Effects of Humic and Fulvic Acids on Poliovirus Concentration from Water by Microporous Filtration

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Because naturally occurring organic matter is thought to interfere with virus adsorption to microporous filters, humic and fulvic acids isolated from a highly colored, soft surface water were used as model organics in studies on poliovirus adsorption to and recovery from electropositive Virosorb 1MDS and electronegative Filterite filters. Solutions of activated carbon-treated tap water containing 3, 10, and 30-mg/liter concentrations of humic or fulvic acid were seeded with known amounts of poliovirus and processed with Virosorb 1MDS filters at pH 7.5 or Filterite filters at pH 3.5 (with and without 5 mM $MgCl_2$). Organic acids caused appreciable reductions in virus adsorption and recovery efficiencies with both types of filter. Fulvic acid caused greater reductions in poliovirus recovery with Virosorb 1MDS filters than with Filterite filters. Fulvic acid interference with poliovirus recovery by Filterite filters was overcome by the presence of 5 mM $MgCl_2$. Although humic acid reduced poliovirus recoveries by both types of filter, its greatest effect was on virus elution and recovery from Filterite filters. Single-particle analyses demonstrated $MgCl_2$ enhancement of poliovirus association with both organic acids at pH 3.5. The mechanisms by which each organic acid reduced virus adsorption and recovery appeared to be different for each type of filter.

As part of the effort to determine the public health significance of human enteric virus contamination of drinking water and other waters, methods to concentrate these viruses from water by adsorption to and subsequent elution from electronegative and electropositive filters have been developed and become widely used (1, 10, 16). Because these methods rely on virus adsorption to filter media surfaces, soluble organic matter in water may interfere with their effectiveness by competing with viruses for adsorption sites, by complexing with virus particles and thereby altering their ability to adsorb, and by accumulating so extensively on filter surfaces that they interfere with virus elution (5–7, 12, 13, 21). Such interference has been tentatively attributed to humic and fulvic acids because of their ubiquity in waste, natural, and finished waters. The ability of soil-derived humic acids to be concentrated from water, using filter adsorption-elution methods employing electronegative filters (6), and of soil-derived fulvic acid to form reversible complexes with MS2 bacteriophage (3) has been previously reported. More recent studies have shown that naturally occurring soluble organic matter in raw surface water and finished tap water reduces the adsorption of poliovirus type 1 and echovirus type 1 to both electronegative (Filterite) and electropositive (Virosorb 1MDS) filters, at least under some operating conditions (18).

In this study the effects of soluble organic matter on poliovirus concentration from water by filter adsorption-elution methods were further investigated in a model system consisting of granular activated carbon (GAC)-treated tap water amended with known amounts of humic and fulvic acids isolated from water.

MATERIALS AND METHODS

Virus. Poliovirus type 1, strain LSc, was used as a model enteric virus. Virus was grown and assayed in buffalo green

monkey kidney cells, as previously described (20). Poliovirus was harvested from infected cells at advanced stages of cytopathology, freeze-thawed, fluorocarbon extracted, and dialyzed against phosphate-buffered saline (PBS). Stock preparations were confirmed as monodispersed by single-particle analysis (SPA) (8).

Filter media. (i) Filterite. Fiber glass-epoxy filters with nominal porosities of 0.25 and 0.45 μm were used as 25-mm-diameter disks in a double layer to simulate the 0.25-μ cartridge configuration used for large volumes of water.

(ii) Virosorb 1MDS. The more electropositive filter consisted of 0.20-μm-porosity, surface-modified fiber glass-cellulose media (Cuno Div., AMF Corp.). Twenty-five-millimeter-diameter disks were used in a double layer to stimulate the cartridge configuration.

Filter preparation. Filters were loaded into 25-mm-diameter polypropylene holders (Millipore Corp.) and sterilized by autoclaving.

Suspending water. Because the primary interest of this study was the effects of soluble and colloidal organics on concentration of viruses from tap water, organics-free tap water was used as the suspending medium. Chapel Hill tap water was GAC treated, filtered through a 0.20-μm polycarbonate filter (Nucleopore), and dechlorinated by sodium thiosulfate addition to a 50-mg/liter final concentration. The GAC-treated water used in this study had total organic carbon (TOC) concentrations consistently <0.5 mg/liter. The physical and chemical characteristics of Chapel Hill tap water and GAC-treated water have been previously described (18, 19).

Isolation of humic and fulvic acids. One hundred twenty gallons (ca. 454 liters) of water from Bay Tree Lake, a highly colored, soft surface water located 40 miles (ca. 64 km) southeast of Fayetteville, N.C., was filtered through a 1.0-μm Fulflo polypropylene filter (Commercial Filter Div., Carborundum Co.), collected, and acidified to pH 2.0 in

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TABLE 1. Effect of humic acid on adsorption and recovery of poliovirus with Virosorb 1MDS and Filterite filters[a]

Humic acid concn (mg of TOC/liter)	Virosorb 1MDS at pH 7.5		Filterite at pH 3.5			Filterite at pH 3.5 with 5 mM MgCl		
	Adsorbed (%)[b]	Recovered (3.5ml) (%)[c]	Adsorbed (%)	Recovered (%)		Adsorbed (%)	Recovered (%)	
				3.5 ml[c]	7.0 ml[d]		3.5 ml	7.0 ml
0	91 (±21)	24 (±17)	99 (±0.1)	67 (±25)	78 (±49)	99 (±0.1)	38 (±66)	60 (±15)
3	94 (±8.2)	18 (±12)	85 (±11)	7.8 (±0.6)	8.8 (±1.4)	97 (±6.4)	37 (±50)	38 (±53)
10	65 (±20)	11 (±8.1)	71 (±31)	1.1 (±1.3)	1.1 (±1.4)	90 (±14)	11 (±11)	11 (±11)
30	35 (±6.9)	8.0 (±0.6)	76 (±47)	0.7 (±0.9)	0.7 (±0.9)	83 (±9.0)	4.6 (±2.5)	4.8 (±2.2)

[a] Values are means from three or four replicate experiments; values in parentheses are 95% confidence limits.
[b] Percentage of initial (input) viruses adsorbed. Value was obtained by subtracting the percentage of unadsorbed (filtrate) virus from 100%.
[c] Percentage of initial (input) viruses recovered in the first 3.5-ml eluate volume.
[d] Percentage of initial (input) viruses recovered in the combined first and second eluates.

50-gallon (ca. 189-liter) polypropylene drums. After 48 h of settling, the humic acid precipitate was collected by removing the supernatant water containing fulvic acid and further concentrated by centrifugation. The precipitate was resuspended and reprecipitated in deionized, distilled water and partially resuspended for continuous-flow dialysis against deionized water for 5 days. The molecular weight cutoff of dialysis material was 1,000 (Spectrapor; Fisher Scientific Co.). The dialysate was lyophilized and its ash content was determined to be 13% by muffle furnace combustion. The concentrated, purified humic acid remained 75 to 80% particulate at pH 7.5, and attempts to further solubilize the material by adjusting the pH to extremely alkaline levels followed by pH readjustment to experimental levels resulted in a unfilterable suspension. Therefore, humic acid stocks were used without further attempts to solubilize at high pH levels.

The fulvic acid fraction was concentrated by adsorption to XAD-8 resin (Rohm and Haas), a macroporous methylmethacrylate copolymer, at pH 2. Fulvic acid was eluted with 0.1 N NaOH, adjusted to pH 7.0, and concentrated by ultrafiltration, using a 500-molecular-weight cutoff membrane (UM 05; Amicon Corp.). The fulvic material was lyophilized and the ash content was determined to be 6%. Fulvic acid was completely soluble at all pH values studied.

Analysis of humic and fulvic acids. Quantitation of humic and fulvic acids was done by TOC analysis, using a Dohrman Envirotech DC-54 Ultralow Level Total Organic Carbon Analyzer. Concentrations of organic acids are expressed as milligrams of TOC per liter.

Virus concentration procedures. Humic or fulvic acid was added to 300-ml volumes of GAC-treated tap water to a final concentration of 3, 10, or 30 mg/liter. These concentrations were considered representative of TOC levels in finished, raw surface, and highly colored raw surface waters, respectively. Control samples of GAC-treated water contained no added organic matter. Samples were then seeded with poliovirus to a concentration of 10^3 PFU/ml. The samples were adjusted to pH 7.5 for Virosorb 1MDS and to pH 3.5 for Filterite, using 1.0 N HCl or NaOH. Magnesium chloride was also added to a series of Filterite water samples to a final concentration of 5 mM (18). A small portion of each sample was taken and neutralized in 0.2 M PBS containing 100 μg of gentamicin and 500 μg of kanamycin per ml (sample diluent) for assay of input virus titers. Water samples were placed in 600-ml-capacity polycarbonate pressure vessels and filtered through 25-mm-diameter filters at a flow rate of 50 ml/min. This corresponds to a hydraulic loading rate of 61.1 ml/cm² of filter surface area and a hydraulic flux of 10.2 ml/cm² per min. Filtrates were collected and sampled for subsequent virus assay. The filtrate titer was divided by the input titer to

determine the proportion of viruses not adsorbed to the filter. The difference between unadsorbed viruses and 1.0 was taken as the proportion of input viruses adsorbed to the filter. Poliovirus was eluted from filters with 0.3% beef extract in 50 mM glycine at pH 9.5. Two successive 3.5-ml volumes were used for all filter elutions, except for Virosorb 1MDS filtrations with humic acid, where only a single 3.5-ml volume of eluent could be passed through the filters. Samples of eluates were assayed to determine the amount of input virus recovered.

SPA. The SPA test (8) was used to differentiate between single virus particles and those associated with either other viruses or other sample components on the basis of altered sedimentation rates during ultracentrifugation. Samples of control (GAC-treated) water, water containing organic acids, PBS, and sterile filtered tap water were seeded with 5 × 10^2 to 1 × 10^4 PFU of poliovirus per ml at pH levels and ionic conditions corresponding to those used in filtration studies. Mixtures were stirred 30 min before ultracentrifugation at 27,000 rpm for 76 min in an SW27 rotor (Beckman Instruments, Inc.). Unassociated virions remained in the supernatant fraction whereas others sedimented more rapidly towards the bottom of the tube. Percentages of single virions were determined by the ratios of supernatant/initial virus titers. Observed losses in virus titer could not be attributed to virion-virion aggregation, as initial virus concentrations were too low and the duration of experiments was too short for this to occur.

Statistical analyses. Statistical computations were done with the Statistical Analysis System (15). Differences were considered significant when $P < 0.05$ for the appropriate test.

RESULTS

Organic acid effects on cell culture viability and poliovirus infectivity. Preliminary experiments revealed that humic and fulvic acid concentrations of up to 30 mg/liter were not cytotoxic to Buffalo green monkey kidney cell cultures used for poliovirus plaque titrations. On the basis of comparisons with poliovirus titers in PBS controls, no consistent losses in poliovirus titers could be attributed to either organic acid at any pH level tested in sample volumes ranging from 30 to 600 ml (data not shown).

Poliovirus adsorption and recovery efficiencies in the presence of humic acid. Increasing humic acid concentrations decreased poliovirus adsorption and recovery efficiencies for all three filter schemes (Table 1). It should be noted that Virosorb 1MDS filtration experiments with humic acid utilized only one 3.5-ml volume of eluent, whereas Filterite experiments used two successive 3.5-ml volumes of eluent. The virus recovery efficiencies obtained with the first 3.5 ml

of eluent for both Filterite conditions are shown in Table 1 for comparison with Virosorb 1MDS efficiencies. Also shown in Table 1 are the Filterite recovery efficiencies with the total 7 ml of eluent. Statistical comparisons by paired t-tests of adsorption and recovery efficiencies between humic acid samples for each filtration condition are shown in Table 2.

High humic acid concentrations significantly reduced adsorption and recovery efficiencies of both Virosorb 1MDS and Filterite filters, but the reductions were not always statistically significant at the 5% level. Reductions in virus recovery efficiency were greatest for Filterite filters used without $MgCl_2$. For Virosorb 1MDS, adsorption with the parameter most affected by humic acids, but recovery efficiency was also somewhat reduced at 10 and 30 mg of humic acid per liter. Although Filterite used with 5 mM $MgCl_2$ gave the greatest adsorption efficiencies in the presence of humic acid, recovery efficiencies were significantly reduced in the presence of 10 and 30 mg of humic acid per liter. At the highest humic acid concentration tested (30 mg/liter), Virosorb 1MDS gave the highest recovery efficiency despite an adsorption efficiency of only 35%.

Poliovirus adsorption and recovery efficiencies in the presence of fulvic acid. In experiments with fulvic acid, two successive 3.5-ml volumes of eluent were used for all filters. Adsorption and recovery efficiencies for the three filtration schemes in the presence of fulvic acid are given in Table 3. Results of statistical analyses of these data by paired t-tests are given in Table 4. Adsorption and recovery efficiencies of Virosorb 1MDS and Filterite filters used without $MgCl_2$ were significantly reduced at increased concentrations of fulvic acid, with Virosorb 1MDS filters being the most seriously affected (Tables 3 and 4). Filterite used with $MgCl_2$ achieved the highest adsorption and recovery efficiencies for the range of fulvic acid concentrations tested (Table 3) and, on the basis of paired t-tests, was not significantly affected by fulvic acid.

Comparison of humic and fulvic acid effects on poliovirus adsorption and recovery. Two-sample t-tests were used to compare organic acid effects on each filter and filtration condition. Poliovirus adsorption efficiencies of Virosorb 1MDS filters were reduced to a significantly greater extent by fulvic acid than by humic acid, but recovery efficiencies were similarly reduced by both organic acids. For Filterite used without $MgCl_2$, adsorption efficiencies were also reduced to a greater extent by fulvic acid than by humic acid, but the difference was statistically significant ($P < 0.05$) only at 3 mg of organic acid per liter. In contrast, recovery efficiencies for Filterite used without $MgCl_2$ appear to be reduced to a greater extent by humic acid than by fulvic acid, but these differences were not statistically significant at the 5% level. For Filterite used with $MgCl_2$, adsorption efficiencies were similarly affected by both organic acids, but recovery efficiencies were reduced to a significantly greater extent by humic acid than by fulvic acid.

It is interesting to note that in fulvic acid samples poliovirus recovery efficiencies from Filterite filters were only slightly higher for the two successive 3.5-ml volumes of eluent than for the first 3.5-ml volume of eluent (Table 3). In contrast, poliovirus recovery efficiencies from Virosorb 1MDS were considerably greater for the two successive eluent volumes than for only the first eluent volume. This finding suggests that in the presence of fulvic acid poliovirus is more easily eluted from electronegative Filterite filters than from electropositive Virosorb 1MDS filters.

SPA. Results of SPA are given in Table 5. The percentage of single particles reflects the amount of interaction between

initially monodispersed poliovirus and various components in the samples. No interaction is assumed to have occurred if the percentage of single virus particles was 100%. PBS, GAC-treated water, tap water, humic acid, and fulvic acid, all at pH 7.5, had high percentages of single or unassociated virions. Percentages of single virus particles were somewhat lower in pH 3.5 tap water with and without $MgCl_2$ and in pH 3.5 humic and fulvic acid samples without $MgCl_2$. However, the greatest decreases in single virus particles were in pH 3.5 humic and fulvic acid samples containing 5 mM $MgCl_2$. The addition of 5 mM $MgCl_2$ significantly increased poliovirus sedimentation rates, thus suggesting the formation of large complexes involving magnesium ions, organic acids, and poliovirions.

DISCUSSION

Naturally occurring aquatic organics have been suspected of interfering with the concentration of viruses from water

TABLE 2. Statistical analyses of the effects of humic acids on poliovirus adsorption and recovery efficiencies[a]

Filter and adsorption conditions	Sample comparison	% Adsorbed	% Recovered in eluent vol of:	
			3.5 ml	7 ml
Virosorb 1MDS, pH 7.5	Control vs 3 mg/liter	0.588	0.439	ND[b]
	Control vs 10 mg/liter	0.126	0.140	ND
	Control vs 30 mg/liter	0.003	0.061	ND
	3 mg/liter vs 10 mg/liter	0.026	0.076	ND
	3 mg/liter vs 30 mg/liter	0.001	0.091	ND
	10 mg/liter vs 30 mg/liter	0.032	0.280	ND
Filterite, pH 3.5	Control vs 3 mg/liter	0.031	0.010	0.028
	Control vs 10 mg/liter	0.057	0.008	0.021
	Control vs 30 mg/liter	0.163	0.007	0.021
	3 mg/liter vs 10 mg/liter	0.137	0.004	0.003
	3 mg/liter vs 30 mg/liter	0.397	0.002	0.004
	10 mg/liter vs 30 mg/liter	0.503	0.314	0.268
Filterite, pH 3.5, with 5 mM $MgCl_2$	Control vs 3 mg/liter	0.165	0.974	0.208
	Control vs 10 mg/liter	0.086	0.213	0.013
	Control vs 30 mg/liter	0.014	0.159	0.003
	3 mg/liter vs 10 mg/liter	0.096	0.200	0.202
	3 mg/liter vs 30 mg/liter	0.053	0.107	0.111
	10 mg/liter vs 30 mg/liter	0.216	0.161	0.168

[a] Sample means were considered significantly different if $P < 0.05$ by paired t-test analysis; significant P values are underlined.

[b] ND, No data for this parameter because no statistical test could be performed.

TABLE 3. Effect of fulvic acid on adsorption and recovery of poliovirus with Virosorb 1MDS and Filterite filters[a]

Fulvic acid concn (mg of TOC/liter)	Virosorb 1MDS at pH 7.5			Filterite at pH 3.5			Filterite at pH 3.5 with 5 mM MgCl$_2$		
	Adsorbed (%)[b]	Recovered (%)		Adsorbed (%)	Recovered (%)		Adsorbed (%)	Recovered (%)	
		3.5 ml[c]	7.0 ml[d]		3.5 ml	7.0 ml		3.5 ml	7.0 ml
0	99 (±0.8)	10 (±0.8)	32 (±8.6)	100 (±0)	83 (±29)	92 (±29)	100 (±0)	47 (±54)	53 (±55)
3	59 (±24)	9.2 (±7.5)	23 (±2.5)	56 (±34)	33 (±46)	39 (±50)	99 (±1.4)	54 (±36)	63 (±48)
10	19 (±15)	6.4 (±5.0)	12 (±10)	59 (±25)	20 (±33)	26 (±39)	92 (±11)	82 (±30)	89 (±29)
30	7.4 (±67)	3.4 (±3.7)	6.9 (±3.3)	54 (±13)	21 (±43)	25 (±52)	87 (±30)	50 (±5.7)	58 (±22)

[a] Values are means from three or four replicate experiments; values in parentheses are 95% confidence limits.
[b] Percentage of initial (input) viruses adsorbed. Value was obtained by subtracting the percentage of unadsorbed (filtrate) virus from 100%.
[c] Percentage of initial (input) viruses recovered in the first 3.5-ml eluate volume.
[d] Percentage of initial (input) viruses recovered in the combined first and second eluates.

by microporous filter methods. In this study humic and fulvic acids, isolated from a surface water, were found to reduce the ability of three filtration procedures to adsorb and recover poliovirus. Humic and fulvic acids reduced poliovirus adsorption and recovery efficiencies of both the electropositive and the electronegative filters tested. However,

TABLE 4. Statistical analyses of the effects of fulvic acids on poliovirus adsorption and recovery efficiencies[a]

Filter and adsorption conditions	Sample comparison	% Adsorbed	% Recovered in eluent vol of:	
			3.5 ml	7 ml
Virosorb 1MDS, pH 7.5	Control vs 3 mg/liter	0.009	0.538	0.035
	Control vs 10 mg/liter	0.002	0.060	0.008
	Control vs 30 mg/liter	0.028	0.010	0.008
	3 mg/liter vs 10 mg/liter	0.011	0.175	0.026
	3 mg/liter vs 30 mg/liter	0.127	0.028	0.001
	10 mg/liter vs 30 mg/liter	0.585	0.052	0.109
Filterite, pH 3.5	Control vs 3 mg/liter	0.031	0.101	0.102
	Control vs 10 mg/liter	0.019	0.049	0.052
	Control vs 30 mg/liter	0.005	0.064	0.071
	3 mg/liter vs 10 mg/liter	0.787	0.071	0.056
	3 mg/liter vs 30 mg/liter	0.855	0.044	0.080
	10 mg/liter vs 30 mg/liter	0.656	0.980	0.945
Filterite, pH 3.5, with 5 mM MgCl$_2$	Control vs 3 mg/liter	0.076	0.420	0.120
	Control vs 10 mg/liter	0.097	0.103	0.092
	Control vs 30 mg/liter	0.177	0.866	0.786
	3 mg/liter vs 10 mg/liter	0.106	0.135	0.165
	3 mg/liter vs 30 mg/liter	0.186	0.730	0.738
	10 mg/liter vs 30 mg/liter	0.323	0.044	0.121

[a] Sample means were considered significantly different if $P < 0.05$ by paired t-test analysis; significant P values are underlined.

the extent to which humic and fulvic acids reduced poliovirus adsorption and recovery efficiency was different for each filter and filtration condition. These results suggest differences in the ways humic and fulvic acids interact with poliovirus and with the adsorbent filters tested.

For Virosorb 1MDS filters, lower recoveries in the presence of both humic and fulvic acids at pH 7.5 were partly due to reduced virus adsorption (Tables 1 and 3). Presumably, reduced adsorption was due to the interaction of the negatively charged organic acids with the positively charged filter surfaces, which reduced the number of adsorptive sites on the filter available to viruses.

In contrast, the ability of Filterite filters to adsorb poliovirus was not greatly reduced by organic acids at pH 3.5. This finding is consistent with that of Farrah et al. (6), who found that pretreatment of Filterite filters with 10-mg/liter humic acid solutions did not decrease subsequent adsorption of poliovirus in tap water. Because humic and fulvic acids are negatively charged, little sorptive interaction between organic acids and these filters would be expected.

Despite relatively efficient poliovirus adsorption to Filterite filters in the presence of both organic acids, recovery efficiency was greatly reduced, especially in humic acid samples. It should be remembered that humic acid is larger than fulvic acid, and in this study humic acid was only partially soluble whereas fulvic acid was completely soluble. Humic acid accumulated as an unfilterable, insoluble layer on Filterite filters whereas fulvic acid did not. It is possible

TABLE 5. SPA of poliovirus in test waters[a]

Test water	Single virus particles (%)	
	Mean[b]	95% Confidence interval
PBS	170	130–200
Sterile filtered tap water, pH 7.5	134	114–154
GAC-treated tap water, pH 7.5	106	59–153
Humic acid, 30 mg/liter, pH 7.5	125	99–151
Fulvic acid, 30 mg/liter, pH 7.5	161	129–193
GAC-treated tap water, pH 3.5	10	7–13
GAC-treated tap water with 5 mM MgCl$_2$, pH 3.5	37	39–45
Humic acid, 30 mg/liter, pH 3.5	29	16–41
Fulvic acid, 30 mg/liter, pH 3.5	27	13–40
Humic acid, 30 mg/liter, with 5 mM MgCl$_2$, pH 3.5	0.03	0–0.06
Fulvic acid, 30 mg/liter, with 5 mM MgCl$_2$, pH 3.5	0.03	0.01–0.05

[a] Humic and fulvic acid samples were prepared in GAC-treated tap water.
[b] Mean values were computed from plaque counts on six or more replicate cultures.

that observed reductions in poliovirus elution efficiency in the presence of humic acid were due to strong interactions of positively charged (at pH 3.5) polioviruses with the insoluble layer of negatively charged humic acid that accumulated on Filterite filter surfaces. Such interactions could result in efficient adsorption but inefficient elution. Fulvic acid, being soluble, was less likely to accumulate on negatively charged Filterite filters and, therefore, would not interfere with virus elution in the manner hypothesized for humic acid. Because only one eluent was used in this study, further studies on humic acid interference with virus elution from adsorbent filters should be done with other eluents in an attempt to improve virus elution efficiencies.

The results of SPA tend to support the notion of electrostatic interactions among viruses, organic acids, cations, and filter surfaces. It is likely that the organic acids can associate with the positively charged Virosorb 1MDS filters and thereby cause the observed reductions in poliovirus adsorption at pH 7.5. Because the organic acids and poliovirus are electronegative at pH 7.5, electrostatic interactions between them were not expected to occur. The SPA results support this assumption, as neither organic acid decreased the percentage of single virus particles at pH 7.5.

Decreasing the sample to pH 3.5, especially in the presence of $MgCl_2$, resulted in virus-organic acid interactions. For humic acid, this interaction was apparently of more importance than the organic acid-filter interactions that were responsible for decreases in Virosorb 1MDS adsorption efficiencies at pH 7.5. The accumulation of insoluble virus-humic acid complexes on Filterite filter surfaces may account for the efficient virus retention but poor virus elution that was observed. Although the SPA results also indicate the formation of poliovirus-fulvic acid complexes, especially in the presence of $MgCl_2$, the smaller size and greater solubility of fulvic acid may account for the more efficient virus elution from Filterite filters that was obtained in fulvic acid samples. When Virosorb 1MDS was used to filter organic acids at pH 7.5, organic acid association with filters was an important mechanism of interference with virus adsorption and recovery. However, interactions between virus and organic acid, especially humic acid, with or without $MgCl_2$ at pH 3.5, appeared to be most important in influencing virus adsorption and recovery by Filterite filters.

The organic acid concentrations of 3, 10, and 30 mg/liter used in this study were selected as representative concentrations for tap waters, surface waters, and highly colored surface waters, respectively. Although both humic and fulvic acids are present in natural, waste, and tap waters, fulvic acids usually predominate, especially in tap water (2, 4, 9, 11).

The observation that organic acids interfere with poliovirus adsorption and recovery by Virosorb 1MDS and Filterite filters is generally consistent with the results of a previous study concerning the effects of soluble organic matter on poliovirus adsorption and recovery from natural surface water, tap water, and GAC-treated tap water (18). In the previous study poliovirus adsorption to Virosorb 1MDS at pH 7.5 and to Filterite without $MgCl_2$ at pH 3.5 was reduced by soluble organics in natural and tap waters, whereas adsorption to Filterite with $MgCl_2$ was reduced to a lesser extent. Poliovirus recovery efficiency for Filterite without $MgCl_2$ was also appreciably reduced by increased levels of soluble organics, but virus recoveries for Virosorb 1MDS and Filterite with $MgCl_2$ were not appreciably affected. The mean TOC levels in the tap and natural waters of the previous study were 2.4 and 4.8 mg/liter respectively,

and therefore are perhaps best represented by the results for the 3-mg/liter organic acid samples of the present study.

The results of this study suggest a number of implications for the use of microporous filter methods to concentrate enteric viruses from water. Because poliovirus recoveries from GAC-treated water and 3-mg/liter organic acid samples were usually not significantly different, organic acid levels in most tap waters are not likely to seriously impair enteric virus recoveries by microporous filter methods. However, the observation that virus recoveries for Filterite without $MgCl_2$ were considerably lower from 3-mg/liter organic acid samples than from GAC-treated water supports the results of previous studies which demonstrate the benefits of multivalent cation addition when concentrating viruses with electronegative filters (5, 22).

The considerably lower poliovirus recovery from 10- and 30-mg/liter organic acid samples versus GAC-treated water suggests that organic acid levels in some natural and wastewaters can seriously reduce virus recoveries by microporous filter methods. Because the organic acid content of natural and wastewaters may vary over time, the degree of reduction in virus recovery efficiency from these waters cannot be accurately predicted. For this reason, it may be necessary to concurrently process seeded samples of the water being examined whenever field samples are processed in order to estimate virus recovery efficiency (16).

The results of this study also indicate the need for improved virus concentration methods that are less susceptible to interference from soluble organic matter in water. The development of such improved methods will require greater understanding of the mechanisms of virus adsorption to filter surfaces and of virus interactions with organic matter in water.

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