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Removal of pathogenic human viruses by insoluble pyridinium-type resin

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SUMMARY

Cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide) (BVP resin) was found to be very efficient in removing pathogenic human viruses from aqueous solution. In batch removal experiments using 50 g/l of BVP resin at 35 °C, the level of infectivity in suspensions of enterovirus, herpes simplex virus, poliovirus, and human immunodeficiency virus was reduced 1000–100000 fold during a 2 h period. Those of coxsackievirus and echovirus were reduced 60–600 fold during 1 h contact. The haemagglutination titres of solutions of human rotavirus, influenza virus, human adenovirus, and Japanese encephalitis virus were reduced 16–256 fold during 30 min of contact. In removal experiments by a continuous flow column method for poliovirus, enterovirus, and coxsackievirus with initial infectivities of less than 10^5/ml, the infectivity of these viruses was no longer detectable in the effluent solution. For poliovirus, coxsackievirus, and echovirus with initial infectivities higher than 10^6, 99·8–99·9998 % of the input viruses was removed as indicated by the reduction of infectivity.

INTRODUCTION

It is difficult to over-emphasize the importance of an adequate supply of wholesome drinking water to any community. Conventional treatment processes for water supplies have been thought to inactivate or remove viruses adequately. However, detection of infectious human viruses in treated drinking water [1–4] has raised concern that conventional methods may not always inactivate or remove viruses from water designated for human consumption. Viruses are reported to be more resistant than bacteria to chlorine disinfection [5, 6]. For example, poliovirus resists free chlorine residuals in excess of 1 mg/l of water [5]. The virucidal effect of chlorinated water is reported to be reduced by cyanuric acid [7]. Published reports deal with the removal of viruses by adsorption on soils, minerals, activated sludge flocks, membrane filters, and other solid materials [8–12]. However, the adsorption is not strong, and viruses are easily eluted from the adsorbents.

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, development of an effective and reliable new method to remove pathogenic human viruses from tap water and other water sources, as well as from air, is of special importance in the field of public health and hygiene, since at present administration of vaccines is the only way to protect humans from diseases caused by viruses. The problem is especially serious in a large city like Tokyo with a growing population, where the principal water resource is the water recovered from sewage treatment plants. Considerable amounts of pathogenic human viruses were detected in sewage, as well as in sludge and treated water obtained from sewage treatment plants [13].

A recent report has shown that cross-linked poly(*N*-benzyl-4-vinylpyridinium halide) (BVP resin), an insoluble pyridinium-type resin, strongly removes bacteriophage T4 from aqueous solution [14]. In a batch removal experiment using 95 g/l of BVP resin, the plaque-forming units of the phage solution were reduced 1000000 fold. The Freundlich isotherm plot of the results gave an equation:

$$\log X = 0{\cdot}13 \log C_e + 5{\cdot}6$$

where X is the amount of adsorbed T4 phage per unit weight of the resin, and C_e is the equilibrium concentration of free T4 phage in solution. This result indicated an extraordinarily strong removal of the virus by BVP resin. In a study using a continuous flow column, no plaque-forming units in the original phage suspension were found in the effluent solution. In the present study, pathogenic human viruses were used in testing for removal by BVP resin. The resin was found to have an excellent capability to remove these pathogenic human viruses from aqueous solution.

MATERIALS AND METHODS

Insoluble pyridinium-type resin

BVP resin, an insoluble pyridinium-type resin, was used. The resin was prepared in powder form as previously described [14] with minor modifications. Resin particle diameters in the wet state were 0·2–0·3 mm. Prior to the removal experiments, the resin was disinfected using 70% ethanol and washed extensively with sterilized physiological saline to remove the ethanol.

Viruses

Poliovirus type 2 (P-2) Sabin strain was allowed to multiply in lesur monkey kidney (LLC-MK2) cells. The cells were cultured in a 70 ml plastic flask (Pyrex Corp.), infected with P-2 seed virus, and incubated at 37 °C for 5 days in Eagle's minimal essential medium (MEM) supplemented with 2% fetal bovine serum. They were disrupted by freezing and thawing, and the supernatant was used as partially purified viral fluid after clarifying centrifugation at 7000 g for 20 min at 4 °C.

Enterovirus type 70 (EV-70) J-670/71 strain, coxsackievirus type A-9 (CA-9) Bozek strain, coxsackievirus type B-2 (CB-2) Ohio-1 strain, coxsackievirus type

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B-5 (CB-5) Faulkner strain, echovirus type 7 (E-7) Wallace strain, echovirus type 21 (E-21) Farina strain, and echovirus type 22 (E-22) Harris strain were all prepared from infected LLC-MK2 cells as described above for P-2 virus.

Herpes simplex virus type 1 (HSV-1) HF strain was propagated in human epithelial cells (HEp-2) of human laryngeal carcinoma. The cells were cultured in a 70 ml plastic flask (Pyrex Corp.), infected with HEp-2 grown seed virus, and incubated at 37 °C for 5 days in MEM supplemented with 2% fetal bovine serum. They were disrupted by freezing and thawing, and the supernatant was used as partially purified viral fluid after clarifying centrifugation at 7000 g for 20 min at 4 °C.

Human adenovirus type 37 (Ad-37) GW strain was prepared from infected human amnion (FL) cells in a similar manner.

The haemagglutinating antigen of Japanese encephalitis virus (JEV) JaGAr-01 strain was obtained from the brain of a 3–4 day-old infected suckling mouse by extraction with an acetone-ether mixture. Dilutions of this antigen were made with borate saline containing 0·5% egg albumin at pH 9·0.

Influenza virus type A (Flu-A) Yamagata/96/85 (H3N2) strain was inoculated allantoically into 10-day-old embryonated chicken eggs and incubated for 48 h at 39 °C. The infected allantoic fluid was used after purification by centrifugation at 7000 g for 20 min at 4 °C.

Tall-1 cells infected with human immunodeficiency virus (HIV) of type 1 LAV-1 strain were incubated at 37 °C for 3 days in RPMI-1640 medium supplemented with 10% fetal bovine serum. A 10 ml sample of supernatant was centrifuged at 13000 g for 30 min at 4 °C, and the pellet resuspended in 10 ml of phosphate-buffered saline (PBS) at pH 7·2.

Human rotavirus (HRV) was freshly isolated from faecal specimens from diarrhoeal patients infected with HRV. The specimens were added to PBS to give a 15% wt/vol. suspension and were homogenized by stirring. The supernatant of this suspension was the source of virus. It was purified by centrifugation at 100000 g for 90 min at 4 °C. The virus suspension was prepared by resuspending the pellet in PBS at pH 7·2.

Removal procedures

All removal experiments were carried out under aseptic conditions and performed in two different ways. Batch studies were done using glass vials 24·3 mm in diameter, and 55 mm high. The test virus (5·0 ml) in PBS and 0·125–1·00 g (dry weight, wet weight was 0·33–2·67 g) BVP resin were placed in the glass vials. The mixture was stirred magnetically at 240 rev/min at 35 °C. After a prescribed time, the mixture was allowed to settle, and 0·5 ml portions of the supernatant of the treated virus solutions were removed and quickly mixed with 4·5 or 0·5 ml of PBS; then two- or ten-fold serial dilutions were made by adding a 0·5 ml aliquot into 4·5 or 0·5 ml, respectively, of sterile physiological saline. The residual concentrations of the virus in each diluted solution were measured. These batch removal experiments were repeated three times unless otherwise stated.

Column studies of the removal experiments were carried out in a glass column 5 mm in diameter and 5 cm long at 25 °C. The column was filled with already wetted 0·25 g (dry weight, wet weight was 0·67 g) BVP resin. The resin bed was

5 mm in diameter and 5 cm long, with a resin bed volume of 1·0 ml. The test virus in PBS was passed through the resin bed in the column with a flow rate of three bed volumes per hour. Aliquots of the effluent solution were tested for infectivity or haemagglutination (HA) levels of the test viruses as described for the batch experiments.

Titration of virus solution

Infectivities of EV-70, P-2, HSV-1, CA-9, CB-2, CB-5, E-7, E-21, and E-22 were assayed as 50% tissue culture infectious doses ($TCID_{50}$) by the Kärber method. The same kinds of cells used for growing these viruses were cultured in 96 well plastic microplates (Greiner) and infected with 40 μl of each virus suspension. After incubation at 37 °C for 1 h, 0·1 ml of MEM was added to the cell suspensions. Incubation was continued at 33 °C for EV-70, or at 37 °C for the other viruses, for 5 days in a CO_2 incubator. Values of $TCID_{50}$/ml were calculated after observing virus infection of the cells under a microscope.

Infectivity of HIV was determined by estimating $TCID_{50}$/ml on an MT-4 cell line carrying HTLV-1. The cells were propagated in RPMI-1600 medium. A 100 μl sample of the cell suspension, containing 2×10^4 cells of MT-4, and 100 μl of the virus solution were added to the microplate wells, and the mixture was incubated at 37 °C for 6 days in a CO_2 chamber. Values of $TCID_{50}$ were calculated after observing infection of cells under a microscope.

The HA titre of Ad-37 was determined by using 0·5% rat red blood cells (RBC) in PBS containing 0·2% bovine serum albumin (fraction V). The haemagglutination reaction was carried out at 37 °C for 1 h. The HA titre of Flu-A was determined at room temperature for 1 h using a 0·5% suspension in PBS of RBC from a 1-day-old chicken. The HA titre of JEV was determined using 0·33% suspension in PBS of RBC from a 1-day-old chicken. The HA reaction was carried out at 37 °C for 1 h.

Recessive passive haemagglutination (R-PHA) was used for evaluating HRV concentration. In this case, a commercial R-PHA kit for HRV (Nissei Corp., Tokyo, Japan) was used for the detection and titration of HRV.

The titrations were done in quadruplicate every time.

RESULTS

Batch removal of pathogenic human viruses by BVP resin

Batch experiments on the removal of various pathogenic human viruses by BVP resin were performed at 35 °C in PBS, and the results are summarized in Tables 1 and 2.

Removal of EV-70 by BVP resin (runs 1–5) showed that infectivity of the virus solution was reduced 100–1000 fold after 10 min and 1000–100000 fold after 120 min. On the other hand, no appreciable change in infectivity was observed in the absence of BVP resin even after 120 min (runs 6 and 7).

The rate of reduction of the infectivity largely depended upon the amount of added BVP resin and the initial concentration of the virus. For example, when the initial $TCID_{50}$ was $1·0 \times 10^4$, the infectivity was reduced 1000 fold after 120 min with 25 g/l of BVP resin (run 5). With 200 g/l of BVP resin, however, the infectivity was reduced 20000 fold after 120 min (run 1). Thus the rate of

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Table 1. *Reduction of infectivity of solutions of pathogenic human viruses by adsorption on BVP resin**

Run no	Virus	BVP resin (g/l)	Infectivity ($TCID_{50}$) at the following contact time (min)				
			0	10	30	60	120
1	Enterovirus type 70	200	$1{\cdot}0 \times 10^{4}$	$4{\cdot}4 \times 10^{1}$	$4{\cdot}0 \times 10^{0}$	$5{\cdot}0 \times 10^{-1}$	$5{\cdot}0 \times 10^{-1}$
2	Enterovirus type 70	100	$1{\cdot}0 \times 10^{4}$	$1{\cdot}0 \times 10^{2}$	$1{\cdot}0 \times 10^{1}$	$4{\cdot}0 \times 10^{0}$	$2{\cdot}5 \times 10^{0}$
3	Enterovirus type 70	50	$1{\cdot}0 \times 10^{4}$	$7{\cdot}8 \times 10^{1}$	$1{\cdot}8 \times 10^{1}$	$1{\cdot}4 \times 10^{1}$	$1{\cdot}0 \times 10^{1}$
4	Enterovirus type 70	50	$1{\cdot}0 \times 10^{5}$	$1{\cdot}0 \times 10^{2}$	$1{\cdot}0 \times 10^{2}$	$1{\cdot}0 \times 10^{1}$	$1{\cdot}0 \times 10^{0}$
5	Enterovirus type 70	25	$1{\cdot}0 \times 10^{4}$	$1{\cdot}0 \times 10^{2}$	$7{\cdot}5 \times 10^{1}$	$5{\cdot}6 \times 10^{1}$	$1{\cdot}0 \times 10^{1}$
6	Enterovirus type 70	0	$1{\cdot}0 \times 10^{4}$	—	—	—	$1{\cdot}0 \times 10^{4}$
7	Enterovirus type 70	0	$1{\cdot}0 \times 10^{5}$	—	—	—	$1{\cdot}0 \times 10^{5}$
8	Herpes simplex virus type 1	50	$5{\cdot}6 \times 10^{4}$	$1{\cdot}0 \times 10^{1}$	$1{\cdot}0 \times 10^{0}$	—	0
9	Herpes simplex virus type 1	0	$5{\cdot}6 \times 10^{4}$	—	—	—	$5{\cdot}6 \times 10^{4}$
10	Poliovirus type 2	50	$1{\cdot}8 \times 10^{5}$	—	$1{\cdot}8 \times 10^{1}$	—	—
11	Poliovirus type 2	0	$1{\cdot}8 \times 10^{5}$	—	$1{\cdot}8 \times 10^{5}$	—	—
12	Human immunodeficiency virus type 1†	50	$1{\cdot}8 \times 10^{7}$	—	$3{\cdot}2 \times 10^{2}$	$3{\cdot}2 \times 10^{2}$	$5{\cdot}0 \times 10^{2}$
13	Human immunodeficiency virus type 1†	0	$1{\cdot}8 \times 10^{7}$	—	$1{\cdot}8 \times 10^{6}$	$1{\cdot}0 \times 10^{6}$	$3{\cdot}2 \times 10^{5}$
14	Coxsackievirus type A-9†	125	$5{\cdot}6 \times 10^{5}$	—	—	$5{\cdot}6 \times 10^{3}$	—
15	Echovirus type 21†	125	$3{\cdot}2 \times 10^{6}$	—	—	$5{\cdot}6 \times 10^{4}$	—
16	Echovirus type 22†	125	$3{\cdot}2 \times 10^{6}$	—	—	$5{\cdot}6 \times 10^{3}$	—

* Performed at 35 °C in phosphate-buffered saline of pH 7·2.

† Results obtained in a single experiment.

Table 2. *Reduction of haemogglutination titres of pathogenic human viruses by adsorption in BVP resin**

Run no.	Virus	BVP resin (g/l)	Haemagglutination (HA) titre at the following contact time (min) 0	10	30	60	120
17	Human rotavirus†	50	2^9	—	2^3	—	2^2
18	Influenza virus type A	50	2^{10}	—	2^2	—	—
19	Human adenovirus type 37	50	2^8	—	2^3	—	—
20	Japanese encephalitis virus†	50	2^5	2^2	$< 2^1$	$< 2^1$	$< 2^1$

* Performed at 35 °C in phosphate-buffered saline of pH 7·2.
† Results obtained by a single experiment.

reduction of the infectivity was higher with a larger amount of BVP resin. When the amount of added BVP resin was 50 g/l and the initial $TCID_{50}$ was $1{\cdot}0 \times 10^5$, the infectivity was reduced 1000 fold after 10 min and 100000 fold after 120 min (run 4). On the other hand, when the initial $TCID_{50}$ was $1{\cdot}0 \times 10^4$, the infectivity was reduced 128 fold after 10 min and 1000 fold after 120 min (run 3). Thus the rate of reduction of the infectivity was higher when the initial concentration of the virus was higher.

The course of removal of EV-70 by 50 g/l of BVP resin was followed at different concentrations of the virus by monitoring the loss of virus infectivity. Results are shown in Fig. 1. When the initial $TCID_{50}$ was $3{\cdot}2 \times 10^3$, the infectivity disappeared after 120 min. When the initial $TCID_{50}$ was $3{\cdot}2 \times 10^2$ or $1{\cdot}0 \times 10^1$, the infectivity disappeared after 10 min contact time. The rate of reduction of the infectivity tended to decrease with lower initial concentrations of virus as can be seen in Fig. 1. However, BVP resin was still able to remove the virus even at low concentration.

These results indicate that the extent of contact between the virus and BVP resin is important in the reduction of infectivity. The greater the contact, the greater the rate of reduction of infectivity, a finding further confirmed by the continuous flow column method. In the continuous removal experiment with a flow rate of three bed volumes per hour and an initial infectivity of $5{\cdot}6 \times 10^4$ $TCID_{50}$, virus infectivity was not detectable in the effluent solution. This result can be explained by the magnitude of the contact between virus and BVP resin, because the column was packed with the resin.

This excellent removal of EV-70 from aqueous solution by BVP resin prompted us to perform further removal experiments with other pathogenic human viruses. In a batch removal experiment using 50 g/l of BVP resin, infectivity of HSV-1 was reduced 5600 fold after 10 min, and disappeared after 120 min (run 8). On the other hand, no appreciable change in infectivity was observed even after 120 min in the absence of the resin (run 9). With 50 g/l of BVP resin, infectivity of P-2 was reduced 10000 fold after 30 min of contact (run 10). Using 50 g/l of BVP resin, infectivity of the HIV solution was reduced 56000 fold after 30 min (run 12), although infectivity decreased to some extent even in the absence of resin (run 13). HIV showed a spontaneous decrease in infectivity under the experimental conditions.

With 125 g/l of BVP resin, infectivity of CA-9 was reduced 100 fold after 60 min

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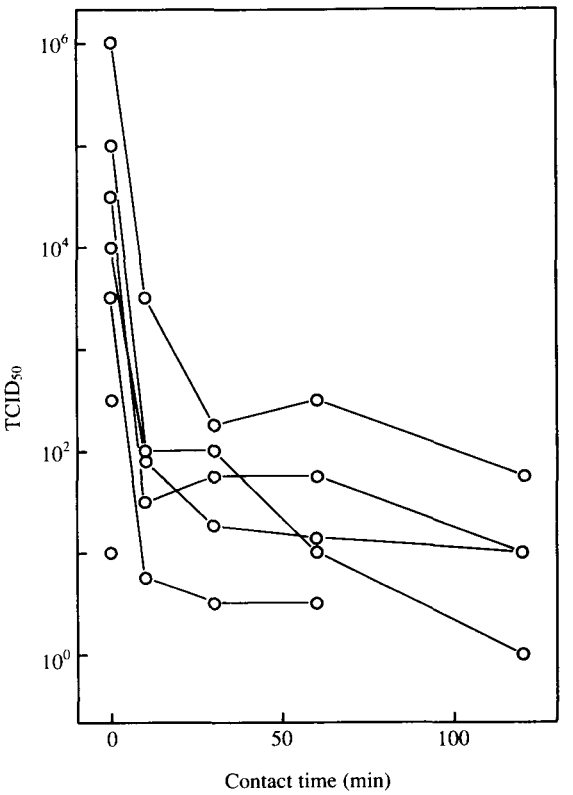

Fig. 1. Course of reduction of infectivity of EV-70 during contact with 50 g/l of BVP resin at 35 °C.

(run 14). With 125 g/l of BVP resin, the infectivities of E-21 and E-22 were reduced 60 and 600 fold, respectively, after 60 min (runs 15 and 16).

The HA titre of HRV was reduced 64 fold after contact with 50 g/l of BVP resin for 30 min, and was reduced 128 fold after 120 min (run 17), while that of Flu-A was reduced 256 fold after contact with 50 g/l of BVP resin for 30 min (run 18). The HA titre of Ad-37 was reduced 32 fold with 50 g/l of BVP resin after 30 min (run 19), and that of JEV was reduced 16 fold after contact with 50 g/l of BVP resin for 30 min (run 20).

Removal of pathogenic human virus using BVP resin by a continuous flow column method

The continuous flow column method is preferable for effective removal of pollutants by adsorption, because a high degree of contact between the pollutants and the surface of adsorbent can be expected. Studies on the removal of pathogenic human viruses from aqueous solutions using a column of BVP resin were carried out at 35 °C with a flow rate of three bed volumes per hour. In the experiments on the removal of EV-70, CB-5 and P-2 from PBS solutions with initial infectivities of $5{\cdot}6 \times 10^4$, $1{\cdot}0 \times 10^5$, and $3{\cdot}2 \times 10^4$, respectively, no infectious virus was detected in the effluent solutions. In order to obtain the rate of removal of viruses by BVP resin more precisely, continuous removal experiments for P-2,

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Table 3. *Removal of pathogenic human viruses from suspensions using BVP resin in a continuous flow column method**

Total volume passed through column (ml)	Poliovirus type 2		Coxsackievirus type B-2		Echovirus type 7	
	$TCID_{50}$	% Removal	$TCID_{50}$	% Removal	$TCID_{50}$	% Removal
Reservoir	$3{\cdot}2 \times 10^6$	—	$1{\cdot}0 \times 10^6$	—	$3{\cdot}2 \times 10^6$	—
1	0	—	0	—	0	—
2	$5{\cdot}6 \times 10^1$	99·998	$1{\cdot}8 \times 10^3$	99·8	$1{\cdot}0 \times 10^3$	99·97
3	$5{\cdot}6 \times 10^1$	99·998	$5{\cdot}6 \times 10^2$	99·94	$3{\cdot}2 \times 10^2$	99·99
4	$1{\cdot}0 \times 10^2$	99·997	$3{\cdot}2 \times 10^1$	99·997	$3{\cdot}2 \times 10^2$	99·99
5	$5{\cdot}6 \times 10^1$	99·998	$3{\cdot}2 \times 10^1$	99·997	$1{\cdot}0 \times 10^2$	99·997
6	$1{\cdot}8 \times 10^1$	99·9994	$5{\cdot}6 \times 10^1$	99·994	$3{\cdot}2 \times 10^1$	99·999
7	$1{\cdot}8 \times 10^1$	99·9994	$5{\cdot}6 \times 10^1$	99·994	$1{\cdot}0 \times 10^1$	99·9997
8	$1{\cdot}0 \times 10^1$	99·9997	$1{\cdot}0 \times 10^2$	99·99	$3{\cdot}2 \times 10^1$	99·999
9	$5{\cdot}6 \times 10^0$	99·9998	$3{\cdot}2 \times 10^1$	99·997	$1{\cdot}8 \times 10^1$	99·9994
10	$5{\cdot}6 \times 10^1$	99·998	$5{\cdot}6 \times 10^1$	99·994	$3{\cdot}2 \times 10^1$	99·999

* Performed by passing solutions of virus in phosphate-buffered saline of pH 7·2 at 25 °C with a flow rate of three bed volumes per hour. Bed dimensions, 5 mm in diameter and 5 cm long. Volume of resin bed, 1·0 ml.

CB-2, and E-7 were performed at initial infectivities in the order of 10^6. Results are summarized in Table 3. The rate of removal of these human viruses by BVP resin was 99·8–99·9998 %. In the removal of poliovirus type 1 and the simian rotavirus SA-11 by a quaternary ammonium-type anion-exchange resin in the triiodide form, the rate of removal was reported to be 91–99·9 % [15]. A comparison of these results leads us to conclude that the removal of viruses by BVP resin is greatly stronger than that by the above anion-exchange resin, although the strain of viruses used was different in these experiments.

DISCUSSION

As has been mentioned above, BVP resin was shown to have an excellent ability to remove not only bacteriophage T4 but also various types of pathogenic human viruses. Batch removal experiments showed 1000–100000 fold reduction in infectivity, or 16–256 fold reduction in haemagglutination titres after contact with 25–200 g/l of BVP resin at 35 °C for 30–120 min. In removal experiments with a continuous flow column method having a flow rate of three bed volumes per hour and with initial infectivities of less than 10^5, no infectivity remained in the effluent solution. Although the viruses were detected in the effluent solution when the initial infectivities were higher than 10^6, removal of these human viruses reached 99·8–99·9998 %. The observation showed that removal of viruses by BVP resin is much greater than by a quaternary ammonium-type anion-exchange resin in the triiodide form which was reported to be 91–99·9 % [15].

BVP resin showed good removal not only of unenveloped viruses such as EV-70, P-2, CA-9, CB-2, CB-5, E-7, E-21, E-22, and Ad-37, but also of enveloped viruses such as HSV-1, HIV, Flu-A, and JEV. A new, reliable and highly effective method can be developed with the use of this novel synthetic resin to remove

pathogenic human viruses from water supplies designated for human consumption.

A strongly basic quaternary ammonium anion-exchange resin in the triiodide form has been reported to show virucidal activity [15, 16]. The chemical structure of the anion-exchange resin is closely similar to BVP resin, because the pyridinium group is a type of quaternary ammonium group. However, BVP resin removed bacteriophage T4 while keeping the bacteriolytic activity [14], in sharp contrast to the virucidal activity of the anion-exchange resin in the triiodide form. The strong action of BVP resin in removing viruses is a specific character of the insoluble pyridinium-type resin and is not generally observed with all types of quaternary ammonium resin. Further research is required to elucidate the mechanism of the powerful removal of viruses by BVP resin. However, if the mechanism is similar to the capture of bacterial cells by the resin [17], then the following factors appear to play important roles in the removal of viruses: the electrostatic interaction between the positive charge of the resin and the negative charge of viruses, the hydrophobic interaction between the resin and virus surfaces [18], the strong hydrophilicity and the considerable swelling of the resin matrix of BVP resin in water [19] (which is quite different from conventional anion-exchange resins).

The insoluble pyridinium-type resin captures microbial cells alive on the surface [17], and the microbial cells immobilized on the resin surface form a useful bioreactor system [20]. However, the resin does not show similar adsorption characteristics against proteins such as enzymes. Thus, it would be difficult to explain the removal of viruses by the BVP resin by the formation of bonds by the resin with proteins.

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REFERENCES

1. American Water Works Association Committee. Viruses in drinking water. J Am Water Works Assoc 1979; **71**: 441–4.
2. Payment P. Isolation of viruses from drinking water at the Pont-Viau water treatment plant. Canadian J Microbiol 1981; **27**: 417–20.
3. Murphy AM, Grohmann GS, Sexton MFH. Infectious gastroenteritis in Norfolk Isoland and recovery of viruses from drinking water. J Hyg 1983; **91**: 139–46.
4. Keswick BH, Gerba CP, DuPont HL, Rose JB. Detection of enteric viruses in treated drinking water. Appl Environ Microbiol 1984; **47**: 1290–4.
5. Bates RC, Shaffer PTB, Sutherland SM. Development of poliovirus having increased resistance to chlorine inactivation. Appl Environ Microbiol 1977; **34**: 849–53.
6. Shaffer PTB, Metcalf TG, Sproul OJ. Chlorine resistance of poliovirus isolants from drinking water. Appl Environ Microbiol 1980; **40**: 1115–21.
7. Yamashita Y, Sakae K, Ishihara Y, Isomura S, Inoue H. Virucidal effect of chlorinated water containing cyanuric acid. Epidemiol Infect 1988; **101**: 631–9.
8. Wallis C, Melnick JL, Fields JE. Concentration and purification of viruses by adsorption to and elution from insoluble polyelectrolytes. Appl Environ Microbiol 1971; **21**: 703–9.
9. Farrah SR, Britton G. Elution of poliovirus adsorbed to membrane filters. Appl Environ Microbiol 1978; **36**: 982–4.

10. Landry EF, Vaughn JM, Thomas MZ, Beckwith CA. Adsorption of enterovirus to soil cores and their subsequent elution by artificial rain water. Appl Environ Microbiol 1979; **38**: 680–7.
11. Murray JP, Laband SJ. Degradation of poliovirus by adsorption on inorganic surfaces. Appl Environ Microbiol 1979; **37**: 480–6.
12. Moore RS, Taylor DH, Sturman LS, Reddy MMM, Fuhs GW. Poliovirus adsorbed by 34 minerals and soils. Appl Environ Microbiol 1981; **42**: 963–75.
13. Taguchi F, Yano K. Viruses in water resources. J Antibact Antifungal Agents 1988; **16**: 233–47.
14. Kawabata N, Hashizume T, Matsumoto T. Adsorption of bacteriophage T4 by cross-linked poly(vinylpyridinium halide). Agricult Biol Chem 1986; **50**: 1551–5.
15. Gerba CP, Janauer GE, Costello M. Removal of poliovirus and rotavirus from tap water by a quaternary ammonium resin. Water Research 1984; **18**: 17–9.
16. Fina LR, Hassouna N, Horacek GL, Lambert JP, Lambert JL. Virucidal capability of resin-triiodide demand-type disinfectant. Appl Environ Microbiol 1982; **44**: 1370–3.
17. Kawabata N, Hayashi T, Matsumoto T. Removal of bacteria from water by adhesion to cross-linked poly(vinylpyridinium halide). Appl Environ Microbiol 1983; **46**: 203–10.
18. Kawabata N, Ueno Y, Torii K, Matsumoto T. Capturing interaction between insoluble pyridinium-type polymer and bacterial cells. Agricult Biol Chem 1987; **51**: 1085–90.
19. Kawabata N, Hayashi T, Nishikawa M. Effect of the structure of insoluble pyridinium-type polymer on its ability to capture bacteria in water. Bull Chem Soc Japan 1986; **59**: 2861–3.
20. Kawabata N, Nishimura S, Yoshimura T. New method of immobilization of microbial cells by capture on the surface of insoluble pyridinium-type resin. Biotech and Bioeng 1990; **35**: 1000–5.