Transport of Human Adenoviruses in Water Saturated Laboratory Columns

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ORIGINAL PAPER

Transport of Human Adenoviruses in Water Saturated Laboratory Columns

P. Kokkinos · V. I. Syngouna · M. A. Tselepi · M. Bellou · C. V. Chrysikopoulos · Apostolos Vantarakis

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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, α, values decreased with decreasing flow rate, Q, and pore water velocity, U, but no significant effect of U on α 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.

Keywords Adenovirus · Transport · Groundwater contamination · Public health

Introduction

Enteric viruses are important pathogens, which can have severe impacts on health. They are responsible for significant economic and societal burdens. Enteric viruses are shed in extremely high quantities in feces and have very low infectious doses, in the order of tens to hundreds of virions (Borchardt et al. 2003; Wong et al. 2014). They are frequently isolated from contaminated water and have been linked to numerous waterborne outbreaks (Flynn et al. 2004; Zhuang and Jin 2008; Fong et al. 2010). Pathogenic viruses have been found in aquifers, groundwater wells, and drinking water (Zhuang and Jin 2003a, b; Weaver et al. 2013; Sadeghi et al. 2013; Frohnert et al. 2014). The EPA estimated that in the US annually 16 people are at risk of death and 168,000 people are at risk of viral illness from consuming groundwater contaminated with pathogenic viruses (USEPA 2000;

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Zhuang and Jin 2003a, b). Main causes of the occurrence of these viruses in water supplies are improper wastewater disposal operations (e.g., septic tanks, wastewater infiltration basins) (Zhuang and Jin 2008). The extremely small size (25–100 nm) of viruses allows them to infiltrate soils, and eventually to invade aquifers. Depending on factors such as rainfall, temperature, soil structure, organic carbon content, soil water pH, cation concentrations, ionic strength, virus taxon-specific factors (e.g., capsid diameter and isoelectric point), and the presence of other biota, viruses can migrate over considerable distances in the subsurface (Dowd et al. 1998; Borchardt et al. 2007; Davies et al. 2006; Cao et al. 2010; Horswell et al. 2010; Syngouna and Chrysikopoulos 2013). Viruses persist for several months in soils and groundwater when temperatures are low and soils are moist (Borchardt et al. 2003), and they are less efficiently eliminated during soil passage than other microorganisms (Frohnert et al. 2014).

To determine groundwater vulnerability and develop protective regulations, the factors that affect the transport and fate of viruses in porous media must be fully understood (Sadeghi et al. 2013; Zhuang and Jin 2003a, b). Due to the potential threat to public health, numerous studies have focused on microorganism transport and fate in fractured and porous media (Flynn et al. 2004; Masciopinto et al. 2008; Cao et al. 2010; Chrysikopoulos et al. 2010). Theoretical and experimental studies have examined the effect of pore water solution chemistry, fluid velocity, matrix structure, moisture content, temperature, grain size, and presence of surface coatings on viral transport and retention in porous media (Syngouna and Chrysikopoulos 2011, 2013; Anders and Chrysikopoulos 2006, 2009; Bolster et al. 2001; Sim and Chrysikopoulos 2000; Jin et al. 1997; Sadeghi et al. 2011; Walshe et al. 2010; Katzourakis and Chrysikopoulos 2014). Most viral transport studies focus on bacteriophages, with MS2 being the most common (Powelson et al. 1991; Dowd et al. 1998; Schijven et al. 2002; Weaver et al. 2013; Chu et al. 2003; Zhuang and Jin 2003a, b; Han et al. 2006; Zhuang and Jin 2008; Syngouna and Chrysikopoulos 2011; Wong et al. 2014). Other frequently used bacteriophages are: phiX174 (Schijven et al. 2002; Kenst et al. 2008; Zhuang and Jin 2008; Han et al. 2006; Chu et al. 2003; Zhuang and Jin 2003a, b; Syngouna and Chrysikopoulos 2011), PRD1 (Schijven et al. 2002; Abudalo et al. 2005; Foppen et al. 2006; Sadeghi et al. 2013; Davies et al. 2006), and T7 (Flynn et al. 2004). Bacteriophages were widely employed as surrogates for human viruses, because they share many properties and features such as size, structure, morphology, and composition. Moreover, they are nonpathogenic to human, specific to host bacteria, simple to assay, and have good survival characteristics (Cao et al. 2010). However, relatively less attention has been given to pathogenic viruses (Pancorbo et al. 1988; Quanrud et al. 2003; Attinti et al. 2010), and specifically to adenoviruses as indicator viruses (Davies et al. 2006; Horswell et al. 2010; Wong et al. 2012, 2014). Fluorescent polystyrene nanospheres (FPNS) have also been used as virus surrogates in studies of virus transport in porous media, where 70-nm sized silica nanoparticles were covalently coupled with specific proteins and a DNA marker for sensitive detection (Pang et al. 2014).

Adenoviruses fit most criteria for an ideal indicator of viral pathogens (Pina et al. 1998; Bofill-Mas et al. 2000; Fong et al. 2010; Wyn-Jones et al. 2011; Kokkinos et al. 2012; Rusiñol et al. 2014). They are known to cause numerous infections including gastroenteritis, and have been implicated in disease outbreaks (Mena and Gerba 2009). Interestingly, estimates show that more than 90 % of the human population is seropositive for one or more serotypes of adenoviruses. Human adenoviruses (hAdVs) are excreted in high concentrations from infected patients (up to 10^{11} viral particles per gram of feces), similarly to other enteric viruses, but are more frequently detected in sewage than other enteric viruses (Fong et al. 2010). They are widespread in the environment and are found in marine, river, ground, drinking, recreational, and waste waters (Wong et al. 2014). Due to their high prevalence and environmental stability, hAdVs have been included in the U.S. Environmental Protection Agency's contaminant candidate lists (Davies et al. 2006; Wong et al. 2014). Adenoviruses are more UV light and heat resistant, compared to other enteric viruses (Horswell et al. 2010), and remain infectious for at least one year, in groundwater, with their DNA being detectable for as long as 22 months (Pang et al. 2014). Quantitative detection of hAdVs as indicators of human fecal contamination is essential because of the frequent reuse of treated wastewater (Fong et al. 2010). Nevertheless, the available literature on hAdVs transport in porous media is limited (Wong et al. 2014).

Despite the previous research on virus transport in porous media, our understanding of how hAdV is transported in aquifers is far from complete, and additional investigation is needed for the development of effective regulations and disinfection strategies and the consequent protection of public health. 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.

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; Gibco,

Grand Island, NY, U.S.), containing 4.5 g/L D-glucose, L-glutamine, and pyruvate, supplemented with 10 % heat inactivated Fetal Bovine Serum (FBS; Gibco). A549 cells support the replication of most human AdV serotypes, except the fastidious serotypes 40 and 41. For the preparation of virus stocks, A549 cells were cultured to 80–90 % confluence in 175-cm^2 flasks (37 °C, 5 % CO_2), and infected with hAdV35. Viruses were released from cells by three cycles of freezing and thawing, and cell debris were removed by low-speed centrifugation (3,000×*g* for 20 min). Finally, the supernatant was ultracentrifuged at 34,500×*g* for 1 h, re-suspended in PBS, quantified and stored in 10 mL aliquots at −80 °C until used. The initial concentration of hAdV35 stock was quantified by QPCR to 10^6 genome copies/mL.

HAdV 35 Infectivity Assay

All A549 cell monolayers were incubated overnight in 12-well plates (Cellstar, Greiner bio–one) at 37 °C, in an atmosphere of 5 % CO_2 to 90–100 % confluence. Thirty microliters (30 µL) of direct and diluted samples were inoculated for 90 min at 37 °C on a rocking table (Environmental shaker incubator ES-20, Biosan). Media with inoculate were discarded, and DMEM media supplemented with 1 % FBS were added. The flasks were incubated for 3–4 days at 37 °C in 5 % CO_2 and then examined for cytopathic effects (CPE). Cytotoxicity effects were determined 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. All assays were performed in triplicate and negative and positive controls were included.

DNase I Protection Assay and Extraction of Viral DNA

A volume of 2.5 µL of DNase I (RNase-free, 2,000 units/mL, New England BioLabs Inc.) was added to 137.5 µL of each sample and then all aliquots were incubated at 37 °C for 2 h. DNase I should degrade any viral DNA that is no longer protected by the viral capsid. Following the enzymatic digestion step, all samples were immediately processed for nucleic acids extraction. In detail, 140 µL of each sample was added (separately) to 560 µL of Lysis Buffer AVL, with 5.6 µL of carrier RNA (QIAamp viral RNA mini kit, Qiagen). The DNase I enzyme was inactivated after the addition of the lysis buffer. The viral DNA was extracted according to the kit manufacturer's instructions into a final volume of 100 µL. The extracts were immediately stored at −80 °C.

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 applied. 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. For each reaction, 10 µL of nucleic acids was used to obtain a final reaction volume of 25 µL. All samples were tested both neat and at a 10-fold dilution, in duplicate.

Electrokinetic Measurements

Virus surface charge plays a significant role in various sorption processes, as well as in adhesion and transport phenomena of viruses in the subsurface (Gerba 1984; Schijven and Hassanizadeh 2000). Note that in water treatment plants, the surface charge of virus particles is used for the design of proper flocculation, and granular filtration in order to obtain safe drinking water (Wegmann et al. 2008a, b). All these processes are governed by electrostatic interactions. The pH value at which the net surface charge of a particle suspended in an aqueous solution changes from positive to negative is referred to as the isoelectric point (IEP). Moreover, the IEP provides initial information about the virus' surface charge in a certain environment, and thus about its sorption and transport behavior. Only a few studies have reported the IEP of hAdVs: the IEP of hAdV serotypes 4 and 5 were reported as 2.6 (Salo and Mayor 1978) and 4.5 (Trilisky and Lenhoff 2007), respectively. Wong et al. (2012) reported that the IEP of hAdV in the range of 3.5–4.0, and varied minimally with I_s (ionic strength). Based on the review by Michen and Graule (2010), the IEP of most microorganisms is in the range of 3.5–7.0.

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, Southborough, MA), following the procedure outlined by Syngouna and Chrysikopoulos (2010), and was found to be equal to -21.78 ± 1.39 mV. The sterile DNase I reaction buffer solution at pH 7.6 was prepared with 0.1 mM Tris–HCl, 0.025 mM $MgCl_2$, 0.005 mM $CaCl_2$, which corresponds to $I_s \approx 1.4$ mM. Furthermore, the zeta potential of glass beads was determined to be -54.6 ± 2.4 mV (Syngouna and Chrysikopoulos 2013). All zeta potential measurements were obtained in triplicates. For soft particles as viruses, the electrical double layer is formed not only outside but also inside the surface charge layer; consequently, the zeta potential becomes less important and for some cases, loses its physical meaning (Duval and Gaboriaud 2010). In this study, the electrokinetic zeta potentials were used instead of the surface potentials. Note that all experiments were performed with sterile DNase

I reaction buffer (pH 7.6, $I_s = 1.4$ mM), conditions that do not interfere with virus viability/inactivation. Furthermore, 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. Glass beads were used for the packing of the columns in order to eliminate possible experimental difficulties associated with real soil, which may provide numerous uncertainties that can considerably complicate the analysis of the experimental data. Following the procedure previously described by Syngouna and Chrysikopoulos (2013), the glass beads were soaked in concentrated 0.1 M HNO_3 (70 %) for a 3 h time period to remove surface impurities. Next, the beads were rinsed with sterile ddH_2O until the water conductivity, as determined by a conductivity meter, was negligible. Subsequently, the glass beads were soaked in 0.1 M NaOH for a 3 h time period, and rinsed repeatedly with ddH_2O until the ionic strength (I_s) of the water was negligible ($I_s = 10^{-4}$ M). The glass beads were dried in an oven at 105 °C, and then stored in screw cap sterile beakers until use in the column experiments. The column was packed with glass beads under standing sterile DNase I reaction buffer solution (pH 7.6, $I_s \approx 1.4$ mM) to minimize air entrapment. The dry bulk density was estimated to be $\rho_b = 1.61$ g/cm^3 and the porosity $\theta = 0.42$. The column was placed horizontally to minimize gravity effects (Chrysikopoulos and Syngouna 2014). A fresh column was packed for each experiment. Also, three pore volumes (PVs) of sterile DNase I reaction buffer solution were passed through the column prior to each transport experiment. The entire packed column and glassware used for the experiments were sterilized in an autoclave at 121 °C for 20 min. 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 $U = q/\theta = 1.22 \pm 0.02$, 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). It should be noted that the pore water velocities employed in this work are quite low and thus representative of slow sand filter and field conditions (Syngouna and Chrysikopoulos 2011). 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 ($\sim$25 °C).

Theoretical Considerations

Transport Data Analysis

Classical colloid filtration theory (CFT) was used to quantitatively compare the attachment of viruses onto glass beads. CFT assumes that the removal of virus particles is described by first-order kinetics with a spatially and temporally constant rate of virus deposition, and the concentrations of retained viruses decrease log-linearly with distance. However, in the absence of straining (i.e., the trapping of viruses in pores that are too small to pass through), an hyper-exponential decrease with distance of virus retention could be observed due to the concurrent existence of both favorable and unfavorable viral interactions with glass bead surfaces suggesting that the attachment rate coefficient is not constant (Tufenkji and Elimelech 2004; Tong and Johnson 2007). Virus deposition is termed favorable in the absence of repulsive interaction energies, whereas unfavorable deposition refers to the case where repulsive viral interactions predominate.

The dimensionless collision efficiency, α (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

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

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

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

The mass recovery, $M_{r(v)}$, of the suspended viruses was quantified by the following expression (James and Chrysikopoulos 2011):

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

where L is the length of the packed column.

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) $A_{123} = 7.5 \times 10^{-21}$ (kg m^2/s^2) (Murray and Parks 1978), Boltzman

constant $k_B = 1.38 \times 10^{-23}$ (kg m^2)/(s^2 K), fluid absolute temperature $T = 298$ K, hAdV particle diameter $d_p = 7 \times 10^{-8}$ m (Wong et al. 2012), hAdV particle density $\rho_p = 1{,}340$ kg/m^3 (Shabram et al. 1997; Vellekamp et al. 2001), fluid density $\rho_f = 999.7$ kg/m^3, absolute fluid viscosity $\mu_w = 8.91 \times 10^{-4}$ 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 $I_s = 1.4$ 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 ($I_s = 1.4$ mM, pH 7.6). Note that the measured electrokinetic zeta potentials were used instead of the surface potentials for DLVO calculations.

Results and Discussion

Although bacteriophages have been widely used as surrogates for viral pathogens in environmental studies, it has become clear through different studies, that they cannot represent the wide range of pathogenic viruses, for their retention and transport in porous media. For example, MS2 has been reported to be less stable than AdV in groundwater at 20 °C, while in transport experiments, eluted AdV concentrations were 2–3 orders of magnitude lower than that of MS2 and ΦX174, with AdV being markedly more retarded (Pang et al. 2014). Although hAdVs have been widely recognized as important indicator viruses of human fecal contamination (Pina et al. 1998; Bofill-Mas et al. 2000), their use in transport experiments has been only recently recorded in very few manuscripts (Davies et al. 2006; Horswell et al. 2010; Wong et al. 2012, 2014). In this work, hAdV35 was used as a more realistic virus surrogate to evaluate the effect of pore water velocity on virus transport in water saturated laboratory-scale columns.

One of the advantages of laboratory-scale studies is that the experimental conditions may be highly controlled, and thus the fundamental processes influencing microorganism transport can be identified (Flynn et al. 2004). In particular, column experiments offer the advantage that permission from local authorities is not required, the transport and removal processes can be studied under well-defined physico-chemical conditions, and it has been reported that there are no major advantages of doing batch experiments for viruses as compared to column experiments (Sadeghi et al. 2013).

The use of sensitive molecular assays for virus detection and quantification offers speed and precision in conducting transport experiments, as has been shown in recent relevant studies (Wong et al. 2012). These molecular assays are based on the detection of nucleic acids. To degrade DNA not protected from viral capsids, a well-recognized experimental strategy of a DNase I enzymatic step addition to purify the viral suspension used for the transport experiments was applied.

Column studies of hAdV transport can provide critical parameters for modeling the transport behavior of hAdV in the field (Wong et al. 2014). Certainly, such information may impact the protection of valuable water resources from viral contamination, and finally the protection of Public Health.

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 $M_{r(v)}$ values, as calculated with Eq. (3), are listed in Table 1, and are illustrated graphically in Fig. 2. The peak concentrations and estimated average $M_{r(v)}$ 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 $M_{r(v)}$ 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.

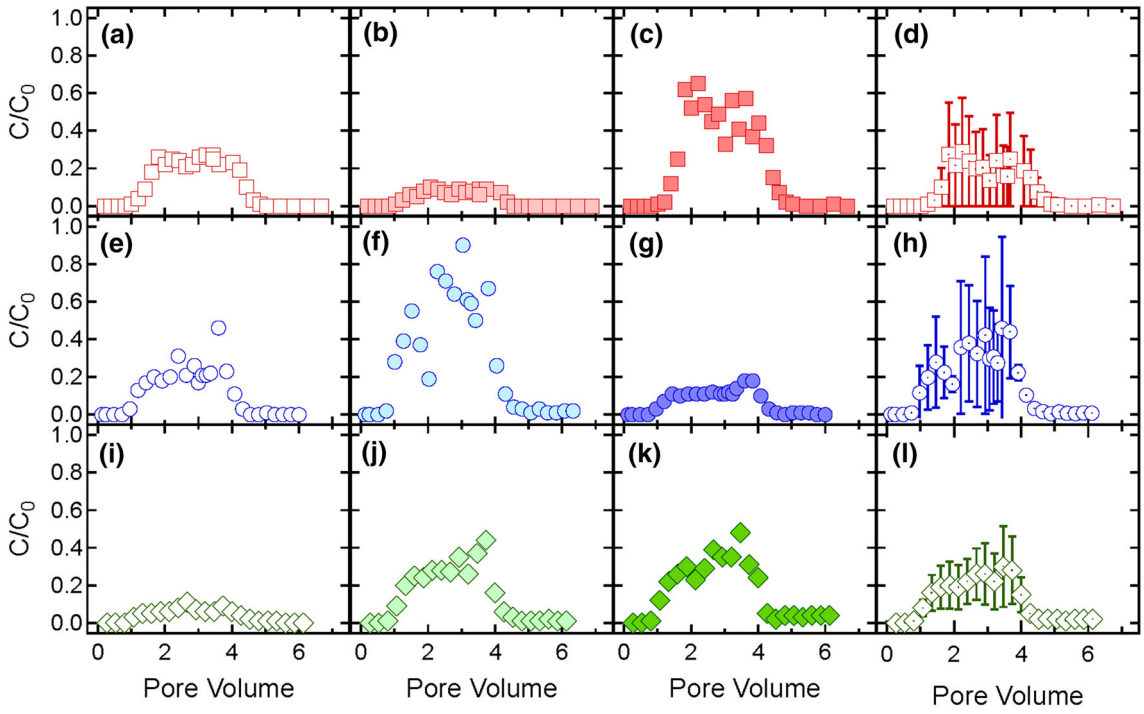

Fig. 1 Experimental hAdV breakthrough data for volumetric flow rates Q of 2.5 mL/min (*squares*-**a**, **b**, **c**, **d**), 1.5 mL/min (*circles*-**e**, **f**, **g**, **h**) and 0.8 mL/min (*diamonds*-**i**, **j**, **k**, **l**) in water-saturated columns packed with glass beads and average breakthrough concentration data (**d**, **h**, **l**) for Q equal to 2.5, 1.5 and 0.8 mL/min, respectively

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

According to the colloid filtration theory, particle attachment is governed by the collision efficiency (Rajacopalan and Tien 1976; Tufenkji and Elimelech 2004). Figure 3 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. The calculated α values (see Table 1) exhibit some variability

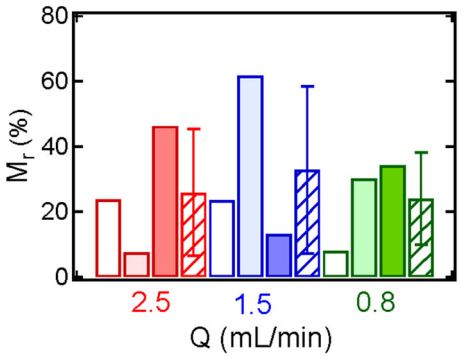

Fig. 2 Calculated mass recovery values, based on hAdV35 virus concentrations in the effluent, from the transport experiments with flow rate equal to: **a** 2.5 mL/min, **b** 1.5 mL/min, and **c** 0.8 mL/min. The *cross shaded columns* are the average M_r values from three experiments conducted under identical conditions

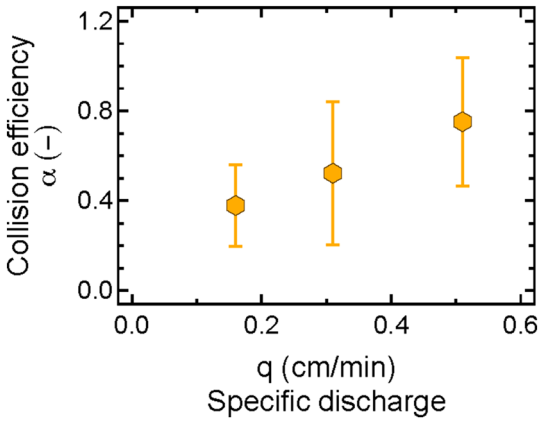

Fig. 3 Average experimental collision efficiency for hAdV35 as a function of specific discharge

because they depend on a variety of parameters [e.g., nature of the grain surface (Mills et al. 1994), the ionic strength of the solution (Jewett et al. 1995), the presence of natural organic matter (Johnson and Logan 1996), and virus surface properties (Jin et al. 1997)]. The α values hold information about the hAdV35 adsorption onto glass beads. Note that, α average values decreased with decreasing Q and q (see Fig. 3). According to the CFT, it is expected to observe an increase of virus retention in porous media with decreasing q or U, because the number of collisions between viruses and collectors (glass beads) is expected to increase too (Camesano and Logan 1998; Choi et al. 2007; Pang 2009). Note that the CFT assumes that the column packing is clean, and that deposited colloids do not affect the rate of colloid removal. If a collector becomes partly blocked by the presence of attached viruses, then α may either increase or decrease when additional viruses are added, depending on whether virus–virus attachment is favorable or unfavorable (Walshe et al. 2010). Although detachment rate constants and inactivation of hAdV35 in the laboratory-scale column experiments of this study were assumed to be relatively negligible due to short residence time, certainly this may not be the case for hAdV35 transport at the field-scale where the detachment rate and inactivation constants of suspended and attached viruses could be significant.

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 $I_s = 1.4$ mM), are shown in Fig. 4. In this study, hAdV35 was considered to follow the principles of colloid chemistry, despite the fact that hAdV35 is more complex than abiotic colloids (van Loosdrecht et al. 1989). Note that the interaction energy between two approaching surfaces versus their separation distance typically consists of a primary minimum, Φ_{min1}, a primary maximum, Φ_{max1}, and the secondary minimum, Φ_{min2}. For the case of hAdV35-glass beads interaction (Fig. 4a), the DLVO interaction energy is repulsive for relatively long separation distances. That repulsion is negligible at a distance of 90 nm, but it continuously increases as the separation distance decreases. A maximum repulsion (Φ_{max1}) is observed at 4 nm separation distance. At separation

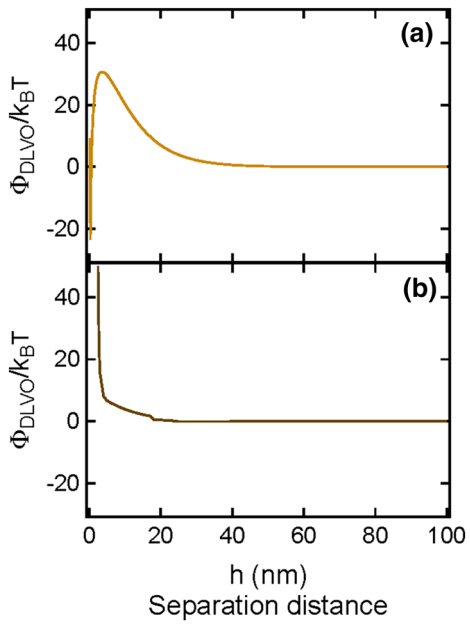

Fig. 4 Predicted normalized Φ_{DLVO} interaction energy profiles for: **a** hAdV35-glass beads treated as sphere-plate, and **b** hAdV35-hAdV35 treated as sphere–sphere, as a function of separation distance (Here $I_s = 1.4$ mM, and pH 7.6)

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

distances below 4 nm, the Φ_{vdW} energy starts to dominate over the Φ_{dl} energy and the interaction between the hAdV35 and glass beads is attractive, suggesting that hAdV35 particles could adhere onto glass beads at Φ_{min1} if they have sufficient kinetic energy to overcome the potential energy barrier. All calculated Φ_{max1}, Φ_{min1}, and Φ_{min2} are listed in Table 2.

The Φ_{DLVO} interaction energy profile for hAdV35-hAdV35 interaction at pH 7.6 and low ionic strength $I_s = 1.4$ mM is shown in Fig. 4b. Clearly, the DLVO theory suggests that no aggregation between virus particles is expected to occur under the experimental conditions. However, hAdV35 aggregation could be seen at high I_s values. Surprisingly, Trilisky and Lenhoff (2007) showed that hAdV5 did not aggregate at I_s as high as 4 M even after a month. One possible explanation for the absence of aggregation at high I_s could be that the virus particles are strongly hydrated, which reduces the effect of van der Waals attraction (Paliwal et al. 2005). The presence of fibers on the surface of the adenovirus could also add some repulsion, but steric effects are not likely to be the major cause of hAdV5 stability against aggregation because the virus has shown to aggregate at pH values near the IEP (Trilisky and Lenhoff 2007).

Summary and 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, α, 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.

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