*	-	-	2.1	638	86
H3_C ₁₆ 20	4.0*	-	-	4.0	736	75
H3_C ₁₆ 30	4.0*	-	-	5.8	711	73

2. We proved, that the aerogels in Section 1. are able to adsorb different low water solubility drugs from supercritical CO₂, and their release can be tuned by altering the surface properties of the solid matrices.

2.1 Hybrid aerogels were impregnated with three low water solubility drugs (triflusal, ketoprofen, ibuprofen).

We showed that both normal and surface functionalized hybrid aerogels can adsorb a large amount of drug (18 – 29 wt.% TRF, 14 – 24 wt.% IBU and 7 – 18 wt.% KET, Table 2) from supercritical medium. The comparison of the infrared (IR) spectra of the pure drugs, the unloaded and the loaded hybrid aerogels clearly show the successful impregnation of the matrices.

2.2 X-ray diffraction (XRD) and differential scanning calorimetry (DSC) showed, that the adsorbed drugs are amorphous in the matrices.

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. 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. The pure drugs melt in the 80 – 120 °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 a molecular level, rather than present in a crystalline form. The pristine hybrid aerogels are highly hydrophilic, having a high number of surface –OH and –NH₂ 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 above

2.3 The composition and the surface properties of the hybrid aerogels have a significant effect on the loadings.

The area specific loadings, that reflect the affinity of the drug molecules to the surface, increase with increasing gelatin content (Table 2). Gelatin has numerous –OH and –NH₂ groups which can serve as potential hydrogen bonding sites, and thus can facilitate the adsorption of 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 C₁₆ modification reduces the adsorbed amount of TRF.

Table 2. Drug loading values of hybrid silica-gelatin aerogels. (* tentatively assigned)

Sample	Gelatin (wt.%)	(CH ₃) ₃ (wt.%)	Triflusal (wt.%)	Ibuprofen (wt.%)	Ketoprofen (wt.%)	Triflusal (μmol m ⁻²)	Ibuprofen (μmol m ⁻²)	Ketoprofen (μmol 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

2.4 It was proven that the hydrophobicity of the aerogel matrix alters the dissolution of the drugs, thus both immediate and sustained release can be realized.

For all the studied drugs, drug release is very fast from hydrophilic aerogels. The aerogel structure disintegrates due to the fast wetting and the drug can make contact with the dissolution medium via a large surface. This results in a very fast release where the majority of the adsorbed drug dissolves in the first 10 – 20 minutes. The wetting of the hydrophobic matrix is very slow, which leads to sustained drug dissolution (Figure 2).

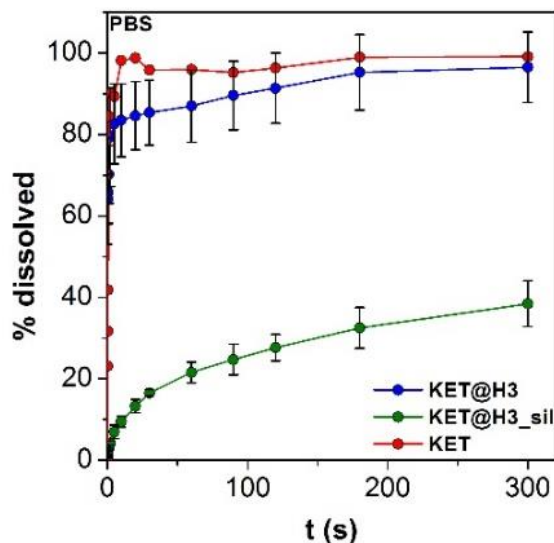


Figure 2. Ketoprofen release from microcrystalline drug (red), drug@H3 (blue) and drug@H3_sil (green) systems at pH=7.4 PBS; $37,0 \pm 0,5$ °C; 60 rpm.

3. Based on release experiments and NMR diffusimetry and cryoporometry measurements, we proposed a mechanism for drug release from hybrid aerogels with 3.7 wt.% gelatin content.

3.1 A fast, on-line dissolution testing method was developed.

We proved, that the method is capable of recording release curves from 0.30 – 1.90 mg of hydrophilic hybrid aerogels with good reproducibility. The 1 s time resolution makes high precision curve analysis possible.

3.2 The behavior of hybrid aerogel with 3.7 wt.% gelatin content was characterized in different solvents with laser diffraction, NMR cryoporometry and NMR diffusimetry.

Using laser diffraction particle sizing we showed that the silica-gelatin hybrid aerogel disintegrates to smaller particles ($d = 10 - 30 \mu\text{m}$) than normal silica aerogels. The particle size distribution of the resulted suspension is also narrower. NMR cryoporometry showed that

the pore structure of silica-gelatin aerogel is not deformed significantly when the material is dispersed in cyclohexane. On the other hand, when dispersed in water, the hybrid aerogel backbone deforms and gives a hydrogel like structures. NMR relaxometry showed the presence of a water layer which interacts strongly with the pore walls. In spite of the deformation and the hydration of the backbone, the porous structure remains permeable for water molecules.

3.3 We proposed a drug release mechanism based on the release profiles and the structural changes during wetting.

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. 3 right). 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. IBU is released much faster in PBS, than in HCl (Fig. 3 left). 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. The shape of the release profiles of KET are markedly different in PBS and in HCl (Fig. 3 right). 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.

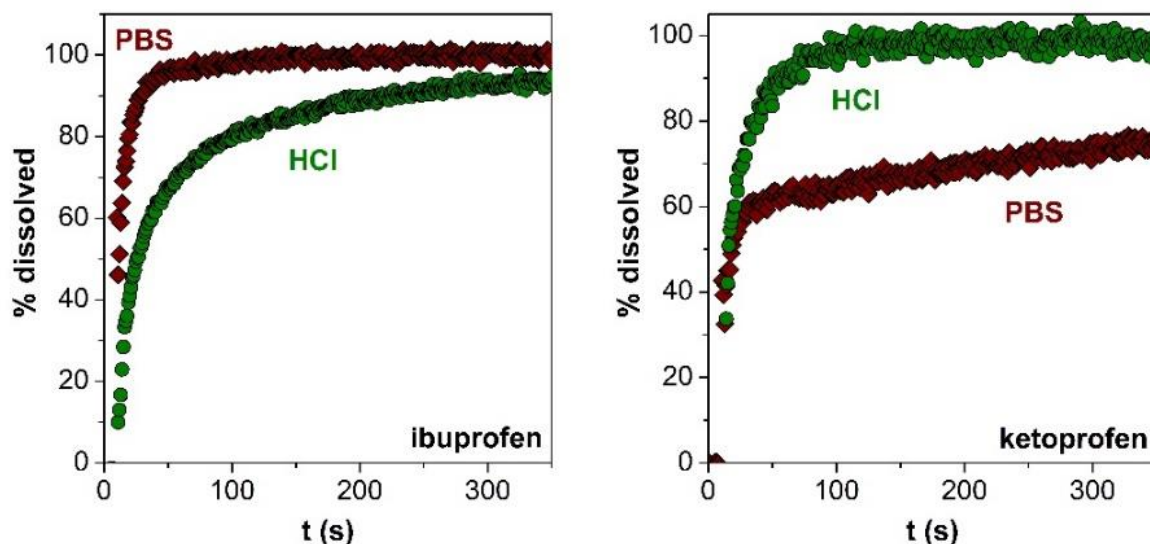


Figure 3. Drug release experiments. Dissolution of ibuprofen (left) and ketoprofen (right) from loaded silica-gelatin aerogel (@H3) in pH 2.0 HCl (green) and in pH 7.4 PBS (red). Mass of loaded aerogel: 1.50 mg, volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring.

4. We proved that increasing gelatin content slows down drug release due to the restricted diffusion of the drug molecules through the hydrogel structure.

4.1 Using NMR diffusimetry and cryoporometry we showed that the amount of incorporated gelatin has a significant effect on the hydration of the aerogels.

The dependence of the self-diffusion coefficients on water content was studied above 40 ms observation time, where the apparent diffusion coefficients are independent of the observation time (Fig. 4). Two diffusion domains were detected for lower gelatin content (3–11 wt.%) and lower water content. As we previously showed, the slower diffusion domain is characteristic for water molecules in the close hydration sphere, while the faster domain belongs to 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. 4). These results suggest that with increasing gelatin content the hybrid aerogels form denser hydrogel like structures in water, that can significantly alter the drug release properties of the matrix.

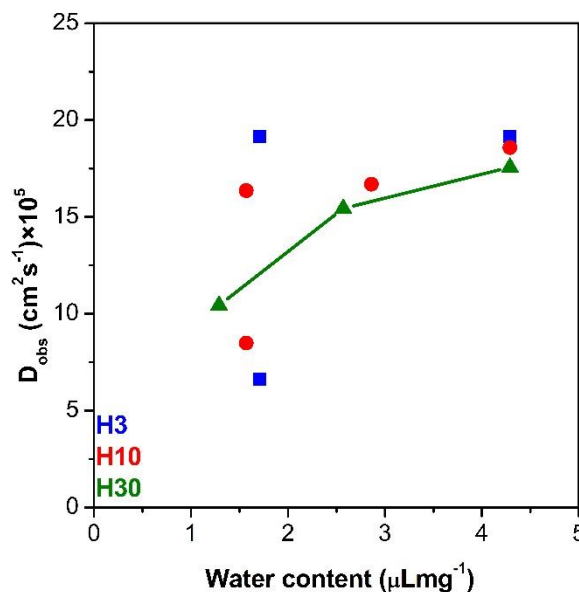


Figure 4. Dependence of the self-diffusion coefficient (D_{obs}) of water on water content in suspensions of silica-gelatin aerogel samples of different gelatin content. Observation times ≥ 40 ms.

4.2 We demonstrated that the rate of drug release from aerogels with higher gelatin content is limited by the restricted diffusion of the drug molecules from the matrix towards the bulk liquid.

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 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 takes place, but now the gelatin content is high enough to

form a dense hydrogel in the presence of water. Finally, drug diffusion through this hydrogel structure becomes the controlling parameter, which limits the release rate.

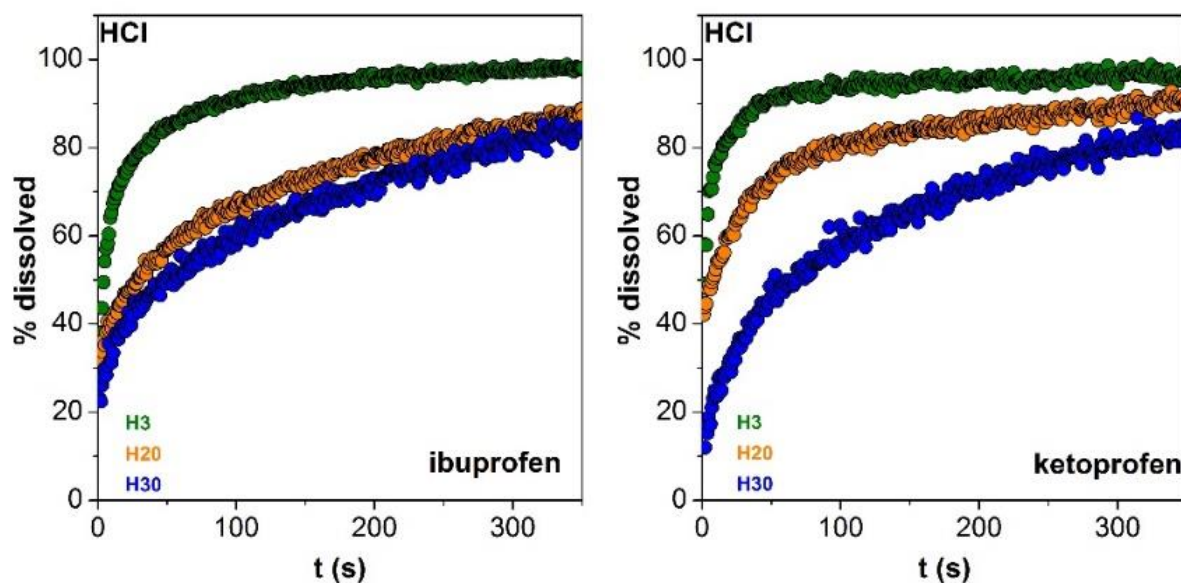


Figure 5. Drug release experiments. The dissolution of ibuprofen (left) and ketoprofen (right) from silica-gelatin hybrid aerogels containing 3-24 wt.% gelatin in pH 2.0 HCl and in pH 7.4 PBS. Mass of loaded aerogel: 1.50 mg, volume of release medium: 3.0 mL, $T = 37.0\text{ }^{\circ}\text{C}$, 300 rpm stirring. pH = 2,0 HCl; $37,0 \pm 0,1\text{ }^{\circ}\text{C}$; 300 rpm.

POSSIBLE APPLICATIONS OF THE RESULTS

Aerogels are excellent candidates for drug delivery systems due to their huge specific surface area and easily tunable composition and surface characteristics. Utilizing their unique features safer and more effective drug administration is achievable. However this research field is still in its early years, thus in the light of the new results more and more possibilities and challenges emerge. Whilst in the early 2000's inorganic aerogels were studied, nowadays the attention of the scientific community is directed towards biodegradable and hybrid materials. A lot of energy is invested into the synthesis and deep characterization of new mesoporous matrices.

During my doctoral research we were able to develop a new synthetic route to produce silica-gelatin hybrid aerogels that combine the advantageous properties of inorganic and organic components. This provides a great potential in the application of controlled release drug delivery systems. The newly developed on-line release testing technique is able to record release curves with 1 s time resolution and high reproducibility. Using the dissolution curves provided by this method high precision curve analysis is applicable, thus the mechanism of drug dissolution can be determined. The technique can be applied for other mesoporous matrices as well. Based on the results, we gained deeper insights into the processes occurring during drug release.

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Mechanism of drug release from silica-gelatin aerogel - Relationship between matrix structure and release kinetics

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PhD Publikációs Lista

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MTMT ID: 10051016

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Veres, P.**, Kéri, M., Bányai, I., Lázár, I., Fábián, I., Domingo, C., Kalmár, J.: Mechanism of drug release from silica-gelatin aerogel-relationship between matrix structure and release kinetics. *Colloid Surf. B-Biointerfaces*. 152, 229-237, 2017. ISSN: 0927-7765.
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List of other publications

Hungarian scientific articles in Hungarian journals (1)

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DOI: <http://dx.doi.org/10.1016/j.jnoncrysol.2017.07.016>
IF: 2.124 (2016)

Total IF of journals (all publications): 14,816

Total IF of journals (publications related to the dissertation): 7,881

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

16 February, 2018

