APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1980, p. 892-896 Vol. 40, No. 5
0099-2240/80/11-0892/05$02.00/0

Capture of Latex Beads, Bacteria, Endotoxin, and Viruses by Charge-Modified Filters

KENNETH HOU,[1] CHARLES P. GERBA,[2]* SAGAR M. GOYAL,[2] AND KATHERINE S. ZERDA[2]

AMF/CUNO, Meriden, Connecticut 06450,[1] and Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030[2]

This report demonstrates how electropositive filters can be used to enhance the removal of microorganisms and other negatively charged particles from water. It was shown that electropositive depth filters were capable of adsorbing viruses and endotoxins many times smaller than the average pore size of the filter. Electronegative filters of similar porosity or electropositive filters that had been treated to destroy the positive charge were almost ineffective under similar conditions for the removal of viruses and small latex spheres. The results of this study indicate that electropositive filters are highly effective in the removal of a wide range of contaminants over a wide range of pH values and ionic conditions.

Removal of microorganisms and other particulate contaminants from liquids by filtration is of major industrial importance in manufacturing processes and in quality control. In addition to contaminant removal, filtration media have applications in the concentration and analysis of microorganisms and other particulate matter from water. Most filters have been designed to remove contaminant material by having pore sizes that would exclude the contaminant material. This creates problems in many types of applications because of the often rapid clogging of pores and low flow rates. In addition, many viruses and soluble endotoxins cannot be removed without the use of ultrafiltration. Obviously, the development of filters that would retain by a combination of adsorption and filtration would offer a major advantage over other forms of filtration media. Such filters could extend the size range of particles and the types of material that could be removed by a given porosity filter. In this report we show how charge modification of filters can be used to enhance removal of microorganisms from water.

MATERIALS AND METHODS

Contaminants and their assays. Poliovirus type 1 (strain LSc) assays were performed by the plaque-forming unit (PFU) method using the BGM cell line, which was passaged, grown, and maintained by previously described methods (8). The PR-8 strain of influenza virus was grown in 11-day-old chicken eggs and was assayed by a hemagglutination test using a microtiter procedure and 0.5% chicken erythrocytes (3).

Bacteriophage MS-2 was grown and assayed by the PFU method, using *Escherichia coli* B ATCC 15597 (1). Cultures of *E. coli* B 15597 grown overnight in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) were used in the bacteria removal studies. Bacterial assays were performed on Levine eosin methylene blue agar. All bacterial samples were diluted in tris(hydroxymethyl)aminomethane-buffered saline.

To evaluate endotoxin removal, purified *E. coli* serotype O111:B4 lipopolysaccharide was obtained from Sigma Chemical Co. (St. Louis, Mo.). Samples for endotoxin determination were serially diluted in pyrogen-free distilled water (Cutter Laboratories, Berkeley, Calif.) or 0.9% NaCl (Travenol Laboratories, Inc., Deerfield, Ill.) and were tested for the presence of endotoxin by using Pyrotell (*Limulus* amoebocyte lysate; Associates of Cape Cod, Inc., Woods Hole, Mass.), which had a sensitivity of 0.03 ng/ml. The test was carried out according to the manufacturer's instructions. The amount of endotoxin present in a given sample was calculated as the sensitivity of the lysate in the solution being tested multiplied by the highest dilution of that sample giving a positive *Limulus* test. All filters were prewashed with 20 to 30 ml of 0.9% NaCl solution and tested with *Limulus* amoebocyte lysate to ensure the absence of endotoxin from the filter.

Uniform polystyrene (latex) beads in monodispersed form were obtained from Dow Chemical Co. (Indianapolis, Ind.). To assess removal, turbidity of the suspended particles was measured with a turbidimeter (model 2100A, Hach Chemical Co., Ames, Iowa).

Filters. The following filters were used in this study: nitrocellulose (type GS, 0.22-μm pore size; Millipore Corp., Bedford, Mass.); epoxy-fiber glass (Duo-Fine series, 0.25-μm nominal pore size; Filterite Corp., Timonium, Md.); and charge-modified cellulose diatomaceous earth filter media (Zeta-plus 05S, 30S, 50S, 90S, and 50C; AMF/CUNO Division, Meriden, Conn.). The M and D filters were also charge-modified filters especially fabricated for this project. The nominal pore size of the Zeta-plus media ranged from 0.2 to 2.0 μm. The manufacturer utilizes a progressively smaller code prefix to represent a progressively larger nominal pore size. All filters were used as circular disks housed in polypropylene or stainless-steel holders at 47- or 25-mm diameter. Since no exacting porosity (p) data are available on the Zeta-plus and Filterite filters, a comparison of flow rates at constant Δp was determined to gain a rough comparison of porosity of the filters (Table 1).

Filtration. To evaluate removal efficiency, the contaminant under study was placed in dechlorinated Houston tap water (average of 450 mg of total dissolved solids per liter), double-distilled water, or solutions of distilled water containing various concentrations of NaCl. When polystyrene beads were used, a peristaltic pump was used to pass the solution through the filter material. In the case of microorganisms and endotoxin, the contaminated solutions were placed in a stainless-steel pressure vessel and passed through the filter media with positive pressure at a constant flow rate. Usually from 50 to 2,000 ml was passed through the filter media, with samples being collected after 50, 100, 500, 1,000, and 2,000 ml had passed through the filter. No significant change in the amount of contaminant removed was observed. All values represent average values for all filtrate samples collected.

Electrophoresis of filter media. The electrophoretic mobility of the filter media as a function of pH was determined by use of a Zeta-Meter (Zeta-Meter, Inc., New York, N.Y.), according to the manufacturer's instructions (10). Filter media were extracted by pulling the filter apart with a pair of forceps. Particles of a suitable size were placed in buffered solutions of ionic strength of 0.2, prepared according to Miller and Golder (9).

RESULTS

Retention of poliovirus, influenza virus, MS-2, and *E. coli* by filters of various porosities and net charges is shown in Table 2. All of the filters retained significant numbers of bacteria, except for the largest-porosity S-grade and the Filterite fiberglass filters. Bacteria were removed by a combination of physical filtration and adsorption. Virus removal by the filters tested depended most on adsorption, since their diameter is many times smaller than the pore size of the filters. The electropositive 50S and M grades were more efficient in removal of all the viruses studied than were the less positively charged C-grade and the negatively charged fiber glass, D-grade, and cellulose nitrate filters. The fiber glass and cellulose nitrate filters are thin-sheet media, but the D grade is a depth filter similar to the C, M, and S grades. The most electropositive material, M, also appeared to be the best at removal of the viruses studied. The electropositive M filters were also the best for removal of endotoxin (Table 3). The effect of filter surface charge on the removal of latex beads by filter media is also indicated in Fig. 1. The electropositive S and M grades were highly effective in removal of latex beads many times smaller than the porosity of the filters being tested. Even the large-pore-sized 05S grade was capable of excellent removal of 0.1-μm latex beads. The less electropositive C grade and the electronegative D grade were not as effective in removal of the smaller-sized latex beads.

TABLE 1. *Comparison of filter flow rates*[a]

Filter	Flow rate (ml/min) at Δp of	
	6 lb/in^2 (41 kPa)	15 lb/in^2 (10 kPa)
05S	461	1,200
50S	63	200
90S	21	60
M	82	
50C	48	150
D	46	150
Cellulose nitrate, 0.22 μm		3.8
Fiberglass, 0.25 μm	133	389

[a] Double-glass-distilled water. Nephelometric turbidity units <1.0.

Autoclaving of the C-grade filters resulted in destruction of the electropositive charge on the filter and its ability to efficiently remove latex beads with a diameter of less than 0.6 μm (Fig. 1). After autoclaving, only mechanical straining resulted in the removal of the latex beads. The

TABLE 2. *Removal of microorganisms by charged filter media*[a]

Filter	% Removal from tap water at pH 8.0			
	MS-2	Poliovirus	Influenza virus	*E. coli*
05S	0	74		73
30S	95	96	50	90
50S	92	99	86	99.9
90S	>99.99	>99.99	>99.99	>99.99
M	99.7	99.99	50	>99.99
50C	0	99.99	62	>99.99
D	0	35	75	>99.99
Fiberglass	0	20	0	74
Cellulose nitrate, 0.22 μm		54		>99.99

[a] Initial concentrations of microorganisms were: MS-2, 10^6/ml; poliovirus, 10^5/ml; influenza, 10^4/ml; and *E. coli*, 10^4/ml.

TABLE 3. *Removal of endotoxin by charged filter media*

Filter	% Removal of *E. coli* endotoxin[a]
D	60
M	>99.7
Cellulose nitrate, 0.22 μm	<10

[a] 0.9% saline, pH 6.7 (Travenol Laboratories), for injection. The initial concentration of endotoxin was 10,000 to 12,000 pg/ml.

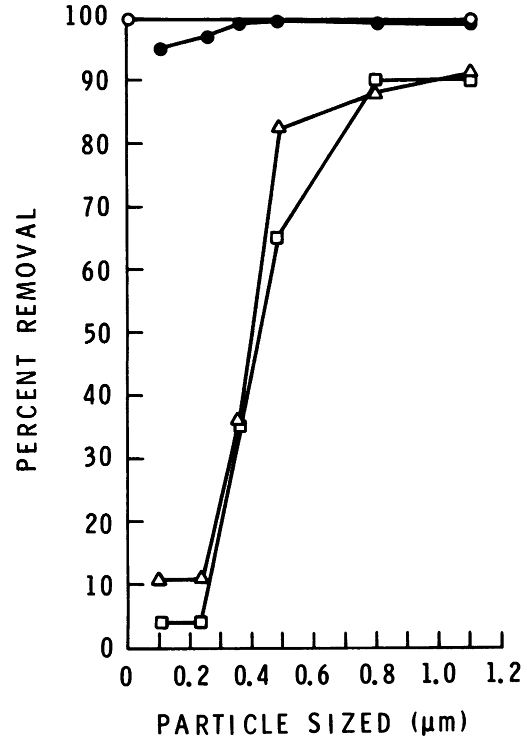

FIG. 1. *Removal of polystyrene beads by different pore sizes and differently charged media. Symbols: (○) 05S, M, 50S; (●) 50C; (△) 50C autoclaved to destroy positive charge; (□) D.*

importance of the electrokinetic effect in the removal of latex beads is shown in Fig. 2. In this experiment, the electropositive charge on the S grade filters was destroyed by alkaline treatment (Fig. 3). Comparison between the sizes of particles removed before and after destruction of the positive charge indicates that electrokinetic effects play a major role in the removal of particles below 1 µm in size with this type of filter.

The pH and salt concentration of the fluid that is being filtered can greatly affect the charge not only on the substance being filtered but also on the filter media. The effect of pH on the removal of bacteriophage MS-2 from tap water by the various filter media is shown in Table 4 and on latex beads by the S-grade media in Fig. 4. All of the filters performed best at lower pH, but only the electropositive filters performed well above pH 6.0. The electropositive S- and M-grade filters retained far greater amounts of virus than the negatively charged filters at all pH values tested. As indicated by the effect of pH on removal of latex beads by an S-grade filter, the capacity of the filters is also reduced at high pH values.

Varying concentrations of NaCl had no appar-

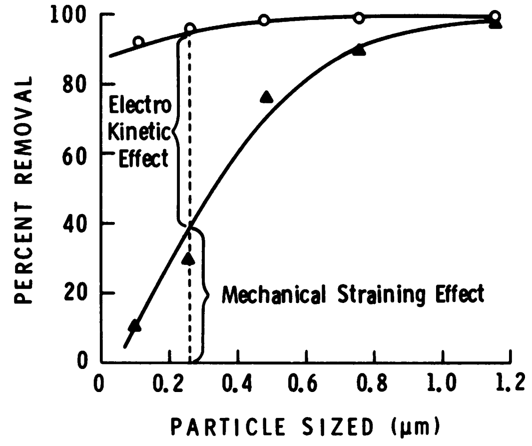

FIG. 2. *Demonstration of mechanical straining and electrokinetic effect by 90S filter media by using polystyrene beads. Symbols: (○) 90S; (▲) 90S alkaline-treated to destroy positive charge.*

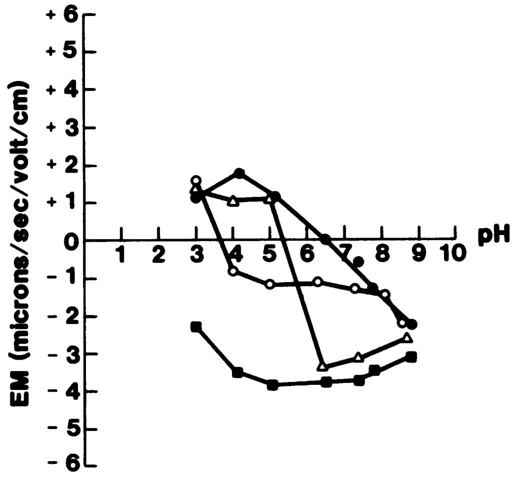

FIG. 3. *Electrophoretic mobility of filter media as a function of pH. Symbols: (●) 50S; (△) M; (■) D; (○) 50C.*

TABLE 4. *Effect of pH on removal of MS-2 from Houston tap water*[a]

Filter	% Removal of MS-2 at pH of:					
	3.5	5.0	6.0	7.0	8.0	9.0
50S	99.8	99.95	99.4	99.99	92	94
M	99.99	99.996	99.9995	99.96	99.7	71
50C	99.99	99.996	72	90	0	1
D	13	8	0	0	0	0
Fiberglass	80	21	0	5	0	0

[a] Initial concentration of MS-2 was 10^6 PFU/ml.

ent effect on the removal of bacteriophage MS-2 by the electropositive M-grade filter, but did slightly enhance adsorption at lower concentrations (Table 5). Increasing concentrations of salt, however, did tend to reduce the retention capacity of latex beads by S-grade filters (Fig. 5).

DISCUSSION

As recently emphasized by Zierdt (12), the adherence of particles to filters with pore sizes larger than the particles to be removed is of major importance in all applications of filter technology. Large-porosity-size filters delay clogging and extend the operational life of the filters. In this study, we attempted to show how modification of the net charge on a filter surface can be used to enhance the removal of suspended particulates and microorganisms.

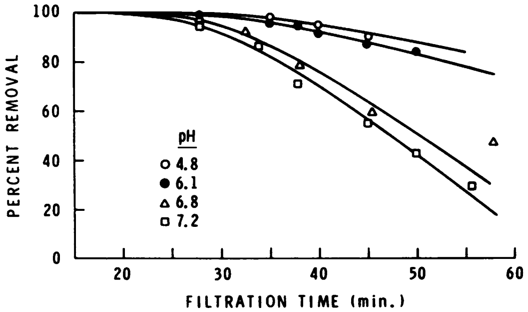

FIG. 4. *Effect of pH on removal of polystyrene beads by 60S filters. Symbols:* (○) *pH 4.8;* (●) *pH 6.1;* (△) *pH 6.8;* (□) *pH 7.2.*

TABLE 5. *Effect of NaCl concentration on removal of MS-2*[a]

NaCl concn (%)	% Virus removal by:	
	D	M
0	3	>99.99
0.00001	21	>99.99
0.0001	25	>99.99
0.001	0	>99.99
0.01	0	>99.99
0.1	0	>99.99

[a] Initial concentration of MS-2 was 10^6 PFU/ml.

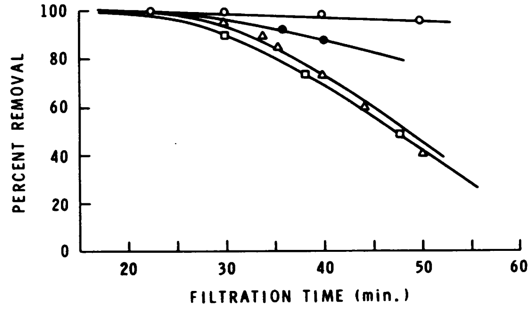

FIG. 5. *Effect of NaCl concentration on removal of polystyrene beads by 60S filters. Symbols:* (○) *0.140 M NaCl;* (●) *0.148 M;* (△) *0.250 M;* (□) *0.353 M.*

In most fluids, microorganisms and other organic and inorganic matter exhibit a net negative charge. Therefore, the use of electropositive filters would be expected to enhance the removal of suspended matter in water by electrokinetic phenomena. An example of this is seen in the study of Sobsey and Jones (11), who showed that poliovirus adsorbed to a greater extent at near-neutral pH in tap water to electropositive than to electronegative filters.

Results of studies on the depth filters indicated that for particles larger than 1 μm, physical phenomena, e.g., mechanical straining, were the most important, whereas particles smaller than 0.5 μm were removed by electrokinetic effects. For intermediate particles (0.5 to 1.0 μm), both types of mechanisms were important.

The existence of both mechanical and electrochemical filtration mechanisms in Zeta-plus filters was demonstrated by using different sizes of monodispersed polystyrene latex beads as contaminants. The mechanical straining efficiency of Zeta-plus C and S grades was demonstrated after destroying the positive charge on these filters. The results reported here (Fig. 1 and 2) and those observed previously (2) strongly suggest that electrokinetic mechanisms are the dominating factor for removing particles less than 0.1 μm in size. Thus, removal of viruses by these filters must depend almost totally on electrokinetic effects. The electrokinetic effects on virus and endotoxin removal were futher illustrated by their ability to easily pass through the electronegative fiber glass, D-grade, and cellulose nitrate filters. Both the fiber glass and the cellulose nitrate filters have been previously shown to be highly electronegative between pH 2 and 8 (7).

A qualitative picture of filtration by electrokinetic phenomena can be described by using the same concepts in colloidal chemistry. If the charges of the filter media and particulates are of opposite signs, electrostatic attraction will allow the particles to deposit on the media. If they are of the same sign, repulsion will occur and deposition will be hindered. Thus, in our case, where grades M, S, and C were positively charged and most particulates were negatively charged, deposition readily occurred onto the filter surface.

The charge on the surface of the filter is altered by changes in pH and the electrolyte concentration of the solution being filtered. This phenomenon is explained by the electrical double-layer theory of colloidal chemistry. A particle such as a virus immersed in an aqueous solution develops a surface charge by adsorbing ions on its surface. A fixed layer of oppositely charged ions develops around the surface of the filter. To maintain the electrically neutral system, there is a diffused layer containing a sufficient number of counterions extended for some distance into the solution. If the bulk solution of counterions increases by addition of cationic salts or increasing pH, the thickness of this layer decreases because less volume is required to contain enough counterions to neutralize the surface charge. The reduction of the thickness of this layer facilitates the approach of the two surfaces, allowing van der Waal's forces to have an effect (7). Lowering the pH or addition of cationic salts thus reduces the electronegativity of the Filterite, cellulose nitrate, and D-grade filters and allows for some adsorption to occur under these conditions (Tables 4 and 5; Fig. 4) (4, 7).

The results of this study indicate that electropositive filters are effective in the removal of a wide range of microorganisms over a wide range of pH values and ionic conditions. On the other hand, electronegative filters of similar porosity were effective over only a limited range of pH values and salt concentrations. The use of electropositive filters would appear to have widespread application in the removal of microorganisms from water, for the concentration of both bacteria and viruses from water (9) and harvesting (5) for removal of endotoxins from contaminated parenterals and foods (6), and for immobilization of microbial cells and antigens.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, New York.
2. **Fiore, J. V., and R. A. Babineau.** 1979. Filtration: an old process with a new look. Food Technol. **33:**67-72.
3. **Francis, T., Jr., and J. E. Salk.** 1942. A simplified procedure for the concentration and purification of influenza virus. Science **96:**499-500.
4. **Gerba, C. P., S. R. Farrah, S. M. Goyal, C. Wallis, and J. L. Melnick.** 1978. Concentration of enteroviruses from large volumes of tap water, treated sewage, and seawater. Appl. Environ. Microbiol. **35:**540-548.
5. **Goyal, S. M., K. S. Zerda, and C. P. Gerba.** 1980. Concentration of bacteriophage lysates by filter chromatography. J. Virol. Methods **1:**79-85.
6. **Haska, G., and R. Nystrand.** 1979. Determination of endotoxins in sugar with the *Limulus* test. Appl. Environ. Microbiol. **38:**1078-1080.
7. **Kessick, M. A., and R. A. Wagner.** 1978. Electrophoretic mobilities of virus adsorbing filter materials. Water Res. **12:**263-268.
8. **Melnick, J. L., H. A. Wenner, and C. A. Phillips.** 1979. Enteroviruses, p. 471-534. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Inc., Washington, D.C.
9. **Miller, G. L., and R. H. Golder.** 1950. Buffers of pH 2 to 12 for use in electrophoresis. Arch. Biochem. **29:**420-423.
10. **Riddick, T. M.** 1968. Control of colloid stability through zeta potential, vol. 1. Zeta-Meter Inc., New York.
11. **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.
12. **Zierdt, C. H.** 1979. Adherence of bacteria, yeast, blood cells, and latex spheres to large-porosity membrane filters. Appl. Environ. Microbiol. **38:**1166-1172.