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Recovery of Poliovirus from Turbid Estuarine Water on Microporous Filters by the Use of Celite

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The application of a new step for recovering poliovirus from moderately to highly turbid estuarine water by the filter virus-adsorption technique was investigated. The experiments were conducted under both (i) laboratory-based conditions (200-ml volumes) where the turbidity was controlled and (ii) simulated field conditions (15- to 100-gal volumes) where the turbidity varied depending upon the hydrology of the raw estuarine water. The new step consisted of adding Celite to the turbid water prior to sampling for virus. In the experiments, the pH of the water was first adjusted to 3.5 and then $AlCl_3$ was added to 0.0005 M. Celite was added to a concentration of 0.01% and mixed thoroughly. Either an HE Cox M-780 microfilter (Cox Instrument, Div. of Lynch Corp., Detroit, Mich.) or an MF-membrane filter (Millipore Corp., Bedford, Mass.) was used as the virus adsorbent. Virus was eluted from the Celite-filter complex in situ at pH 9 with 5× nutrient broth. In the laboratory-based experiments, when turbidity ranged from 5.0 to 30.0 Jackson turbidity units (JTU), virus recovery ranged from 66 to 89%. In the simulated field experiments, when the turbidity ranged from 8.5 to 80.0 JTU, virus recovery ranged from <1 to 74%, depending upon the multiplicity of virus input and the level of turbidity. The new step greatly improved the filtration-flux of turbid water and significantly reduced the premature clogging problem usually observed with microporous filters.

The distribution of enteric viruses in the estuarine environs has not been adequately defined because of a de facto lack of suitable methodology for examining large volumes of water. The epidemiological incrimination of shellfish as a potential source of infectious hepatitis outbreaks, on the other hand, has been well documented (4, 11, 15). Consequently, a need exists for a reliable method that can be adapted to the examination of shellfish-growing waters for the occurrence of enteric viruses. Of the several methods available, adsorption of viruses to microporous filters within a flow-through sampling system, i.e., the filter virus-adsorption technique, appears to be the most promising for detecting viruses in 25- to 100-gal volumes of water (1, 6, 7, 18, 19). However, examination of raw waters containing suspended particulate matter has presented problems due to clogging of the filter surfaces. This has limited the volume of water that could be processed and negates the applicability of the filter virus-adsorption technique for detecting low multiplicities of virus in moderately to highly turbid waters. The use of Tween 80-treated and untreated fiberglass prefilter pads (e.g., AP 20 prefilter pads, Millipore Corp., Bedford, Mass.) has been used to overcome very low turbidity problems with varying degrees of success (13, 18). More recently, an apparatus consisting of several yarn-wound clarifying filters coupled with an MF-membrane filter (Millipore Corp.) as the virus adsorbent has been described for recovering enteric viruses from large volumes of tap water heavily contaminated with suspended solids (19). Microporous filters, particularly MF-membrane filters, therefore, have been shown to be effective as virus-adsorbents for recovering virus from water (1, 7, 13, 14, 18). We previously reported on a technique that consisted of first adsorbing virus to MF-membrane filters followed by aqueous polymer two-phase separation of the eluates (7). This was a highly sensitive and simple proce-

dure for quantitatively recovering low multiplicities of poliovirus from 100-gal volumes of prefiltered waters. We have now examined the efficacy of recovering virus from turbid estuarine water by the use of Celite (a filter aid) in combination with the filter virus-adsorption technique. This third report of a series (7, 10) describes our observations.

MATERIALS AND METHODS

Virus. The LSc2ab strain of poliovirus type 1 was used throughout the study. The virus stock was kindly supplied by the Lederle Research Laboratories, American Cyanamid Co., Inc. The virus was propagated in HEp-2 cells. The virus was filtered to remove viral aggregates by a technique modified after Ver et al. (17). A 10-ml volume of 10% fetal calf serum in sterile distilled water was filtered through a 47-mm, 0.22-μm porosity membrane filter and made isotonic with 10× BME. The filtrate was passed through a 47-mm, 50-nm porosity membrane filter, and the serum-treated filter was then rinsed with 5 ml of sterile distilled water. The filter was then used to prepare monodispersed poliovirus stock by filtration. The monodispersed virus was stored in 1-ml quantities at −80 C until used.

Cell culture. HEp-2 cells were used for plaque assays. The procedures for cultivating the cells, preparing cell monolayers, and handling the cell overlay have been described previously (8).

Plaque assay. The plaque assay procedure as modified by Hsiung and Melnick (9) was used throughout the study. Counts were expressed as plaque-forming units (PFU). For purposes of assay, serial 10-fold dilutions of virus were made in nutrient broth (5), and 1 ml of each virus dilution was inoculated onto cell monolayers. All samples were assayed for virus by conducting five subsample replicate titrations. Assays were repeated when the expected variability of the mean of replicate subsamples exceeded by 1.5 standard deviations that were predicted by the Poisson distribution.

Virus adsorbents. Two types of microporous filters were used throughout the study as virus adsorbents: (i) MF membranes composed primarily of cellulose nitrate (1, 3) with a 0.45-μm porosity and (ii) HE Cox M-780 microfilters composed of microsized glass and asbestos fibers bound in epoxy saturant with a 0.45-μm porosity (Cox Instrument, Div. of Lynch Corp., Detroit, Mich.). Filters with a diameter of 47 mm (9.6 cm^2 surface area) were used in the laboratory-based experiments (200-ml volume runs). Filters with a diameter of 293 mm (486 cm^2 surface area) were used in all the large volume simulated field experiments (15- to 100-gal volume runs). In some of the experimental setups, an AP20 Microfiber Glass Disc prefilter (Millipore Corp.) placed on top of the virus-adsorbent filter was used also as an adjunct to the Celite as a clogging preventive. An MF-membrane filter holder was used in all the experimental setups.

Water supply. The raw estuarine water used in the simulated field experiments was pumped into the laboratory from Dauphin Island Bay, Alabama. Details of the pumping system have been described previously (6, 8). Turbidity was measured with a Hach Turbidimeter Model 2100A (Hach Chemical Co., Ames, Iowa) and expressed as Jackson turbidity units (JTU). Salinity was measured with an AO T/C refractometer (AO Instrument Corp., Buffalo, N.Y.) and expressed as parts per thousand (g/kg).

Celite. Celite analytical filter aid, a product of Johns-Manville, was used to facilitate the filtration of turbid estuarine water through the filters. The Celite was used at a concentration of 0.01 to 0.06% depending upon the experimental conditions (see Results, Table 5).

Experimental. In the laboratory-based experiments, the desired turbidity was produced by adding "marine silt" which consisted of natural estuarine bottom mud to the seawater. In the simulated field experiments, raw estuarine water as occurred in the Bay at the time of the experimental runs was used. Consequently, these experiments were conducted under changing hydrologic conditions whereby salinity and turbidity of the seawater varied. In all the experiments, the acidity of the water was first adjusted to pH 3.5 ± 0.1 with HCl. $AlCl_3$ was added to the water to a final concentration of 0.0005 M (18) when salinity of the water was less than 20 g/kg. The Celite was then added to the desired concentration and mixed thoroughly. Monodispersed virus was thawed and diluted in distilled water to the desired multiplicity immediately before use. Virus was then added to the test water. The virus-contaminated water was then pressure filtered through the virus-adsorbent filters. Virus was eluted from the Celite-filter complex in situ at pH 9 with 5× nutrient broth in 0.05 M carbonate buffer (8). When the 47-mm size filter system was used, elution was carried out under negative pressure by using 10 ml of eluant. When the 293-mm size filter system was used, elution was carried out under positive pressure using 1,000 ml of eluant. The latter was done by connecting a 1-gal (3.785-liter) pressure vessel to the MF-membrane filter holder. Approximately 200 ml of eluant was then carefully forced into the holder. This essentially filled the void area. Eluant was allowed to remain in contact for 15 min before forcing the remaining eluant through the setup. The pH of all eluates was adjusted to 7.2 for either (i) direct virus assay when the virus input was high, i.e., ≥1 PFU per ml or (ii) further concentrated by the aqueous polymer two-phase separation technique (16) when the virus input was low, i.e., 1 to 20 PFU per gal. The aqueous polymer two-phase separation technique was conducted as previously described (8) with the exception that sodium dextran sulfate 500 (Sigma Chemical Co.) was substituted for the sodium dextran sulfate 2000 which is no longer commercially available.

RESULTS

Effects of Celite on filtration-flux on turbid estuarine water. Twenty-two filtration-flux runs were conducted by using 293-mm size

filters to ascertain the efficacy of the Celite-filter systems to filter large volumes of highly turbid estuarine water. Both types of virus-adsorbent filters (see Materials and Methods) were subjected to testing. Turbidities tested ranged from 3.5 to 30.0 JTU. At the lower turbidities, e.g., 3.5 to 8.0 JTU, both filter types exhibited comparable filtration capabilities when Celite was added to a final concentration of 0.01%. When Celite was not added, severe clogging of all the filters occurred rapidly. For example, within a 30-min period, when turbidity was 8.0 JTU, the addition of the Celite caused approximately a sixfold increase in the total volume of water filtered through the MF-membrane filter system, i.e., 5.9 gal versus 34.9 gal (Table 1).

The results of several 30-min MF-membrane filter runs are also illustrated in Fig. 1. It is quite obvious that the addition of 0.01% Celite to the turbid water prior to filtration increased the filtration capacity of the system significantly. Additionally, at turbidity levels as high as 8.5 JTU, no serious clogging problems were observed while filtering 100-gal quantities of water even though it took more than 2 h to filter this volume. At turbidities of 15.0 to 30.0 JTU, however, it was necessary to include an AP20 prefilter in addition to the Celite in order to filter 50 to 100 gal of water through the filter setups within the same time period. Conversely, at the turbidity level of 7.0 JTU conducted without Celite, the MF-membrane filter system was virtually clogged after 30 min. Similar results were also observed with the HE Cox M-780 microfilter system.

TABLE 1. *Filtration of turbid (8 JTU) estuarine water by use of Celite*

Experimental setup	Time (min)	Gal filtered[a]	Accumulated gal filtered
MF-membrane Celite added[b] (0.01%)	5	7.4	7.4
	10	6.1	13.5
	15	6.0	19.5
	20	5.6	25.1
	25	5.0	30.1
	30	4.8	34.9
MF-membrane without Celite[c]	5	3.2	3.2
	10	0.9	4.1
	15	0.5	4.6
	20	0.5	5.1
	25	0.4	5.5
	30	0.4	5.9

[a] Values indicate volume measured at 5-min intervals.

[b] Total gallons filtered, 102.6 (2.40 h):

[c] Virtually clogged after 30 min.

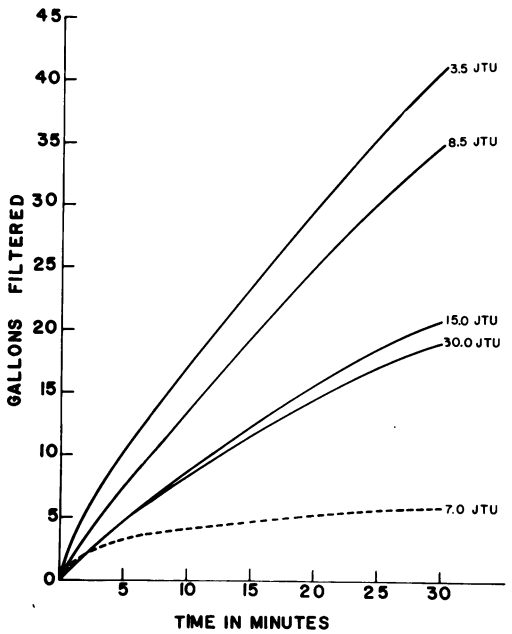

FIG. 1. *Filtration-flux characteristics of turbid estuarine water with and without the addition of Celite. Solid lines, 0.01% Celite added; dashed line, no Celite added. The 293-mm size MF-membrane filter was used.*

Recovery of poliovirus from turbid estuarine water: laboratory-based experiments. To evaluate virus recovery efficiency from the Celite-filter complex, nine experiments were conducted in which 200 ml of virus-contaminated turbid estuarine water containing 0.01% Celite was filtered through a 47-mm size microporous filter. The total virus input per 200-ml sample was 10,674 PFU. Turbidities tested were 5.0, 15, 0, and 30.0 JTU. Cation as Al^{3+} was not added to the water in these experiments because the salinity exceeded 20 g/kg. Elution was conducted with 10 ml of eluant. The results are shown in Table 2. When the MF-membrane filter was used, virus recovery ranged from 67 to 79% with a mean virus recovery of 73% for all turbidities tested. When the HE Cox M-780 microfilter was used, virus recovery ranged from 72 to 89% with a mean virus recovery of 78%. When the HE Cox M-780 microfilter combined with the AP20 prefilter was used, virus recovery ranged from 66 to 81% with a mean virus recovery of 72%. Efficiency of virus recovery was similar with all three setups conducted under all three turbidity levels. The overall virus recovery was 74%, a value considered to be good to excellent.

Recovery of poliovirus from large volumes of turbid estuarine water: simulated field experiments. The application of the filter virus-adsorption technique to field situations requires that large volumes of at least 25 to 100 gal of water be sampled, particularly when viruses occur at low multiplicities in the water. Therefore, a need existed for evaluating the Celite-filter system under simulated field conditions, using large volumes of raw water artificially contaminated with virus. This was done in two series of experiments. One series of experimental runs was conducted at a high multiplicity of virus input ($\geq$1 PFU per ml), and one series was conducted at a low multiplicity of virus input (1 to 20 PFU per gal). The high virus input experiments were conducted in order to examine the efficiency of virus recovery from 50- to 100-gal volumes of turbid estuarine water. The low virus input experiments were conducted to examine the sensitivity of the method. In all the experiments, Celite was added to the turbid water at a final concentration of 0.01% before filtration and virus seeding. The results of runs with a high multiplicity of virus input are shown in Table 3. At a turbidity level of 8.5 JTU, virus recovery was 48% when the MF-membrane filter was used as the virus adsorbent and 59% when HE Cox M-780 microfilter was used. At a turbidity level of 15.0 JTU, virus recovery was 63% when the MF-membrane filter was used, 74% when the HE Cox M-780 microfilter was used, and 35% when the combination of the MF-membrane filter and the AP20 prefilter was used. From an efficiency standpoint, these virus recoveries were considered good to excellent. This is particularly true considering the highly turbid conditions and the volumes of water examined. When the turbidity reached 15.0 JTU, filter clogging prevented filtration of volumes exceeding 50 gal within a 2.5-h period even with the use of Celite. The use of an AP20 prefilter in conjunction with the membrane filter, however, facilitated the filtration of the higher turbid water at greater volumes, i.e., approximately 100 gal in 2.5 h. With waters having a turbidity of 8.5 JTU or below, no clogging occurred that would prevent the filtration of 100 gal even though the filtration-flux was considerably decreased toward the end of the experimental period.

TABLE 2. *Recovery of poliovirus from turbid estuarine water by use of Celite:laboratory-based experiments*[a]

Experimental setup	Turbidity (JTU)[b]			
	5.0	15.0	30.0	Mean
MF-membrane filter	79[c]	67	74	73
HE Cox M-780 microfilter	72	89	72	78
HE Cox M-780 + AP20 prefilter	66	69	81	72
Mean	72	75	76	74

[a] Salinity: 23.3 g/kg. Celite analytical filter aid: 0.01%.
[b] Total virus input: 10,674 PFU/200 ml of sample.
[c] Percent of virus recovered.

The results of the low multiplicity of virus input runs are shown in Table 4. Virus recovery was very low, i.e., <1 to 2.2%. Despite this low virus recovery, nonetheless, virus was detected in all experiments, a remarkable finding considering that the turbidity of the raw water had increased several orders of magnitude to a high of 35.0 JTU. It should also be noted that the quantity of water that could be effectively filtered in a reasonable time was also diminished. Filter clogging became essentially prohibitive after 25 to 30 gal had been filtered when the turbidity was 35.0 JTU.

Effect of various concentrations of Celite on recovery of poliovirus from highly turbid

TABLE 3. *Recovery of high levels of poliovirus from large volumes of turbid estuarine water by use of Celite: simulated field experiments*[a]

Experimental setup	Vol of water[b] (gal)	Turbidity of water (JTU)	Total virus input (PFU $\times 10^5$)	Total virus recovered (PFU $\times 10^5$)	Total virus recovered (%)
MF-membrane filter	103	8.5	3.22	1.54	48
293 mm	50	15.0	3.37	2.12	63
MF-membrane 293 mm + AP20 prefilter	97	15.0	6.52	2.30	35
HE Cox M-780 microfilter	102	8.5	3.22	1.90	59
293 mm	47	15.0	3.17	2.34	74

[a] Celite analytical filter aid: 0.01%.
[b] Salinity of the water samples ranged from 6.0 to 20.0 g/kg.

TABLE 4. *Recovery of low levels of poliovirus from large volumes of turbid estuarine water by use of Celite:simulated field experiments*[a]

Expt no.[b]	Vol of water[c] (gal)	Turbidity of water (JTU)	Calculated total virus input[d] (PFU)	Total virus recovered (PFU)	Total virus recovered (%)
1	90	8.5	56	1	1.8
2	50	18.0	692	7	1.0
3	50	18.0	692	4	0.6
4	50	23.0	45	1	2.2
5	30	35.0	554	9	1.6
6	30	35.0	554	2	0.4
7	24	35.0	444	5	1.1

[a] Celite analytical filter aid: 0.01%.

[b] An AP20 prefilter was used in combination with the MF-membrane filter in all runs except experiment 1.

[c] Salinity of water ranged from 0.0 to 12.0 g/kg.

[d] Virus was initially added to 100 gal of water. The total virus input was then calculated based upon the volume filtered through the system.

estuarine water. During one of the filtration-flux runs, when the raw estuarine water had a turbidity of 30 JTU, the Celite concentration was doubled to 0.02% in a parallel experiment. The results indicated approximately a 35% increase in total volume of water filtered during a 40-min filtration run. Consequently, it was decided to determine the influence of greater concentrations of Celite for filtering water having higher turbidities and concurrently evaluating virus recovery. Turbidity of the raw estuarine water was 60.0 and 80.0 JTU. Virus inputs ranged from 570 to 1,500 PFU per 100 gal; therefore, these studies were considered to be low multiplicity of virus input type of experimental runs. The results are shown in Table 5. When the turbidity of the water increased to levels as high as 60.0 and 80.0 JTU, the systematic increase in Celite concentration from 0.02 to 0.06% effected only a marginal increase in filtration capacity. The recovery of poliovirus from these experiments was also low, ranging from less than 1 to approximately 4%. Despite this, virus was detected in every experiment even though the virus input was as low as 6 PFU per gal of water.

DISCUSSION

The present study concerned the successful introduction of a new step for filtering turbid water into the filter virus-adsorption technique reported by Cliver (3), Wallis et al. (18), Rao and Labzoffsky (13), and adapted to large volumes of water by Hill et al. (8). One of the major limitations of the filter virus-adsorption technique has been the problem of filter clogging when waters to be examined contained suspended particulate matter. This problem became increasingly more acute when the need arose for examining 50 to 100 gal of water, for when viruses occur at low multiplicities in water, large volumes must be processed to effect their detection.

The addition of various filter aids for increasing filtration efficiency of filters is not a new concept. In fact, Celite has been used to aid membrane filtration of influenza virus in aqueous suspensions (12) and also in turbid water samples for bacteriological examination (M. Presnell, personal communication; 2). The major departure of our studies concerned the evaluation of a Celite-filter system for recovering virus from large volumes of moderately to highly turbid estuarine water. The Celite apparently functioned to form a type of "pre-filter" in situ during the filtration process. In any event, large volumes of poliovirus-seeded waters having turbidities of 8.5 to 15.0 JTU were readily filtered in the system and, as shown in the results, virus was efficiently recovered from the Celite-filter complex. When the turbidity of the estuarine water occurred at levels at 30.0 to 80.0 JTU, difficulty was observed in filtering volumes of water greater than 15 to 30 gal. In addition, recovery of poliovirus was also significantly reduced.

During the course of the study, no significant difference was observed between the MF-membrane filter and the HE Cox-780 microfilter to adsorb poliovirus. Additionally, the filtration flux was not improved by either of the virus-

TABLE 5. *Effect of various concentrations of Celite on recovery of poliovirus from highly turbid estuarine water*[a]

Celite concn (%)	Vol of water[b] (gal)	Turbidity of water (JTU)	Calculated total virus input[c] (PFU)	Total virus recovered (PFU)	Total virus recovered (%)
0.02	23	60.0	349	5	1.4
0.03	30	60.0	455	4	0.9
0.03	15	80.0	227	2	0.9
0.04	18	80.0	103	4	3.9
0.05	15	80.0	86	1	1.2
0.06	23	80.0	132	1	0.8

[a] In all experiments, an AP20 prefilter was used in combination with an MF-membrane filter.

[b] Salinity of the water ranged from 0.0 to 4.0 g/kg.

[c] Virus was initially added to 100 gal of water. The total virus input was then calculated based upon the volume filtered through the system.

adsorbents used. We did note, however, that the HE Cox-780 microfilter failed to seal in the 293-mm MF-membrane holder on occasion, and some water leakage occurred during the filtration process. Consequently, in the majority of simulated field experiments, the MF-membrane filter was used as the virus-adsorbent.

There is little doubt that Celite effectively facilitated the filtration of large volumes (50 to 100 gal) of water when the turbidity levels ranged from 3.5 to 15.0 JTU. At turbidities exceeding 23.0 JTU, filtering 50-gal quantities was prohibitive because of the running time and the obvious clogging that occurred during a 2-h filtration run. The systematic increase of the Celite concentration above 0.01% to a level as high as 0.06% did not appreciably influence the filtration capacity at the extreme turbidity levels of 60.0 and 80.0 JTU. Of equal importance, it was also noted that the void space unique to the MF-membrane filter holder placed a limitation on the total quantity of Celite that could be effectively impinged onto the surface of the microporous filters. Consequently, in our opinion, 0.01 to 0.02% Celite concentration would be maximal when attempting to filter 50 to 100 gal of water having turbidities up to 23.0 JTU.

The decrease in virus recovery efficiency at the higher turbidity levels observed in our experiments was somewhat disappointing even though the test virus was detected at all levels of turbidity examined. Wallis et al. (19) observed a similar decrease in virus recovery efficiency while filtering progressively larger volumes of Houston city tap water which contained high levels of suspended solids. Our low virus recoveries seemed to be primarily a function of elution efficiency from the Celite-AP20 prefilter-membrane filter complex. Other investigators using our technique (F. M. Wellings, personal communication) have realized greater virus recoveries from the Celite-filter complexes by grinding the complexes and then centrifuging to remove the debris. This approximately doubles virus recovery. Additionally, some virus loss could have occurred as a result of using the AP 20 prefilter in conjunction with the microporous filter system as a clogging preventive. The loss of virus on prefilters has been reported by others (14). On the other hand, we did not observe a significant virus loss when high multiplicities of virus input were used (see Tables 2 and 3). Perhaps, the use of sonication as proposed by Berg et al. (1) or pretreating the prefilters with Tween-80 (18) would aid recovery of virus from the particulate-filter complexes. In any event, further work is needed to resolve the problem of recovering small amounts of virus from waters having very high turbidities.

It should also be noted that we used monodispersed virus in all the experiments reported. This seemed to eliminate recovering greater than 100% (exceeding random variability) of virus input. This observation has been attributed to viral aggregation and disaggregation phenomenon. With crude stock virus (clarified of host cell debris), we have observed greater than 100% recovery of virus on numerous occasions. Consequently, we were prompted to use monodispersed virus in all subsequent experiments concerning virus recovery efficiency.

We do not propose that the use of Celite in conjunction with microporous filters will solve all the conceivable problems that may be encountered in recovering virus from turbid waters. We do feel, however, that our studies represent a definitive improvement in and an extension of the application of the filter virus-adsorption technique for recovering viruses from raw waters that contain various levels of suspended particulate matter.

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