Transport of human adenoviruses in porous media

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Abstract

Groundwater may be contaminated with infective human enteric viruses from various wastewater discharges, sanitary landfills, septic tanks, agricultural practices, and artificial groundwater recharge. Coliphages have been widely used as surrogates of enteric viruses, because they share many fundamental properties and features. Although a large number of studies focusing on various factors (i.e. pore water solution chemistry, fluid velocity, moisture content, temperature, and grain size) that affect biocolloid (bacteria, viruses) transport have been published over the past two decades, little attention has been given toward human adenoviruses (hAdVs). The main objective of this study was to evaluate the effect of pore water velocity on hAdV transport in water saturated laboratory-scale columns packed with glass beads. The effects of pore water velocity on virus transport and retention in porous media was examined at three pore water velocities (0.39, 0.75, and 1.22 cm/min). The results indicated that all estimated average mass recovery values for hAdV were lower than those of coliphages, which were previously reported in the literature by others for experiments conducted under similar experimental conditions. However, no obvious relationship between hAdV mass recovery and water velocity could be established from the experimental results. The collision efficiencies were quantified using the classical colloid filtration theory. Average collision efficiency, a, values decreased with decreasing flow rate, Q, and pore water velocity, U, but no significant effect of U on a was observed. Furthermore, the surface properties of viruses and glass beads were used to construct classical DLVO potential energy profiles. The results revealed that the experimental conditions of this study were unfavorable to deposition and that no aggregation between virus particles is expected to occur. A thorough understanding of the key processes governing virus transport is pivotal for public health protection.

Materials and Methods

Preparation of Cell Cultures and Virus Stocks

Human Adenovirus serotype 35 (hAdV35) (kindly donated by Dr. Annika Allard, University of Umea, Sweden), was cultivated in human lung carcinoma cell line A549, growing in Dulbecco's modified Eagle's medium (DMEM), containing 4.5 g/L D-glucose, L-glutamine, and pyruvate, supplemented with 10 % heat inactivated Fetal Bovine Serum (FBS). The initial concentration of hAdV35 stock was quantified by QPCR to 10^6 genome copies/mL.

HAdV 35 Infectivity Assay

Cytopathic effects were examined by visual inspection under the optical microscope. The final results were expressed as the geometric mean of the most probable number of cytopathic units (MPNCU) per milliliter, calculated for two independent replicates.

DNase I Protection Assay and Extraction of Viral DNA

A volume of 2.5 μL of DNase I (RNase-free) was added to 137.5 μL of each sample and then all aliquots were incubated at 37 °C for 2 h. Following the enzymatic digestion step, all samples were immediately processed for nucleic acids extraction, by QIAamp viral RNAmini kit, Qiagen.

Human Adenovirus QPCR

The qPCR assay for the detection and quantification of hAdV35 used the primers and conditions described by Hernroth et al. (2002), with the inclusion of a carryover contamination prevention system consisting of uracil N-glycosylase (UNG). TaqMan Universal PCR Master Mix (Applied Biosystems) was used. The thermocycling conditions were 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C.

Electrokinetic Measurements

The zeta potential of the hAdV35s, was measured at pH 7.6 in sterile DNase I reaction buffer by the zetasizer (Nano ZS90, Malvern Instruments), following the procedure outlined by Syngouna and Chrysikopoulos (2010), and was found to be equal to -21.78 ± 1.39 mV. The zeta potential of glass beads was determined to be -54.6 ± 2.4 mV. In this study, the electrokinetic zeta potentials were used instead of the surface potentials. The experimental conditions do not interfere with virus viability/inactivation. The conventional Derjaguin–Landau–Verwey–Overbeek (DLVO) interaction models were used, assuming that viruses behave as hard spheres.

Column Experiments

All flow through experiments were conducted using a 30 cm long glass column with 2.5 cm diameter, which was packed with 2-mm diameter glass beads. The column was packed with glass beads under standing sterile DNase I reaction buffer solution (pH 7.6, Is & 1.4 mM) to minimize air entrapment. The dry bulk density was estimated to be ρ_b = 1.61 g/cm^3 and the porosity θ = 0.42. The column was placed horizontally to minimize gravity effects. Three pore volumes (PVs) of sterile DNase I reaction buffer solution were passed through the column prior to each transport experiment.

Constant flow of buffer solution at three flow rates of Q = 2.5, 1.5, and 0.8 mL/min, corresponding to specific discharge or approach velocities of q = 0.51, 0.31, and 0.16 cm/min, and pore water (interstitial) velocities of U = q/h = 1.22 ± 0.02, 0.75 ± 0.02, and 0.39 ± 0.01 cm/min, respectively, was maintained through the packed column with a peristaltic pump (Masterflex L/S, Cole-Palmer). For each experiment, the virus suspensions were injected into the packed column for three PVs, followed by three PVs of buffer solution. All experiments were carried out at room temperature (≈25 C).

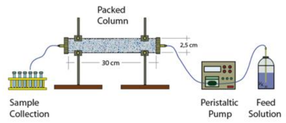

Figure 1. Schematic illustration of the experimental apparatus.

Transport Data Analysis

The dimensionless collision efficiency, a (the ratio of the collisions resulting in attachment to the total number of collisions between viruses and glass bead surfaces), was calculated from each hAdV35 breakthrough curve by the Rajagopalan and Tien (1976) model:

$$\alpha = -\frac{2d_c \ln(\mathrm{RB})}{3(1-\theta)\eta L} \qquad (1)$$

where d_c [L] is the average collector diameter, η_0 [–] is the dimensionless single-collector removal efficiency for favorable deposition (in the absence of double layer interaction energy), and RB [–] is the ratio of mass recovery of suspended virus particles, $M_{r(v)}$ [%], relative to the tracer mass recovery, $M_{r(t)}$ [%], in the outflow

$$\mathrm{RB} = \frac{M_{r(v)}}{M_{r(t)}} \qquad (2)$$

The mass recovery, Mr(v), of the suspended viruses was quantified by the following expression (James and Chrysikopoulos 2011), where L is the length of the packed column:

$$M_r(L) = \frac{\int_0^\infty C_i(L,t)\mathrm{d}t}{\int_0^{t_p} C_i(0,t)\mathrm{d}t} \qquad (3)$$

The collision efficiency of hAdV35 was calculated for the experimental conditions of this study using Eq. (1), where the η_0 values were obtained from an existing correlation (Tufenkji and Elimelech 2004), with the following parameter values for the complex Hamaker constant of the interactive media (virus–water–glass beads) A_{123} = 7.5 x 10^{-21} (kg m^2/s^2) (Murray and Parks 1978), Boltzman constant k_B = 1.38 x 10^{-23} (kg m^2)/(s^2 K), fluid absolute temperature T = 298 K, hAdV particle diameter d_p = 7 x 10^{-8} m (Wong et al. 2012), hAdV particle density ρ_p = 1,340 kg/m^3 (Shabram et al. 1997; Vellekamp et al. 2001), fluid density ρ_f = 999.7 kg/m^3, absolute fluid viscosity μ_w = 8.91 x 10^{-4} kg/(m s), and acceleration due to gravity g = 9.81 m/s^2.

DLVO Interaction Energy Calculations

Virus retention by the packed column and adsorption onto glass beads greatly depends on the total DLVO interaction energy. To better understand the observed virus and glass beads interactions in the column experiments conducted in this study at pH 7.6 and Is = 1.4 mM, the interaction energy between hAdV35-glass beads was calculated following the procedure described in Chrysikopoulos and Syngouna (2012) for the sphere-plate geometry approximation. Note that the total interaction energy Φ_{DLVO} [J] equals the sum of the van der Waals, Φ_{vdW} [J], the electrostatic double layer, Φ_{dl} [J] and the Born, Φ_{Born} [J] interaction energies over the separation distance h [L] between the approaching surfaces (Loveland et al. 1996). Moreover, in order to evaluate the possibility of particle aggregation, the Φ_{DLVO} interaction energy profiles for the case of sphere–sphere approximation as applied to identical virus–virus interactions were constructed under the experimental conditions (Is = 1.4 mM, pH 7.6). Note that the measured electrokinetic zeta potentials were used instead of the surface potentials for DLVO calculations.

Results and Discussion

Transport experiments

Figure 1 presents the normalized hAdV35 breakthrough data for three different flow rates (*Q* = 2.5, 1.5, 0.8 mL/min). The corresponding *Mr*(v) values, as calculated with Eq. (3), are listed in Table 1, and are illustrated graphically in Fig. 2. The peak concentrations and estimated average *Mr*(v) values for hAdV35 were similar for all *Q* employed in this study. High hAdV35 retention was observed for all cases examined. With no exception, all estimated average *Mr*(v) values for hAdV35 were lower than those of MS2 and ΦX174, which were reported by Syngouna and Chrysikopoulos (2013) under similar experimental conditions. Pang et al. (2014) observed lower mass recovery values for hAdV type 41 (VR-930 strain) than MS2. Note that hAdV35 is less negatively charged than coliphages MS2 and ΦX174 at the experimental conditions of this study. Therefore, hAdV35 was expected to attach onto the solid matrix more than coliphages. Straining (virus trapping in pore throats that are too small to allow virus passage) and wedging (virus attachment onto surfaces of two or more collector grains in contact) are not considered important mechanisms of mass loss in the packed columns examined in this study because the virus to collector diameter ratios (d_p/d_c) were well below the suggested threshold of 0.004 (Johnson et al. 2010) or 0.003 (Bradford and Bettahar 2006) for all cases examined. However, other factors (e.g., collector surface heterogeneity) may have contributed to the observed hAdV35 retention.

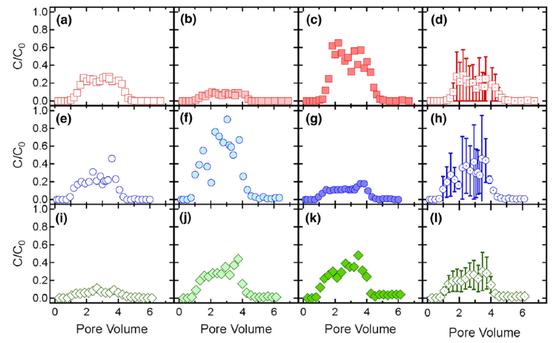

Figure 2. Experimental hAdV35 breakthrough data for volumetric flow rates Q of 2.5 mL/min (squares-a, b, c, d), 1.5 mL/min (circles-e, f, g, h) and 0.8 mL/min (diamonds-i, j, k, l) in water-saturated columns packed with glass beads and average breakthrough concentration data (d, h, l) for Q equal to 2.5, 1.5 and 0.8 mL/min, respectively

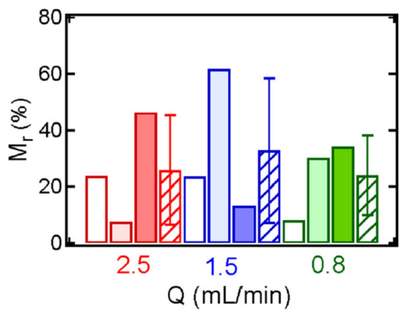

Figure 3. Calculated mass recovery values, based on hAdV35 virus concentrations in the effluent, from the transport experiments with flow rate equal to: a 2.5 mL/min, b 1.5 mL/min, and c 0.8 mL/min. The cross shaded columns are the average Mr values from three experiments conducted under identical conditions

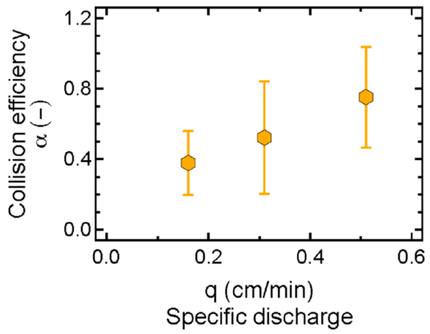

Figure 4. Average experimental collision efficiency for hAdV35 as a function of specific discharge

Table 1. Data analysis for the hAdV35 transport experiments

Exp no.	*q* (cm/min)	C_0 (GC/mL) (viruses)	θ (–)	*U* (cm/min)	$M_{r(v)}$ (%)	η_0 (–)	α
	Q = 2.5 mL/min						
1	0.51	3,125,483	0.42	1.21	23.86	0.013	0.817
2	0.51	2,880,561	0.41	1.24	7.59	0.014	1.000
3	0.51	386,159	0.42	1.21	46.23	0.013	0.440
Average ± SD			0.42 ± 0.01	1.22 ± 0.02	25.89 ± 19.40	0.014 ± 0.000	0.752 ± 0.286
	Q = 1.5 mL/min						
4	0.31	402,241	0.42	0.74	23.57	0.019	0.577
5	0.31	328,009	0.4	0.78	61.71	0.020	0.179
6	0.31	719,443	0.42	0.74	13.17	0.019	0.810
Average ± SD			0.41 ± 0.01	0.75 ± 0.02	32.82 ± 25.56	0.019 ± 0.000	0.522 ± 0.319
	Q = 0.8 mL/min						
7	0.16	2,128,402	0.4	0.4	7.9	0.032	0.586
8	0.16	609,031	0.41	0.39	30.07	0.031	0.288
9	0.16	546,243	0.41	0.39	34.08	0.031	0.258
Average ± SD			0.41 ± 0.01	0.39 ± 0.01	24.02 ± 14.10	0.032 ± 0.000	0.378 ± 0.182

Calculation of Parameter Values

Figure 4 presents the average collision efficiencies for hAdV35 as calculated with Eq. (1) for all three q (0.51, 0.31, 0.16 cm/min), using the previously calculated η_0 values. Also, average collision efficiencies are listed in Table 1. Note that, a average values decreased with decreasing Q and q (see Fig. 4).

Calculations of Virus–Glass Beads and Virus–Virus Interactions

The results of DLVO interaction energy profiles for hAdV35-glass beads and hAdV35-hAdV35, for the experimental conditions (DNase I reaction buffer solution at pH 7.6 with Is = 1.4 mM), are shown in Fig. 5. All calculated Φ_{max1}, Φ_{min1}, and Φ_{min2} are listed in Table 2.

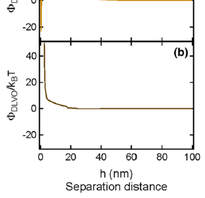

Figure 5. Predicted normalized UDLVO interaction energy profiles for: a hAdV35-glass beads treated as sphere-plate, and b hAdV35-hAdV35 treated as sphere–sphere, as a function of separation distance (here Is = 1.4 mM, and pH 7.6)

Table 2. Estimated values of Φ_{min1}, Φ_{min2}, and Φ_{max1} for the hAdV35-glass beads and hAdV35-hAdV35 interactions.

DLVO interactions	Φ_{min1} (k_BT)	*h* (nm)	Φ_{max1} (k_BT)	*h* (nm)	Φ_{min2} (k_BT)	*h* (nm)
Sphere-plate approximation						
hAdV35-glass beads	−4.41	0.5	30.63	4	−0.00712	90
Sphere-sphere approximation						
hAdV35-hAdV35	na	na	na	na	−0.07768	56

Conclusions

A full set of column experiments was carried out in order to investigate the effects of water velocity on hAdV35 transport. The results of this study indicated that although the virus mass recovery and degree of velocity enhancement were affected by the interstitial water velocity, no clear trends could be determined. Note that the average experimental collision efficiency, *a*, values decreased with decreasing flow rate, *Q*, and specific discharge, *q*. However, no significant effect of specific discharge on the collision efficiency was observed. Moreover, using classical DLVO theory, the results revealed that the experimental conditions were unfavorable to deposition, and that no aggregation between virus particles is expected to occur under the experimental conditions. It is possible that factors such as collector surface heterogeneities, angularity, and roughness may have contributed to physicochemical filtration and virus retention.

References

Bradford, S. A., & Bettahar, M. (2006). Journal of Contaminant Hydrology, 82, 99–117.
Chrysikopoulos, C. V., & Syngouna, V. I. (2012). Colloids and Surfaces B: Biointerfaces, 92, 74–83.
Hernroth, B. E., Conden-Hansson, A. C., Rehnstam-Holm, A. S., Girones, R., & Allard, A. K. (2002). Applied and Environmental Microbiology, 68(9), 4523–4533.
James, S. C., & Chrysikopoulos, C. V. (2011). Advances in Water Resources, 34(10), 1249–1255.
Loveland, J. P., Ryan, J. N., Amy, G. L., & Harvey, R. W. (1996). Colloids and Surfaces A: Physicochemical and Engineering Aspects, 107, 205–221.
Murray, J. P., & Parks, G. A. (1978). In M.C. Kavanaugh, J.O. Leckie (Eds.), Particulates in water: Characterization, fate, effects and removal, Adv. Chem. Ser. 189, Washington, DC: American Chemical Society.
Rajagopalan, R., & Tien, C. (1976). AIChE Journal, 22, 523–533.
Shabram, P. W., Giroux, D. D., Goudreau, A. M., Gregory, R. J., Horn, M. T., Huyghe, B. G., et al. (1997). Human Gene Therapy, 8, 453–465.
Syngouna, V. I., & Chrysikopoulos, C. V. (2010). Environmental Science and Technology, 44, 4539–4544.
Syngouna, V. I., & Chrysikopoulos, C. V. (2013). Colloids and Surfaces A: Physicochemical and Engineering Aspects, 416, 56–65.
Tufenkji, N., & Elimelech, M. (2004). Environmental Science and Technology, 38, 529–536.
Vellekamp, G., Porter, F. W., Sutjipto, S., Cutler, C., Bondoc, L., Liu, Y. H., et al. (2001).Gene Therapy, 12, 1923–1936.
Wong, K., Mukherjee, B., Kahler, A. M., Zepp, R., & Molina, M. (2012). Environmental Science and Technology, 46(20), 11145–11153.

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