# Filtration of Recombinant Norwalk Virus Particles and Bacteriophage MS2 in Quartz Sand: Importance of Electrostatic Interactions

**ARTICLE** *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · DECEMBER 1997

Impact Factor: 5.33 · DOI: 10.1021/es961071u



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Environ. Sci. Technol. **1997**, *31*, 3378−3383

# Filtration of Recombinant Norwalk Virus Particles and Bacteriophage MS2 in Quartz Sand: Importance of Electrostatic Interactions

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Norwalk virus is known to be transmitted through groundwater, yet the environmental factors that facilitate its interstitial transport in subsurface systems are not yet clear. This paper investigates the filtration and surface charge of recombinant Norwalk virus (rNV) particles that are morphologically and antigenically similar to live Norwalk strains but lack nucleic acid and are therefore noninfectious. In contrast to bacteriophage MS2, a common surrogate for waterborne viral pathogens, the surface charge of rNV particles and their filtration in packed beds of quartz sand are strongly influenced by pore water pH over the environmentally important range of pH 5−7. From a mechanistic perspective, these results suggest that the physicochemical filtration of the Norwalk virus is highly dependent on the nature and magnitude of electrostatic interactions that develop between the virus and filter media. Furthermore, because MS2 and the rNV particles differ significantly with respect to their electrostatic properties, MS2 may not mimic the subsurface filtration of Norwalk virus in natural systems.

## Introduction

Viral gastroenteritis is the leading cause of infectious diarrhea in the United States and elsewhere, accounting for more than 3.5 million cases of illness and hundreds of deaths in the United States per year (*1*−*3*). Norwalk and Norwalk-like viruses are the disease-causing agents in many of these cases and are responsible for ≥50% of epidemic nonbacterial gastroenteritis associated with contaminated food and water in the United States (*4*, *5*). The Norwalk virus (NV) genome consists of a single-stranded RNA molecule about 7.7 kb in length that is encapsulated by a spherically shaped protein shell approximately 38 nm in diameter (*6*, *7*). Many outbreaks of NV in the United States are spread by drinking water, and a large fraction of these outbreaks can be traced to the contamination of groundwater supplies by inadequate filtration of sewage effluent from private or community septic tank systems (*4*, *8*). To limit the spread of microbial pathogens through groundwater, the U.S. EPA has proposed a new set of rules under the 1996 reauthorization of the Safe Drinking Water Act that would require public water systems to disinfect source water from each groundwater well unless "natural disinfection" can be demonstrated or a variance can be obtained (*9*). With respect to NV, however, the efficacy of these proposed rules is questionable because the environmental variables that control the natural disinfection of this particular viral pathogen in subsurface systems are largely unknown.

In this study, we have overcome long-standing barriers to conducting filtration experiments with NV by utilizing recombinant Norwalk virus (rNV) particles as a model system. These particles are created by a biochemical procedure in which the structural protein gene for NV is cloned into a baculovirus expression system. When the single recombinant NV capsid protein is expressed, it spontaneously self-assembles into virus-like particles that are morphologically and antigenically similar to the native Norwalk virus (*7*, *10*−*13*). The resulting rNV particles differ from live NV in only one known but important respect: *they lack the genetic material (in particular, RNA) necessary for replication in the host*. Thus, while the rNV particles "look" like a real Norwalk virus, they are harmless protein particles that cannot initiate a human infection (see Figure 1). As a model system for filtration studies, the rNV particles are ideal because they can be grown to high concentration (20 mg of protein or about $10^{15}$ particles/200 mL of cell culture), and their noninfectious character implies that filtration experiments at the field scale may be possible. The only source of NV, on the other hand, is the stools of human volunteers infected with the virus, and so few particles are shed during an active infection that it would be difficult, if not impossible, to purify enough NV to conduct a single filtration experiment. In this report, we demonstrate the feasibility of using rNV particles to investigate the environmental factors—in particular, pore water pH—that influence the filtration of NV in porous media. Because the bacteriophage MS2 is often used as a surrogate for human enteric viruses, we also compare the surface chemical properties and filtration behavior of the rNV particles with those of MS2.

## Methods

**Preparation and Detection of rNV Particles.** Radiolabeled rNV particles used in the filtration experiments were prepared by infection of *Spodoptera frugiperda* insect cells with the rNV-baculovirus recombinant at a multiplicity of infection of 10 in Hink's medium (GibcoBRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum. Twenty-eight hours postinfection, the cells were starved for 30 min in Grace's methionine-free medium (GibcoBRL), followed by the addition of 10 $\mu$Ci/mL of $^{35}$S-trans label (ICN, Irvine, CA). Thirty-three hours postinfection, cold methionine was added, and the infection was allowed to continue for 4 days. $^{35}$S-labeled rNV particles released into the medium were harvested by pelleting for 2 h at 26000 rpm in an SW28.1 rotor (Beckman, Fullerton, CA), followed by isopycnic centrifugation on a cesium chloride gradient. The particles were collected as a band on the cesium chloride gradient, diluted into water and pelleted. The pelleted rNV particles were then suspended in 500 $\mu$L of sterile MilliQ water and then characterized by electron microscopy and BSA protein assay (Pierce, Rockford, IL). These particles were detected in liquid samples by scintillation counting with a Beckman LS6000IC scintillation counter. Denatured rNV protein used in the filtration and sucrose gradient experiments (described below) was prepared by boiling radiolabeled rNV protein suspended in the electrolyte solution of interest for approximately 10 min. The procedure for generating unlabeled rNV particles used for the electrophoretic mobility measurements was the same as described above, except that no radioactive precursors were

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S0013-936X(96)01071-1 CCC: $14.00

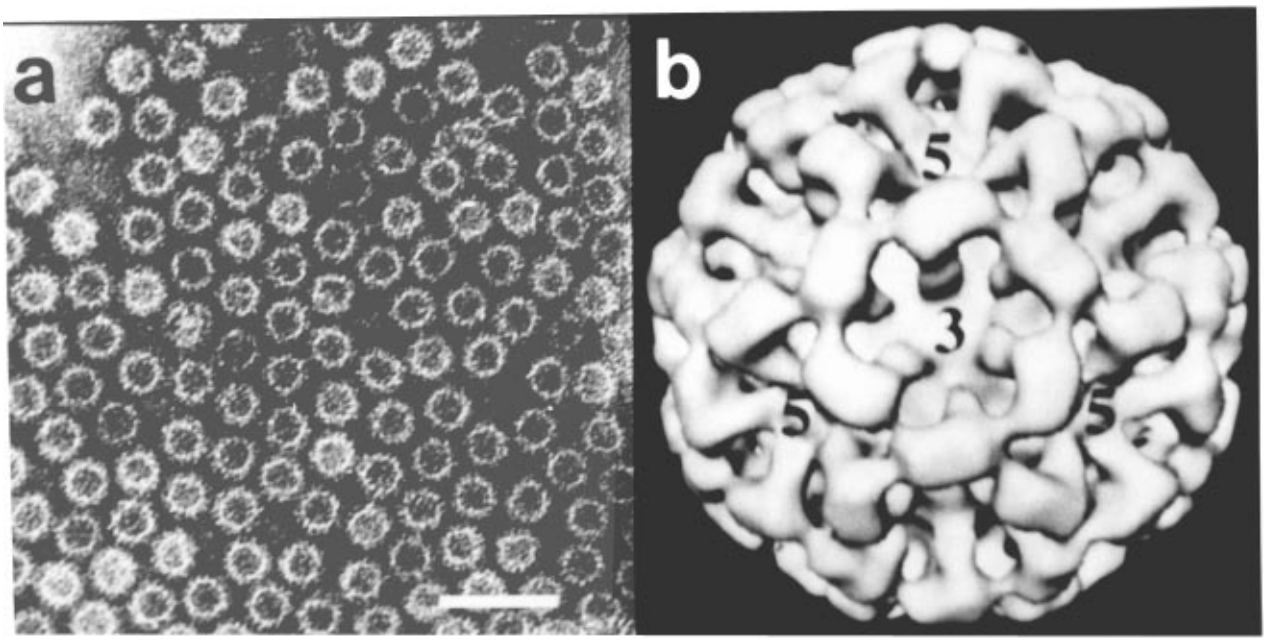


**FIGURE 1. Structure of recombinant Norwalk virus particles. (a) Negative-stain electron micrograph of rNV particles and (b) the three-dimensional structure of rNV particles viewed along the icosahedral 3-fold axis. This structure was determined by image processing of the rNV particles shown in panel a. These figures are a composite of data reported in refs 6 and 7. The scale bar corresponds to 100 nm.**

added during the infection step that was carried out in Grace's media supplemented with 0.5% fetal calf serum.

**Growth and Measurement of Bacteriophage MS2.** The bacteriophage MS2 was grown on an *Escherichia coli* host (ATCC 15597) following the procedure outlined in Bales et al. (*14*). Stocks of MS2 were stored at 4 °C in Tris buffer [0.04 M tris(hydroxymethyl)aminomethane (base), 0.22 M NaCl, 0.008 M KCl, and 0.0006 M $Na_2HPO_4$ adjusted to pH 7.4 with 12 N HCl]. Concentrations of infective MS2 particles were determined by the plaque-forming unit (PFU) assay using the agar overlay method (*14*, *15*). Briefly, dilutions of each sample were mixed with host cells, plated on nutrient agar, and incubated overnight. The resulting plaque counts were converted to PFU/mL.

**Microelectrophoresis Measurements.** Electrolyte solutions for electrophoresis measurements were prepared from deionized water (Milli-Q, 18.2 MΩ·cm, Millipore, Inc., Bedford, MA) and analytical-grade NaCl, $NaHCO_3$, and HCl. The pH of the electrolyte solution was adjusted by the addition of HCl and $NaHCO_3$. The ionic strength of the electrolyte solution increased by less than 2% when the pH was adjusted in the range of 4−7, by approximately 10% when the pH was adjusted to 8, and by approximately 100% when the pH was adjusted to 9. Seventy microliters of purified unlabeled rNV particles (at a concentration of 1 mg of rNV protein dissolved in 1 mL of deionized water) was added to 7 mL of filtered (0.22 mm pore size, Nalgene, Rochester, NY) electrolyte solution for a final rNV concentration of 0.01 mg/mL. Measurements were conducted using a Rank Brothers Mark II apparatus (Cambridge, U.K.) equipped with a 0.5-mW green 544-nm wavelength He-Ne laser (Melles Griot Model O5 SGR 851, Melles Griot, Carlsbad, CA), and a capillary cell in a four electrode operation. The capillary cell was flushed three times with distilled water and four times with rNV-free electrolyte solution. The capillary was then filled with the electrolyte solution containing rNV particles, and mobility measurements were carried out at temperatures between 6 and 8 °C. The reported values of electrophoretic mobility represent the average of at least 40 measurements, 20 at both the upper and lower stationary levels. Exceptions were at pH 5 where the rNV particles had no measurable velocity and at pH 9 where only 25 measurements at the lower stationary level were taken. Differences between measurements at the upper and lower stationary levels were generally less than 20%.

**Filtration Experiments.** The quartz sand was purchased from Unimin (New Canaan, CT), fractionated by size using a wet sedimentation/flotation technique, and then cleaned to remove metal and organic contaminants (*16*, *17*). The cleaning steps included soaking the sand in 12 N HCl for at least 24 h, washing with deionized water (Milli-Q), and baking the sand at 800 °C overnight. Cleaned sand was stored under vacuum. Before conducting column experiments, the sand was rehydrated by boiling for at least 1 h in filtered deionized water. The filtration experiments were carried out with glass columns (Pharmacia LKB C16, Piscataway, NJ) packed by allowing the quartz to settle in filtered deionized water. The packed columns were equilibrated by pumping (Pharmacia LKB P1 peristaltic pump) at least 10 pore volumes of the electrolyte solution through the column prior to each filtration experiment. Influent solutions were prepared by diluting radioactively-labeled rNV particles directly into 250 mL of 0.01 M NaCl solution, pH adjusted to either 5 or 7 with HCl or $NaHCO_3$. In the case of MS2, lysates were purified by isopycnic centrifugation on CsCl gradients (*18*), dialyzed repeatedly against 3 L of 0.01 M NaCl solution, and diluted into 250 mL of pH-adjusted electrolyte solution. Dialysis was carried out using a Slide-A-Lyzer dialysis system (Pierce, Rockford, IL). Column effluent was collected in 9-mL fractions (Pharmacia LKB FRAC-200).

**Sucrose Gradient Analysis.** To assess whether radioactively-labeled rNV capsid protein was present as intact particles, 9 mL of column influent or effluent was concentrated to a final volume of less than 0.2 mL using Microcon 3 microconcentrators (Amicon, Inc., Beverly, MA). The membranes in these concentrators have a molecular weight cutoff of 3000, which is substantially below the MW of a single recombinant NV capsid protein (58 000 Da); therefore, the concentrators should retain individual capsid protein as well as intact particles. The concentrated solution was layered on top of a continuous 10−50% sucrose gradient (8 mL total volume) and centrifuged for 70 min at 35 000 rpm in a Beckman SW41 rotor at 4 °C (*12*). Fractions were collected by puncturing the bottom of the centrifuge tube with a 21-gauge needle. The fractions obtained were then analyzed for rNV protein by scintillation counting. Sucrose gradients were also carried out on samples of purified rNV particles and

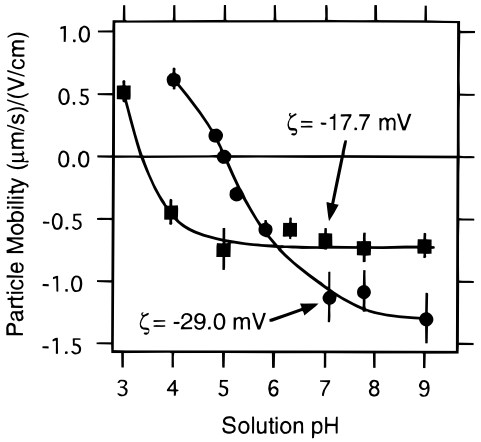


**FIGURE 2. Electrophoretic mobility of rNV particles (circles) and MS2 (squares) as a function of solution pH in the presence of 0.01 M NaCl. The electrophoretic mobility represents the observed particle velocity normalized by the voltage drop over the length of the capillary cell. Error bars represent 1 standard deviation. The electrophoretic mobility measurements were carried out using non-radiolabeled rNV particles; mobility values reported for MS2 are reproduced from a previous report (*30*). Also shown in this figure is the zeta potential (ζ) of MS2 and rNV particles at neutral pH calculated using Henry's equation (see text).**

boiled rNV particles as positive and negative controls, respectively.

## Results

**Electrophoretic Mobility of MS2 and rNV Particles.** When a virus is suspended in water, ionization of weakly acidic and basic functional groups associated with the coat protein gives rise to a net charge on the viral surface that is pH dependent (*19*). This surface charge is often invoked to explain the pH and ionic strength dependence of virus removal from water by physicochemical processes like coagulation and filtration (*20*−*25*), although direct evidence for the involvement of electrostatic forces has been obtained in only a few cases (*26*−*29*). The method of choice for characterizing the surface electrical potential of colloidal particles is whole-particle microelectrophoresis in which the velocity of individual particles (or electrophoretic mobility) is measured in the presence of an applied electric field. Recently, we developed a new approach for conducting these measurements that permits the electrophoretic characterization of very small particles the size of Norwalk virus (*30*).

Figure 2 shows the results of microelectrophoresis measurements carried out on the rNV particles (circles) and on the bacteriophage MS2 (squares) in the presence of 0.01 M NaCl. The bacteriophage MS2 is a spherically shaped virus with a diameter of approximately 27 nm (*31*)—similar in size and shape to Norwalk virus. The point of zero charge (pzc) of rNV particles occurs at pH 5, roughly 1.5 pH units higher than the pzc of MS2. Estimates of the electrostatic potential, or ζ potential can be made from these electrophoretic mobility measurements using Henry's equation, which is valid for small potentials (*32*). At neutral pH and in 0.01 M NaCl, the ζ potential of the rNV particles (−29.0 ± 5.1 mV) is almost twice the ζ potential of MS2 (−17.7 ± 2.3 mV), implying that the former particle is significantly more electronegative at this particular pH. Importantly, the rNV particles develop their negative surface charge over the environmentally relevant pH range of 5−7. Hence, the electrostatic character of the rNV particles, and by extension Norwalk virus itself, may be strongly influenced by small changes in the pH of interstitial waters in natural subsurface systems. In contrast, the electrophoretic mobility of MS2 is approximately constant for all pH values greater than pH 5.

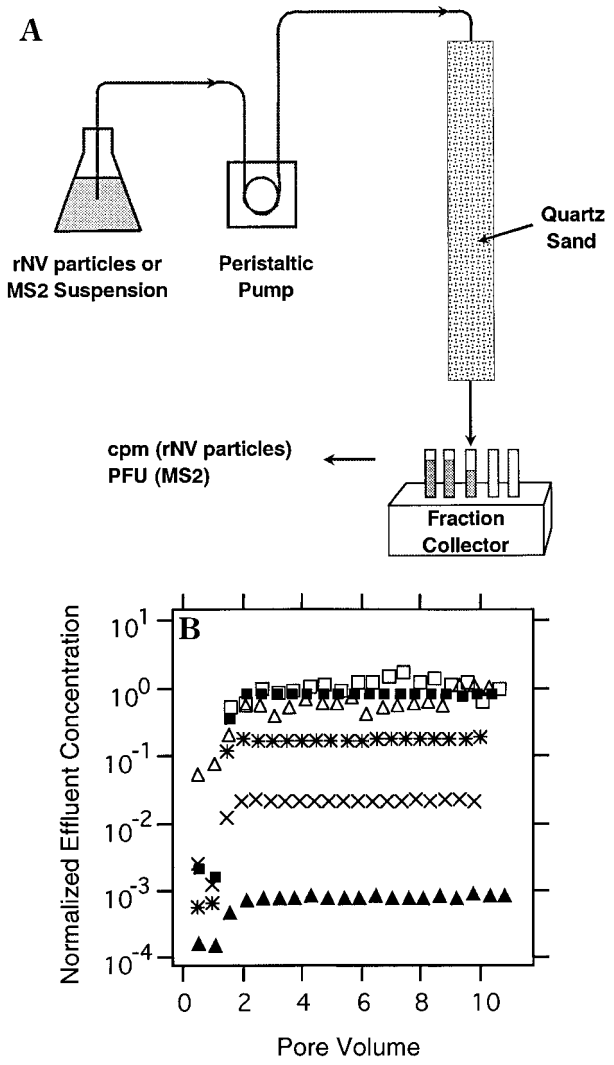


**FIGURE 3. (A) Experimental apparatus used in the filtration experiments. (B) Breakthrough curves for rNV particles (closed symbols) and MS2 (open symbols) at pH 5 (triangles) and pH 7 (squares) and for denatured rNV capsid protein at pH 5 (crosses) and pH 7 (stars). Control experiments were carried out to ensure that MS2 inactivation did not occur over the time scale of the experiments, as reported in a previous study (*40*).**

**Packed Bed Filtration of MS2 and rNV Particles.** The apparatus used in the filtration experiments is illustrated in Figure 3A. Suspensions of either radiolabeled rNV particles or infective MS2 particles were pumped through a column saturated with a 0.01 M NaCl electrolyte solution and packed with well-cleaned quartz sand. Fractions of the column effluent were collected over time and analyzed for either radioactivity (in the case of rNV particles) or virus infectivity (in the case of MS2). These "breakthrough" data were then normalized relative to the influent concentration and plotted against the number of pore volumes passed through the column (see Figure 3B). Separate experiments were conducted using electrolyte solutions pH adjusted to either pH 5 or pH 7, corresponding to where the rNV particles possess a net zero or net negative charge, respectively. The pzc of the quartz sand used in these experiments occurs at a solution pH of between 2 and 3, so the surface of the quartz was negatively charged at both pH 5 and pH 7 (*17*).

At pH 5, the normalized steady-state breakthrough concentration of rNV particles in the column effluent is $7.6 \pm 0.3 \times 10^{-4}$ ($\pm 1\ \sigma$), implying that 99.92% of the rNV particles that enter the column are retained on the quartz grains under these conditions (solid triangles in Figure 3B). When the

**TABLE 1. Characteristics of Filtration Experiments Conducted with either rNV Particles or MS2[a]**

| description of experiment | column length, *L* (cm) | influent concn[b] |
|---|---|---|
| MS2, pore water pH 5 | 18.2 | $1 \times 10^4$ PFU/mL |
| MS2, pore water pH 7 | 17.3 | $8 \times 10^3$ PFU/mL |
| rNV particles, pore water pH 5 | 16.9 | 30 808 CPM/mL ($5.6 \times 10^{10}$ particles/mL) |
| rNV particles, pore water pH 7 | 17.7 | 4931 CPM/mL ($9.0 \times 10^9$ particles/mL) |
| denatured rNV capsid protein, pore water pH 5 | 18.7 | 5213 CPM/mL |
| denatured rNV capsid protein, pore water pH 7 | 18.4 | 7168 CPM/mL |

[a] For all experiments, the concentration of NaCl in the pore fluid was 0.01 M, the superficial velocity was $U = 1.5 \pm 0.01$ cm/min, the inner diameter of the column was 1.6 cm, the average radius of the quartz packing material was $a = 0.011 \pm 0.002$ cm, the bed porosity was $\epsilon = 0.49 \pm 0.02$, and the temperature was approximately 4 °C. The specific activity of the intact rNV particles and denatured rNV protein was $3.1 \times 10^4$ and $5.3 \times 10^3$ CPM/$\mu$g, respectively. [b] Numbers in parentheses represent the calculated number of rNV particles per mL, assuming all protein is associated with intact particles.

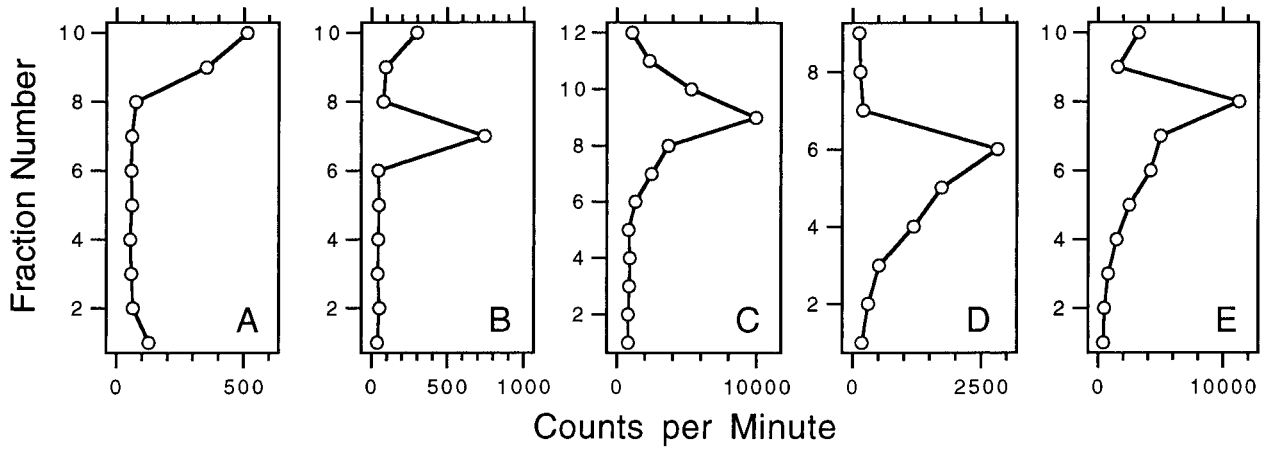


**FIGURE 4. Sucrose gradient analysis of (A) denatured rNV capsid protein, (B) intact rNV particles in stock preparation, (C) rNV particles in the pH 7 column influent, (D) rNV particles in the pH 7 column effluent, and (E) rNV particles suspended in pH 5 electrolyte solution after 7 days of incubation at 4 °C. Intact rNV particles migrated roughly one-third the length of the gradient, while little or no migration is observed for the denatured protein.**

solution pH is raised to pH 7, the normalized steady-state breakthrough concentration of rNV particles increases more than 1000-fold (or 3 $\log_{10}$ units) to $0.83 \pm 0.01$ (solid squares in the figure). In this case, only about 17% of the rNV particles that enter the column are retained by the quartz matrix. The dramatic decrease in filter performance going from pH 5 to pH 7 coincides with the change in the electrostatic character of the rNV particle surface from no net charge at pH 5 to fully charged at pH 7. At the higher pH, repulsive electrostatic interactions apparently inhibit the deposition of the rNV particles onto the quartz sand, resulting in higher concentrations of the rNV particles in the pore fluid and more rapid transport down the column. MS2, on the other hand, exhibits near-complete breakthrough at both pH 5 ($0.7 \pm 0.2$) and pH 7 ($1.1 \pm 0.3$) (open triangles and open squares in Figure 3B, respectively). The surface of the MS2 virus is negatively charged at both pH values, and repulsive electrostatic interactions probably contribute to the poor filtration observed in this case as well.

The steady-state breakthrough concentrations of rNV particles plotted in Figure 3B may be translated into filtration rate constants using a simple one-dimensional mathematical model of particle filtration known as the "clean-bed filtration model" (*33*):

$$n^* = \exp\left[\frac{-3(1-\epsilon)k_{exp}L}{Ua}\right] \quad (1)$$

where $n^*$, $\epsilon$, $L$, $a$, $U$, and $k_{exp}$ represent, respectively, the normalized steady-state breakthrough concentration of rNV particles, bed porosity, the distance over which the filtration occurs, collector radius, superficial velocity of the pore fluid, and the experimental filtration rate constant; values for $\epsilon$, $L$, $a$, and $U$ specific to our system are summarized in Table 1. In general, the magnitude of the rate constant $k_{exp}$ will depend on the detailed nature of the filtration experiment, including the superficial velocity employed, the grain size distribution of the packed bed, the bed porosity, pore water chemistry, ambient temperature, etc. With the exception of pore water pH, all of these attributes were held constant in the set of filtration experiments involving the rNV particles. Hence, differences in the magnitude of the filtration rate constants calculated from the rNV particle breakthrough curves can be directly related to the influence of pore water pH on the deposition kinetics of the rNV particles. Filtration rate constants estimated from the steady-state breakthrough concentrations of rNV particles at pH 5 and pH 7 are $k_{exp}^{pH5} = 7.7 \pm 1.1 \times 10^{-5}$ cm s$^{-1}$ and $k_{exp}^{pH7}$ $1.9 \pm 0.3 \times 10^{-6}$ cm s$^{-1}$, respectively.

**Breakdown of the rNV Particles.** In the filtration experiments described above, the rNV particles were detected by measuring for a radioactive $^{35}$S-methionine label incorporated into the capsid protein. In principle, this label could be associated with either intact rNV particles or isolated polypeptides generated from the breakdown of intact particles. There are several lines of evidence to suggest that breakdown of the rNV particles did not occur over the course of the filtration experiments. Sucrose gradient analysis of samples collected from the column influent and effluent at pH 7 indicated that most of the radioactivity (≥86%) was associated with intact particles (see Figure 4). Because of the high levels of rNV particle filtration that occurred at pH 5, it was not possible to concentrate enough protein in the column effluent to carry out a sucrose gradient analysis. However, sucrose gradient analysis of rNV particles suspended in an electrolyte solution identical to the one used in the pH 5 filtration experiments indicated that the particles did not breakdown over a period of 7 days at 4 °C (panel E in Figure 4). For comparison, the

filtration experiments conducted with the rNV particles at this pH were completed in less than 2 h. Finally, filtration experiments conducted with denatured rNV structural protein exhibited breakthrough concentrations that were significantly different from those observed for intact particles (crosses and stars in Figure 3B).

## Discussion

**Extrapolation of Filtration Experiments to Field Scales.** The breakthrough data plotted in Figure 3B reflect the degree of rNV particle and MS2 filtration that occurs over a distance of 18 cm, the approximate length of the packed columns employed in our study. It is interesting to consider how these breakthrough results might extrapolate to length scales more typical of groundwater basins. In general, virus fate and transport in the subsurface depends on a combination of biological and physicochemical processes including loss of viruses through viral inactivation and, possibly, grazing by higher trophic levels, pore water advection, reversible and/or irreversible filtration, and dilution of the viral plume with ambient groundwater by hydrodynamic dispersion. Which of these processes dominate in a particular subsurface system depends on many factors that are not well understood at the present. We used eq 1 together with the parameter values applicable to our column experiments (see Table 1) to estimate how physicochemical filtration alone might influence the NV concentration at various distances from a hypothetical source, such as a septic tank or groundwater recharge basin. At a distance of only 1 m, eq 1 predicts a reduction in NV concentration of 19 $\log_{10}$ units if the pore water pH is 5, compared to only 0.5 $\log_{10}$ units if the pore water pH is 7. The predicted reduction in NV concentration at 10 m from the source grows to an astronomical 190 $\log_{10}$ units at pH 5, compared to 5 $\log_{10}$ units at pH 7. These calculations underscore the significant influence that pore water pH, in particular, and pore water chemistry, in general, can exert on the filtration dynamics of NV. Additional geochemical features of a site that might influence the subsurface transport of NV include the presence of natural or sewage-derived organic matter and/or divalent cations in the pore fluid and the mineralogical heterogeneity associated with the sediment or soil (*34*). Experiments are currently under way in our laboratories to assess how these additional factors influence rNV particle filtration in model systems.

On the basis of our filtration data and the simple analysis presented above, we can formulate two "working hypotheses" regarding the subsurface transport of viral pathogens such as NV. First, under the right conditions, physicochemical filtration can be an extremely effective treatment process for the removal of viral pathogens from contaminated groundwater. In its draft of the Groundwater Disinfection Rule (*35*), the EPA suggested that the setback distance between a groundwater pumping well and any source of human waste should be sufficient to ensure a reduction in viral concentration at the well of 11 $\log_{10}$ units. With respect to NV, our results indicate that physicochemical filtration alone can exceed this level of treatment at very short setback distances (approximately 1 m) if there is no electrostatic barrier to deposition, filtration occurs irreversibly, and particle deposition is not inhibited by previously deposited particles. Second, the ability of physicochemical filtration to attenuate virus concentrations in the subsurface is strongly influenced by the electrostatic character of the viral particles and filter media, both of which depend on the pH and the electrolyte composition of the pore fluid. In fact, taken at face value, our results suggest that the chemical makeup of the pore water may determine the capacity of groundwater systems to provide natural disinfection by physicochemical filtration.

**Deposition Mechanisms.** At pH 5, there is no electrostatic barrier to the deposition of the rNV particles on the quartz grains; however, it not clear whether the filtration rate observed at this particular pH is transport-limited or if other (nonelectrostatic) forces retard the deposition rate. To address this issue, we compared the filtration rate constant calculated above for the rNV particles at pH 5 to a theoretical estimate, which assumes that particle deposition onto the grain surfaces is transport-limited (*36*, *37*):

$$k_{\text{theor}} = [A_s U]^{1/3}[k_B T/(12\pi a \mu r)]^{2/3} \qquad (2)$$

where the Happel's cell parameter $A_s$ accounts for the three-dimensional packing of collectors in the filter, $k_B$ is Boltzmann's constant, $\mu$ is the dynamic viscosity of the pore fluid, and $r$ is the radius of the particles. Using the set of parameter values listed in Table 1 and a value for the Happel cell parameter appropriate for our system of $A_s = 23.1 \pm 0.4$ (*38*), the theoretical rate constant predicted by eq 2 is $k_{\text{theor}} = 1.8 \pm 0.2 \times 10^{-4}$ cm s$^{-1}$. This theoretical rate constant is $2.3 \pm 0.4$ times larger than $k_{\text{exp}}^{\text{pH5}}$, or slightly outside of the factor of 2 agreement reported by other researchers for the filtration of polystyrene latex particles in packed beds of glass beads or quartz grains when there is no electrostatic barrier to particle deposition (*16*, *33*, *39*).

In a previous report, we examined the packed bed filtration of MS2 through the same quartz packing material used in the present study and found that the filtration rate coefficient estimated from eq 2 was eight times larger than the filtration rate coefficient estimated from the breakthrough concentration of MS2 at its pzc, $k_{\text{exp}}^{\text{pH3.5}}$ (*29*). Because the deposition of MS2 on the quartz grains should not be retarded by electrostatic double-layer forces at pH 3.5, one or more nonelectrostatic forces must be responsible for the fact that $k_{\text{theor}} \gg k_{\text{exp}}^{\text{pH3.5}}$. We suggested that this nonelectrostatic force was steric in nature, arising from hydrophilic polypeptide loops that extend a maximum of 1 nm off the MS2 surface. The fact that the filtration rate of rNV particles at pH 5 more closely conforms to eq 2 suggests that nonelectrostatic forces play a less important role in the deposition kinetics of this particular virus. More generally, these results indicate that different repulsive forces may control the deposition rates of different viruses in granular media, even if the viruses in question are structurally and morphologically similar, as is the case for MS2 and the rNV particles.

**Suitability of MS2 as a Surrogate for NV.** What can we say about the suitability of MS2 as a surrogate for NV in field and column filtration studies? Compared to the rNV particles, MS2 has a significantly lower pzc and a less negative $\zeta$ potential at neutral pH and above. Because electrostatic interactions appear to be an important factor in the transport of the rNV particles through porous media, MS2 will probably not mimic the filtration of NV in many subsurface systems. On the other hand, our data do support the use of MS2 as a "conservative" indicator of fecal contamination in sandy aquifers because this bacteriophage exhibited less filtration than the rNV particles at both pH 5 and pH 7. Indeed, the fact that MS2 was less filtered than the rNV particles at pH 7 is unexpected, given that the rNV particles are more electronegative than MS2 at neutral pH. Perhaps the enhanced transport of MS2 at pH 7 is due to the existence of nonelectrostatic repulsive forces acting between the virus and collector surface, as discussed in the last section. It should also be noted that the overall suitability of MS2 as a surrogate virus for NV also depends on the relative inactivation rates of these two viruses in groundwater—an issue that the rNV particle system is not well-suited to address.

## Future Prospects

The use of rNV particles as a model system for filtration studies makes possible, for the first time, a systematic investigation of the environmental factors that influence the interstitial transport of NV in porous media. The data presented in this paper demonstrate the importance of repulsive electrostatic

interactions in facilitating the transmission of NV through unconsolidated quartz-rich sands. From a practical point of view, our results suggest that pore water chemistry may significantly impact the capacity of groundwater systems to provide natural disinfection by physicochemical filtration. Ultimately, the rNV particles may provide a direct and simple methodology for individual water districts to determine if specific production wells are protected from virus intrusion. In particular, the rNV particles and necessary antibody-based detection systems could be made available in "kit" form, and the results of standardized groundwater filtration tests could be used as an evaluation method for granting variances to Federal and/or State groundwater disinfection regulations. The unique application of recombinant virus-like-particle technology presented in this report represents a major step forward in our understanding of the capabilities and limitations of natural groundwater disinfection as a barrier against the transmission of waterborne diarrheal disease.

## Acknowledgments

We thank the following individuals for reading the manuscript and offering helpful advice: L. B. Grant, P. Berger, W. A. Yanko, and O. Ogunseitan. This work was sponsored by the NSF/EPA Partnership for Environmental Research, under Contract DEB95-24481.

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*Received for review December 30, 1996. Revised manuscript received May 8, 1997. Accepted September 17, 1997.*[⊗]

ES961071U

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1997.