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Influence of Water Quality on Enteric Virus Concentration by Microporous Filter Methods

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Four enteric viruses, poliovirus type 1, echovirus type 1, reovirus type 3, and simian adenovirus SV-11, were concentrated from seeded 1.3-liter volumes of raw, finished, and granular activated carbon-treated waters by adsorption to 47-mm-diameter (17 cm^2), electropositive (Virosorb 1MDS) filters at pH 7.5 or electronegative (Filterite) filters at pH 3.5 with and without 5 mM added $MgCl_2$, followed by elution with 0.3% beef extract in 50 mM glycine at pH 9.5. Removal of particulates from raw and finished waters by 0.2-μm prefiltration before virus addition and pH adjustment had little effect on virus concentration efficiencies. Soluble organic compounds reduced virus adsorption efficiencies from both raw and finished waters compared with granular activated carbon-treated water, but the extent of interference varied with virus type and adsorption conditions. For electropositive 1MDS filters, organic interference was similar with all virus types. For Filterite filters, organic interference was evident with poliovirus and echovirus, but could be overcome by adding $MgCl_2$. Reovirus and SV-11 were not adversely affected by organics during adsorption to Filterite filters. Elution of reovirus and adenovirus was inefficient compared with that of poliovirus and echovirus. None of the three adsorption schemes (1MDS at pH 7.5 and Filterite with and without 5 mM $MgCl_2$ at pH 3.5) could be judged superior for all viruses and water types tested.

Evaluating the role of drinking water in the transmission of enteric viruses to humans requires the use of reliable methods to detect small quantities of these viruses in large volumes of raw and finished waters. Such virus concentration methods are especially needed to investigate outbreaks of waterborne viral disease, to evaluate wastewater reclamation systems, and to study the removal and destruction of viruses in water and wastewater treatment processes under field conditions. The most widely used and accepted concentration methods use electronegative or electropositive microporous adsorbent filters to accumulate viruses from large volumes of water (1, 6, 8). The adsorbed viruses are then eluted with small volumes of eluent fluid and further concentrated by one of several alternative methods for subsequent virus assays in cell cultures (2, 7).

The effectiveness of microporous filter and other methods for virus concentration from water and wastewater is limited by interference from dissolved and suspended matter in the sample water. Suspended matter clogs adsorbent filters, thereby limiting the volumes that can be processed and possibly interfering with virus elution (17, 18). Dissolved and colloidal organic matter interferes with virus adsorption to filters, apparently by competing for adsorption sites (3, 14, 16). Despite the recognition that both suspended matter and dissolved and colloidal organic matter probably interfere with virus concentration by microporous filter methods, the effects of such interference have not been systematically studied under controlled conditions. The purpose of this study was to quantitatively determine the effects of naturally occurring suspended solids and soluble organic matter in the same surface water source on enteric virus concentration efficiency with electropositive and electronegative adsorbent filters. Because all of the waters were derived from the same source, the composition and concentrations of their dissolved microsolutes were similar.

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MATERIALS AND METHODS

Viruses. Four enteric viruses were used: poliovirus type 1, strain LSc; echovirus type 1, strain V239 (a gift from Charles P. Gerba, Baylor College of Medicine); reovirus type 3, Dearing strain; and simian adenovirus SV-11. Viruses were cultivated and assayed as previously described (10).

Filters. The electropositive adsorbent filters medium was Virosorb 1MDS (charge-modified fiber glass, 0.2-μm nominal porosity; AMF Corp., Cuno Division, Meriden, Conn.). To correspond to the medium configuration in Virosorb 1MDS cartridges, two layers of 1MDS medium were used in each filter housing.

The representative electronegative filter medium was Filterite (fiber glass-epoxy, 0.25-μm nominal porosity; Filterite, Inc., Timonium, Md.) One layer of Filterite medium was used in each filter housing because Filterite cartridges contain only one layer of 0.25-μm porosity medium.

Adsorbent filter disks, 47 mm in diameter, were loaded into polypropylene housings, along with a 10-μm-porosity polypropylene prefilter (Gelman Instrument Co., Ann Arbor, Mich.). Housings were assembled with media and sterilized by autoclaving.

Waters. Samples of raw and finished water were collected from the Orange County water treatment plant in Carrboro, N.C., and dechlorinated with 50 mg of sodium thiosulfate per liter. The source of raw water is University Lake, and the finished water is produced from this source by a conventional treatment scheme consisting of alum coagulation-flocculation, sedimentation, mixed-media (anthracite-sand) filtration, and chlorination. The characteristics of both raw and finished water have been described previously (10).

Sampling times were arranged such that the same batch of water was sampled before and after treatment. Half of each water type was prefiltered through a 0.2-μm-porosity poly-

TABLE 1. Physical and chemical quality of test waters[a]

Test water	Turbidity (NTU)	Color (cobalt units)	Total organic carbon (mg/liter)	Hardness (mg of $CaCO_3$/liter)	Conductivity (μmho/cm)	Alkalinity (mg of $CaCO_3$/liter)	pH
Raw	13 (9.4)	32 (4.8)	4.3 (1.6)	28 (7.4)	118 (8.0)	27 (5.6)	6.8 (0.3)
Filtered, raw	0.02	No data	4.8 (1.9)	No data	No data	No data	6.8 (0.3)
Finished	0.24 (0.22)	0	2.7 (1.0)	27 (7.8)	182 (34)	35 (6.3)	7.4 (0.3)
Filtered, finished	0.02	0	2.4 (0.6)	No data	No data	No data	7.4 (0.3)
GAC treated, finished	0.02	0	0.4 (0.5)	No data	No data	No data	7.6 (0.3)

[a] Data are from analyses of water samples on days of collection for virus experiments: 37 sample collections, approximately weekly, over an 11-month period. Total organic carbon data are based on only 12 samples collected monthly. Initial values are means; values in parentheses are standard deviations. NTU, Nephelometric turbidity units; GAC, granular activated carbon.

carbonate filter (Nuclepore Corp., Pleasanton, Calif.) to remove suspended solids. Polycarbonate prefilters were chosen because their low affinity for soluble organic compounds and their inert chemical composition would not affect the soluble organic levels in water samples.

In addition, a sample of tap (finished) water was pretreated by granular activated carbon (Filtrasorb 400; Calgon, Inc., Pittsburgh, Pa.) filtration (adsorption) followed by filtration through a 0.2-μm-porosity polycarbonate filter to further reduce levels of soluble organics and to remove suspended solids and carbon fines.

General characteristics of the water types used in this study are summarized in Table 1.

Virus concentration experiments. A 1,300-ml volume of each water type was seeded with test virus to about 5×10^3 PFU/ml. With 1 N HCl or NaOH, each seeded sample was adjusted to the desired adsorption pH: pH 3.5 for Filterite filters or pH 7.5 for Virosorb 1MDS filters. For trials with Filterite filters, replicate volumes of seeded water types were processed for virus concentration with and without 5 mM added $MgCl_2$.

After sampling for determination of input virus levels, each seeded sample was filtered through a sterile adsorbent filter at 50 ml/min, using regulated air as a source of positive pressure. Filtrates were collected and sampled for assay of viruses not adsorbed by the filter. Adsorbed viruses were eluted from filters with two successive 7.5-ml volumes of 0.3% beef extract in 50 mM glycine, pH 9.5, as previously described (9, 13). Eluates were neutralized with dilute HCl and sampled for assay of recovered viruses. All samples were initially diluted in an equal volume of double-strength phosphate-buffered saline with 0.4% beef extract and antibiotics (500 μg of kanamycin, 100 μg of gentamycin, 200 μg of streptomycin, and 200 U of penicillin per ml) and stored at −30°C until assayed. Subsequent dilutions were made in single-strength phosphate-buffered saline containing 0.2% beef extract and antibiotics.

RESULTS

Effects of suspended solids. Enteric virus adsorption and recovery efficiencies from untreated and prefiltered raw and finished waters are summarized in Tables 2 and 3. In general, removal of suspended solids from raw (Table 2) and finished (Table 3) water by 0.2-μm prefiltration had little or no effect on subsequent virus recovery efficiency. Comparison by paired *t*-tests showed no significant difference in virus recovery efficiencies between untreated and prefiltered waters for any virus type in either raw or finished water ($P > 0.1$ in all cases).

With Filterite filters at pH 3.5 without added $MgCl_2$, significant differences ($P < 0.05$) were observed between virus adsorption efficiencies in untreated and prefiltered raw water for both poliovirus and echovirus (Table 2). For both viruses, removal of suspended solids by prefiltration resulted in a significant decrease in adsorption efficiency. Thus, for the two enteroviruses, suspended solids at the levels in raw water enhanced virus adsorption to or retention by

TABLE 2. Influence of suspended solids on enteric virus concentration from raw water

Virus type	Filter type, adsorption conditions	Adsorption efficiency (%)[a]		Recovery efficiency (%)[a]	
		Untreated	Prefiltered	Untreated	Prefiltered
Poliovirus	Virosorb 1MDS, pH 7.5	62 (±29)	57 (±46)	36 (±13)	48 (±42)
	Filterite, pH 3.5	55 (±32)	29 (±23)[b]	31 (±36)	22 (±17)
	Filterite, pH 3.5 + $MgCl_2$	70 (±89)	82 (±66)	47 (±65)	75 (±61)
Echovirus	Virosorb 1MDS, pH 7.5	32 (±28)	29 (±21)	14 (±16)	38 (±58)
	Filterite, pH 3.5	53 (±39)	23 (±35)	23 (±17)	12 (±7.4)
	Filterite, pH 3.5 + $MgCl_2$	70 (±94)	76 (±67)	21 (±51)	57 (±100)
Reovirus	Virosorb 1MDS, pH 7.5	74 (±19)	58 (±40)	13 (±14)	15 (±14)
	Filterite, pH 3.5	97 (±6.1)	99 (±0.4)	1.2 (±2.6)	1.2 (±2.9)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.4)	99 (±1.4)	2.1 (±8.2)	2.1 (±8.8)
Adenovirus	Virosorb 1MDS, pH 7.5	54 (±36)	54 (±31)	22 (±18)	17 (±27)
	Filterite, pH 3.5	77 (±53)	100 (±0.1)	18 (±52)	38 (±20)
	Filterite, pH 3.5 + $MgCl_2$	98 (±2.7)	99 (±1.0)	25 (±38)	32 (±60)

[a] Mean percentages from three to five replicate experiments; 95% confidence intervals inside parentheses. Adsorption percentages were obtained from the difference in virus concentrations between the initial water before filtration and the filtrate. Recovery percentages were obtained by dividing the total viruses in the eluate by the total viruses in the initial water.

[b] Boxed values are significantly different at the 5% level in a paired *t* test.

TABLE 3. Influence of suspended solids on enteric virus concentration from finished water

Virus type	Filter type, adsorption conditions	Adsorption efficiency (%)[a]		Recovery efficiency (%)[a]	
		Untreated	Prefiltered	Untreated	Prefiltered
Poliovirus	Virosorb 1MDS, pH 7.5	79 (±32)	68 (±41)	57 (±17)	44 (±18)
	Filterite, pH 3.5	48 (±26)	47 (±17)	33 (±16)	41 (±18)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.9)	99 (±1.6)	76 (±135)	79 (±19)
Echovirus	Virosorb 1MDS, pH 7.5	71 (±39)	61 (±58)	53 (±30)	56 (±48)
	Filterite, pH 3.5	61 (±36)	37 (±42)	22 (±22)	31 (±28)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.2)	99 (±1.2)	36 (±84)	62 (±4.9)
Reovirus	Virosorb 1MDS, pH 7.5	76 (±34)	80 (±12)	19 (±11)	22 (±23)
	Filterite, pH 3.5	99 (±0.4)	99 (±0.4)	0.6 (±0.7)	0.6 (±1.4)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.4)	99 (±1.4)	1.8 (±6.9)	2.1 (±8.6)
Adenovirus	Virosorb 1MDS, pH 7.5	71 (±20)	69 (±20)	17 (±18)	13 (±22)
	Filterite, pH 3.5	99 (±4.1)	97 (±3.7)	34 (±41)	24 (±33)
	Filterite, pH 3.5 + $MgCl_2$	100 (±1.0)	99 (±1.6)	28 (±33)	32 (±35)

[a] Mean percentages from three to five replicate experiments; 95% confidence intervals inside parentheses. Adsorption percentages were obtained from the difference in virus concentrations between the initial water before filtration and the filtrate. Recovery percentages were obtained by dividing the total viruses in the eluate by the total viruses in the initial water.

electronegative adsorbent filters when used at acidic pH levels with no added $MgCl_2$. Differences in virus adsorption efficiencies between prefiltered and unprefiltered finished waters were not observed, perhaps because suspended solids levels in finished water are already so low without prefiltration. The mechanism of adsorption enhancement by suspended solids in raw water is not clear from these experiments, but it may involve reduced flow rates due to filter clogging or additional virus adsorption sites provided by the solids that accumulate in the filter matrix or both. It is interesting to note that suspended solids-mediated enhancement of enterovirus adsorption from raw water was observed only with Filterite filters without $MgCl_2$ amendment. Therefore, as might be expected, both pH and ionic conditions seem to influence enterovirus interactions with suspended solids in water.

Notably, suspended solids had no discernible effect on the adsorption of the reovirus and SV-11 to both types of filter. This observation suggests that there may be differences among virus types with respect to interactions with suspended solids in water.

Effects of soluble organic compounds. To examine the influence of soluble organic compounds on microporous filter methods for virus concentration, viruses were concentrated from solids-free (i.e., 0.2-μm-prefiltered) waters containing different levels of naturally occurring organic compounds: raw water (high levels of soluble organics), finished water (moderate levels of soluble organics), and granular activated carbon-treated tap water (low levels of soluble organics). Adsorption and recovery efficiencies for the three adsorbent/adsorption conditions and the four virus types are summarized in Table 4.

TABLE 4. Influence of naturally occurring soluble organics on enteric virus concentration from prefiltered waters

Virus type	Filter type, adsorption conditions	Adsorption efficiency (%)[a]			Recovery efficiency (%)[a]		
		Raw	Finished	GAC[b]	Raw	Finished	GAC
Poliovirus	Virosorb 1MDS, pH 7.5	57 (±46)	68 (±41)	92 (±21)	48 (±42)	44 (±18)	36 (±30)
	Filterite, pH 3.5	29 (±23)	47 (±17)	94 (±8.8)[c]	22 (±17)	41 (±18)	67 (±39)
	Filterite, pH 3.5 + $MgCl_2$	82 (±66)	99 (±1.6)	100 (±0)	75 (±61)	79 (±19)	75 (±46)
Echovirus	Virosorb 1MDS, pH 7.5	29 (±21)	61 (±58)	100 (±0.1)	38 (±58)	56 (±48)	83 (±24)
	Filterite, pH 3.5	23 (±35)	37 (±42)	90 (±11)	12 (±7.4)	31 (±28)	65 (±36)
	Filterite, pH 3.5 + $MgCl_2$	76 (±67)	99 (±1.2)	100 (±0.4)	57 (±110)	62 (±4.9)	79 (±21)
Reovirus	Virosorb 1MDS, pH 7.5	58 (±40)	80 (±12)	96 (±11)	15 (±14)	22 (±23)	27 (±25)
	Filterite, pH 3.5	99 (±0.4)	99 (±0.4)	99 (±0.4)	1.2 (±2.9)	0.6 (±1.4)	0.2 (±0.2)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.4)	99 (±1.4)	99 (±1.4)	2.1 (±8.8)	2.1 (±8.6)	0.9 (±2.1)
Adenovirus	Virosorb 1MDS, pH 7.5	54 (±31)	69 (±20)	82 (±33)	17 (±27)	13 (±22)	16 (±30)
	Filterite, pH 3.5	100 (±0.1)	97 (±3.7)	85 (±39)	20 (±38)	24 (±33)	40 (±78)
	Filterite, pH 3.5 + $MgCl_2$	99 (±1.0)	99 (±1.6)	73 (±40)	32 (±60)	32 (±35)	6.4 (±9.2)

[a] Mean percentages from three to five replicate experiments; 95% confidence intervals inside parentheses. Adsorption percentages were obtained from the difference in virus concentrations between the initial water before filtration and the filtrate. Recovery percentages were obtained by dividing the total viruses in the eluate by the total viruses in the initial water.

[b] GAC, Granular activated carbon-treated, finished water.

[c] Boxed values are significantly different at the 5% level by analysis of variance.

Interference by soluble organics with adsorption to electropositive Virosorb 1MDS filters at pH 7.5 was observed for all virus types tested. Although differences were not always statistically significant, virus adsorption efficiencies were generally greater from waters with lower concentrations of soluble organics.

As suggested by previous work (9), soluble organics caused generally greater reductions in adsorption efficiencies of poliovirus and echovirus on electronegative Filterite filters used with no added $MgCl_2$ than on electropositive Virosorb 1MDS filters, possibly because of pH-associated changes in the physicochemical characteristics of the interfering compounds or the viruses or both. However, as previously reported (11, 13), addition of 5 mM $MgCl_2$ to the water before filtration largely overcame adsorption interference for Filterite filters by soluble organics (Table 4).

Recovery efficiencies for poliovirus and echovirus followed a general pattern similar to adsorption efficiencies. Virosorb 1MDS filters showed less susceptibility to interference by soluble organics than did Filterite filters without $MgCl_2$ amendment, whereas Filterite used with added $MgCl_2$, showed little or no evidence of organic interference.

In contrast to the enteroviruses, reovirus adsorbed very efficiently (99%) to Filterite filters at pH 3.5, regardless of the soluble organic or ionic content of the water. However, adsorbed reoviruses were poorly recovered from Filterite filters (2.1%) compared with Virosorb 1MDS filters (15 to 27%) and were poorly recovered in general compared with the enteroviruses. Because reovirus has been shown (10) to be stable in the range of pH 3.5 (the adsorption pH used for Filterite filters) to pH 9.5 (the elution pH used in this study), virus inactivation in solution probably cannot account for the high adsorption efficiencies and low recovery efficiences observed. Poor elution and recovery of reovirus adsorbed to Filterite filters from tap water have been observed previously (13). A possible explanation for the observed results is stronger adsorption of reovirus than poliovirus and echovirus to Filterite filters, thus making subsequent reovirus elution more difficult. Another possible explanation is reovirus inactivation on the surface of the adsorbent filter.

Simian adenovirus SV-11 showed an unusual dependence on soluble organic compounds during adsorption to Filterite filters. Adsorption efficiency of SV-11 decreased with decreasing organic load. Thus, it would appear that, unlike either the two enteroviruses or reovirus, naturally occurring organic compounds in water enhance SV-11 adsorption to electronegative filter surfaces. SV-11 recoveries were generally somewhat greater with Filterite filters than with Virosorb 1MDS filters. However, like reovirus, SV-11 was recovered somewhat less efficiently from either adsorbent than were the enteroviruses, and recovery efficiencies were not significantly different among the three types of water.

DISCUSSION

The results of this study indicate that differences exist among enteric virus types with respect to their interactions with adsorbent filters in the presence of suspended solids and soluble organics. Each of the three representative enteric virus groups, enteroviruses, reoviruses, and adenoviruses, exhibited a unique pattern of adsorption and recovery efficiencies, especially with electronegative Filterite filters.

The two enteroviruses, poliovirus and echovirus, behaved much alike, despite the fact that the V239 strain of echovirus has been shown to adsorb poorly to soils (5). Both viruses showed improved adsorption to Filterite filters in the presence of the high suspended solids concentrations in untreated raw water. Both were similarly influenced by adsorption interference from soluble organics, and they showed similar improvements in adsorption and recovery efficiencies with Filterite filters when $MgCl_2$ was added to the water. Overall, few differences were observed between the concentration efficiencies of these two members of the enterovirus group.

The enhancement by suspended solids of enterovirus adsorption to Filterite filters observed in this study is consistent with results of previous work (10), in which echovirus type 7 was recovered more efficiently from solids-clogged electronegative adsorbent filters than from unclogged filters. However, in the present work no significant differences in enterovirus recoveries were observed between untreated and prefiltered waters, suggesting that variations in elution efficiencies of different virus strains or types from solids on filters may also be an important means by which suspended solids influence virus concentration from water.

Reovirus was apparently unaffected by suspended solids or soluble organics during adsorption to electronegative filters. However, it responded much like the enteroviruses during adsorption to electropositive 1MDS filters, with improved adsorption at lower levels of soluble organics. Reovirus was poorly recovered from either filter type compared with the enteroviruses, with especially poor recovery from electronegative filters as observed in a previous study with tap water (13).

Adenovirus SV-11 was unaffected by suspended solids during adsorption to either type of filter. SV-11 responded similarly to the enteroviruses during adsorption to electropositive 1MDS filters, with improved adsorption from waters with lower soluble organics levels. However, it responded dissimilarly during adsorption to electronegative Filterite filters in that adsorption efficiencies were lower from waters with lower levels of soluble organics. Recovery efficiencies for SV-11 were greater with Filterite than with Virosorb 1MDS, greater overall than for reovirus, and somewhat lower than for the enteroviruses.

The observed differences in adsorption and recovery efficiencies among viruses in this study are generally consistent with differences noted for three of these same viruses (poliovirus, echovirus, and reovirus) in previous studies with tap water (13). In the previous tap water studies virus recoveries with both filter types were highest for poliovirus, somewhat lower for echovirus, and lowest for reovirus.

It is likely that the considerable differences observed among adsorption efficiencies for the different virus types with Filterite filters is at least partly a result of virion surface charge differences at pH 3.5. Differences in isoelectric points are likely to have a considerable effect on the polarity and surface charge density of the various virus types at pH 3.5. The important role of electrostatic interactions in virus adsorption to charged surfaces has been described previously (15).

In the case of electropositive Virosorb 1MDS filters, all four viruses exhibited similar adsorption efficiencies. This observation is not unexpected, because at pH 7.5 all four virus types have net electronegative surface charges. Therefore, adsorption to the electropositive filter surface at this pH is probably less influenced by specific electrostatic characteristics of the virions than is their adsorption to electronegative filter surfaces at an acidic pH of 3.5.

The observed differences in recovery efficiencies for the four viruses probably reflect differences in the ability of the eluent to desorb attached virions from filter surfaces. The results of this study suggest that there may be some differences in the mechanisms of interaction with adsorbent filter

surfaces among the virus types tested. Specifically, reovirus and SV-11 seem to adhere somewhat more tenaciously to filter surfaces than do the two enteroviruses, poliovirus and echovirus. Several mechanisms for virion adsorption to surfaces have been proposed, and there is evidence for both electrostatic and hydrophobic interactions (4, 12). It is likely that the specific physicochemical configuration of the virus capsid as well as the pH and solute composition of the water determine the most influential attachment mechanism(s).

From the foregoing discussion, it follows that no single combination of adsorbent/adsorption conditions can be expected to give optimum recovery efficiencies for all virus types from all water types. This conclusion is supported by the results of the present study.

Virus recovery efficiencies are certainly influenced by water quality. However, significant interference with virus concentration methods seems to be limited to conditions of high soluble organics and suspended solids loadings characteristic of untreated, raw surface water.

Considerable variation can be seen among recovery efficiencies for the three virus groups examined. Enteroviruses were recovered more efficiently than the adenovirus, which in turn was recovered more efficiently than the reovirus. These observations point up the risks involved in extrapolating data from investigations with any single enteric virus group to other virus groups or water types. Further studies are needed to specifically determine which soluble organic compounds interfere with virus adsorption to filters and which suspended solids mediate improved virus adsorption to filters. The need for improved eluents for reoviruses, adenoviruses, and possibly other enteric viruses is also indicated. Although both reovirus and adenovirus models were recovered less efficiently than the two enterovirus models from all of the waters studied, the results suggest that it should be possible to detect these former viruses in contaminated raw and finished waters with current microporous filter methods, albeit with lower efficiency. However, further work is needed to increase reovirus and adenovirus recovery efficiencies using microporous filter methods, perhaps by the use of improved elution procedures.

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