Removal of Virus from Water by Filtration Using Microporous Membranes Made of Poly(*N*-benzyl-4-vinylpyridinium chloride)

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SYNOPSIS

Two types of microporous filter materials were developed for removing virus from water by using poly(*N*-benzyl-4-vinylpyridinium chloride) that captures virus in water. Conventional ultrafiltration using one to three sheets of 145-μm-thick cellulose nitrate membrane with a pore size of 0.45 μm and coated with 1.7 mg/g of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) showed 99.4–99.998% removal (2.2–4.7 $\log_{10}$-unit reduction in concentration) of bacteriophage T4, whereas the control experiments using noncoated membrane showed 91–96% removal (1.0–1.4 $\log_{10}$-unit reduction in concentration) of the virus. A composite 360-μm-thick microporous membrane with a pore size of 20 μm was prepared that consisted of connected minute beads of 1.7 μm in diameter made of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by a nonwoven cloth. Simple filtration using one sheet of the composite membrane at 34.2 cm/h showed 99.96–99.9995% removal (3.4–5.3 $\log_{10}$-unit reduction in concentration). The virus was not detected in the filtrate when two sheets of the composite membrane were used. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

It is difficult to overemphasize the importance of an adequate supply of drinking water to any community. Conventional treatment processes for water supplies have been thought to inactivate viruses adequately. However, detection of infectious human viruses in treated drinking water[1,2] has raised concern that conventional processes may not always inactivate viruses. Viruses are reported to be more resistant than bacteria to chlorine disinfection.[3,4] For example, poliovirus resists free chlorine residuals in excess of 1 mg/L of water.[3] In diseases caused by viruses, there are few drugs whose effect is equivalent to that of the antibiotics developed for diseases caused by bacteria, although extensive research is now focused on new chemicals with antiviral activity. Therefore, the development of an effective method to remove viruses from drinking water is of special importance in the field of public health and hygiene.

During the course of a study to develop an alternative method of chlorination, we found remarkable ability of crosslinked poly(*N*-benzyl-4-vinylpyridinium halide) to remove bacteriophage T4[5] and pathogenic human viruses[6] from water. Because filtration is a convenient method in practical water treatment, and effective removal of virus by filtration using microporous cellulose and cellulose acetate membranes was reported,[7–9] we attempted to develop new filter materials based on poly(*N*-benzyl-4-vinylpyridinium halide) for effective removal of virus. In the preceding report, we demonstrated effective removal of bacteria, yeasts, and spores of fungi by simple filtration using nonwoven cloth coated with a small amount of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene).[10] However, the filtration was not very effective for removing bacteriophage T4, and the virus appeared to be smaller than the crevice of the coated nonwoven cloth. In this work, therefore, we attempt to develop micro-

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porous membranes made of the pyridinium-type polymer.

EXPERIMENTAL

Materials

4-Vinylpyridine was purified by distillation under reduced pressure. Styrene and 55% divinylbenzene were purified by washing with 5% aqueous sodium hydroxide solution, followed by distillation under reduced pressure. 2,2'-Azobisisobutyronitrile (AIBN) and other chemicals and solvents were used without further purification. Microporous cellulose membranes were provided by Toyo Roshi Kaisha Ltd. (Tokyo, Japan). Nonwoven cloth, 0.5 mm thick, made of pure 1.5-denier rayon was provided by Japan Vilene Co. Ltd. (Tokyo, Japan).

Poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene)

Polymerizations were carried out in a 500-mL, round-bottomed, three-necked flask equipped with a mechanical stirrer, a reflux condenser, and a gas inlet. A mixture of 4-vinylpyridine (28.1 g, 0.267 mol), styrene (76.3 g, 0.733 mol), and AIBN (0.73 g, 4.5 mmol) was added to 250 mL of ethanol under a nitrogen atmosphere and heated at 80°C with stirring for 6 h. After cooling to room temperature, benzyl chloride (33.8 g, 0.267 mol) was added and allowed to react at 80°C for 5 h. The polymer was isolated by pouring the content of the flask into ethyl acetate and was dried *in vacuo* to constant weight. The intrinsic viscosity was 0.25 dL/g when determined in ethanol containing 10 g/L of $MgCl_2 \cdot 6H_2O$ at 30°C. The polymer contained 2 : 5 molar ratio of *N*-benzyl-4-vinylpyridinium chloride and styrene and 1.77 mmol/g of the pyridinium group.

Microporous Cellulose Membranes Coated with Poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene)

Microporous cellulose membranes coated with the polymer were prepared by soaking the commercial membranes in 0.5 wt % solution of the polymer in 6/4 (v/v) methano–acetone mixture at room temperature overnight and were dried at room temperature in a hood. This soaking procedure was repeated several times.

Composite Microporous Membrane Made of Crosslinked Poly(*N*-benzyl-4-vinylpyridinium chloride) and Reinforced by a Nonwoven Cloth

A 15-cm-long and 15-cm-wide sample of the nonwoven cloth was soaked in a monomer mixture containing 4-vinylpyridine (30 g, 0.285 mol), 55% divinylbenzene (3.0 g, 0.013 mol), AIBN (326 mg, 2.0 mmol), toluene (25 g), and acetone (7 g) for 7 min at room temperature. The treated cloth was placed on a Teflon sheet supported by a mirror glass of 7 mm thick, and several drops of the monomer mixture were added. After degassing, the treated cloth was covered by another Teflon sheet supported by a mirror glass. Thus, sandwiched cloth was fastened using clippers and placed in a water bath. Polymerization performed by heating the water bath at 121°C for 30 min using an autoclave gave a composite membrane made of crosslinked poly(4-vinylpyridine). After washing with deionized water and drying *in vacuo* at room temperature, the membrane was placed in a 2-L Erlenmeyer flask containing benzyl chloride (50 g, 0.39 mol) and ethanol (1.5 L) at pH 7. The mixture was allowed to react at 70°C for 6 h to afford the title membrane. After drying to constant weight *in vacuo* at room temperature, the membrane was extensively washed by passing deionized water through the membrane until total organic carbon disappeared in the filtrate. The membrane contained 0.36 mol/m^2 of the pyridinium group.

Virus

Bacteriophage T4 IFO 20,004 was used as a test virus for this work and was propagated in *Escherichia coli* strain B in a medium prepared by dissolving peptone (10.0 g), meat extract (3.0 g), yeast extract (5.0 g), NaCl (2.5 g), and KH_2PO_4 (8.0 g) into 1000 mL of water at pH 7.2. The virus was inoculated into *E. coli* culture at the logarithmic growth phase, and the culture was maintained at 37°C overnight. After multiplication of the virus, the suspension was centrifuged at 3000 rev/min at room temperature for 10 min. After filtration through a membrane made of 125-μm-thick cellulose acetate with a pore size of 0.45 μm, the supernatant was used as the virus suspension.

Bacteriophage T4 was assayed using plates of peptone soft agar. This assay procedure was repeated five times every time. The virus concentration was evaluated by the plaque-forming unit (PFU).

Bacteriophage can be counted by a modification of the plating procedure used for counting bacteria.

Table I Removal of Virus from Water by Conventional Ultrafiltration Using a Microporons Cellulose Membrane Coated with Poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene)

Run Number	Pore Size (μm)	Amount of Coated Polymer (mg/g)	Number of Piled Sheets	Concentration (PFU/mL)		Removal (%)
				Influent	Effluent	
			Cellulose nitrate membrane[a]			
1	0.45	0	1	2.79×10^7	2.44×10^6	91
2	0.45	0	1	6.44×10^6	5.62×10^5	91
3	0.45	0	1	7.22×10^5	5.56×10^4	92
4	0.45	0	2	2.79×10^7	1.91×10^6	93
5	0.45	0	3	2.79×10^7	1.25×10^6	96
6	0.45	1.7	1	2.79×10^7	1.61×10^5	99.4
7	0.45	1.7	1	6.44×10^6	5.34×10^3	99.92
8	0.45	1.7	1	7.22×10^5	9.80×10^1	99.99
9	0.45	1.7	2	2.79×10^7	8.42×10^3	99.97
10	0.45	1.7	3	2.79×10^7	5.68×10^2	99.998
			Cellulose acetate membrane[b]			
11	0.45	0	1	2.79×10^7	1.95×10^7	30
12	0.45	1.9	1	2.79×10^7	1.28×10^7	54
13	0.8	0	1	2.79×10^7	2.53×10^7	9
14	0.8	2.2	1	2.79×10^7	1.54×10^7	45

Ultrafiltration was carried out by supply of 0.5 kg/cm^2 of nitrogen at room temperature using bacteriophage T4 as a test virus. The rate of filtration was about 50 cm/h.

[a] Thickness was 145 μm.

[b] Thickness was 125 μm.

About 10^8 bacteria are mixed with melted agar and phage particles, and this mixture is then poured onto a solid agar layer; the liquid agar cools and hardens, forming a layer about 1 mm thick. The bacteria in the thin agar layer grows for three to five generations and produces 10^8 microcolonies. Because the colonies are in contact, such growth produces a confluent layer of bacteria, which is visibly turbid. If a phage particle is present in the thin agar layer, it can grow in one of the bacteria initially added and produce progeny phage that can infect many nearby bacteria. Many cycles of phage growth can occur, producing 10^8–10^9 phage particles in a region no more than 1 mm across. Because the bacteria are lysed in this region, the phage particle initially present will produce a clear zone in the turbid layer of bacteria. This clear region is called a *plaque*. Because one phage produces one plaque, phage can be counted by this procedure. Therefore, concentration of the bacteriophage can be evaluated by the PFU, and the percentage decrease of PFU reflects the percentage removal of the bacteriophage.

Removal of Virus from Water by Membrane Filtration

Removal of virus by conventional ultrafiltration using a microporous cellulose membrane coated with a small amount of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) was performed by supply of 0.5 kg/cm^2 of nitrogen at room temperature using bacteriophage T4 as a test virus.

Removal of virus by simple filtration using the composite microporous membrane made of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by a nonwoven cloth was carried out using a glass column, 25 by 30 mm, with two silicone-rubber stoppers connected by a glass inlet to the virus suspension. The composite microporous membrane was placed in the glass column, and the virus suspension was passed through the filtration apparatus at room temperature using a peristaltic pump.

The removal efficiency was evaluated based on the difference between PFU of influent and effluent suspensions. Percentage of the virus removal was calculated based on the following equation:

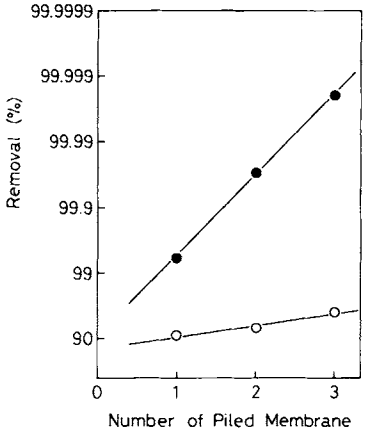


Figure 1 Effect of the number of piled sheets of membrane on the percentage of removal of virus from water by conventional ultrafiltration using a microporous cellulose nitrate membrane. Pore size of the membrane, 0.45 μm; test virus, bacteriophage T4; influent concentration of the virus, 2.79×10^7 PFU/mL. (●) Ultrafiltration using a membrane coated with 1.7 mg/g of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene); (○) control experiment using a membrane that was not coated with the polymer. The ultrafiltration was carried out by supply of 0.5 kg/cm^2 of nitrogen at room temperature, and the rate of filtration was about 50 cm/h.

$$\text{Removal (\%)} = (\text{PFU}_{\text{INF}} - \text{PFU}_{\text{EFF}})/\text{PFU}_{\text{INF}} \times 100$$

Here, PFU_{INF} and PFU_{EFF} are concentrations of the virus in the influent and effluent suspensions, respectively, in the unit of PFU/mL.

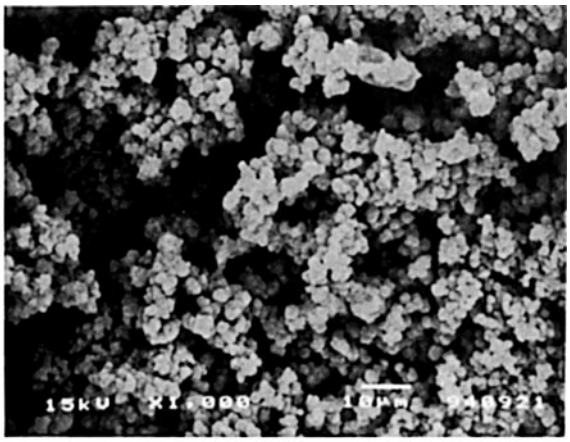
RESULTS AND DISCUSSION

Removal of Virus from Water by Conventional Ultrafiltration Using a Cellulose Membrane Coated with Poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene)

This work was aimed at development of an effective filter material for removing virus from water by using poly(*N*-benzyl-4-vinylpyridinium chloride) that captures virus in water.[5,6] Although the filtration using a nonwoven cloth coated with a small amount of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) exhibited effective removal of bacteria, yeasts, and spores of fungi, the method was not effective for removing bacteriophage T4.[10] The virus appeared to be smaller than the crevice of the nonwoven cloth.

In this work, therefore, we attempted to use a microporous filter material instead of the coated nonwoven cloth. At first, we used microporous cellulose membranes coated with a small amount of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene). Because hydrophilicity is an important factor in the capture of microorganisms by the pyridinium-type polymer,[11] we anticipated that cellulose membrane is preferred to polypropylene membrane and other hydrophobic membranes as the base of the filter material. Bacteriophage T4 was used as a test virus.

Conventional ultrafiltration using one to three sheets of microporous cellulose membrane coated with a small amount of poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) was performed by supply of 0.5 kg/cm^2 of nitrogen at room temperature.



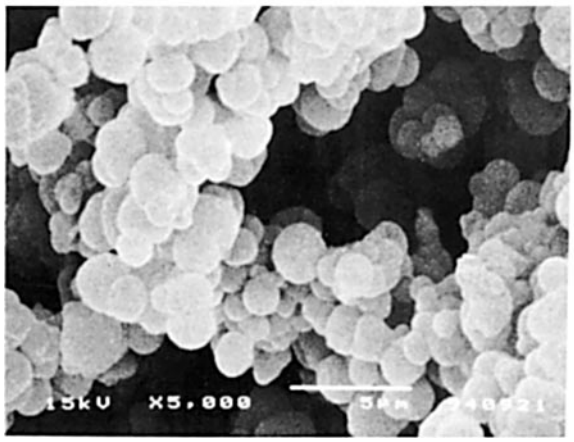


Figure 2 Electron micrographs of the composite microporous membrane made of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by a nonwoven cloth. Scale bars are 10 μm (upper) and 5 μm (lower), respectively.

Table II Removal of Virus from Water by Simple Filtration Using a Composite Microporous Membrane Made of Cross-linked Poly(*N*-benzyl-4-vinylpyridinium chloride) and Reinforced by a Nonwoven Cloth

Run Number	Number of Piled Sheets	Rate of Filtration (cm/h)	Concentration (PFU/mL)		Removal (%)
			Influent	Effluent	
15	1	3.4	2.97×10^7	0	100
16	1	8.5	1.20×10^7	4.7×10^1	99.9996
17	1	17.1	2.82×10^7	2.7×10^3	99.99
18	1	25.7	1.51×10^7	1.2×10^3	99.992
19	1	34.2	3.27×10^7	1.2×10^4	99.96
20	1	34.2	4.42×10^6	4.2×10^1	99.999
21	1	34.2	3.95×10^5	2.0×10^0	99.9995
22	2	8.5	2.06×10^7	0	100
23	2	17.1	3.91×10^7	0	100
24	2	25.7	2.37×10^7	0	100
25	2	34.2	2.21×10^7	0	100

Filtration was carried out by passing the virus suspensions through a composite microporous membrane using a peristaltic pump at room temperature using bacteriophage T4 as a test virus.

Under the conditions, the rate of filtration was about 50 cm/h. Results are summarized in Table I.

Coated cellulose nitrate membrane showed more effective removal of virus than coated cellulose acetate membrane under the conditions where pore size and amount of the coated polymer were similar. For example, ultrafiltration using one sheet of the coated cellulose nitrate membrane showed 99.4% removal (2.2 $\log_{10}$-unit reduction in concentration) of the virus when the influent concentration was 2.79×10^7 PFU/mL (run 6). However, ultrafiltration using the coated cellulose acetate membrane performed under the corresponding conditions showed only 64% removal (0.4 $\log_{10}$-unit reduction in concentration) of the virus (run 12). Difference in hydrophilicity appeared to play an important role in the removal efficiency.

The removal efficiency remarkably increased with the number of piled sheets of the coated cellulose nitrate membrane as shown by closed circles in Figure 1. Ultrafiltration using one sheet of the coated membrane gave 99.4% removal (2.2 $\log_{10}$-unit reduction in concentration). Ultrafiltration using two and three sheets of the coated membrane gave 99.97 and 99.998% removal (3.5 and 4.7 $\log_{10}$-unit reduction in concentration), respectively. As shown by open circles in Figure 1, control ultrafiltration using one to three sheets of noncoated cellulose nitrate membrane gave 91–96% removal (1.0–1.4 $\log_{10}$-unit reduction in concentration). In this case, the rate of removal insignificantly increased with the number of piled sheets of the noncoated membrane.

The removal efficiency increased when decreasing the influent concentration. For example, the rate of removal was 99.4, 99.92, and 99.99% (2.2, 3.1, and 4.0 $\log_{10}$-unit reduction in concentration) when the influent concentration was 2.79×10^7, 6.44×10^6, and 7.22×10^5 PFU/mL (runs 6, 7, and 8), respectively. Ultrafiltration using the coated cellulose nitrate membrane seems to be more effective at lower concentration of the virus.

Ultrafiltration using the coated cellulose nitrate membrane showed much more effective removal of the virus than simple filtration using the coated nonwoven cloth described in the preceding report.[10] For example, ultrafiltration at about 50 cm/h using one sheet of cellulose nitrate membrane coated with 1.7 mg/g of the polymer showed 99.4% removal (2.2 $\log_{10}$-unit reduction in concentration) when the influent concentration was 2.79×10^7 PFU/mL (run 6). On the other hand, simple filtration at 20 cm/h using 40 sheets of nonwoven cloth coated with 222 mg/g of the polymer showed 72% removal (0.6 $\log_{10}$-unit reduction in concentration) of the virus when the influent concentration was 2.79×10^7 PFU/mL.[10] The virus appeared to be smaller than crevice of the nonwoven cloth but not smaller than pore of the cellulose membrane.

Preparation of a Composite Microporous Membrane Made of Crosslinked Poly(*N*-benzyl-4-vinylpyridinium chloride)

As described above, the conventional ultrafiltration using a microporous cellulose nitrate membrane coated with poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) effectively removed virus from water, making a sharp contrast to the simple filtration

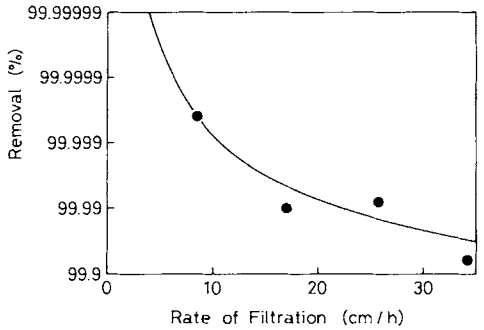


Figure 3 Influence of the rate of filtration on the percentage of removal of virus from water by simple filtration using a composite microporous membrane made of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by a nonwoven cloth. The filtration was carried out by passing the virus suspension through the membrane using a peristaltic pump at room temperature. Influent concentration of the virus, 1.20–3.27 × 10^7 PFU/mL.

using a nonwoven cloth coated with the pyridinium-type polymer. The result indicates utility of a filter material made of the pyridinium-type polymer for removing virus from water when possessed micropores of appropriately small size. However, because the conventional ultrafiltration is not always suitable for practical water treatment of large scale, we attempted a further development of a new filter material made of poly(*N*-benzyl-4-vinylpyridinium chloride) having a larger pore size than that of the above cellulose membrane for removing virus from water. We prepared a composite microporous membrane made of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride) reinforced by a nonwoven cloth by solution copolymerization of 4-vinylpyridine with divinylbenzene in the presence of a nonwoven cloth followed by the reaction with benzyl chloride.

The most appropriate molar ratio of 4-vinylpyridine to divinylbenzene was 10 : 1. When too much amount of divinylbenzene was used, a brittle membrane was obtained, probably because the degree of crosslinking was too high. On the other hand, when a too small amount of divinylbenzene was used, a rubberlike sheet was obtained that showed strong resistance to the flow of water through the membrane. In this case, the porosity was probably too low for easy filtration due to the poor crosslinking.

An appropriate amount of solvent was used for the copolymerization to make the resulted membrane microporous. During the drying procedure, the solvent was removed and left micropores in the membrane. A mixture of toluene and acetone in a weight ratio of 5 : 1 was used as the solvent. The ratio of monomer mixture to solvent mixture was an important factor in making the membrane microporous. When the ratio in weight of monomer mixture to solvent mixture was about 2 : 1, the resulted membrane strongly resisted against the flow of water. In this case, the amount of solvent was not enough, and the porosity was probably too low for easy filtration. On the other hand, when the ratio was about 1 : 1 or 1 : 2, the resulted membranes enabled the filtration by simple natural fall without giving pressure. Although both membranes enabled easy filtration, the membrane prepared by using about 1 : 1 mixture of monomers to solvents showed higher removal efficiency. Pore size of the membrane appeared to be too large for effective removal of virus when prepared by using about 1 : 2 mixture of monomers to solvents. Results of the experiments of removing virus from water by simple filtration using the composite membrane are described later.

Figure 2 shows electron micrograph of the 360-μm-thick composite microporous membrane prepared under the most appropriate conditions. Based on the micrograph, porosity of the composite membrane was evaluated to be 50%. Pore diameter was 2–60 μm, and the mean value was 20 μm. The easy filtration through the composite microporous membrane can be explained in terms of the large pore diameter compared with those of cellulose membranes.

As can be seen in Figure 2, the composite microporous membrane consisted of connected minute beads of crosslinked poly(*N*-benzyl-4-vinylpyridinium chloride). Particle diameter of the minute beads was 1.2–2.5 μm, and the mean value was 1.7 μm. Surface area of the inside of pores of the membrane would be tremendously large and favorable for the effective virus removal.

Removal of Virus from Water by Simple Filtration Using the Composite Microporous Membrane Made of Crosslinked Poly(*N*-benzyl-4-vinylpyridinium chloride)

Removal of bacteriophage T4 from water by simple filtration using the composite microporous membrane prepared in this work was performed by passing suspensions of the virus using a peristaltic pump. The filtration was easily accomplished, and exertion of pressure was not necessary. Results are summarized in Table II.

Figure 3 shows influence of the rate of filtration on the percentage of removal when one sheet of the composite membrane was used. In the case of slow filtration at 3.4 cm/h, the virus was not detected in the filtrate (run 15), although it is impossible to ex-

press the result of 100% removal in Figure 3. The percentage of removal decreased with an increase in the rate of filtration. However, Figure 3 suggests that it is not difficult to ensure 99.9% removal (3 $\log_{10}$-unit reduction in concentration) of the virus by using only one sheet of the composite microporous membrane at a high rate of filtration.

The percentage of removal increased with the number of piled sheets of the composite membrane. When influent concentration was 10^7 PFU/mL, the filtration using one sheet of the membrane gave 99.96–99.9996% removal (3.4–5.4 $\log_{10}$-unit reduction in concentration) of the virus (runs 16–19), but the virus was not detected in the effluent suspension when two sheets of the membrane were used (runs 22–25).

The percentage of removal increased with decrease of influent concentration of the virus. For example, the filtration gave 99.96, 99.999, and 99.9995% removal (3.4, 5.0, and 5.3 $\log_{10}$-unit reduction in concentration) of the virus when influent concentration was 3.27×10^7, 4.42×10^6, and 3.95×10^5 PFU/mL, respectively (runs 19, 20, and 21).

Simple filtration using the composite microporous membrane was more effective for removing virus from water than conventional ultrafiltration using the coated cellulose nitrate membrane despite the fact that pore size of the composite membrane (2–60 μm; 20 μm in average) was 5–130 times larger than that of the coated cellulose nitrate membrane (0.45 μm). For example, when the influent concentration was 10^7 PFU/mL, the simple filtration at 34.2 cm/h using one sheet of the composite microporous membrane gave 99.96% removal (3.4 $\log_{10}$-unit reduction in concentration) of the virus (run 19), and the virus was not detected in the filtrate (infinite $\log_{10}$-unit reduction in concentration) when two sheets of the composite membrane were used (run 25). On the other hand, conventional ultrafiltration using one and two sheets of the coated cellulose nitrate membrane at about 50 cm/h gave 99.4 and 99.97% removal (2.2 and 3.5 $\log_{10}$-unit reduction in concentration) of the virus, respectively (runs 6 and 9).

Simple filtration using the composite microporous membrane was thus demonstrated to be useful for effective removal of virus from water. The filtration was easily accomplished without exertion of pressure that was indispensable for the conventional ultrafiltration. Pore of the composite microporous membrane contains a tremendous number of connected minute beads of a pyridinium-type polymer that captures viruses on the surface (Figure 2). Effective removal of the virus can be explained in terms of the large surface area of pores of the composite membrane made of the polymer that captures viruses.

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