MS-2 and Poliovirus Transport in Porous Media: Hydrophobic Effects and Chemical Perturbations

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In a series of pH 7 continuous-flow column experiments, removal of the bacteriophage MS-2 by attachment to silica beads had a strong, systematic dependence on the amount of hydrophobic surface present on the beads. With no hydrophobic surface, removal of phage at pH 5 was much greater than at pH 7. Release of attached phage at both pH values did occur, but was slow; breakthrough curves exhibited tailing. Poliovirus attached to silica beads at pH 5.3 much more than at pH 7.0, and attachment was also slowly reversible. Time scales for phage and poliovirus attachment were of the order of hours. The sticking efficiency factor (α), reflecting microscale physicochemical influences on virus attachment, was in the range of 0.0007-0.02. Phage release was small but measurable under steady state conditions. Release was enhanced by lowering ionic strength and by introducing beef extract, a high-ionic-strength protein solution. Results show that viruses experience reversible attachment/detachment (sometimes termed sorption), that large chemical perturbations are needed to induce rapid virus detachment, and that viruses should be quite mobile in sandy porous media. Even small amounts of hydrophobic organic material in the porous media (≥0.001%) can retard virus transport.

INTRODUCTION

The occurrence of human enteric viruses in groundwater near surface waste discharges and septic tanks has been well documented [Keswick and Gerba, 1980]. Such studies indicate that viruses can travel distances over 1 km under certain conditions. Use of bacteriophages as laboratory and field tracers of human-virus transport has great appeal owing to the lack of infection risk, shorter assay times and commensurate lower cost [Bales et al., 1989; Yates et al., 1987; Gerba, 1985]. The fate of viruses in soil and groundwater is governed by attachment to and release from immobile substrates and by inactivation [Yates et al., 1987]. Several factors contribute to the adhesion of viruses and other colloids to soil particles, including electrostatic attraction and repulsion, van der Waals forces, covalent-ionic interactions, hydrogen bonding, and hydrophobic effects [Murray and Parks, 1980].

While several studies of virus transport have been reported [e.g., Dubois et al., 1976; Goyal and Gerba, 1979; Landry et al., 1980; Gerba et al., 1981; Vaughn et al., 1981; Bales et al., 1989, 1991], few have been done under sufficiently well-controlled conditions to allow observing and modeling the effects of solution and surface chemistry on transport. Solution pH and soil-surface hydrophobicity have been shown to be important for phage attachment and release from surfaces [Bales et al., 1991]. However, the systematic dependence of phage removal on important porous-media properties, such as mass-fraction organic carbon (fOC) has yet to be shown. Also, the reversibility of virus adsorption has been studied for only a few cases [Murray and Parks, 1980]. Changes in pH and solution composition can result in significant release of attached phage [Bales et al., 1991]. While several mathematical models are available for describing transport of virus and other colloids in soil and groundwater [e.g., Yates et al., 1987; Harvey and Garabedian, 1991; Corapcioglu and Haridas, 1984], all lack sufficient data for validation.

The research described in this paper is part of our ongoing studies of virus transport in porous media. We previously reported significant attachment of MS-2 phage to silica beads having a partial organic coating, but little attachment to unbonded silica [Bales et al., 1991]. Our primary objective in the current work was to systematically examine the role of the hydrophobicity (i.e., mass-fraction organic carbon, fOC) of the porous media in attenuating MS-2 phage transport at pH 7, where attachment to silica surfaces is small. A second objective was to examine the role of pH and ionic strength on the attachment and detachment of viruses to well-characterized silica surfaces under steady state conditions, in contrast to our previous transient conditions [Bales et al., 1991]. A third objective was to compare the transport of MS-2 and poliovirus, a human enterovirus. Phages are less costly and safer to use in laboratory studies than animal viruses. However, it is important to demonstrate that phage behavior is representative of pathogenic human enteric viruses so that results of this and similar studies can be applied to the development of protection zones around drinking water wells [Yates et al., 1987].

MATERIALS AND METHODS

Nine continuous-flow column experiments were conducted with the bacteriophage MS-2 at pH 7.0 and three at pH 5 (Table 1). Experimental methods were described previously [Bales et al., 1991]. From 2.4 to 6.6 pore volumes of virus-containing solution were introduced into a column.
TABLE 1. Experimental Conditions and Results

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Percent Hydrophobic*</th>
<th>pH</th>
<th>(10^{-1} \text{ cm s}^{-1})</th>
<th>(\log C_{0}), pfu mL(^{-1})</th>
<th>Pulse, Pore Volume</th>
<th>(\log C_{p}/C_{0})(^\dagger)</th>
<th>95% CI for (\log C_{p}/C_{0})</th>
<th>(\alpha)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>292.3</td>
<td>&lt;-5.85</td>
<td>...</td>
<td>0.016</td>
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</table>

**Poliovirus**

| 13         | 0                    | 7.0| 7.81             | 3.93                   | 2.5              | -1.10           | -1.95/-0.83     | 0.0040 |
| 14         | 0                    | 7.0| 5.47             | 5.57                   | 2.7              | -2.49           | -2.97/-2.27     | 0.0072 |
| 15         | 0                    | 5.5| 6.20             | 5.46                   | 3.0              | -4.4            | -4.92/-4.17     | -0.014 |

*Percent of bonded-silica (hydrophobic) beads in column; the bonded-silica beads had a mass-fraction organic carbon of 0.00005, versus 0.00002 for the unbonded silica.

\(^\dagger\)Steady state value of column-outlet concentration following initial rise in concentration.

To initiate an experiment; the amounts were chosen to assure that the column outlet reached a constant value. In two cases (experiments 10 and 12), phages were introduced for much longer times to determine if surface binding sites on the silica beads could be fully saturated. Phage detachment was studied in some, but not all, of the experiments by subsequently introducing a phage-free solution into the column. In two of the experiments (3 and 4), ionic strength was lowered to examine its role in inducing phage detachment. A beef-extract-containing solution (1-2.5%; BBL, Cockeysville, Maryland) was subsequently introduced in about half of the experiments to more fully detach phages and to verify that attached phages were still viable. Beef extract is a high-ionic-strength enzyme digest of beef proteins composed of polypeptides, polypeptides, and amino acids made from hydrolyzed hamburger. Experiments were done at 7 ± 3°C using a 15-cm × 0.9-cm inside-diameter precision-bore glass chromatography column (Spectrum Medical Industries, Inc., Los Angeles, California) packed with 45-90 µm glass beads. The resulting porosity was 0.35 and bulk density was 1.60 g cm\(^{-3}\). At 7°C, inactivation of the MS-2 and polio virus over the few-day-long experiments was negligible, less than 1.5% day\(^{-1}\) [Yahya et al., 1993]. Column fittings were Teflon, and Teflon tubing was used everywhere in the system except for a length of tygon tubing in the pump. In order to take columns inlets samples throughout an experiment without disrupting the flow, a parallel feed tubing was set up. Feed-reservoir virus titers remained constant during an experiment, within analytical uncertainty (±20%). Sodium phosphate (0.02 M) was used to buffer pH, with Ca\(^{2+}\) added as CaCl\(_2\) (10\(^{-6}\) M). In experiments 3 and 4, the sodium-phosphate concentration was only 0.002 M, and 0.5 M NaCl concentration was added as an ionic-strength buffer during attachment; the NaCl concentration was lowered to 0.005 M for the detachment portion of the experiment. The lower phosphate buffer concentration was needed to permit lowering the ionic strength to induce detachment.

Bacteriophage MS-2 is an icoshedral phage with a diameter of 26.0 to 26.6 nm [VanDuin, 1988] and pH\(_{\text{rep}}\) of 3.9 [Zerda, 1982]. The materials, MS-2 phage, chemicals, and MS-2 assay methods were the same as described previously [Bales et al., 1991]. Samples were assayed for MS-2 on the same day as the experiment using the plaque-forming-unit (pfu) method [Adams, 1959]. All virus assays were performed in duplicate.

Poliovirus type 1 strain LSc is an animal virus with a diameter of 23 nm and pH\(_{\text{rep}}\) of 6.6 [Zerda and Gerba, 1984]. Poliovirus was obtained from the standard collection of the Department of Microbiology and Immunology, College of Medicine, University of Arizona. Poliovirus was grown and assayed in the Buffalo-Green-Monkey kidney well line (BGM) by previously described methods [Smith and Gerba, 1982]. The pfu method was used to quantitate the polivirus. Cell culture harvests treated with Freon were purified by centrifugation for 3 hours at 100,000 g to pellet the virus. The virus was then resuspended in sterile distilled water. The virus preparations were then assayed and frozen (-20°C) until used in an experiment.

Silica for column experiments was Spherosil glass 2530 beads (Potters Industries, Inc., Hasbrouck Heights, New Jersey), and was cleaned by washing with NH\(_4\)OH and HCl as described previously [Bales et al., 1991]. To prepare hydrophobic silica, beads were suspended in octadecylchlorosilane solution, giving a patchy C\(_{18}\) coating on the surface [Bales et al., 1991; Sceceody and Bales, 1989]. The measured mass-fraction organic carbon content of the beads, measured by elemental pyrolysis, was 0.00005; for the unbonded beads it was 0.00002. To vary the mass-fraction organic carbon in the media, bonded and unbonded beads were mixed together before being packed into an experimental column. Thus organic carbon was heterogeneously distributed, both within the column and on the bead surfaces.

**Results**

Column-outlet concentrations in most experiments exhibited a rise in virus concentration after about one pore volume of virus-containing solution had passed into the column,
followed by a steady state value until the eluent was switched to a virus-free solution (Figures 1–5). Experiments showed declines in \( C/C_0 \), the ratio of virus concentration at column outlet to that at the inlet, about one pore volume after the feed was switched. A small fraction of attached viruses were then released, with subsequent detachment peaks due to chemical perturbations in the feed. Viruses were still detaching after each experiment was terminated, from 1 to 15 days later.

**MS-2 Column Experiments**

Experiment 1, with unbonded silica at pH 7, showed a rapid rise in \( C \) to 10% of \( C_0 \), then a slowly rising concentration on the breakthrough curve until the feed was switched to phage-free solution after 2.4 pore volumes. Significant release then occurred for one pore volume, and a slowly dropping concentration followed thereafter through 12 pore volumes (Figure 1a). There was bacteriophage retention in the column, and the breakthrough curve failed to reach \( C_0 \). The first phages were detected after 0.9–1.0 pore volumes of solution had passed through the column. Addition of 2.5% beef extract to the eluent at 12 pore volumes resulted in an additional outlet peak, showing that phages were still viable. However, only a small fraction of the retained phages were released. Even with this chemical perturbation, detachment was slow; retained phages are expected to be released over several days to weeks.

Experiment 2, with unbonded silica at pH 5, showed no breakthrough of phages (Figure 1b); essentially all were retained in the column. There was some release upon introducing beef extract at pH 7 into the column, about the same peak concentration as in experiment 1. Both the pH 7 and 5 results are consistent with those previously reported in the same experimental system [Bales et al., 1991]. In experiments 3 and 4, also carried out at pH 5, there was little to no phage breakthrough, and little phage release from the column under steady state chemical conditions. Upon changing the eluent ionic strength from 0.5 to 0.005 M, phage release was enhanced, as shown by the peaks in the column outlet. The magnitude of these detachment peaks was similar to those observed with beef-extract addition.

Addition of beads with hydrophobic groups bonded to the surface resulted in more phage retention in the column. Mixing 1–4% hydrophobic-bonded beads with unbonded beads resulted in a maximum steady state \( C/C_0 \) less than 1.0, and as low as 0.01 (Figures 2 and 3). Experiments with 1–3% hydrophobic beads were terminated after the retention portion of the breakthrough curves. Experiments 8 and 9, with 4% hydrophobic beads, exhibited similar breakthrough curves, though the steady state \( C/C_0 \) was somewhat lower in experiment 9. There was a small amount of phage release in experiment 9 following the change to phage-free eluent, then
Further release upon addition of beef extract after 15.5 pore volumes. Addition of this high-ionic-strength protein solution showed that phages were viable and can be detached from hydrophobic (experiment 9) as well as hydrophilic (experiments 1-4) surfaces.

Two experiments with 5% hydrophobic beads (10-11) were reproducible, showing nearly the same removals of phage (Figure 4). The longer duration of experiment 10 showed that total phage attachment was less than that needed to fully occupy available attachment sites. Both experiments had a small amount of phage release following changing to phage-free eluent, and significantly more release following addition of beef extract. Introduction of a 1% Tween 80 solution, a nonionic detergent, after 243 pore volumes in experiment 10 resulted in further phage release; calcium phosphate buffer alone (before and after beef extract and Tween 80 additions) resulted in smaller detachment rates. These large phage releases 7-8 days after the last phages were introduced into the column do demonstrate that attached phages remain viable.

In experiment 12 with 10% hydrophobic beads, there was no phage breakthrough. Addition of the beef-extract solution resulted in phage release, showing that retained phages were viable.

Poliovirus Column Experiments

Experiment 13, with unbonded silica at pH 7, showed a rapid rise in C to 10% of $C_0$, then a small rise in concentration just prior to switching the feed to virus-free solution, and a lower concentration thereafter (Figure 5). Addition of 2.5% beef extract at 12 pore volumes resulted in a detachment peak that exceeded $C_0$. Comparing the areas above and under the curve gives about 20% more virus release than actually fed into the column; it thus appears that virus detachment was essentially complete. Experiment 14 showed the same rapid rise in C, but to only 0.1% of $C_0$. Virus release in phosphate buffer was small; release upon adding beef extract was qualitatively similar to that of experiment 13, but was about 13% of attached virus. The poliovirus-removal portion of the breakthrough curve looks similar to that for MS-2 (experiment 1), but with more tailing, i.e., slower release.

Experiment 15 with unbonded silica at pH 5.5 showed little breakthrough of virus, with C values only reaching $10^4$-$10^5$ during the experiment; values were $\leq 10^3$ during the detachment portion of the experiment. This pH dependence was thus similar to that observed for MS-2.

Discussion

Breakthrough curves with 1-5% hydrophobic-bonded silica were attenuated due to virus attachment to the silica beads, but did not have the gradual approach to $C/C_0$ of the unbonded-silica curves seen on Figure 1 and in previous work [Bales et al., 1991]. Rather, they reached a steady state value reflecting a constant rate of phage removal. The rate of phage release can be assumed to be slow relative to the removal rate; otherwise, the breakthrough curve would show a gradual increase rather than a constant value at the later times. The relative heights of the steady state plateaus decreased as the mass fraction of hydrophobic beads increased (Figure 6). The columns with 4-5% hydrophobic
beads showed a consistent trend, and were replicated. The 1–3% columns showed less removal and were not replicated.

Column–outlet concentrations in experiments 5–11 reached steady state values about one pore volume into the experiment. These steady values suggest that detachment rates are low relative to rates of attachment, with no “equilibrium adsorption” achieved. Rate coefficients, the inverse of which indicate time scales for attachment, can be estimated from the breakthrough curves.

Since the breakthrough curves reached constant concentration values, reflecting a constant rate of colloid removal, the rate of phage attachment to surfaces can be estimated using a steady state model. That is, a simplified form of the governing equations for one-dimensional colloid transport in porous media [Bales et al., 1991] can be used. In the steady state case, neglecting dispersion, detachment, inactivation, and equilibrium (reversible) binding sites, one simply has first-order removal in an advecting fluid:

$$\frac{dC}{dz} = -k_1 C$$

where $C$ is the colloid (virus) concentration in the aqueous phase, $u$ is the average interstitial velocity, and $k_1$ is a pseudo-first-order rate coefficient for attachment, which depends on the colloid’s diffusion coefficient, the media geometry, and the sticking efficiency (i.e., net energy of interaction between colloid and collector). The parameter $k_1$ is related to the sticking efficiency ($\alpha$) in a single-collector model [Bales et al., 1991]:

$$\eta = 2 \frac{k_1 d}{u} \frac{1}{1 - \theta}$$

where $\eta$ is single-collector removal efficiency and $d$ is the collector diameter. Substituting, one gets the steady state single-collector model that is widely used to describe particle removal in water filtration [Yao et al., 1971]:

$$\frac{dC}{dz} = \frac{3}{2} \frac{1 - \theta}{d} \eta \alpha C$$

Integrating, one gets

$$\log \frac{C}{C_0} = -\frac{1}{2.303} \frac{k_1}{u} \frac{1}{2} \frac{3}{2} \frac{1 - \theta}{d} \eta \alpha L$$

where $L$ is the column length. For the virus colloids, Brownian diffusion is the primary mechanism for particle transport to the collector, and

$$\eta = 0.9 A_s^{1/2} \left[ \frac{\mu d_p}{kT} \right]^{2/3}$$

where $\mu$ is fluid viscosity, $d_p$ is the colloid diameter, $k$ is the Boltzmann constant, $T$ is temperature, and $A_s$ is a parameter that accounts for the effects of adjacent grains on the flow about a collector [O'Melia, 1985]. For a spherical collector, $A_s$ has been given as

$$A_s = \frac{1 - \varepsilon^5}{1 - 1.5 \varepsilon^5 + 1.5 \varepsilon^5 - \varepsilon^5}$$

where $\varepsilon = (1 - \theta)^{1/3}$ [Tien and Payatakes, 1979].

Estimates of $k_1$ from the steady state $C/C_0$ values of Table 1 are 0.0002–0.006 s$^{-1}$. That is, the time scale for phage attachment to the surfaces is of the order of a few hours. For $A_s = 52.5$ (from equation (6)), estimates of $\alpha$ from the same breakthrough curves are 0.0007–0.02. Using the single-collector model of Yao et al. [1971], which does not include the $A_s$ term, gives $\alpha$ values of 0.003–0.07.

For $pH$ 5.5, the nearly complete MS-2 removal on unbonded silica (experiments 2–4) suggests that $\alpha \approx 0.02$. That is, for $C/C_0$ to equal to the detection limit in these experiments, $\alpha$ would be equal to 0.02. So for $C/C_0$ less than the detection limit, $\alpha$ would need to be greater than 0.02. The $\alpha$ value for unbonded silica at $pH$ 7 (0.0022) is the same as that estimated from previous nonsteady experiments ($\alpha$ of 0.019 at $pH$ 7, using the above definition of $\eta$) [Bales et al., 1991]. We consider the current value to be a more reliable estimate of $\alpha$, owing to the greater certainty in parameter estimation in the steady state versus unsteady model. In the unsteady case [Bales et al., 1991], the estimate of $k_1$ depends on an independent estimate of the dispersion coefficient, and $k_1$ is estimated from the transient part of the breakthrough curve. In the current case it is not necessary to use the initial transient part of the breakthrough curve; and thus dispersion did not enter into the estimate of $k_1$.

Experiments with 1 and 3–10% bonded silica showed consistently greater $\alpha$ with a greater fraction of hydrophobic surface (Figure 6). The experiments with 2 and 4% bonded silica had similar $\alpha$ values; the unbonded silica experiment had an $\alpha$ value equal to that from 3% bonded silica. The overall trend is for a higher $\alpha$ with more organic carbon in the porous medium. Dividing the $\alpha$ values in Figure 6 by the fraction of bonded silica beads gives $\alpha = 0.14 \pm 0.07$ (mean ± standard deviation), the estimated $\alpha$ for a column packed entirely with hydrophobic beads. This sticking efficiency can be compared with the fraction of the surface that is actually hydrophobic. Using the measured $f_{oc}$ of 0.00003 g C g$^{-1}$, a mean $d_p$ of 62 μm, a solid density of 2.5 g cm$^{-3}$, and an assumed area covered by one octadecyl molecule of 0.06 nm$^2$ (based on silica geometry), suggests that 13% of the surface of bonded-silica beads is carbon covered. That is, the probability of striking a “hydrophobic” point $\alpha$ on the surface is 0.13. These two numbers (0.14 and 0.13) are the same, but are not directly comparable. For example, the
actual distribution of octadecyl molecules on the surface is probably patchy rather than random, and a virus particle may strike several or strike few bonded organic molecules per collision.

The phage release from unbounded silica accompanying a drop in ionic strength (experiments 3–4) was associated with an expansion of the electrical double layer. Though the change in ionic strength was severe, it does illustrate that phages detach in response to large changes in colloid-collector electrostatic repulsion. This is consistent with previous observations of virus release from soil accompanying simulated rainfall [Funderburg et al., 1981].

Poliovirus demonstrated the same dependency of pH on attachment as did MS-2, although a somewhat greater amount of attachment of poliovirus occurred to the unbound silica at pH 7. This was probably a reflection of the difference in the isoelectric point of the two viruses (i.e., pH_{iep} 6.6 for polio and pH_{iep} 3.9 for MS-2). These results suggest that electrostatic interactions are more important for poliovirus than MS-2 to the unbound silica. However, the virus removal portion of the breakthrough curve was similar for both viruses, although poliovirus release was slower. Beef extract was passed through the columns primarily to assess the viability of the attached virus. Protein solutions such as beef extract are commonly used to deattach viruses from surfaces [Gerba, 1984]. Some of these molecules apparently compete with the silica or hydrophobic-silica-surfaces for viruses and are effective in displacing viruses bound at the surface. The beef-extract solution also has a high ionic strength, which would reduce electrical-double-layer extent and could facilitate adsorption of the organic molecules. The high ionic strength and change in electrical double layer results in a higher charge density very near the surface, which may also alter the configuration of hydrophobic, octadecyl groups bound to the silica by creating a less hydrophobic environment. This could induce release of viruses that were retained due to hydrophobic effects. Addition of the beef extract confirmed that viruses attached to the silica were viable and were not inactivated during attachment/detachment. Virus inactivation on some types of surfaces is known to occur at a fairly rapid rate [Gerba, 1984] and it is important to establish the fate of the viruses in an assessment of virus interaction with surfaces.

Conclusions

MS-2 and poliovirus retention in silica-bead columns at flow rates of about 0.005 cm s^{-1}, typical for rapid groundwater velocities, was reversible, but both attachment and detachment were kinetically controlled. As time scales were estimated to be of the order of days or shorter, one can expect phage removal to be at steady state in many natural systems. Because of the variability in column-outlet concentrations and phage assays, the experiments in which C reached a steady state value provide better estimates of virus attachment rates than do the short-pulse experiments reported previously [Bales et al., 1991].

Hydrophobic effects were important in attachment of MS-2 to surfaces and may be orders of magnitude more important than electrostatic forces in soil. Phage removal increased with f_{oc} of the media. Changing pH from 5 to 7 also exerted a major control on the attachment and transport. Lowering ionic strength and adding beef extract were both effective in releasing retained phage and illustrating that attached phages were viable.

Poliovirus attachment to unbound silica was comparable at pH 5–5.5 and greater at pH 7 relative to MS-2, suggesting that electrostatic forces played a role in its attachment. However, chemical perturbations had the same effect on poliovirus, although poliovirus exhibited more tailing. These results suggest that animal viruses have a similar response to changing chemical conditions as phage. Thus MS-2 and perhaps other similar phages can be useful as conservative models of animal virus behavior because they exhibit lower attachment under identical chemical conditions.

Our results suggest that chemical perturbations associated with rainfall may cause more release of colloids than would occur over long periods at constant chemical conditions. On the other hand, slow detachment under steady state conditions could result in long-term release of viruses into groundwater.

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