Journal of Chromatography A, 1381 (2015) 118–124

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Methacrylate monolith chromatography as a tool for waterborne virus removal

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ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form
12 December 2014
Accepted 1 January 2015
Available online 10 January 2015

Keywords:
Monolith chromatography
Waterborne
Virus
Removal
Wastewater
qPCR

ABSTRACT

Enteric viruses are commonly present in environmental waters and represent the major cause of waterborne infections and outbreaks. Since traditional wastewater treatments fail to remove enteric viruses in the water purification process, they are released daily into environmental waters. Monolithic supports have enabled chromatography to enter the field of virology. They have been successfully used in virus purification and concentration. In this work quaternary amine (QA) methacrylate monoliths were exploited to remove enteric viruses from wastewater treatment plant effluent. Expectedly, chromatographic processing of such a complex medium was troublesome, even for monoliths, characterized by extremely large pore dimensions. This problem was solved by introducing a pre-step chromatography using hydroxyl (OH) methacrylate monoliths. This way, molecules, that would hinder virus binding to the anion-exchanger monolith, were removed. As a result, the OH pre-column reduced backpressure increase on the subsequent anion-exchanger column, and increased both QA column binding capacity and life time. Wastewater effluent samples were successfully purified from five waterborne enteric viruses (rotavirus, norovirus genogroup I and II, astrovirus, sapovirus), below the detection limit of RT-qPCR. The breakthrough of the rotavirus binding capacity was not reached for concentrations that significantly exceeded those expected in effluent waters. The obtained results confirm that methacrylate monoliths can be a valuable tool for simultaneous removal of different waterborne viruses from contaminated water sources.

1. Introduction

Enteric viruses are a major cause of waterborne infections and outbreaks [1,2]. The viruses are shed via feces from infected individuals at concentrations up to 10^{13} virus particles per gram of stool [3], leading to the release of large amounts of viruses into raw sewage. Despite wastewater treatment, infectious viruses are released daily into the environment through the discharge of treated water and biosolids [4,5]. The lack of viral envelope increases the resistance of enteric viruses to environmental inactivation [6,7]. This helps them to stay infective for longer time periods thus finding their way into recreational, irrigation and even drinking waters [5]. Even highly diluted enteric viruses still pose a threat to humans due to their extremely low infectivity dose. In some cases 10 ingested virus particles were suggested to be enough to cause an infection [8].

Water (drinking and wastewater) treatment usually consists of a series of barriers or steps to remove contaminants from water [9,10]. During these steps virus concentration is reduced by physically removing viruses or by inactivating them with different physicochemical treatments. Separation of viruses from contaminated waters traditionally consists of virus flocculation and their settling out by gravity and pressure driven membrane processes like filtration, ultrafiltration, nanofiltration and reverse osmosis [9]. On the other hand, chlorination, ozonation and UV-light irradiation represent virus inactivation treatments, where virus infectivity is reduced or abolished by modification of viral surface proteins or its genome [10–12].

Due to the small size of viruses the efficiency of virus physical removal procedures is often poor; thus, disinfection by

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http://dx.doi.org/10.1016/j.chroma.2015.01.003

physicochemical inactivation is typically the main and most efficient barrier for the viruses [9] but it is not without shortcomings. Disinfection efficiency can be highly dependent on the water parameters (pH, salt concentration, turbidity, etc.) and contact time. Moreover, chemicals like chlorine used as disinfectants can react with naturally occurring materials in drinking waters to form by-products detrimental to human health [13].

Viral pollution in treated water is highly dependent on the water source and water treatment [14]. Changes of natural fresh water distribution due to climate change and human activities in both developing and industrialized countries are responsible for ever scarcer clean water sources [15]; therefore, drawbacks of existing treatments are more evident, pushing the need for new and specialized steps to implement existing waterborne virus removal strategies, that will enable safe and efficient water reuse [15,16].

Monolithic chromatography columns traded by the name Convective Interaction Media (CIM) [17] have presented a breakthrough in fast and efficient separation and concentration of large biomolecules. Highly porous material with interconnected flow-through channels (average diameter of 1.5, 2 or even 6 μm) enables convective mass transport of the molecules (in contrast to the diffusive transport of bead based supports), leading to flow independent dynamic binding capacity and separation [18,19]. Anion exchange monoliths (QA and DEAE) have proven to be very effective for fast separation or concentration of different viruses such as tomato mosaic virus [20,21], potato virus Y [22], orthoreovirus [23], rotaviruses [24,25], hepatitis A virus and caliciviruses [26], rubella virus [27], influenza virus [28], adenovirus [29], adeno-associated virus [30], lenti virus [31] bacteriophages [32–34] and virus-like particles [35,36]. They have also been successfully applied to the concentration of waterborne viruses [25,26] where fast flow rates and large binding capacity are essential.

The present study evaluates CIM monolithic columns for their waterborne virus removal capabilities. Their removal performance was evaluated using effluent from wastewater treatment plant and monitoring five different enteric viruses (rotavirus, norovirus genogroup I and II, astrovirus, sapovirus) that occur in those waters. During the experiments the CIM based removal method was optimized with the purpose to prolong column life time, to increase virus binding capacity and to lower pressure increase during sample processing.

2. Materials and methods

2.1. Samples

Samples (5–10 L) of wastewater treatment plant effluent (effluent) were obtained from local wastewater treatment plant (Central Wastewater Treatment Plant Domžale-Kamnik, Ihan, Slovenia). The tap water sample was taken from the tap in National institute of biology (Ljubljana, Slovenia). After sampling, samples were stored at 4 °C until processing.

2.2. Chromatography columns and instrumentation

Chromatography experiments were performed on CIM monolithic columns (BIA Separations, Ajdovščina, Slovenia) of quaternary amine (QA) chemistry. All experiments were performed on CIM 8 ml tube monolithic columns, except binding capacity experiments that were performed on CIM disk monolithic columns (bed volume of 0.34 ml). Some samples (wastewater effluents) were subjected to a pre-column purification using a hydroxyl (OH) CIM 8 ml tube monolithic column. Before each run, columns were equilibrated with equilibration buffer and after the run regenerated according to the manufacturer's instructions. Columns were mounted on a FPLC system ÄKTA Purifier 100 (GE Healthcare, Uppsala, Sweden) equipped with P-900 pumps and a UV/conductivity detector UPC-900. The software used for control of the equipment and data acquisition was Unicorn 5.11 (GE Healthcare, Uppsala, Sweden). The pH of each loaded sample before the chromatography run was measured using SevenMulti pH meter (Mettler Toledo, Schwerzenbach, Switzerland).

2.3. Virus RNA isolation and detection

Viral RNA from different samples and chromatographic fractions was isolated using the QIAamp Viral RNA Kit (QIAGEN, Foster city, CA, USA) according to the manufacturer's instructions. Purified RNA was eluted with 45 μl of molecular grade RNAse-free water (Sigma, Steinheim, Germany). Extraction buffer was spiked with (2 ng per sample) luciferase RNA (Promega, Madison, WI, USA) prior to RNA isolation and served as an extraction control [37]. A negative control (ultra pure reverse osmosis filtered water) for the extraction procedure was included in each isolation round. Isolated viral RNA was stored at −20 °C. RT-qPCR assays for detection of enteric viruses used in this study were all previously described: rotaviruses (RoV) [38]; norovirus genogroup I and II (NoV-I and NoV-II) [39]; astrovirus (AsV) and sapovirus (SaV) [40]. RT-qPCR reactions were done using AgPath-ID™ One-Step RT-PCR kit (Life Technologies, Foster city, CA, USA) and the 7900HT Fast Real-Time PCR system or the StepOne™ real-time qPCR device (both from Applied Biosystems, Foster city, CA, USA). The thermal cycling conditions were as recommended in the AgPath kit manual. RTqPCR results are represented in quantification cycles (Cq) that are inversely proportional to the concentration of nucleic acid target in the sample. The difference of 1 Cq between measurements represents a 2-fold difference in target copies. Therefore, 10-times difference in nucleic acid target copy number is represented as 3.32 Cq difference between samples.

2.4. Virus removal

CIM QA monolithic column capabilities for waterborne virus removal were tested with effluent samples collected monthly during one year (from April 2012 to May 2013). During effluent sample preparation larger particles were removed by Wathman paper filtration followed by filtration through low protein binding 0.8 μm cellulose acetate filter (Sartorius Stedim Biotech, Goettingen, Germany).

First two samples (April and May 2012) were buffered to a final 50 mM HEPES, pH 7. June sample was tested twice: once buffered and once unbuffered. Since unbuffered sample performed excellently, subsequent samples were processed without buffering the effluent.

Flow rate during sample loading on a CIM QA 8 ml tube monolithic column was adjusted so that back pressure did not increase over the 2 MPa limit and ranged from 80 to 30 ml/min depending on the number of cycles the column went through. Volume of loaded samples was between 4500 and 5000 ml. Aliquots of loading material and flow-through fraction of the processed effluent samples were collected and analyzed for the presence of nucleic acids from RoV, NoV-I, NoV-II, AsV and SaV.

2.5. CIM OH pre-column

Effluent samples were filtered as described in Section 2.4 and split into two aliquots of 5 L. One aliquot was directly processed on a CIM QA monolithic column and the second was first passed through CIM OH monolithic pre-column and the flow-through was then processed on CIM QA monolithic column. Two different CIM QA monolithic columns were used for CIM OH treated and non-treated effluent samples. All CIM OH treatments were done on the

same CIM OH monolithic column. Flow rate was 60 ml/min. Loading volume per each run was 4500 ml. Pressure and flow-through were monitored throughout the runs.

2.6. Dynamic binding capacity

The dynamic binding capacity (DBC) of CIM QA monolith for rotavirus (RoV) was measured using different samples. A RoV clarified suspension was derived from routine rotavirus positive clinical stool samples collected at the Institute for Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia. Virus concentration was estimated using electron microscopy and latex particle counting (particles were of known size and concentration) with a JEM 1200 EXII instrument (Jeol, Tokyo, Japan). The estimated rotavirus concentration was 9.87×10^{10} particles/ml. Effluent, buffered effluent (50 mM HEPES, pH 7), CIM OH pretreated effluent, and tap water were filtered through 0.8 μm cellulose acetate filter (Sartorius Stedim Biotech, Goettingen, Germany) and spiked with RoV clarified suspension to a final concentration of 8×10^8 RoV particles/ml. Each spiked sample was loaded on CIM QA disk with a flow rate of 4 ml/min. Using Fraction Collector F-920 (GE Healthcare, Uppsala, Sweden), the flow-through was divided into 5 ml fractions that were later analyzed for the presence of RoV. Last negative fraction was considered as breakthrough point, and the capacity of rotavirus particles/ml of monolithic support was calculated accordingly.

2.7. Mechanism of virus removal

An effluent sample naturally containing rotaviruses was filtered through Whatman paper and 0.8 μm cellulose acetate filter and then purified first using CIM OH pre-column and afterwards using CIM QA column. For both columns flow rate of 60 ml/min was used. Everything that was bound to the QA columns during purification run was eluted with ca. 15 ml of elution buffer (1 M NaCl, 50 mM HEPES, pH 7) at a flow rate of 20 ml/min. Chromatography fractions were collected at each step of the experiment and analyzed by RT-qPCR for the presence of rotavirus.

2.8. Comparison between CIM monolithic column and Aquafilter

Effluent water sample (10 L) was filtered through Wathman paper and split into two 5 L aliquots. Both were spiked with RoV clarified suspension to a final concentration of 8×10^8 RoV particles/ml. 4.5 L of the first aliquot was processed with Aquafilter family (The Safe water trust ltd, UK) and operated according to the instruction's manual. The 4.5 L of the second aliquot was processed with a CIM removal method, including CIM OH pre-column: the sample first passed through 0.8 μm cellulose acetate filter, then through CIM OH 8 ml column and finally it was processed on CIM QA 8 ml column. Flow rate was 60 ml/min. Aliquots of the effluent, spiked effluent, and purified sample were collected for isolation of RNA and RT-qPCR analysis.

3. Results and discussion

3.1. Virus removal in effluent from WWTP

Effluent waters were chosen specifically as a specially challenging sample to process because of their chemical and biological diversity. Most of the raw effluent samples contained three of the five viruses that were monitored, three samples contained four viruses and one of them (October sample) contained all five viruses. The most prevalent was RoV which was present throughout all the samples. Undetectable signal for the presence of RoV, NoV-I, NoV-II, SaV and AsV in majority of the CIM QA monolithic column purified samples compared to raw (unpurified) ones, demonstrating that CIM QA monoliths are capable of enteric virus removal from wastewater treatment plant effluent samples (Table 1). As a starting point for the development of a CIM based virus removal method, the same buffer and chemistry conditions previously optimized by Gutierrez Aguirre et al. [24] for the binding of RoV from buffered environmental waters were selected: CIM QA monolithic column and 50 mM HEPES, pH 7 as a binding buffer. Consequently, purification of the first two samples (May and June) was done using buffered conditions. However, in a real scenario, if purification of large water volumes is pursued, buffering would be too expensive and added salts would introduce unwanted chemicals into the purified water. Therefore, the July sample was subjected to comparison: efficiency of CIM monolithic column virus removal from buffered and unbuffered effluent. No difference in performance between the two samples was observed (Table 1) and therefore it was concluded that buffering of the sample can be omitted. Measurements of pH and conductivity, both being parameters that influence virus binding ability to the CIM QA columns, additionally confirmed that no buffering is needed. pH of the effluent remained slightly basic throughout the year with minimal pH at 7.13 and maximal pH at 7.83 (excluding the samples from May and June that were buffered). Conductivity of the samples was between 0.87 mS/cm and 1.49 mS/cm. Both parameters indicate that conditions in the

Table 1
Performance of virus removal in effluent samples throughout one year.

Sample		May[a]	Jun[a]	Jul[a]	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Consecutive no. of use of the column		1	2	3	4	5	6	7	8	9	1	3	4	1
pH		7.00	7.07	7.02	7.48	7.33	7.13	7.42	7. 34	7.41	7.34	7.53	7.63	7.83
Cond. (mS/cm)		1.25	1.12	1.31	0.87	1.04	1.09	0.91	1.00	0.98	1.49	0.92	0.99	1.00
Raw	RoV	++++	+++	+++	+++	+++++	+++++	++	+++	++++	+++++	+++++	+++++	+++
	NoV-I	++	–	–	–	–	+++	+++	++	++++	+++	–	+	+++
	NoV-II	–	–	++	++	+++	–	++	–	–	–	–	–	–
	AsV	++	++	–	–	–	–	++	–	+++	++++	+++	++	+++
	SaV	–	+++	++	++	+++	+++	+++	+++	++++	++++	+++	+++	+++
Purified using CIM QA	RoV	–	–	–	–	–	++++	–	–	+++	–	–	–	–
	NoV-I	–	–	–	–	–	–	–	–	+	–	–	–	–
	NoV-II	–	–	–	–	–	–	–	–	–	–	–	–	–
	AsV	–	–	–	–	–	–	–	–	–	–	–	–	–
	SaV	–	–	–	–	–	++	–	–	-	–	–	–	–

For easier interpretation Cq values were clustered together: $29 \leq Cq < 32.3$ = +++++; $32.3 \leq Cq < 35.6$ = ++++; $35.6 \leq Cq < 38.9$ = +++; $38.9 \leq Cq < 42.2$ = ++; $Cq \geq 42.2$ = + the span between the beginning of two consecutive clusters is 3.3 Cq which corresponds to approximate difference of one order of magnitude; Cq of 42.2 means approximately 1–10 virus copies in the reaction, – = not detected; RoV = rotavirus; NoV-I = norovirus group I; NoV-II = norovirus group II; AsV = astrovirus; SaV = sapovirus.

[a] Buffered samples; raw – unpurified sample.

effluent are in favor of the CIM QA removal method since enteric viruses are negatively charged in near neutral solutions [41] (QA is positively charged and binds negatively charged particles) and conductivity is very close or below the conductivity of buffered samples (Table 1).

None of the 5 monitored viruses were detected in the purified effluent samples with the exception of samples from September and December (Table 1).

It was observed that after certain number of purification cycles performed on the same QA column, despite regeneration with 1 M NaOH after each cycle, removal performance of CIM QA column decreased. This is probably the reason for the presence of virus signal in purified September sample, for purification of which the column was already used six times (Table 1). After occurrence of this first virus signal, the CIM QA column was not excluded from the tests. It was used for purification of three more samples (Oct., Nov. and Dec.). Concentration of virus load in October and November samples was lower compared to September or December; for this reason column performance seemed to be unaffected (Oct and Nov.), but as soon as virus load became higher (Dec.) the virus signal was observed again. With increasing removal-regeneration cycles, an increasingly faster build-up of backpressure was observed during the purification runs, which ultimately led to a decline of the virus removal performance, indicating that the column life time was coming to an end. In response to this, whenever the rate of pressure build-up started to increase during the purification the column was substituted with a new one. From this moment on virus signal was never observed again in any of the processed samples independently of the virus load (Table 1).

3.2. Solving back pressure issue

The reason for the pressure build-up is likely due to particles or compounds that bind progressively and nonspecifically to the column and are not removed during the regeneration of CIM columns, resulting in more pressure build-up every successive run. This ultimately leads to the inability to bind all virus particles present in a given sample, i.e., the virus signal observed in September and December. First attempt to solve the issue was to use a CIM QA column with bigger average pore diameter, 6 μm instead of 1.5 μm. The back pressure build-up was removed completely, but virus removal capability was poor (results not shown), probably due to a smaller surface area of the monolith with larger pores and therefore a different solution was necessary.

Effluent waters from wastewater plant contain a substantial amount of lipids [42], which, together with other potential molecules such as polysaccharides and nucleic acids, could be responsible for the pressure build-up observed during purification cycles due to nonspecific binding. Using a pre-column step has been shown to reduce interfering compounds and substances influencing the capacity of anion exchange monoliths previously [43]. Therefore, we decided to use this strategy by including a CIM OH monolithic pre-column in order to remove such compounds from the effluent and to assess the effect on the pressure build-up observed in the subsequent CIM QA monolithic column. The back pressure build-up generated during virus removal with CIM QA in the presence and absence of a CIM OH pre-column was compared. In the absence of a CIM OH pre-column, effluent samples generated a substantial backpressure increase already in the initial run (Fig. 1). After passing 3700 ml of sample through the CIM QA column, flow rate had to be decreased from 60 ml/min to 50 ml/min in order to stay below the recommended 2 MPa backpressure limit (Fig. 1A). Despite regeneration, the back pressure in subsequent runs increased even faster. Several reductions of flow rate per run were necessary to maintain the pressure below the limit. After 9 runs, the QA column, used to remove viruses from CIM OH non-treated samples, could no longer retain viruses (purified sample was positive for RoV with RT-qPCR). On the other hand, in the presence of a CIM OH pre-column step, samples generated very little backpressure increase, even after 9 runs. No flow rate reduction was necessary in any of the performed runs (Fig. 1B) and most important, virus removal performance remained unaltered (no virus detected in purified sample). This confirmed that inclusion of a CIM OH pre-column substantially decreases the backpressure build-up problem and prolongs the life time of the CIM QA monolithic column used for virus removal. During the CIM OH step itself, no back pressure increase was observed (Fig. A.1).

3.3. Capacity

To assess the impact of buffering the effluent and including a CIM OH pre-column step on the virus removal efficiency, the capacity of CIM QA for RoV particles was measured with three RoV spiked samples (non buffered effluent, buffered effluent and non-buffered effluent including CIM OH pre-column step) as described in Section 2.6. DBC was determined to be 3.06×10^{11} for non-buffered effluent, 3.88×10^{11} for buffered effluent and 4.94×10^{11} RoV particles/ml for non buffered effluent including CIM OH pre-column step. Although no qualitative difference was detected in the virus removal performance when processing in parallel buffered and unbuffered effluent from July (Table 1), the capacity measurements revealed that buffering of the effluent sample increases the binding capacity by 27% compared to unbuffered effluent (Table 2). The difference is non-negligible, but as mentioned above (Section 3.1), adding salts to the water is not acceptable for a water purification method. The inclusion of a CIM OH pre-column step overcomes this situation by allowing a 60% increase in the RoV binding capacity of CIM QA monolith even in the absence of buffering, compared to the raw effluent. Moreover, no chemicals are added to the sample. Dynamic binding capacity of CIM QA monolith for RoV in tap water was also measured as an indication of the method performance in more defined and pure water samples. The breakthrough of the RoV in tap water sample was not reached with the available sample, but the obtained values (>9.41×10^{11} RoV particles/ml of monolith) exceeded by far the values obtained in effluent waters (more than 3× times compared to non-treated effluent waters), confirming that effluent waters contain a lot of molecules that compete with viruses for binding sites on CIM monolithic supports.

Table 2
Binding capacity of CIM QA monolithic column for rotavirus in different water samples.

Sample	Volume at the last negative fraction (ml)	Capacity of the QA disk (RoV par./disk)	Relative capacity of QA monolith (RoV par./ml of monolith)	Comparison of capacities between samples relative to effluent	Theoretical volume of water that can be purified[b] (L of water/ml of monolith)
Effluent	130	1.04×10^{11}	3.06×10^{11}	1.00	306
Buffered effluent	165	1.32×10^{11}	3.88×10^{11}	1.27	388
OH column pre-step	210	1.68×10^{11}	4.94×10^{11}	1.62	494
Tap water[a]	Not breached	>3.20×10^{11}	>9.41×10^{11}	>3.08	>942

[a] After 400 ml of sample loaded, capacity was not breached. All the values for this sample are given for 400 ml of loaded sample.
[b] Considering that water sample contains on average 10^9 RoV particles/L.

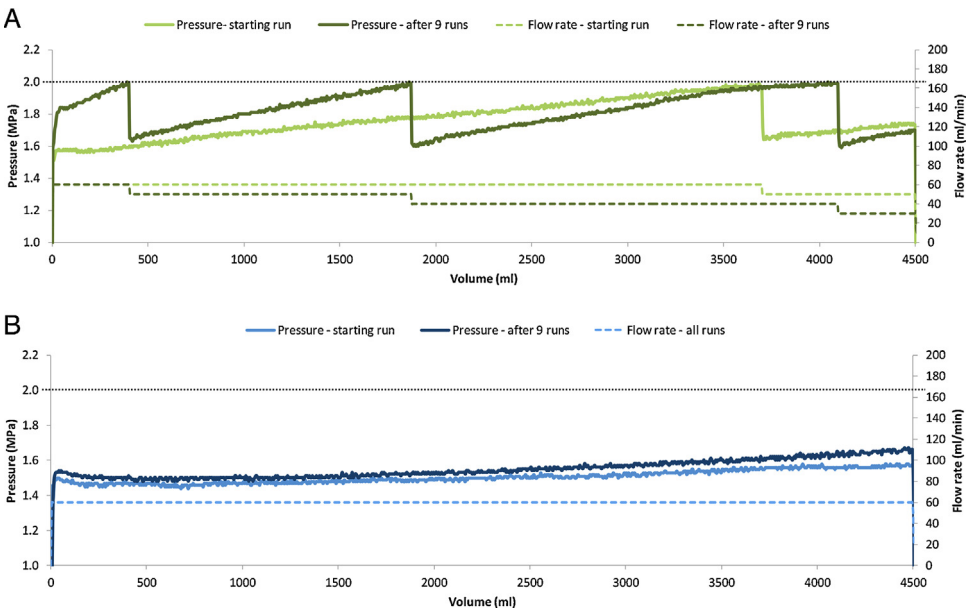

Fig. 1. Impact of CIM OH pre-column on the back pressure build-up during waste water effluent purification with CIM QA column. (A) No CIM OH pre-column step; (B) with CIM OH pre-column step; dotted line denotes maximal allowed pressure for the CIM 8 ml column, if it was reached, flow rate was decreased for 10 ml/min.

Table 3
Dissection of monolith waterborne virus removal procedure.

Step of the procedure	Detected rotavirus by RT-qPCR
Raw effluent	+++
After pre-filtration[a]	+++
Flow through from CIM OH column	+++
Flow through from CIM QA column	–
Elution from CIM QA column	+++++

For easier interpretation Cq values were clustered together: 29 ≤ Cq < 32.3 = +++++; 32.3 ≤ Cq < 35.6 = ++++; 35.6 ≤ Cq < 38.9 = +++; the higher the Cq, the lower the virus concentration; the span between the beginning of two consecutive clusters is 3.3 Cq, which corresponds to approximate difference of one order of magnitude; – = not detected.

[a] After filtration by Whatman paper and cellulose acetate filter.

3.4. Mechanism of virus removal

Fraction analysis after each single step during effluent treatment clearly demonstrates that the main virus removal occurs on CIM QA column. Pre-filtration (Whatman paper, cellulose acetate filter) and CIM OH pre-column steps have little impact on virus concentrations during the process, whereas after the effluent passes the CIM QA column virus is no longer detected by RT-qPCR (Table 3).

The release (elution) of the viruses from the column (after the removal process), upon washing the column with buffer with high salt (NaCl) concentration, proves that viruses were bound due to electrostatic interactions (Fig. 2 and Table 3).

3.5. CIM vs. tangential flow filtration

The final experimental procedure for the removal of viruses from waters based on CIM monoliths consists of pre-filtration and two CIM monolithic columns: OH chemistry, for back pressure increase reduction and longer life time of the QA column by depleting substances that bind non-specifically; and QA chemistry that binds and removes viruses. The waterborne virus removal performance of the CIM based procedure was compared to a commercially available tangential filtration system for water purification (Aquafilter family). Both Aquafilter and CIM showed similar performances and were able to reduce the RoV signal from spiked effluent sample for 4.88 log and 5.03 log, respectively. It should be noted that such high rotavirus concentrations as the one present in the spiked sample are not expected to occur naturally (Table 4).

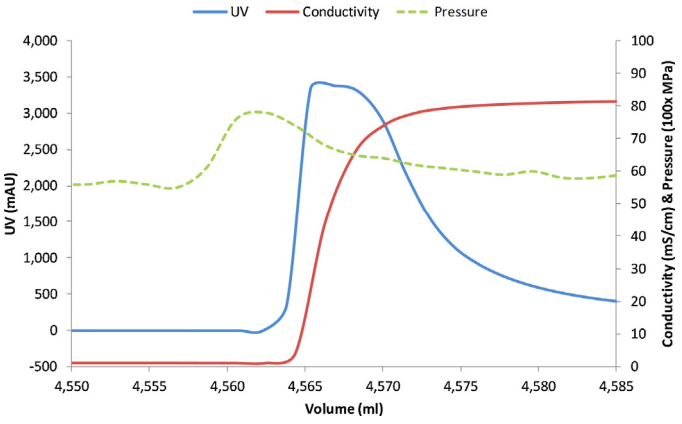

Fig. 2. After virus removal procedure viruses that were removed from the effluent can be eluted from the CIM QA column using buffer with high salt concentration. Flow rate was 20 ml/min.

Table 4
CIM vs. tangential flow filtration.

Sample	CIM OH-QA method RoV (Cq)	Aquafilter RoV (Cq)
Spiked effluent	24.1	23.8
Purified using CIM	40.8	40.0

Effluent was spiked with RoV clarified suspension to a final concentration of 8×10^8 RoV particles/ml (spiked effluent); n.d. – not detected; NK negative control of isolation; NTC – no template control; Cq – quantification cycle is an output of qPCR, and is inversely proportional to the concentration of nucleic acid target in the sample. The difference of 1 Cq between measurements represents a 2-fold difference in RoV genome copies. The difference of 3.32 Cq therefore represents 10-times difference in target copy number.

3.6. Conclusions

The present study demonstrated that CIM monoliths have great potential to be used for removal of waterborne viruses even in such a complex sample as the effluent from wastewater treatment plant.

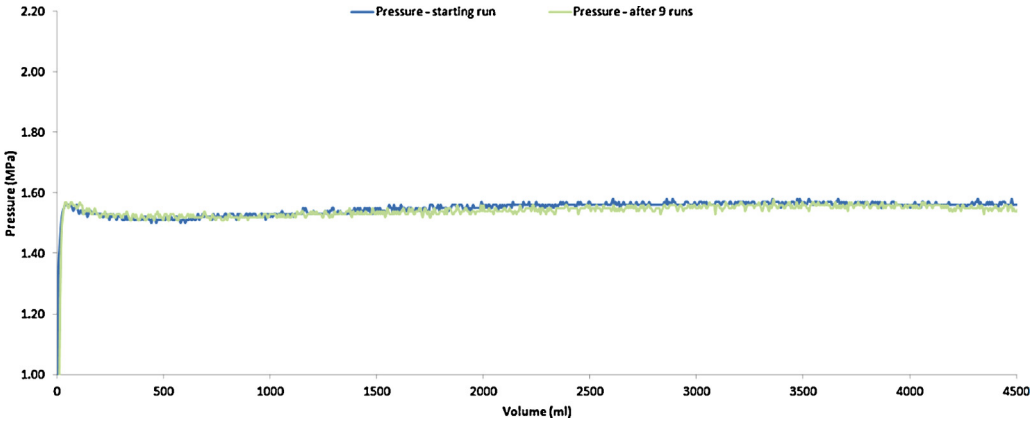

Fig. A.1. Monitoring of the back pressure during the CIM OH pre-column step. Flow rate was 60 ml/min.

The method was able to eliminate or significantly reduce the virus signal in water samples. Combination of different chemistries provides flexibility to adapt the CIM based virus removal procedure to a specific sample. This was demonstrated by combination of CIM OH and QA chemistry for effluent water sample. Moreover, comparison of CIM removal method with on-site use tangential filtration water purification device confirmed that CIM could be a standalone method. The method could have a great impact as a complement to existing purification methods where it would focus specifically on virus removal. It could be especially helpful in final polishing steps of water purification where other impurities are already removed and full capacity of CIM monolithic columns for viruses would be used.

Acknowledgments

The authors are grateful to Gill Griffith from the Safe Water Trust Ltd., UK, for providing the Aquafilter family. We also thank Barbara Brajer Humar and Marjeta Stražar from the Domžale-Kamnik central wastewater treatment plant for providing the wastewater effluent samples. This study was financially supported by the Slovenian Research Agency (contract no. L2-4314 and 34504).

Appendix A.

Fig. A.1

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