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Concentration of Viruses from Large Volumes of Tap Water Using Pleated Membrane Filters

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A method is described for the efficient concentration of viruses from large volumes of tap water in relatively short time periods. Virus in acidified tap water in the presence of aluminum chloride is adsorbed to a 10-inch (ca. 25.4 cm) fiberglass depth cartridge and a 10-inch pleated epoxy-fiberglass filter in series at flow rates of up to 37.8 liters/min (10 gallons/min). This filter series is capable of efficiently adsorbing virus from greater than 19,000 liters (5,000 gallons) of treated tap water. Adsorbed viruses are eluted from the filters with glycine buffer (pH 10.5) and the eluate is reconcentrated using an aluminum flocculation process. Viruses are eluted from the aluminum floc with glycine buffer (pH 11.5). Using this procedure, viruses in 1,900 liters (500 gallons) of tap water can be concentrated 100,000-fold in 3 h with an average recovery of 40 to 50%.

Wallis and Melnick (11) determined parameters that influence virus adsorption to membrane filters. These workers noted the enhancing effect of salts on virus adsorption and developed a virus concentrator using a membrane virus adsorber, clarifying filters, and $MgCl_2$ to enhance virus adsorption (10). Later work by Wallis et al. (9) indicated that $AlCl_3$ could replace $MgCl_2$ and could be used in much lower concentrations. While salts were required for optimal recovery of virus from surface water (7), low pH without added salts was found sufficient for recovery of virus from clean water (8). Modifications of the Wallis-Melnick virus concentrator have been described (2) and include the use of different filters as primary virus adsorbents (5) and the use of a proportioner pump for addition of salts and acids (3).

In the present study, additional modifications of the Wallis-Melnick virus concentrator that permit processing of large volumes of tap water at high flow rates are described.

MATERIALS AND METHODS

Virus and viral assays. Plaque-purified poliovirus type 1 (strain LSc) was used to seed tap water. The BGM cell line (1) was kindly supplied by Gerald Berg (Environmental Protection Agency, Cincinnati, Ohio) and was used for viral assays. Samples were assayed after being made isotonic and after fetal calf serum (FCS) was added to make a final concentration of 2% or after dilution in tris(hydroxymethyl)aminomethane-buffered saline containing 2% FCS. Samples (0.4 ml/bottle containing 25-cm^2 cell surface) were placed on cell layers for 30 min at 37 C before being overlaid with agar. Plaque-forming units were determined as previously described (6).

Virus concentrator. The virus concentrator used in these experiments has been described previously (8) and was supplied by the Carborundum Co. (Niagara Falls, N.Y.).

Virus adsorbents. Nitrocellulose filters (type HA, Millipore Corp., Bedford, Mass.), epoxy-fiberglass filters (series AA, Cox Instrument Corp., Detroit, Mich.), acrylonitrile polyvinylchloride copolymer filters (Acropor series, Gelman Instrument Co., Ann Arbor, Mich.), 10-inch (ca. 25.4 cm) glass fiber, melamine-impregnated paper, epoxy filters (Duo-Fine series, Filterite Corp., Timonium, Md.), and 10-inch honeycomb, fiberglass depth filters (model K-27, Commercial Filters Division, Carborundum Co., Lebanon, Ind.) were used. The characteristics of the honeycomb depth filters have been described previously (10).

Virus elution from membrane filters. Virus adsorbed to filters was eluted by passing 2,000 ml of 0.05 M glycine adjusted to pH 10.5 by addition of 10 N NaOH through the filters five times. The glycine was permitted to remain in contact with the filters for approximately 1 min during each passage. Eluates were neutralized with 0.05 M glycine adjusted to pH 2 with 12 N HCl.

Reconcentration procedure using flocculation. Neutralized eluates were made to 0.003 M $AlCl_3$. This resulted in a lowering of the pH to between 4 and 5. The eluates were then adjusted to pH 7.5 with 1 M sodium carbonate while the acidified fluids were stirred with a magnetic bar. The resultant floc was allowed to settle for 30 min. The supernatant was then removed by siphoning and the remaining floc was pelleted by centrifugation at 2,000 rpm for 5 min. Virus in the floc was eluted by mixing the floc with an equal volume of 1 M glycine adjusted to pH 11.5 with 10 N NaOH. This produced

a viscous suspension with a pH of 10.5. This mixture was then centrifuged, the supernatant was saved, and the floc was resuspended in another equal volume of glycine (pH 11.5). The mixture was centrifuged, the supernatants were pooled, and the remaining floc was discarded. The pooled supernatants were adjusted to pH 6 with 1 M glycine which had previously been adjusted to pH 2 with 12 N HCl. The floc formed was recovered by centrifugation and the supernatant was discarded. Virus in the floc was recovered by mixing it with an equal volume of 1 M glycine (pH 11.5), and then neutralizing the mixture with 1 M glycine (pH 2). FCS was added to make a final 2% concentration, and the sample was assayed directly or after necessary dilutions were made.

Virus sampling procedure. Tap water in a 500-gallon (1,900-liter) plastic container was acidified to pH 3.5 with 12 N HCl, dechlorinated with a final concentration of 0.05 mg/liter of sodium thiosulfate, and made to 0.0005 M $AlCl_3$ before addition of virus. The virus-seeded water was pumped through the filter system under test with a Jabsco pump (Jabsco Pumps, Costa Mesa, Calif.) powered by a 3/4 horsepower electric motor.

RESULTS

The virus concentrator developed in this laboratory and described previously (8) is capable of processing 378 liters of tap water with an average efficiency of recovery of added virus of 75%. When this concentrator is used to process larger volumes of tap water, the initial flow rate of 12 liters/min is rapidly reduced to 4 liters/min or less. Consequently, processing of 1,900 liters of water is a time-consuming process requiring over 6 h plus additional time for reconcentration of eluates from the primary filters. Since the factor limiting flow rates was found to be the 142-mm Cox filter rather than the K-27 prefilter, different filter types and configurations were considered as replacements for the Cox filter.

Cartridge filters have larger surface areas than flat filters and would therefore be expected to process larger amounts of water before clogging (Table 1). The components of different filters housed in 47-mm filter holders were tested for their ability to process large volumes of tap water before clogging and for their ability to adsorb virus. Tap water at pH 3.5 was passed through different 0.45-μm porosity 90-mm filters at initial flow rates of 2 to 9 liters/min until the flow rate decreased to approximately 1 liter/min. At intervals the filters were challenged with virus contained in 1 liter of tap water at pH 3.5. Virus in the influent and filter effluent samples was assayed to determine virus adsorption. The Acropor, Cox, Filterite and Millipore filters all adsorbed greater than 90% of the virus present in tap water at pH 3.5. However, the Acropor, Cox, and Millipore filters clogged after processing less than 20 liters of water, whereas the Filterite processed 150 liters of water before clogging (Fig. 1). Since the surface area of a 47-mm filter (approximately 10 cm^2) is 1/280th times the area of the 10-inch Filterite filter, the 10-inch filter would be expected to process over 40,000 liters of similar water before clogging. A partially unfolded 10-inch Filterite filter is shown in Fig. 2.

To determine optimum conditions for virus adsorption to Filterite filters, virus in 30 ml of tap water at different pH values was passed through a 25-mm, 0.25-μm Filterite filter at a flow rate of approximately 1 ml/min. Low pH greatly enhanced virus adsorption (Table 2). A pH of 3.5 resulted in greater than 90% adsorption and was used in subsequent experiments.

Since Filterite filters are capable of processing water at high flow rates, the effect of flow rate on virus adsorption was studied. Virus in 200 liters of dechlorinated tap water at pH 3.5 with and without a final concentration of

TABLE 1. *Surface areas of membrane filters*

Filter	Surface area (cm^2)	Relative surface area compared to a 142-mm flat filter
Cartridge filters		
Acropor, 10-inch (ca. 25.4 cm)	5,600	60
Filterite, 10-inch	2,800	30
Millipore, 22-inch (ca. 55.88 cm)	850	8
Flat filters		
293 mm	470	5
142 mm	97	1

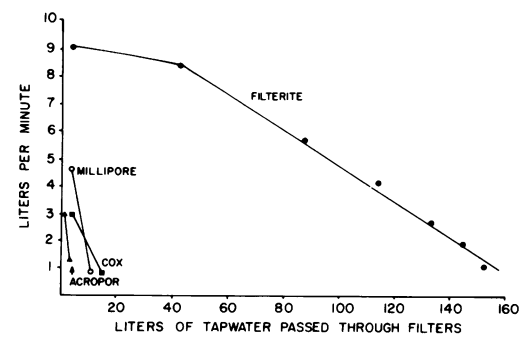

FIG. 1. *Comparison of Acropor, Cox, Filterite, and Millipore filters: flow rate with tap water at pH 3.5.*

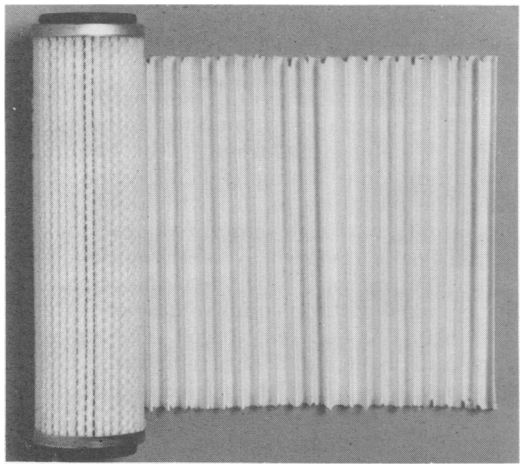

FIG. 2. *A partially unfolded 10-inch Filterite filter showing the pleated nature of the filter.*

TABLE 2. *Influence of pH on virus adsorption to Filterite filters*[a]

pH	Virus adsorbed (%)
6	10
5	10
4	90
3.5	>90
3.0	>90
2.5	>90

[a] Virus (3×10^5 plaque-forming units/ml) in 30 ml of 0.05 M glycine was passed through 0.25-μm Filterite filters in 25-mm holders at 60 ml/min.

0.0005 M $AlCl_3$ was passed through 10-inch, 0.25-μm porosity Filterite filters at flow rates of 5 to 40 liters/min. Virus in the filter influent and effluent was measured to determine virus adsorption. As shown in Fig. 3, virus in tap water at pH 3.5 without added $AlCl_3$ was adsorbed less efficiently at higher flow rates. Addition of 0.0005 M $AlCl_3$ permitted adsorption of virus at the maximum flow rates obtainable with the holders and filters employed.

The presence of a K-27 prefilter had little effect on the ability of Filterite filters to adsorb virus from tap water at pH 3.5. In a series of trials with and without a prefilter, between 50 and 75% of virus added to 1,900 liters of tap water could be recovered in the filter eluates. The K-27 did protect the Filterite from clogging. The protective effect of the prefilter was especially noted when 3,780 liters or more of tap water were processed. Protection of filters by K-27 prefilters has been noted before (10).

Tap water at pH 3.5 was passed through a 0.25-μm, 10-inch Filterite filter preceded by a K-27 prefilter to determine the maximum amount of water the combination could process before the filters clogged or lost their ability to adsorb virus. The flow rate was measured at intervals, and virus in 19 liters of tap water at pH 3.5 was passed through the filters. Virus in the effluent was measured to determine adsorption by the filters. As shown in Table 3, the filters were capable of adsorbing poliovirus in tap water after the filters had processed 19,000 liters of tap water. A flow rate of 26 liters/min was maintained throughout the course of the experiment. Since neither the flow rate nor the ability of the filters to adsorb virus diminished, the capacity of the filters remains to be determined. However, the filters appear adequate for processing volumes in excess of 3,780 liters.

Previous work with Cox filters had shown that elution with glycine (pH 11.5) gave optimal recovery of viruses (9). To reduce the possibility of viral inactivation at pH 11.5, elution with glycine (pH 10.5) was attempted. Elution with glycine (pH 10.5) gave lower and more erratic recoveries than elution with pH 11.5 when the eluent was passed through the filters one time (Table 4). Recycling the eluent through the filters five times permitted an average recovery

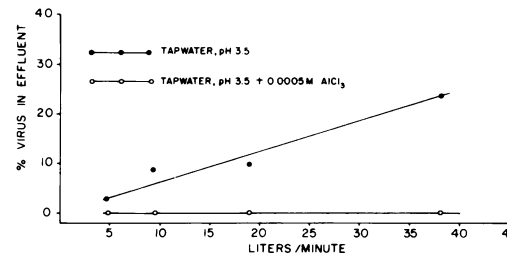

FIG. 3. *Influence of flow rate on adsorption of virus to 0.25-μm Filterite filters.*

TABLE 3. *Adsorption of virus to Filterite filters after passage of large volumes of tap water*

Water passed (liters)	Virus adsorbed to filters (%)	Flow rate (liters/min)
0	80	26
1,900	>90	26
3,800	>90	26
7,600	>90	26
11,400	80	26
15,200	80	26
19,000	90	26

[a] Virus (450 plaque-forming units/ml) in 20 liters of dechlorinated tap water (pH 3.5) was passed through the filters, after the indicated volume of water had previously been passed through the same filters at a flow rate of 26 liters/min.

TABLE 4. *Influence of eluent pH on elution of virus from a K-27/Filterite series*

Eluent pH	Times eluent passed through filters	Virus input/1,900 liters, (PFU)[a]	Virus recovered in eluate	
			PFU	Input (%)
11.5	1	2.4×10^8	1.2×10^8	50
		4.6×10^6	2.8×10^6	61
		1.6×10^7	1.4×10^7	86
		7.5×10^6	4.7×10^6	63
		8.7×10^6	6.2×10^6	78
		3.5×10^7	2.4×10^7	77
				69 = mean
10.5	1	3.1×10^6	1.6×10^6	52
		3.0×10^6	1.3×10^5	4
		4.1×10^6	8.0×10^5	20
		1.2×10^6	6.4×10^5	50
		1.0×10^7	7.3×10^5	7
		1.5×10^7	4.4×10^6	28
		1.8×10^7	1.5×10^5	1
				24 = mean
10.5	5	7.7×10^6	6.1×10^6	80
		8.5×10^6	6.0×10^6	71
		5.8×10^6	3.3×10^6	57
		5.0×10^6	2.4×10^6	48
				64 = mean

[a] PFU, Plaque-forming units.

of 64% of virus initially added to 1,900 liters of tap water.

Reconcentration of virus in initial eluates from filters used to process 380 liters or less of tap water can be accomplished by adjusting the eluates to pH 3.5 and 0.0005 M $AlCl_3$ and filtering the treated eluates through 0.47-μm epoxy-fiberglass filters. Under these conditions virus is adsorbed to the filters and can be eluted with glycine (pH 11.5) (8). When larger volumes of tap water are processed, the organic compounds concentrated in the initial virus-adsorbing step interfere with reconcentration on smaller filters by acting as membrane-coating components that interfere with virus adsorption and by forming flocs at low pH that clog the smaller filters.

Since previous work by Wallis and Melnick (12) had shown that virus readily adsorbs to inorganic floc, adsorption of virus in eluates to aluminum floc was studied. Addition of $AlCl_3$ to neutralized eluates lowered the pH to between 4 and 5. Adjustment of samples to pH 7.5 with 1 M sodium carbonate resulted in formation of a floc. The samples were mixed for 5 min and then the floc was allowed to settle for 30 min. The supernatant was removed by siphoning and discarded. The remaining floc was pelleted by centrifugation at 2,000 rpm for 5 min. A final $AlCl_3$ concentration of 0.003 M was the lowest that permitted a 2-log reduction in titer in the supernatant and was routinely used for reconcentration of initial filter eluates (Table 5).

Using the above procedure, approximately 50 ml of floc is obtained from 3,000 ml of initial eluate. Virus is eluted from the floc by mixing it with an equal volume of 1 M glycine at pH 11.5. The pH drops to 10.5 with mixing. The floc is only partially solubilized, and unsolubilized floc is removed by centrifugation. The clear supernatant is saved and the remaining floc is mixed with an equal volume of 1 M glycine (pH 11.5) and centrifuged. The supernatants are pooled and the remaining floc is discarded. The pH of the pooled supernatants is lowered using 1 M glycine (pH 2). A floc forms at approximately pH 9 and redissolves at pH 4. The maximum amount of virus is adsorbed to the floc at pH values between 5.5 and 6.0 (Fig. 4). The floc (5 to 10 ml from 3,000 ml of eluate) is collected by centrifugation and then the supernatant is discarded. Virus in the floc is recovered by mixing it with an equal volume of 1 M glycine (pH 11.5) and neutralizing with 1 M glycine (pH 2). Approximately 30 ml of eluate is produced. Before plating on cell monolayers, 2% FCS is added to the neutralized

TABLE 5. *Adsorption of virus to aluminum flocs formed in filter eluates*

Concn of $AlCl_3$ (mol/liter)	Virus in supernatant	
	PFU[a]	%
0	1.1×10^6	100
0.0005	6.9×10^5	63
0.001	9.0×10^4	8
0.003	$<1.0 \times 10^4$	<1
0.005	$<1.0 \times 10^4$	<1
0.01	$<1.0 \times 10^4$	<1

[a] PFU, Plaque-forming units.

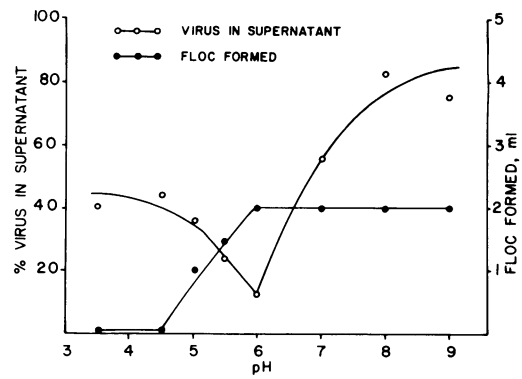

FIG. 4. *Influence of pH on floc formation and virus adsorption to floc.*

eluate. In comparative experiments, elution of virus from the floc with undiluted FCS resulted in lower virus recovery than did elution with glycine (pH 11.5) (Table 6).

The complete method for isolating virus from tap water is as follows: (i) adsorption of virus in tap water at pH 3.5 with 0.0005 M $AlCl_3$; (ii) elution of virus off the filters with glycine (pH 10.5); (iii) formation of an aluminum floc in the initial eluate; (iv) elution of virus from the gel with 1 M glycine (pH 11.5); (v) formation of a second floc by lowering the pH of the eluate from the first floc to between 5.5 and 6.0; and (vi) recovery of virus in the second floc by mixing with 1 M glycine (pH 11.5), neutralizing the mixture, and adding FCS to a final concentration of 2%. The complete procedure requires approximately 3 h for processing 1,900 liters. Each additional 1,900 liters would require an additional hour.

In a series of tests with high and low inputs of virus, a concentration of 100,000-fold with an average efficiency of recovery of 40 to 50% was obtained (Table 7). Since all of the final eluate can be plated, it should be possible to detect one virus particle present in 800 liters of tap water.

An advantage of Filterite filters is their ability to be reused. After elution with glycine (pH 10.5), the filters are treated with 0.1 N NaOH for 5 min to inactivate residual virus and to remove additional adsorbed organics. In the present work, both fresh filters and those that had been reused up to five times were tested. No apparent decrease in virus-adsorbing ability or flow rate was noted with the reused filters. Additional information on filter reuse will be published elsewhere.

DISCUSSION

The Wallis-Melnick virus concentrator (10) has been the basic model for most methods developed to recover viruses from large volumes of water. A recent model of this concentrator was found capable of efficiently concentrating virus from 380 liters of tap water (8). Our initial attempts to use this virus concentrator to sample larger volumes met with two problems: (i) low flow rates and clogging of the initial virus adsorbent, and (ii) concentration of organic compounds along with virus on the initial virus adsorbent. Elution of virus from the initial virus adsorbent also results in elution of the organics which interfere with virus adsorption to smaller filters and form flocs at low pH that clog smaller filters used for reconcentration. The first problem was overcome by replacing the flat 142-mm Cox filter with a 10-inch pleated Filterite cartridge filter as the primary virus adsorbent. The second was solved by eliminating filters in the reconcentration step and reconcentrating the initial filter eluate with an aluminum flocculation procedure.

Filterite cartridge filters provide larger surfaces for virus adsorption than flat filters without a corresponding increase in filter size. In addition, Filterite filters are able to process larger volumes of tap water than similar size Acropor, Cox, or Millipore filters. Besides being able to process large volumes of water, Filterite filters are capable of adsorbing virus in tap water at flow rates up to 38 liters/min. This flow rate is faster than that reported for the virus concentrator previously described from this laboratory (8) and for a recently described system that uses cartridge filters (4). Another advantage of Filterite filters is their ability to be reused, thus reducing operating costs.

Sobsey et al. (8) found that elution from filters used to process 380 liters of tap water resulted in elution of components that interfered with virus adsorption to small filters used for reconcentration. Addition of 0.0005 M $AlCl_3$ overcame the effects of the interfering components and permitted adsorption of virus in initial filter eluates to 47-mm Cox filters. Virus could then be recovered in small volumes by eluting the 47-mm filters with glycine (pH 11.5). This reconcentration method was used by Jakubowski et al. (4) for reconcentrating eluates from filters used to process 380 and 1,900 liters of water. However, we found that reconcen-

TABLE 6. *Elution of virus from aluminum floc*

Total virus added (PFU)[a]	Eluate (PFU)	
	Undiluted fetal calf serum	Glycine (pH 11.5)
445	220	
445		410

[a] PFU, Plaque-forming units.

TABLE 7. *Recovery of virus from 1,900 liters of tap water*

Virus added to 1,900 liters (PFU)[a]	Virus recovered	
	PFU	%
8.5×10^6	4.0×10^6	47
1.1×10^4	5.0×10^3	45
5.3×10^2	2.1×10^2	40
13	7	54
6	4	67
5	2	40

[a] Plaque-forming units.

trating eluates from filters used to process more than 380 liters of tap water on membrane filters was a difficult process. It required such high pressures and long times to filter the eluates through a 47- or 90-mm Cox filter as to be impractical. Previously, Wallis and Melnick (12) demonstrated virus adsorption to preformed aluminum flocs. In the present method, flocs were formed in the eluates rather than being preformed. In both cases, efficient virus concentration was achieved. Flocculation eliminates the problems produced by membrane-coating components and clogging associated with reconcentration procedures that use filters, although it introduces the requirement for centrifugation.

Although this work was concerned with tap water, it is likely that procedures using Filterite filters and reconcentration with aluminum floc will prove valuable in isolating viruses from surface, estuarine, and waste water. Current work in this laboratory is aimed at developing a field unit with Filterite filters that can be used to sample different types of water.

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