MONOLITHIC ADSORPTION FILTRATION FOR WATER SAMPLES

Tuomas Putkinen

Opinnäytetyö
Kesäkuu 2018
Laboratoriotekniikan koulutus

TIIVISTELMÄ

Tampereen ammattikorkeakoulu
Laboratoriotekniikka

PUTKINEN, TUOMAS:
Monoliittiadsorptiosuodatus vesinäytteille
Opinnäytetyö 30 sivua
Kesäkuu 2018

Opinnäytetyön kokeellinen osuus suoritettiin Tampereen teknillisen yliopiston kemian ja biotekniikan laboratoriossa. Opinnäytetyön tavoitteena oli monoliitti adsorptiosuodattimen valmistus ja karakterisointi. Tarkoituksena oli syntetisoida monoliittinen kolonni sekä tutkia kolonnin rakennetta pyyhkäisyelektronimikroskoopilla. Monoliittinen kolonni valmistettiin polyglyseroli-3-glysidyylieetterin itsepolymerointireaktion avulla. Lisäksi tarkoituksena oli testata valmistetun monoliitin toimivuutta 1 l hanavesinäytteen ja 10 l raakavesinäytteen suodatuksella.

Työssä saatiin kehitettyä monoliittikolonnille valmistusmenetelmä, jonka avulla pystyttiin tuottamaan toistettavia kolonneja. Pyyhkäisyelektronimikroskoopilla havaittiin, että kolonnin monoliittinen rakenne oli epähomogeeninen. Vesinäytteen suodatukseen kehitetty menetelmä saatiin onnistuneesti testattu molemmilla vesinäytteillä. Hieman sameampi raakavesinäyte ei tukkinut kolonnia suodattimen aikana.

Tavoitteena ollut monoliittiadsorptiosuodattimen valmistus onnistui. Valmistetulla monoliitilla oli haluttu huokoinen rakenne sekä korkea huokoisuus. Kolonnin epähomogeeninen rakenne johtui luultavasti liian korkeasta reaktio lämpötilasta. Jatkossa reaktiolämpötilaa täytyy seurata, jotta voidaan selvittää epähomogeenisen rakenteen syitä. Raakavesinäytteen onnistunut suodatus viittaa siihen, että menetelmällä voidaan suodattaa suurempia vesinäytteitä.

Asiasanat: monoliittiadsorptiosuodatus, monoliitti, veden suodatus

ABSTRACT

Tampereen ammattikorkeakoulu
Tampere University of Applied Sciences
Degree Programme in Laboratory Engineering

PUTKINEN, TUOMAS:
Monolithic Adsorption Filtration for Water Samples
Bachelor's thesis 30 pages
June 2018

This thesis was done at Tampere University of Technology, Laboratory of Chemistry and Bioengineering. When water is contaminated with viruses, the risk of infection is 10-1000 times greater than with bacteria at same level of exposure. Therefore, a fast and effective method to concentrate viruses in water is needed.

The objective of this work was to prepare a monolithic adsorption filter (MAF) and characterize its structure. The purpose of this work was to produce a monolithic column and characterize its structure with a scanning electron microscope. A macroporous epoxy-based monolithic column was synthesized by self-polymerization of polyglycerol-3-glycidyl ether. The other purpose was to test how the prepared monolith column works with different water samples. The MAF method was tested with two different water samples: 1 l tap water sample and 10 l surface water sample.

As a result of this work, the monolithic column was successfully prepared and preparation protocol for monolithic column was developed. It was found out that the inner structure of the column was inhomogeneous. The method for filtering water was successfully tested with 10 l surface water sample.

The inhomogeneous monolithic structure of the column indicates that reaction temperature was too high during polymerization. The reason for the inhomogeneity of the monolithic structure will need to be found out in future studies. The 10 l surface water test suggest that larger volumes of water can be filtered using this method.

Key words: monolithic adsorption filtration, monolith, water filtration

CONTENTS

1 INTRODUCTION 5
2 THEORETICAL BACKGROUND 6
2.1 Monolithic column 6
2.2 Monolithic materials and reactions 6
2.3 Waterborne viruses 9
2.4 Monolithic adsorption filtration for concentration of viruses 9
2.5 Characterization with scanning electron microscope 11
3 MATERIALS AND METHODS 13
3.1 MAF-disk preparation 13
3.1.1 Chemicals and equipment 13
3.1.2 Preparation 14
3.2 Sulfuric acid activation 15
3.3 Water filtering experiments 16
3.3.1 Filtering equipment and preliminaries 17
3.3.2 Filtering 1 l tap water sample 18
3.3.3 Filtering 10 l surface water sample 20
3.4 Scanning electron microscopy 23
4 RESULTS 24
4.1 Characterization of the monolithic column 24
4.2 Filtering method 26
5 CONCLUSION AND DISCUSSION 27
REFERENCES 29

1 INTRODUCTION

Monolith columns are widely used in chromatography to separate large biomolecules for example proteins, protein aggregates, plasmid DNA and viruses. They have a few advantages compared to conventional packed columns such as bigger molecules have easier access to the surface of the column due to larger pore size of monolith column. (Jungbauer & Hahn 2008)

This thesis was done at Tampere University of Technology. The objective of this work was to prepare monolithic adsorption filter (MAF) and characterize its structure. The purpose of this work was to produce monolithic column and characterize its structure. Macroporous epoxy-based monolithic column was synthesized by self-polymerization of polyglycerol-3-glycidyl ether. Scanning electron microscope was used to characterize the inner structure of the monolithic column because it gives higher magnification and resolution images of the surface than optical microscope.

The other purpose was to test how the prepared monolith column works on different water samples. Produced monolithic column was tested with two different water samples to see how it performs. Monolithic column's performance was tested with 1 l tap water and 10 l surface water samples. Surface water was used to test if more turbid water causes clogging. Elution was also tested with two different method: pumping elution solution with a tube and pumping it with a syringe.

2 THEORETICAL BACKGROUND

2.1 Monolithic column

Monolith is a single block which is made from one continuous piece of material. Monolithic column is made from a single piece of porous material. There are pores connected with each other which form channels inside the column. Monoliths are used as stationary phase in chromatography. Large biomolecules for example proteins, protein aggregates, plasmid DNA and viruses can be separated and purificated with monolithic column (Jungbauer & Hahn 2008).

Monolithic column has a few advantages compared to packed column. Packed column is a tube filled with packing material. Pore size of a macroporous monolith is over 1000 nm while packed column has pore size of 10-100 nm. Molecules have easier access to the surface of the column when the pore size is bigger. Thus, for large molecules a monolithic column has higher binding capacity than a packed column even though its inner surface is lower. (Jungbauer & Hahn 2008)

Main characteristic of monolithic column is high porosity. Porosity is the ratio of void-space divided by the total volume of the column. Monolithic columns have higher porosity (0.25-0.73) compared to packed column in which porosity is limited to 0.4. With higher porosity flow rate can be increased without increasing backpressure. High flow rate with low backpressure enables filtration of large volume samples. (Jungbauer & Hahn 2008; Podgornik et al. 2013)

2.2 Monolithic materials and reactions

Monolithic materials can be synthesized from homogenous mixture that contains a monomer, a porogenic solvent and the radical initiator. Polymerization can be carried out in mold which enables production of different shaped column such as cylinder, disk or tube. (Arrua et al. 2008) Monoliths can be prepared from different material such as synthetic polymers, natural polymers and inorganic materials. Many different monomers can be

used in polymerization for example methacrylate, styrenedivinylbenzene or polyglycerol-3-glycidyl ether. (Jungbauer & Hahn 2008; Janco et al. 2000; Peskoller et al. 2009).

Macroporous epoxy-based monolith can be synthesized by self-polymerization of polyglycerol-3-glycidyl ether. Figure 1 describes the self-polymerization reaction of polyglycerol-3-glycidyl ether. Lewis acid BF_3 is an initiator which activates the epoxy groups for a nucleophilic attack. (Peskoller et al. 2009)

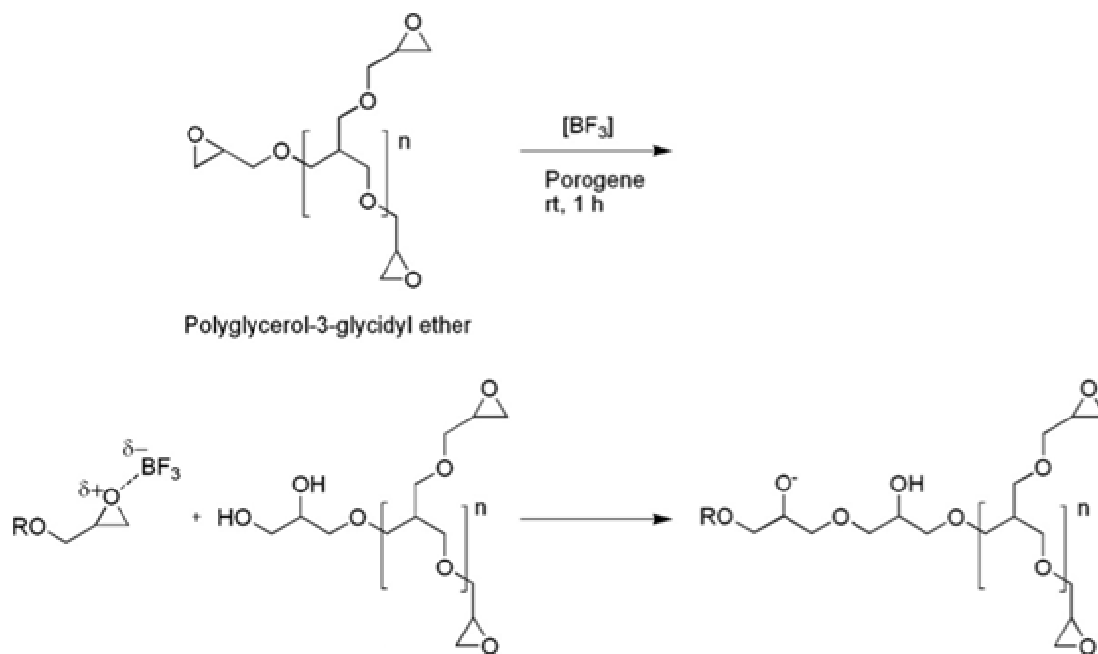

FIGURE 1. Self-polymerization reaction of polyglycerol-3-glycidyl ether (Peskoller et al. 2009, modified)

Monomers bond to each other forming polymer structure. Porogens cause the formation of pores during polymerization process by phase separation. Reaction is carried out at room temperature for 1 hour.

Pore size and porosity of monoliths can be changed by using different porogen compositions. Organic solvents are used as porogens. Table 1 shows the effect of porogen composition on porosity, pore size and shrinkage on epoxy-based monoliths. (Peskoller et al. 2009)

TABLE 1. The effect of porogen composition on porosity, pore size and shrinkage (Peskoller at al. 2009)

Porogen	Porosity (%)	Pore size (µm)	Shrinkage (%)
MTBE/dioxane (3:2)	71	1 ± 0.3	27.5 ± 3.0
Toluene/dioxane (4:1)	73	2 ± 0.4	22.3 ± 2.1
Toluene/MTBE/PEG (57:38:5)	78	7 ± 3.8	5.5 ± 0.6
Toluene/MTBE (3:2)	79	22 ± 9.0	5.0 ± 0.3

Table 1 shows that using toluene/MTBE as porogen, a high porosity and large pore size can be achieved with small shrinkage. Compared to the combination of toluene and methyl tert-butyl ether (MTBE), using MTBE or toluene with dioxane as porogen gives monolith smaller pore size and high shrinkage. As mentioned before large pore size is preferred because then larger molecules have easier access to column’s surface.

Epoxy groups inside the column can be functionalized which makes MAF versatile method for concentration. This enables adsorption of bacteria and viruses to the MAF via hydrophobic and ionic interactions. Using differently functionalized groups for MAF can be used for concentration of different microorganisms. (Karthe et al. 2016) The epoxy group’s ring structure can be opened by hydrolyzation with sulfuric acid (figure 2). (Peskoller et al. 2009)

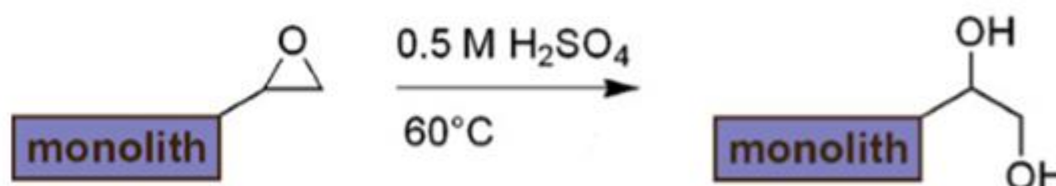

FIGURE 2. Hydrolyzation with sulfuric acid (Peskoller et al. 2009, modified)

This forms hydroxyl groups which are used for concentration of bacteria and viruses. The hydroxyl groups can be used for further surface modification, for example immobilization of ligand or antibodies. (Peskoller et al. 2009)

2.3 Waterborne viruses

Bacteria are usually used as indicators when microbiological quality of water is assessed. However, when water is contaminated with viruses, the risk of infection is 10-10 000 times greater than with bacteria at same level of exposure. (Haas et al. 1993 according to Pei 2015, 5) Most viruses have low infectious dose, 1-10 particles are enough to cause sickness. Because of low infectious dose effective methods of concentration and detection of viruses from drinking water is needed. (Schiff et al. 1984, according to Pei 2015, 6)

Viruses are the smallest pathogens and because of their small size they are difficult to remove from water with physical process such as filtering. Some viruses are more resistant to disinfection than bacteria and parasites such as adenovirus which is more resistant to UV light. Viruses have long-term persistence in water. They are typically species specific for example usually animals do not carry most of the human enteric viruses. (WHO 2011, 126)

Waterborne transmitted viruses mostly infect the gastrointestinal tract. One example is adenovirus which can excreted in large numbers in human feces. It can occur in raw water sources and treated drinking-water supplies. Enteroviruses are a common cause of infections. They also are excreted in feces of infected human. Enteric viruses occur in raw water sources and treated drinking-water supplies. (WHO 2011, 260-264)

2.4 Monolithic adsorption filtration for concentration of viruses

Viruses have pH-dependent surface charge in water. The pH value where the net surface charge is zero is called isoelectric point (IEP). When pH value of the environment is lower than IEP, virus has positive surface charge and when higher virus has negative surface charge. This feature is very important in virus adsorption-elution process. IEP value of the target virus must be considered when developing adsorption-elution method. Viruses can be adsorbed into negatively charged surface in acid conditions and eluted off under basic conditions. IEPs of viruses are found to be between 1.9 and 8.4 most of them are in the range of 3.5 to 7. (Michen & Graule. 2010)

Hydrogen bonding is another reason why viruses are adsorbed on the surface of the monolith. In hydrogen bonding, hydrogen atom is connected to an electronegative atom (for example oxygen or nitrogen) with a lone-pair electron. The monolith contains polarized electron rich alkyl oxygen atoms which have lone pairs of electrons. Protein capsid of the virus has carboxylic acid and ammonium groups which have high affinities toward lone pair of electron on monolith's surface. This leads to strong hydrogen bonding between monolithic column and viruses. The adsorption-elution mechanism between monolithic column and virus is described in figure 3. (Pei 2015, 35-36)

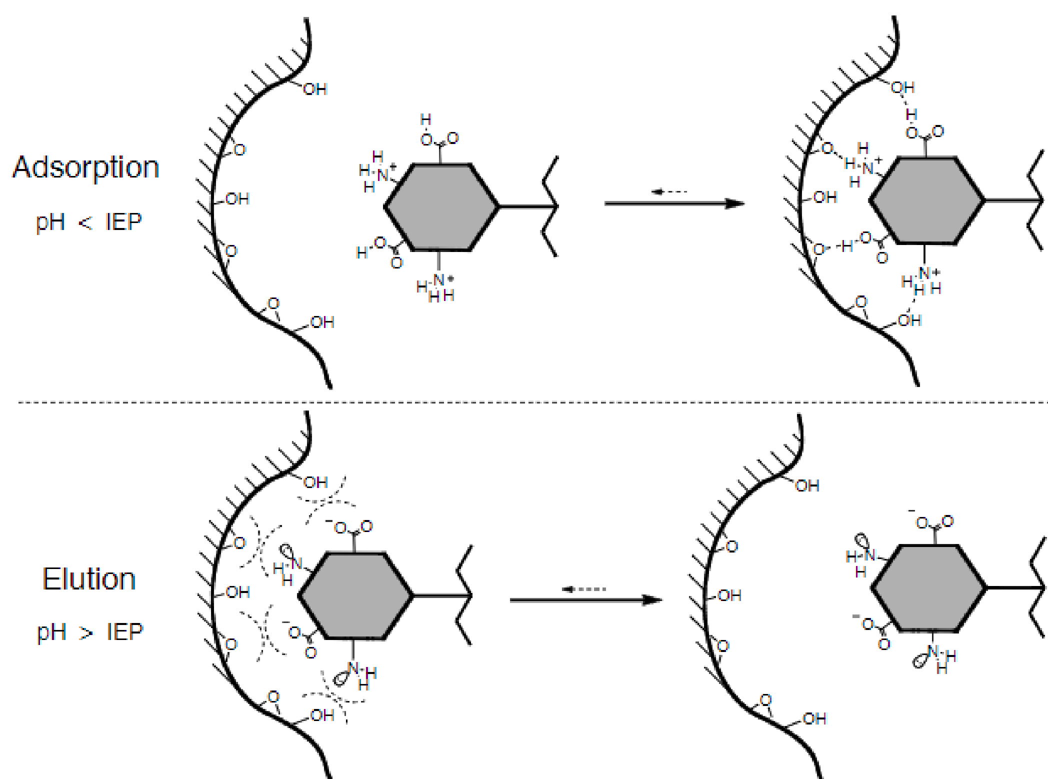

FIGURE 3. The absorption-elution mechanism between monolithic column and virus (Pei 2015, 36)

In the adsorption stage pH value is lowered below IEP of the virus which causes carboxylic acid and ammonium groups on the surface of the virus to protonate. These groups function as hydrogen bond donors. Protonated carboxylic acid and ammonium groups onto the surface of the virus and alkyl oxygen onto surface of the monolithic column are bound through the hydrogen bonding. In the elution stage, the pH is raised above the IEP of the virus. This causes carboxylic acid and ammonium groups to be deprotonated. The hydrogen bonding between monolith and virus is broken and viruses are eluted with elution buffer. (Pei 2015, 35-36)

A few studies on the use of MAF to concentrate pathogens from water have been published. These studies have received high recoveries for different pathogens. Table 2 shows some examples of recovery of bacteria and viruses concentrated by MAF.

TABLE 2. Recovery of bacteria and viruses by MAF

	Recovery (%)	Reference
E. coli	97 ± 3	Peskoller et al. 2009
Bacteriophage MS2	106.1	Pei 2015
Bacteriophage ΦX174	40.2 ± 17.0	Pei 2015
Murine norovirus	67.2 ± 58.8	Pei 2015
Human adenovirus	12.2 ± 5.7	Pei 2015

Pathogens listed in table 2 were concentrated using monolithic column which had prepared from the same epoxy-base material and had the same pore size and porosity. Samples used in these tests were spiked. Viruses were in the same 10 l tap spiked water sample. *E. coli* was concentrated from small spiked ultrapure water sample but the monolithic column used was also small (6 mm x 4.5 mm inner diameter) compared to column in virus concentration test (1.0 cm x 3.86 cm inner diameter). (Peskoller et al. 2009; Pei 2005, 67-69)

2.5 Characterization with scanning electron microscope

Structure of the monolith can be characterized with scanning electron microscope (SEM) (Peskoller et al. 2009). SEM is used because it provides higher magnification (10x to 100 000x) than optical microscopes. Higher magnification is needed because pore size of the monolith is 1-22 µm (table 1). SEM also provides larger depth of field and higher resolution than optical microscopes. SEM uses electrons instead of light to produce a magnified picture from objects. SEM has a large vacuum tube which has electron gun (cathode) at the top of the tube producing the electron beam. Sample is at the bottom of the tube acting as an anode. In the vacuum tube, there are series of electromagnetic lenses that shape and position the electron beam. The beams intensity is controlled by voltage and diameter. Electron beam interacts with sample and these interactions are detected by using different detectors. (Wheeler & Wilson 2008, 323-327)

The main detector of the SEM is a photomultiplier tube. It produces image by dividing sample into a x/y grid and scanning grid sequentially. When electron beam interacts with

sample it causes electron from sample to deflect off. These electrons are collected by photomultiplier tube. When the entire grid is scanned the image of the sample is formed based on its topical features. Because the surface of the monolith is non-conductive it must be coated with conductive material for example gold/palladium alloy. Non-conductive surface traps electrons from the electron beam. This causes surface to charge and creates additional white areas to the images. (Wheeler & Wilson 2008, 323-327)

3 MATERIALS AND METHODS

3.1 MAF-disk preparation

The purpose of this section was to prepare monolith column in a disk-shaped mold. At first monolith disk was made in a polytetrafluoroethylene (PTFE) beaker which was cut shorter. Because result was good but disk was too big, mold was made from a same Dispenser tip as the filtering module so that the disk would fit inside the filtering module. The MAF-disk preparation was made based on the research of Peskoller et al. (2009).

3.1.1 Chemicals and equipment

Monolith disk was prepared in a mold that was specially made for this purpose. Mold's housing was made from 50 ml BRAND PD-tip™ Precision Dispenser Tip. The tip of the Dispenser Tip was cut off and PTFE plugs were fitted in both end of the housing (figure 4). Mold's inner diameter was 3.6 cm and height 1.5 cm.

FIGURE 4. Mold for synthesis

The monolith disk was prepared using 60:40 (v/v) mixture of toluene and tert-butyl methyl ether as a porogen. Polyglycerol-3-glycidyl ether was selected as monomer.
Initiator was boron trifluoride diethyl etherate (Lewis acid BF_3). Lewis acid BF_3 was diluted in 1,4-dioxane (1:10 (v/v) dilution). A fresh initiator solution was used every 24 hours because the solution would not wear for long time.

3.1.2 Preparation

Chemicals were mixed in an oven-dried 20 ml vial. Total volume of the disk was approximately 10,13 ml. At first 8 ml of porogen and 0.13 ml of initiator (1.25% of the total volume) were mixed in the vial with magnetic stirrer. After that 2 ml of polyglycerol-3-glycidyl ether (20:80 v/v monomer/porogen ratio) was added with 1 ml syringe while mixing with magnetic stirrer. The reaction mixture was stirred for 45 seconds (timing was started after the first milliliter of monomer added) and poured in the mold. The mold was sealed with a plug so that there was air space below the plug. Polymerization was allowed to proceed at room temperature (approximately 24 °C) for 1 h.

After the reaction was completed, monolith disk was cut off from the mold. The disk was put into the sintered Buchner funnel and washed with 300 ml of methanol to remove unreacted organic solvents (figure 5).

FIGURE 5. Washing of newly synthetized monolith disk

Cotton wool was put in the bottom of the funnel to reduce the flow of the methanol. After washing the disk was stored in methanol in the refrigerator.

3.2 Sulfuric acid activation

To form hydroxyl groups on the monolith surface, monolith disk was activated with sulfuric acid. Monolith was activated using 500 ml of 0.5 M sulfuric acid at 60 °C. There were two methods of activation: activating disk in sulfuric acid bath and recirculating sulfuric acid through the disk.

Masterflex L/S peristaltic pump with Masterflex Norprene (A 60 G) L/S 16 tubing were used for washing and recirculating. For washing, two disks were placed in 50 ml BRAND PD-tip™ Precision Dispenser Tip. Before activation methanol was washed off with 500 ml of Milli-Q water. Water was pumped through monolith disks at a flow rate of 50 ml/min.

For activating monolith in sulfuric acid bath, disks were put in 500 ml wide-mouth Pyrex bottle. Bottle was placed in 60 °C water bath for 3 h and bottle was gently stirred every 30 minutes. For the recirculating acid through monolith disks, sulfuric acid was placed in 60.5 °C water bath because acid might cool before it reaches disks. Recirculating procedure is shown in figure (6).

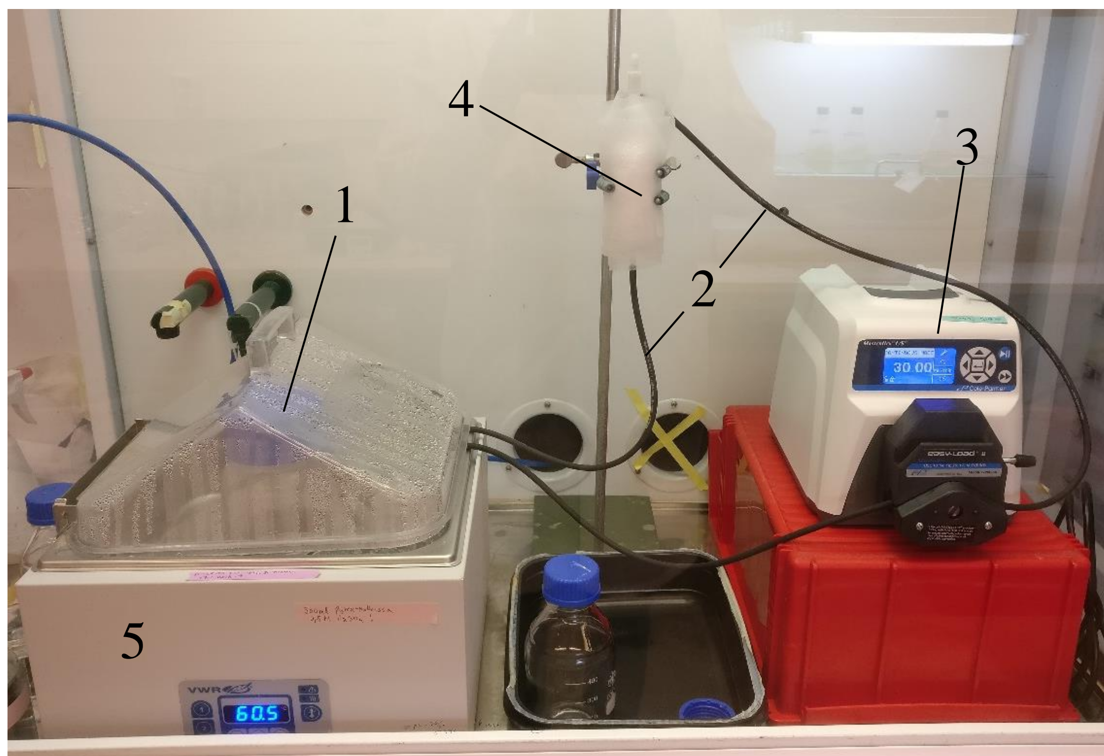

FIGURE 6. Recirculating equipment for sulfuric acid activation: 1) sulfuric acid bottle, 2) Norprene tube, 3) pump, 4) monolith disk inside BRAND PD-tip™ Precision Dispenser Tip and 5) water bath

Sulfuric acid was pumped through disks at a flow rate of 30 ml/min under recirculation for 3 h. Dispenser tip was insulated using aluminum foil and foam (figure 6 section 4). After activation, sulfuric acid was washed off with Milli-Q water. Disks were washed by pumping 1000 ml of Milli-Q water through at a flow rate of 50 ml/min. Activated disks were stored in the refrigerator in Milli-Q water.

3.3 Water filtering experiments

The purpose of these experiment was to test the monolith column with water samples. Column was tested with tap and surface water samples and filtering procedures. At first

the modules tightness and overall performance was tested with 1 l tap water sample. Thereafter, the same MAF disk was used for filtration of 10 l surface water sample. Surface water was used to test if more turbid water would cause clogging. Elution was also tested with two different methods: pumping elution solution with a tube and pumping it with a syringe. Filtering method and module were made based on the research of Kunze et al. (2015).

3.3.1 Filtering equipment and preliminaries

The pumping equipment was Masterflex L/S peristaltic pump with Masterflex L/S 15 silicon tubing. Module was hold with retort stand and clamp. The filtration module's housing was made from 50 ml BRAND PD-tip™ Precision Dispenser Tip (figure 7).

FIGURE 7. The filtering module

A PTFE holder with 2 mm holes was at the bottom of the module. Monolithic disk was sealed with 2 O-rings, one below and one on top of the disk. Top of the module was sealed

with PTFE fitting which had two O-rings to make sure that the module does not leak during filtering. The module was reusable.

Elution buffer was 0.5 M glycine containing 3 % beef extract (w/v). Glycine solution was prepared by weighing 3.7535 g of glycine and diluted to 100 ml with Milli-Q water in volumetric flask. Glycine solution was poured into beaker and 3.0 g of beef extract was added while stirring with magnetic stirrer. After all the beef extract was dissolved, pH was adjusted to 9.5 by adding 3.8 ml of 5 M sodium hydroxide. Elution buffer was divided to five 20 ml batches and autoclaved at 121 °C for 15 minutes.

Silicon tubing, glass dishes and Nalgene containers were sterilized by autoclaving at 121 °C for 15 minutes. The filtering module was disinfected with 10 % chloride solution for 30 minutes. After that the filtering solution was rinsed with Milli-Q water 10 times. Sodium thiosulfate pentahydrate solution (18 mg/ml) was prepared by dissolving 1.8 g sodium thiosulfate pentahydrate to 100 ml of Milli-Q water. Solution was sterile filtered to two 50 ml Falcon tubes and stored in a refrigerator.

3.3.2 Filtering 1 l tap water sample

Monolithic disk was loaded into the filtration module with tweezers. The module was assembled in order: PTFE holder, O-ring, MAF disk, O-ring and fitting. Before filtering monolithic disk was equilibrated with 100 ml of tap water, adjusted to pH 3. Tap water was measured to a sterilized 100 ml Pyrex bottle with measuring cylinder. Tap water was dechlorinated by adding 0.1 ml of sterile sodium thiosulphate solution (18 mg/ml) and then pH was adjusted with 0.38 ml of 0.5 M hydrochloric acid. Filtering module was connected to Pyrex bottle and water was pumped through the disk at a flow rate of 100 ml/min.

In this experiment 1000 ml of tap water was used to test the monolithic column. Tap water sample was measured to 1000 ml with measuring cylinder. Sample was dechlorinated by adding 1 ml of sterile sodium thiosulphate solution (18 mg/ml) and then pH was adjusted with 3.8 ml of 0.5 M hydrochloric acid. The figure 8 shows the filtering set-up for 1 l water sample.

FIGURE 8. Filtering set-up for 1 l water sample: 1) water sample, 2) silicon tube, 3) pump, 4) filtering module and 5) monolith disk

Sample was stirred with magnetic stirrer for 2 minutes before filtration. Sample was pumped through the column at the flow rate of 500 ml/min. After the whole sample had been filtered, air was pumped through the filter to empty it. Elution procedure was tested by adding 20 ml of elution buffer containing 3 % beef extract (w/v) and 0.5 M glycine, adjusted to pH 9.5 with 5 M sodium hydroxide to MAF column. Before elution filtration tube (figure 8 position 2) was disconnected from the filtering module and put in the sample bottle. Elution was done with shorter tube than filtration so that less elution solution would remain in the tube. Elution set-up is shown in figure 9.

FIGURE 9. Elution using peristaltic pump: 1) bottle of elution solution, 2) silicon tube, 3) pump, 4) filtering module and 5) elution solution collecting bottle

Elution buffer was pumped at the flow rate of 20 ml/min. Air was pumped through the tube and filtration unit in order to collect as much elution buffer as possible. Elution solution was collected into 100 ml Pyrex bottle (figure 9 position 5).

3.3.3 Filtering 10 l surface water sample

Before filtration monolithic disk was loaded to the filtering module and equilibrated as described in section 3.3.2. In this experiment monolithic column was tested with 10 l surface water sample. Surface water sample (originated from lake Roine) was collected to sterile 10 l Nalgene container from Tampere Water's Rusko water treatment plant. Sample's pH was lowered to 3 by adding 20 ml of 0.5 M hydrochloric acid. Nalgene container was mixed thoroughly by shaking the container. Filtering module was connected to the sample container (figure 10 position 1) and another tube was put between the bottom of the module and the second Nalgene container (figure 10 position 5).

FIGURE 10. Filtering equipment 10 l filtering procedure: 1) 10 l Nalgene container for sample, 2) silicon tube, 3) pump and 4) filtering module and 5) 10 l Nalgene container for filtered water

At first sample was pumped through the column at the flow rate of 100 ml/min to check for any leakage. Flow rate was then raised to 500 ml/min. After all the water had been filtered, air was pumped through the filter to empty it. Tube was disconnected from filtering module. Elution procedure was tested by adding 20 ml of elution buffer containing 3 % beef extract (w/v) and 0.5 M glycine, adjusted to pH 9.5 with 5 M sodium hydroxide

to MAF column. Elution was carried out with 20 ml syringe and elution solution was collect to sterile 100 ml Pyrex bottle (figure 11).

FIGURE 11. Elution with 20 ml syringe: 1) 20 ml syringe, 2) filtering module and 3) elution solution inside the syringe

Short piece of tubing was used as a seal between syringe and filtration module. Elution buffer was pumped at a flow rate of approximately 20 min/ml. The flow rate was attempted to get as close to 20 ml/min using 60 seconds timer. To make sure that the filter was empty as much as possible, air was pushed through the filter with syringe.

3.4 Scanning electron microscopy

Scanning electron microscope pictures were taken in Tampere University of Technology's Laboratory of Materials Science. Pictures were taken with Zeiss ULTRAplus Ultra high resolution field emission scanning electron microscope (shown in figure 12).

FIGURE 12. Zeiss ULTRAplus Ultra high resolution field emission scanning electron microscope (Tampere University of Technology 2014)

SEM was used to characterize the structure of the monolithic column. The aim was to study how homogenous the column's porous structure was and see was there any structural differences between monolithic column hydrolyzed with sulfuric acid and one that was not hydrolyzed. Two samples were made by cutting small piece of monolithic disk with scalpel. Samples were taken from two different disks: one that was activated with sulfuric acid and other that was not.

Because synthesized monolithic disks were stored in methanol and hydrolyzed disks in Milli-Q water, the disks were dried for three days in fume hood. Two samples were as follows: sample 1 was hydrolyzed monolithic column and sample 2 was non-hydrolyzed monolithic column. Samples were glued on the aluminum stud and were sputter-coated with gold before SEM analysis.

4 RESULTS

4.1 Characterization of the monolithic column

Disk-shape monolithic columns were prepared in a specially made mold. Successful reaction product was a white disk approximately 1 cm thick. It had a sponge-like composition. Disks were quite soft and easy to break. Two of the disk were broken when they were bend too much while they were detached from the mold. After the reaction the disk was same size as the mold but after methanol wash and storage the disk's diameter was reduced approximately by 1-2 mm. Three of the column preparations failed because the disk was really thin and it seemed that only the part of the chemicals reacted. Overall 16 disks were made in this work and only three of them failed which means over 80 % of reactions were successful. SEM was used to characterize the inner structure of the monolithic column. Figure 13 shows image taken from the hydrolyzed monolithic column with SEM.

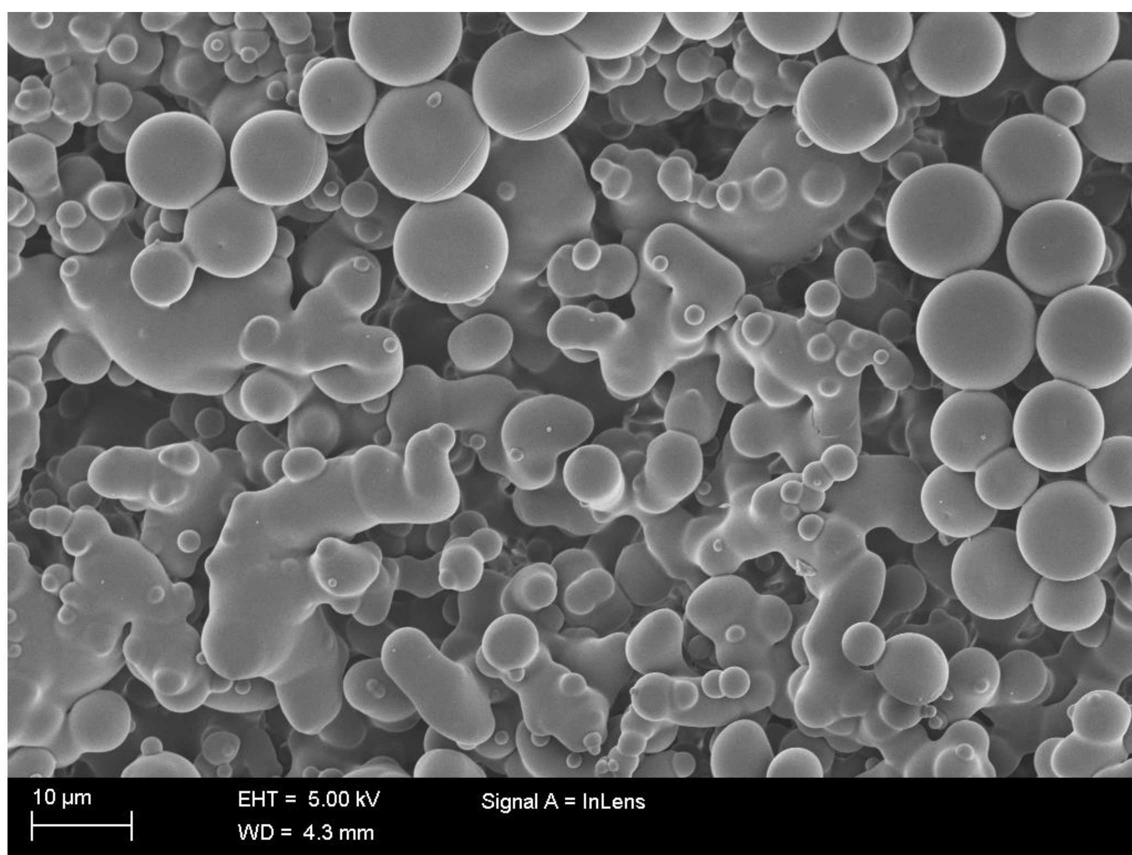

FIGURE 13. SEM image of the inner structure of the hydrolyzed monolithic column

Figure 13 shows that the structure is porous and the porosity is high. Figure (13) also shows that the column's monolithic structure was inhomogeneous. SEM was also used to study structural differences between hydrolyzed (section A) and non-hydrolyzed (section B) monolithic column (figure 14).

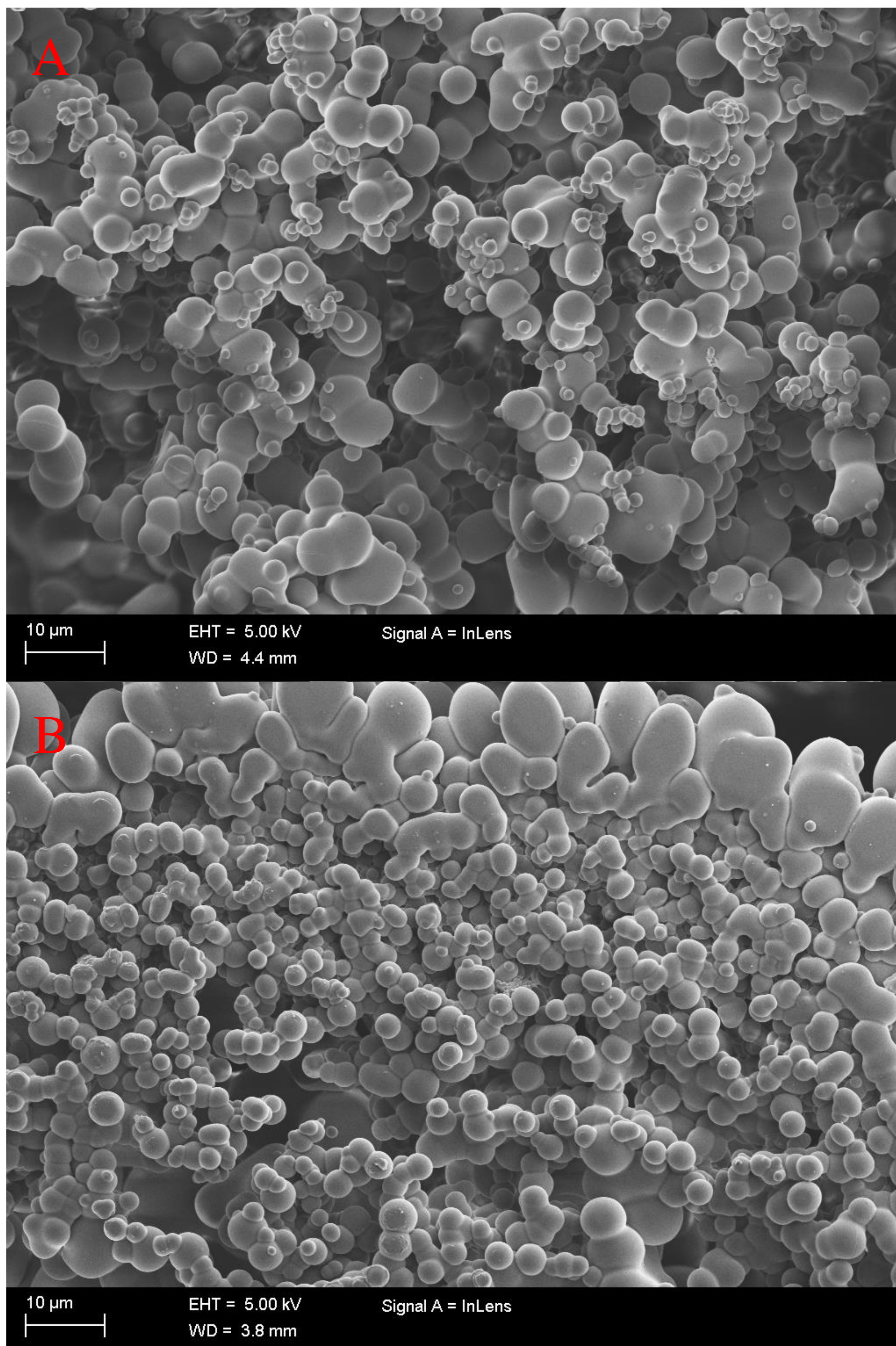

FIGURE 14. SEM image of the monolithic columns (A) hydrolyzed (B) non-hydrolyzed

Figure 14 shows that column's inner surface structure was not changed during hydrolysis. Column's structure was not damaged by sulfuric acid treatment. Structures do not look completely similar because samples were taken from two different disks. Images were also taken from small area of the column. Structure of the column can vary within the column.

4.2 Filtering method

One of the purposes in this work was to test how the prepared monolith column works on different water samples. Column was tested with two different water samples and filtering procedures. Both 1 l and 10 l filtering experiments were successful so that the whole water sample was filtered without the filter being blocked or decreased flow rate. Even though at the beginning of the first filtering test the water sample was pumped through the column at a flow rate of 1000 ml/min. At that flow rate it seemed that the column was slightly flattened and for that reason the flow rate was lowered to 500 ml/min.

Few improvements were made after 1 l experiment. Elution method was the biggest improvement. Switching from tube to syringe when pumping elution buffer through the column was more convenient and time-saving method. Small amount of elution buffer remained in the tube even if air was pumped through the tube after elution. This problem was eliminated with syringe elution. Column was also emptier after elution when syringe was used.

Two changes were made to filtration module. The stainless steel pipe on the module was cut off half to make it easier to handle the module. Tip of the module was also cut off at approximately 1 cm length. This made the tube connecting easier and widened the hole at the bottom of the module which may reduce pressure during pumping. During the 10 l surface water test, the monolithic column was not clogging. Column was just slightly stained. This suggest that larger volumes of surface water can be filtered using this method.

5 CONCLUSION AND DISCUSSION

The objective of this work was to prepare monolithic adsorption filter (MAF) and characterize its structure. The purpose of this work was to produce monolithic column and characterize its structure with scanning electron microscope. Thesis laboratory work included preparation of monolithic column together with testing method for filtration of water samples with the column.

The monolithic column production was successful although at first there were some problems with the reaction and some of the disks were too thin and the reaction was incomplete. Problems were fixed by reducing the mixing time from 60 second to 45 seconds and using a fresh (not older than 24 hours) initiator solution. Mixing time was reduced because reaction started quickly after monomer was added and solution started to precipitate during mixing. Disks were made one at the time because only one mold was made. In future making more molds would be preferred because it will take less time to make more disks.

SEM images showed that the column's monolithic structure was inhomogeneous. This could be due to too a high temperature during reaction. Even if reaction was carried out at room temperature, temperature may have been increased too much inside the reaction volume. This is caused by the exothermic nature of the polymerization reaction. In future the reaction temperature should be monitored. Monolithic structure could also be characterized in terms of porosity in the future. Porosity of the monolith could be determined with the mercury intrusion porosimetry (Peskoller et al. 2009). In research of Bereli et al. (2005) surface area and elemental analysis of the monolith were studied. These tests could also be done in the future to further characterize the produced monolith.

Sulfuric acid activation was performed by two different methods: activating disk in sulfuric acid bath and recirculating sulfuric acid through the disk. Acid bath activation was easier to execute even though acid bath bottle needed to be stirred every 30 minutes. During recirculation the disks were not below the acid surface and it was unclear, was the whole disk activated evenly. In the acid bath the whole disk was in contact with sulfuric acid all the time. Thus, acid bath would be better method for sulfuric acid activation. Although methanol was washed off with same equipment as recirculating was performed

this could be replaced by washing methanol off by the same way as the organic solvents were washed after the reaction.

The other purpose was to test how the produced monolith column works on different water samples. MAF was working without any clogging when filtering 10 l surface water sample. Column was not clogging which suggested that even larger water sample could be filtered. Although surface water from lake Roine was not very turbid so testing with more turbid surface water is preferred in the future. Elution method was improved by pumping the elution buffer with syringe. This saved some time and made the elution easier. Elution could be improved further by figuring out way to pump elution buffer without need to disconnect the tubing after filtration.

All in all, a working method for monolith column production was elaborated during this work. The objective was achieved and this thesis gives a reasonable basis for further research. Filtration was working without problems but more testing is needed with different sources of water. In the future monolith column could be tested by filtration of various pathogens.

REFERENCES

Arrua, R. D., del Barrio, J. S. R., Ruiz, A. G., Strumia, M., Igarzabal, C. I. A. 2008. Preparation of polymeric macroporous rod systems: Study of the influence of the reaction parameters on the porous properties. Materials Chemistry and Physics 112 (3), 1055-1060.

Bereli, N., Uzun, L., Yavuz, H., Elkak, A. and Denizli, A. 2006. Antibody purification using porous metal–chelated monolithic columns. Journal of Applied Polymer Science 101 (1), 395-404.

Haas, C. N., Rose, J. B., Gerba, C., Regli, S. 1993. Risk assessment of virus in drinking-water. Risk Analysis 13 (5), 545-552.

Janco, M., Sykora, D., Svec, F., Frechet, J., M. J., Schweer, J., Holm, R. 2000. Rapid determination of molecular parameters of synthetic polymers by precipitation/redissolution high-performance liquid chromatography using "molded" monolithic column. Journal of Polymer Science: Part A: Polymer Chemistry 38 (15), 2767-2778.

Jungbauer, A. & Hahn, R. 2008. Polymethacrylate monoliths for preparative and industrial separation of biomolecular assemblies.
Journal of Chromatography A 1184, (1-2), 62-79.

Karthe, D., Behrmann, O., Blättel, V., Elsässer, D., Heese, C., Ho, J., Hügle, M., Hufert, F., Kunze, A., Niessner, R., Ho, J., Scharaw, B., Spoo, M., Tiehm, A., Urban, G., Vosseler, S., Westerhoff, T., Dame, G. & Seidel, M. 2016. Modular development of an inline monitoring system for waterborne pathogens in raw and drinking water. Environmental Earth Science 75 (23), 148.

Kunze, A., Pei, L., Elsässer, D., Niessner, R., Seidel, M. 2015. High performance concentration method for viruses in drinking water. Journal Virological Methods 222, 132-137.

Michen, B. & Graule, T. 2010. Isoelectric points of viruses. Journal of Applied Microbiology 109 (2), 388-397.

Pei, L. 2015. Monolithic adsorption filtration (MAF)-Based Methods for Concentrating Viruses from Water. Technical university of Munich. Faculty of Chemistry. Doctoral thesis.

Peskoller, C., Niessner, R., Seidel, M. 2009. Development of an epoxy-based monolith used for the affinity capturing of Escherichia coli bacteria. Journal of Chromatography A 1216 (18), 3794-3801.

Podgornik, A., Yamamoto, S., Peterka, M., Krajnc, N. L. 2013. Fast separation of large biomolecules using short monolithic columns. Journal of Chromatography B 927, 80-89.

Schiff, G. M., Stefanovic, G. M., Young, B., Pennekamp, J. K., 1984. Minimum human infectious dose of enteric viruses (echovirus-12) in drinking water. In Enteric viruses in water, Melnick, J. L., Ed. Karger: Basel, Switzerland, Vol. 15, 222-228.

Tampere University of Technology. 2014. Scanning electron microscopy. Updated on 01.09.2014. Read on 16.04.2018.http://www.tut.fi/en/research/research-fields/materials-science/research-equipment/microscopy/sem/index.htm

Wheeler, B.P & Wilson, L.J. 2008. Practical forensic microscopy: a laboratory manual. Hoboken. NJ Chichester. England: Wiley.

WHO. 2011. Guidelines for drinking water quality. 4th edition. Malta: Gutenberg.