RECOVERY OF ADENOVIRUS FROM TAP AND SURFACE WATER BY CROSSFLOW ULTRAFILTRATION: EXPERIMENTAL DETERMINATION AND XDLVO PREDICTIONS

Hang Shi a*, Irene Xagoraraki a, Kristin N. Parent b, Merlin L. Bruening c, Volodymyr V. Tarabara a

a Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI
b Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI
c Department of Chemistry, Michigan State University, East Lansing, MI

*presenter: shihang2@msu.edu; Ph: 734-355-4180;

Abstract

This study examines the recovery of enteric adenovirus HAdV 40 by crossflow ultrafiltration from several water matrices (deionized water, tap water and surface water) and interprets recovery data by elucidating the physicochemical mechanisms that control virus adhesion on surfaces. In accordance with predictions from the extended Derjaguin-Landau-Verwey-Overbeek theory, pre-elution recovery of HAdV 40, from deionized water was higher with the PEM-coated membranes than with CS-blocked membranes. Eluent containing sodium polyphosphate and tween 80 effectively disrupt electrostatic and hydrophobic interactions between the virus and the membrane, leading post-elution recovery close to 100%. Eluent composition is the most important factor for high virus recovery from complex water matrices. addition of ethylenediamine tetraacetic acid (EDTA) to the eluent greatly improves the elution efficacy even when organic carbon concentration was high.

Introduction

Waterborne diseases are caused by contaminated water containing pathogenic microorganisms. World Health Organization reports that contamination of the water supply is responsible for deaths of 840,000 people annually(WHO). human adenovirus (HAdV) is one of the viral pathogens in EPA contaminant candidate lists and is the second-leading cause of childhood gastroenteritis worldwide(Crabtree, Gerba et al. 1997, Mena and Gerba 2009, Fongaro, do Nascimento et al. 2013). Most cases of adenovirus-associated gastroenteritis were found to be related to HAdV serotypes 40 and 41(Jiang 2006). HAdV 40 and 41 have longer survival time in the environment (Enriquez, Hurst et al. 1995) and are resistant to UV irradiation(Thurston-Enriquez, Haas et al. 2003). Monitoring HAdV 40 and 41 is necessary to better understand their fate and transport in the environment and treatment systems and eventually to prevent HAdV outbreaks. However, the typically low concentration of HAdV reported in various natural water matrices makes detection challenging and highlights the importance of the concentration step for rapid and reliable detection of these viruses.
US EPA recommended method for virus concentration is VIRADEL which involves two steps: (1) adsorption of viruses on either electropositive or electronegative microfilter; and (2) elution of the adsorbed viruses off the filters by a solution with pH adjusted. Although virus recoveries by VIRADEL depend on the specific type of filter and eluent used, and can vary greatly as a function of water composition, the reported VIRADEL recoveries of adenoviruses are low compared with those of other viruses under similar conditions. For example, low elution efficiency of adenovirus (<3%) was reported by Gibbons et al. from NanoCeram filter though retention of >99% for adenovirus by such filter was reported in the same study (Gibbons, Rodriguez et al. 2010). Low recoveries of adenovirus are believed to result from its viral capsid structure: fibers associated with each penton base of the capsid can facilitate the physical entrapment of virus particles in the filter matrix, thus making adenovirus “elution-recalcitrant” (Gibbons, Rodriguez et al. 2010, Ikner, Soto-Beltran et al. 2011).

Low recovery of elution-recalcitrant viruses like adenoviruses by VIRADEL motivates the search for alternative concentration methods. An emerging new method of sample concentration is crossflow ultrafiltration (UF) which relies on size exclusion. Ultrafilter with pores smaller than effective size of viruses can serve as a barrier to ensure that viruses remain suspended in retentate during filtration, whereas water and low molecular weight compounds pass through the membrane. The crossflow should minimize membrane fouling and virion deposition onto the membrane. However, a large fraction of viruses may still be adsorbed on the membrane surface due to hydrophobic interaction leading to lower virus recoveries (Berman, Rohr et al. 1980, Morales-Morales, Vidal et al. 2003, Polaczyk, Narayanan et al. 2008, Ikner, Gerba et al. 2012, Pasco, Shi et al. 2014). To improve efficiency of virus concentration, UF membranes are typically coated with proteinaceous solutions (e.g. calf serum solution) that can block potential virus adsorption sites on the membrane and reduce virus adhesion (Berman, Rohr et al. 1980, Morales-Morales, Vidal et al. 2003, Hill, Kahler et al. 2007, Liu, Hill et al. 2012). Similar to VIRADEL, elution of virus adsorbed to UF membranes can be accomplished using special solutions (eluents). Eluents typically contain sodium polyphosphate (NaPP) and Tween 80 to disrupt electrostatic and non-ionic (hydrophobic) interactions between viruses and the membrane (Hill, Kahler et al. 2007, Polaczyk, Narayanan et al. 2008, Liu, Hill et al. 2012).

Crossflow UF has three major advantages over VIRADEL: (1) lower cost of UF filters than VIRADEL filters, (2) simultaneous concentration of multiple types of pathogens, (3) removal of low molecular weight compound which may inhibit culture/molecular assays. However, long preparation time and possible contamination during storage and transportation limit the practical application of calf serum blocked (CS-blocked) UF membranes especially where rapid response and field sampling are required (Hill, Polaczyk et al. 2005). To improve virus concentration by crossflow UF, we coated membrane with polyelectrolyte multilayer (PEM) film prepared via rapid (<1 h) layer-by-layer adsorption of polycations (chitosan) and polyanions (heparin). Compared with traditional CS-blocked membrane, such PEM-coated membrane showed higher recovery of bacteriophage P22 from both DI water and MBR effluent (Pasco, Shi et al. 2014).

The current study builds on this prior work and extends it to concentrate human pathogens (HAdV 40) from several water matrices (deionized water, tap water and surface water). The study examines the recovery of HAdV 40 by crossflow ultrafiltration and interprets recovery data by elucidating the physicochemical mechanisms that control virus adhesion on surfaces.
Both CS-blocked membrane and PEM-coated membrane were employed to study the effect of membrane surface properties on virus recovery.

**Materials and Methods**

**Water Samples**
DI water with a resistivity of 18.2 MΩ⋅cm was from Barnstead NANOpure System. Tap water was supplied by the East Lansing-Meridian Water and Sewer Authority (East Lansing, MI) and used immediately after collection. Samples of surface water were collected from Lake Lansing in October 2013 (fall) and April 2014 (spring). Surface water samples were filtered through 0.45 μm membrane (mixed cellulose esters, Millipore) immediately after collection, stored at 4 °C and used within one week. All samples were characterized in terms of pH, conductivity, total organic carbon (TOC) contents, and concentrations of key cations (Na⁺, K⁺, Mg²⁺, Ca²⁺).

**Virus Propagation, Purification and Characterization**
HAdV 40 was purchased from ATCC and propagated in A549 cell line. The concentration of propagated virus was ~10⁹ to 10¹⁰ copies/mL measured by quantitative real-time polymerase chain reaction (qPCR). After harvest, HAdV 40 was purified by rate-zonal centrifugation with CsCl density gradient. HAdV 40 from purified stock was used in all characterization tests.

Hydrodynamic diameter of HAdV 40 was determined by dynamic light scattering (DLS) technique. Electrophoretic mobility of HAdV 40 was measured by phase analysis light scattering (PALS) technique and then was used to calculate zeta potential. Surface energy components of HAdV 40 was determined by measuring contact angle of three probe liquids on virus lawn. Detailed information on virus propagation, purification and characterization was described in our published paper (Shi, Xagoraraki et al. 2016).

**XDLVO Energy of Virus-Membrane Interactions**
XDLVO energy profiles were calculated using the surface properties characterized for HAdV 40 and membranes. Membranes were characterized in our previous study (Shi, Xagoraraki et al. 2016). Details was described in our previous publication (Pasco, Shi et al. 2014, Shi, Xagoraraki et al. 2016).

**Membrane Modification**
Polyethersulfone (PES) membrane (Omega, Pall Corp.) with a MWCO of 30 kDa was used as the membrane support. To coat the membrane with a PEM film, 1 mg/mL heparin (HE) and chitosan (CHI) solutions were prepared in the 0.15 M NaCl (pH=5). The surface of the PES membrane was alternately exposed to each polyelectrolyte solution for 5 min with a 1 min DI water rinse in between. PEM film was prepared in 4.5 bilayers with HE as both initial and outmost layers. To block membrane with calf serum, the membrane was placed in a crossflow filtration unit and 500mL of 5% calf serum solution was circulated over the surface of the membrane overnight at the rate of 1.0 L/min at room temperature with no pressure applied. After blocking, the membrane was rinsed with DI water twice at the same rate for 10 min each.
**Virus Concentration and Recovery Tests**

Three different water types were evaluated: DI water, tap water and surface water. Prior to the virus concentration by UF, the membrane was compacted for 90 min at 40 psi. 1 L water sample was spiked with 1 mL of HAdV 40 stock (estimated virus concentration of \( \sim 10^6 \) to \( 10^7 \) GC/mL), pressurized using compressed N\textsubscript{2} gas and delivered to the membrane cell (CF042P, Sterlitech) via a peristaltic pump (model 621 CC, Watson Marlow Pumps Group) at the 2.0 L/min rate. The transmembrane pressure was maintained at \( \sim 20 \) psi. To calculate flux, the permeate mass was recorded using the digital balance in real time. Filtration was stopped when volume of water sample reduced to 100 mL.

After filtration, viruses were eluted off the membrane using a solution (eluent) containing 0.01% NaPP and 0.01% Tween 80. For virus concentration and recovery from lake water, another eluent containing 0.01% NAPP, 0.01% tween 80 and 0.01% EDTA was also employed. Elution was performed at the same crossflow rate as what was used during filtration but without the transmembrane pressure applied. Feed, permeate, retentate and eluate were all sampled for virus quantification. Samples were also taken during membrane compaction to ensure that there was no virus contamination of the feed water prior to the experiment.

**Virus Quantification and Recovery**

qPCR was used to determine HAdV 40 concentration. Virus pre-elution recovery \( (r_{\text{pre}}) \), post-elution recovery \( (r_{\text{post}}) \) were calculated using the following equations:

\[
\begin{align*}
  r_{\text{pre}} &= \frac{C_r V_r}{C_f V_f}, \\
  r_{\text{post}} &= \frac{C_r V_r + C_e V_e}{C_f V_f},
\end{align*}
\]

where \( C_f \), \( C_r \), \( C_e \) represent virus concentration in feed, retentate, eluate and permeate samples respectively and \( V_f \), \( V_r \), \( V_e \) are volumes of these samples.

**Results and Discussion**

**Water Sample Quality**

Water quality parameters for all three types of samples used in this study (DI water, tap water, surface water) are provided in Table 1. The biggest difference among the waters are significantly higher TOC and Ca\textsuperscript{2+} concentrations in the surface water samples than in other two types of water samples.
Table 1. Water Quality Parameters (Shi, Xagoraraki et al. 2016).

<table>
<thead>
<tr>
<th>Water source</th>
<th>pH range</th>
<th>Conductivity (μS/cm)</th>
<th>TOC (mg/L)</th>
<th>Cation concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Na$^+$</td>
</tr>
<tr>
<td>Deionized water</td>
<td>5.7 to 6.0</td>
<td>n/a$^a$</td>
<td>n/a$^a$</td>
<td>0.18 ± 0.00</td>
</tr>
<tr>
<td>Tap water</td>
<td>7.5 to 8.0</td>
<td>319.5 ± 27.6</td>
<td>1.1 ± 0.1</td>
<td>22.18 ± 2.33</td>
</tr>
<tr>
<td>Lake water (fall)</td>
<td>7.0 to 7.5</td>
<td>336.3 ± 12.7</td>
<td>7.5 ± 0.2</td>
<td>16.42 ± 2.53</td>
</tr>
<tr>
<td>Lake water (spring)</td>
<td>7.5 to 7.8</td>
<td>369.3 ± 32.0</td>
<td>9.4 ± 0.1</td>
<td>16.46 ± 2.60</td>
</tr>
</tbody>
</table>

$^a$ below detection limit.
Physicochemical Properties of HAdV 40

The number-based average hydrodynamic diameter of HAdV 40 in 1mM NaCl, pH 5.7 (unadjusted) is 94±3 nm with half width at half maximum (HWHM) of the distribution at 13.3±2.4 nm. The largest population of HAdV 40 has diameter at 95±4 nm (modal diameter).

The number-based average hydrodynamic diameter of HAdV 40 in tap water (pH 7.5~8.0) is 109±14 nm. The measured hydrodynamic diameters of HAdV 40 particles were in the range from 70 nm to 100 nm which is the typical size range of adenovirus reported(Wilhelmi, Roman et al. 2003, Kennedy and Parks 2009, Nemerow, Stewart et al. 2012). Polydispersity index of the HAdV suspension was ~0.06 in 1 mM NaCl, pH 5.7 and ~0.1 in tap water, indicating minimal aggregation in both background solutions.

In 1 mM NaCl, pH 5.7, electrophoretic mobility of HAdV 40 was measured at -1.7 ± 0.5 µm∙S⁻¹∙V⁻¹∙cm and zeta potential was -22 mV. Zeta potential decreased to -17.7 mV in tap water with electrophoretic mobility of -1.40 ± 0.2 µm∙S⁻¹∙V⁻¹∙cm.

Total surface energy of HAdV 40 is 42.4 mJ/m². Apolar surface energy component (γ LW) of HAdV 40 measured in this study is 41.6 mJ/m², which is a value typical for biological materials(Van Oss 2006). Polar component of surface energy of virion equals to 0.84 mJ/m². Our study revealed that polar adhesion energy between HAdV 40 and water (79.7 mJ/m²) was insufficient to overcome the energy of cohesion of water (-102.0 mJ/m²). Thus, HAdV 40 can be categorized as hydrophobic with negative interfacial free energy (ΔG wv =-30.4 mJ/m²).

XDLVO Energy of Virus-Membrane Interfacial Interaction

XDLVO calculation of virus-membrane interactions in DI water were performed for an ionic strength of 0.2 mM which is ionic strength determined from conductivity measurements of DI water spiked with HAdV 40 stock. However, the streaming potential of the membrane could only be measured above 1 mM NaCl electrolyte, so streaming potential in 1 mM NaCl was used to approximate the membrane charge in the HAdV-spiked DI water. The approximation is reasonable because the electrophoretic mobility of virions measured in DI water (-1.65 ± 0.19 µm⋅S⁻¹⋅V⁻¹⋅cm; pH 5.8) and in 1 mM NaCl solution (-1.72 ± 0.48 µm⋅S⁻¹⋅V⁻¹⋅cm; pH 5.8) were not statistically different.

At separation distances > 5 nm, van der Waals and electrostatic interactions dominate the XDLVO energy of interfacial interaction between HAdV 40 and membranes in both DI and tap water. Over a shorter range (from the minimum equilibrium cut-off distance of ~ 0.16 nm to 0.7 nm), however, the acid-base interaction energy is significantly greater than both van der Waals and electrostatic interaction energies. In 1 mM NaCl at pH=5.8 (unadjusted), the PEM-coated membrane is negatively charged with zeta potential at -7.0 ± 3.0 mV(Pasco, Shi et al. 2014). Due to the hydrophilicity of the PEM as well as a repulsive electrostatic interaction between the virus and the membrane surface, the secondary minimum in interaction energy profile is as shallow as -4.2 kT (Figure 1(A), solid line). In tap water, PEM-coated membrane is positive charge with zeta potential of +5.6 ± 0.4 mV. For the membrane-virus interaction energy profile in tap water, electrostatic attraction coupled with van der Waals attraction at large separation distances and a predominant repulsive acid-base interaction at small separation distances yields a -10.3 kT secondary minimum at the separation distance of 3.2 nm (Figure 1(A), dashed line).
This increase in depth of secondary minimum may lead to increased reversible adsorption of HAdV 40 onto the membrane surface during tap water filtration.

The CS-blocked membrane in 1 mM NaCl, pH 5.8 carries a very weak positive charge (3.0 ± 2.0 mV) (Pasco, Shi et al. 2014) though the isoelectric point of bovine serum albumin, a major component of calf serum, is 4.7~5.6 (Kanal, Fullerton et al. 1994, Ge, Kojio et al. 1998, Barbosa, Ortoe et al. 2010). The slightly positive charge of CS-blocked membrane can be attributed to the presence of other components in calf serum such as IgG (PI range from 6.4 to 9.0 (Josephso.Rv, Sinha et al. 1972)). XDLVO energy profile showed a -48.2 kT secondary minimum at the separation distance of 2.4 nm (Figure 1(B), solid line); which may suggest deposition of a significant amount of virus. In tap water, zeta potential of CS-blocked membrane was 2.8 ± 1.0 mV, similar to that in 1mM NaCl. In comparison to experiments in DI water, the reduced magnitude of negative charge for HAdV 40 in tap water leads to a decrease in the virus-membrane electrostatic attraction and depth of the second minimum in tap water reduced to -7.9 kT (Figure 1(B), dashed line).
Figure 1. XDLVO energy of interfacial interaction of HAdV 40 in aqueous media with (A) a PEM-coated membrane and (B) a CS-blocked membrane (Shi, Xagoraraki et al. 2016).
Virus Recovery from DI Water

Pre-elution virus recovery from DI water by PEM-coated membrane (74.8%± 9.7%) was significantly higher than that by CS- blocked membrane (54.1% ± 6.2%), which could stem from negative charge and hydrophilicity of PEM-coated membrane(Pasco, Shi et al. 2014). However, the <100% recovery indicates that some viruses were still adsorbed on the PEM-modified membrane even though the secondary minimum in the XDLVO energy profile was shallow (Figure 1, solid line). The presence of microscale attraction could explain this adsorption(Van Oss 2006, Hori and Matsumoto 2010), whereas XDLVO theory describes only macroscopic interactions. For example, in experiments on B. cepacia adhesion Hwang et al. reported bacterial adhesion in the absence of a secondary minimum in the XDLVO energy profile and suggested the adhesion stems from cell appendages such as pili and flagella(Hwang, Kang et al. 2012). Similarly, fibers of HAdV 40 containing long, thin shaft terminated with a globular knob may penetrate the macroscopic scale repulsion to achieve microscopic attraction. Interactions such as those between electron donor sites on the fiber knob and electron acceptor sites on the membrane may overcome the macroscopic repulsion and lead to local attraction. Moreover, adenovirus capsid contains nearly 1 million amino acids(Reedy, Natchiar et al. 2010, Nemerow, Stewart et al. 2012), so charge heterogeneity is expected on surface of virus particles, which can lead to local electrostatic attraction.

Lower recovery from DI water by CS-blocked membrane could stem from the presence of second minimum in XDLVO energy profile. Second minimum with depth of 48.2 kT was sufficient to capture viruses with average thermal energy of ~0.5 kT(Hahn and O'Melia 2004).

Virus elution was performed after filtration. High post-elution recoveries were achieved for both PEM-coated membrane (99.5 ± 6.6%) and CS-blocked membrane (98.8 ± 7.7%) with eluent containing 0.01% NaPP and 0.01% Tween 80. During elution, adsorption of NaPP on the membranes will impart negative charge to surface, increasing electrostatic repulsion between negatively charged virus and the membrane surface. Tween 80, as a nonionic surfactant with both hydrophilic and hydrophobic regions will minimize the hydrophobic interaction between the membrane surface and adsorbed virus. Both components in eluent could help release the virus from membrane surface.
Figure 2. Recovery of HAdV 40 with CS-blocked and PEM-coated membranes from (A) HAdV-spiked deionized water and (B) tap water (Shi, Xagoraraki et al. 2016).
Virus recoveries from practically-relevant water matrices (tap water and surface water) were also investigated in this study. For tap water, no statistically significant difference in pre-elution virus recovery was observed with PEM-coated membranes (40.5 ± 9.9%) and CS-blocked membranes (38.3 ± 9.3%). Based on XDLVO predictions for CS-blocked membranes (Figure 1B), the pre-elution recovery from tap water should be higher than from DI water. However, the opposite occurred. Average pre-elution recovery from tap water with such membrane reduced approximately 16%, compared with that from DI water (Figure 2). The discrepancy suggests that non-XDLVO effects (e.g. steric interactions(Yuan, Pham et al. 2008) or Ca^2+ bridging(Kim, Shan et al. 2009)) should be considered. Another possible cause for the discrepancy is virion adsorption to dissolved and suspended species present in the tap water (TOC = 1.1± 0.1 mg/L) and on the membrane surface. Significant flux declined (15% ~20%) during crossflow UF of tap water suggested the formation of a fouling layer on the membrane surface. The pre-elution recovery from tap water with the PEM-coated membrane was ~ 34% lower than from DI water (Figure 2). In addition to the possible effects described above for the CS-blocked membranes, charge reversal of the PEM membrane in tap water may decrease the pre-elution recovery.

Surface water is a complex matrix with presence of naturally occurring organic and inorganic species. Pre-elution recoveries from surface water collected in the fall were ~ 40% with both membranes, which is similar to recoveries from tap water, but significantly lower than recoveries from DI water. Permeate flux severely declined over 90 min of filtration for both membranes. Foulants formed a cake layer and masked the anti-adhesive properties of the membrane surface leading to the deposition of virus on membranes. Virus could also be adsorbed to various components suspended in the feed water (e.g. humic acid, clay, silica particles) and deposited simultaneously to membrane with those components. Calcium bridging could also occur between carboxyl groups on natural organic matter and on the HAdV 40 viral capsid (e.g. carboxylic groups on fiber knobs) leading to virus loss to the membrane surface. In general, virus-foulant interactions, rather than virus-membrane interactions, likely govern recovery in UF from complex waters. This is consistent with the result that pre-elution recovery from surface water was not statistically different for the two membrane types (Figure 3).

We also analyzed virus recovery from the surface water collected from the same lake in early spring after ice melted. Severe flux decline also occurred for both membranes during filtration of spring surface water, and pre-elution recoveries with both membranes were reduced to ~20%. Lower recovery from spring surface water than that from fall surface water could stem from higher concentrations of Ca^2+ and TOC in spring lake water.
Figure 3. Recovery of HAdV 40 from surface water after crossflow UF with CS-blocked membranes (A) and PEM-coated membranes (B) (Shi, Xagoraraki et al. 2016).
Post-elution recovery with CS-blocked membranes was 61.0 ± 2.8% and 34.9 ± 10.1% for surface water samples collected in fall and spring, respectively. For PEM-coated membranes, post-elution yielded 62.4 ± 2.2% and 41.6 ± 2.0% recoveries for fall and spring samples. Thus, virus adsorbed from surface water on both membranes were not eluted as effectively (Figure 3) as those adsorbed from DI or tap water (Figure 2) when using an eluent with NaPP and Tween 80 only, suggesting that fouling also decreased the effectiveness of the elution process.

To increase the elution efficiency, 0.01 wt% EDTA was added to the eluent to complex Ca²⁺ (Li and Elimelech 2004). With EDTA in the eluent, the post-elution recovery from spring and fall surface water, averaged over both types of membranes, increased to 84.3 ± 4.5%. The increased elution efficiency is consistent with the hypothesis that calcium binding decreases virus recovery from surface water.

**Conclusions**

Pre-elution recovery of HAdV from DI water was higher with PEM-coated membranes (74.8 ± 9.7%) than with CS-blocked membranes (54.1 ± 6.2%). Aqueous solution of sodium polyphosphate and Tween 80 as eluent was effective for both DI water (~99% recovery) and tap water (~90% recovery) for both membranes. The near 100% efficacy of elution indicates that polyanions and surfactants (e.g. sodium polyphosphate and Tween 80) in the eluent can disrupt electrostatic and hydrophobic interactions between the virion and the membrane. Pre- and post-elution recoveries from surface waters were significantly lower and showed no statistically significant difference between the two membrane types. However, addition of EDTA to the eluent greatly increased the elution efficacy (~88% recovery), possibly by eliminating cation bridging between virions and other components of the feed water matrix in suspension or in the fouling layer on the membrane surface.

Interestingly, the membrane choice is not very important for achieving high virion recoveries. For more complex water matrices such as surface water, the composition of the eluent is the most important factor for achieving high virion recovery. Recoveries of HAdV depend on its interactions with other components in the feed water (either in suspension or deposited on the membrane surface as a fouling layer), and not on virus-membrane interactions. An eluent that includes a polyanion (sodium polyphosphate), a non-ionic surfactant (Tween 80) and a chelating agent (EDTA) recovers HAdV effectively even from high TOC surface water.

**Reference**


