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# Hydrophobic ceramic capillary membranes for versatile virus filtration

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A B S T R A C T

In this study, we present hydrophobic yttria-stabilized zirconia capillary membranes conditioned for virus filtration. These macroporous ceramic filters ($d_{50}$ = 150 nm) efficiently extract viruses regardless of their surface charge with high throughput rates. For hydrophobic functionalization of the ceramic membranes we used two different silanes, n-hexyltriethoxysilane (HTS, C6-chain) and n-octyltriethoxysilane (OTS, C8-chain), in three different molarities. The virus retention of the membranes is tested in dead-end mode by intracapillary virus feeding using two small bacteriophages as model species: MS2 and PhiX174. Virus retention increases most strongly for hydrophobic capillaries functionalized with 0.05 M OTS, showing a virtually complete retention with log-reduction values (LRVs) of ~ 9 for both bacteriophages compared to the non-functionalized membrane with LRVs of 0.3 ± 0.1 for MS2 and 3.4 ± 0.2 for PhiX174. The functionalized membranes allow a high membrane flux of ~ 150 L/($m^2$hbar), with throughput rates up to ~ 400 L/($m^2$ h) while maintaining high filtration efficiency. Even under varying feed conditions using only mono- or divalent salt ions or pH values ranging from 3 to 9, retention capacities of the capillary membranes are high. Accordingly, such hydrophobic ceramic membranes offer a versatile alternative to conventional polymeric membranes for virus removal with greatly improved membrane flux.

## 1. Introduction

Designing versatile and highly effective filtration systems for a wide variety of viruses with both high membrane throughput rates and high retention capacities is very challenging. Due to the small size of virus particles ranging from 20 to 500 nm [1], decontamination via size exclusion is impeded by low water permeate fluxes as a result of the required mesopores [2–4]. Conventional filtration systems adsorb viruses at the membrane surface via electrostatic interactions, but this only works for viruses that are charged oppositely to the membrane surface. Since surface charge varies strongly between different types of viruses, it is crucial to control the interactions of pathogens independently of their electrostatic charge in respect to the material surface.

Tailoring virus-surface interactions has wide-ranging technological applications in purification processes of water [5,6] and food products [7], where the membranes have to fulfill a log reduction value (LRV) of 4, which is given by the World Health Organization (WHO) as a standard for safe and clean water [8,9]. Furthermore, virus filtration plays a major role in biopharmacy [10], where it is mainly used for virus clearance of plasma products and monoclonal antibodies and purification of viral vectors and vaccines [11–13]. Another application for virus filtration systems is the detection and quantification of small virus concentrations [14].

Today, conventional membranes for virus filtration are mainly based on polymeric materials and rely on the size exclusion principle [15,16]. Conventional polymeric filter systems only allow moderate fluxes due to limited pressure tolerances, which could be improved by using ceramic membranes with their characteristic high mechanical strength which allows high pressure loads. Compared to polymeric membranes, ceramic membranes furthermore do not show any swelling behavior during filtration and can be cleaned by back-flushing without affecting the porous structure [17].

Due to the structural diversity of viruses, virus-material interactions are complex and difficult to predict [18]. In the absence of a direct size exclusion effect, electrostatic interactions were demonstrated to play the dominant role in virus-material interactions [12]. E.g. at the pH of ~ 6, many viruses are negatively charged and readily adsorb to a positively charged membrane surface (isoelectric point (IEP) > 9) [19–22]. In our own recent study, we demonstrated the functionalization of ceramic capillary membranes with n-(3-trimethoxysilylpropyl) diethylenetriamine (TPDA, an aminosilane with three amino groups per silane, IEP > 9). Here, the log-reduction value (LRV) could be increased by a factor of ~9 to 9.6 ± 0.3 for the bacteriophage MS2 at a pH of 5.8 [19]. In contrast, no MS2 adsorption was observed for capillaries functionalized with 3-(trihydroxysilyl)-1-propanesulfonic acid (HSPSA), which exhibit the same surface charge as the virus capsid [19]. Electrostatic double layer forces between the virus particle and the membrane surface, as described by the DLVO theory [23–28], are well-known to be central for the electrostatic adsorption and can be influenced by pH or salt concentration. Lukasik et al.[29] demonstrated

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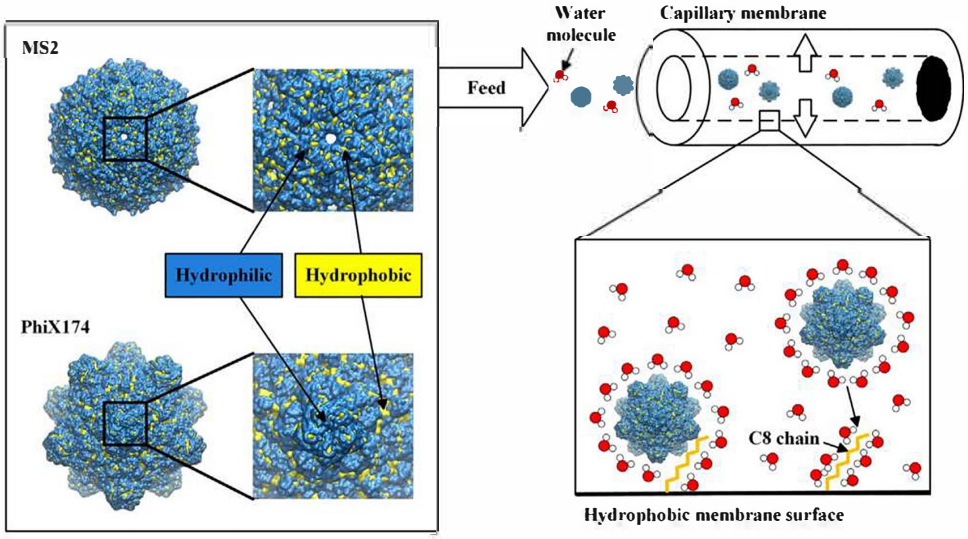


**Fig. 1.** Illustration of the principle of dead-end filtration with hydrophobic ceramic capillary membranes.

that the effects of salt ions further depend on the filter type and the filtration conditions, as $MgCl_2$ promoted, inhibited or had no effect on virus adsorption under different circumstances. In our own work, we showed that the LRV for TPDA functionalized capillary membranes is higher in the presence of divalent salt ($MgCl_2$) when compared to monovalent salt (NaCl), as the electrostatic shielding between surface and amino groups by the adsorbed $Mg^{2+}$ ions leads to an upright orientation of the TPDA molecules and thus to a better accessibility during filtration [30].

Beyond the classic DLVO forces, hydrophobic interactions can significantly influence the adsorption of viruses to membranes. In water, hydrophobic surfaces have a preference to associate with each other, which is caused by the high free energy of the interfacial solute layer of polar water molecules which decreases with a reduction of the water-exposed surface area [31]. This effect is shown in Fig. 1. Various studies reported hydrophobic interactions during virus filtration especially with regard to the removal of viruses by soil passage [18,32]. The significance of hydrophobic interactions for the retention of the bacteriophage MS2 were shown for hydrophobic polymer membranes which were found to be a better barrier for MS2 than hydrophilic polymer membranes [33–35]. Even for relatively hydrophilic viruses the hydrophobic effect is important for adsorption as shown by Bales et al. [36]. Additionally, some salts have a positive effect on virus removal by increasing the ordering of water molecules and the promotion of the sequestering of the hydrophobic entities [23].

The aim of this work is to design a filtration system to remove various kinds of viruses independently of their IEP and their surface charge from liquids based on ceramic capillary membranes. Modern ceramic materials offer a platform of ultra-hard substrates with defined pore size, porosity and tailored surface chemistry, charge and wetting properties which can be engineered by chemical functionalization. In this respect, silanization, the well-known hydrolysis-condensation reaction with silanes, is a versatile and well-established tool [37], as the reaction can be performed in aqueous media [38,39], organic solvents [40–43] or in the vapor phase [44,45] with silanes consisting of different functional groups (e.g. alkyl- [46,47], amino- [48,49] or sulphonate-groups [39]) and spacer lengths [37]. In our previous work with ceramic capillary membranes, we investigated the retention behavior of the bacteriophage MS2 based on electrostatic interaction, while being very efficient, this filtration system is only working for specific viruses which have IEPs lower than the pH of the feed solution [19].

Here, we hydrophobized our well established yttria-stabilized zirconia (YSZ) capillary membranes by silanization with 0.2 M, 0.05 M or 0.0125 M n-hexyltriethoxysilane (HTS) or n-octyltriethoxysilane (OTS). The capillaries are characterized by microstructure analysis with focus on the pore size, the open porosity and the specific surface area and mechanical stability. The functionalization of the filters is investigated by water contact angle measurements, thermogravimetric analysis (TGA) and the long-term stability of the functionalization is evaluated for a period of three weeks. Virus filtration is carried out with the two model bacteriophages MS2 (IEP = 3.5) and PhiX174 (IEP = 6.2) which are selected for their strong differences in surface charge. Both virus species exhibit a fraction of hydrophobic moieties on the virus surface of around 10% [50]. Virus retention capacities are determined by filtration experiments in dead-end mode under varying conditions, e.g. with filtration pressures ranging from 0.5 to 2.5 bar and at a pH range from 3 to 12. With this experimental design we demonstrate the performance of the hydrophobic ceramic membrane at throughput rates up to 400 L/($m^2$ h bar) and with different surface charges of the virus particles.

## 2. Materials and methods

### 2.1. Materials

We used the yttria stabilized zirconia powder TZ-3YS-E (YSZ-90 nm, Lot. S305635P) from Krahn Chemie GmbH, Germany for the fabrication of the capillaries. The following reagents were purchased from Sigma-Aldrich Chemie GmbH, Germany: 3-aminopropyltriethoxysilane (APTES, 99%, product number 440140, Lot. SHBD4935V), hydrogen peroxide solution (purum p.a. ≥ 35%, product number 95299, Lot. BCBH5638V), magnesium chloride hexahydrate ($MgCl_2$, product number M2670, Lot. BCBJ4439V), polyvinyl alcohol (PVA, fully hydrolyzed, product number P1763, Lot. SLBC9027V), sodium chloride (NaCl, product number S7653, Lot. SZBF0350V), sulfuric acid (95–97%, product number 30743, Lot. SZBF0330V), tryptic soy agar (TSA, product number 22091, Lot. BCBR8554V), culture media tryptic soy broth (TSB, product number T8907, Lot. SLBL1497V), N-(3-trimethoxysilylpropyl)diethylenetriamine (TPDA, product number 413348, Lot. MKBW1074V), hydrochloric acid solution (HCl, product number 35328, Lot. SZBD2670V) and sodium hydroxide (NaOH, product number 71692, Lot. SZB82950). N-hexyltriethoxysilane (HTS, 95%, product number AB174350, Lot. 1365286), n-octyltriethoxysilane (OTS, 97%, product number AB110649, Lot. 1350285) and 3-(trihydroxysilyl)-1-propanesulfonic acid (HSPSA, 30–35%, product number AB130830, Lot. 1246512) were obtained from abcr GmbH & Co. KG, Germany.

The bacteriophage MS2 (DSM Cat. No. 13767) and its host bacteria *E. coli* (DSM Cat. No. 5210) as well as the bacteriophage PhiX174 (DSM Cat. No. 4497) and its host bacteria *E. coli* (DSM Cat. No. 13127) were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ), Germany.

Double-deionized water with an electrical resistance of 18.2 MΩ, which was obtained from a Synergy® apparatus (Millipore, Germany), was used for all experiments.

### 2.2. Fabrication of hydrophobic YSZ capillary membranes

The tubular YSZ membranes were prepared by extrusion as described in detail in our previous studies [2,19]. The ceramic slurry consists of 132 g YSZ powder (YSZ-90 nm) as ceramic material, 4 g APTES as dispersant, 20 g PVA-water solution (25 wt%) as binder and 13.5 g double-deionized water. The ingredients were milled in a planetary ball mill (PM400, Retsch), using 50 alumina grinding balls with a diameter of 10 mm, for 3 h at 350 rpm while changing the rotation direction every 5 min. Afterwards, the slurry was shaped with a self-made laboratory extruder which was equipped with a nozzle of 2.0 mm in diameter and a pin of 1.0 mm in diameter with an extrusion speed of 50 cm/min. After drying for at least two days at room temperature (RT) (20 °C, relative humidity ~ 50%) the capillaries were sintered for 2 h at 1050 °C with dwell times at 280 °C (0.5 h) and 500 °C (1 h) (HT40/17, Nabertherm, Lilienthal, Germany).

To generate hydroxyl groups on the surfaces of the capillaries, the sintered YSZ capillary membranes were incubated in Piranha solution (97% $H_2SO_4$: 35% $H_2O_2$, 3:1 (v/v)) for 30 min at RT (Fig. 2A). As shown in our previous work, the membrane microstructure is not affected by an activation with Piranha solution [19]. Afterwards, the capillaries were washed with water until the effluents achieved a neutral pH and dried at 70 °C for 16 h (UT6120, Heraeus, Hanau, Germany). For hydrophobization, five activated capillaries with an individual length of 60 mm were incubated in 10 mL silane solution at 65 °C under slight shaking at 150 rpm (Inkubator 1000/Unimax 1010, Heidolph, Schwabach, Germany) for 24 h (Fig. 2B). The silane solutions based on HTS or OTS were prepared by dilution with water (pH not adjusted) in three different molarities (0.2 M, 0.05 M and 0.0125 M). After functionalization, the hydrophobic capillaries (Fig. 2C, D) were washed until the effluents had a neutral pH and dried at 70 °C for 16 h (UT6120, Heraeus, Hanau, Germany). The activated capillary membranes were silanized in the same way with 0.2 M HSPSA or 0.2 M TPDA to serve as references.

### 2.3. Characterization of silanized membranes

#### 2.3.1. Capillary properties

The determination of structural and mechanical properties of the silanized membranes was performed as described in detail in our previous studies [2,19]. In short, Hg-porosimetry (Mercury Porosimeter Pascal 140 and 440, POROTEC GmbH) was used to obtain the pore size distribution, the average pore size ($d_{50}$) and the open porosity, the specific surface area was measured by nitrogen adsorption according to BET method (Gemini, Micromeritics) after degassing the capillaries at 120 °C for at least 3 h with argon and 3-point bending tests were used according to DIN EN 843-1 (Roell Z005, Zwick) to determine the flexural strength and the Weibull modulus. The microstructure of the outer capillary surface was analyzed by scanning electron microscopy (SEM, Field-emission SEM SUPRA 40, Zeiss). The zeta-potential of the capillary membranes was measured in the pH range between 3 and 9 with a SurPASS (Anton Paar) apparatus which is based on the streaming potential method. Water contact angle measurements were performed with filter platelets (diameter = 12 mm) which were produced with a hydraulic press. The YSZ-90 nm powder was pressed at 10 kN for 30 s and sintered in the same way as the capillaries to have similar structural properties. The water contact angle (Dataphysics, OCA25) was measured with a needle size of 0.5 mm and a drop volume of 0.25 µL.

Thermogravimetric analysis (TGA; STA503, Bähr-Thermoanalyse GmbH, Hüllhorst, Germany) was performed with the capillary membranes in a temperature range between 40 °C and 900 °C with a heating rate of 10 K/min under 2 L/h flowing air.

#### 2.3.2. Membrane flux measurements

Membrane flux measurements of the tubular filters were performed by intracapillary water feeding for 30 min with a peristaltic pump (BVP Standard, Ismatec) in dead-end mode where one end of the capillary was sealed with silicone (Wirosil Dublier-Silikon, Bego Medical GmbH, Bremen, Germany) using pressures between 250 and 1000 mbar (C9500, COMARK). For each capillary type, three individual capillaries in vertical orientation were tested and the membrane flux for 1 bar was calculated by linear regression in L/($m^2$hbar).

#### 2.3.3. Virus retention test

The model bacteriophages MS2 and PhiX174 are not pathogenic for

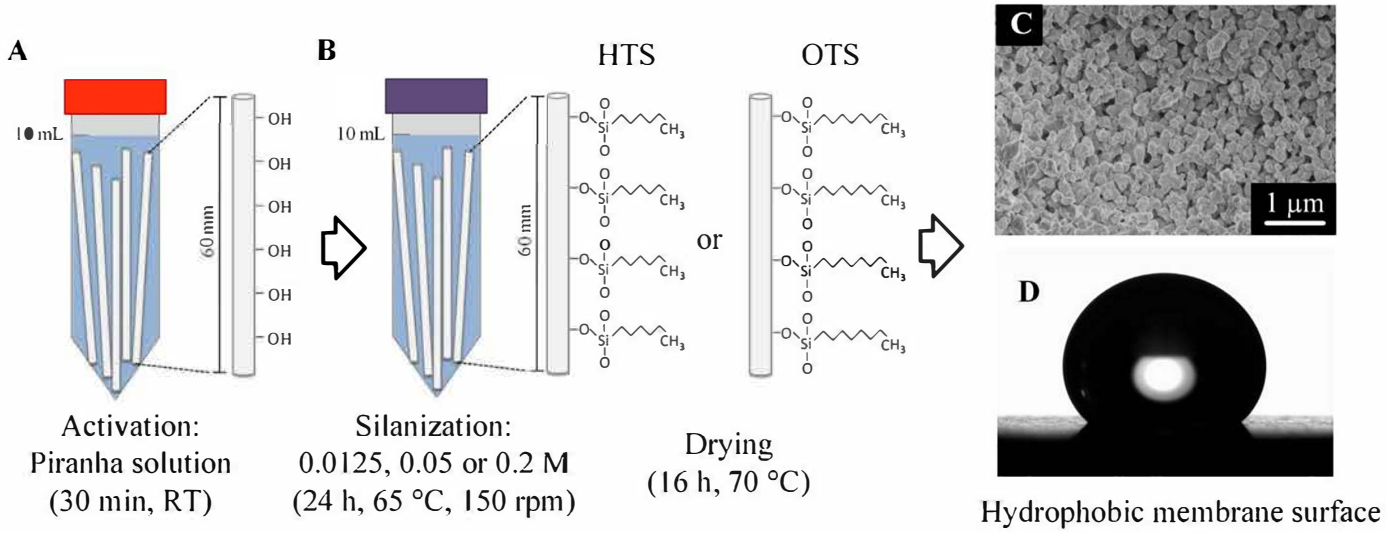


**Fig. 2.** Functionalization strategy of sintered YSZ capillary membranes silanized with HTS or OTS (A, B), a SEM picture of a 0.05 M OTS functionalized membrane surface (C) and a photograph of a water contact angle measurement of a 0.05 M OTS functionalized flat membrane. HTS = n-hexyltriethoxysilane, OTS = n-octyltriethoxysilane.

**Table 1**
Properties of the bacteriophages MS2 and PhiX174.

| | MS2 | PhiX174 |
|---|---|---|
| Nucleic acid[a,b] | ssRNA, 3569 nucleotides | ssDNA, 5386 nucleotides |
| Morphology[a] | Non-enveloped, icosahedral, d = 25–27 nm | Non-enveloped, icosahedral, d = 26–32 nm |
| Host[c] | *E.coli*; F-specific | *E.coli, Salmonella typhimurium*; somatic |
| Multiplication rate ($h^{-1}$)[d] | 669 | 697 |
| Molecular weight[e] (MDa) | 3.6 | 6.2 |
| IEP[f] | 3.5 ± 0.6 | 6.2 ± 1.6 |

[a] [52].
[b] [53].
[c] [3,54].
[d] [55].
[e] [56].
[f] [57].

humans [16,20,51] and are therefore often used in virus inactivation and filtration tests. The bacteriophage properties are summarized in Table 1.

MS2 is a single-stranded RNA virus which is 25–27 nm in diameter and has an isoelectric point (IEP) of 3.5 ± 0.6 and PhiX174 is a single-stranded DNA virus with a diameter of 26–32 nm and an IEP of 6.2 ± 1.6 [3,57]. Both bacteriophages have an excess of positively charged amino acids at pH 7.3, even if their zeta potential is negative which can be explained by the strong influence of the negatively charged nucleic acid in the capsid [50,58,59]. Both bacteriophages can be considered mainly hydrophillic with hydrophobic residues [34,50,60,61]. Both virus species show a fraction of hydrophobic moieties on the virus surface of around 10% [50].

For the preparation of the bacteriophage solution the bacteria were grown in 50 mL of a sterile 30 g/L TSB medium at 37 °C in an incubator shaker (Inkubator 1000/Unimax 1010, Heidolph) at 150 rpm for 4 h to obtain bacteria in an exponential growth phase (4-h culture). Two different *E.coli* strains were used depending on the bacteriophage. For the propagation of the bacteriophage MS2 the host bacteria *E.coli* (DSM Cat. No. 5210) and for the bacteriophage PhiX174 the host bacteria *E.coli* (DSM Cat. No. 13127) were used. The bacteria culture was centrifuged at 3000*g* for 10 min and the supernatant was removed while the bacteria were resuspended in 50 mL of a salt solution containing 0.02 M $MgCl_2$ and 0.15 M NaCl to remove the agents from the TSB medium. The pH of the salt solution was not adjusted (pH 5.8). At the pH of 5.8 the viruses are charged differently according to their IEPs. MS2 with an IEP of 3.5 is negatively charged and PhiX174 with an IEP of 6.2 is positively charged at these conditions. 1 mL of the bacteriophage stock suspension (prepared according to suppliers information) was added and the culture was incubated at 37 °C at 100 rpm for 20 h. After the incubation time, the bacteria are removed by centrifugation at 3000*g* for 30 min at RT and the supernatant which contains the viruses was cleared additionally of bacterial debris and organic matter through sterile 0.2 µm syringe filters. Nevertheless, there will be a proportion of small molecules based on bacteria cell debris in the virus solution used for the experiments. In a pre-experiment we measured the zeta potential of 80 nm $SiO_2$ particles which have nearly the same IEP as the hydrophobic membrane surface at a concentration that results in the same specific surface area as the studied membrane system (0.1 $m^2$ particles in 1 mL solution) with the Zetasizer Nano ZSP (Malvern Instruments) when in contact with only the salt solution containing 0.02 M $MgCl_2$ and 0.15 M NaCl and in contact with the used virus solution which contains organic matter. Our results show no significant difference between the measurements. The zeta potential is −6.5 ± 2.3 for the particles in salt solution and −4.9 ± 2.1 for the particles in virus solution. Therefore, we assume that the proportion of organic matter in the virus solution has no effect on the performed experiments or in other words, that the surface area of the filter membranes is so high that no competitive effects between DOC and virus should be expected.

The virus retention test of the bacteriophages MS2 and PhiX174 was performed by analyzing the feed solution and the permeate using the Plaque Forming Unit (PFU) method. The PFU method (plaque assay) is the most quantitative and useful biological assay for viruses which is based on the ability of a single virus to give rise to a macroscopic area of cytopathology on a monolayer of bacteria cells [52]. For the PFU method, dilution series of 10-fold steps were performed in 0.02 M $MgCl_2$/0.15 M NaCl solution. The diluted phage suspensions were mixed with the specific host bacteria (*E.Coli* 5210 for MS2 and *E.Coli* 13127 for PhiX174) and agar, poured on a Petri dish and incubated for 16 h at 37 °C (Memmert, Modell 100-800). The formed plaques were counted and the log reduction value (LRV) was calculated as shown by Kroll et al. [51]. Retention of microorganisms is expressed in the LRV, which is defined as the logarithm to base 10 of the ratio of viral concentrations in the feed to those in the permeate.

The standard virus retention test was performed by intracapillary virus feeding in dead-end mode with a pressure of 500 mbar until a permeate volume of 15 mL was filtrated. The permeate was obtained after ~85 min of filtration through the active filtering area of 1.4 $cm^2$. After this time the influent and effluent samples were taken to determine the LRV. The used standard feed was based on 0.02 M $MgCl_2$/0.15 M NaCl (pH 5.8, not adjusted) showing initial virus concentrations of around $10^9$ PFU/mL.

Additional to the standard virus retention test, the feed solution was varied by using only one salt component in the feed solution, namely 0.02 M $MgCl_2$ or 0.15 M NaCl or by adjusting the pH to 3 with HCl or to 9 and 12 with NaOH. Furthermore, the standard virus test was performed with increased pressure to up to 2.5 bar.

For each experiment, three individual capillary membranes with a length of 5 cm (active filter area = 1.4 $cm^2$) were tested.

#### *2.3.4. Leaching test*

To analyze wash-out effects of the silane on the capillaries, the capillaries were intracapillary fed in dead-end-mode with a saline solution (0.02 M $MgCl_2$/0.15 M NaCl) at 500 mbar at RT for 21 days. After each week a virus retention test was performed with MS2 and PhiX174 to analyze the LRV of the capillaries. For each time period, three individual capillary membranes with a filter length of 5 cm were tested.

## 3. Results and discussion

### *3.1. Structural and mechanical properties*

Before considering the performance of the ceramic membranes for virus removal, the material properties of the membrane need to be fully characterized. The membrane properties obtained by Hg-porosimetry (pore size range, average pore size and open porosity), BET method (specific surface area), 3-point bending tests (bending strength, Weibull modulus) and water contact angles of the capillary membranes are shown in Table 2. The non-functionalized capillary membranes show an average pore size of 144 nm and a pore size distribution in the range between 5 and 200 nm in combination with a relatively high open porosity of 49.3% and homogeneously distributed pores. The mechanical stability of the capillaries is 29.0 ± 4.4 MPa, which makes them suitable for filtration experiments. The capillaries functionalized with HTS or OTS show the same structural and mechanical properties as the non-functionalized capillary membranes. Only for the capillaries silanized with 0.2 M HTS or OTS the average pore size is decreased to values of 126 nm and 122 nm, respectively.

The wettability of the functionalized material was determined by static water contact angle measurements on flat membrane surfaces.

**Table 2**
Membrane properties of capillary membranes functionalized with HTS and OTS.

| | Hg-porosimetry | | | BET method | 3-point bending test | Water contact angle measurement |
|---|---|---|---|---|---|---|
| Membrane-functionalization | Pore size range in nm | $d_{50}$ in nm | Open porosity in % | Specific surface area in $m^2/g$ | Bending strength in MPa | Water contact angle in deg. |
| Non-functionalized | 5–200 | 144 | 49.3 | 5.4 | 29.0 ± 4.4 | very hydrophilic |
| 0.0125 M HTS | 5–200 | 151 | 47.0 | 5.1 | 25.0 ± 6.3 | 133.1 ± 7.7 |
| 0.05 M HTS | 5–200 | 148 | 46.6 | 4.9 | 22.7 ± 5.0 | 135.1 ± 6.9 |
| 0.2 M HTS | 5–200 | 126 | 50.1 | 5.2 | 27.9 ± 6.7 | 135.9 ± 8.1 |
| 0.0125 M OTS | 5–200 | 149 | 49.9 | 4.8 | 28.7 ± 5.2 | 131.3 ± 6.6 |
| 0.05 M OTS | 5–200 | 153 | 47.8 | 5.1 | 23.5 ± 6.0 | 140.5 ± 7.3 |
| 0.2 M OTS | 5–180 | 122 | 50.1 | 4.9 | 22.4 ± 5.5 | 140.5 ± 3.0 |

The water contact angle for the non-functionalized platelets could not be measured, as the platelets were too hydrophilic and the water was sucked into the highly porous structure by capillary forces. All samples functionalized with either HTS or OTS show a high static water contact angle between 131.3° and 140.5°. Accordingly, the membranes were successfully converted from hydrophilic to hydrophobic with the chosen functionalization approach.

TGA of the capillaries was performed measuring the mass of the samples during a temperature increase to 900 °C. Fig. 3 shows the weight loss of the functionalized HTS capillaries (left) and the OTS capillaries (right) compared to the non-functionalized membranes as a function of temperature. The non-functionalized membrane loses 0.14% of its weight which is most likely related to water desorption. Below 200 °C, the same initial weight-loss observed for the functionalized samples. The two silane types show a nearly identical increased weight loss in the range between 250 °C and 600 °C at the respective molarities when compared to the non-functionalized sample. The weight loss increases with silane concentration used during functionalization. Accordingly, the highest weight loss is attributed to the 0.2 M samples. After 600 °C the 0.0125 M HTS samples have a weight loss of around 0.2% which is attributed to 0.2 molecules/$nm^2$, the 0.05 M HTS samples show a weight loss of 0.33% (0.9 molecules/$nm^2$) and the highest weight loss was determined for 0.2 M HTS with 0.59% (2.1 molecules/$nm^2$). For the OTS samples the weight loss is slightly higher compared to the HTS samples with 0.22% (0.4 molecules/$nm^2$) for the 0.0125 M OTS membranes, 0.46% (1.3 molecules/$nm^2$) for the 0.05 M OTS membranes and 0.67% (2.3 molecules/$nm^2$) for the 0.2 M OTS membranes. The weight loss is not increasing any further after 600 °C for both capillary types.

The TGA results show that the silanization with HTS and OTS was successful for all used molarities and could confirm the hydrophobic functionalization also observed by water contact angle measurements. The contact angles are in the same range for all sample types as discussed previously (Table 2), in contrast to the clear differences in the TGA measurement. We assume that the outer membrane surface is fully silanized for all membrane types, but that the silanization of the internal pore structure is improved at higher molarities.

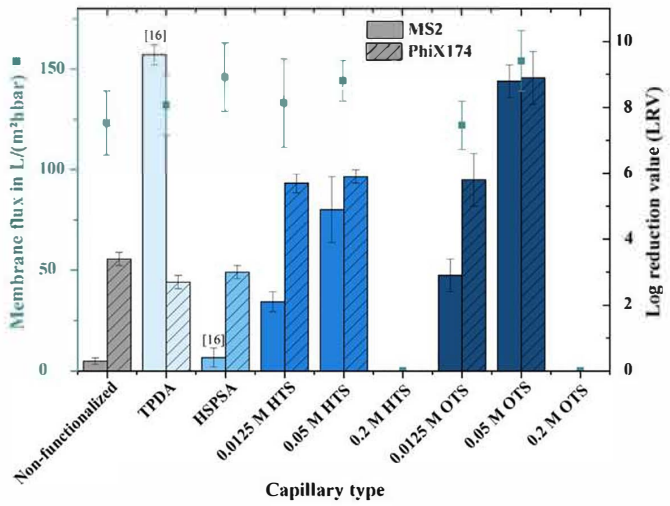


**Fig. 4.** Membrane flux and virus retention of the bacteriophages MS2 and PhiX174 measured in dead-end mode with non-functionalized and functionalized ceramic capillary membranes. The feed was based on a saline solution (0.02 M $MgCl_2$, 0.15 M NaCl, pH 5.8) with a viral concentration of around $10^9$–$10^{10}$ PFU/mL. TPDA and HSPSA retention capacities and membrane fluxes tested under the same conditions were taken from our previous publication [19].

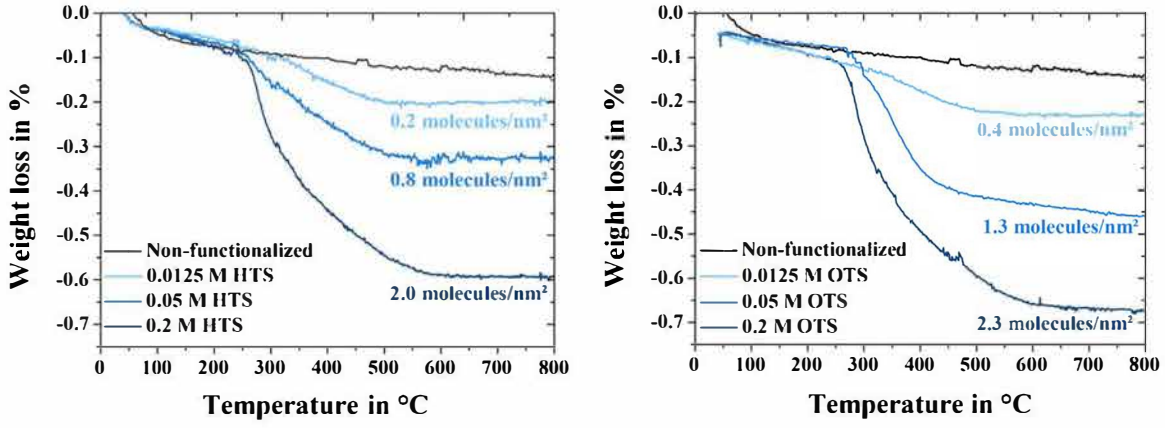


**Fig. 3.** Weight loss determined by TGA measurements with the assessed loading capacities of the capillaries functionalized with HTS (left) and OTS (right) compared with non-functionalized capillary membranes.

### 3.2. Membrane flux and virus retention

Fig. 4 shows the membrane flux and the virus retention capacity of the bacteriophages MS2 and PhiX174 for the non-functionalized and the silanized capillary membranes. The membrane flux of the non-functionalized, as well as the membranes silanized with 0.0125 M and 0.05 M HTS or OTS is around 150 L/($m^2$ h bar). For comparison, membranes silanized with TPDA and the HSPSA, which were investigated in our previous work [30], show the same membrane flux. At concentrations of 0.0125 M and 0.05M HTS or OTS, the hydrophobicity of the functionalized membranes is not significantly influencing the water flux. However, capillaries functionalized with 0.2 M HTS and OTS show no water permeate flux at 1 bar. Due to their high loading with ~ 2.0 silanes/$nm^2$ (Fig. 3) and the slightly reduced pore diameters (Table 2), no water was capable to flow through the pores of the membranes in dead-end mode which renders them unsuitable for filtration applications. The membrane flux can be influenced by a high organic carbon content in the feed which may adsorb onto the membrane due to electrostatic interactions [62] and lead to membrane fouling. But, as the virus size with around 25 nm is much higher compared to the size of the DOCs, the effect is neglectable.

The non-functionalized capillary membranes show relatively low virus retention capacities of LRV 0.3 ± 0.1 for MS2 and of 3.4 ± 0.2 for PhiX174. For MS2, poor retention is caused by repulsion between negatively charged viruses (IEP = 3.9) and negatively charged pore wall surfaces (IEP < 3) [19] at the applied pH of 5.8. PhiX174 (IEP = 6.6) is slightly positively charged at pH 5.8 and therefore adsorbs to the non-functionalized capillary surface. However, the retention is below LRV of 4, which is given by the World Health Organization (WHO) as a standard for safe and clean water [8,9].

As shown in our previous work, the virus retention capacity for MS2 can be strongly increased to LRVs of 9.6 ± 0.3 by using positively charged TPDA-silanized capillaries (IEP > 9). Here, the negatively charged MS2 viruses (at pH 5.8) can adsorb to the positively charged membrane during dead-end filtration [19]. In contrast, the retention capacity for PhiX174 viruses is decreased with the TPDA-functionalized membrane compared to the non-functionalized membrane, down to an LRV of 3.0 ± 0.2 for the TPDA capillaries, as the repulsion between the positively charged PhiX174 and the positively charged pore wall surfaces at the applied pH of 5.8 is hindering adsorption. Nevertheless, an adsorption occurs and we assume that the moderately high filtration efficiency for PhiX174 with the TPDA-functionalized membrane is a result of the complex surface chemistry of the virus membrane that also bears patches or domains with negative charge. The negatively charged HSPSA capillary (IEP < 3) shows a similar virus retention capacity as the non-functionalized capillary membrane at pH 5.8, which can be explained by the nearly identical zeta-potentials of virus and membrane [19].

The virus retention capacities of the HTS functionalized capillaries is increased for both virus types and both used molarities compared to the non-functionalized membranes. The HTS capillaries retain MS2 with LRVs of 2.1 ± 0.3 (0.0125 M HTS) and 4.9 ± 1.0 (0.05 M HTS), which is much lower than the retention with TPDA capillaries. The PhiX174 retention shows an LRV of around 6 for both used molarities which is higher compared to the non-functionalized, the TPDA- and the HSPSA-silanized capillary membranes. Thus, the 0.05 M HTS membrane is fulfilling the virus filter criterion of LRV 4 required by the World Health Organization for clean water for both bacteriophage types [8,9]. However, for the purification of biopharmaceutical products like monoclonal antibodies, a full removal of virus particles is desired [63]. Such a virtually complete virus removal is obtained by the ceramic capillary membranes functionalized with 0.05 M OTS, where LRVs of 8.8 ± 0.5 for MS2 and 8.9 ± 0.8 for PhiX174 are observed. The 0.0125 M OTS capillaries still show an LRV of 2.9 ± 0.5 for the MS2 bacteriophages and of 5.8 ± 0.8 for the PhiX174 bacteriophages. The OTS capillaries most likely have a higher virus retention as a result of the higher functionalization density (Fig. 3) compared to the HTS capillaries.

Both bacteriophages are mainly hydrophilic with hydrophobic residues [34,50,60,61], therefore, they attach to the hydrophobic regions of the capillary membranes. PhiX174 viruses have 11% hydrophobic moieties, which is slightly higher compared to MS2 which have 8% hydrophobic residues [50]. This might explain the stronger attraction of PhiX174 to the hydrophobic capillary surface resulting in higher LRVs compared to MS2, especially at low molarities of HTS and OTS. To a certain extent, electrostatic adsorption can also be used to explain the findings. The HTS and OTS functionalized membranes have IEPs around 4.5, therefore the PhiX174 viruses are positively charged at pH 5.8 and the functionalized membranes are negatively charged, which increases the removal effect when compared to MS2. For the 0.05 M OTS capillaries the electrostatic interactions seem to play only a minor role, as both virus types have nearly the same retention values.

In our previous as well as in the present study, the observed inactivation of viruses is not necessarily equal to virus absorption on the membrane surface. Based on the observed correlation between phage inactivation and surface properties, inactivation and retention occurs due to hydrophobic and electrostatic interactions between membrane surface and virus.

Without post-chemical functionalization, such LRVs are otherwise only reachable by using membranes with significantly smaller pore sizes in the order of the viral target ($d_{50}$ = ~ 25 nm). As shown in our previous work, this pore size reduction is associated with a strong decrease in membrane flux from 150 L/($m^2$hbar) to values of around 3 L/($m^2$ h bar) [2]. Commercially available polymeric membranes obtained from Sartorius, Germany (Virosart CPV) reached the same high virus retention levels [19], but, according to the manufacturer, with a moderate membrane flux of ~ 84 L/($m^2$ h bar).

Stability and wash-out effects of the silane on the capillaries were evaluated under filtration conditions with capillary membranes functionalized with 0.05 M OTS as they show the best virus retention capacities (Fig. 4). Therefore, the capillary membranes were intracapillary fed with a saline solution (0.02 M $MgCl_2$, 0.15 M NaCl, pH 5.8) at 500 mbar for up to 3 weeks in dead-end mode. After each week a PFU test was performed as described above. As shown in Fig. 5, the LRV for both bacteriophages was higher than 8 over the time period of 3 weeks. No wash-out effects are determined, which means that a stable bond of OTS on the hydroxyl-activated membrane surface was formed, which is suitable for virus filtration even under throughflow filtration conditions.

Furthermore, the virus retention of the bacteriophages MS2 and PhiX174 was analyzed in dead-end mode with 0.05 M OTS capillary

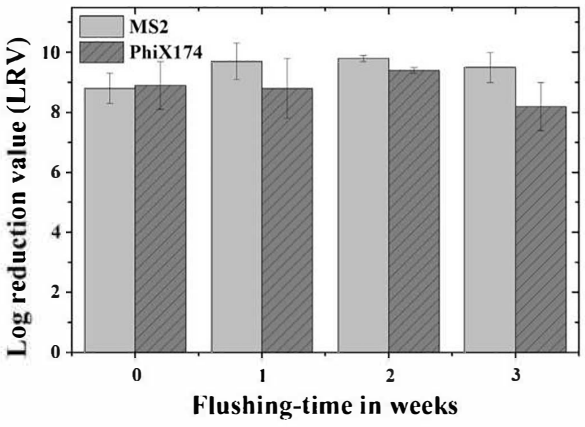


**Fig. 5.** Virus retention of the bacteriophages MS2 and PhiX174 measured in dead-end mode with 0.05 M OTS capillary membranes after flushing with a salt solution (0.02 M $MgCl_2$, 0.15 M NaCl) to evaluate the stability of the immobilized silane. The stability test was performed over a period of 3 weeks.

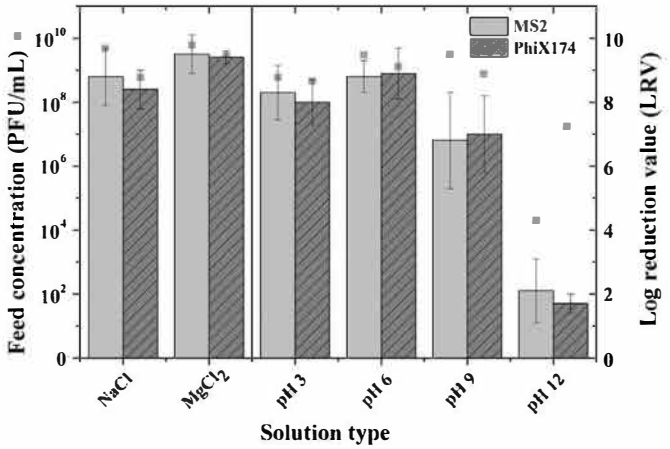


**Fig. 6.** Virus retention of the bacteriophages MS2 and PhiX174 measured in dead-end mode with 0.05 M OTS capillary membranes in salt solutions varying in composition (NaCl and $MgCl_2$) and in pH.

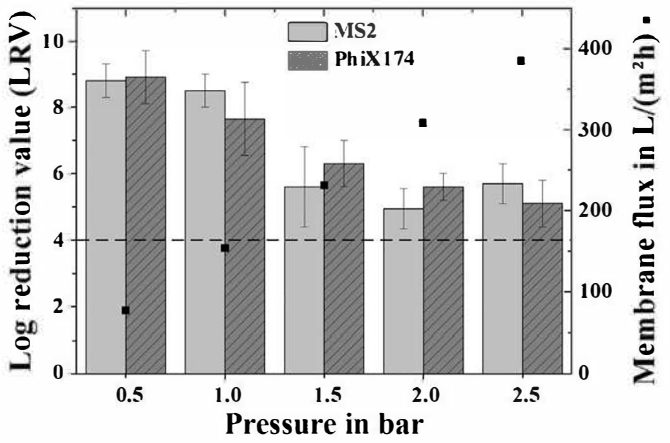


**Fig. 7.** Virus retention of the bacteriophages MS2 and PhiX174 measured in dead-end mode with 0.05 M OTS capillary membranes with pressures ranging from 0.5 to 2.5 mbar.

membranes using varying feed solutions and the results are presented in Fig. 6.

In our previous work, we showed that the LRV for TPDA functionalized capillary membranes is higher in the presence of divalent salt ($MgCl_2$) when compared to monovalent salt (NaCl), as the shielding between surface and amino groups by the adsorbed $Mg^{2+}$ ions leads to an upright orientation of the TPDA molecules and thus to a better accessibility [30]. As shown by Gerba et al., some salts also have a positive effect on virus removal via the hydrophobic effect [23]. However, for the OTS functionalized capillary membranes no significant change in the retention capacity can be observed in the presence of divalent cations (0.02 M $MgCl_2$). Here, virus retention is 9.5 ± 0.6 for MS2 and 9.4 ± 0.2 for PhiX174. The LRV for a feed solution containing 0.15 M NaCl (pH 6.3) is still high with 8.8 ± 0.9 for MS2 and 8.4 ± 0.6 for PhiX174.

Furthermore, the pH stability of the ceramic filter system was analyzed by adjusting the feed solution (0.02 M $MgCl_2$, 0.15 M NaCl) to different pHs with HCl or NaOH. The virus retention for the feed in the acidic and neutral range show LRVs above 8 for both bacteriophages. At pH 9 the virus retention is decreased to around 7 for both bacteriophages. When using pH 12 the bacteriophage feed is reduced from a concentration of $\sim 10^9$ to $\sim 10^4$ even before filtration, which indicates that the viruses are inactivated in this pH regime. The retention of the reduced feed concentration is around 2 for both bacteriophages. To determine if the silane is removed due to the high pH, water contact angle measurements with membrane platelets, which were incubated in pH 9 or pH 12 over 1.5 h, were performed. The results show a slight decrease of the contact angle from 135.1° ± 6.9° to 124.8° ± 6.4° for the incubation in pH 9 and a further decrease to 115.0° ± 10.1° for the incubation in pH 12. Thus, the functionalization is not stable at basic pH for longer periods of time.

The standard virus test, which was previously performed at 500 mbar, was tested with increased pressure to up to 2.5 bar, which is the overall practical limit of the testing setup (Fig. 7). The retention is slightly decreased for experiments performed at 1.0 bar to retentions of around LRV 8 and further decreased for pressures of 1.5 bar to LRVs of around 6 for both bacteriophages. A further increase of the pressure to 2.0 and 2.5 bar only result in a slight decrease of the retention capacity. Thus, at a pressure of 2.5 bar a LRV of 5.7 ± 0.6 for MS2 and of 5.1 ± 0.7 for Phix174 is observed, which still fulfills the required LRV of 4 for clean water. The decrease of virus retention during pressure increase can be explained by the shorter contact time of the virus to react with the hydrophobic parts of the membrane surface. With a pressure of 2.5 bar, a water volume of nearly 400 L/($m^2$ h) can be filtrated which showcases the promising properties of hydrophobic ceramic capillaries for filtration applications. Compared to a commercially available polymeric membrane obtained from Sartorius, Germany (Virosart CPV) a maximal water flux of 168 L/($m^2$ h) can be reached as the working pressure of 2.0 bar should not be exceeded.

## 4. Conclusion

In our study, YSZ capillary membranes with pore sizes 6 times larger than the bacteriophages MS2 and PhiX174 are successfully hydrophobized with HTS and OTS. The functionalization with 0.05 M OTS with a resulting surface coverage of around 1.3 molecules/$nm^2$ can be beneficially utilized for virus retention as a result of hydrophobic interactions, which do not significantly affect the water permeate flux (~ 150 L/($m^2$ h bar)). The hydrophobic membrane and the hydrophobic regions of the viral capsid preferentially associate to each other due to the high free energy of the interfacial solute layer of polar water molecules. At 0.5 bar, an almost complete virus removal is obtained with LRVs of 8.8 ± 0.5 for MS2 and 8.9 ± 0.8 for PhiX174, which is in the same range as commercially available polymeric virus filtration membranes. The ceramic membranes surpass those polymeric membranes in the higher applicable pressures of up to 2.5 bar resulting in high throughput rates of nearly 400 L/($m^2$ h), at which the retention of both viruses is still fulfilling the requirements for safe water of LRV 4. Combined with the general advantages of ceramic membranes like high mechanical stability of the porous structure and long-term stability of the functionalization, the hydrophobic ceramic capillary membranes open promising perspectives for filtration applications of diverse viruses and related biological entities.

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