



Modeling Virus Adsorption in Batch and Column Experiments

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Abstract. Experiments with batch suspensions, recirculating columns and flow-through columns have been carried out involving a sandy soil and five bacteriophages: MS2, PRD1, ϕ X174, Q β and PM2. In batch and recirculating column experiments, attachment and detachment rate coefficients were determined by fitting a two-parameter (attachment and detachment) model. In general, attachment and detachment rate coefficients were not found to be significantly different between the two kinds of experiments. There was one exception, however: MS2 appeared to detach faster in the presence of strong advective flow. In the case of flow-through column experiments, it is shown that a two-site model, with adsorption to equilibrium and kinetic sites, fits the breakthrough curves of all the phages, except PM2, satisfactorily. A one-site kinetic model was found to be appropriate for phage PM2. A small proportion of bacteriophages MS2, PRD1, and Q β adsorbed to equilibrium sites, whereas a large proportion of ϕ X174 adsorbed to equilibrium sites. Such a distinction between adsorption to equilibrium and kinetic sites cannot be made in the case of batch or recirculating column experiments. Kinetic attachment rate coefficients were found to be significantly higher for the bacteriophages with presumably stronger negative charge. This may be ascribed to the presence of multivalent cations. Under these conditions, bacteriophage ϕ X174 appears to behave more conservatively than more negatively charged viruses, and may then be a better choice as a relatively conservative tracer for virus transport through the subsurface.

Key words: bacteriophages, adsorption, batch experiments, column experiments

1. Introduction

Pathogens of major threat to human health are pathogenic viruses. Viruses are a significant cause for waterborne disease outbreaks attributed to the consumption of contaminated groundwater (Craun, 1985). Groundwater is an important source for drinking water that

needs to be protected from contamination with viruses. Surface water is also a source for drinking water and is becoming increasingly important. Surface water is contaminated with viruses, mainly due to discharges of wastewater. Viruses can be removed effectively by passage of the surface water through soil, provided travel times and travel distances are adequate.

Processes of major importance that affect virus concentrations during soil passage are adsorption and inactivation (Keswick and Gerba, 1980; Yates *et al.*, 1987). Viruses are removed from the liquid phase by the combined effect of adsorption and inactivation, and, in addition, advection and dispersion cause spreading of viruses and thereby result in the attenuation of virus concentrations (Yates and Yates, 1991). In the case of reversible adsorption, one may have equilibrium and/or kinetic adsorption sites.

Equilibrium sites are sites where detachment of viruses is relatively fast compared to attachment and advection, allowing an apparent equilibrium to be reached between free and attached viruses within a short time scale. For some other sites, adsorption is kinetically limited relative to flow velocity. Generally speaking, both kinds of adsorption may occur in a given medium.

Interactions of viruses with soil are studied at laboratory scale by means of batch and column experiments. In batch experiments, water containing a known number of viruses is mixed with soil and the change in concentration of free virus particles is measured. Initially, free virus concentrations decline with time, but after a short time, they remain almost constant. At that point, equilibrium partitioning of viruses between solid and liquid phase is attained, because of reversible adsorption. This apparent equilibrium is rapidly reached but not instantaneously and depends on the actual attachment and detachment rates (Gerba, 1984). The kinetic behavior that is operative before apparent equilibrium is reached can be described by virus attachment and detachment from soil. In the case of irreversible attachment, virus concentrations will decline constantly. Over longer periods of time, concentrations can also decline due to inactivation of free and attached viruses (Grant *et al.*, 1993).

A shortcoming of batch experiments is that estimates of adsorption parameters from batch experiments appear to be of limited use in the prediction of virus adsorption in column or field experiments and field situations. For the reasons described momentarily, attachment is overestimated and detachment underestimated. Due to the stirring in a batch experiment, the number of accessible sites for attachment is much higher than in a column or in the field. At the same time, detachment rates in a batch system are presumably lower than under transport conditions influenced by advective flow. Detachment in a soil matrix is limited by diffusion over an energy barrier resulting from virus–soil interactions and by diffusion across a boundary layer near the solid surface (Ryan and Elimelech, 1996). The diffusion coefficient of the virus and the thickness of the boundary layer control the rate of transport across the diffusion boundary layer. Primarily the velocity of the fluid controls diffusion during transport. If this velocity increases, the thickness of the diffusion boundary layer will decrease and detachment of viruses may increase.

In a flow-through column experiment, the effect of adsorption to equilibrium sites is mainly retardation of virus transport and little attenuation of virus concentrations. The effect of adsorption to kinetically limited sites is different however. Due to attachment,

virus concentrations are attenuated and due to usually even slower detachment, virus transport is retarded, resulting in long tailing of virus breakthrough. A third kind of experiment, in which features of batch and column experiments are combined, is the recirculating column experiment. Here, after introducing an initial amount of viruses, the column effluent is continuously fed as influent to the column. Thus, because of viruses circulating through such a column, eventually equilibrium is reached between attached and detached viruses, similarly as in a batch suspension. At the same time, because viruses are transported through a porous medium, attachment and detachment rate coefficients should resemble more those estimated from a flow-through column experiment.

Recently, an extensive set of batch, flow-through column, and recirculating column experiments were carried out by Corapcioglu *et al.* (1997). These experiments were carried out using five bacteriophages: MS2, PRD1, ϕ X174, Q β and PM2. These bacteriophages differ in size and isoelectric point. Dowd *et al.* (1998) analyzed the data from the recirculating and flow-through column experiments, but not the data from the batch experiments. Their primary goals were to identify the role of isoelectric points and sizes of the viruses on their adsorption and transport in a sandy soil. The experiments were short enough in duration so that viral inactivation could be neglected. It was found that in the recirculating columns, the percentage viral adsorption correlated negatively with the isoelectric point of the viruses. Similar results were obtained in the flow-through columns for the three smaller viruses (MS2, ϕ X174 and Q β). The absorption was found to be stronger for PRD1 and PM2, which are larger phages, suggesting a possible correlation between virus size and adsorption. Apparently, viruses that are supposed to be more negatively charged, attached more. Dowd *et al.* (1998) argued that more attachment of viruses with lower pI could be explained by their attraction to the positively charged diffuse Gouy-layer that surrounds negatively charged soil particles.

The isoelectric point of a virus does not really provide information on its actual electric surface charge. Penrod *et al.* (1995) found strong indications that surface charge of virus particles determines attachment and that this charge is a function of pH. In accordance with DVLO theory, negatively charged viruses attach less at higher pH because of increased electrostatic repulsion (Ryan and Elimelech, 1996; Loveland *et al.*, 1996). At near neutral pH, a virus that has a low pI, is generally expected to have a larger net negative charge, but there are exceptions. At pH-values above 5, the surface charge of MS2 remains constant (Penrod *et al.*, 1995). At pH-values above 6, surface charges of vaccinia virus, reovirus and phage λ are also relatively insensitive to changes in pH (Penrod *et al.*, 1995). At higher pH's, PRD1 (Loveland *et al.*, 1996) and recombinant Norwalk-like virus particles (Redman *et al.*, 1997), however, show a further decrease in their negative charge.

Also contrary to the findings of Dowd *et al.* (1998), most studies have shown that attachment of MS2 is less than or equal to that of most other viruses, including ϕ X174 (Goyal and Gerba, 1979; Herbold-Paschke *et al.*, 1991; Bradford *et al.*, 1993; Farrah and Preston, 1993; Bales *et al.*, 1993; Sobsey *et al.*, 1995; Penrod *et al.*, 1996; Jin *et al.*, 1997; Redman *et al.*, 1997). For an extensive review see Schijven and Hassanizadeh (2000).

Dowd *et al.* (1998) fitted observed breakthrough curves from flow-through column experiments with two different models, a one-site kinetic and an equilibrium model; they did not consider the combination. Because both types of sites seem to be present, it is more

appropriate to model the experiments with a model combining both equilibrium and kinetic adsorption sites. Concentrations of both PRD1 and PM2 were attenuated more than other phages in the flow-through column experiments, whereas in the recirculating columns percentage of adsorption was found to be the highest for MS2. No explanation was given for these apparently contradictory results.

In the present paper, the results of the batch and circulating column experiments have been used to obtain values for attachment and detachment rate coefficients. These values were compared in order to test the following hypotheses:

- i) Attachment rate coefficients in batch experiments are larger than in column experiments, due to the larger number of accessible sites for attachment under batch conditions, and
- ii) Detachment rate coefficients in batch experiments are smaller than in column experiments, due to the thinner diffusion boundary layer associated with advective flow.

By comparison of batch experiments and recirculating columns, it was investigated whether these experiments are useful for predicting viral transport in flow-through columns. Re-evaluation of the breakthrough curves was carried out by fitting the data to a two-site model in order to assess the contribution of equilibrium and kinetic adsorption sites. Finally, soil and groundwater adsorption characteristics are compared with that of other studies in order to understand why supposedly more negatively charged viruses attached more, contrary to expectations.

2. Conceptual Model

Consider a situation where viruses can adsorb to two different kinds of sites on solid grains: fast and slow sites (see e.g. Bales *et al.*, 1991, 1997; McCaulou *et al.*, 1994). If inactivation of viruses is neglected, the governing equations describing virus transport are as follows (Toride *et al.*, 1995):

$$\left(1 + \frac{\rho_B}{n} k_{\text{eq}}\right) \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - k_{\text{att}} C + k_{\text{det}} \frac{\rho_B}{n} S^k, \quad (1)$$

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

where C is the number of free viruses per unit volume in the aqueous phase, [L^{-3}]. The attached virus concentration is given in terms of number of viruses per unit mass of soil, [M^{-1}]. The symbol S^k is used to denote the concentration of viruses attached to slow, i.e. kinetic adsorption sites. Further, ρ_B is the bulk density of the saturated soil, [$\text{M} \cdot \text{L}^{-3}$]; n is the porosity, [-]; D is the hydrodynamic dispersion coefficient, [$\text{L}^2 \cdot \text{T}^{-1}$]; v is the pore water velocity, [$\text{L} \cdot \text{T}^{-1}$]; k_{eq} is a distribution coefficient for equilibrium adsorption,

$[\text{M}^{-1} \cdot \text{L}^3]$; k_{att} and k_{det} are the attachment and detachment rate coefficients for the kinetic sites, respectively, $[\text{T}^{-1}]$.

For convenience we define a dimensionless distribution coefficient for equilibrium adsorption:

$$k_{\text{eq}}^* = \frac{\rho_{\text{B}}}{n} k_{\text{eq}}. \quad (3)$$

3. Materials and Methods

3.1. Soil, groundwater and bacteriophages

Sediment and groundwater were originally obtained from a sandy aquifer underlying the Brazos Alluvium. Some major characteristics of the soil and groundwater are summarized in Table 1. A detailed description of this aquifer sediment and groundwater was given in Munster *et al.* (1996). The soil contains virtually no organic matter.

As mentioned earlier, five bacteriophages were used in various experiments: MS2, PRD1, ϕ X174, Q β and PM2. The origin and characteristics of the bacteriophages, as well as methods for their enumeration were described in Dowd *et al.* (1998).

3.2. Batch experiments

Batch experiments were performed using 50 ml centrifuge tubes (Falcon 2098) containing 5 grams of aquifer material and 9 ml of groundwater to which 1 ml with 10^6 – 10^{10} plaqueforming units (pfu) of bacteriophages were added. Viral lysate was diluted in

Table 1. Soil and groundwater characteristics (Munster *et al.*, 1996).

<i>Brazos alluvium</i>		
Clay		3%
Silt		2%
Sand		95%
Porosity (columns)		0.35
Grain size (lab experiments)	mm	0.2–0.5
<i>Groundwater</i>		
Alkalinity (CaCO ₃)	mM	3.0
HCO ₃ ⁻	mM	11
Ca ²⁺	mM	3.7
Cl ⁻	mM	1.2
Dissolved solids	mg/L	694
Hardness (CaCO ₃)	mM	5.4
Fe (dissolved)	mM	0.011
Mg ²⁺	mM	1.7
Conductivity	$\mu\text{S}/\text{cm}$	1088
pH		7.1

groundwater to reduce the amount of the suspending media (Tryptone Soy Broth). One ml of the groundwater diluted stock was then added to a time-zero tube which was vortexed, serial diluted, and the virus enumerated to provide an initial phage concentration (C_0). The remaining tubes containing groundwater and sediment were inoculated and immediately placed in a shaking incubator at 21°C. One tube at a time was removed at 10 min, 20 min, 40 min, and 90 min and centrifuged at $1000 \times g$ for 2 min in order to sediment the soil. The supernatant was then sampled and assayed to determine remaining virus concentrations.

3.3. Column experiments

The recirculating and flow-through column experiments have already been described in detail in Dowd *et al.* (1998). Briefly, polyvinyl chloride columns with an inner diameter of 0.05 m were filled with sand up to 0.78 m and saturated with groundwater. The flow-through columns were seeded with two pore volumes of the bacteriophages. Effluent samples were obtained for about 90 min. MS2 and PRD1 were introduced together, but the other phages were introduced in separately prepared flow-through columns. One column was made recirculating by connecting the outlet to the inlet of the column. Bacteriophages were injected using a syringe with 20-gauge needle. Here, MS2 and PRD1 were also injected together, but the other phages were each introduced separately, i.e. back to back. Because Q β cross-reacts with the host of MS2, Q β was injected after MS2 could not be detected anymore. Samples were obtained by injecting 1 ml of phage-free groundwater into the sampling port, waiting for several seconds and then withdrawing a 1-ml aliquot. Samples were obtained at 2.5, 5, 10, 20, 40, 60, 120 and 240 min after injection. The pore-water velocity in the recirculating column experiments was 0.17 m/min and in the flow-through column experiments 0.11 m/min.

3.4. Parameter estimation from batch and recirculating column experiments

To describe adsorption in experiments with batch suspensions and recirculating columns, the same two-site model given by Eqs. (1) and (2) may be applied. However, because in both batch and recirculating experiments, the concentration is spatially uniform, dispersion and advection fluxes may be neglected.

Then, the reduced forms of Eqs. (1) and (2) have the following simple solution:

$$C = C_0 \frac{b + a \exp[-(a + b) t]}{a + b}, \quad (4)$$

where

$$a = \frac{k_{\text{att}}}{1 + k_{\text{eq}}^*} \quad \text{and} \quad b = k_{\text{det}}.$$

From Eq. (4), it can be seen that observed concentrations in time from batch and recirculating column experiments can be fitted with only two parameters, namely a for attachment and b for detachment. Due to the absence of advective flux, it is not possible to identify retardation, therefore, we cannot distinguish between equilibrium and kinetic adsorption in batch and recirculating column experiments.

Because free virus concentrations in a batch or recirculating column experiment span several orders of magnitude and because lower concentrations show much larger variation than higher concentrations, Eq. (4) and the measured concentrations were logarithmically transformed. A non-linear fitting procedure according to the Levenberg-Marquardt iteration method has been used in Mathematica version 4.0.0.0 to obtain the values for a and b .

In order to compare the estimated values for a and b between batch and recirculating column experiments, parameters a and b in Eq. (4) were substituted by:

$$a = a_1 + a_2 y, \quad (5)$$

$$b = b_1 + b_2 y, \quad (6)$$

where y is a variable that was introduced to distinguish between the observations of the two types of experiments. In the case of the batch experiment, $y = 0$ and in the case of the recirculating column experiment, $y = 1$. The estimated values of a_2 and b_2 indicate whether the estimated values for a and b were significantly different between batch and recirculating column experiments.

In the case that a showed no significant difference, all batch and recirculating column data were combined, a common value for a was estimated and separate values for b were estimated by substitution of the following equation into Eq. (4):

$$b = b_1(1 - y) + b_2 y. \quad (7)$$

Similarly, in the case that b showed no significant difference, all batch and recirculating column data were combined, a common value for b was estimated and separate values for a were estimated by substitution of the following equation into Eq. (4):

$$a = a_1(1 - y) + a_2 y. \quad (8)$$

In the case that neither a nor b showed significant differences, all batch and recirculating column data were combined and common values for a and for b were estimated.

3.5. Parameter estimation from flow-through column experiments

For fitting the breakthrough curves from the flow-through column experiments, the computer code CXTFIT (Toride *et al.*, 1995) was used. This code is based on analytical

solutions of Eqs. (1) and (2). This way, estimated values for k_{eq}^* , k_{att} and k_{det} were obtained. The fraction of adsorption sites that are always at equilibrium is denoted by f and was calculated from the adsorption rate coefficients as follows:

$$f = \frac{k_{\text{eq}}^*}{k_{\text{eq}}^* + \frac{k_{\text{att}}}{k_{\text{det}}}}. \quad (9)$$

4. Results

4.1. Batch and recirculating column experiments

Figures 1a–e show the fitted adsorption curves for the five phages in the batch and recirculating column experiments. The corresponding parameter values are given in Table 2.

For two of the model viruses (ϕ X174 and Q β), the attachment and detachment rate coefficients did not appear to be significantly different between the batch and recirculating column experiments. In the case of PRD1 and PM2, attachment in the column experiment was found to be significantly different from that seen in the batch experiment, but each phage behaved in the opposite manner that is lower and greater, respectively. Apparently, there was an effect of site accessibility, causing slower attachment of PRD1 in a packed column as compared to a soil suspension. This effect of site accessibility may be related to the larger size of PRD1. There is no obvious explanation for the greater attachment of PM2 in the column experiment. Possibly there was a difference in soil characteristics between the two kinds of experiments. Only with MS2, detachment was significantly greater in the recirculating column experiment, probably caused by the flow of water in the column.

4.2. Flow-through column experiments

Figure 2a–e show the observed breakthrough curves together with best-fit curves obtained from a two-site model (Eqs. (1) and (2)). The corresponding parameter values are given in Table 3. It was assumed that the dispersivity of all columns was the same: 6.4 cm. This value produced the best fitting results for all phages. This is a rather high value and tends to indicate a wide grain size distribution, it may have also been influenced by the very large flow velocity.

Initial breakthrough of all bacteriophages, except PM2, was retarded. Also, all breakthrough curves exhibit long tailing. Therefore, a two-site model was needed to fit the breakthrough curves. In the case of PM2, a one-site kinetic model was found to be more appropriate. The value of k_{eq}^* ranged between 0.6 and 1 for the retarded phages. In the case of MS2, PRD1, and Q β , the values of f indicated that only a minor part of these phages

adsorb to equilibrium sites, whereas $\phi X174$ adsorbed for most part to equilibrium sites. In fact, we found that with a one-site equilibrium model, an equally good breakthrough curve could be simulated for $\phi X174$. In both cases the tailing was underestimated (not shown). In order to fit the tail part of the breakthrough curve of $\phi X174$ the value of k_{det} was set to 0.033 and the other parameters were evaluated using CXTFIT. In the case of PM2, the measured concentrations along the tail are relatively low and do not have much weight in the fitting procedure. This resulted in overestimation of the tail concentrations and a small estimate for k_{det} . A visually better fit of the breakthrough tail was obtained by setting the value of k_{det} to 0.00021 and evaluating the other parameters using CXTFIT.

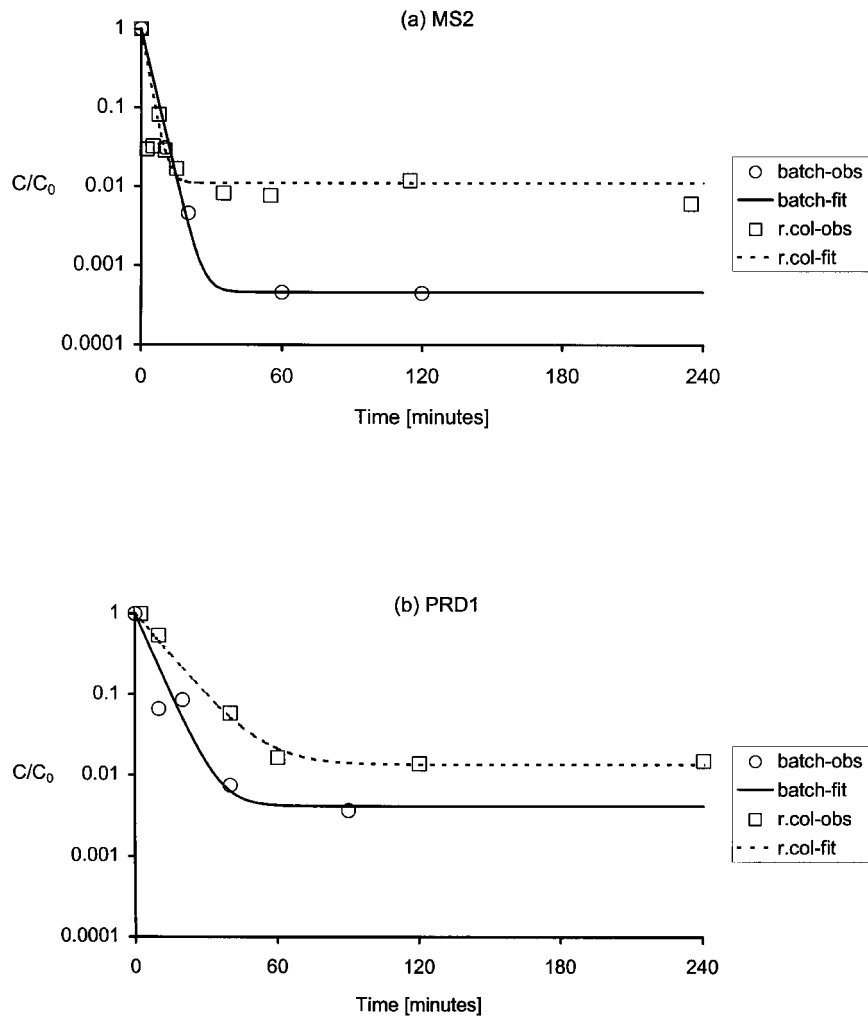


Figure 1a-b. Observations and fitted model in batch and recirculating column-experiments.

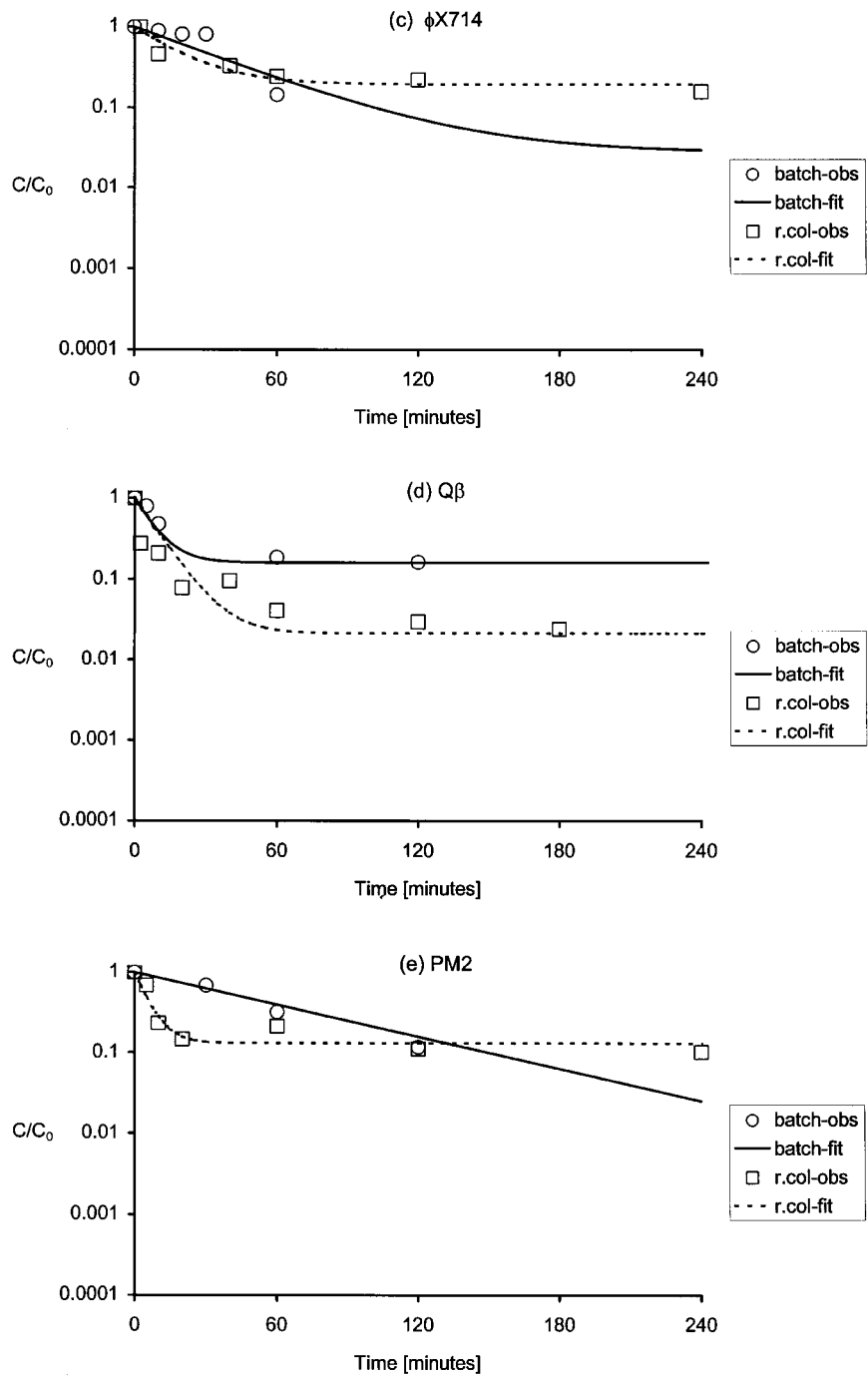


Figure 1c-e. (continued)

Table 2. Estimated parameter values of batch and recirculating column experiments.

			Estimate	SE	p (%)
MS2	3-parameter model	a	0.31	0.033	7.3×10^{-5}
		b (batch)	0.00015	0.000086	10
		b (r. col)	0.0030	0.0012	2.4
PRD1	3-parameter model	a (batch)	0.16	0.022	0.0081
		a (r. col)	0.076	0.011	0.013
		b	0.00089	0.00029	1.5
ϕ X174	2-parameter model	a	0.030	0.0069	0.15
		b	0.0043	0.0021	6.6
Q β	2-parameter model	a	0.12	0.038	0.84
		b	0.0055	0.0030	8.9
PM2	3-parameter model	a (batch)	0.037	0.015	3.1
		a (r. col)	0.12	0.034	0.83
		b	0.014	0.0060	4.5

Dimensions of a and b is min^{-1} .

Table 3. Estimated values of model parameters from flow through column experiments.

	MS2	PRD1	ϕ X174	Q β	PM2
k_{eq}^*	0.96	0.75	0.67	0.67	0
k_{att}	0.089	0.26	0.017	0.087	0.20
$a = k_{\text{att}}/(1 + k_{\text{eq}}^*)$	0.045	0.15	0.010	0.053	0.20
$b = k_{\text{det}}$	0.0035	0.0042	0.033	0.020	0.00021
f	0.036	0.011	0.56	0.13	0

Dimensions of a , b , k_{att} and k_{det} is min^{-1} ; k_{eq}^* and f are dimensionless.

When comparing the five phages with each other, it can be seen that the value of k_{att} was lowest for ϕ X174. The values of k_{att} for MS2 and Q β were not significantly different. The value of k_{att} for PRD1 was not significantly different from that for PM2, but was significantly higher than that of the three other phages. Detachment of PRD1 was found to be one order of magnitude higher than that of PM2.

4.3. Flow-through column experiments versus batch and recirculating column experiments

The values of the (a) attachment and (b) detachment rate coefficients from the flow-through column experiments can be compared with those from the batch and recirculating column experiments. Similar values for the attachment rate coefficient were found for the

three kinds of experiments in the case of PRD1 and ϕ X174. But in the case of MS2 and Q β these values were lower and in the case of PM2 higher in the flow-through column experiments compared to the batch and recirculating column experiments. The values of the detachment coefficients were similar between the experiments only in the case of MS2. They were smaller in the flow-through column experiment in the case of PM2 and greater in the case of the other three phages.

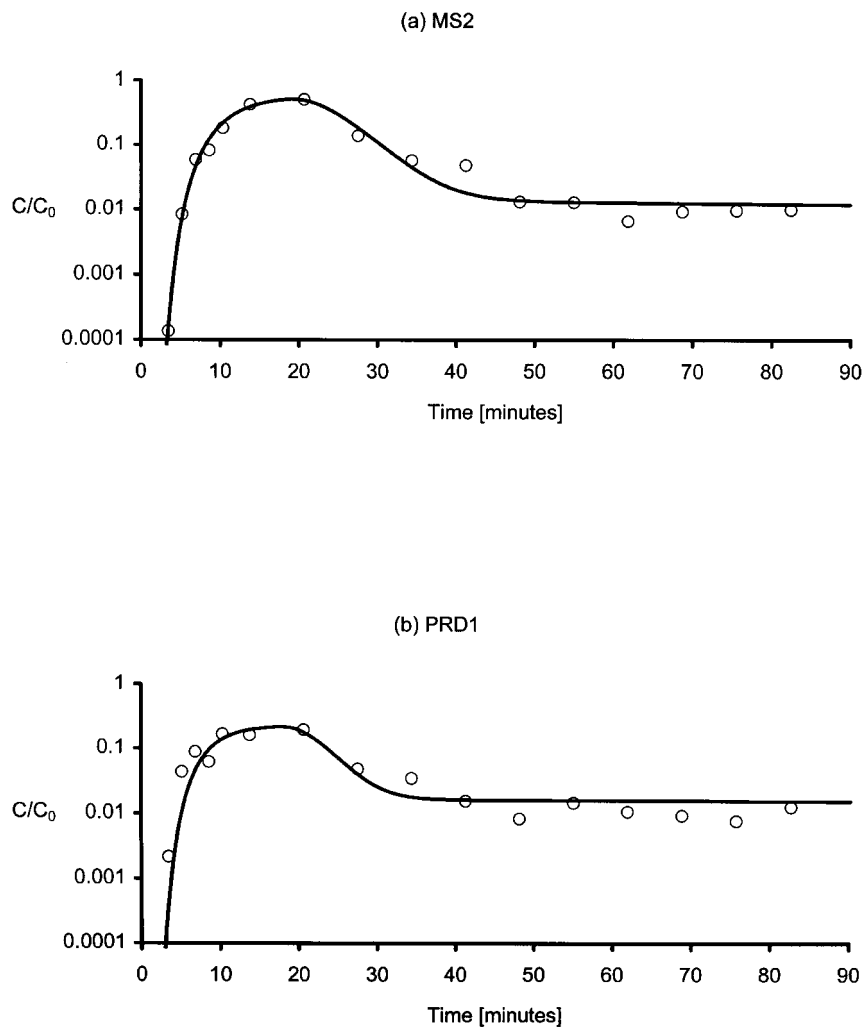


Figure 2a-e. Breakthrough curves of flow-through column experiments. The circles represent observations and the lines a 2-site model fit.

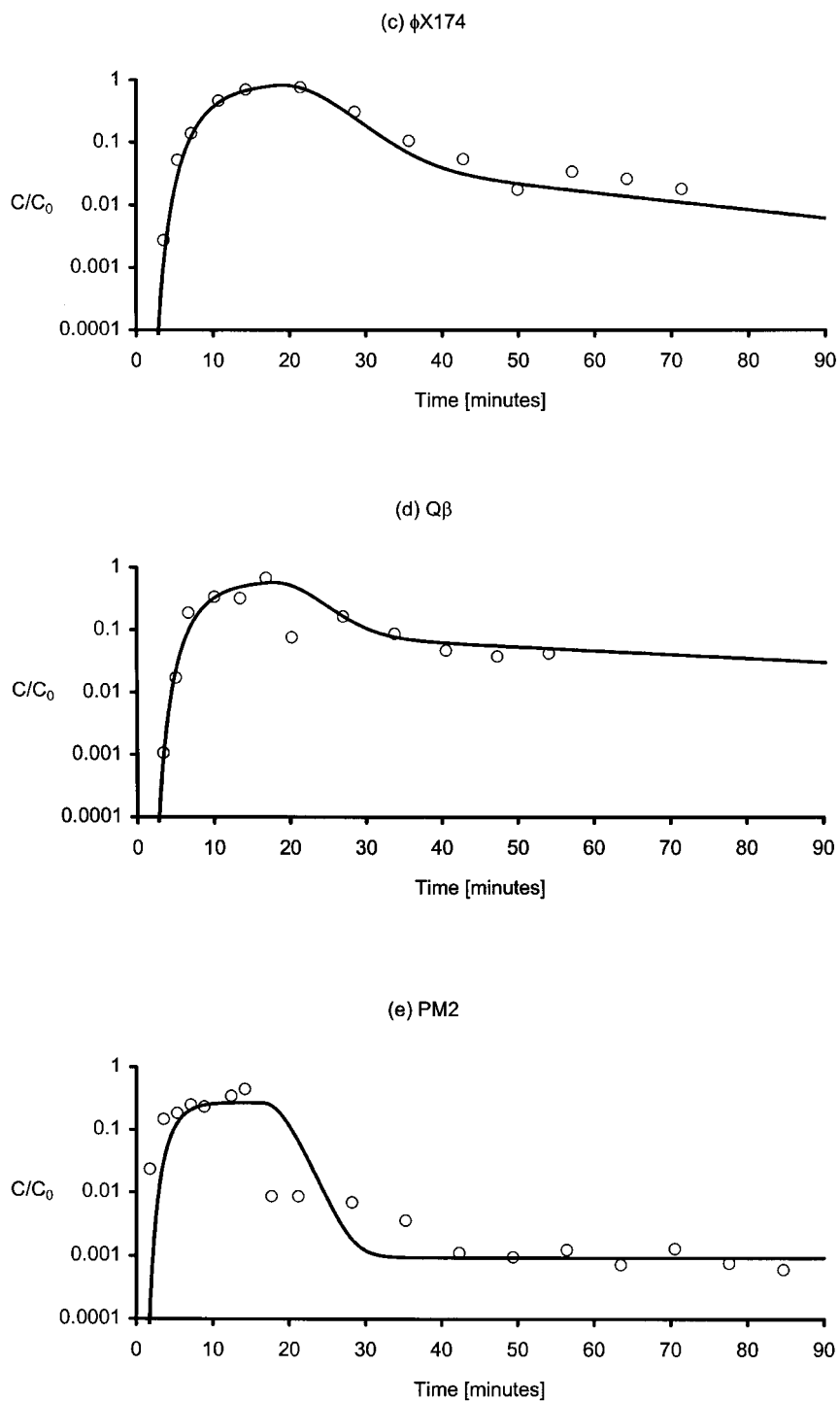


Figure 2c-e. (continued)

5. Discussion and Conclusions

Mostly, values of attachment and detachment obtained from experiments with batch suspensions were not found to be significantly different from those obtained from the recirculating column experiments. There were a few exceptions however.

Bacteriophage MS2 was found to detach faster under the influence of advective flow. PRD1 was found to attach slower in a recirculating column, than in the batch experiment. However, in the flow-through column, the value of attachment for PRD1 was similar to that obtained with the batch experiment suggesting that the difference found between the batch and the recirculating column experiment may not be caused by the presence or absence of advective flow.

To conclude, the first hypothesis, that attachment rate coefficients in batch experiments are larger than in column experiments, could not be verified. Also, the second hypothesis, that detachment rate coefficients in batch experiments are smaller than in column experiments is not verified. Only in case of MS2, the detachment rate increased as a consequence of the advective flow of water. This implies that similar attachment and detachment rate coefficients for certain virus-soil combinations from batch and recirculating column experiments may be found, but there may be exceptions, dependent on the type of virus.

One would expect similar values of attachment and detachment to be obtained from the two kinds of column experiments. However, here some inconsistencies were found. For example, the attachment rate of MS2 was found to be considerably lower, and the detachment rate of PRD1 was found to be four times higher in the flow-through column than in the recirculating column. The inconsistencies between the two types of column experiments can neither be explained by the characteristics of the bacteriophages, nor by the type of column experiment. It is more plausible to ascribe such inconsistencies to the irreproducibility of column experiments. This may be due to differences in soil characteristics and/or packing of the soil in the different columns. The recirculating column experiments were all carried out using one column, whereas the flow-through column experiments were carried out using four different columns.

In the batch and recirculating column experiments that are presented here, it was found that attachment decreased in the order of MS2, PRD1, Q β , ϕ X174 and PM2. A plausible explanation for the higher attachment of the presumably more negatively charged viruses may be found in the presence of positively charged sites; for example, in the form of ferric oxyhydroxides (Ryan *et al.*, 1999), or in the fact that concentrations of bivalent cations are rather high (Table 1). Bales *et al.* (1991) showed that Ca²⁺-concentrations of 1–100 μ M at pH 7 are sufficient to promote attachment of viruses. Multivalent cations can link viruses and adsorbents of like charge by forming salt bridges between them (Sobsey *et al.*, 1980; Moore *et al.*, 1982; Lipson and Stotzky, 1983) or by charge reversal (Grant *et al.*, 1993). Of course, high concentrations of monovalent cations may also promote attachment by compressing double layers (Lipson and Stotzky, 1983; Grant *et al.*, 1993; Redman *et al.*, 1999), but multivalent cations are believed to be much more effective. The findings from the present study suggest that multivalent cations promote attachment of the more negatively charged viruses even more. Hydrophobic interactions

probably do not play a role here, because of the low organic matter content of the soil in this study.

Additional support for the explanation that more negatively charged viruses attach more than less negatively charged viruses, at high concentrations of multivalent cations, may be found by comparing the present study with the field studies of Pieper *et al.* (1997) and Schijven *et al.* (1999). In these studies, values of k_{att} for MS2 and PRD1 were found to be 10 to 100 times lower than those from the flow-through column experiments in the present study. Compared to the present study, in the study of Pieper *et al.* (1997), pH was 5.0–5.7, electrical conductivity was about two times lower, and concentrations of multivalent cations were less than one-tenth. So, attachment may be lower in the study of Pieper *et al.* (1997), because of lower concentrations of monovalent and multivalent cations. In the study of Schijven *et al.* (1999) compared to the present study, pH was higher (8.3), electrical conductivity was comparable and concentrations of multivalent cations were about half. Attachment may be lower in the study of Schijven *et al.* (1999), because of higher pH and two times lower concentrations of multivalent ions.

This study has shown that similar attachment and detachment rate coefficients for a virus may be found in batch and recirculating column experiments, but there are exceptions, dependent on the type of virus. In batch and recirculating column experiments, no clear distinction can be made between equilibrium and kinetic sites, whereas this is possible in flow-through column experiments. It appeared that ϕ X174 attached for the larger part to equilibrium sites, but MS2, PRD1 and Q β for a minor part. PM2 attached only to kinetic sites. The inconsistencies that were found between the two types of column experiments may be due to soil characteristics and/or packing of the soil in the different columns. Therefore, when carrying out column experiments, soil heterogeneities need to be taken into consideration.

Under conditions of high pH in most sandy soils, MS2 can be regarded as a relatively conservative tracer virus, however, in the presence of multivalent cations, bacteriophage ϕ X174 may attach less than MS2. This implies that in the case of soils, at near neutral pH, in the presence of high concentrations of multivalent cations, bacteriophage ϕ X174 may be the better choice for a relatively conservative tracer virus in field and column studies than MS2.

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