ground water

Systematic Study of Effects of pH and Ionic Strength on Attachment of Phage PRD1

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Abstract

Objectives of this work are to investigate effects of pH and ionic strength (IS) on virus transport in saturated soil and to develop a quantitative relationship for these effects. A series of 50-cm column experiments with clean quartz sand under saturated conditions and with pH values of 5, 6, 7, 8, and IS values of 1, 10, and 20 mM were conducted. Bacteriophage PRD1 was used as a model virus. Applying a one-site kinetic model, attachment, detachment, and inactivation rate coefficients were determined from fitting breakthrough curves using the software package Hydrus-1D. Attachment rate coefficients increased with decreasing pH and increasing IS, in agreement with DLVO theory. Sticking efficiencies were calculated from the attachment rate coefficients and used to develop an empirical formula for sticking efficiency as a function of pH and IS. This relationship is applicable under unfavorable conditions for virus attachment. We compared sticking efficiencies predicted by the empirical formula with those from field and column experiments. Within the calibrated range of pH and IS, the predicted and observed sticking efficiencies are in reasonable agreement for bacteriophages PRD1 and MS2. However, the formula significantly overestimates sticking efficiencies for IS higher than 100 mM. In addition, it performs less well for viruses with different surface reactivity than PRD1 and MS2. Effects of pH and IS on detachment and inactivation rate coefficients were also investigated but the experimental results do not allow constraining these parameters with sufficient certainty.

Introduction

Groundwater is a major source for drinking water, because of its good microbial quality in its natural state as compared with fresh surface water. Nevertheless, it may be contaminated with pathogenic microorganisms (Schijven et al. 2010), especially viruses, and that may hamper drinking water production (Schijven and Hassanizadeh 2000). Groundwater can be protected from contamination with pathogenic microorganisms by applying adequate setback distances between sources of contamination and production wells, using soil as a barrier (Schijven and Hassanizadeh 2002). In order to establish protection zones around abstraction wells, data need to be collected and deterministic relationships need to be developed for quantifying the removal processes during subsurface transport. In that regard, removal processes (attachment and inactivation) largely determine the size of the required protection zones.

According to current regulations in the Netherlands, protection zones of shallow unconfined sandy aquifers should ensure a travel time of 60 d (Schijven et al. 2006). However, using conservative estimates of removal processes, Schijven et al. (2006) found that a travel time

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of 1 to 2 years may be required for shallow, unconfined sandy aquifers. Nevertheless, to date, the uncertainties with respect to the extent of removal processes are large and therefore the required extension of protection zones cannot be estimated accurately. Greater certainty about the requirements for protecting drinking water wells from virus contamination is urgently needed as the enlargement of the protection zones has great consequences for spatial planning. To identify and reduce these uncertainties, the relationship between removal processes and physicochemical conditions of the groundwater and sediments needs to be quantified at different scales. The two most significant processes controlling virus mobility in the subsurface environment are virus attachment and inactivation (Schijven et al. 2006). Based on previous studies, many factors have been identified that impact these processes, such as the size and isoelectric point of viruses (Dowd et al. 1998), ionic strength (IS) and pH of the groundwater (Fontes et al. 1991; Torkzaban et al. 2006), organic content of the groundwater (Pieper et al. 1997; Zhuang and Jin 2003; Foppen et al. 2006), aquifer substrate grain size (Fontes et al. 1991), and soil water content (Jin et al. 2000; Torkzaban et al. 2006). Other hydrological factors are also important such as flow velocity and the heterogeneity of the aquifer substrate (Bhattacharjee et al. 2002; Walshe et al. 2010).

Among previously mentioned factors, pH and IS seem to have the largest influence on virus removal (Schijven and Hassanizadeh 2000). As Schijven and Hassanizadeh (2000) reviewed, in many studies it has been shown that virus attachment is generally much less at higher pH. This can be explained, according to the DLVO theory, by the increased electrostatic repulsion at higher pH. In addition, it is in agreement with DLVO theory that attachment rates of viruses to solid particles increase with increasing IS because of compression of double layers around soil grains (Israelachvili 1992). Comprehensive studies on the effect of pH on virus attachment have been conducted by Grant et al. (1993) and Loveland et al. (1996) and on the effect of IS by Grant et al. (1993) and Penrod et al. (1996). Nevertheless, so far, a quantitative relationship for the combined effect of pH and IS on virus attachment for a wide range of values of pH and IS is not yet established.

The objectives of this work have been to investigate the effects of pH and IS on virus removal in saturated porous media and to develop quantitative relationships for these effects. In order to do so, a systematic study in columns with clean sand under saturated conditions at various pH and IS values, within the range of field conditions typically encountered in sand aquifers, was conducted using bacteriophage PRD1 as a model virus.

Materials and Methods

Column Setup and Operation

A cylindrical polymethylmethacrylate column with an inner diameter of 5 cm and length of 50 cm was packed with quartz sand. Top and bottom lids were made of polyoxymethylene with an inlet in the middle for the water flow. Between lid and column two 80 to 130 mesh hydrophilic polyethylene screens were placed to distribute the water evenly over the entrance/exit area.

Quartz sand (H31, Sibelcoo, Belgium) with an average grain size of 0.44 mm was used to pack the column. In order to remove potential impurities, the procedure used by Foppen et al. (2007) was adopted to clean the sand. The sand was heated to 850 ± 50 °C for 4 h followed by acid washing in 12 N HCl for 48 h and rinsing with de-ionized water until electrical conductivity of rinse water was less than 1 μS/cm. Before packing, the sand was boiled in de-ionized water to facilitate hydration of the sand grain surfaces and remove air. Columns were packed incrementally under saturated conditions. During column packing the sand was stirred with a steel spatula to prevent particle size separation during settling. To support settling, vibration via a rubber mallet was applied to each increment of sand. Columns were packed with new sand for each experiment.

Preparation of Solution

For the preparation of the solution used in batch and column experiments, the following procedure was used. With the aid of thermodynamic equilibrium calculations (MINEQL+ 4.6), the amount of bicarbonate required to achieve a desired value of pH in equilibrium with the atmospheric CO_2 pressure was calculated. Then, NaCl was added to adjust IS by taking into consideration the amount of $NaHCO_3$. In total, 12 different solutions with various combination of pH values of 5, 6, 7, 8 and IS values of 1, 10, and 20 mM were used in experiments. The composition of the experimental solutions is listed in Table 1. Prior to the column experiments, the solution was equilibrated open to the atmosphere over several days in which the pH was regularly readjusted with NaOH or HCl. The additionally added amounts of NaOH and HCl did not alter IS significantly.

Columns were flushed with several pore volumes of water of the desired pH and IS until the difference in pH and electric conductivity of the influent and effluent was not more than 0.05 and 10 μS/cm, respectively. Columns were operated under saturated conditions and at steady-state flow. Flow rate was measured just before seeding the column and a second time before the arrival of breakthrough curve. Effluent samples were collected in 20-mL glass tubes using a fraction collector. All column

Table 1
The Composition of Inflow Solution

	$NaHCO_3$			NaCl		
pH	IS 1 (mM)	IS 10 (mM)	IS 20 (mM)	IS 1 (mM)	IS 10 (mM)	IS 20 (mM)
5				1.00	10.00	20.00
6	0.01	0.01	0.01	0.99	9.99	19.99
7	0.07	0.08	0.08	0.93	9.92	19.92
8	0.72	0.76	0.79	0.28	9.24	19.21

experiments were conducted in a temperature controlled room (11 ± 1 °C) to mimic typical conditions in Dutch aquifers.

A pulse of 10 mM NaCl solution with the length of 0.25 to 0.40 pore volumes in the inflow was used as a tracer in order to determine dispersivity and porosity of each column. Salt breakthrough data were analyzed using Hydrus-1D (Simunek et al. 2005).

Bacteriophage PRD1

Bacteriophage PRD1 was used as a model virus in our experiments. PRD1 is an icosahedral phage with a diameter of 62 nm and an isoelectric point between pH 3 and 4, implying it is strongly negatively charged at pH values of 5 and higher (Loveland et al. 1996). A suspension of 10^{13} plaque forming particles (pfp)/mL was prepared as described in ISO 10705-1 and stored at 5 ± 3 °C. For each column experiment, seeding suspensions of bacteriophage PRD1 containing about 10^5 pfp/mL were prepared from the stock suspension by diluting with the solution set to the pH and IS that was used for running the column experiment. Each seeding suspension was introduced into the column for about 7 h at a constant flow rate. The influent was then switched to a bacteriophage-free solution after a given number of pore volumes as listed in Table 2.

Salmonella typhimurium LT-2 was the host bacteria for PRD1. Host bacteria and bacteriophage were obtained from the National Institute of Public Health and the Environment, Bilthoven, The Netherlands (RIVM). The samples were assayed using the plaque forming technique described by ISO 10705-1 (1995), with the omission of nalidixic acid. All samples were analyzed within 24 h of collection and samples with anomalous values were retested the following day.

Inactivation Experiments

Inactivation of PRD1 in water, defined as gradual loss of the ability to infect its bacterial host, was measured in batch experiments under controlled room temperature (11 ± 1 °C). For this purpose, 100-mL glass bottles were filled with water at IS of 1 mM and five different pH values ranging from 4 to 8 ($NaHCO_3$/NaCl). The initial concentration of bacteriophages was about 4000 pfp/mL. The concentration of active bacteriophages was monitored over a period of 3 weeks by regularly taking subsamples. From these data, the inactivation rate coefficient in water, μ_l, was calculated for different pH values at IS = 1 mM. In addition, inactivation experiments were conducted at IS of 20 mM but only at pH 7. The same μ_l values as for IS = 20 mM were used for IS = 10 mM, as they are expected to be close to each other. Values for the inactivation rate coefficients, μ_l, were estimated by means of linear regression analysis.

Modeling of Transport and Fate of Viruses in Saturated Sand Columns

The governing equations for modeling virus transport, including advection, dispersion, attachment, detachment, and inactivation are (Bales et al. 1991):

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(\alpha_L v \frac{\partial C}{\partial x}\right) - v\frac{\partial C}{\partial x} - k_{att}C - \mu_l C + k_{det}\frac{\rho_B}{n}S \quad (1)$$

$$\frac{\partial S}{\partial t} = \frac{n}{\rho_B}k_{att}C - k_{det}S - \mu_s S \quad (2)$$

where C is the number density of viruses in water [no. of virus particles L^{-3}], S is the number of attached viruses per unit mass of soil [no. of virus particles M^{-1}], ρ_B is the dry bulk density [ML^{-3}], α_L is dispersivity [L], v is the pore water velocity [LT^{-1}], n is the porosity [−], μ_l and μ_s are the inactivation rate coefficients for bacteriophages in water and attached to the solid surface, respectively [T^{-1}]. k_{att} and k_{det} are the attachment and detachment rate

Table 2
Measured and Estimated Parameter Values for Various pH and IS

pH	IS (mM)	n	α_L	v (cm/h)	PD[1] (PV)	k_{att} (h^{-1})	95% CI (h^{-1}) Low	95% CI (h^{-1}) High	k_{det} (h^{-1})	95% CI (h^{-1}) Low	95% CI (h^{-1}) High	μ_l (h^{-1})	μ_s	R^2	α
8	1	0.37	0.20	2.09	1.12	0.0045	0	0.0093	0.20	0	0.89	0.003	0.015	98%	0.00011
7	1	0.36	0.20	2.17	1.08	0.041	0.023	0.060	0.0019	0	0.015	0.005	0.025	95%	0.0011
6	1	0.36	0.22	2.11	1.05	0.070	0.044	0.095	0.0009	0	0.0091	0.004	0.025	92%	0.0018
5	1	0.36	0.20	1.95	1.22	0.11	0.10	0.12	0.0031	0	0.0068	0.006	0.035	97%	0.0030
8	10	0.37	0.20	2.11	1.02	0.038	0.028	0.049	0.0036	0	0.011	0.007	0.040	97%	0.0010
7	10	0.37	0.20	2.05	1.04	0.040	0.028	0.052	0.0026	0	0.0094	0.007	0.035	97%	0.0011
6	10	0.37	0.20	2.16	1.57	0.14	0.12	0.16	0.0030	0	0.0073	0.007	0.035	92%	0.0036
5	10	0.36	0.20	2.09	1.50	0.80	0.79	0.82	0.0070	0.0059	0.081	0.007	0.035	95%	0.021
8	20	0.36	0.20	2.09	1.28	0.11	0.089	0.12	0.0045	0	0.0094	0.007	0.027	95%	0.0027
7	20	0.36	0.20	2.12	1.41	0.21	0.19	0.22	0.014	0.011	0.017	0.007	0.027	94%	0.0053
6	20	0.36	0.20	2.04	1.51	0.55	0.54	0.57	0.0036	0.0026	0.0046	0.007	0.027	94%	0.015
5	20	0.36	0.20	2.11	1.40	2.08	2.02	2.14	—	0	0.0095	0.007	0.035	65%	0.054

[1]Pulse duration in pore volumes (PV).

coefficients [T^{-1}], respectively. The following boundary conditions were applied:

$C = C_0$ at $x = 0$ and $\frac{\partial C}{\partial x} = 0$ at $x = L$, where L is the column length.

Commonly, the attachment coefficient is assumed to be related to the average flow velocity. The formula that is often used is (Tufenkji and Elimelech 2004):

$$k_{att} = \frac{3}{2}\frac{1-n}{d_c} v \alpha \eta_0 \quad (3)$$

where d_c is the diameter of grain size [L], α is the sticking efficiency [−], and η_0 is the single-collector contact efficiency [−], representing the ratio of the rate of particles approaching the collector to the rate of particles striking a collector (Tufenkji and Elimelech 2004). The single-collector contact efficiency η_0 can be calculated independently (Tufenkji and Elimelech 2004). Hence, for a given k_{att} the sticking efficiency parameter (α) can be calculated from Equation 3. The sticking efficiency is defined as the ratio of the number of collisions that result in attachment vs. the total number of collisions. Essentially, α represents the probability that collision will end in attachment. The significance of the sticking efficiency is that, in contrast to k_{att}, it is considered to be independent of hydrologic conditions.

Parameter Estimation

Breakthrough curves were fitted for parameter estimation using Hydrus-1D (Simunek et al. 2005). Values for medium porosity (n) and dispersivity (α_L) were obtained by fitting the salt breakthrough curves. Pore water velocity v was calculated from n and the measured superficial velocity. The inactivation rate of free phage was determined directly from laboratory measurements as described in the *Inactivation Experiment* section. Using v and α_L from the salt breakthrough curves as fixed values, values for k_{att}, k_{det}, and μ_s were obtained from fitting the virus breakthrough curves to resident concentration values. The concentration values of the tails of the breakthrough curves are 2 to 3 orders of magnitude lower than the maximum breakthrough concentrations. In order to ensure their influence in the fitting procedure, they were therefore, given weights of ten in order to achieve proper fitting of the tails, while maintaining proper fitting of the maximum breakthrough concentrations.

The k_{att} values that were obtained from fitting the breakthrough curves were used to calculate sticking efficiencies using Equation 3. An empirical formula was then developed to relate α to pH and IS using the function NonlinearModelFit in Mathematica (version 7, Wolfram Research Inc., Champaign, Illinois).

Results and Discussion

Figure 1 presents the measured and fitted breakthrough curves from the column experiments with clean quartz sand conducted at the various pH and IS values. In these graphs, the normalized effluent concentration is plotted vs. the number of pore volumes passed through the column. Breakthrough of the injected bacteriophage was measured in the effluent after approximately 0.70 pore volumes. In all cases, a tail was observed except for the experiment at pH 5 and IS 20 mM, where there was a rapid decline to concentrations below the detection limit for 1 mL of sample. This tail shows that detachment of bacteriophage particles takes place. The height of this tail is mainly determined by the detachment rate, whereas the slope of the tail is mainly determined by the inactivation rate of attached phage particles (Schijven et al. 1999). There was no time difference between the arrival of the NaCl tracer and the bacteriophage. Parameter values for k_{att}, k_{det}, and μ_s obtained from fitting the breakthrough curves with the one-site kinetic model are listed in Table 2.

From Figure 1, it can be seen that for a given IS (1, 10, and 20 mM) the breakthrough concentration decreases with decreasing pH. While for a given pH, the breakthrough concentration increases with decreasing IS. The effect of pH on the breakthrough concentration is stronger at higher IS. Because the breakthrough concentration is mainly determined by attachment (Schijven et al. 1999), it implies that attachment increases with decreasing pH and increasing IS. These trends are also apparent from the k_{att} values given in Table 2.

The values of μ_l at IS of 1 mM and pH values from 5 to 8, varied between 0.003 and 0.006 h^{-1}, whereas the value at pH 7 and IS of 20 mM was 0.007 h^{-1}. These values are so low that inactivation within the time span of the column experiments was negligible. Nevertheless, a μ_l of 0.007 h^{-1} was used in the fitting of breakthrough curves with IS of 10 and 20 mM.

In a number of cases, tailing was measured up to 8 to 10 pore volumes. In those cases, the slopes of the tails are similar. Because the slope of the tail is mainly determined by the inactivation rate of attached viruses (Schijven et al. 1999), apparently, pH and IS have little effect on this process. The values of μ_s varied between 0.015 and 0.04 h^{-1}, with the majority being near 0.035 h^{-1} with no obvious effect of pH or IS. However, the value of μ_s is 5 to 6 times higher than that of μ_l. The heights of the tails are mainly determined by detachment (Schijven et al. 1999). The estimates of k_{det} are very uncertain (Table 2). When taking the 95% confidence interval into consideration, with the exception of pH 8 and IS of 1 mM, there is no apparent effect of pH and IS on the value of the detachment rate coefficient.

From non-linear model fitting, sticking efficiencies were determined as a function of pH and IS. R^2, the square of the sample correlation coefficient, was 99.2%. The following empirical formula was obtained:

$$\alpha(\mathrm{pH}, \mathrm{IS}) = \mathrm{Exp}(a_0 - a_1 \mathrm{pH} + a_2 \mathrm{IS}) \quad (4)$$

The values of coefficients a_0, a_1, and a_2 are given in Table 3, including their standard error.

Within the range of experimental conditions, the logarithm of the sticking efficiency appears to depend

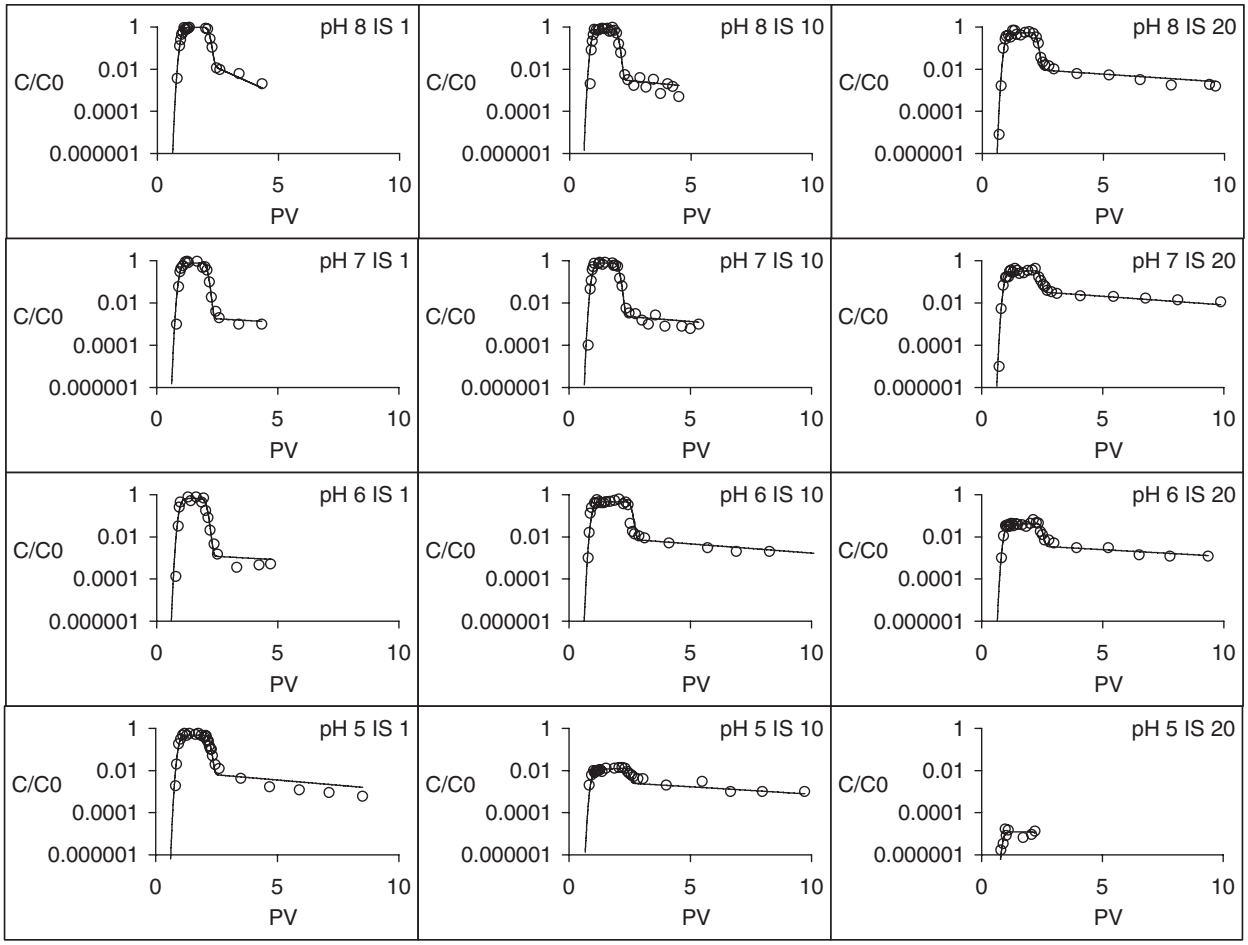

Figure 1. Measured breakthrough concentration of bacteriophage PRD1 (open circles) fitted with a one-site kinetic model (solid line).

Table 3
Values of a_0, a_1, and a_2 in Equation 4

	a_0	a_1	a_2
Estimate	1.29	1.28	0.11
Standard error	0.53	0.099	0.0082

linearly on pH and IS. Figure 2 shows the estimated sticking efficiencies and the fitted formula. This empirical equation can be rationalized based on the interaction force boundary layer (IFBL) model (Cail and Hochella 2005). According to this model, the sticking efficiency is exponentially related to the intersurface potential energy. This explains the exponential form of Equation 4 when relating the terms inside the exponential function to the intersurface potential energy. This energy depends, according to DLVO theory, on the surface potentials of the particles and the Debye length (Israelachvili 1992). Both bacteriophage and quartz have variable-charge surfaces and they are both negatively charged in the investigated pH range. Consequently, the absolute value of their surface charge densities increases with pH. At constant

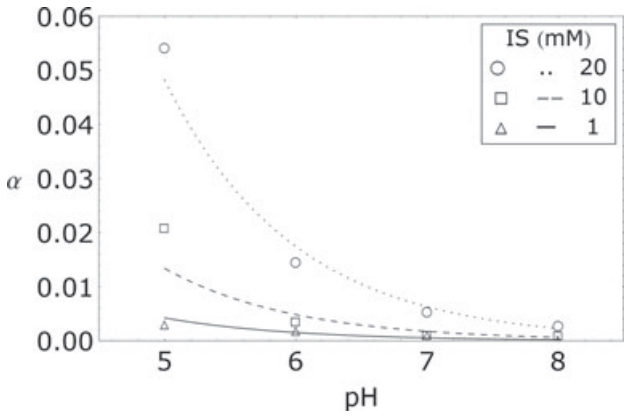

Figure 2. Sticking efficiency of bacteriophage PRD1 as a function of pH and ionic strength.

IS, an increase in surface charge density is accompanied by an increase in surface potential. This, in turn, implies that the intersurface potential energy, hence the energy barrier, becomes larger which has a negative effect on the sticking efficiency. This relationship is reflected in the negative pH term in the exponential function given in Equation 4.

The effect of IS is less straightforward. On one hand, the Debye length is a square root function of IS and the compression of the double layer reduces the intersurface potential energy according to DLVO theory. On the other hand, diffuse double layer theory predicts that the suppression of electrostatic effects at elevated IS facilitates the dissociation of functional groups at the particle surfaces (Stumm 1992). As a consequence, the surface charge densities are expected to increase but this increase has little effect on the surface potentials. This is because the relationship between surface charge density and surface potential is also IS dependent for variable-charge surfaces (Stumm 1992). In summary, the decrease in Debye length according to the DLVO theory is the dominating effect of increasing IS on sticking efficiency. The positive term in the exponential function accounts for this effect.

In conclusion, Equation 4 captures the main trends predicted by theory, even though it is not derived from rigorous combination of IFBL model, DLVO and double layer theory. It is also evident that only a relationship integrating the effects of both pH and IS on the sticking efficiency can be of general applicability when dealing with particles and collectors with variable-charge surfaces. The latter is usually the case for the transport of viruses in aquifer material. Hence, we assume that Equation 4, in its general form, can be widely applied to relate sticking efficiencies for viruses in aquifers to pH and IS. However, the general form of Equation 4 is inappropriate when virus and mineral surfaces are oppositely charged. When particle and collector are oppositely charged, particle deposition is not controlled by the effects of electrostatic repulsion but by transport limitation (Spielman and Friedlander 1974) and Equation 4 fails to account for this. In the relevant pH range, bacteriophage PRD1 surfaces are generally negatively charged (Bales et al. 1991). In addition, the main components of sedimentary aquifers, silicates and carbonates, tend to be negatively charged at neutral pH (Stumm 1992). However, iron and aluminum oxides have typically a pH_{pzc} (point of zero charge) above

Table 4
Comparison Between Sticking Efficiency from Previously Published Literature and Empirical Formula Used in This Study

	Microbe	Soil	pH	IS (mM)	α measured	Reference	α model
Column	PRD1	Sand Cape Cod	5.7	100	0.94	Kinoshita et al. (1993)	>1
			7	100	0.82		>1
			8.2	100	0.58		>1
	MS2	Sand Cape Cod	5.7	100	0.007	Kinoshita et al. (1993)	>1
			7	100	0.01		>1
			8.2	100	0		>1
		Quartz	3.5	10	0.12	Penrod et al. (1996)	0.124
			3.5	300	0.16		>1
			5	10	0.009		0.018
			5	100	0.09		>1
			5	300	0.04		>1
	λ	Quartz	3.9	10	1.25	Penrod et al. (1996)	0.074
			5	10	0.045		0.018
			5	100	0.53		>1
			5	300	0.65		>1
	Polio1	Silica beads	5.5	50	0.014	Bales et al. (1993)	0.779
			7	50	0.004		0.114
			7	50	0.0072		0.114
Field	PRD1	Sand	7.4	5.0	0.0029	Bales et al. (1997)	0.0005
		(Borden)	8.4	5.0	0.0012		0.0001
		Sand	5–5.7	1.3	0.011	Pieper et al. (1997)	0.0028–0.0070
		Cape Cod	6–6.7	6.4	0.002		0.0007–0.0034
		Sand	5.4–5.6	0.6	0.0032 ± 0.0016	Ryan et al. (1999)	0.0030–0.0038
		Cape Cod	5.8–6	4.6	0.0016 ± 0.005		0.0028–0.0036
		Dune sand	7.3–8.3	14.4	0.0024	Schijven et al. (1999)	0.0004–0.0015
			7.3–8.3	14.4	0.00043		0.0004–0.0015
	FRNAPH's	Dune sand	7.3–8.3	14.4	0.02	Schijven et al. (1998)	0.0004–0.0015
			7.3–8.3	14.4	0.00078		0.0004–0.0015
	ϕX174	Sand/gravel	7.2	4.6	0.006–0.311	DeBorde et al. (1998)	0.0006
		Missoula	7.2	4.6	0.007–0.319		0.0006
	Polio 1	Sand/gravel	7.2	4.6	0.047–2.108	DeBorde et al. (1998)	0.0006
			7.2	4.6	0.019–0.866		0.0006

7.0 implying that the surface charge is positive at neutral pH. Even when present in low amounts, these phases might account for a significant part of the reactive surface area in aquifers when occurring as surface coatings or as the gibbsite layer in kaolinite. Under these conditions, pH and IS might be less important regarding virus attachment.

In order to evaluate the utility of this formula for application to field situations, we have compiled values of sticking efficiency reported in the literature. In particular, we have collected data from sandy aquifers, where sticking efficiencies have been determined with reasonable certainty. Data is given in Table 4. The calculated sticking efficiencies by using Equation 4 are given in the last column of the table. At high IS ($\geq$100 mM) high sticking efficiencies ($>$1) are predicted by using Equation 4 and therefore overestimates the observed values. Equation 4 has only been calibrated until an IS of 20 mM and apparently fails when extrapolating to higher IS. In Figure 3, sticking efficiencies obtained from field and column experiments under conditions within the calibrated pH and IS range are plotted against those calculated using our empirical formula. Figure 3 shows that the calculated and reported sticking efficiencies are in reasonable agreement for MS2 from column experiments and for PRD1 from field studies. Like PRD1, MS2 is a strongly negatively charged virus (Schijven and Hassanizadeh 2000). Our empirical formula underestimates the sticking efficiencies of bacteriophage ϕX174 and of poliovirus, but this is evident from the fact these viruses are not so strongly negatively charged as MS2 and PRD1 (Schijven and Hassanizadeh 2000). Nevertheless, from Figure 3 it can also be seen that there is a similar trend between measured sticking efficiency and calculated sticking efficiency for PRD1, MS2, and phage λ. The latter is an icosahedral bacteriophage with a tail.

Most of the measured and predicted sticking efficiencies listed in Table 4 are above 4×10^{-4}. However, in other field studies under anoxic conditions (Schijven et al. 2000; Wielen et al. 2008), sticking efficiencies as low as 10^{-5} were estimated. The observation of very low sticking efficiencies can be attributed to scale effects. A relationship between column length and sticking efficiencies has been reported for *E. coli* strains (Lutterodt et al. 2009). In longer columns, it is possible to determine attachment under unfavourable conditions with greater accuracy. In addition, the existence of possible subpopulations of the bacteriophages with lower sticking efficiency would become apparent in experiments with longer columns.

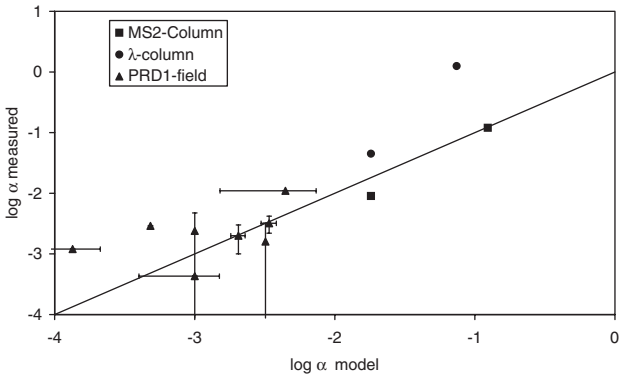

Figure 3. Comparison between sticking efficiency from previously published literature and empirical formula used in this study.

Summary and Conclusions

An empirical formula for the sticking efficiency as a function of pH and IS has been developed for bacteriophage PRD1 in clean quartz sand columns for the pH range of 5 to 8 and IS of 1 to 20 mM. This is the first time that such a quantitative formula has been presented. Sticking efficiencies were found to increase with decreasing pH and increasing IS, in agreement with DLVO theory. The comparison of values of the sticking efficiency reported in literature with those calculated using the empirical formula shows reasonable agreement for the range of ISs we have tested. Effects of pH and IS on the detachment rate coefficient as well as on the inactivation rate coefficient of attached virus particles still need to be determined with higher certainty.

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