Supplemental Material for the manuscript

Elution is a critical step for human adenovirus 40 recovery from tap and surface water by crossflow ultrafiltration

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S.1 HAdV 40 propagation procedures

The HAdV 40 propagation procedure was adapted from protocols by Wong et al. (1) and Mautner (2). A549 cells, which are susceptible to a wide range of enteric viruses, were used as the HAdV 40 host. These cells were cultured in 50 mL of cell growth medium in a culture flask with a surface area of 150 cm$^2$ until 90% confluence was achieved. Medium in the culture flasks was decanted, and cell cultures were inoculated with 2 mL of virus stock. The flask was incubated at 37 °C for 60 min and rocked gently at 15 min intervals. After 60 min, 2 mL of viral inoculums was discarded and ~25 to 30 mL of cell maintenance medium was added to the flask. The tightly sealed flask was incubated at 37 °C and the cytopathic effect (CPE) was checked daily. As HAdV 40 is a fastidious virus, 4 days are usually needed to observe a good CPE. Viruses should be harvested when cells round up and detach from the flask surface. Harvesting too early leads to low virus production, and harvesting too late may lead to virus release to medium. To harvest viruses, the remaining cells were gently pipetted off the flask surface. Cell suspension was centrifuged at 900 g for 10 min at 4 °C, and supernant was then discarded. Cell pellets were resuspended in maintenance medium and then subjected to a freeze/thaw procedure (-70 °C / 37 °C) to release virions from cells. After 3 freeze/thaw cycles, cell resuspension was centrifuged at 12,000 g for 10 min at 4 °C. Supernatant containing viruses was collected, aliquoted, and stored at -70 °C for further use.

Viruses were further purified using a CsCl density step gradient (density steps from the bottom up: 1.45 g/cm$^3$ CsCl, 1.38 g/cm$^3$ CsCl, 1.32 g/cm$^3$ CsCl, 40% glycerol cushion). HAdV 40 stock was then added to the top and a virus band was obtained by ultracentrifugation at 80,000 g (AH-650 rotor, Sorvall, Thermo Scientific, Waltham, MA) and 4 °C for 3 h. The band was collected by side puncture of the tube with a syringe. CsCl was removed by dialysis against the desired buffer at 4 °C with gentle stirring.
Figure S1. HAdV 40 band obtained by CsCl density gradient centrifugation.

Figure S2. Transmission electron microscopy image of HAdV 40 viruses.
S.2 HAdV 40 quantification by qPCR

A MagNA Pure Compact System (Roche Diagnostics USA, Indianapolis, IN) was used to extract HAdV 40 DNA from 390 μL samples collected immediately after filtration experiments. 10 μL of carrier RNA (1 mg/mL) was added to increase DNA recovery during DNA extraction. Extracted DNA was stored in 100 μL eluates and at -20 °C prior to qPCR analysis. Each extract was quantified by qPCR in triplicate, and qPCR was performed using the LightCycler 1.5 system (Roche Diagnostics USA, Indianapolis, IN) with the primer and probe sequences described in Table S1. The volume of each PCR reaction mixture is 20 μL containing 5 μL of extracted DNA sample, 0.8 μL of forward primer (10 mM), 0.4 μL of each of two reverse primers (10 mM), 0.6 μL of Taqman Probe (10 mM), 10 μL of probe master mix (2X, Light Cycler 480 Probes, Roche) and 2.8 μL of PCR-grade water. qPCR mixtures were heated at 95°C for 15 min for DNA denaturation prior to 45 cycles of DNA amplification (95°C for 10 s, 60°C for 30 s and 72°C for 12 s). Finally, mixtures were cooled for 30 s at 40°C.

To prepare a standard curve (qPCR crossing-point (CP) values versus number of HAdV 40 DNA copies), plasmid DNA carrying cloned HAdV 40 hexon gene served as standards and was prepared following the method described earlier (3).

Table S1. Primer and probe sequences used in qPCR for HAdV 40 detection

| Forward Primer (for serotype 40 and 41) | HAdV-F4041-hex157f | ACC-CAC-GAT-GTA-ACC-ACA-GAC |
| Reverse primer 1 (for serotype 40) | HAdV-F40-hex245r | ACT-TTG-TAA-GAG-TAG-GCG-GTT-TC |
| Reverse primer 2 (for serotype 41) | HAdV-F41-hex246r | CAC-TTT-GTA-AGA-ATA-AGC-GGT-GTC |
| Taqman probe | HAV-F4041-hex214rprobe | 6-FAM-CGA-CKG-GCA-CGA-AKC-GCA-GCG-T-BHQ-1 |
S.3 Crossflow ultrafiltration (UF) apparatus

Figure S3. Crossflow filtration apparatus.
S.4 Permeate flux in HAdV 40 recovery tests.

Figure S4 shows the normalized permeate flux as a function of time during virus recovery from different water matrices. Flux values were normalized by the flux value at $t = 0$.

**Figure S4.** Normalized permeate flux as function of filtration time for CS-blocked membranes (A) and PEM-coated membranes (B).
S.5 Testing of potential inhibition of qPCR in complex water matrices.

We evaluated whether chemicals present in complex water matrices inhibit DNA extraction and qPCR detection. One liter samples of DI water, tap water and surface water were spiked with 1 mL of HAdV 40 stock. Log concentrations of DNA from complex water matrices (tap water and surface water) as well as DI water were calculated based on cross point (CP) values of DNA extracts obtained by qPCR. Similar experiments were conducted to evaluate the potential inhibition in the retentate produced by filtering tap water and surface water samples. One liter samples were filtered using the protocol for virus recovery tests, but no virus was introduced into the feed. 100 mL retentate samples were collected and spiked with 1 mL of HAdV 40 stock. The same procedure was followed for DI water samples. Log concentration of DNA from complex water matrices (tap water and surface water) as well as DI water were calculated. Our results (Figure S5) show no significant difference among the DNA concentrations determined in all of the feed and retentate matrices. This indicates there is either no inhibition or the same inhibition level for all water types in both feed and retentate.

![Graph showing log concentrations of DNA in DI, Tap, Lake (Fall), and Lake (Spring) feed and retentate matrices.](image-url)
Figure S5. Log concentration of DNA detected by qPCR in feed and retentate water matrices spiked with the same concentrations of virions.

S.6 Calculation of the virus-virus interaction energy and virus-membrane interaction energy using the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory

The total XDLVO interfacial interaction energy, $U^{XDLVO}$, is given by:

$$U^{XDLVO} = U^L + U^E + U^A$$

(S1)

where $U^L$, $U^E$, and $U^A$ correspond to Lifshitz - van der Waals, electrical double layer, and Lewis acid-base interactions, respectively. Equations for each interaction as a function of separation distance are introduced below.

S6.1 The case of virus-virus (sphere-sphere) interaction in water ($U_{vwv}$)

$U_{vwv}$ is the total interfacial interaction energy between two virions ($v$) immersed in water ($w$). As stated by eq. (S1), $U_{vwv}$ includes three components: $U^{LW}_{vwv}$, $U^{EL}_{vwv}$, and $U^{AB}_{vwv}$.

S6.1.1 Lifshitz - van der Waals (LW) interaction energy, $U^{LW}_{vwv}$

$$U^{LW}_{vwv} = -\frac{A}{6} \left[ \frac{2a^2}{\ell(4a + \ell)} + \frac{2a^2}{(\ell + 2a)^2} + \ln \frac{\ell(4a + \ell)}{(\ell + 2a)^2} \right]$$

(S2)

where $a$ is the radius of the virion, $\ell$ is the separation distance and $A$ is the Hamaker constant:

$$A = 12\pi \ell_o^2 \Delta G^{LW}_{\ell_o}$$

(S3)

where $\ell_o$ is the minimum equilibrium cut-off distance, which is estimated as 0.158 nm (4.6) and $\Delta G^{LW}_{\ell_o}$ is the LW adhesion energy per unit area at $\ell_o$:

$$\Delta G^{LW}_{\ell_o} = -2\left( \gamma^{LW}_v - \gamma^{LW}_w \right)^2$$

(S4)
where $\gamma_v^{LW}$ and $\gamma_w^{LW}$ are LW (apolar) components of surface energy for virus and water respectively.

S6.1.2 Electrical double layer (EL) interaction energy, $U_{vwv}^{EL}$

$$U_{vwv}^{EL} = 32\pi \varepsilon_r \varepsilon_0 a \left(\frac{kT}{ze}\right)^2 \gamma^2 \exp(-\kappa \ell)$$  \hspace{1cm} (S5)

where

$$\kappa = \sqrt{\frac{2N_A e^2 I}{\varepsilon_0 \varepsilon_r kT}}$$  \hspace{1cm} (S6)

where $N_A$ is Avogadro’s constant and $I$ is ionic strength. The dimensionless $\gamma$ is given by:

$$\gamma = \tanh \left(\frac{ze \psi_0}{4kT}\right)$$  \hspace{1cm} (S7)

where $\psi_0$ is the surface potential of the virion, which can be estimated from the $\zeta$–potential of the virion, $\zeta_v$:

$$\psi_0 = \zeta_v \left(1 + \frac{d}{a}\right) \exp(\kappa d)$$  \hspace{1cm} (S8)

$d$ is the distance from the particle’s surface to the slipping plane and is usually 0.3 to 0.5 nm. We used $d$ of 0.5 nm. The difference in $\exp(\kappa d)$ due to the choice of $d = 0.3$ nm rather than $d = 0.5$ nm, is at most 7% (seen at pH 7.6).

Eq. (S5) is valid for $\kappa a > 5$ and $\ell < a$ (7). In our study, $0.5\kappa d_{Adv}^h \geq 5.8$ in 1mM NaCl (Table S2) making eq. (S5) applicable.

Equation (S8) is valid for $\zeta$–potential values up to ~50 mV (5). In our study, the absolute value of $|\zeta_{Adv}|$ is $\leq 45.1$ (Table S2), making eq. (S8) applicable.
S6.1.3 Lewis acid-base (AB) interaction energy, $U_{vwv}^{AB}$

$$U_{vwv}^{AB} = a\pi\lambda\Delta G_{\ell_0}^{AB} \exp\left(\frac{\ell_0 - \ell}{\lambda}\right)$$ (S9)

where $\lambda$ is the decay length for water. The commonly used value of $\lambda$ is 0.6 nm (4).

$\Delta G_{\ell_0}^{AB}$ is the AB adhesion energy per unit area at $\ell_0$:

$$\Delta G_{\ell_0}^{AB} = -4\left(\sqrt{\gamma_v^+} - \sqrt{\gamma_w^+}\right)\left(\sqrt{\gamma_v^-} - \sqrt{\gamma_w^-}\right)$$ (S10)

where $\gamma_v^+$ and $\gamma_w^+$ are electron-acceptor parameters of surface energy for virion and water respectively, $\gamma_v^-$ and $\gamma_w^-$ are electron-donor parameters of surface energy for virion and water respectively.

S6.2 The case of virion-membrane (sphere-plate) interaction ($U_{vwm}$)

$U_{vwm}$ is the total interfacial interaction energy between virions ($v$) and membrane ($m$) immersed in water ($w$).

S6.2.1 Lifshitz - van der Waals (LW) interaction energy, $U_{vwm}^{LW}$

For the sphere-plate case, Lifshitz – van der Waals (LW) interaction energy is given by (8)

$$U_{vwm}^{LW} = -\frac{A}{6} \left[ a + \frac{a}{\ell + 2a} + \ln \frac{\ell}{\ell + 2a} \right]$$ (S11)

For the virus-membrane interaction, $\Delta G_{\ell_0}^{LW}$ can be calculated as:

$$\Delta G_{\ell_0}^{LW} = 2\left(\sqrt{\gamma_v^{LW}} - \sqrt{\gamma_m^{LW}}\right)\left(\sqrt{\gamma_v^{LW}} - \sqrt{\gamma_w^{LW}}\right)$$ (S12)

where $\gamma_m^{LW}$ is the LW component of surface energy for the membrane.

S6.1.2 Electrical double layer (EL) interaction energy, $U_{vwm}^{EL}$
\[ U_{vwm}^{EL} = \pi \varepsilon_r \varepsilon_0 a \left[ 2 \zeta_v \zeta_m \ln \left( \frac{1 + e^{-\kappa \ell}}{1 - e^{-\kappa \ell}} \right) + (\zeta_v^2 + \zeta_m^2) \ln(1 - e^{-2\kappa \ell}) \right] \]  

(S13)

where \( \zeta_m \) is the \( \zeta \)-potential of the membrane.

**S6.1.3 Lewis acid-base (AB) interaction energy, \( U_{vwm}^{AB} \)**

\[ U_{vwm}^{AB} = 2\pi a \lambda \Delta G_{\ell_0}^{AB} \exp \left( \frac{\ell_0 - \ell}{\lambda} \right) \]  

(S14)

For the virus-membrane interaction, \( \Delta G_{\ell_0}^{AB} \) is calculated as:

\[ \Delta G_{\ell_0}^{AB} = 2 \sqrt{\gamma_m^+ (\sqrt{\gamma_m^- + \sqrt{\gamma_v^-} - \sqrt{\gamma_w^-}) + 2 \sqrt{\gamma_m^- (\sqrt{\gamma_m^- + \sqrt{\gamma_v^-} - \sqrt{\gamma_w^-})} } \right) - 2 \left( \sqrt{\gamma_m^- \gamma_v^+} + \sqrt{\gamma_m^- \gamma_v^-} \right) \]  

(S15)

\( \gamma_m^+ \) and \( \gamma_m^- \) are electron-acceptor and electron-donor parameters of surface energy for membrane.

To calculate each component of surface energy for the membrane and the virus, we measured contact angles of DI water, glycerol and diiodomethane on both solid surfaces (membrane surface and virus lawn) using a goniometer. We obtained the components of surface energy for the solids by substituting contact angles and surface energy components of probe liquids into eq. (S16):

\[ (1 + \cos \theta) \gamma_I^{TOT} = 2 \left( \sqrt{\gamma_s^{LW} \gamma_I^{LW}} + \sqrt{\gamma_s^+ \gamma_I^-} + \sqrt{\gamma_s^- \gamma_I^+} \right) \]  

(S16)

\[ \gamma^{AB} = 2 \sqrt{\gamma^+ \gamma^-} \]  

(S17)

\[ \gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \]  

(S18)

where \( \theta \) is the contact angle of the probe liquid on the solid, \( \gamma^{TOT} \) is total surface energy, \( \gamma^{LW} \) is the LW component of surface energy, \( \gamma^+ \) is the electron-acceptor parameter of surface energy, and \( \gamma^- \) is the electron-donor parameter of surface energy. Subscripts \( l \) and \( s \) refer to probe liquid and solid surface respectively.
The free energy of interfacial interaction between two virions when immersed in water ($\Delta G_{vvw}$) can be determined using the following equation (9):

$$\Delta G_{vvw} = -2 \left( \sqrt{\gamma_v^{LW}} - \sqrt{\gamma_w^{LW}} \right)^2 - 4 \left( \sqrt{\gamma_v^+ \gamma_v^-} + \sqrt{\gamma_w^+ \gamma_w^-} - \sqrt{\gamma_v^+ \gamma_w^-} - \sqrt{\gamma_v^- \gamma_w^+} \right)$$

(S19)

S.7. HAdV 40 size distribution in tap water.

**Figure S6.** Normalized number-based size distribution of HAdV 40 in tap water (pH = 7.5 to 8.0). The vertical dashed red line indicates the average modal diameter (98.5 nm) in the buffer recommended for the storage of purified HAdV 40 (10 mM Tris-HCl and 1 mM EDTA, pH 7.6). The vertical dash-dot blue line denotes the average HAdV diameter (~ 80 nm) determined from TEM.
S.8 XDLVO energy of virus-membrane interfacial interaction: Relative contributions from van der Waals, electrostatic and acid-base interactions.

**Figure S7.** XDLVO energies of interfacial interaction of HAdV 40 with CS-blocked membranes in (A) DI water ($I \cong 0.2$ mM after spiking with HAdV 40) and (B) tap water.
Figure S8. XDLVO energies of interfacial interaction of HAdV 40 with PEM-coated membranes in (A) DI water ($I \approx 0.2$ mM after spiking with HAdV 40) and (B) tap water.
S9. On the determination of $\zeta$–potential based on measured electrophoretic mobility

Figure S9 present the results of electrophoretic mobility measurements.

![Figure S9: Electrophoretic mobility of HAdV 40 as a function of pH.](image)

To convert measured mobilities into $\zeta$–potential values, we used an expression derived by Ohshima (10):

$$
\mu = \frac{2 \varepsilon_r \varepsilon_0}{3 \eta} \zeta \left[ 1 + \frac{1}{2 \left[ 1 + \frac{2.5}{\kappa \alpha \left( 1 + e^{-\kappa} \right)} \right]^3} \right]
$$

(S20)

where $\kappa$ (nm$^{-1}$) is the Hückel parameter ($\kappa^{-1}$ (nm) is the Debye length), $\eta$ is the viscosity of the electrolyte, in which particles are suspended, and $\alpha$ is the radius of the particle. Equation (S20) provides an accurate (<1% error) estimate of $\zeta$–potential for any
value of \( \kappa a \). This is in contrast to Smoluchowski's (eq. (S21)) and Hückel's (eq. (S22)) expressions for electrophoretic mobility, which are applicable for \( \kappa a \gg 1 \) and \( \kappa a \ll 1 \), respectively.

\[
\mu = \frac{\varepsilon_r \varepsilon_0}{\eta} \zeta \quad (\text{S21})
\]

\[
\mu = \frac{3 \varepsilon_r \varepsilon_0}{2 \eta} \zeta \quad (\text{S22})
\]

**S.10 On the determination of \( \zeta \)-potential of virus aggregates.**

At pH values where virions aggregate, the \( \zeta \)-potential measured is that of an aggregate of virions, and not a single virion. We calculated the \( \zeta \)-potential of individual (non-aggregated) virions using eq. (S23), which connects the surface charge density of a particle (\( \sigma \)) with its \( \zeta \)-potential, diameter (\( d_p \)) and Debye length (\( \kappa^{-1} \)) (11):

\[
\sigma = \frac{2 \varepsilon_r \varepsilon_0 k T}{z e} \sinh \left( \frac{z e \zeta}{2 k T} \right) \sqrt{1 + \frac{1}{\kappa d_p} \cosh^2 \left( \frac{z e \zeta}{4 k T} \right) + \frac{1}{\left( \kappa d_p / 2 \right)^2} \frac{8 \ln \left[ \cosh \left( \frac{z e \zeta}{4 k T} \right) \right]}{\sinh^2 \left( \frac{z e \zeta}{2 k T} \right)}} \quad (\text{S23})
\]

This calculation assumes that \( \sigma \) is an intensive property and does not depend on the aggregation state of the virion. To determine the \( \zeta \)-potential of an individual virion from the \( \zeta \)-potential measured for virion aggregates, we first used eq. (S23) to calculate \( \sigma \) based on the measured \( \zeta \)-potential and average size of virus aggregates. With this value of \( \sigma \), we then used eq. (S23) again with the hydrodynamic diameter of an individual virion to calculate the \( \zeta \)-potential of an individual virus (Table S2). Because \( \zeta \)-potential measurements involved adding acid or base to adjust pH, the ionic strength was different at different pH values. To account for these differences we estimated the ionic strength at each pH from measured values of conductivity.

At pH values where virus aggregation occurred, the difference between \( \zeta \)-potentials of aggregated virions (measured \( \zeta \)-potential) and that of an individual virion (calculated \( \zeta \)-potential) was relatively minor (Table S2).
Table S2: Charge characteristics of HAdV 40 at different pH values. Shaded (gray) areas denote pH values where significant aggregation occurs and $\zeta_{HAdV}^{agg} \neq \zeta_{HAdV}$.

<table>
<thead>
<tr>
<th>Water matrix</th>
<th>1 mM NaCl</th>
<th>buffer $^a$</th>
<th>tap water</th>
<th>1 mM NaCl</th>
<th>DI water $^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.8</td>
<td>4.0</td>
<td>4.3</td>
<td>4.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Ionic strength (mM)$^b$</td>
<td>7.5</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>$\sigma_{HAdV}$ (mC/m²)</td>
<td>6.49</td>
<td>0.77</td>
<td>-0.89</td>
<td>-1.84</td>
<td>-3.02</td>
</tr>
<tr>
<td>$\zeta_{HAdV}^{agg}$ (mV)$^c$</td>
<td>n/a$^d$</td>
<td>6.8</td>
<td>-7.8</td>
<td>-14.9</td>
<td>n/a$^d$</td>
</tr>
<tr>
<td>$\zeta_{HAdV}$ (mV)</td>
<td>29.4</td>
<td>7.5</td>
<td>-8.8</td>
<td>-17.8</td>
<td>-28.0</td>
</tr>
<tr>
<td>Debye length, $\kappa^{-1}$ (nm)</td>
<td>3.5</td>
<td>7.2</td>
<td>7.4</td>
<td>7.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Hückel parameter, $\kappa$ (nm$^{-1}$)</td>
<td>0.285</td>
<td>0.139</td>
<td>0.136</td>
<td>0.133</td>
<td>0.128</td>
</tr>
<tr>
<td>$0.5\kappa d_{HAdV}^b$</td>
<td>16.0</td>
<td>19.8</td>
<td>16.1</td>
<td>9.9</td>
<td>6.0</td>
</tr>
</tbody>
</table>

$^a$ 10 mM Tris-HCl and 1 mM EDTA (buffer recommended for the storage of purified HAdV 40).

$^b$ Ionic strength at each pH is estimated based on electrical conductance measurements.

$^c$ Values of $\zeta_{HAdV}^{agg}$ are also reported in Figure 3. The values are included here for the ease of comparison with $\zeta_{HAdV}$.

$^d$ There is no detectable aggregation of HAdV 40 at this pH.

$^e$ After spiking DI water with HAdV 40 stock

$^f$ Not measured
Table S3: Additional parameters of HAdV 40 size distribution at different pH values \(^a\). Shaded (gray) areas denote pH values where significant aggregation occurs.

\(^a\) 10 mM Tris-HCl and 1 mM EDTA (buffer recommended for the storage of purified HAdV 40).

\(^b\) Half width at half maximum.

<table>
<thead>
<tr>
<th>Water matrix</th>
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<th>buffer (^a)</th>
<th>tap water</th>
<th>1 mM NaCl</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>2.8</td>
<td>4.0</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>HWHM (^b) (nm)</td>
<td>11.8 ± 5.7</td>
<td>31.3 ± 17.3</td>
<td>22.0 ± 1.4</td>
<td>18.8 ± 7.5</td>
</tr>
<tr>
<td>(d_{HAdv}^{mod}) (nm)</td>
<td>101.7 ± 7.9</td>
<td>203.4 ± 81.4</td>
<td>233.2 ± 89.7</td>
<td>149.8 ± 8.1</td>
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References


