

1 **Effect of dissolved calcium on the removal of bacteriophage PRD1 during soil**
2 **passage: the role of double-layer interactions**

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18 **Keywords** Colloids, solute transport, bacteriophage PRD1, Calcium

1 **Abstract**

2

3 The objective of this work was to investigate and obtain quantitative relations for
4 the effects of Ca^{2+} concentration on virus removal in saturated soil. In order to do so, a
5 systematic study was performed with a range of calcium concentrations corresponding to
6 natural field conditions. Experiments were conducted in a 50-cm column with clean
7 quartz sand under saturated conditions. Inflow solutions were prepared by adding CaCl_2 ,
8 NaCl and NaHCO_3 to de-ionized water. Values of pH and ionic strength were fixed at 7
9 and 10 mM, respectively. Bacteriophage PRD1 was used as a conservative model virus
10 for virus removal. The samples were assayed using the plaque forming technique.

11 Attachment, detachment and inactivation rate coefficients were determined from fitting
12 breakthrough curves. Attachment rate coefficients were found to increase with increasing
13 calcium concentration. Results were used to calculate sticking efficiency, for which an
14 empirical formula as a function of Ca^{2+} was developed. Numerical solutions of the
15 Poisson-Boltzmann equation were obtained to evaluate the effect of Ca^{2+} on the double-
16 layer interactions between quartz and PRD1. Based on these results, the DLVO
17 interaction energies were calculated. It turned out that the experimental findings cannot
18 be explained with the distance profiles of the DLVO interaction. The discrepancy
19 between theory and experiment can be attributed to underestimation of the van der Waals
20 interactions, chemisorption of Ca^{2+} onto the surfaces, or by factors affecting the double-
21 layer interactions, which are not included in the Poisson-Boltzmann equation.

22 When abruptly changing from inflow solution containing Ca^{2+} to a Ca^{2+} -free solution,
23 pronounced mobilization of viruses was observed. This indicates virus removal is not

1 irreversible and that chemical perturbations of the groundwater can cause a burst of
2 released viruses.

3

4 **Introduction**

5

6 Groundwater is the main source of drinking water for the world's population. It
7 usually is of excellent microbial quality and often directly underfoot at low capital cost.
8 Nevertheless, it may become contaminated with pathogenic microorganisms, especially
9 viruses, and that may pose a public health risk. Therefore, it is extremely important to be
10 able to describe and quantify all processes which affect virus transport through soil and
11 aquifers. The two most significant processes controlling virus mobility in the subsurface
12 environment are virus attachment and inactivation (Schijven and Hassanizadeh, 2000).
13 Based on earlier studies, many factors have been identified that impact these processes,
14 such as ionic strength (IS) and pH of the ground water (Fontes et al., 1991; Sadeghi et al.
15 2011) and organic content of the ground water (Foppen et al., 2006; Pieper et al., 1997;
16 Zhuang and Jin, 2003a). Also, presence or absence of bivalent cations, in particular
17 calcium (Redman et al., 1999) as one of the major cations in groundwater, could be of
18 great importance (Gerba and Schaiberger, 1975). Dissolved calcium is a weathering
19 product of almost all rocks and is, consequently, ubiquitous in most ground waters. Many
20 waters from limestone areas may contain 30–100 mg l⁻¹ of calcium and those associated
21 with gypsiferous shale may contain several hundred milligrams per liter. In Dutch
22 groundwaters, calcium concentrations range from 20 to 150 mg l⁻¹ (Vermooten et al.,
23 2007).

1 Numerous field-scale, laboratory-based and theoretical-based studies have
2 documented the influence of divalent cations on deposition of colloid and microbial
3 particles (e.g. Ryan and Elimelech, 1996; Redman et al., 1999; McCarthy et al.,
4 2002&2004; Zhuang and Jin, 2003b; Xiqing et al., 2004). Bales et al. (1991) showed in a
5 batch experiment at pH 5 that attachment of MS2 to silica beads was at least 10 times
6 higher in the presence of Ca^{2+} . Redman et al.(1999) found that the attachment of
7 bacteriophage SJC3 to quartz was more pronounced when the ionic strength was
8 increased by using a multivalent (Ca^{2+} or Mg^{2+}) instead of a monovalent (Na^+) cation.
9 Carlson et al. (1968) demonstrated that virus adsorption was greatly enhanced, when the
10 surface charge of the mineral changes from negative to positive as a result of presence of
11 multivalent cations such as Ca^{2+} and Al^{3+} .

12 The effect of multivalent cations on virus attachment can be attributed to the
13 change in electrostatic interactions between virus and mineral surfaces but other
14 interactions and inner sphere complexation of the cations at the particle surfaces might
15 also contribute to the observed effects. Furthermore, the relationship between the ionic
16 composition of the solution and the electrostatic interactions of charged particles can be
17 complex (Israelachvili, 1992). The lack in exact mechanistic understanding of the
18 underlying mechanism explains why the consequences of replacing monovalent by
19 multivalent cations on virus attachment have been interpreted in various ways and using
20 different terminology including: salt bridging (Pham et al., 2009; Bales et al., 1991;
21 Lukasik et al., 2000; Chu et al., 2003), charge neutralization (Bales et al., 1991; Lukasik
22 et al., 2000), screening of repulsive surface interaction energies between colloids and
23 grain surfaces (McCarthy and McKay, 2004), reduction of the net charge within the

1 electrokinetic shear plane (Simoni et al., 2000), and compression of the double-layer
2 (Huysman and Verstraete, 1993).

3 Although there are many studies that demonstrate qualitatively the relation
4 between bivalent ion concentration and deposition of bacteriophages, to our knowledge,
5 there is no quantitative relationship between the amount of bivalent cations and virus
6 attachment to the sand grains and virus inactivation. Such a quantitative relationship will
7 be needed for predicting virus transport under various geochemical conditions.

8 In this work, the influence of Ca^{2+} on the transport of bacteriophage PRD1 in saturated
9 quartz sand was investigated and an empirical formula for the effect of Ca^{2+}
10 concentration on virus attachment in saturated soil was developed. Furthermore,
11 numerical solutions of the Poisson-Boltzmann equation were used in order to determine
12 the change in double-layer interactions upon varying Ca^{2+} concentration. Based on these
13 calculations, the agreement of the observed trends with predictions from DLVO theory
14 was evaluated.

15

16 **Material and methods**

17

18 *Column set-up and operation*

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

1 Quartz sand (H31, Sibelcoo, Belgium) with an average grain size (d_{50}) of 0.42, d_{10} of 0.28
2 and d_{90} of 0.63 mm was used. In order to remove potential impurities, the procedure used
3 by Foppen et al. (2007) was adopted to clean the sand. The cleaning procedure included
4 heating at 850 ± 50 °C for four hours followed by washing with 12N HCL for 48 hours
5 and rinsing with de-ionized water until the electrical conductivity of rinse water was less
6 than $1 \mu\text{Scm}^{-1}$. Columns were packed incrementally under saturated conditions following
7 the procedure described in Sadeghi et al. (2011).

8 Inflow solutions were prepared by adding CaCl_2 , NaCl and NaHCO_3 to de-ionized
9 water. The amount of added bicarbonate was sufficient to achieve equilibrium with the
10 atmospheric CO_2 pressure at pH 7 and ionic strength (IS) of 10 mM. NaCl was used to
11 adjust IS by taking into consideration the amount of NaHCO_3 as well as the amount of
12 CaCl_2 . Prior to column experiments, the solution was equilibrated with open atmosphere
13 for several days, during which the pH was regularly readjusted with NaOH . The added
14 amounts of NaOH did not alter IS significantly. All chemicals were of analytical grade
15 and purchased from Merck. In total four different experiments have been conducted with
16 calcium concentrations ranging from 0 to $120 \text{ mg l}^{-1} \text{ Ca}^{2+}$, and are referred to as Ca0,
17 Ca20, Ca60 and Ca120. The molar composition of the experimental solutions is given in
18 table 1.

19 Columns were flushed with several pore volumes (PV) of water of the desired pH,
20 Ca^{2+} and IS until the differences in pH and electric conductivity of the influent and
21 effluent were less than 0.05 and $10 \mu\text{S/cm}$, respectively. Columns were operated under
22 saturated conditions and at steady-state flow. Flow rate was measured just before seeding
23 the column and after breakthrough of viruses and were found to be constant. Measured

1 flow rates were used to calculate the pore volumes of solution that had passed through the
2 column (table 2). Outflow samples were collected in 20-ml glass tubes using a fraction
3 collector. All column experiments were conducted in a temperature-controlled room at
4 9.5 ± 0.5 °C, representing typical groundwater temperature in the Netherlands.

5 A solution of 10 mM NaCl was input for 0.25 – 0.40 pore volumes as a tracer in
6 order to determine dispersivity and porosity of each column. Salt breakthrough data were
7 analyzed using Hydrus-1D (Simunek et al., 2005) to check the packing of the column and
8 to estimate column porosity and dispersivity.

9

10 *Bacteriophage PRD1*

11 Bacteriophage PRD1 was used as a model virus in our experiments. PRD1 is an
12 icosahedral phage with a diameter of 62 nm and an isoelectric point between pH 3 and 4,
13 implying it is negatively charged at pH 5 to 8 (Loveland et al., 1996). PRD1 may be
14 considered as a worst-case model virus because of its low inactivation rate between 10-23
15 °C (Blanc and Nasser, 1996). Because of its larger size, PRD1 is of interest as a
16 representative of rotaviruses and adenoviruses (Sinton et al., 1997). A suspension of
17 bacteriophage PRD1 containing about 10^5 - 10^6 plaque-forming particles per millilitre
18 (pfp/ml) was introduced into the column for about one pore volume at a constant flow
19 rate. The seeding suspension with PRD1 was prepared in the solution used as inflow in
20 the corresponding experiment. The influent was then switched to a bacteriophage-free
21 solution keeping the same flow rate. In two experiments (Ca60 & Ca120) after 25 pore
22 volumes, the inflow was switched to calcium-free solution with ionic strength of 1 mM
23 and the same pH value. This steady state condition was kept for about 40 PV.

1 The samples were assayed using the plaque forming technique described by ISO
2 10705-1 (1995), with the omission of nalidixic acid. Nalidixic acid was omitted because
3 *Salmonella typhimurium* LT-2 was the host bacteria used for the counting of PRD1 in
4 samples and this organism is sensitive to nalidixic acid. Nalidixic acid is added in order
5 to suppress the growth of other bacteria in the assay. This was not necessary in our
6 experiments as the solutions were clean and did not contain other bacteria. Host bacteria
7 and bacteriophage were obtained from the National Institute of Public Health and the
8 Environment, Bilthoven, The Netherlands (RIVM). All samples were analyzed within 24
9 hours of collection and samples with anomalous values were retested the following day.

11 *Inactivation experiments*

12 Inactivation of PRD1 in water was measured in batch experiments at a controlled
13 temperature of 9.5 ± 0.5 °C. For this purpose, 200-ml glass bottles were filled with water
14 at pH 7, IS of 10mM, and four different Ca^{2+} concentrations (0, 20 ,60 and 120 mg l⁻¹).
15 The initial concentration of bacteriophages was about 10^5 pfp ml⁻¹. The concentration of
16 active bacteriophages was monitored over a period of three months by regularly taking
17 subsamples. The length of the inactivation experiments exceeded that of the column
18 experiments because inactivation rates are low and a long duration was necessary to
19 obtain accurate values for the rate coefficient. Values for the inactivation rate
20 coefficients, μ_i , were estimated by means of linear regression analysis.

22 **Modelling of transport and fate of viruses in saturated sand columns**

1 The governing equations for modelling virus transport, including advection,
 2 dispersion, attachment, detachment and inactivation are (e.g. Bales et al., 1991; Schijven
 3 et al., 2000):

$$5 \quad \frac{\partial C}{\partial t} = \frac{\partial}{\partial x} \left(\alpha_L v \frac{\partial C}{\partial x} \right) - v \frac{\partial C}{\partial x} - k_{att} C - \mu_l C + k_{det} \frac{\rho_B}{n} S \quad (1)$$

$$6 \quad \frac{\rho_B}{n} \frac{\partial S}{\partial t} = k_{att} C - k_{det} \frac{\rho_B}{n} S - \mu_s \frac{\rho_B}{n} S \quad (2)$$

7
 8 where C [# of virus particles L^{-3}] is the number density of viruses in water, S [# of virus
 9 particles M^{-1}] is the number of attached viruses per unit mass of soil, ρ_B [ML^{-3}] is the dry
 10 bulk density, α_L [L] is dispersivity, v [LT^{-1}] is the pore water velocity, n [-] is the
 11 porosity, μ_l and μ_s [T^{-1}] are the inactivation rate coefficients for free and attached
 12 bacteriophages, respectively. k_{att} and k_{det} [T^{-1}] are the attachment and detachment rate
 13 coefficients, respectively. The following boundary conditions were employed:

$$14 \quad C=C_0 \text{ at } x=0 \text{ and } \frac{\partial C}{\partial x} = 0 \text{ at } x=L, \text{ where } L \text{ is the column length.}$$

15 Commonly, the attachment coefficient is assumed to be related to the average
 16 flow velocity (e.g. Tufenkji and Elimelech, 2004):

$$18 \quad k_{att} = \frac{3(1-n)}{2} \frac{v\alpha\eta_0}{d_c} \quad (3)$$

19

1 where d_c [L] is diameter of grain size, α [-] is the sticking efficiency and η_0 [-] is the
2 single-collector contact efficiency representing the ratio of the number of particles
3 approaching the collector to the number of particles striking a collector (Tufenkji and
4 Elimelech, 2004). The sticking efficiency parameter (α) is defined as the ratio of the
5 number of collisions that result in attachment versus the total number of collisions.
6 Essentially, α represents the probability that collision will end in attachment. The
7 significance of the sticking efficiency is that, in contrast to k_{att} , it is considered to be
8 independent of flow velocity.

9

10 **Parameter estimation**

11

12 Breakthrough curves were fitted for parameter estimation using Hydrus-1D
13 (Simunek et al., 2005). Values for medium porosity (n) and dispersivity (α_L) were
14 obtained fitting the salt breakthrough curves. Pore water velocity v was calculated from
15 n and the measured superficial velocity. The inactivation rate of free phage was
16 determined directly from laboratory measurements as described in inactivation
17 experiment section. Using v and α_L from the salt breakthrough curves as fixed values ,
18 values for k_{att} , k_{det} and μ_s were obtained from fitting the virus breakthrough curves. The
19 concentration values of the tails of the breakthrough curves are orders of magnitude lower
20 than the maximum breakthrough concentrations. Therefore, the tail concentrations were
21 given a weight of ten in the fitting process. This ensured a better fitting of the tail, whilst
22 maintaining proper fitting of the peak breakthrough concentrations.

1 The k_{att} values that were obtained from fitting the breakthrough curves were used to
2 calculate sticking efficiency, using equation (3). An empirical formula was then
3 developed to relate α to Ca^{2+} concentration by means of regression analysis.

4

5 **Evaluating the effect on double-layer interactions by replacing Na^+ with Ca^{2+}**

6

7 The DLVO theory provides a conceptual framework for rationalizing virus
8 attachment to geological media (Harvey and Ryan, 2004). According to this theory, virus
9 interaction with soil particles is largely governed by double layer interactions and van der
10 Waals forces (Murray and Parks, 1980). Replacing Na^+ with Ca^{2+} in a solution is
11 expected to affect double-layer interactions. In order to quantify this effect, an approach
12 based on the treatment of interparticular electrostatic interactions, was used as described
13 in Chapter 12 of *Intermolecular & Surface forces* (Israelachvili, 1992).

14 First, the influence of Ca^{2+} on the surface potential Ψ_0 was investigated based on
15 the diffuse double-layer (DDL) concept. The surface charge of PRD1 is predominately
16 controlled by the reaction of amino and carboxyl groups of the protein capsid (Harvey
17 and Ryan, 2004), which presumably have a broad range of intrinsic acidity constants.
18 There are no values reported for intrinsic equilibrium constants of reactive groups on the
19 PRD1 surface or for reactive site densities. However, the measured change in the zeta
20 potential of PRD1 upon pH variation shows a similar trend as that of quartz (Bales et al.,
21 1991). These curves further show that PRD1 is negatively charged at neutral pH similar
22 to quartz. Hence, it can be assumed that the surface of PRD1 has a variable charge and
23 surface complexation models are, in general, suitable for describing the change in surface

1 potential of PRD1 due to changes in pH and ion composition of the solution. The
2 application of a surface complexation model to describe the surface chemistry of
3 microorganisms is well established for bacteria (e.g. Yee & Fein, 2003). It is also difficult
4 to find parameterized surface complexation models for quartz which are based on
5 comprehensive surface titration data (Duval et al., 2002), therefore, a generic simplified
6 model was used to determine the effect of Ca^{2+} on the surface potentials of both
7 constituents, PRD1 and quartz.

8 For simplicity, the change in surface potential of both surfaces upon replacing Na^+
9 with Ca^{2+} , was calculated for a surface site S , which contains one type of functional
10 group, forming a negatively-charged surface site upon deprotonation according to the
11 reaction



13
14
15 The effect of Ca^{2+} solution on surface potential was calculated as a function of the
16 difference between the intrinsic acidity constant ($K_{a, \text{int}}$) and the pH in the solution for a
17 surface with a site density (Γ_{sites}) of 2.43 sites nm^{-2} . This value has been derived by
18 Schindler and Kamber (1968) for silicagel but the principal trends derived from these
19 calculations do not depend on the exact value for the site density.

20
21 Commonly-used computer programs based on equilibrium thermodynamics,
22 which include surface complexation by applying the DDL concept, usually do not
23 account for the valences of the ions in the solution. In these programs, the relationship

1 between surface potential and surface charge density are calculated for a 1:1 electrolyte.
 2 Hence, standard computer programs are not suitable for calculating the change in surface
 3 charge density and surface potential. However, bivalent counterions are more efficient in
 4 shielding the surface charge than monovalent ions. The surface potential of a variable
 5 charge surface can change considerably if only a small fraction of Na^+ is replaced in the
 6 solution with the corresponding number of moles of Ca^{2+} (Israelachvili 1992, Tables
 7 12.1). For this reason, the Grahame (1953) equation was used to relate the surface
 8 potential to the surface charge density σ (Cm^{-2}) as follows:

$$10 \quad \sigma = \sqrt{8\epsilon\epsilon_0 kT} \sinh(e\psi_0 / 2kT) \{ [Na^+]_{\infty} + [Ca^{2+}]_{\infty} (2 + \exp^{-e\psi_0/kT}) \}^{1/2} \quad (5)$$

11
 12 where ϵ is the permittivity of water, ϵ_0 is the permittivity of free space, k (JK^{-1}) stands for
 13 the Boltzmann's constant, e (C) is the electronic charge, Ψ_0 (V) is the electrostatic surface
 14 potential and T ($^{\circ}\text{K}$) is the temperature.

15 However, the surface charge density of a variably-charged surface is not constant but
 16 may vary due to a change in surface potential. This phenomenon can be described for this
 17 surface by the following equations:

$$18 \quad \sigma = F \frac{\Gamma_{sites} K_{a,app}}{K_{a,app} + \{H^+\}} \quad (6)$$

$$19 \quad K_{a,app} = K_{a,int} \exp\left(-\frac{\Delta z \Psi_0 F}{RT}\right) \quad (7)$$

20
 21 where $K_{a,app}$ [-] is the apparent acidity constant, F [C mol^{-1}] is the Faraday constant, and
 22 Δz is the change in charge of the surface group due to the reaction, which is -1 for the

1 deprotonation reaction (Eq. 4). For different electrolyte compositions and $pK_{a,int}$ values,
2 the surface potential was obtained by minimizing the difference between the surface
3 charge densities calculated with Eq. (5) and Eq. (6) in an iterative procedure.
4 For determining the free energy of electrostatic interactions between two planar surfaces,
5 the electrical potential and the concentration of dissolved ions as a function of distance
6 between the planes was calculated. Analytical solutions for 1:1 and 2:1 electrolytes can
7 be found in the literature, but, for mixed electrolytes with dissimilar valences, a
8 numerical procedure is required (Grahame, 1953). Hence, the decrease in electrical
9 potential with distance from the surface and the corresponding ion distribution was
10 approximated with an explicit numerical scheme. This was done by simultaneously
11 integrating Eq. (8) and Eq. (9) with a step size of 2×10^{-10} m starting at the surface with
12 the surface potential obtained from Eq. (5). Equation 8 is an integrated form of the
13 Poisson equation relating the change in potential at a given distance from the surface to
14 the corresponding number density of dissolved ions. The Boltzmann equation, Eq. (9), is
15 used to calculate the number density of dissolved ions in an electric field with the
16 potential ψ :

17

$$18 \quad \sum_i \rho_{xi} = \sum_i \rho_{\infty i} + \frac{\epsilon \epsilon_0}{2kT} \left(\frac{d\psi}{dx} \right)_x^2 \quad (8)$$

$$19 \quad \rho_{0i} = \rho_{\infty i} \exp^{-ze\psi_0/kT} \quad (9)$$

20

1 From this, the excess osmotic pressure P between two planar surfaces was calculated
2 from the concentrations of the ions in the middle between the two surfaces:

3

$$4 \quad P = kT \left[\sum_1 \rho_{(x/2)i} - \sum_1 \rho_{\infty i} \right] \quad (10)$$

5

6 For this, the mid-plane potential was assumed to be simply the sum of the potentials from
7 each surface at half distance. That is, the surface potential was assumed not to change
8 when the two surfaces approach each other. The electrostatic interaction free energy for a
9 given distance was then obtained by numerically integrating the excess osmotic pressure
10 from a distance of 40 nm to the distance of interest with a step size of 4×10^{-11} m.

11 Finally, the DLVO interaction potential was calculated by adding the interaction free
12 energy due to van der Waals interactions using a Hamaker constant A of 4×10^{-21} J (Eq.
13 11). This value has been reported for van der Waals interactions between poliovirus and
14 quartz (Penrod et al., 1996; Murray and Parks, 1980):

15

$$16 \quad W_{\text{vdw}} = - A / (12 \pi x^2) \quad (11)$$

17

18

19 **Results and discussion**

20

21 Figure 1 presents the measured and fitted breakthrough curves from the column
22 experiments with clean quartz sand conducted at the pH value of 7 and ionic strength of
23 10 mM and the four different concentrations of Ca^{2+} . In these graphs, the normalized

1 effluent concentration is plotted versus the number of pore volumes passed through the
2 column. The peak of breakthrough curves is related to the removal of PRD1, which is
3 determined by attachment and inactivation. The maximum PRD1 concentration in the
4 breakthrough curve for a calcium concentration of 120 mg l⁻¹ is almost three orders
5 smaller than that without Ca²⁺ at identical ionic strength. In all cases, a tail of the
6 breakthrough curves was observed, demonstrating slow detachment of attached phage
7 particles.

8 The fitted parameter values for k_{att} , k_{det} , μ_s and the corresponding experimental
9 conditions are listed in table 2. The effect of Ca²⁺ on the maximum PRD1 concentration in
10 the outflow is also reflected in values of the optimized transport parameters. The value
11 for k_{att} is more than 30 fold larger in the presence of 120 mg l⁻¹ Ca²⁺ than without Ca²⁺,
12 when both solutions have the same ionic strength (Table 2). In all cases, fitted values for
13 k_{det} were small. The values ranged from 0.0010 to 0.0020 h⁻¹. Regression analysis
14 revealed that there was no significant relation between k_{det} and [Ca²⁺] based on our data.
15 In all experiments μ_s was larger than μ_l .

16 Figure 2, shows that over the range of 0-120 mg l⁻¹ of [Ca²⁺], both attachment rate
17 coefficient (k_{att}) and sticking efficiency (α) increase linearly with Ca²⁺ concentration. By
18 means of regression analysis the following empirical formulas was obtained:

19

$$20 \quad k_{att} \text{ (h}^{-1}\text{)} = 0.011[\text{Ca}^{2+}] \quad (12)$$

$$21 \quad \alpha = 0.00048[\text{Ca}^{2+}] \quad (13)$$

22

1 wherein $[Ca^{2+}]$ is the calcium concentration in $mg\ l^{-1}$ in water. Intercepts were not
2 significantly different from zero and R^2 was almost 1.

3

4 Obviously, removal of PRD1 during its passage through quartz sand is not only
5 determined by pH and ionic strength (Sadeghi et al., 2011) but the valence of the
6 counterions can significantly change the rates of PRD1 attachment. An increase of virus
7 removal in the presence of bivalent cations has been reported (McCarthy and McKay,
8 2004). Here, an ionic strength of 10 mM was maintained to isolate the effect of the
9 valence of the counterions on PRD1 mobility. Equations 12 and 13 suggest, that the
10 attachment rate of PRD1 increases linearly when the concentrations of bivalent cations
11 increase and the ionic strength remains approximately constant. This is, for example,
12 typically the case when water percolates through soils. The concentration of bivalent
13 cations in soil solutions often increases with depth along the vertical weathering-leaching
14 gradient (Sverdrup and Warfvinge, 1993).

15

16 It needs to be evaluated in future experiments whether the linear relationship
17 holds for different substrates and under different conditions. It can be assumed that the
18 slope of the regression line depends on pH, ionic strength, and the mineral composition of
19 the substrate. Relating the empirical relationship to a mechanistic model might help to
20 extrapolate the relationship to other conditions. Below, the possibility to relate the effect
21 of Ca^{2+} on the double-layer interaction between PRD1 and quartz to the change in k_{att} is
22 discussed. Until a mechanistic model will be established, the empirical relationship

1 provides a first approximation for quantitatively implementing the effect of changing
2 concentrations of bivalent cations for predicting virus transport.

3

4 Figure 3 demonstrates that switching to calcium-free water with an ionic strength
5 of 1 mM causes high release of retained phage particles. This was observed in the
6 experiments with Ca^{2+} of 60 and 120 mg l^{-1} . Virus concentrations in the observed pulse of
7 remobilized exceed the peak values of breakthrough concentration in the presence of
8 Ca^{2+} . From this observation, one can conclude that the introduction of a lower IS and
9 lower $[\text{Ca}^{2+}]$ water causes fast detachment of attached viruses. Such chemical
10 perturbations and the resulting peak concentrations may also occur in natural systems.
11 For example, a rain event can dilute ionic strength levels near the surface and cause a
12 burst of released viruses. For these reasons, adsorption to soils cannot be considered as a
13 process of absolute immobilization of viruses in the water. Given the fact that viruses
14 may survive long periods of time (De Roda Husman et al., 2009), infective viruses may
15 be released from soil particles after immobilization for long periods of time.

16 An increase of k_{att} (or sticking efficiency) with increasing Ca^{2+} concentration is, in
17 the first instance, not unexpected. Ca^{2+} is more efficient to shield the charge of the
18 surfaces than Na^+ , which is intuitively expected to reduce the repulsive double-layer
19 interactions between surfaces with identical charge.

20 Replacing Na^+ with Ca^{2+} in the solution at constant ionic strength has a significant
21 effect on the surface potential when its absolute value exceeds about 30 mV. When the
22 pK_{int} is lower than the pH of the solution, the surface charge density and the absolute
23 value of the surface potential are low (Fig. 4). In this case, changing the Ca^{2+}

1 concentration has only little effect of the surface potential. However, when the pK_{int} is
2 lower than the pH, the surface potential strongly depends on Ca^{2+} concentration. When
3 the $pK_{\text{a,int}}$ is 1.2 units lower than the pH, the surface potential changes from -112 to -88
4 mV when a NaCl solution is replaced with a CaCl_2 solution of identical ionic strength. In
5 contrast, the surface potential only changes from -14.2 to -13.3 mV when the $pK_{\text{a,int}}$ is 1.8
6 units larger than the pH. The same effect can be observed when changing the site density
7 (data not shown). The effect of Ca^{2+} concentration is more pronounced when the site
8 densities are high, which leads to higher charge densities and higher absolute values of
9 surface potential. Hence, presence of bivalent counterions can have a pronounced effect
10 on the surface potential of variable charge surfaces, but the difference between 1:1 and
11 2:1 electrolytes with identical ionic strength is only relevant when the absolute value of
12 the surface potential is larger than about 30 mV. The effect of Ca^{2+} on surface potential
13 increases with increasing Ca^{2+} concentration but the greatest relative effect is obtained
14 when a small fraction of Na^+ is replaced with Ca^{2+} .

15
16 Although the absolute value of the surface potential decreases when replacing Na^+
17 with Ca^{2+} , the repulsive double-layer interaction energy can be higher at close distances.
18 With increasing Ca^{2+} concentration, the absolute value of the surface potential decreases
19 and the electric field extends less far into the solution (Fig. 5a). When two surfaces
20 approach each other, the absolute sum of the potentials of their electric fields at a given
21 distance is lower in the presence of Ca^{2+} than without it. It seems counterintuitive that the
22 electrostatic interaction energy can be higher in the presence of Ca^{2+} (Fig. 5b). This is
23 because the relative enrichment of Ca^{2+} in an electric field with a given negative potential

1 is larger than that of Na^+ (Eq. 9). Consequently, the osmotic pressure between two
2 surfaces can be larger when Ca^{2+} is added to the solution, although the absolute value of
3 the potential in the mid-plane is lower. For the $\text{pK}_{a,\text{int}}$, pH , and site density used in the
4 calculations shown in Fig. 5, the increase in surface potential and the compression of the
5 double-layer by adding Ca^{2+} results in lower double-layer interaction energies when the
6 distance between the surfaces is larger than about 2 nm. At closer distance, the double-
7 layer interaction energy increases with increasing Ca^{2+} concentration. This ambivalent
8 effect of Ca^{2+} has consequences when rationalizing virus attachment based on the DLVO
9 theory.

10

11 The effect of Ca^{2+} on the height of the energy barrier between the approaching
12 negatively charged virus and quartz surfaces also depends on the strength of the van der
13 Waals interactions. The energy barrier reflects the point at which the energy gain due to
14 van der Waals interactions upon further approach overcompensates the increase in
15 repulsive double-layer interactions. This implies that the height of the energy barrier
16 increases with increasing Ca^{2+} concentration when the barrier is located at distances
17 below 2 nm (Fig. 5c). This is the case when using a Hamaker constant of 4×10^{-21} J,
18 because the energy barrier is located at distances below 2 nm and the height of the energy
19 barrier increases with increasing Ca^{2+} concentration. An about ten times higher Hamaker
20 constant is required to invert this trend (data not shown).

21

22 The value and the position of the secondary minimum also depend on Ca^{2+} (Fig.
23 5d). The value of the secondary minimum decreases with increasing Ca^{2+} concentration.

1 This is because the secondary minima are located at a distance at which the repulsive
2 double-layer energy is lowered by adding Ca^{2+} . The trend of decreasing secondary
3 minima with increasing Ca^{2+} concentration is consistent for the range of evaluated $\text{pK}_{\text{a,int}}$
4 values (Fig. 6a) reflecting surface potentials between -13 to -78 mV.

5
6 The height of the energy barrier slightly decreases with increasing Ca^{2+}
7 concentration when the $\text{pK}_{\text{a,int}}$ is 1.8 larger than the pH and the absolute value of the
8 surface potential is relatively small between values around 13 to 14 mV, (Fig. 6b).
9 However, the decrease is not very significant and the height of the energy barrier is only
10 6% lower at a Ca^{2+} concentration of 120 mg l^{-1} than without Ca^{2+} . At higher pH values
11 and consequently larger absolute values for the surface potential, the trend is opposite and
12 the height of the energy barrier increases with increasing Ca^{2+} concentration. Hence,
13 replacing 1:1 by 2:1 electrolyte with identical ionic strength has only marginal effect on
14 the height of the energy barrier when the absolute values of the surface potentials are
15 small ($|\Psi| < 15 \text{ mV}$). This is the case when the $\text{pK}_{\text{a,int}}$ values are significantly lower than
16 the pH of the solution or the site densities are low. However, the calculations indicate that
17 the energy barrier can increase with increasing Ca^{2+} concentration when the absolute
18 values of the surface potentials are high unless the Hamaker constant is significantly
19 higher than $4 \times 10^{-21} \text{ J}$. Although intuitively not expected, an increase in repulsive double-
20 layer interaction upon replacing NaCl with CaCl_2 in the electrolyte at close distances can,
21 therefore, lead to an increase in the energy barrier.

22

1 The observed increase in PRD1 attachment with increasing Ca^{2+} is in line with the
2 increasing depth of the secondary minima of the interaction energy. However, when
3 putting the interacting surface area at about 3000 nm^2 based on the dimensions of PRD1
4 (Olsen et al., 1974), the absolute values of the secondary minima are about 10 times
5 smaller than one kT . Hence, the energy difference seems to be too small to cause
6 significant attachment of PRD1. Also the dependency of k_{att} on Ca^{2+} concentration argues
7 against the idea that trapping of PRD1 in the secondary minimum is the predominate
8 retardation mechanism. If this would be the case, k_{att} should reflect the kinetics of
9 diffusive transport into the secondary minimum and be independent on Ca^{2+} concentration
10 while k_{det} would depend on the depth of the minimum. Although the exact values for k_{det}
11 could not be determined from the experimental data, k_{det} seems not to vary between the
12 different experiments. Hence, it is unlikely that the dependence of PRD1 attachment on
13 Ca^{2+} concentration can be attributed to the effect of Ca^{2+} on the depth of the secondary
14 minimum. This conclusion is in agreement with results of Loveland et al. (1996) who
15 found that the shallowness of the secondary minima does not support the assertion that
16 the depth of the secondary minima affects PRD1 attachment behavior.

17

18 When PRD1 is attached in the primary minimum, the logarithm of k_{att} should
19 inversely correlate with the height of the energy barrier (Schijven and Hassanizadeh,
20 2000). According to our calculations, k_{att} increases with increasing Ca^{2+} concentration
21 although the height of the energy barrier increases. Hence, the experimental observations
22 are diametrically opposed to the predictions of the DLVO theory when the double-layer
23 interactions are derived from numerical integration of the Poisson-Boltzmann equation.

1 This contradiction between experimental findings and theory can be explained in multiple
2 ways. First, the Hamaker constant for PRD1 interaction with quartz could be higher than
3 the value reported for the polio virus. When the van der Waals interactions increase, the
4 energy barrier can be located at larger distances than the intersection point of the double-
5 layer energy curves for different Ca^{2+} concentrations. In this case, the height of the
6 energy barrier decreases with increasing Ca^{2+} concentration. Second, the enrichment of
7 Ca^{2+} close to the surface can lead to charge regulating interactions. That is, chemisorption
8 of Ca^{2+} can neutralize or even overcompensates the negative surface charge caused by the
9 dissociation of functional groups at the quartz or PRD1 surface. Third, the double-layer
10 interactions might not be accurately described by the Poisson-Boltzmann equation. The
11 accuracy of the Poisson-Boltzmann equation can be limited by several factors
12 (Israelachvili, 1992). In particular, ion-correlation effects can be significant for bivalent
13 counter-ions. Ion correlation attraction can exceed double layer repulsion at distances
14 below 2 nm when Ca^{2+} is present in the solution (Israelachvili, 1992). Before refining the
15 theory, better knowledge of the surface properties of PRD1 is mandatory. However, our
16 results demonstrate that quantitative assessment of virus mineral interactions based on the
17 DLVO theory can lead to intuitively unexpected results. Qualitatively attributing
18 enhanced virus attachment in the presence of Ca^{2+} to its shielding effect of the surface
19 charge or the compression of the double-layer might be too simplistic and quantitative
20 calculations are necessary to identify the underlying mechanism.

21

22

23

1 **Acknowledgments**

2

3 The first author acknowledges the Iranian Ministry of Health and Medical Education for
4 support of his PhD studies at Utrecht University. Pieter J. Kleingeld is greatly
5 acknowledged for his technical support in the column experiments. The authors thank Jan
6 Gerritse, Alette Langenhoff, Fredericke Langenhoff, at the Geo Environment Research
7 Laboratory for their support.

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