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# COMPARISON OF POSITIVELY CHARGED MEMBRANE FILTERS AND THEIR USE IN CONCENTRATING BACTERIOPHAGES IN WATER

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**Abstract**—Virus adsorption–elution studies and physical measurements were performed on four electropositive membrane filters (Virosorb 1MDS, Posidyne N66, Zeta plus C-30 and Seitz S). The relative hydrophobicity of the membrane filters was determined by measuring the contact angle of carbon tetrachloride with the filters and by measuring the rate of rise of hexadecane and water in the filters. Viruses adsorbed to the least hydrophobic filter (Virosorb 1MDS) could be eluted by using a solution of salt (NaCl or NaEDTA) alone to disrupt electrostatic interactions between the viruses and the filter. Solutions containing both salt and detergent were required to elute virus adsorbed to two of the more hydrophobic filters (Posidyne N66 and Zeta plus C-30), indicating that both electrostatic and hydrophobic interactions were responsible for viral adsorption to these filters. A two-step procedure for recovering bacteriophages from water was developed that employed different filters for each step. Virosorb 1MDS was used to adsorb indigenous phage in water samples. Adsorbed virus could be recovered by treating the filter with a solution of 1.0 M NaCl. Phages in this solution could be further concentrated by a second adsorption–elution step that used Seitz S filters.

*Key words*—bacteriophage, positively-charged membrane filters, phage concentration

## INTRODUCTION

In recent years, investigators have studied procedures for concentrating bacteriophages in natural waters. This work has been done for three main reasons. First, the efficiency of a wastewater treatment plant has been studied using bacteriophage as a model for virus removal (Safferman and Morris, 1976). Secondly, several investigators have shown that concentration of bacteriophages is essential in determining more about the ecology and distribution of phage in natural water systems (Seely and Primrose, 1979, 1982). Finally, several investigators have advanced the idea of using bacteriophages as indicators of fecally contaminated water (Drury and Wheeler, 1982; Hilton and Stotzky, 1973; Keswick, 1982; Kott *et al.*, 1974).

In recent years, methods for the detection and concentration of animal virus from water samples has greatly improved. Adsorption to and subsequent elution from membrane filters is still considered to be among the best methods for detecting small numbers of animal viruses in large volumes of water (Goyal and Gerba, 1982). Two general types of filters have been used. Electronegative filters have net negative charges at pH values between 4 and 6. In contrast electropositive filters have net positive charges or slight negative charges over the same pH range (Sobsey and Glass, 1980; Sobsey and Jones, 1979). Both types of filters have been used to recover enteroviruses from water. Negatively charged filters have been shown to be unsuitable for bacteriophage concentration, primarily because phages are not as stable as enteroviruses at the low pH values required for adsorption of viruses to these filters. Investigators have recently explored the use of electropositive membrane filters, which allow for the concentration of viruses without exposing the phages to extremes in pH (Seely and Primrose, 1982; Singh and Gerba, 1983).

Several positively-charged filters of varying composition are now commercially available. In this report, the association of phage to several types of filters was studied under different conditions. It was found that the filters were similar in their ability to adsorb phage in tapwater. However differences were observed in the ability of certain solutions to elute phage adsorbed to the filters. These differences in the elution of adsorbed phage were related to differences in the relative strength of the electrostatic and hydrophobic interactions between the phages and the filters and were found to correlate well with physical parameters of the filters such as the contact angle of a nonpolar liquid with the filter. The differences in adsorption–elution characteristics of the filters were used to develop a two-stage procedure for concentrating bacteriophage from water using dissimilar membrane filters for each stage. Bacteriophages in water were first adsorbed to and eluted from one

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Table 1. Filter characterization

| Filter | Pore size | Contact angle* | Major components | Source |
|---|---|---|---|---|
| Virosorb 1MDS | 0.45 μ | 151° | -Fiberglass/cellulose/ "surface-modified resin" | AMF Cuno, Inc., Meriden, Conn. |
| Posidyne N66 | 0.45 μ | 128° | Nylon 66 (polyamide) resin | Pall Trinity Micro Corp., Cortland, N.Y. |
| Zeta plus C-30 | 0.6–2.0 μ | 128° | Cellulose/diatomaceous earth/"charge-modified resin" | AMF Cuno, Inc., Meriden, Conn. |
| Seitz S | 0.5 μ | 113° | Asbestos–cellulose | Republic Filters, Milldale, Conn. |

*Contact angle of carbon tetrachloride on water-saturated filters.

membrane filter, and then further concentrated by adsorption to a smaller filter of different composition. Phages adsorbed to this filter were then recovered in a relatively small volume of eluting solution.

## MATERIALS AND METHODS

### *Phage and phage assays*

Bacteriophage T7, obtained from the department of Microbiology and Cell Science, University of Florida, was used in laboratory studies on phage adsorption and elution. T7 phage was ennumerated by a soft agar plaque procedure previously described (Farrah, 1982) using *Escherichia coli* B(ATCC 11303) as the host bacterium. Indigenous bacteriophages were studied similarly using both *E. coli* B and *E. coli* C-3000 (ATCC 15597) as the host bacterium.

### *Filters*

The filters used in this study, their characterization and sources, are shown in Table 1.

### *Chemicals*

The chemicals used in this study and their sources were as follows: chloroform, orthotolidine, imidazole and sodium chloride (Fisher Scientific Co., Fair Lawn, N.J.); sodium thiosulfate, Tween 80 and ethylenediaminetetraacetic acid (EDTA) (Sigma Chemical Co., St Louis, Mo.); sodium sulfate (Mallinckrodt, Inc., Paris, Ky); and beef extract (Inolex Corp., Glenwood, Ill). Solutions were adjusted to the desired pH by the addition of hydrochloric acid or sodium hydroxide.

### *Water samples*

The water samples used for indigenous phage concentration and their sources are described in Table 2.

### *Capillary rise measurements*

Filters were cut in strips measuring 20 cm long and 1 cm wide. These strips were then dipped in distilled water or hexadecane 1 cm deep. The time at which the strip was dipped in the liquid was taken as the zero reference time. The rise of liquid on the filter was measured at one minute intervals for 12 min. The height of liquid of the filter vs time was plotted using the mean values of two trials (Hiemenz, 1977).

### *Contact angle measurements*

A cubic cell (3 × 3 × 3 cm) was filled with distilled water and a filter was placed in the cell and allowed to stand for 1 h in order to be saturated with water. A small drop of carbon tetrachloride was injected in the water just above the filter surface using a 50 μl syringe (Hamilton Co., Reno, Nev.). The contact angle between the filter and droplet was measured using a NRL Goniometer Model A-100, Rame-Hart, Inc. (Osipow, 1977).

### *Adsorption and elution studies using T7*

Tap water was dechlorinated by the addition of sodium thiosulfate; the absence of residual chlorine was determined using the orthotolidine method (APHA, 1975). The tap water was adjusted to pH 4, 7 or 9 using 0.1 N NaOH or HCl. Approximately $10^7$ PFU of T7 phage was added to the treated tap water and 10 ml passed through the filters, which were held in 25 mm holders, at a rate of 1 ml $s^{-1}$. Viruses in the tap water and in the filter effluent were assayed to confirm retention by the filters. Next, 10 ml of a test eluent (see Tables) was passed through the filters, and the eluted viruses were assayed. The virus eluted was expressed as a percentage of the virus present in the initial tap water solution. Values were obtained in 2–3 trials and represent the means of 4–6 determinations.

### *Concentration of indigenous bacteriophages*

Samples of water were collected in pressure vessels and brought back to the laboratory for immediate processing. A five ml aliquot was removed to determine the input concentration of bacteriophage. For the one-step procedure, samples were passed through an AP20 prefilter (Millipore Corp., Bedford, Mass.) and 3 layers of Virosorb 1MDS filters without pH adjustment. The filtrates were collected and assayed to determine bacteriophage adsorption. Next, 1.0 M NaCl in 0.05 M imidazole buffered at pH 7 was used to elute the adsorbed phages. The eluate volume was 5 ml when filters were held in 25 mm holder, 10 ml for 47 mm filter and 40 ml for 90 mm filters. The two-step concentration procedure started as the one-step procedure described above, with 90 mm filters used. The 40 ml sodium chloride eluate was pulled through a 47 mm Seitz S filter using vacuum filtration without pH adjustment and collected to check for phage adsorption. Next, 10 ml of a 4% beef extract and 0.1% Tween 80 solution at pH 7 was placed on the filter and 2–3 ml was pulled through using a vacuum to displace residual fluid and ensure that the beef extract/detergent eluate completely soaked the filter. The remaining 7–8 ml of the eluate remained on the filter, soaking for 5 min, then was pulled through and assayed for phage elution.

Table 2. Characterization of natural water samples*

| Water sample | pH | Turbidity (NTUs) | Conductivity (mhos) | Organics† |
|---|---|---|---|---|
| Cypress strand | 6.9–7.2 | 46–61 | 350–500 | 0.48–0.58 |
| Secondary effluent | 6.9–7.4 | 2.8–3.1 | 350–460 | 0.09–0.16 |
| Holding pond | 7.0–7.5 | 52–60 | 600–720 | 0.46–0.51 |
| Land runoff | 7.6–7.9 | 2.4–3.7 | 420–630 | 0.37–0.41 |

*Range of each measurement is indicated.
†Absorption at 254 nm, u.v. light (Dobbs *et al.*, 1972).

Table 3. Elution of T7 adsorbed to positively-charged membrane filters by solutions* at pH 7

| Filter | % Adsorbed virus eluted by | | | |
|---|---|---|---|---|
| | Buffer alone | 0.1% Tween 80 | 0.5 M EDTA | 0.1% Tween 80 +0.5 M EDTA |
| Virosorb 1MDS | 7 ± 6 | 8 ± 8 | 74 ± 7 | 105 ± 20 |
| Zeta plus C-30 | 0 | 49 ± 9 | 0 | 72 ± 2 |
| Posidyne N66 | 8 ± 3 | 37 ± 9 | 5 ± 1 | 78 ± 14 |
| Seitz S | 0 | 0 | 0 | 22 ± 8 |

*All solutions contained 0.05 M imidazole and were adjusted to pH 7.0.

## RESULTS

Initial studies were conducted to determine the adsorption of bacteriophage T7 to the four membrane filters. In these experiments, tapwater was dechlorinated and adjusted to three different pH values (4, 7 and 9). The results of these studies indicated that adsorption of T7 to these filters was efficient at all three pH values (99% adsorbed). Since it permitted adsorption of virus over a wide pH range, tapwater was used as the adsorbing solution in subsequent studies.

The results of preliminary studies on the ability of detergents and salts to elute T7 phage adsorbed to the four positively charged membrane filters are shown in Table 3. A solution of the buffer alone eluted <10% of the adsorbed phage from all of the filters tested. A solution containing a neutral detergent (Tween 80) at pH 7 did not elute any T7 adsorbed to the Seitz filters and less than 10% of the phage adsorbed to the Virosorb 1MDS filters. This detergent solution eluted greater than 35% of the T7 adsorbed to the Zeta plus C-30 or Posidyne N66 filters. A solution of EDTA eluted 74% of the virus adsorbed to the Virosorb 1MDS filter but less than 5% of the virus adsorbed to the other filters. It has been previously shown that solutions containing salts and detergents are very efficient at eluting viruses adsorbed to electronegative filters (Shields and Farrah, 1983). When a solution containing 0.5 Na EDTA as the salt and 0.1% Tween 80 as the detergent was used with the positively charged filters, greater than 70% of the T7 adsorbed to the Zeta plus C-30, Posidyne N66 and Virosorb 1MDS filters was eluted. However, only 22% of the T7 adsorbed to the Seitz S filters was eluted.

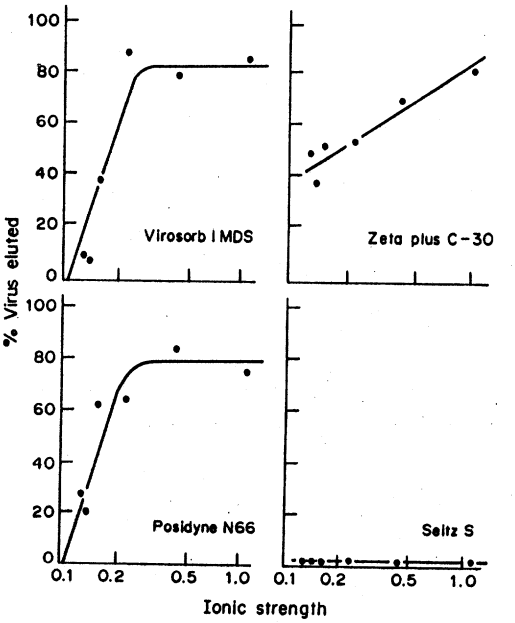


Fig. 1. Elution of bacteriophage T7 adsorbed to various filters by solutions of Tween 80 and NaCl or $Na_2SO_4$ at pH 7.

Preliminary results indicated that the ionic strength of the eluting solution effected the amount of virus eluted from these filters. Figure 1 shows the influence

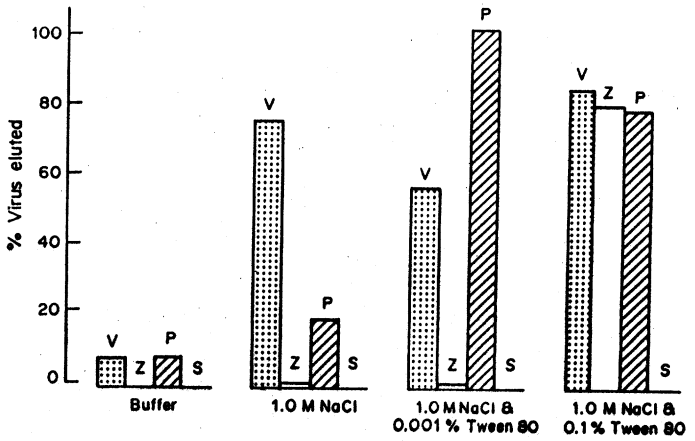


Fig. 2. Elution of bacteriophage T7 adsorbed to membrane filters by solutions of NaCl and Tween 80 at pH 7.

of ionic strength in solutions of detergent at pH 7 on the elution of T7 adsorbed to membrane filters. In the presence of detergent, solutions with ionic strengths of 0.4 or above efficiently eluted the bacteriophage adsorbed to the Zeta plus C-30 and the Posidyne N66 filters, while solutions with ionic strengths below 0.4 eluted the virus adsorbed to the Virosorb 1MDS filters. Again little or no virus adsorbed to the Seitz S filters was eluted.

The effect of detergent concentration in the presence of 1 M NaCl on viral elution was determined (Fig. 2). Again, a solution of buffer alone at pH 7 eluted less than 10% of the T7 adsorbed to all filters tested. A solution of 1 M NaCl at pH 7 did not elute any virus adsorbed to either the Zeta plus C-30 or Seitz S filters, while less than 20% of the adsorbed T7 was eluted from the Posidyne N66 filter. However, greater than 75% of the T7 adsorbed to the Virosorb 1MDS was eluted using the salt alone. Tween 80 concentrations of 0.001% in the presence of NaCl greatly increased the amount of phage eluted from the Posidyne N66 yet had no effect on the elution of phage adsorbed to Zeta plus C-30 or Seitz S filters. A solution of 0.1% Tween 80 in presence of salt eluted greater than 75% of the T7 adsorbed to the Virosorb 1MDS, Posidyne N66 and Zeta plus C-30 filters, yet had no effect on elution of T7 adsorbed to the Seitz S filters. Higher concentrations of Tween 80 are not soluble in the salt solution.

In order to determine the relative hydrophobic–hydrophilic nature of the filters, the contact angle of carbon tetrachloride on membrane filters submerged under water was measured (Table 1). The contact angle of carbon tetrachloride on the Seitz S filter was the smallest (113°), the contact angle on the Virosorb 1MDS was the largest (151°), while the contact angles on the Zeta plus C-30 (128°) and Posidyne N66 (128°) were intermediate.

The filters were further characterized using the capillary rise method. As shown in Fig. 3, the most rapid rise of water was observed in the Virosorb 1MDS filters, while the smallest rate of water rise was observed for the Seitz S filters. Again, Zeta plus C-30 filters were intermediate in nature. Similar studies in which hexadecane was used in place of water (data not shown) gave exactly opposite results in that the rate of rise of hexadecane was fastest for the Seitz filters and slowest for the 1MDS filters. The rate of rise in Zeta plus C-30 filters was again intermediate between that of the other two filters.

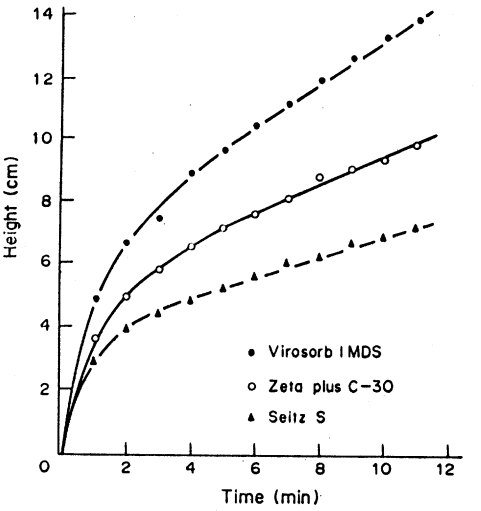


Fig. 3. The rise of water on various membrane filters.

Indigenous bacteriophages from several water sources were concentrated using a one step adsorption elution procedure involving Virosorb 1MDS filters. Table 4 shows the % recovery of indigenous bacteriophages using filters of various sizes and input volume of water. In general, recovery of virus was better when the smaller filter holders were used. In addition, there was no significant difference in viral recovery using *E. coli* B or *E. coli* C-3000 as the host bacteria. The average recovery of the 41 samples from 4 different sources was 55% using *E. coli* B as the host and 61% using *E. coli* C-3000 as the host.

In samples with relatively high numbers of phages a one-stage concentration procedure may be sufficient to detect viruses. However, where low levels are found, a two-stage procedure may be required to provide a sufficient concentration factor to detect indigenous phages. The results of this study have shown that some solutions that permit virus ad-

Table 4. One-step concentration procedure for the recovery of indigenous bacteriophage from natural water samples

| Water source | Filter size (dia, mm) | Volume filtered (ml) | % Recovery *E. coli* B Mean | *E. coli* B SD | *E. coli* C-3000 Mean | *E. coli* C-3000 SD | Replications |
|---|---|---|---|---|---|---|---|
| Cypress strand | 25 | 50–100 | 133 | 23 | 105 | 19 | 4 |
| | 47 | 100–500 | 84 | 38 | 66 | 12 | 6 |
| | 90 | 500–1500 | 63 | 33 | 53 | 16 | 4 |
| Secondary effluent | 25 | 250–400 | 47 | 22 | 60 | 11 | 3 |
| | 47 | 500–1000 | 26 | 1 | 58 | 10 | 4 |
| | 90 | 2000–7000 | 26 | 5 | 68 | 23 | 3 |
| Holding pond | 25 | 50–75 | 40 | 3 | 89 | 16 | 3 |
| | 47 | 375–475 | 32 | 7 | 61 | 30 | 4 |
| Land runoff | 25 | 50–100 | 82 | 19 | 59 | 9 | 3 |
| | 47 | 300–700 | 30 | 7 | 34 | 16 | 5 |
| | 90 | 2000 | 13 | 4 | 19 | 1 | 2 |
| Totals | | | 55 | 40 | 61 | 27 | 41 |

Table 5. Recovery of bacteriophages from treatment plant effluents

| Source, type of effluent | Volume sampled (l.) | Initial PFU $l^{-1}$ using: | | % Bacteriophages recovered using | |
|---|---|---|---|---|---|
| | | *E. coli* B | *E. coli* C-3000 | *E. coli* B | *E. coli* C-3000 |
| Tallahassee, Fla chlorinated effluent | 66.3 | $6.7 \times 10^4$ | $4.3 \times 10^4$ | 43 | 39 |
| University of Florida unchlorinated effluent | 3 | $5.0 \times 10^3$ | $6.9 \times 10^4$ | 15 | 54 |
| University of Florida unchlorinated effluent | 4 | $6.7 \times 10^4$ | $6.9 \times 10^4$ | 21 | 59 |
| University of Florida unchlorinated effluent | 4 | $8.3 \times 10^4$ | $9.1 \times 10^4$ | 24 | 100 |
| Total | | | | $26 \pm 10$ | $63 \pm 23$ |

sorption to one filter are capable of eluting virus adsorbed to another filter. This raises the possibility that a simple two-step virus concentration procedure can be developed in which virus adsorbed to one filter can be eluted using a solution that permits virus adsorption to a second smaller filter. The elution–adsorption step can be accomplished with little or no modification of the solution. Based on the results of this study, several two step concentration procedures using two different filters in series are possible (Table 5). Figure 4 outlines one two-step concentration procedure we have used to recover viruses from several secondary effluent samples. Using this procedure, the average recovery of bacteriophages was 26% using *E. coli* B as the host bacteria, and 63% using *E. coli* C-3000 as the host. In two samples, bacteriophage was detected in the final concentrated sample when none was detected in the initial sample (data not shown).

## DISCUSSION

Methods which employ adsorption to and subsequent elution from microporous filters are still among the most promising for the concentration of viruses from water samples. Several concentration procedures developed for animal viruses use electronegative filters as the primary adsorbing material (Goyal and Gerba, 1982). Adsorption of viruses to these filters requires the lowering of the pH of the water sample and/or the addition of cations (Wallis and Melnick, 1967). Since it has been previously shown that these concentration procedures are unsuitable for bacteriophage concentration (Seely and Primrose, 1979, 1982), investigators have explored the possibility of the use of electropositive filters for the concentration of bacteriophages (Goyal *et al.*, 1980; Seely and Primrose, 1982; Singh and Gerba, 1983). These filters are more positively charged at the pH range of most natural water samples (pH range 6.5–8) than are the electronegative filters, and so adsorption to the electropositive filters can be accomplished with little or no manipulation of the water sample.

In this study, we have examined four filters, Virosorb 1MDS, Zeta plus C-30, Posidyne N66 and Seitz S filters. All four are more electropositive at pH 5–8 than are negatively charged filters and adsorb virus in water at these pH values. However, their composition is quite varied. It was the purpose of this study to determine what effects these differences in composition would have on the association of bacteriophage with the filters.

Although all of the filters adsorbed T7 in tapwater at pH 4–9, differences in the ability of certain solutions to elute the adsorbed phage were noted. Previous studies have shown that solutions of a neutral detergent, such as Tween 80, can disrupt hydrophobic interactions between viruses and membrane filters (Farrah, 1982; Farrah and Shields, 1982;

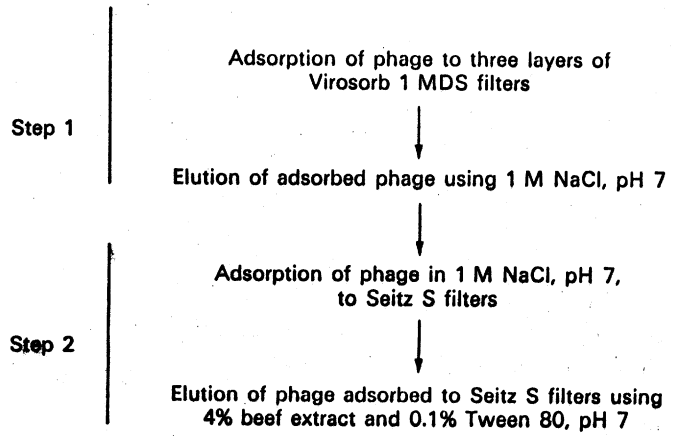


Fig. 4. One possible two-step procedure for the concentration of bacteriophages from water.

Wallis and Melnick, 1967). Once the hydrophobic interactions have been disrupted, the addition of certain salts to this solution will disrupt electrostatic interactions and result in the elution of adsorbed viruses (Farrah and Shields, 1982; Shields and Farrah, 1983). When hydrophobic interactions were disrupted at pH 7 by the addition of detergent, only T7 adsorbed to Posidyne N66 and Zeta plus C-30 filters was eluted to any appreciable extent. If salt was added to the detergent solution, thus disrupting both the hydrophobic and electrostatic interactions, most of the adsorbed phage was eluted from all filters tested except the Seitz S filter. These findings indicated that there were distinct differences in the relative strength of hydrophobic and electrostatic interactions with the phage among these four filters.

It was found that the ionic strength of the eluting solution, in the presence of a detergent, affected the amount of adsorbed phage that was eluted. Solutions of detergent with sodium chloride or sodium sulfate with ionic strengths greater than 0.4 eluted most of the adsorbed virus from all filters tested except the Seitz S. It is assumed that increasing the ionic strength decreases the strength of the electrostatic interactions between the virus and the filter (Shields and Farrah, 1983). Therefore, the filters that form relatively strong electrostatic associations with viruses require solutions with relatively high ionic strength to elute adsorbed viruses.

A titration of the amount of detergent necessary to elute adsorbed T7 in the presence of 1 M sodium chloride provided the most striking results. A solution of sodium chloride alone eluted most of the T7 adsorbed to the Virosorb 1MDS filter. Since no detergent was added, it can be assumed that no hydrophobic interactions were disrupted. Since salts can disrupt electrostatic interactions but promote hydrophobic interactions (Farrah, 1982; Farrah *et al.*, 1981), this result can be explained by assuming that hydrophobic interactions are not important in the association of phage to the Virosorb 1MDS filter. Similarly, since a solution of a salt alone did not elute viruses adsorbed to the other three filters, some hydrophobic interactions are probably involved in maintaining viral association to these filters. Since less detergent in the presence of salt was necessary to elute phage adsorbed to the Posidyne N66 filter than was required to elute phage adsorbed to the Zeta plus C-30, it can be assumed that hydrophobic interactions are relatively stronger for the Zeta plus C-30 filters than for the Posidyne N66 filters. Again, these specific solutions eluted little or no phage adsorbed to the Seitz S filters.

These results led us to characterize further these four electropositive filters. In this report, we used two means of assessing these filters which can be done without destruction of the filter surface. One such test was contact angle measurement. The contact angle determines the affinity of a liquid (in this case carbon tetrachloride) for a surface submerged under water. If carbon tetrachloride preferentially wets the submerged filter, the resulting contact angle between the filter surface and carbon tetrachloride droplet will be small. This is an indication of the preferential hydrophobicity of the filter. It was found that Virosorb 1MDS filters had the largest contact angle, indicating that it was the least hydrophobic of the filters tested. This was consistent with elution studies which had previously shown that hydrophobic interactions were not important in phage association with the Virosorb 1MDS filters. The Seitz S filters had the smallest contact angle, and therefore were found to be the most hydrophobic. This may be one reason why elution from these filters using the defined solutions employed in this study was so poor. Consistent with elution studies, Zeta plus C-30 and Posidyne N66 filters were found to be intermediate in the hydrophobic nature of their filters.

The second method used for characterization of the filters was the capillary rise method. In this case, the movement of water through the filters is measured. The rate of rise is related to the hydrophilicity of a filter. Those filters which are more hydrophilic will have a correspondingly higher rate of rise of water. The results of this test were in agreement with both the elution studies and contact angle measurements. The Virosorb 1MDS filters, previously shown to be the least hydrophobic were found to be the most hydrophilic. The Seitz S filters were found to be less hydrophilic, while the Zeta plus C-30 filters were once again found to be intermediate in their hydrophilicity.

The recent development of the Virosorb 1MDS filters has renewed interest in the more electropositive filters. This filter is one of the few electropositive filters available in a pleated-sheet cartridge that allows for large volumes of water to be processed. Sobsey and Glass (1980) found that the concentration of poliovirus from seeded tap water was equally efficient using Virosorb 1MDS filter cartridges or the highly electronegative Filterite filters. Moreover, they found that the Virosorb 1MDS filters were much simpler to use. The Virosorb 1MDS filters have also been used to concentrate indigenous virus from sewage, well water and chlorinated tap water collected during an outbreak of gastroenteritis (Hejkal *et al.*, 1982). The use of Virosorb 1MDS filters in the concentration of animal viruses led us to develop a method for concentrating bacteriophages with them. These filters were found to adsorb indigenous phage from the four natural water samples tested without any modification or treatment of the sample, and concentration of the indigenous phage was accomplished by using sodium chloride at pH 7 for elution. The results of this study indicated that sodium chloride, which could be used to elute virus adsorbed to the Virosorb 1MDS filters, was ineffective for eluting viruses adsorbed to any of the other three electropositive filters used in this study. This raises the possibility of using two different filters in series for

a simple two-step concentration procedure for bacteriophages based on differences in the elution characteristics of the filters used. This method has the advantage of maintaining a constant pH value throughout the concentration procedure, thus minimizing inactivation of the bacteriophages due to pH extremes. In addition, the method is relatively simple and requires no costly instrumentation, and therefore, is suitable for field use. One possible combination of filters was used successfully in this study to concentrate indigenous bacteriophages. It is likely that other filters and eluting solutions may be used in improved procedures.

Work in this laboratory has shown that, in addiiton to filters, viruses vary in the relative strength of their electrostatic and hydrophobic interactions. As more is learned about these differences, simple methods may be developed for separating virus types (Farrah and Shields, 1982). Further studies should be done to explore these possibilities.

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