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Poliovirus Concentration from Tap Water with Electropositive Adsorbent Filters

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Simple, reliable, and efficient concentration of poliovirus from tap water was obtained with two types of electropositive filter media, one of which is available in the form of a pleated cartridge filter (Virozorb 1MDS). Virus adsorption from tap water between pH 3.5 and 7.5 was more efficient with electropositive filters than with Filterite filters. Elution of adsorbed viruses was more efficient with beef extract in glycine, pH 9.5, than with glycine-NaOH, pH 11.0. In paired comparative studies, electropositive filters, with adsorption at pH 7.5 and no added polyvalent cation salts, gave less variable virus concentration efficiencies than did Filterite filters with adsorption at pH 3.5 plus added $MgCl_2$. Recovery of poliovirus from 1,000-liter tap water volumes was approximately 30% efficient with both Virozorb 1MDS and Filterite pleated cartridge filters, but the former were much simpler to use. The virus adsorption behavior of these filters appears to be related to their surface charge properties, with more electropositive filters giving more efficient virus adsorption from tap water at higher pH levels.

The need for a simple, reliable, and efficient method to quantitatively concentrate enteric viruses from large volumes of water has been frequently expressed (12, 14, 15, 20, 26). Research toward this goal has produced methods based primarily on adsorption of viruses from water onto microporous filters and subsequent desorption or elution of adsorbed viruses with small volumes of elution media (5, 6, 9, 10, 15, 16, 26, 29-31). Adsorption-elution methods of this type appear in the 14th and 15th editions of *Standard Methods* (1, 2).

Most workers exploring adsorption-elution methods have used microporous adsorbent filters which carry a strong net negative electrostatic surface charge (18, 25). Because most virus types also carry a net negative surface charge at neutral pH (3, 19; R. R. Jacobs, Ph.D. thesis, University of North Carolina, Chapel Hill, 1980), it has been necessary to acidify virus-containing water so that virus adsorption can occur, presumably by reducing or eliminating electrostatic repulsive forces between virions and filter surfaces. Also, it has often been necessary to add exogenous multivalent cation salts to water in order to promote virus adsorption to filter surfaces (15, 23, 30).

The need for extensive conditioning of water before virus adsorption to strongly electronegative filters requires expensive equipment and contributes to the complexity of the concentration method. Moreover, previous work in this and other laboratories has revealed that virus adsorption to strongly electronegative filters can be impaired by soluble or colloidal materials in water (6-8, 22, 23, 31).

Many of the described difficulties with present adsorption-elution methods may be avoided by the use of microporous adsorbent filters which carry a less negative net charge in the pH range of most natural and tap waters, pH 5 to 9 (23, 25). Electrostatic repulsion between virions and filter surfaces would be minimized or eliminated, making preconditioning of the water unnecessary and greatly simplifying the method. Furthermore, less negatively charged filters may be less sensitive to adsorption-interfering components of water than are strongly electronegative filters (25). Previous work in this laboratory has provided information which supports the utility of less electronegative filter media as virus adsorbents (23, 25).

In the present study, two electropositive filter types were rigorously evaluated for their applicability to adsorption-elution virus concentration methods. Comparisons were made between poliovirus concentration efficiencies by these less electronegative filters and by a widely used strongly electronegative fiber glass-epoxy filter.

MATERIALS AND METHODS

Poliovirus. All experiments were performed with poliovirus type 1, strain LSc. Viruses were grown and assayed in Buffalo green monkey kidney cells, as previously described (24). Virus stock suspensions were subjected to Freon extraction (22, 24), after which

they were shown by electron microscopy to be monodispersed (M. D. Sobsey, W. A. Rutala, R. R. Jacobs, and J. S. Glass, unpublished data).

Filter media. (i) Filterite. Fiber glass-epoxy filters, nominal porosity 0.45 μm, were obtained in sheets and in 25.4-cm (10-inch)- by 7.6-cm (3-inch)-diameter pleated-sheet cartridges (Duofine) from Filterite, Inc., Timonium, Md. Filter disks of 47-mm diameter were cut from the sheet material.

(ii) AMF Cuno. Electropositive filters, manufactured by AMF Corp., Cuno Division, Meriden, Conn., were as follows: Zeta Plus 50S, 47-mm-diameter disks of surface-modified cellulose and filter aid mixtures, nominal porosity 0.75 μm; and Virozorb 1MDS, supplied as 25.4- by 7.6-cm-diameter pleated-sheet cartridges containing two identical layers of a thin-sheet medium consisting of surface-modified fiber glass and cellulose mixtures, nominal porosity 0.2 μm. For small-scale experiments, the 1MDS filter material was also tested as 47-mm-diameter disks with either one or two layers of thin-sheet medium.

Filter preparation and use. Filter disks of 47-mm diameter were loaded into polypropylene holders (Millipore Corp., Bedford, Mass.) and sterilized by autoclaving. Pleated-sheet cartridge filters were sterilized by autoclaving and loaded into sterilized holders. New filters, disk or cartridge, were used in each experiment.

Concentration experiments. (i) Small-volume experiments. Tap water (3.8-liter volumes) was dechlorinated with 50 mg of sodium thiosulfate per liter and adjusted to the required adsorption pH with small volumes of 1.0 or 0.1 N HCl or NaOH. Poliovirus was then added to a final concentration of approximately 10^4 plaque-forming units (PFU) per ml of tap water. After mixing, a small sample was immediately neutralized by dilution in an equal volume of sample diluent (0.2 M phosphate-buffered saline [PBS] containing 4% heat-inactivated fetal calf serum, 0.04% $MgCl_2 \cdot 6H_2O$, 400 U of penicillin per ml, and 400 μg of streptomycin sulfate per ml) for subsequent virus assay. Seeded tap water volumes were then placed in 3.8-liter-capacity stainless-steel pressure vessels (Amicon Corp., Lexington, Mass.) and forced through 47-mm-diameter filter disks at flow rates of 1.5 to 3.0 ml/min per cm^2 of filter surface area, using a regulated nitrogen gas cylinder as a source of positive pressure. Hydraulic loading was about 220 ml of tap water per cm^2 of filter surface area. Filtrates were collected and thoroughly mixed, and small samples were diluted in an equal volume of sample diluent for subsequent virus assay to determine the unadsorbed virus fraction.

Adsorbed viruses were eluted from filters with two successive 7.5-ml volumes of either 0.05 M glycine-NaOH at pH 11.0 or 0.3% (dry wt/vol) beef extract (Lablemco powder, Oxoid, K. C. Biologicals, Lenexa, Kan.) in 0.05 M glycine-NaOH (BE-GLY), pH 9.5. Eluates were collected dropwise from the filters and then adjusted to pH 7.5 with 0.05 M glycine-HCl, pH 2.0, or with 0.1 N HCl. Small samples of the neutralized eluates were diluted in equal volumes of sample diluent for subsequent virus assay, and each final eluate volume was measured. All samples were stored at −30°C until assayed.

A portion of the same stock virus that was added to the water was also routinely diluted in PBS containing 2% heat-inactivated fetal calf serum, 0.02% $MgCl_2 \cdot 6H_2O$, 200 U of penicillin per ml, and 200 μg of streptomycin sulfate per ml for subsequent virus assay. This was done to make sure that the amount of virus detectable in the initially seeded tap water corresponded to the amount of virus in the seed stock. No statistically significant differences between them were ever observed, indicating that there were no initial losses of virus titer in the seeded water due to inactivation, aggregation, or other phenomena. A portion of the stock virus used in large-volume experiments was similarly assayed.

(ii) Large-volume experiments. Tap water was drawn from the Chapel Hill distribution system via a four-place manifold, as previously described (22). Poliovirus was added to the flowing tap water along with the sodium thiosulfate solution used for dechlorination via one proportioner of a Triplex fluid proportioning pump (Johannsen and Son Machine Corp., Clifton, N.J.). For Filterite filters, the remaining two portioners, operating reciprocally, added HCl and $MgCl_2$ to bring the water to pH 3.5 and a final $MgCl_2$ concentration of 5×10^{-3} M for virus adsorption enhancement (23). For AMF Cuno filters, the remaining two proportioners added HCl or NaOH when required to maintain water at pH 7.5.

After conditioning, the seeded tap water was filtered through 25.4- by 7.6-cm-diameter (~4×10^3-cm^2 surface area) pleated-sheet fiber glass cartridge filters (Filterite Duofine or Virozorb 1MDS) at flow rates of about 2 ml/min per cm^2 of filter surface area. Hydraulic loading was about 250 ml of tap water per cm^2 of filter surface area. When virus inputs were high, samples of filtrate water were collected after volumes of 10, 100, 300, and 1,000 liters had passed through the filters. The filtrate samples were pooled and mixed thoroughly, and a small portion was diluted in an equal volume of sample diluent for subsequent determination of the unadsorbed virus fraction.

When 1,000 liters of water had been processed through an adsorbent, the proportioning pump was stopped and the remaining water was forced out of the filter cartridges with air pressure. Adsorbed viruses were eluted from each cartridge by recirculating about 1,000 ml of pH 9.5 BE-GLY through the filter for 5 min by means of a peristaltic pump. The eluate was mixed and adjusted to pH 7.5 with 1.0 N HCl and, when virus inputs were high, a small sample was taken and immediately diluted in an equal volume of sample diluent for subsequent virus assay.

For reconcentration by organic flocculation (17, 23), the primary eluate was brought to 1.5% beef extract by adding an appropriate volume of 30% beef extract solution and then adjusting to pH 3.5 with 1.0 N HCl. After gently stirring for 15 to 30 min, the resulting floc was recovered by centrifugation at $1,300 \times g$ for 15 min. When virus inputs were high, the centrifugal supernatant was decanted and adjusted to pH 7.5 with 1.0 N NaOH, and a small sample was diluted in an equal volume of sample diluent for subsequent determination of the unadsorbed virus fraction. Sedimented floc was resuspended in a small volume (35 to 70 ml) of 0.15 M Na_2HPO_4 and, if necessary, adjusted to pH 7.5 with additional buffer. When virus inputs were high, a small sample of this dissolved floc was diluted

in an equal volume of sample diluent for subsequent virus assay. When virus inputs were low, the dissolved floc was adjusted to 0.1 M PBS, 0.02% $MgCl_2 \cdot 6H_2O$, 200 U of penicillin per ml, and 200 μg of streptomycin sulfate per ml by addition of appropriate amounts of concentrated stock solutions. Final concentrate volumes were measured, and all samples were stored at −30°C until assayed.

At the end of each experiment, the volume of sodium thiosulfate-virus dosing solution drawn by each proportioning pump was measured. From this volume, the volume of virus stock added to the dosing solution, and the virus stock titer (assayed when experimental samples were assayed), the total virus input was calculated for each adsorbent.

Electrophoresis. Electrophoresis of filter media particles was performed with a Zeta Meter (Zeta Meter, Inc., New York), using a Zeta Meter type II-UVA electrophoresis cell. A 47-mm-diameter disk of each filter medium was shredded, placed in approximately 200 ml of cold tap water, and blended at low speed for 2 to 4 min. The resulting slurry was diluted in tap water to a particle concentration suitable for use in the electrophoresis apparatus as determined by microscopic observation of the diluted suspension. Separate 50-ml portions of the diluted suspension were adjusted to desired pH levels by the addition of small volumes (≤1 ml) of 0.05 N HCl or NaOH.

After pH adjustment, a portion was poured into the electrophoresis cell. A molybdenum cathode and platinum-iridium anode were then fitted, and any trapped air bubbles were removed from the cell. Electrophoresis was done at room temperature (25 ± 2°C), and observation times were always less than 3 min to avoid thermal overturn in the electrophoresis cell (33). In the pH range examined, pH 3.5 to 10.5, specific conductivities of the samples ranged from 180 to 350 μS (μmhos)/cm, and all samples were electrophoresed in a 300-V electric field. Voltage was applied across the electrophoresis cell, and the average rate of migration (micrometers/second) of 10 particles was measured. Although the suspended particles were heterogeneous, consisting of roughly spherical and fibrous morphologies, it was observed that all particles migrated at similar rates in the same direction at a given pH level. Three replicate migration rate measurements were made at each pH level examined. Electrophoretic mobility (EM) was determined (33) by using the mean migration rate for the three replicate measurements and expressed as micrometers/second per volt/centimeter.

Statistical analyses. Statistical computations and tests were done with a Texas Instruments SR-52 calculator, using the Texas Instruments ST1 statistics software library (Texas Instruments, Inc., Dallas, Tex.). Differences were considered significant when $P \leq 0.05$ for the appropriate comparative test.

RESULTS

Comparison of Filterite and Zeta Plus 50S for poliovirus concentration. Over a period of 5 months, a series of small-volume experiments was performed to directly compare virus concentration efficiencies of Zeta Plus 50S and Filterite filters (Table 1). Adsorption was at pH 3.5 for Filterite filters and at pH 7.5 for 50S filters. Adsorbed viruses were eluted with glycine-NaOH at pH 11.0.

Adsorption efficiencies are expressed as percentage of input viruses adsorbed, elution efficiencies are expressed as percentage of adsorbed viruses eluted, and recovery efficiencies are expressed as percentage of input viruses recovered in the eluate. Over the 5-month period of experimentation, the mean adsorption efficiencies of Filterite and Zeta Plus 50S filters were 86 and 93% of input viruses, respectively. Recovery efficiencies averaged 60 and 56% of input viruses for Filterite and 50S, respectively. A paired *t*-test with adsorption data showed no significant difference between mean efficiencies for Filterite and 50S ($P = 0.097$). In addition, there was no significant difference between mean recovery efficiencies for Filterite and 50S filters by paired *t*-test ($P = 0.482$).

When virus adsorption and recovery efficiency data for 50S and Filterite filters were plotted as bimonthly mean histograms, both filter types showed an increase in adsorption efficiency during the spring and summer months (Fig. 1). A similar increase in recovery efficiency was apparent with 50S. However, Filterite seemed to show a maximum in recovery efficiency during

TABLE 1. *Comparative poliovirus concentration efficiencies with Filterite and Zeta Plus 50S filters*[a]

Date	Filterite, 0.45 μm[b]			Zeta Plus 50S		
	% ADS	% EL	% REC	% ADS	% EL	% REC
3/22/79	53	7	4	86	35	30
3/30/79	73	70	51	88	50	44
4/6/79	40	56	22	75	62	46
4/13/79	47	62	29	94	96	90
5/3/79	99	79	78	92	77	71
6/28/79	82	100	82	82	45	37
6/28/79	88	82	72	89	54	48
7/6/79	97	47	46	100	59	59
7/6/79	91	90	82	100	57	57
7/12/79	99	80	79	98	65	64
7/12/79	96	40	38	97	42	41
7/19/79	97	54	52	82	59	48
7/19/79	99	58	57	100	47	47
8/2/79	100	94	94	99	74	73
8/2/79	100	80	80	100	71	71
8/10/79	100	82	82	100	69	69
8/10/79	100	72	72	100	54	54
Mean[b]	86 ± 10	68 ± 12	60 ± 13	93 ± 4	60 ± 8	56 ± 8

[a] See text for complete description of adsorbent filters. Tap water volume, 3.8 liters; filter diameters, 47 mm; adsorption flow rate, 1.5 to 3.0 ml/min per cm^2; poliovirus input, 10^4 PFU/ml; elution with glycine-NaOH, pH 11.0. Adsorption was at pH 3.5 for Filterite and pH 7.5 for Zeta Plus 50S. Abbreviations: ADS, input viruses adsorbed; EL, adsorbed viruses eluted; REC, input viruses recovered.

[b] 95% confidence interval.

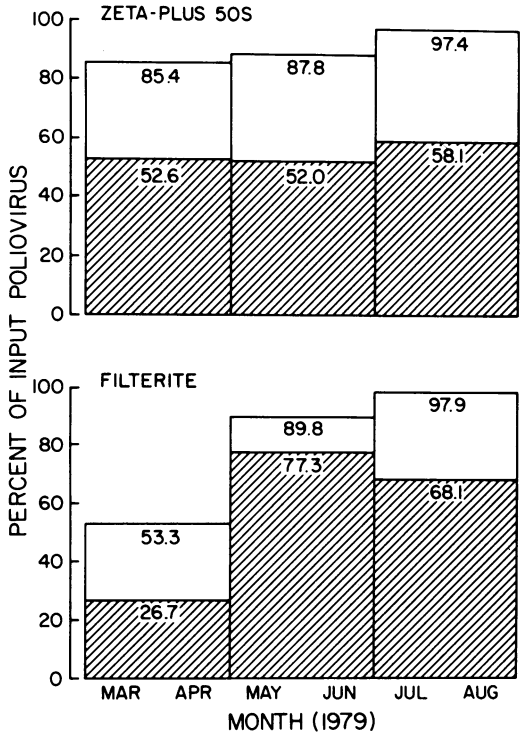

FIG. 1. *Histogram of bimonthly mean poliovirus adsorption (□) and recovery (▨) efficiencies from tap water with Zeta Plus 50S and Filterite 0.45-μm-porosity filters. Poliovirus input, 10^4 PFU/ml; tap water volume, 3.8 liters; filter disk diameter, 47 mm; adsorption flow rate, 1.5 to 3.0 ml/min per cm^2 of filter surface; adsorption at pH 7.5 for Zeta Plus 50S and at pH 3.5 for Filterite; elution with 0.05 M glycine-NaOH, pH 11.0.*

May and June, followed by a slight decline thereafter. In general, recovery efficiency variations, as indicated by 95% confidence intervals about mean values (Table 1), were more pronounced with Filterite than with 50S.

Analyses of variance showed significant variation among bimonthly mean adsorption efficiencies for both filter types (50S, $P = 0.009$; Filterite, $P = 5.6 \times 10^{-7}$). No significant variation was found among bimonthly mean virus recovery efficiencies for 50S ($P = 0.777$), but Filterite recovery efficiencies varied significantly ($P = 0.002$).

Effect of pH on poliovirus adsorption efficiency to different AMF Cuno filters. Because poliovirus concentration efficiency from tap water with Zeta Plus 50S at pH 7.5 was comparable to that with Filterite filters at pH 3.5, a series of experiments was conducted to determine whether other types of electropositive filters were capable of concentrating viruses from water. Small-volume experiments were conducted to evaluate poliovirus adsorption efficiency from tap water by AMF Cuno filters over a wide range of adsorption pH levels. Volumes (3.8 liters) of dechlorinated tap water were adjusted to pH 3.5, 5.5, 7.5, or 9.5 and seeded with 10^4 PFU of poliovirus per ml. One volume at each pH level was then filtered through a replicate 47-mm-diameter, single-layer AMF Cuno filter disk. Total filtrates were collected, sampled, and assayed to determine the fraction of input PFU not adsorbed by the filter. Eighteen different types of AMF Cuno media were evaluated, and single layers of Zeta Plus 50S and Virozorb 1MDS proved to be the most efficient virus adsorbents.

Results for these two filter types are shown in Fig. 2 as percentage of input viruses adsorbed as a function of pH. For comparison, similar data for Filterite filters, obtained from Sobsey and Jones (25), have been included. Both AMF Cuno filter types adsorbed poliovirus with efficiencies of ≥80% between pH 3.5 and 7.5, but above pH 7.5, adsorption efficiencies greatly declined. Virus adsorption by the Filterite filter type has been previously shown to be efficient only at pH 3.5 (25).

Single layers of Virozorb 1MDS medium were less efficient as virus adsorbents at pH 5.5 and 7.5 than were Zeta Plus 50S filters. A single layer of 1MDS medium is much thinner (ca. 1 mm thick) than is the Zeta Plus 50S (ca. 5 mm thick), and therefore opportunities for virion contact with filter surfaces may be fewer with 1MDS than with 50S. This suggestion was supported by the results of a series of small-volume exper-

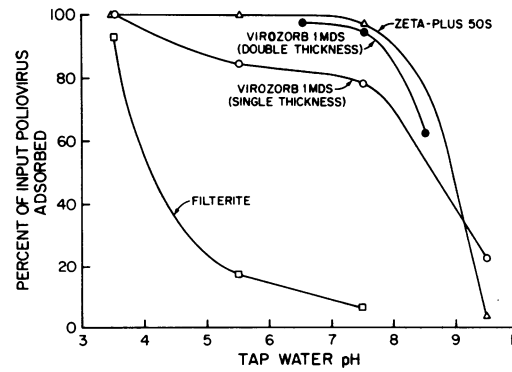

FIG. 2. *Effect of tap water pH on poliovirus adsorption efficiencies for AMF Cuno and Filterite 0.45-μm-porosity filters. AMF Cuno: Poliovirus input, 10^4 PFU/ml; tap water volume, 3.8 liters; filter disk diameter, 47 mm; adsorption flow rate, 1.5 ml/min per cm^2 of filter surface. Filterite: Poliovirus input, 10^5 PFU/ml; tap water volume, 0.5 liter; filter disk diameter, 47 mm; adsorption flow rate, 10 ml/min per cm^2 of filter surface; data from Sobsey and Jones (25).*

iments in which poliovirus adsorption efficiency by parallel 50S filters was evaluated at flow rates of 1.5 and 15.0 ml/min per cm^2 of filter surface area. Adsorption was at pH 7.5, and the filtrates were sampled for determination of the unadsorbed virus fraction. Virus adsorption at the higher flow rate (62%) was significantly less efficient than at the lower flow rate (86%) by paired *t*-test ($P = 0.006$). Presumably, the higher flow rate allowed fewer opportunities for virion contact with the filter surface than did the lower flow rate.

The 1MDS filter medium, because of its thin and pliable nature, can be produced as a double-layer, pleated-sheet cartridge filter, whereas the 50S medium cannot. In fact, commercially available Virozorb 1MDS cartridges always contain two layers of 1MDS medium. Because of the need for a large-surface-area, pleated-sheet cartridge filter for use in large-volume experiments, the virus adsorption efficiency of the 1MDS medium was further evaluated.

To increase the probability of virion contact with filter surfaces and to evaluate the 1MDS medium in a form corresponding to the commercially available cartridges, two layers of 1MDS filter medium as 47-mm-diameter disks in a single filter holder (double 1MDS) were evaluated as virus adsorbents in small-volume experiments. The experimental procedure was as described above, with the exception that poliovirus adsorption was evaluated from tap water at pH 6.5, 7.5, and 8.5. At pH 6.5 and 7.5, two layers of 1MDS adsorbed poliovirus more efficiently than did one layer and nearly as efficiently as did 50S (Fig. 2). As with single layers of 1MDS medium and Zeta Plus 50S, adsorption efficiencies of double 1MDS decreased above pH 7.5.

Comparison of AMF Cuno filter types for poliovirus adsorption and elution efficiencies. Zeta Plus 50S, single-layer 1MDS, and double-layer 1MDS filter disks (all 47-mm diameter) were evaluated for poliovirus adsorption and elution efficiencies in a series of small-volume experiments. Adsorption was at pH 7.5 for all filter types, and adsorbed viruses were eluted either with glycine-NaOH at pH 11.0 or with BE-GLY at pH 9.5. All experiments were done during September and October 1979 to minimize effects of water quality-associated temporal changes in adsorption and elution efficiencies (22). Poliovirus adsorption efficiencies for 50S, single 1MDS, and double 1MDS averaged 99, 76, and 95%, respectively (Table 2). Analysis of variance showed significant variation among adsorption efficiencies for the three filter types ($P = 4.6 \times 10^{-6}$). Adsorption efficiency for each filter type was significantly different from efficiencies for each of the other two filter types by two-sample *t*-tests ($P < 0.001$ in all cases). 50S was the most efficient virus adsorbent, followed by double 1MDS and single 1MDS.

TABLE 2. *Comparative concentration efficiencies by AMF Cuno filters for poliovirus*[a]

Adsorbent	Mean % ADS[b]	0.05 M Glycine-NaOH, pH 11.0		BE-GLY, pH 9.5	
		No. of trials	% EL[c]	No. of trials	% EL
50S	99 ± 1	6	57	NT[d]	
Single 1MDS	76 ± 9	8	51	2	67
Double 1MDS	95 ± 2	2	62	8	66
Mean % EL[c]		54 ± 6		66 ± 9	

[a] Experiments were performed during September and October 1979. Virus input, 10^4 PFU/ml; water volume, 3.8 liters; filter diameter, 47 mm; adsorption flow rate, 3.0 ml/min per cm^2; adsorption at pH 7.5. See text for complete description of adsorbents.

[b] Mean percentage of input viruses adsorbed (95% confidence interval).

[c] Mean percentage of adsorbed viruses eluted (95% confidence interval).

[d] Not tested.

Glycine-NaOH at pH 11.0 gave mean elution efficiencies of 57, 51, and 62% of the adsorbed viruses from 50S, single 1MDS, and double 1MDS, respectively. No significant variation among mean elution efficiencies by glycine-NaOH for the three filter types was found by analysis of variance ($P = 0.314$).

BE-GLY at pH 9.5 gave mean elution efficiencies of 67 and 66% of the adsorbed viruses from single and double 1MDS, respectively. A two-sample *t*-test showed no significant difference between mean elution efficiencies by BE-GLY for the two filter types ($P = 0.979$). However, disregarding filter types, BE-GLY at pH 9.5 was a significantly more efficient eluent than was glycine-NaOH at pH 11.0 by two-sample *t*-test ($P = 0.015$). Overall mean elution efficiencies were 54 and 66% of adsorbed viruses for glycine-NaOH and BE-GLY, respectively.

Comparison of Filterite and Virozorb 1MDS cartridges for poliovirus concentration from large tap water volumes. In an attempt to verify the results of the preceding small-volume experiments with large water volumes, a series of experiments was conducted in which polioviruses were concentrated from 1,000-liter volumes of tap water using Filterite or Virozorb 1MDS pleated-sheet cartridge adsorbent filters. Primary adsorption conditions used for the Filterite filters were the optimal ones previously used in this laboratory (23): pH 3.5 and 5×10^{-3} M added $MgCl_2$. For the Viro-

zorb 1MDS filters, adsorption was at pH 7.5 with no added cation salts. Approximate virus inputs were either 10^8 or 200 to 400 PFU per 1,000 liters. Two replicate cartridges of each filter type were used in each experiment.

Adsorbed viruses were eluted with BE-GLY, pH 9.5, by recirculation through the filter cartridges for 5 min. Primary eluates were adjusted to 1.5% beef extract and reconcentrated by organic flocculation at pH 3.5. Final concentrate volumes ranged from 40 to 75 ml.

The results of four experiments are summarized in Table 3. When virus inputs were high (about 10^8 PFU), average adsorption efficiencies for Filterite and Virozorb 1MDS were 86 and 90%, respectively. An average of 62 and 78% of the adsorbed viruses were eluted from the Filterite and 1MDS filters, respectively. Overall mean virus recoveries were 34% for Filterite and 48% for 1MDS.

Adsorption and elution efficiencies could not be determined when virus inputs were low because titers in intermediate samples were below minimum detectable levels. Average overall recoveries of input viruses in large-volume experiments were 33 and 30% for Filterite and Virozorb 1MDS, respectively. There was no significant difference between mean recovery efficiencies for the two filter types by two-sample t-test ($P = 0.755$).

EM of filter media particles. Because of the presumed importance of adsorbent surface potentials for virus adsorption to filter surfaces and the marked differences in optimum pH and ionic conditions for virus adsorption between AMF Cuno and Filterite filters, the electrophoretic mobilities of the filter particles were investigated. Figure 3 illustrates the relationships between tap water pH and EM for Filterite, Zeta Plus 50S, and Virozorb 1MDS filter media. All three media, as particle suspensions in tap water, were electronegative between pH 3.5 and 10.5. However, both AMF Cuno media were considerably less electronegative in the pH range examined than was Filterite medium.

As expected, both AMF Cuno media exhibited increasing electronegativity with increasing pH. The 1MDS medium was slightly more electronegative at all pH levels than was 50S medium. Both media followed a similar pattern of EM change with increasing pH, showing apparent plateaus of EM stability around pH 5.5 and 10.0. 50S medium was isoelectric at pH 3.5, whereas the extrapolated apparent isoelectric point for 1MDS medium was approximately pH 2.5. Maximum EMs of -1.7 μm/s per V/cm for 50S and -2.0 μm/s per V/cm for 1MDS were obtained at pH 10.5.

The EM curve for Filterite medium was considerably different in shape and magnitude than the curves for AMF Cuno media. Filterite particle EM increased sharply between pH 3.5 and

TABLE 3. *Concentration of poliovirus from 1,000-liter volumes of tap water with pleated-sheet cartridge filters*[a]

Date	Filter[b]	pH[c]	Total PFU input	Primary		Org. floc.		Overall		Mean % REC
				% ADS	% EL	% ADS[d]	% REC[e]	PFU REC	% REC	
10/25/79	Filterite	3.5	1.0×10^8	86	83	95	52	3.5×10^7	35	34
			8.5×10^7	85	40	99	100	2.9×10^7	34	
	1MDS	7.5	8.6×10^7	95	77	99	65	4.1×10^7	47	48
			8.8×10^7	86	78	99	72	4.2×10^7	48	
11/8/79	Filterite	3.5	258	ND	ND	ND	ND	44	17	16
			239	ND	ND	ND	ND	36	25	
	1MDS	7.5	193	ND	ND	ND	ND	33	17	18
			188	ND	ND	ND	ND	38	20	
11/20/79	Filterite	3.5	216	ND	ND	ND	ND	193	89	65
			199	ND	ND	ND	ND	81	41	
	1MDS	7.5	232	ND	ND	ND	ND	68	29	34
			204	ND	ND	ND	ND	80	39	
12/28/79	Filterite	3.5	392	ND	ND	ND	ND	64	16	17
			326	ND	ND	ND	ND	60	18	
	1MDS	7.5	323	ND	ND	ND	ND	49	15	20
			295	ND	ND	ND	ND	71	24	

[a] Abbreviations: Org. floc., organic flocculation in 1.5% beef extract, pH 3.5; ADS, input viruses adsorbed; EL, adsorbed viruses eluted with 1,000 ml of BE-GLY, pH 9.5 (5-min filter/eluent contact); REC, recovered; ND, not determined (titer below detectable limits).

[b] See text for complete descriptions of adsorbent filters and procedures.

[c] Adsorption pH; tap water supplemented with 5×10^{-3} M $MgCl_2$ for Filterite.

[d] Percentage of viruses in primary eluate removed with organic floc.

[e] Percentage of adsorbed viruses recovered by resolution of organic floc in 0.15 M Na_2HPO_4.

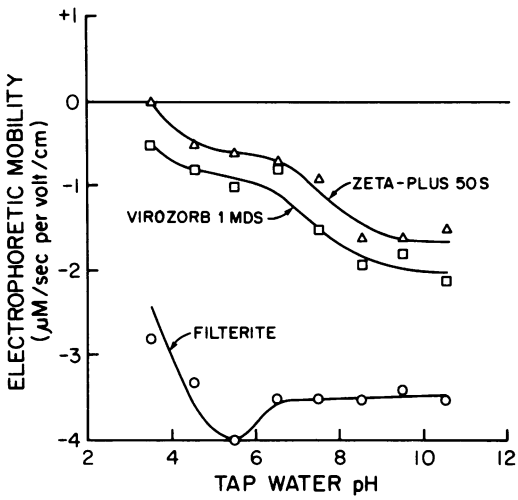

FIG. 3. *Effect of tap water pH on EM values of AMF Cuno and Filterite filter particles. Filter disks disrupted by low-speed blending in tap water; pH adjustment with 0.05 N Hcl or NaOH; particles electrophoresed in 300-V field.*

5.5, reaching a maximum of −4.0 μm/s per V/cm at pH 5.5. Above pH 5.5, particle EM stabilized at approximately −3.5 μm/s per V/cm. The minimum observed EM for Filterite particles was −2.8 μm/s per V/cm at pH 3.5, which was somewhat greater than the maximum EM for either AMF Cuno medium. Extrapolation of the Filterite EM curve yielded an isoelectric point at about pH 1.5.

DISCUSSION

The results of this study confirm that AMF Cuno filters offer clear advantages over Filterite and probably other strongly electronegative filters in adsorption-elution methods for concentrating viruses from water. Virozorb 1MDS medium is especially promising because it is available as a double-layer pleated-sheet cartridge which is simple to use and contains a large filter surface area that allows for high flow rates and hydraulic loadings.

One important advantage of AMF Cuno filters is their ability to efficiently adsorb viruses at much higher pH levels than can more strongly electronegative filters. In this study, both Zeta Plus 50S and Virozorb 1MDS filters adsorbed 80% or more of input polioviruses between pH 3.5 and 7.5 (Fig. 2). In a previous study, Sobsey and Jones (25) observed a substantial decrease in poliovirus adsorption efficiency by Zeta Plus 50S at pH 3.5. This apparent inconsistency cannot readily be explained. One possibility is related to the fact that the 50S medium used in the pH experiments of the previous study was prototype material, and perhaps it differed somewhat from the 50S medium now commercially available. However, it is clear from Fig. 2 that the current 50S medium efficiently adsorbs viruses between pH 3.5 and 7.5. It should also be noted that in the present study all of the 18 AMF Cuno filter types examined gave maximum poliovirus adsorption efficiencies at pH 3.5 (although some of them gave similarly high adsorption efficiencies at higher pH levels) (Sobsey et al., unpublished data).

In small-volume concentration experiments (Tables 1 and 2), Zeta Plus 50S adsorbed 93 to 99% of input viruses at pH 7.5 compared with 86% adsorption for Filterite at pH 3.5 At an adsorption pH of 7.5, double-layer 1MDS adsorbed 95% of input viruses, whereas single-layer 1MDS adsorbed only 76%. In experiments with 1,000-liter tap water volumes, Virozorb 1MDS cartridge filters adsorbed about 90% of input viruses at pH 7.5, whereas Filterite cartridge filters adsorbed about 85% at pH 3.5 (Table 3). Such close agreement between small-volume and large-volume experiments is not unexpected, since flow rate and hydraulic loading were similar in both types of experiment.

In large-volume experiments, the addition of 5×10^{-3} M $MgCl_2$ to the tap water yielded no apparent improvement in virus adsorption to Filterite filters over that observed in small-volume experiments where no $MgCl_2$ was added. This observation, in apparent contradiction to previous studies (15, 23, 30), may be explained in the light of recent findings in this laboratory (22). During an evaluation of the tentative standard method (1, 2) for concentrating enteric viruses from drinking water, temporal changes in efficiency of enteric virus recovery from 100-gal (ca. 380-liter) volumes of tap water were observed. Virus recovery efficiencies were generally poor during fall and winter (October through March) but improved during spring and summer (April through September). Further experimentation suggested that the observed variation in virus concentration efficiency may have been related to changes in the concentrations of unidentified adsorption-interfering soluble or colloidal components in Chapel Hill tap water.

In the present study, similar temporal changes were observed for poliovirus adsorption and recovery efficiencies (Fig. 1). The general pattern of recovery efficiency variation observed with Filterite filters in this study is in close agreement with the pattern observed in the previous study with other strongly electronegative filter types (22).

Because of the considerable temporal variability in virus adsorption efficiency, direct comparison of the overall mean adsorption efficien-

cies for Filterite in small-volume experiments with those in large-volume experiments is not justified. In fact, on the basis of the previously observed pattern and cause of temporal changes in virus recovery efficiency (22), one could expect adsorption efficiency to decrease during the months in which the large-volume experiments were conducted (October, November, and December). Thus, the 85% adsorption efficiency observed for Filterite in large-volume experiments probably reflects virus adsorption enhancement by $MgCl_2$ during a time period when water quality interferences with virus adsorption were at yearly maximum levels.

Comparison of the levels of adsorption interference by tap water components on Zeta Plus 50S and Filterite filters (Fig. 1) demonstrates the second important advantage of AMF Cuno filters for adsorption-elution virus concentration. During the months in which adsorption-interfering components were at apparent maximum levels in tap water (March and April), 50S showed considerably less reduction in virus adsorption efficiency than did Filterite. This observation supports the hypothesis that less electronegative AMF Cuno filters may be less affected by virus adsorption-interfering soluble or colloidal components of tap water than are strongly electronegative filters such as Filterite. It is possible that acidification of tap water activates the interfering components in some way so that adsorption at neutral pH prevents them from affecting AMF Cuno filters. Humic acids are a likely candidate in this regard since they become insoluble at acidic pH levels (21) and have been previously suggested as virus adsorption-interfering compounds (7, 22, 31). Alternatively, the surface of AMF Cuno filters may somehow be less susceptible to the compounds that interfere with virus adsorption than is the more strongly electronegative Filterite surface, either because the AMF Cuno filters react with the interfering compounds to a lesser extent or in a different way or because they have a greater capacity for the compounds.

Elution of adsorbed poliovirus from AMF Cuno filters was found to be more efficient with pH 9.5 BE-GLY than with pH 11.0 glycine-NaOH. In comparative trials, BE-GLY eluted 66% of adsorbed viruses from AMF Cuno filters, whereas glycine-NaOH eluted only 54%. This result supports previous findings on the efficiency of proteinaceous media as virus eluents (5, 17, 23, 25) and the instability of poliovirus at pH 11.0 (5, 23; Jacobs, Ph.D. thesis, 1980). Viruses in proteinaceous eluates can, with possible modifications for certain virus types (23), be efficiently reconcentrated by organic flocculation (17). In the present study, the organic flocculation technique was about 70% efficient for recovery of poliovirus, which is consistent with previous findings (13, 23).

No significant difference was found between Filterite and Virozorb 1MDS cartridge filters for recovery efficiency of small numbers of poliovirus from 1,000-liter volumes of tap water. However, the concentration method was considerably simplified by using the 1MDS cartridge, because acidification of tap water and addition of cation salts were not required. Disregarding filter type, virus recovery efficiency in large-volume experiments averaged 32%. Thus, it should be possible to detect poliovirus at a level of about 3 PFU/1,000 liters or about 1 PFU/100 gal.

EM values observed for Filterite and AMF Cuno filter media in the present study are generally consistent with previous findings, although particles of Zeta Plus 50S and Filterite media showed more electronegative EM values than previously reported (18, 25). However, the referenced studies used particle-suspending media of considerably higher ionic strength than tap water. Specifically, Kessick and Wagner (18) suspended Filterite particles in 0.02 M KCl, and Sobsey and Jones (25) suspended Zeta Plus 50S and Filterite particles in 0.05 M NaCl. In contrast, the average total dissolved solids concentration of Chapel Hill tap water is 80 mg/liter as NaCl or about 0.001 M. It is known that increasing ionic strength reduces EMs and can reverse the apparent charge of particles in colloidal suspension, due to compression of the diffuse electric double layer surrounding each particle (18, 28). Thus, the EM values for filter media observed in this study may more accurately depict filter surface charges in a tap water environment. It should be noted, however, that EM values observed with suspensions of disrupted media may not exactly correspond to the surface charge on intact filters, because disruption may expose fiber ends or other electronegative particle surfaces normally coated with binding resin in an intact filter, thereby making the particles less electropositive. In fact, streaming potential measurements of intact Zeta Plus filter media indicate that they are indeed electropositive (R. A. Knight and E. A. Ostreicher, paper presented at the 71st Annu. Am. Inst. Chem Eng. Meet., Miami, Fla., 12-16 Nov. 1978). However, it is clear from Fig. 3 that AMF Cuno filter media are much less electronegative between pH 3.5 and 10.5 than is the Filterite medium, even with disrupted media particles.

For all filter types studied, a general relationship between filter particle EM and poliovirus

adsorption efficiency was observed. Specifically, decreasing filter medium electronegativity corresponded to increasing virus adsorption efficiency (Fig. 2 and 3). The phenomenon is particularly evident for Filterite and single-layer 1MDS, where filter pad depths are comparable.

Poliovirions have two isoelectric points, a major one at pH 6.8 to 7.2 and a minor one at pH 3.5 to 4.0 (19, 32). Between pH 3.5 and 7.5, the virion surface charge is probably composed of heterogeneous point charges. In this range of pH, the virion surface charge is probably relatively weak and depends on the exact pH of the suspending medium (19). Thus, it is likely that the relatively strong filter surface charge exerts a major influence on virus adsorption in this pH range.

Valentine and Allison (27) have suggested that the diffusion, by Brownian motion, of fowl plague virus and vaccinia virus toward negatively charged adsorbent surfaces in low- to moderate-ionic-strength environments is inhibited by electrostatic repulsive forces between the counterion layer covering the adsorbent surface and like charges associated with the virions. When the counterion layers were compressed by increasing the ionic strength of the medium, reducing the electrostatic repulsive forces, virus attachment to the adsorbent surfaces occurred at rates consistent with collision frequencies predicted by diffusion theory.

The relationship between filter particle EM and virus adsorption efficiency observed in this study is consistent with the explanation of Valentine and Allison (27). Decreasing filter surface electronegativity in a relatively constant ionic environment, as in tap water, should result in decreasing thickness and charge density of the counterion layer covering the surface. Hence, electrostatic repulsive forces between the filter counterion layer and like charges associated with the virions should decrease, and the diffusion of virions toward the filter surface should be facilitated. When virions can diffuse to within close proximity of the filter surface, adsorption can occur by Van der Waals forces (27), virion-cation-filter bridging (28), interactions between specific surface functional groups (4), or other mechanisms.

From the foregoing discussion, it appears that optimal virus adsorbent filters should possess a minimal negative or even a small positive charge in the pH range of natural waters. Obviously, AMF Cuno filters approach this requirement more closely than do other adsorbent filters presently in use (1, 2, 18, 25). In order to develop virus adsorbent filters with optimal surface properties, further studies are needed to elucidate the influence of filter surface charge on virus adsorption and the actual mechanisms of virus adsorption to filters.

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LITERATURE CITED

1. **American Public Health Association, American Water Works Association, and Water Pollution Control Federation.** 1976. Detection of enteric viruses in water and wastewater, section 913, p. 968–975. *In* Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
2. **American Public Health Association, American Water Works Association, and Water Pollution Control Federation.** 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
3. **Brinton, C., and M. Lauffer.** 1959. The electrophoresis of viruses, bacteria and cells, and the microscopic method of electrophoresis, p. 427–492. *In* M. Bier (ed.), Electrophoresis. Academic Press, Inc., New York.
4. **Cookson, J. T.** 1969. Mechanisms of virus adsorption to activated carbon. J. Am. Water Works Assoc. **61**:45–50.
5. **Farrah, S. R., and G. Bitton.** 1978. Elution of poliovirus adsorbed to membrane filters. Appl. Environ. Microbiol. **36**:982–984.
6. **Farrah, S. R., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. **31**:221–226.
7. **Farrah, S. R., S. M. Goyal, C. P. Gerba, C. Wallis, and P. T. B. Shaffer.** 1976. Characteristics of humic acids and organic compounds concentrated from tap water using the aquella virus concentrator. Water Res. **10**: 897–901.
8. **Farrah, S. R., C. Wallis, P. T. B. Shaffer, and J. L. Melnick.** 1976. Reconcentration of poliovirus from sewage. Appl. Environ. Microbiol. **32**:654–658.
9. **Fattal, B., E. Katzenelson, T. Hostovsky, and H. I. Shuval.** 1977. Comparison of adsorption-elution methods for concentration and detection of viruses in water. Water Res. **11**:955–958.
10. **Fields, H. A., and T. G. Metcalf.** 1975. Concentration of adenovirus from seawater. Water Res. **9**:357–364.
11. **Gerba, C. P., M. D. Sobsey, C. Wallis, and J. L. Melnick.** 1974. Enhancement of poliovirus adsorption in wastewater onto activated carbon, p. 115–126. *In* J. Malina and B. Sagik (ed.), Virus survival in water and wastewater systems. University of Texas Press, Austin.
12. **Gerba, C. P., C. Wallis, and J. L. Melnick.** 1975. Viruses in water: the problem, some solutions. Environ. Sci. Technol. **9**:1122–1126.
13. **Glass, J. S., R. J. Van Sluis, and W. A. Yanko.** 1978. Practical method for detecting poliovirus in anaerobic digester sludge. Appl. Environ. Microbiol. **35**:983–985.
14. **Hill, W. F., E. W. Akin, and W. H. Benton.** 1971. Detection of viruses in water: a review of methods and application. Water Res. **5**:967–995.
15. **Hill, W. F., E. W. Akin, and W. H. Benton.** 1972. Virus in water. II. Evaluation of membrane cartridge filters for recovering low multiplicities of poliovirus from water. Appl. Microbiol. **23**:880–885.

16. **Jakubowski, W., W. F. Hill, and N. A. Clarke.** 1975. Comparative study of four microporous filters for concentrating viruses from drinking water. Appl. Microbiol. **30**:58-65.
17. **Katzenelson, E., B. Fattal, and T. Hostovsky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. **32**:638-639.
18. **Kessick, M. A., and R. A. Wagner.** 1978. Electrophoretic mobilities of virus-adsorbing filter materials. Water Res. **12**:263-268.
19. **Mandel, B.** 1971. Characterization of type 1 poliovirus by electrophoretic analysis. Virology **4**:554-568.
20. **Melnick, J. L.** 1971. Detection of virus spread by the water route, p. 114-124. *In* V. Snoeyink (ed.), Proceedings of the 13th Water Quality Conference. University of Illinois, Urbana.
21. **Schnitzer, M.** 1971. Metal-organic matter interactions in soils and waters. *In* S. S. Faust and J. V. Hunter (ed.), Organic compounds in aquatic environments. Marcel Dekker, New York.
22. **Sobsey, M. D., J. S. Glass, R. J. Carrick, R. R. Jacobs, and W. A. Rutala.** 1980. Evaluation of the tentative standard method for enteric virus concentration from large volumes of tap water. J. Am. Water Works Assoc. **72**:292-299.
23. **Sobsey, M. D., J. S. Glass, R. R. Jacobs, and W. A. Rutala.** 1980. Modifications of the tentative standard method for improved virus recovery efficiency. J. Am. Water Works Assoc. **72**:350-355.
24. **Sobsey, M. D., H. R. Jensen, and R. J. Carrick.** 1978. Improved methods for detecting enteric viruses in oysters. Appl. Environ. Microbiol. **37**:121-128.
25. **Sobsey, M. D., and B. L. Jones.** 1979. Concentration of poliovirus from tap water using positively charged microporous filters. Appl. Environ. Microbiol. **37**:588-595.
26. **Sobsey, M. D., C. Wallis, M. Henderson, and J. L. Melnick.** 1973. Concentration of enteroviruses from large volumes of water. Appl. Microbiol. **26**:529-534.
27. **Valentine, R. C., and A. C. Allison.** 1959. Virus particle adsorption. I. Theory of adsorption and experiments on the attachment of particles to non-biological surfaces. Biochim. Biophys. Acta **34**:10-23.
28. **Van Olphen, H.** 1977. An introduction to clay colloid chemistry. John Wiley & Sons, New York.
29. **Wallis, C., M. Henderson, and J. L. Melnick.** 1972. Enterovirus concentration on cellulose membranes. Appl. Microbiol. **23**:476-480.
30. **Wallis, C., and J. L. Melnick.** 1967. Concentration of enteroviruses on membrane filters. J. Virol. **1**:472-477.
31. **Wallis, C., and J. L. Melnick.** 1967. Concentration of viruses from sewage by adsorption to millipore membranes. Bull. W.H.O. **36**:219-225.
32. **Yeager, J. G., and R. T. O'Brien.** 1979. Structural changes associated with poliovirus inactivation in soil. Appl. Environ. Microbiol. **38**:702-709.
33. **Zeta-Meter, Inc.** 1900. Zeta-Meter manual ZM-77, 4th ed. Zeta-Meter, Inc., New York.