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# New Electropositive Filter for Concentrating Enteroviruses and Noroviruses from Large Volumes of Water▽

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**The U.S. Environmental Protection Agency's information collection rule requires the use of 1MDS electropositive filters for concentrating enteric viruses from water, but unfortunately, these filters are not cost-effective for routine viral monitoring. In this study, an inexpensive electropositive cartridge filter, the NanoCeram filter, was evaluated for its ability to concentrate enteroviruses and noroviruses from large volumes of water. Seeded viruses were concentrated using the adsorption-elution procedure. The mean percent retention of seeded polioviruses by NanoCeram filters was 84%. To optimize the elution procedure, six protocols, each comprising two successive elutions with various lengths of filter immersion, were evaluated. The highest virus recovery (77%) was obtained by immersing the filters in beef extract for 1 minute during the first elution and for 15 min during the second elution. The recovery efficiencies of poliovirus, coxsackievirus B5, and echovirus 7 from 100-liter samples of seeded tap water were 54%, 27%, and 32%, respectively. There was no significant difference in virus recovery from tap water with a pH range of 6 to 9.5 and a water flow rate range of 5.5 liters/min to 20 liters/min. Finally, poliovirus and Norwalk virus recoveries by NanoCeram filters were compared to those by 1MDS filters, using tap water and Ohio River water. Poliovirus and Norwalk virus recoveries by NanoCeram filters from tap and river water were similar to or higher than those by the 1MDS filters. These data suggest that NanoCeram filters can be used as an inexpensive alternative to 1MDS filters for routine viral monitoring of water.**

Viruses that primarily infect and replicate in the gastrointestinal tract are known as enteric viruses. More than 140 different enteric viruses are known to infect humans. These include the enteroviruses, rotaviruses, hepatitis A virus, noroviruses, adenoviruses, and reoviruses, among others. Enteric viruses are capable of causing a wide range of illnesses, including gastroenteritis, paralysis, aseptic meningitis, herpangina, respiratory illness, fevers, myocarditis, etc. Given the potential public health impact of the enteric viruses, enteroviruses (echovirus and coxsackievirus), adenoviruses, and caliciviruses are on the U.S. Environmental Protection Agency's contaminant candidate list 2 for regulatory consideration for drinking water (11). Within the *Caliciviridae* family, noroviruses are the primary viruses of concern for drinking water.

Contaminated drinking water is considered to be a potential transmission route, and an infectious dose in humans may consist of only a small number of virus particles. Enteric viruses are introduced in aquatic environments through natural or human activities, such as leaking sewage and septic systems, urban runoff, landfills, injection of treated wastewater into aquifers, wastewater discharge, sewage outfall, etc. These viruses have been found in surface water, groundwater, and drinking water (1, 6, 13, 22, 26). Between 1971 and 2004, 789 drinking water outbreaks and 575,207 cases of illness were reported in the United States, and 8% of the reported outbreaks were due to enteric viruses (2, 5, 28, 29, 30, 46).

The levels of enteric viruses in natural waters are often low, and as such, typical virus sampling involves a primary concentration of viruses from large volumes of water (hundreds to thousands of liters). Unlike other waterborne pathogens (such as bacteria and parasites), viruses are smaller, and thus, size exclusion filtration is often not practical, especially for turbid waters. In addition, viruses are negatively charged in natural environments and can be adsorbed onto a number of different matrices by electrostatic and hydrophobic interactions (16). Consequently, different types of matrices have been used to isolate enteric viruses from water. These include negatively and positively charged membranes or cartridge filters (10, 17, 32, 34, 35, 39), gauze pad (31), and glass powder or glass wool (14, 27). Of all of these methods, electronegative and electropositive filters are most commonly used. In the case of electronegative filters, the acidification of the water and addition of multivalent cations are required for optimal virus adsorption. Because of this need to condition the water to attain acceptable recoveries, it is difficult to use electronegative filters for field sampling. In contrast, electropositive filters do not require conditioning of the water. Among all the filters, 1MDS electropositive filters (Cuno, Meriden, CT) are the most commonly used filter for fresh and drinking water sampling; however, they are not cost-effective for routine viral monitoring of water and require pH adjustment for waters with pH values exceeding 8.0 (12).

Viruses adsorbed on the filter are usually eluted and recovered using 1 to 1.6 liters of eluting solution (6, 12). Many different procedures are described in the literature to elute

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viruses from filters. These procedures include the use of different eluting solutions, such as 0.3%, 1.5% or 3% beef extract, urea-arginine phosphate buffer, glycine buffer, etc. (10, 12, 24, 37). There are also different elution processes, such as single elution, recirculation of eluents, or successive elution of filters (6, 8, 15, 43). Sobsey and Hickey (40) used only one elution with 0.3% beef extract in 50 mM glycine. Sobsey et al. (43) suggested that 1 liter of 1.5% beef extract be recirculated through the filters for 5 min. Dahling and Wright (8) reported that the highest virus recoveries were obtained by three elutions, each using 1.6 liters of 3% beef extract. Dahling (6) reported that the highest virus recoveries were obtained with two separate beef extract elutions, one being an overnight filter immersion in beef extract.

Although methods for concentration of many enteric viruses have been developed, limited studies have been conducted for concentrating noroviruses from water. Huang et al. (21) described a norovirus concentration method using porcine calicivirus (Pan-1) as a surrogate. Pan-1 was sensitive to the high pH (9.5) of the eluting solution, which is commonly used. Myrmel et al. (33) described a method of norovirus concentration using feline calicivirus as a surrogate organism. The method used electronegative filters, and the recovery of virus was 5 to 10%. Many other studies reported detection of human noroviruses in environmental waters (18, 19, 25); however, none of these studies evaluated the recovery efficiencies of human noroviruses from large volumes of water.

The objective of this study was to evaluate the NanoCeram (Argonide, Sanford, FL) cartridge filter for the concentration of enteroviruses and noroviruses from large volumes of water. NanoCeram filters have an active component of nano alumina (AlOOH) fibers, which give them a naturally occurring electropositive charge.

## MATERIALS AND METHODS

**Viral stock preparation.** Poliovirus 1 (Mahoney strain), coxsackievirus B5, echovirus 7, and Norwalk virus were used in this study. Poliovirus 1, coxsackievirus B5, and echovirus 7 were grown in the laboratory by inoculation into a confluent monolayer of Buffalo green monkey kidney (BGM) cells. Viruses were stored in 1-ml amounts at −70°C, thawed shortly before each experiment, and diluted in sterile Milli-Q water. Norwalk virus was extracted from stool samples, using an organic solvent solution. Briefly, 2 g of fecal sample was added to 18 ml of phosphate-buffered saline containing 0.1% bovine serum albumin (U.S. Biochemical Corp., Cleveland, OH) and 10 ml of solvent mixture. The solvent mixture was freshly prepared by mixing 0.4 ml of 0.01% dithiozone (diphenylthiocarbazone; Fisher catalog no. D90) in chloroform (Acros, NJ), 3.6 ml of 0.01 M 8-hydroxyquinoline (Fisher catalog no. 0261) in chloroform, 4 ml of butanol (Fisher Scientific, Fair Lawn, NJ), 1 ml of methanol (Baxter, Muskegon, MI), and 1 ml of trichloroethane (Spectrum Chemical, Gardena, CA). The mixture was vortexed for 2 to 3 min and centrifuged at 4,080 × *g* for 10 min. The supernatant was collected and centrifuged at 3,824 × *g* for 5 min. The supernatant was collected and filtered through a 0.2-μm serum acrodisc 37-mm syringe filter (Pall Corporation, Ann Arbor, MI) pretreated with 1.5% beef extract. The stool specimen was from a volunteer study funded by the National Marine Fisheries Service and was kindly provided by Gary Richards (Charleston Laboratory, National Marine Fisheries Service). Extracted virus was stored at 4°C prior to use.

**Filters used.** NanoCeram (Argonide, Sanford, FL) and Zeta Plus Virosorb 1MDS (Cuno, Meriden, CT) electropositive cartridge filters were used in this study. NanoCeram cartridge filters contain nano alumina fibers that are dispersed throughout a microglass fiber matrix, resulting in a nonwoven medium with 2-μm average pore size. The alumina fibers are 2 nm in diameter, approximately 0.3 μm long, and have a surface area of 500 to 600 $m^2/g$ (44), providing a very large area for adsorbing electronegative particles. Zeta Plus Virosorb 1MDS (Cuno, Meriden CT) is a charged modified glass and cellulose medium electropositive filter. NanoCeram and 1MDS cartridge filters used in this study were 12.7 cm (5 in.) and 25.4 cm long, respectively.

**Evaluation of virus retention and recovery by NanoCeram filters.** Deionized water (100-liter) samples were seeded with $2 \times 10^5$ to $9 \times 10^5$ PFU (~2 to 9 PFU/ml) of poliovirus and mixed. Virus inoculums were added to 200 ml of deionized water and mixed for several minutes to disrupt aggregates before seeding 100 liters of water. Water samples were then filtered through NanoCeram cartridge filters at an average flow rate of 5.6 (±0.17) liters per minute. A composite sample of 100 ml was collected from the filtrate to determine the virus retention by the filters. Six elution procedures were evaluated for elution of viruses retained by the filters. Each elution procedure was composed of two separate elutions of the viruses from the filters with 500 ml each of 1.5% beef extract (Adam Scientific catalog no. 4900-107; pH 9.0) containing 0.05 M glycine (Fisher Scientific, NJ). Each elution was performed by initially filling the cartridge housing with the buffered beef extract solution. The entire 500-ml portion of the solution was then pushed through the filter after a contact time of 1 min for the first elution and at variable times of 1, 15, 30, 60, or 120 min or overnight for the second elution. The U.S. Environmental Protection Agency's information collection rule (ICR) (12) contained a standardized virus method that requires two elutions of viruses from the 1MDS filter, each requiring 1 minute of contact time between the eluent and the filter. In this study, we evaluated whether a longer contact time for the second elution results in higher virus elution from NanoCeram filters. Viruses present in each filter eluent were concentrated using a modification of the celite elution method described by Dahling and Wright (9). Briefly, this was performed by adding 0.5 g of celite (Fisher Scientific catalog no. C211), adjusting the pH to 4.0, stirring for 30 min at room temperature, and collecting the celite onto sterile 75-cm-diameter prefilters (Millipore Corporation catalog no. AP20 075 00) by vacuum filtration. Adsorbed viruses were eluted by allowing 40 ml of 0.15 M sodium phosphate (pH 9.0 to 9.5) to flow through the filter with no vacuum. Each filter eluent from the first and second elutions was analyzed separately by plaque assay (see below) to determine the virus recovery efficiency.

**Comparison of virus recoveries at different water pH levels.** Tap water (100 liters) was dechlorinated by adding 50 mg of sodium thiosulfate per liter, and the pH was adjusted to 6, 7, 8, 8.5, 9, and 9.5 using 1 N HCl or NaOH. The pH-adjusted water was seeded with $10^5$ PFU of poliovirus 1 and mixed. Virus inoculums were added to 200 ml of dechlorinated tap water and mixed for several minutes to disrupt aggregates before seeding 100 liters of water. Water samples were then filtered through NanoCeram cartridge filters at an average flow rate of 5.3 (±0.4) liters per minute. Viruses retained by the filters were eluted using the optimal filter elution method, which used the 1-minute contact time for the first elution and the 15-min contact time for the second elution. Filter eluents were concentrated as described above, and the sample concentrates from the first and second elutions were analyzed separately using the plaque assay.

**Comparison of virus recoveries at different flow rates.** One hundred liters of dechlorinated tap water (pH adjusted to 7.0) was seeded with $10^5$ PFU of poliovirus and mixed. Water samples were then passed through the NanoCeram cartridge filters at variable flow rates of 5.5, 12, and 20 liters per minute. After filtration, viruses retained by the filters were eluted and concentrated using the optimal method as described above. Sample concentrates from the first and second elutions were analyzed separately using the plaque assay.

**Evaluation of different enteric virus recoveries from tap water.** One hundred liters of dechlorinated tap water (pH adjusted to 7.0) was seeded with $10^5$ PFU of either poliovirus, coxsackievirus B5, or echovirus 7 and mixed. Virus-seeded water was then filtered through the NanoCeram cartridge filters at a flow rate of 5.5 liters per minute. After filtration, viruses retained by the filters were eluted and concentrated using the optimal method. Sample concentrates from the first and second elutions were analyzed separately using the plaque assay.

**Comparison of poliovirus and Norwalk virus recoveries by 1MDS and NanoCeram filters from the Ohio River and tap water samples.** Ohio River water samples were collected in 40-liter carboys and kept at 4°C. One day prior to initiating virus-seeding experiments, the river water samples were brought into the laboratory and kept at room temperature. Water pH was measured using a standard pH meter (Corning model 440), and turbidity was measured using a turbidimeter (HACH 2100N; Loveland, CO). Dechlorinated tap water (pH adjusted to 7.0) or river water samples of either 10 or 100 liters were seeded with 94 to 318 PFU of poliovirus and $1.15 \times 10^6$ most probable number of reverse transcription (RT)-PCR units of Norwalk virus and mixed. Virus-seeded water samples were then filtered through either 1MDS or NanoCeram cartridge filters, at an average flow rate of 5.2 (±0.3) liters per minute. After filtration, viruses retained by the filters were eluted and concentrated as described above. To determine the recovery efficiencies of poliovirus, 10-ml sample concentrates from the first and second elutions were analyzed separately using the plaque assay.

TABLE 1. Retention of poliovirus 1 by NanoCeram filters

| No. of replication | Seed titer (PFU)[a] | Titer in the filtrate (PFU)[b] | Virus retention (%)[c] |
|---|---|---|---|
| 1 | $5.1 \times 10^5$ | $5.0 \times 10^4$ | 90 |
| 2 | $9.4 \times 10^5$ | $1.1 \times 10^5$ | 88 |
| 3 | $5.4 \times 10^5$ | $8.0 \times 10^4$ | 85 |
| 4 | $7.7 \times 10^5$ | $6.0 \times 10^4$ | 92 |
| 5 | $7.6 \times 10^5$ | $1.8 \times 10^5$ | 76 |
| 6 | $3.7 \times 10^5$ | $9.0 \times 10^4$ | 76 |
| 7 | $2.4 \times 10^5$ | $7.0 \times 10^4$ | 71 |
| 8 | $5.0 \times 10^5$ | $1.0 \times 10^5$ | 80 |
| 9 | $4.0 \times 10^5$ | <DL | 100 |
| 10 | $6.0 \times 10^5$ | $1.0 \times 10^5$ | 83 |

[a] Total virus PFU in 100 liters of deionized water.
[b] DL, detection limit.
[c] The mean is 84% (standard deviation of ±9). The range is 71 to 100%.

The additional 30 ml sample concentrates were processed for PCR inhibitor removal following the procedure described by Fout el al. (13). After inhibitor removal, portions of the sample concentrates were stored at either −20°C until assayed for poliovirus by RT-PCR or 4°C until assayed for Norwalk virus by RT-PCR.

**Plaque assay.** Concentrated water sample volumes of 1 to 10 ml were inoculated onto BGM cells in 25- or 75-$cm^2$ flasks, using inoculum volumes of 0.5 ml per 25-$cm^2$ flask or 2 ml per 75-$cm^2$ flask, and then overlaid according to Dahling and Wright (7). Flasks were incubated at 37°C and observed for 1 week for plaque formation. The plaque assay was used to determine the titers of the poliovirus, coxsackievirus B5, and echovirus 7 stocks and to evaluate the recovery efficiencies of the NanoCeram and 1MDS filters.

**RT-PCR for poliovirus and Norwalk virus.** Primers for poliovirus (MRD 13 and MRD 14) and Norwalk virus (MON 432 and MON 434) have been described elsewhere (13, 36). All samples were assayed using a serial dilution series. Each RT reaction was performed by adding 5 μl of a serial dilution to a mix containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.67 mM of each deoxyribonucleotide triphosphate, and 50 pmol of downstream primers (MRD 13 for poliovirus and MON 434 for Norwalk virus) in a final reaction volume of 30 μl. Viral RNA was released by heating at 99°C for 5 min. After quenching the viral RNA suspension on ice, 30 U of recombinant RNasin (Promega catalog no. N251B) and 50 U of murine leukemia virus reverse transcriptase (Applied Biosystems catalog no. N8080018) were added, and cDNA was prepared by incubating at 43°C for 60 min. Reverse transcriptase was then inactivated by a step at 94°C for 5 min. PCR was performed by adding 20 μl of a mix containing 10 mM Tris (pH 8.3), 50 mM KCl, 4.5 mM $MgCl_2$, 50 pmol of upstream primer, and 5 U of AmpliTaq Gold polymerase (Applied Biosystems catalog no. N8080245). Viral cDNA was amplified with 40 cycles of 94°C for 30 s, 50°C for 1 min and 30 s, and 72°C for 1 min. Following the 40 cycles, samples were incubated at 72°C for 7 min and then kept at 4°C or at −20°C for long-term storage. To determine the RT-PCR inhibition by samples, two RT-PCRs were performed for each dilution, for each sample. One 50-μl reaction mixture (total reaction volume) contained 5 μl of sample, while the other 50-μl reaction mixture contained 5 μl of sample plus 100 RT-PCR units of Norwalk virus or 80 to 100 PFU of poliovirus. The quality assurance guidelines described by the U.S. Environmental Protection Agency (45) were followed during PCR analysis of the samples.

**Agarose gel electrophoresis.** Four microliters of the RT-PCR product was added to 1 μl of 0.04% bromophenol blue, 0.04% xylene cyanol, and 50% glycerol and run on 3% agarose gel in 40 mM Tris, 5 mM sodium acetate, 1 mM EDTA (pH 8.0) (TAE buffer) at 100 to 110 V for 60 to 90 min. Gels were stained with TAE buffer containing 1 μg of ethidium bromide per microliter. Results were recorded by the Kodak gel electrophoresis documentation system (Rochester, NY).

**Norwalk virus recovery.** Norwalk virus recovery was calculated from the total RT-PCR units recovered based upon the endpoint of serial dilutions from each sample. The titer of the stock Norwalk virus was determined using a serial dilution series using 10 replicates of each dilution. A titer of $1.15 \times 10^6$ most probable number of RT-PCR units was calculated using the MPNV calculator (http://www.epa.gov/microbes). Recovery is expressed as the mean percent recovery and standard deviation of the results for three trials.

**Statistical analysis.** Virus recovery data were analyzed using a generalized linear model for overdispersed Poisson data, represented by a negative binomial probability mass function (20). This describes Poisson counts with an added variance component to account for higher variance of PFU counts than would be expected from a completely random dispersion of virus particles. Analysis was performed using PROC GENMOD of the SAS statistical software (SAS Institute Inc., Cary, NC). Fixed factors considered were pH, flow rate, and elution method in evaluating recovery efficiency.

TABLE 2. Poliovirus recovery by NanoCeram filters using six different elution procedures[a]

| Method | Elution | Virus recovery (%) | | | |
|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 | Mean (±SD) |
| 1 | First elution[b] | 50 | 38 | 35 | 41 (±8) |
| | Second elution for 1 min | 8 | 36 | 18 | 21 (±14) |
| | Combined % recovery | 58 | 74 | 53 | 62 (±11) |
| 2 | First elution | 53 | 62 | 31 | 48 (±16) |
| | Second elution for 15 min | 9 | 32 | 44 | 28 (±17) |
| | Combined % recovery | 62 | 93 | 74 | 77 (±16) |
| 3 | First elution | 57 | 60 | 32 | 50 (±15) |
| | Second elution for 30 min | 11 | 33 | 25 | 23 (±11) |
| | Combined % recovery | 68 | 93 | 58 | 73 (±18) |
| 4 | First elution | 42 | 57 | 47 | 48 (±8) |
| | Second elution for 60 min | 10 | 31 | 24 | 21 (±11) |
| | Combined % recovery | 52 | 88 | 71 | 70 (±18) |
| 5 | First elution | 35 | 41 | 41 | 39 (±3) |
| | Second elution for 120 min | 10 | 23 | 28 | 21 (±9) |
| | Combined % recovery | 45 | 64 | 70 | 60 (±13) |
| 6 | First elution | 33 | 27 | 37 | 32 (±5) |
| | Second elution for overnight | 9 | 13 | 10 | 11 (±2) |
| | Combined % recovery | 42 | 40 | 47 | 43 (±4) |

[a] Poliovirus was seeded in 100 liters of deionized water and filtered through NanoCeram filters.
[b] First elution was done after 1 minute of contact of beef extract-glycine and the filter.

## RESULTS

The NanoCeram filter was first evaluated for its ability to bind viruses and then release them during elution. These experiments were conducted using seeded deionized water. The mean retention of seeded polioviruses by the cartridge filters was 84% (Table 1). Six filter elution procedures were evaluated, using various contact times between the filter and eluent at room temperature. In every case, an initial elution was done using a contact time of 1 min, followed by a second elution using a contact time of 1, 15, 30, 60, or 120 min or overnight at room temperature. Table 2 shows the average poliovirus recoveries by different elution methods. There was a significant difference ($P < 0.05$) between method 6 and either method 2 or 3. That is, recoveries for methods 2 and 3 were significantly higher than those for method 6. However, none of the differences among other elution procedures were significant. The highest virus recovery (77%) was obtained by immersing the filter in beef extract for 15 min during the second elution (method 2). A third elution of the filters (1 min contact of beef extract-glycine and filter) did not yield substantial additional recovery of virus (<3%; $n = 3$) (data not shown). Using 100 liters of seeded dechlorinated tap water, method 2 was then compared with a single elution from filter using a contact time of 16 min. The recovery of viruses using method 2 was 58%, while the recovery for a single elution was 28% ($n = 3$). Thus, two elutions from filters resulted in a significantly higher recovery of viruses from seeded dechlorinated tap water than

TABLE 3. Recovery of poliovirus 1, coxsackievirus B5, and echovirus 7 from dechlorinated tap water using NanoCeram filters

| Virus[a] | Elution[b] | Mean recovery (±SD) (%) |
|---|---|---|
| Poliovirus 1 | First elution | 35 (±9) |
| | Second elution | 19 (±5) |
| | Combined percent recovery | 54 (±8) |
| Coxsackievirus B5 | First elution | 18 (±12) |
| | Second elution | 9 (±6) |
| | Combined percent recovery | 27 (±17) |
| Echovirus 7 | First elution | 14 (±6) |
| | Second elution | 18 (±9) |
| | Combined percent recovery | 32 (±8) |

[a] For poliovirus 1, $n = 6$; for coxsackievirus B5 and echovirus 7, $n = 3$.
[b] Contact time was 1 min for the first elution and 15 min for the second elution.

that with a single elution ($P < 0.0001$). Because method 2 had the highest virus recovery, and would facilitate rapid processing of a sample, this method was used for filter elutions in subsequent experiments.

Viruses differ in their electronegativity and thus in their binding efficiency to electropositive filters. The recovery efficiencies of different enteric viruses using the NanoCeram filter were therefore evaluated. The recovery efficiencies of poliovirus, coxsackievirus B5, and echovirus 7 from seeded tap water were 54%, 27%, and 32%, respectively (Table 3).

The effect of water pH and of the flow rate on virus recovery by the NanoCeram filter was then evaluated. Figure 1 shows that there was no significant difference ($P = 0.36$) in poliovirus recovery by NanoCeram filters from tap water at pH values of 6 to 9.5. The ICR virus method (12) called for sampling using a maximum flow rate of 11.4 liters/min (3 gallons/min) with 1MDS filters. Because there are no empirical data to support the flow rate used for the ICR, we tested whether flow rate would affect virus recovery. There was no significant difference ($P = 0.08$) in poliovirus recovery at water flow rates of 5.5 liters/min, 12 liters/min, and 20 liters/min (data not shown).

Virus recoveries by NanoCeram filters were compared with those by 1MDS filters using tap water and the Ohio River water samples. The mean pH of the river water samples was 7.7 (range 7.6 to 7.8; $n = 6$). The mean turbidity of the river water samples during 100-liter and 10-liter volume experiments was 41 nephelometric turbidity units (NTU; range, 26 to 90 NTU; $n = 6$) and 1.2 NTU (range, 0.17 to 2.75 NTU; $n = 6$), respectively. The turbidity of water varied because river water samples were collected on different days. Except for in one instance, the turbidity of the river water samples was below 75 NTU, and thus, no prefilter was used during this study. The mean recoveries of polioviruses from 10- or 100-liter samples of tap and river water are presented in Table 4. Virus recovery from tap and river water samples on the NanoCeram filters was similar to or higher than that on the 1MDS filters. There was no significant difference in virus recovery from tap and river water between the filter types using a 100-liter sample volume ($P = 0.24$). However, for the 10-liter samples, there was a significant difference in recovery between the two filter types (for river water, $P = 0.015$; for tap water, $P < 0.001$). Virus recoveries by NanoCeram filters with 10-liter sample volumes were higher than those by the 1MDS filters. Virus recoveries by the 1MDS filters from tap water were higher than those reported in some of the previous studies (3, 39), and the recoveries from river water were lower than those described in a previous study (3).

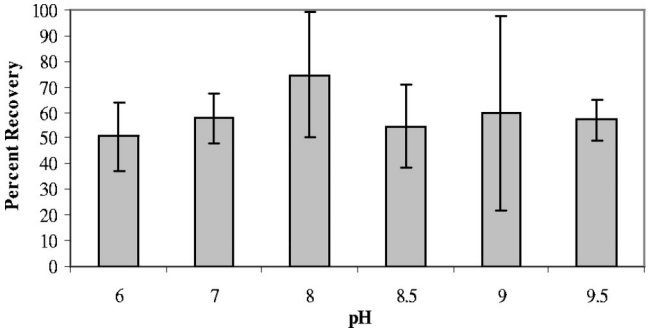


FIG. 1. Poliovirus recovery at different pHs of dechlorinated tap water. Error bars represent the standard deviation. For pHs 6, 7, and 8.5, $n = 3$; for pHs 8, 9, and 9.5, $n = 6$.

TABLE 4. Comparison of poliovirus recovery by NanoCeram and 1MDS filters from seeded dechlorinated tap water and river water

| Filter type | Elution[a] | Mean virus recovery (%) for indicated samples[b] | | | |
|---|---|---|---|---|---|
| | | 100 liters[c] | | 10 liters[d] | |
| | | Tap water | River water | Tap water | River water |
| NanoCeram | First elution | 23 ± 14 | 21 ± 18 | 182 ± 42 | 30 ± 16 |
| | Second elution | 28 ± 13 | 16 ± 15 | 95 ± 64 | 25 ± 15 |
| | Combined % recovery | 51 ± 26 | 38 ± 35 | 277 ± 22 | 65 ± 22 |
| 1MDS | First elution | 39 ± 4 | 25 ± 20 | 31 ± 14 | 13 ± 4 |
| | Second elution | 28 ± 6 | 11 ± 4 | 13 ± 13 | 17 ± 9 |
| | Combined % recovery | 67 ± 6 | 36 ± 21 | 44 ± 9 | 30 ± 11 |

[a] Contact time was 1 min for the first elution and 15 min for the second elution.
[b] For all experiments, $n = 3$.
[c] There was no significant difference between filters using 100-liter samples ($P = 0.24$).
[d] For the 10-liter samples, there was a significant difference in recovery between the two filter types (for river water, $P = 0.015$; for tap water, $P < 0.001$).

To determine whether there is any difference in RT-PCR inhibition between NanoCeram and 1MDS filter concentrates, 10-fold dilutions of sample concentrates were seeded with 80 to 100 PFU of poliovirus or 100 RT-PCR units of Norwalk virus. Neither tap nor river water concentrates from 1MDS and NanoCeram filters were inhibitory to RT-PCRs after the inhibitor removal process (data not shown). The abilities of RT-PCR to detect enteroviruses in NanoCeram and 1MDS filter concentrates were compared. Poliovirus was detected more frequently in NanoCeram filter concentrates compared to 1MDS filter concentrates (Table 5).

Because Norwalk virus cannot be grown in established cell lines, the recovery efficiency of Norwalk virus by the NanoCeram and 1MDS filters was determined using RT-PCR. Based upon three trials and endpoint dilutions, norovirus recovery on NanoCeram filters was at least 3.6% ± 0.6% from tap water and 12.2% ± 16.3% from river water. Recoveries from the 1MDS filters were lower than those from the NanoCeram

TABLE 5. RT-PCR detection of poliovirus from seeded tap and river water samples

| Filter type | Elution[a] | Poliovirus detection in 100-liter seeded samples of[b]: | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tap water | | | River water | | |
| | | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 |
| NanoCeram | First | + | − | + | − | + | + |
| | Second | + | + | + | + | + | + |
| 1MDS | First | − | + | + | − | − | − |
| | Second | − | + | + | + | + | − |

[a] The first elution was done for 1 min, and the second elution was done for 15 min.

[b] + indicates RT-PCR-positive detection, and − indicates RT-PCR-negative detection.

filters: 1.2% ± 1.4% from tap water and 0.4% ± 1.8% from river water.

## DISCUSSION

A major challenge for detection of enteric viruses in drinking water is the efficient concentration of viruses from large volumes of water. This study describes a new electropositive filter (NanoCeram filter) for concentrating enteric viruses from large volumes of water. The adsorption of poliovirus by NanoCeram filters was comparable to that found for other electropositive filters (19, 23, 38, 39, 42, 43). Sobsey and Glass (39) reported that 93% of seeded poliovirus was adsorbed by Zeta Plus 50S disc filters at pH 7.5. Similarly, Sobsey et al. (43) reported that 83% of the input poliovirus was adsorbed by 1MDS filters. In a third study, Sobsey and Jones (42) examined poliovirus adsorption by three different electropositive filters (Zeta Plus 50S, Zeta Plus 60S, and Seitz filters) and found that both Zeta Plus filters adsorbed 61 to 99% of input viruses at pH range 7 to 7.5, while the Seitz filters (composed of asbestos-cellulose) adsorbed 100% of seeded virus at pH range 3.5 to 9. Finally, Sobsey and Glass (38) reported 62 and 79% of poliovirus absorption by 1MDS disc filters from raw and finished water, respectively. These experiments were conducted using small volumes of water (0.5 to 3.8 liters), small filters (47-mm-diameter disc filters), a flow rate of 1.5 to 10 ml/min per square centimeter of filter surface area, and a high titer ($10^3$ to $10^4$ PFU/ml) of input virus (39, 42, 43). In contrast, the present study was conducted using large volumes of water (100 liters), cartridge filters (12.7 cm by 6.35 cm; 316 $cm^2$ surface area), and a flow rate of 5.6 liters per minute (18 ml/min per square centimeter of filter surface area), as normally employed for large-scale virus concentration in field sampling. Also, the input virus concentration in the present study was much lower (~2 to 9 PFU/ml) than those in the previous studies. Under the experimental conditions tested, NanoCeram filters adsorbed a significant amount of input poliovirus (mean 84%; range, 71 to 100%) with a low level of variability (±9%; $n$ = 10) suggesting that these filters are capable of adsorbing a low concentration of viruses from large volumes of water. In a previous study, Sobsey and Glass (39) reported that 90% of seeded poliovirus was adsorbed by 1MDS cartridge filters (flow rate of 2 ml/min per square centimeter, and input virus titer of ~$10^2$ PFU/ml) using a large sample volume (1,000 liters). Although, NanoCeram cartridge filters used in this study are half the size of the 1MDS cartridge filters, virus adsorption by NanoCeram filters was comparable to those by 1MDS filters (39).

Different procedures to elute adsorbed viruses from filters are described in the literature. These procedures include use of different eluting solutions and use of single or multiple elutions of the filters. The ICR (12) virus protocol requires two successive elutions of filters with beef extract, each requiring 1 min of contact of beef extract with the filter. In this study, six filter elution procedures were evaluated. The highest virus recovery (77%) was obtained by two separate elutions, which included immersing the filter in beef extract for 1 min during the first elution and 15 min during the second elution. Two elutions resulted in a >2-fold recovery of virus compared to that resulting from a single elution. Dahling (6) reported that the highest virus recoveries from 1MDS cartridge filters (95%) were obtained with two separate beef extract elutions, one being an overnight filter immersion in beef extract. However, the Dahling study (6) did not report virus recovery by each elution. In addition, the author reported that a single overnight elution of the filter gave the lowest recovery (38%) of viruses. In the present study, recovery efficiencies of both first and second elutions were separately determined, and the data indicated that overnight elution during the second elution decreased virus recovery from the filters. The overnight elution in this study had the lowest virus recovery (43%), which is plausible due to the inactivation of viruses by the extended contact of viruses with the eluting solution at pH 9.0 or from the incubation at room temperature. Although poliovirus was found to be stable for up to 2 hours at pH 9.5 (41), the overnight stability of this virus in an alkaline pH is unknown. In a previous study, Dahling and Wright (8) reported that highest virus recoveries were obtained with three successive elutions from filters with 1.6 liters of 3% beef extract. However, in this study, a third elution of NanoCeram filters did not yield substantial additional virus recovery (<3%; $n$ = 3) (data not shown), suggesting that two elutions are sufficient for eluting the adsorbed viruses from these filters. The filter elution method outlined in this study would facilitate rapid detection of viruses in environmental samples compared to the methods suggested for overnight elution of filters.

To reduce the impact of test water variability, initial experiments for the present study were conducted using deionized water; however, because water chemistry can significantly affect virus recovery from water (38), the efficiency of virus recovery from tap water using NanoCeram filters was also determined. The recovery efficiency of poliovirus from seeded tap water (54%) (Table 3) was similar to those from some of the previously reported studies (10, 38, 39, 43). Sobsey and Glass (38) reported 36% and 57% of seeded virus recovery from small volumes (1.3 liters) of raw and finished water, respectively, using 1MDS electropositive disc filters. In another study, virus recovery by 1MDS disc filters from 1.3 liters of water was 52% (43), while Sobsey and Glass (39) reported an average of 30% virus recovery from 1,000 liters of tap water using 1MDS cartridge filters. Poliovirus recovery by NanoCeram filters was lower than that reported in the Dahling study (6) in which the author reported 95% seeded virus recovery from 120 liters of river water using 1MDS cartridge filters. However, the higher recovery observed in the Dahling study

(6) could be due to the use of a sensitive roller bottle virus enumeration method.

With regard to the impact of pH, there was no significant difference in poliovirus recoveries by NanoCeram filters at tap water pHs of 6 to 9.5 (Fig. 1). Previous studies reported that poliovirus adsorptions by electropositive filters are similar between pH 3.5 and 7.5, but above pH 7.5, adsorption efficiency greatly decreases (39). Contrary to the previous studies (39, 42), the present study suggests that NanoCeram filters can efficiently recover viruses between pH 6.0 and 9.5. The pH of drinking water and natural water is unlikely to be greater than 9.5, so the virus recovery efficiency by NanoCeram filters at a pH above 9.5 was not evaluated.

With regard to flow rate, the standardized virus method in the ICR (12) requires sampling using a maximum flow rate of 11.4 liters/min (3 gallons/min) with 1MDS filters. Because there are no empirical data to support the flow rate used for the ICR, the effect of flow rate on virus recovery by NanoCeram filters was investigated. There was no significant difference in poliovirus recovery at water flow rates between 5.5 liters/min and 20 liters/min. The flow rates examined were equivalent to 17 ml/min, 38 ml/min, and 63 ml/min per square centimeter of the filter surface area. A previous study using electropositive filters indicated no difference in virus recovery between flow rates of 3.8 and 26.4 ml/min/cm$^2$ (43).

The recovery efficiencies of other enteric viruses by NanoCeram filters were lower than those of poliovirus. The recovery efficiencies of coxsackievirus B5 and echovirus 7 from seeded tap water were 27% and 32%, respectively (Table 3). Other studies have also reported low recoveries of coxsackievirus and echovirus from environmental samples. The mean recoveries of coxsackievirus B3 and A9 from tap water using cellulose ester filters were reported to be 0.7% and 27%, respectively (41). Chang et al. (4) reported 44% coxsackievirus B3 recovery from 19 liters of activated sludge effluent using positively charged (Zeta Plus 30S) filters. The higher recovery of coxsackievirus in the Chang et al. study (4) compared to that in this study could be due to the difference in virus type or water matrix. Recovery of echovirus 7 from tap water was reported to be 2.5 to 10% using four different filters (41). The low recoveries for coxsackievirus B5 and echovirus 7 compared to the recoveries of poliovirus could be due to the differences in virus surface charges.

The recovery of poliovirus by NanoCeram cartridge filters from tap and river water was compared with those from the 1MDS cartridge filters. In 10-liter water samples, poliovirus recovery was much higher using NanoCeram filters than that using 1MDS filters for both tap and river water samples. Virus recovery from 10-liter tap water samples was higher than 100% (Table 4), but the reason for this is not clear. Virus recoveries from 100-liter samples of river water were similar for both types of filters ($P = 0.24$). These data suggest that NanoCeram filters can be used as an alternative to 1MDS filters for concentrating environmental water samples. However, in highly turbid water (90 NTU), NanoCeram filters tend to clog more rapidly than do 1MDS filters (experimental observation), thus the use of a prefilter may be necessary for these waters. Virus attached to suspended solids may be retained by the prefilter. If a prefilter is used, the eluent should be passed through both the prefilter and the NanoCeram filter (12).

Due to the absence of an infectivity assay using established cell lines, the presence of noroviruses in environmental waters is often measured with molecular methods. One of the problems of virus detection by molecular methods is the presence of humic and other organic substances in concentrated water samples that can inhibit enzymes used in RT-PCR. Thus, the coconcentration of PCR inhibitory substances by NanoCeram filters was investigated. The data from this study suggest that neither the NanoCeram nor 1MDS filter concentrates were inhibitory to RT-PCRs for Norwalk virus and poliovirus. The inhibitor removal process used in this study (13) effectively removed the PCR inhibitory substances from both NanoCeram and 1MDS filter concentrates. The recovery of Norwalk virus from tap and river water using NanoCeram filters was higher than the recovery using 1MDS filters. Norwalk virus recovery efficiencies by both filters were lower than the recovery efficiency using glass wool filters (27). However, the recovery values were generated using conventional RT-PCR and endpoint dilutions. Due to the nature of endpoint dilutions, recoveries are likely to be higher than those reported herein; therefore, future studies are needed to examine recovery using quantitative RT-PCR.

The ability to detect poliovirus in NanoCeram and 1MDS filter concentrates was compared using RT-PCR. Poliovirus was detected more frequently in NanoCeram filter concentrates than in 1MDS filter concentrates (Table 5), especially in the case of river water. This is not surprising as the recovery efficiencies of NanoCeram filters for river water were higher than those of 1MDS filters.

The presence of enteric viruses in drinking water is a potential public health risk. Electropositive filters are frequently used for concentration of enteric viruses from water. However, the most commonly used electropositive filters, the 1MDS cartridge filters, are expensive, making them prohibitive for routine monitoring of virus. In contrast, NanoCeram cartridge filters are comparatively economical, and the present study suggests that NanoCeram filters can be used as an inexpensive alternative to 1MDS filters for routine viral monitoring of water.

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