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Assessment of the efficacy of membrane filtration processes to remove human enteric viruses and the suitability of bacteriophages and a plant virus as surrogates for those viruses

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Abstract

Here, we evaluated the efficacy of direct microfiltration (MF) and ultrafiltration (UF) to remove three representative human enteric viruses (i.e., adenovirus [AdV] type 40, coxsackievirus [CV] B5, and hepatitis A virus [HAV] IB), and one surrogate of human caliciviruses (i.e., murine norovirus [MNV] type 1). Eight different MF membranes and three different UF membranes were used. We also examined the ability of coagulation pretreatment with high-basicity polyaluminum chloride
(PACI) to enhance virus removal by MF. The removal ratios of two bacteriophages (MS2 and φX174) and a plant virus (pepper mild mottle virus; PMMoV) were compared with the removal ratios of the human enteric viruses to assess the suitability of these viruses to be used as surrogates for human enteric viruses. The virus removal ratios obtained with direct MF with membranes with nominal pore sizes of 0.1 to 0.22 µm differed, depending on the membrane used; adsorptive interactions, particularly hydrophobic interactions between virus particles and the membrane surface, were dominant factors for virus removal. In contrast, direct UF with membranes with nominal molecular weight cutoffs of 1 to 100 kDa effectively removed viruses through size exclusion, and >4-log_{10} removal was achieved when a membrane with a nominal molecular weight cutoff of 1 kDa was used. At pH 7 and 8, in-line coagulation–MF with nonsulfated high-basicity PACIs containing Al_{30} species had generally a better virus removal (i.e., >4-log_{10} virus removal) than the other aluminum-based coagulants, except for φX174. For all of the filtration processes, the removal ratios of AdV, CV, HAV, and MNV were comparable and strongly correlated with each other. The removal ratios of MS2 and PMMoV were comparable or smaller than those of the three human enteric viruses and MNV, and were strongly correlated with those of the three human enteric viruses and MNV. The removal ratios obtained with coagulation–MF for φX174 were markedly smaller than those obtained for the three human enteric viruses and MNV. However, because MS2 was inactivated after contact with PACI during coagulation pretreatment, unlike AdV, CV, MNV, and PMMoV, the removal ratios of infectious MS2 were probably an overestimation of the ability of
coagulation–MF to remove infectious AdV, CV, and caliciviruses. Thus, PMMoV appears to be a suitable surrogate for human enteric viruses, whereas MS2 and φX174 do not, for the assessment of the efficacy of membrane filtration processes to remove viruses.

Keywords: Electrostatic interaction, Hydrophobic interaction, Microfiltration, Nonsulfated high-basicity PACl, Pepper mild mottle virus, Ultrafiltration

1. Introduction

The provision of safe drinking water is essential for ensuring public health. However, increases in the global populations of humans and domestic animals have resulted in increased demand for safe drinking water, which has led to the use of alternative water sources, sometimes of compromised quality, in many parts of the world (Bosch, 2007; Ferrer et al., 2015). These population increases have also led to increased fecal contamination of water sources, and because large numbers of pathogenic microorganisms are shed in the feces of infected people and animals, water sources receiving sewage discharge are often contaminated with those microorganisms (Rose et al., 1991; Albinana-Gimenez et al., 2006; Bosch, 2007). Therefore, water safety plans built on the principles of multiple barriers, hazard analyses, and critical control points are crucial not only for preventing
the contamination of source water via human and animal waste but also for providing adequately
treated and disinfected drinking water (WHO, 2011).

Membrane filtration, particularly low-pressure membrane filtration, is commonly used as an
absolute barrier to microorganism contamination in the production of drinking water. However,
although complete removal of bacteria and protozoa has been reported for the low-pressure
membrane filtration processes of direct microfiltration (MF) and ultrafiltration (UF) (Jacangelo et
al., 1995; Howe 2006), varying virus removal ratios have been reported for these processes
(Jacangelo et al., 1995; Madaeni et al., 1995; Urase et al., 1996; van Voorthuizen et al., 2001;
Langlet et al., 2009; Boudaud et al., 2012; ElHadidy et al., 2013; Matsushita et al., 2013; Ferrer et
al., 2015). The mechanisms underlying the removal of viruses by low-pressure membrane filtration
include size exclusion and adsorptive interactions (i.e., hydrophobic and electrostatic interactions)
between the virus particles and the membrane surface. The relative contribution of these
mechanisms to the removal of viruses depends on the characteristics of the virus particles and
membrane such as the relative size of the virus particles to the size of the membrane pores and the
hydrophobicity and surface charge of the virus particles and membrane surface (Urase et al., 1996;
van Voorthuizen et al., 2001; ElHadidy et al., 2013). Indeed, since the pore sizes of membrane
filters are generally larger than the size of virus particles, there is almost no removal of viruses via
direct MF when the relative contribution of absorptive interactions to virus removal is negligible
(van Voorthuizen et al., 2001; Matsushita et al., 2013).
Coagulation pretreatment prior to membrane filtration, particularly in MF processes, is widely used in the water industry to improve the quality of drinking water, and the combination of coagulation pretreatment and MF processes (coagulation–MF) also shows promise as an effective means of removing virus particles. Indeed, under appropriate coagulation conditions, the virus removal performances of coagulation–MF with membrane with a nominal pore size larger than the size of the virus particles are similar or greater than the performance of direct UF with membrane with a nominal molecular weight cutoff (MWCO) smaller than the size of the virus particles (Matsui et al., 2003; Matsushita et al., 2013). Aluminum-based coagulants such as polyaluminum chloride (PACl) and alum are common coagulants in coagulation–MF processes, and coagulation conditions such as coagulation pH, coagulant dosage, and coagulation time have been shown to affect virus removal performance (Matsushita et al., 2005; Shirasaki et al., 2009; Tanneru et al., 2013). In addition, coagulant properties, such as the distribution of the aluminum hydrolyte species in the coagulant, which might be partly controlled by basicity ([OH⁻]/[Al³⁺]), have also been shown to affect membrane permeability and the quality of water processed by means of coagulation–MF (Zhao et al., 2010; Kimura et al., 2015). Kimura et al. (2015) reported that increasing the basicity of PACl coagulants from the typical basicity of 1.5 to a high basicity of 2.1 not only increased the removal of dissolved organic carbon and reduced the concentration of residual aluminum in the filtrate but also mitigated irreversible membrane fouling, which is a major cause of reduced membrane permeability that increases operating costs, in coagulation–MF processes. Thus, the
integration of pretreatment with high-basicity PACls into MF processes may increase the removal of virus particles.

The virus removal performances of direct MF, direct UF, and coagulation–MF processes are often evaluated by using bacteriophages such as MS2, Qβ, and φX174 as surrogates of human enteric viruses because these viruses do not infect humans and are easier to cultivate than human enteric viruses (Jacangelo et al., 1995; Urase et al., 1996; van Voorthuizen et al., 2001; Matsui et al., 2003; Matsushita et al., 2005; Langlet et al., 2009; Shirasaki et al., 2009; Boudaud et al., 2012; Tanneru and Chellam 2012; ElHadidy et al., 2013; Matsushita et al., 2013; Tanneru et al., 2013; Ferrer et al., 2015). However, data on the removal of human enteric viruses via membrane filtration is limited, particularly regarding fundamental principles such as the contributions of the size exclusion and adsorptive interaction mechanisms to virus removal via direct MF or UF and the effects of coagulation conditions and coagulant properties on virus removal via coagulation–MF, partly due to the need for bio-containment facilities for the safe handling of viruses and the fact that virus cultivation is labor intensive and time consuming (Ryu et al., 2010). Thus, whether these bacteriophages are adequate surrogates for human enteric viruses in membrane filtration processes remains unknown.

Recently, a metagenomic analysis revealed that pepper mild mottle virus (PMMoV; genus Tobamovirus, family Virgaviridae), which infects pepper species, was the most abundant viral RNA in human feces (concentrations up to $10^9$ virus particles/g of feces; Zhang et al., 2006). In addition,
because PMMoV is more frequently detected at higher concentrations in environmental waters, including drinking water sources, than are human enteric viruses (Hamza et al., 2011; Haramoto et al., 2013), PMMoV may be a potential indicator of fecal contamination of surface water. Thus, if the removal efficiencies of PMMoV and human enteric viruses are comparable, PMMoV could be a useful surrogate for evaluating the efficacy of membrane filtration processes to remove human enteric viruses.

In the present study, we used eight types of MF membranes and three types of UF membranes to investigate the efficacy of direct membrane filtration processes to remove the representative human enteric viruses (i.e., adenovirus [AdV], coxsackievirus [CV], and hepatitis A virus [HAV]) included in the Fourth Drinking Water Contaminant Candidate List (CCL4) published by the US Environmental Protection Agency (2016), and murine norovirus (MNV) as a surrogate of human caliciviruses. In addition, we examined the effects of membrane pore size and membrane material, which are related to the hydrophobic and electrostatic interactions between virus particles and the membrane surface, on virus removal. Next, we examined the potential of pretreatment with a high-basicity PACl to enhance virus removal via membrane filtration by comparing virus removal efficiencies obtained with several PACls and alum under various coagulation conditions. Finally, we examined removal efficiencies of the bacteriophages MS2 and φX174, and of the plant virus PMMoV, to assess the suitability of these viruses as surrogates for human enteric viruses.
2. Materials and methods

2.1. Source water, coagulants, and membranes

On 30 September 2015, river water was sampled from the Edo River (Tokyo, Japan; see Table 1 for water quality), which is the source water for the Kanamachi Water Purification Plant (Tokyo, Japan). The source water samples were stored at 4 °C until use and brought to 20 °C immediately prior to use.

To investigate the effect of coagulant type (i.e., effects of coagulant basicity and sulfate content) on virus removal via in-line coagulation pretreatment and MF, we used five aluminum-based coagulants (provided by Taki Chemical Co., Kakogawa, Japan). Specifications of the coagulants are shown in Table 2 and are described in Supplementary Information.

To investigate the effects of membrane pore size and membrane material on virus removal via membrane filtration, we used eight commercially available MF membranes and three commercially available UF membranes (provided by Millipore Corp., Billerica, MA, USA, and 3M Corp., Maplewood, MN, USA). Specifications of the membranes are shown in Table 3.

2.2. Characterization of coagulants and membranes

The aluminum hydrolyte species in the coagulants were analyzed by using liquid $^{27}$Al nuclear magnetic resonance (NMR) spectroscopy after the coagulants were diluted with Milli-Q water to a
concentration of 54 g-Al/L (i.e., 2.0 M-Al). The details of the liquid $^{27}$Al NMR analysis are described in our previous report (Shirasaki et al., 2014).

The hydrophobicities of the membranes were determined by means of water contact angle measurement. The surface charges (i.e., zeta potential) of the membranes were determined with a Zetasizer Nano ZS (50-mW, 532-nm green laser; Malvern Instruments, Malvern, Worcestershire, UK) equipped with a surface zeta potential cell kit (ZEN1020; Malvern Instruments). Details of the hydrophobicity and zeta potential measurements are provided in Supplementary Information.

2.3. Human enteric viruses, MNV, bacteriophages, and PMMoV

AdV type 40 Dugan strain (ATCC VR-931), CV B5 Faulkner strain (ATCC VR-185), HAV IB HM175/18f strain (ATCC VR-1402), and MNV type 1 CW1 strain (ATCC PTA-5935) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and propagated in human lung carcinoma epithelial cells (A549 cells; ATCC CCL-185, obtained from ATCC), buffalo green monkey kidney epithelial cells (BGM cells; kindly supplied by Dr. Daisuke Sano, Hokkaido University, Sapporo, Japan), fetal rhesus monkey kidney epithelial cells (FRhK-4 cells; ATCC CRL-1688, obtained from ATCC), and murine macrophage cells (RAW264.7 cells; ATCC TIB-71, obtained from ATCC), respectively. The details of the propagation and purification of AdV, CV, HAV, and MNV are described in our previous reports (Shirasaki et al., 2016a; Shirasaki et al., 2017). The concentrations of AdV, CV, HAV, and MNV in the purified solutions were
approximately $10^{5-6}$, $10^7$, $10^{5-6}$, and $10^6$ plaque-forming units (PFU)/mL, respectively, as evaluated by means of plaque assays (Shirasaki et al., 2016a; Shirasaki et al., 2017).

F-specific RNA bacteriophage MS2 (NBRC 102619) and somatic DNA bacteriophage φX174 (NBRC 103405) were obtained from the National Institute of Technology and Evaluation Biological Research Center (Kisarazu, Japan), as were the *Escherichia coli* bacterial hosts in which the bacteriophages were propagated (NBRC 13965 for MS2, NBRC 13898 for φX174). The details of the propagation and purification of the bacteriophages are described in our previous report (Shirasaki et al., 2016a). The concentrations of MS2 and φX174 in the purified solutions were approximately $10^{10}$ and $10^{7-8}$ PFU/mL, respectively, as evaluated by means of plaque assays (Shirasaki et al., 2016a).

PMMoV pepIwateHachiman1 strain (MAFF 104099) was obtained from the National Institute of Agrobiological Sciences Genebank (Tsukuba, Japan) and propagated in *Nicotiana benthamiana* (seeds kindly supplied by Dr. Kenji Nakahara, Hokkaido University). The details of the propagation of PMMoV are provided in Supplementary Information. The concentration of PMMoV in the stock solution was approximately $10^7$ lesions/mL, as evaluated by using a local lesion count assay with *N. tabacum* cv. *Xanthi-nc* (seeds also kindly supplied by Dr. Kenji Nakahara).

2.4. Direct membrane filtration experiments and coagulation–MF experiments

Direct membrane filtration experiments and coagulation–MF experiments were conducted with
virus-spiked river water at 20 °C. The details of the experimental procedures are provided in Supplementary Information.

2.5. Virus assay

The real-time polymerase chain reaction (real-time PCR), which can detect all viruses regardless of their infectivity or the existence of aggregates, was used to quantify viral DNA and RNA. Specifically, viral DNA of AdV or φX174 was quantified by means of real-time PCR, and viral RNA of CV, HAV, MNV, MS2, or PMMoV was quantified by means of real-time reverse-transcription PCR (real-time RT-PCR). The details of the real-time PCR and real-time RT-PCR methods are provided in Supplementary Information.

2.6. Characterization of PMMoV

The electrophoretic mobility of PMMoV was measured in prepared Milli-Q water. The hydrophobicity of PMMoV was estimated by using the bacterial adherence to hydrocarbon method (Rosenberg et al., 1980), with some modifications. Stock solution of PMMoV was suspended in specially prepared Milli-Q water (for the measurement of the electrophoretic mobility) or phosphate-buffered saline (for the measurement of the hydrophobicity) at approximately $10^{4-5}$ lesions/mL. The details of the electrophoretic mobility and hydrophobicity measurements are described in our previous reports (Shirasaki et al., 2016a; Shirasaki et al., 2017).
3. Results and discussion

3.1. Virus removal by means of direct MF or UF

Figure 1 shows the removal ratios ($\log_{10}[C_f/C_0]$) of the viruses via direct MF with eight different membrane filters. The surface is typically considered hydrophobic (low wettability) if the water contact angle is greater than 90° and hydrophilic (high wettability) if the water contact angle is less than 90° (Wang et al., 2016). Thus, the surfaces of PVDF-0.1-HP and PVDF-0.22-HP were deemed to be hydrophobic, the surface of PTFE-0.1 was deemed to be neutral, and those of the remaining membranes were deemed to be hydrophilic, as assessed by means of water contact angle measurement (Table 3). The surface of ZP-0.1 was positively charged whereas the surfaces of the other membranes were negatively charged in Milli-Q water at pH 7 (Table 3). The virions of AdV, CV, HAV, MNV, MS2, and φX174 are spherical with diameters of 70‒90 nm, 22‒30 nm, 22‒30 nm, 27‒40 nm, 26 nm, and 28 nm, respectively, and the virions of PMMoV are elongated, rigid cylinders with a diameter of 18 nm and a length of 300‒310 nm (Fauquet et al., 2005). The virus particles were negatively charged and stably monodispersed by electrical repulsion at pH 7; the isoelectric points of AdV, CV, HAV, MNV, MS2, and φX174 ranged from 1.6 to 3.8 (Shirasaki et al., 2016a; Shirasaki et al., 2017) and that of PMMoV was 3.2 (Figure S1). Thus, if the adsorptive interactions between the virus particles and membrane surface were negligible, the viruses were
expected to pass through the MF membranes because the diameters of the viruses were smaller than the nominal pore size of the MF membranes (0.1–0.22 µm). Indeed, limited removal ratios (<0.5-log₁₀) were obtained for AdV, CV, HAV, and MNV when the hydrophilic, negatively charged MF membranes (i.e., PVDF-0.1-LP, PVDF-0.22-LP, PTFE-0.1, and PC-0.1) were used at pH 7, although MCE-0.1 was an exception (range, 0.5–2.1-log₁₀). In contrast, the hydrophobic or positively charged MF membranes effectively removed the viruses from the water; the removal ratios of AdV, CV, HAV, and MNV for PVDF-0.1-HP, PVDF-0.22-HP, and ZP-0.1 were 3.6- to 4.6-log₁₀, 2.8- to >4.2-log₁₀, and 1.8- to 2.7-log₁₀, respectively. Similar results were obtained for MS2, φX174, and PMMoV. These results suggest that hydrophobic and electrostatic interactions are the main mechanisms by which viruses are removed during MF and that the contribution of size exclusion was negligible. Madaeni et al. (1995) reported 1- to 4-log₁₀ removal of MS2 was achieved with PVDF-0.22-HP at pH 7, whereas almost no removal was observed with PVDF-0.22-LP, which is in agreement with our results. In addition, because the removal ratios of AdV, CV, HAV, and MNV obtained with the hydrophobic and negatively charged membranes PVDF-0.1-HP and PVDF-0.22-HP were higher than those obtained with the hydrophilic and positively charged membrane ZP-0.1, the contribution of hydrophobic interactions was likely greater than that of electrostatic interactions to the removal of the viruses at pH 7.

Figure 2 shows the removal ratios of the viruses via direct UF with three different filters. Because the relative molecular masses of AdV, CV, HAV, MNV, MS2, φX174, and PMMoV (150–180 × 10⁶,
8–9 × 10^6, 8–9 × 10^6, 15 × 10^6, 4 × 10^6, 6 × 10^6, and 40 × 10^6 Da, respectively; Sinsheimer 1959; Fauquet et al., 2005) were much larger than the nominal MWCOs of the three UF membranes (1–100 kDa), effective virus removal by all of the filters was predicted for all of the viruses. As expected, 3- to >5-log_{10} removal of AdV, CV, HAV, and MNV were observed when the UF membrane with a nominal MWCO of 100 kDa (i.e., RC-100k) was used at pH 7. In addition, the virus removal ratio increased as the nominal MWCO of the UF membranes was decreased from 100 kDa to 1kDa; >4-log_{10} removal of all the viruses used in the present study was achieved with the UF membrane with a nominal MWCO of 1 kDa (i.e., RC-1k). These results indicated that direct UF is effective for the removal of different types of virus, including three types of human enteric viruses. Other groups have also reported the usefulness of direct UF for the removal of bacteriophages, and have suggested that the bacteriophage removal ratio is strongly dependent on the physicochemical characteristics of the membrane (Jacangelo et al., 1995; Langlet et al., 2009; Boudaud et al., 2012). Because the hydrophobicities and surface charges of the three UF membranes were comparable (Table 3) and the roles of hydrophobic and electrostatic interactions in virus removal were likely negligible, size exclusion must have been the main mechanism by which the viruses were removed. Thus, the difference in the virus removal ratios of RC-1k, RC-10k, and RC-100k was considered to be due to the differences in the nominal MWCOs of these UF membranes.
3.2. Effect of adsorptive interactions on virus removal

As described in section 3.1, adsorptive interactions between the virus particles and membrane surface were likely the dominant factors for virus removal via direct MF at pH 7. To confirm this finding, we conducted additional filtration experiments with PVDF-0.1-LP, PVDF-0.1-HP, MCE-0.1, and ZP-0.1 where the filtration volume of the virus-spiked river water was increased from 50 mL up to 250 mL (Figure 3a–g) or 1000 mL (Figure 3h–n). The virus removal ratios of all of the viruses obtained with PVDF-0.1-LP were very low and remained constant up to the maximum filtration volume of 1000 mL. Jacangelo et al. (1995) reported that the removal ratio of MS2 obtained with a polysulfone MF membrane with a pore size of 0.2 µm increased with increasing mass of kaolinite deposited on the membrane surface at pH 7. Although the source water used in the present study contained suspended solids (turbidity = 2.8 NTU; Table 1), the removal ratios obtained did not increase with filtration volume, possibly due to differences in the mass of suspended solids deposited on the membrane surface between our study and that of Jacangelo et al. (1995).

The virus removal ratios obtained with PVDF-0.1-HP after 50 mL of filtration were similar to those obtained in the direct MF experiments (Figure 1); however, the removal ratios gradually decreased as the filtration volume was increased from 50 to 250 mL and from 200 to 600 mL (Figure 3). These results indicate that the most accessible hydrophobic adsorption sites for virus adsorption on the PVDF-0.1-HP membrane were gradually saturated and were almost exhausted at
600 mL of filtration. Thus, hydrophobic adsorptive interactions between the virus particles and hydrophobic membrane surface were the dominant factors for virus removal via direct MF until the adsorption sites were saturated with hydrophobic substances present in the source water. In addition, because the virus removal ratios obtained with ZP-0.1 also gradually decreased with increasing filtration volume, except in the case of HAV, electrostatic adsorptive interactions between the virus particles and the positively charged membrane surface also likely contributed to the virus removal ratios obtained. In contrast, the change in the virus removal ratios obtained with MCE-0.1 and increasing filtration volume differed, depending on the type of virus in the spiked water; the virus removal ratios of AdV, HAV, MNV, and PMMoV decreased with increasing filtration volume, whereas those of CV, MS2, and φX174 remained constant during 1000 mL of filtration. Because the contact angle and surface charge of MCE-0.1 were similar to those of PTFE-0.1 and PC-0.1, respectively, and the virus removal ratios of PTFE-0.1 and PC-0.1 were <0.5-log_{10}, as described in section 3.1, the virus removal ratios observed for MCE-0.1 could not be explained simply by the contact angle and surface charge of the membranes. Further investigation, such as elucidation of the pore size distribution of the membranes, is needed to determine why the hydrophilic, negatively charged membrane MCE-0.1 more effectively removed viruses compared with the other hydrophilic, negatively charged MF membranes.

Natural organic substances present in surface water are known to adsorb onto the membrane during filtration and cause a decline in flux (i.e., membrane fouling). Indeed, the fouling rate for
PVDF-0.22-HP is considerably larger than that for PVDF-0.22-LP when filtration is performed with water containing natural organic substances (Fan et al., 2001). We further investigated the competitive adsorption between viruses and natural organic substances in the source water by using PVDF-0.1-HP (Figure S2). The virus removal ratios obtained after prefiltration with 200 or 400 mL of the unspiked river water were smaller than those obtained without prefiltration, indicating that natural organic substances in the source water used in the present study competed with the viruses for the hydrophobic adsorption sites on PVDF-0.1-HP. Therefore, the reduction of virus removal performance by adsorption competition between virus and natural organic substances should be considered when hydrophobic interactions are the dominant removal mechanism during membrane filtration.

3.3. Virus removal by means of coagulation‒MF

Figure 4 shows the removal ratios obtained with in-line coagulation‒MF. As described in section 3.1, limited virus removal (<0.2-log₁₀) was obtained with PVDF-0.1-LP without coagulation pretreatment at pH 7 (Figure 1). However, the removal ratios of AdV, CV, HAV, and MNV were improved when the water was subjected to coagulation pretreatment with 0.54 mg-Al/L of coagulant prior to MF (Figure 4a), suggesting that during coagulation pretreatment the virus particles were incorporated into the aluminum floc, which was larger than the nominal pore size of the MF membrane, and the virus-containing floc was then effectively removed by size exclusion.
The virus removal ratios obtained were dependent on coagulant type; virus removal ratios of 0.5- to 2-\log_{10} were obtained with in-line coagulation–MF with alum or PACl-1.5s as the coagulant, whereas virus removal ratios of 1- to >4-log_{10} were obtained with the high-basicity PACls. In particular, with the nonsulfated, high-basicity PACls (i.e., PACl-2.1ns and PACl-2.5ns), virus removal ratios of >4.3-log_{10} for AdV, >3.5-log_{10} for HAV, and 3.3–3.6-log_{10} for MNV were obtained, and CV, MS2, and PMMoV were removed more efficiently than with the other coagulants. However, the virus removal ratios of φX174 were comparable, regardless of the coagulant used.

The virus removal ratios increased as coagulant dosage was increased from 0.54 to 1.08 mg-Al/L at pH 7. For AdV, HAV, and MNV, virus removal ratios of >3.9-log_{10} were obtained with in-line coagulation–MF, irrespective of the type of coagulant. In addition, PACl-2.1ns and PACl-2.5ns had the highest virus removal ratios, irrespective of the type of virus, except in the case of φX174 (range, >4.0- to >5.7-log_{10}).

The virus removal ratios obtained with pretreatment with alum, PACl-1.5s, or PACl-2.1s (dose 1.08 mg-Al/L) markedly decreased when the pH of the treated water was increased from 7 to 8 (Figure 4b) and roughly corresponded to those obtained with a coagulant dose of 0.54 mg-Al/L at pH 7 (Figure 4a). Even when the coagulant dosage was increased from 1.08 to 2.16 mg-Al/L at pH 8, almost no or only a slight improvement in the virus removal ratios was observed for alum, PACl-1.5s, or PACl-2.1s. In contrast, the high virus removal ratios obtained with PACl-2.1ns or PACl-2.5ns were retained (range, >4.1- to >6.0-log_{10}) even at pH 8, although the case of φX174 was...
an exception. These results indicate that the type of coagulant strongly affected the virus removal
performance of in-line coagulation‒MF, and that nonsulfated, high-basicity PACls were the most
effective for removing viruses not only at neutral pH but also under weakly alkaline pH. In addition,
the coagulation conditions (i.e., coagulation pH and coagulant dosage) also strongly affected the
virus removal performance of in-line coagulation‒MF, although the magnitude of the effects of the
coaugulation conditions on virus removal performance was dependent on the type of coagulant; the
virus removal performances of alum, PACl-1.5s, and PACl-2.1s were more sensitive to changes in
the coagulation pH and coagulant dosage than were those of PACl-2.1ns and PACl-2.5ns.

We previously reported that PACl-2.1ns has a higher colloid charge density than do alum,
PACl-1.5s, and PACl-2.1s, probably due to its higher colloidal aluminum content and absence of
sulfate (Shirasaki et al., 2014). In addition, we previously reported that the Al30 species
\([Al_{30}O_8(OH)_{56}(H_2O)_{24}]^{18+}\), which is classified as a colloidal aluminum species, as assessed by the
ferron method (Chen et al., 2007), probably plays a major role in the removal not only of
bacteriophages (Shirasaki et al., 2014) but also of human enteroviruses (Shirasaki et al., 2016b)
during coagulation. To confirm whether Al30 species were present in the nonsulfated, high-basicity
PACls used in the present study, we analyzed the coagulants by using $^{27}$Al NMR (Figure 5). In the
$^{27}$Al NMR spectra, the signals at 0, 4, 10 to 12 (broad peak), 63, 70 (broad peak), and 80 ppm were
attributed to the Al monomer species, the Al dimer and trimer species, the octahedral Al of the
external shells in Al13 species \([AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}\) and Al30 species, the central tetrahedral Al
in Al$\textsubscript{13}$ species, the central tetrahedral Al in Al$\textsubscript{30}$ species, and the internal standard, respectively (Allouche et al., 2000; Chen et al., 2007). Whereas no signal for Al$\textsubscript{30}$ species was found in the spectra of alum and PACl-1.5s, the spectra for PACl-2.1s contained a clear broad peak for the octahedral Al of external shells in Al$\textsubscript{13}$ and Al$\textsubscript{30}$ species at 10 to 12 ppm and a very weak signal at 70 ppm for Al$\textsubscript{30}$ species (Figure 5). These results indicated that PACl-2.1s, but not alum or PACl-1.5s, contained Al$\textsubscript{30}$ species. Therefore, the presence of the Al$\textsubscript{30}$ species in PACl-2.1s probably led to the higher virus removal performances observed compared with alum and PACl-1.5s. The spectra of PACl-2.1ns and PACl-2.5ns showed clear broad peak at 70 ppm, which indicate the presence of Al$\textsubscript{30}$ species, whereas that of PACl-2.1s did not (Figure 5). These results indicate that PACl-2.1ns and PACl-2.5ns a greater amount of Al$\textsubscript{30}$ species than did the other coagulants examined. Because Al$\textsubscript{30}$ species are the highest-charged polycations ever characterized among PACls, and they confer a stronger floc formation capacity over a broader pH range and wider coagulant dosage compared with other Al species in PACls (Chen et al., 2006; Zhang et al., 2008), the presence of the Al$\textsubscript{30}$ species in PACl-2.1ns and PACl-2.5ns likely resulted in the virus removal performances being the highest obtained under the coagulation conditions examined in the present study (Figure 4). The high virus removal performances obtained with PACl-2.1ns and PACl-2.5ns at a dose of 1.08 mg-Al/L were comparable with those achieved with the UF membrane with a nominal MWCO of 1 kDa (Figure 2). Therefore, in-line coagulation–MF with a nonsulfated, high-basicity PACl is a potential alternative to direct UF with membranes with a relatively low MWCO for the removal of
human enteric viruses. In addition, because the cake layer of aluminum floc particles generated by the coagulation of the river water with PACl-2.5ns was more effective for the removal of human enteric viruses than was that formed with PACl-1.5s (Figure S3; see Supplementary Information for details of the effect of the cake layer on virus removal), further increases in virus removal by the formation and growth of the cake layer is predicted when the filtration volume during in-line coagulation–MF is increased, particularly when a nonsulfated, high-basicity PACl is used.

Prevost et al., (2016) reported that most human enteric viruses, including AdV and NV, detected in river water had an intact capsid and were still infectious, as assessed by means of an intercalating dye pretreatment coupled with PCR method. In addition, because some serotypes of viruses are highly resistant to treatments such as UV disinfection (e.g., AdV type 40) and free-chlorine disinfection (CV B5) (Nwachuku et al., 2005; Cromeans et al., 2010), multiple-barrier approaches that both remove and inactivate viruses are important for preventing infection by human enteric viruses in drinking water (Shannon et al., 2008). Thus, in-line coagulation–MF with a nonsulfated, high-basicity PACl could be an effective physical barrier for human enteric viruses such as AdV type 40 and CV B5.

3.4. Relationship between enteric virus removal and bacteriophage or PMMoV removal

To investigate whether the bacteriophages MS2 and φX174, and the plant virus PMMoV, are suitable surrogates for AdV, CV, HAV, and caliciviruses, Pearson’s correlation coefficients ($r$) for
the removal ratios of viruses obtained with direct filtration (MF and UF), or in-line coagulation–MF were calculated by using the Excel Toukei BellCurve software (Social Survey Research Information Co., Tokyo, Japan; Tables 4 and 5). Virus concentrations that were below the quantification limit of the PCR method were assigned the quantification limit when calculating the correlation coefficients. The removal ratios obtained with AdV, CV, HAV, and MNV were strongly correlated with each other in the direct MF and UF \((r = 0.91–0.96)\) and the in-line coagulation–MF experiments \((r = 0.76–0.95)\). Although these viruses have similar isoelectric points \((3.6–3.8)\), they have different surface hydrophobicities; HAV and MNV are more hydrophobic than are AdV and CV, as estimated by means of the bacterial adherence to hydrocarbon method (Shirasaki et al., 2016a; Shirasaki et al., 2017). Therefore, it was likely that the similarities in the surface charge properties of the viruses resulted in the comparable removal ratios obtained for AdV, CV, HAV, and MNV, and the differences in the surface hydrophobicities of the viruses did not affect the virus removal performances of membrane filtration processes.

The removal ratios of MS2 were strongly correlated with those obtained for the three human enteric viruses and MNV in the direct MF and UF \((r = 0.85–0.97)\) and the in-line coagulation–MF experiments \((r = 0.81–0.95)\); the removal ratios of MS2 and of the three human enteric viruses and MNV were comparable (Figure S4a,d). These results suggest that MS2 is a potential surrogate for AdV, CV, HAV, and caliciviruses in membrane filtration processes when the virus removal ratios are evaluated by means of PCR. However, some groups, including ours, have reported that MS2 is
inactivated after contact with PACl during coagulation (Matsushita et al., 2011; Kreissel et al., 2014), whereas AdV, CV, and MNV were not (Shirasaki et al., 2016a; Shirasaki et al., 2017), as assessed by means of a combination of plaque assay and PCR method. These results imply that MS2 is also inactivated during coagulation–MF with PACl, unlike AdV, CV, and MNV, and therefore that the removal ratios of MS2, as determined by means of plaque assay, which can detect only infectious viruses, probably resulted in overestimation of the ability of coagulation–MF to remove infectious AdV, CV, and caliciviruses. Therefore, if the virus removal performances of membrane filtration processes, including coagulation–MF, are evaluated by using MS2 as a surrogate of human enteric viruses, the PCR method is more appropriate than the plaque assay for assessing virus removal efficacy.

The removal ratios obtained for φX174 were also strongly correlated with those of the three human enteric viruses and MNV in the direct MF and UF experiments ($r = 0.89–0.97$), and these removal ratios had an approximately 1:1 correlation (Figure S4b). However, the removal ratios of φX174 were clearly smaller than those of the three human enteric viruses and MNV in the coagulation–MF experiment and the range was limited (0.7–1.9-log$_{10}$), unlike that for the other viruses (Figure S4e), although moderate or strong correlations between were found among the removal ratios of φX174 and those of the three human enteric viruses and MNV ($r = 0.51–0.83$). Therefore, φX174 appears to be an appropriate surrogate for AdV, CV, HAV, and caliciviruses during direct MF or UF, but it does not appear to be an appropriate surrogate for those viruses
The removal ratios of PMMoV were strongly correlated with those of the three human enteric viruses and MNV in the direct MF and UF ($r = 0.92–0.98$) and the in-line coagulation–MF experiments ($r = 0.73–0.96$), and the removal ratios of PMMoV were comparable with or somewhat smaller than those of the three human enteric viruses and MNV (Figure S4c,f). PMMoV has an isoelectric point of 3.2 (Figure S1), which is similar to that of AdV, CV, HAV, and MNV (i.e., 3.6–3.8; Shirasaki et al., 2016a; Shirasaki et al., 2017). The degree of surface hydrophobicity of PMMoV, as estimated by means of the bacterial adherence to hydrocarbon method, is probably intermediate between the viruses with a relatively low hydrophobic surface (i.e., AdV and CV) and the viruses with a relatively high hydrophobic surface (i.e., HAV and MNV) because PMMoV was transferred to the solvent phase only when $p$-xylene was used as the solvent (Figure S5); AdV and CV remained completely in the water phase after mixing with the test solvents, whereas HAV and MNV were transferred to the solvent phase when $n$-octane or $p$-xylene were used as the solvent (Shirasaki et al., 2016a; Shirasaki et al., 2017). Therefore, given that there were no marked differences between the surface hydrophobicity of PMMoV and those of AdV, CV, HAV, and MNV, it must have been the similar surface charges among the viruses that led to the comparable removal ratios obtained. In addition, we confirmed that, unlike MS2, PMMoV was not inactivated by contact with PACl during coagulation (data not shown), as evaluated by means of a combination of a local lesion count assay and a PCR method. These results suggest that PMMoV is potentially a suitable
surrogate for AdV, CV, HAV, and caliciviruses during membrane filtration. Moreover, because PMMoV has been detected at higher concentrations in drinking water sources than have human enteric viruses (Haramoto et al., 2013), PMMoV could be a useful target virus for evaluating the virus removal performances of drinking water treatment plants that use membrane filtration processes. Evaluating virus removal efficiencies in this way may be a useful means of determining the efficacy of membrane filtration processes for the removal of human enteric viruses as part of the management of the spread of waterborne diseases caused by exposure to human enteric viruses in drinking water.

4. Conclusions

(1) Different membranes had different virus removal performances during direct MF, even though the nominal pore sizes of the membranes (0.1–0.22 µm) were larger than the diameters of the virus particles used in the present study. Adsorptive interactions, in particular hydrophobic interactions between the virus particles and the membrane surface, were the dominant factor for virus removal until the adsorption sites were saturated with viruses and natural organic substances.

(2) Direct UF with membranes with nominal MWCOs of 1 to 100 kDa effectively removed viruses via size exclusion because the nominal MWCOs of the UF membranes were smaller than the
relative molecular masses of the viruses used in the present study; >4-log_{10} removal was achieved for all of the viruses examined when a 1-kDa membrane was used.

(3) In-line coagulation–MF with nonsulfated, high-basicity PACls containing Al_{30} species removed viruses more efficiently than did in-line coagulation–MF with the other aluminum-based coagulants; high virus-removal performances (i.e., >4-log_{10} at pH 7 and pH 8) were obtained.

(4) The removal ratios of AdV, CV, HAV, and MNV were strongly correlated with each other, and comparable removal ratios were observed for those viruses with membrane filtration.

(5) Although comparable removal ratios were obtained with direct MF and UF for the bacteriophages MS2 and φX174 and the three human enteric viruses and MNV, MS2, unlike AdV, CV, MNV, and PMMoV, was inactivated after contact with PACl during coagulation. In addition, the removal ratios obtained with coagulation–MF for φX174 were clearly smaller than those of the three human enteric viruses and MNV. Therefore, MS2 and φX174 do not appear to be appropriate surrogates for human enteric viruses for the assessment of the efficacy of coagulation–MF processes.

(6) The removal ratios of PMMoV obtained not only with direct MF or UF but also with coagulation–MF were strongly correlated with those of the three human enteric viruses and MNV, and were similar to or somewhat smaller than those of the three human enteric viruses and MNV. Thus, PMMoV is a potential surrogate for human enteric viruses for the assessment of the efficacy of membrane filtration processes.
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References


Tanneru, C.T., Rimer, J.D. and Chellam, S. (2013) Sweep flocculation and adsorption of viruses on
aluminum flocs during electrochemical treatment prior to surface water microfiltration. Environmental Science and Technology 47(9), 4612-4618.


Table 1. Edo River water quality.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>2.8</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>1.0</td>
</tr>
<tr>
<td>UV260 (cm⁻¹)</td>
<td>0.020</td>
</tr>
<tr>
<td>Alkalinity (mg-CaCO₃/L)</td>
<td>36.0</td>
</tr>
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</table>
Table 2. Specifications of the aluminum-based coagulants used in the present study.

<table>
<thead>
<tr>
<th>Coagulants</th>
<th>Product name</th>
<th>Basicity</th>
<th>Aluminum concentration (% [w/w] as Al₂O₃)</th>
<th>Sulfate concentration (% [w/w])</th>
<th>Relative density at 20°C</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum</td>
<td>Aluminum sulfate</td>
<td>0.0</td>
<td>8.1</td>
<td>22.6</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>PACI-1.5s</td>
<td>PACI250A</td>
<td>1.5</td>
<td>10.1</td>
<td>2.9</td>
<td>1.2</td>
<td></td>
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<tr>
<td>PACI-2.1s</td>
<td>PACI700A</td>
<td>2.1</td>
<td>10.1</td>
<td>2.0</td>
<td>1.2</td>
<td>Taki Chemical Co.</td>
</tr>
<tr>
<td>PACI-2.1ns</td>
<td>–</td>
<td>2.1</td>
<td>10.4</td>
<td>0.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>PACI-2.5ns</td>
<td>Takbain #1500</td>
<td>2.5</td>
<td>23.2</td>
<td>0.0</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Specifications of the filtration membranes used in the present study.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Membranes</th>
<th>Product name</th>
<th>MF or UF</th>
<th>Nominal pore size or nominal molecular weight cutoff</th>
<th>Material</th>
<th>Contact angle (°)</th>
<th>Zeta potential (mV)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVDF-0.1-LP</td>
<td>Durapore VVLP</td>
<td>MF</td>
<td>0.1 µm</td>
<td>Hydrophilic polyvinylidene fluoride</td>
<td>20.2 ± 2.0</td>
<td>-39.1 ± 0.7</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>PVDF-0.22-LP</td>
<td>Durapore GVWP</td>
<td>MF</td>
<td>0.22 µm</td>
<td>Hydrophilic polyvinylidene fluoride</td>
<td>ND</td>
<td>ND</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>PVDF-0.1-HP</td>
<td>Durapore VVHP</td>
<td>MF</td>
<td>0.1 µm</td>
<td>Hydrophobic polyvinylidene fluoride</td>
<td>136.5 ± 4.6</td>
<td>-33.6 ± 4.2</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>PVDF-0.22-HP</td>
<td>Durapore GVHP</td>
<td>MF</td>
<td>0.22 µm</td>
<td>Hydrophobic polyvinylidene fluoride</td>
<td>ND</td>
<td>ND</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>PTFE-0.1</td>
<td>Omnipore JVWP</td>
<td>MF</td>
<td>0.1 µm</td>
<td>Hydrophilic polytetrafluoroethylene</td>
<td>87.9 ± 3.1</td>
<td>-50.2 ± 1.2</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>MCE-0.1</td>
<td>Millipore VCWP</td>
<td>MF</td>
<td>0.1 µm</td>
<td>Mixed cellulose esters</td>
<td>55.9 ± 2.0</td>
<td>-50.1 ± 4.0</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>PC-0.1</td>
<td>Isopore VCTP</td>
<td>MF</td>
<td>0.1 µm</td>
<td>Polycarbonate</td>
<td>44.6 ± 1.5</td>
<td>-33.0 ± 0.7</td>
<td>Millipore Corp.</td>
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<tr>
<td>ZP-0.1</td>
<td>Zeta Plus 90S</td>
<td>MF</td>
<td>0.1–0.2 µm</td>
<td>Cellulose, etc.</td>
<td>Very low</td>
<td>12.0 ± 7.1</td>
<td>3M Co.</td>
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<tr>
<td>RC-1k</td>
<td>Ultrace PLAC</td>
<td>UF</td>
<td>1 kDa</td>
<td>Regenerated cellulose</td>
<td>14.6 ± 1.7</td>
<td>-25.9 ± 0.4</td>
<td>Millipore Corp.</td>
</tr>
<tr>
<td>RC-10k</td>
<td>Ultrace PLGC</td>
<td>UF</td>
<td>10 kDa</td>
<td>Regenerated cellulose</td>
<td>12.2 ± 1.4</td>
<td>-12.7 ± 7.0</td>
<td>Millipore Corp.</td>
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<tr>
<td>RC-100k</td>
<td>Ultrace PLHK</td>
<td>UF</td>
<td>100 kDa</td>
<td>Regenerated cellulose</td>
<td>15.8 ± 0.8</td>
<td>-28.7 ± 4.7</td>
<td>Millipore Corp.</td>
</tr>
</tbody>
</table>

\textsuperscript{a} ND, not determined. Contact angle and zeta potential of PVDF-0.22-LP and PVDF-0.22-HP are almost the same as those of PVDF-0.1-LP and PVDF-0.1-HP, respectively, according to the manufacturer’s information. Because ZP-0.1 has very high wettability, we could not accurately measure the contact angle of this membrane.
Table 4. Pearson’s correlation coefficient matrix for the removal ratios obtained with direct MF and UF (n = 11).a

<table>
<thead>
<tr>
<th></th>
<th>AdV</th>
<th>CV</th>
<th>HAV</th>
<th>MNV</th>
<th>MS2</th>
<th>φX174</th>
<th>PMMoV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdV</td>
<td>1.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CV</td>
<td>0.91**</td>
<td>1.00</td>
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<tr>
<td>HAV</td>
<td>0.93**</td>
<td>0.96**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNV</td>
<td>0.93**</td>
<td>0.96**</td>
<td>0.91**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS2</td>
<td>0.85**</td>
<td>0.97**</td>
<td>0.90**</td>
<td>0.91**</td>
<td>1.00</td>
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</tr>
<tr>
<td>φX174</td>
<td>0.97**</td>
<td>0.92**</td>
<td>0.89**</td>
<td>0.95**</td>
<td>0.85**</td>
<td>1.00</td>
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<tr>
<td>PMMoV</td>
<td>0.98**</td>
<td>0.92**</td>
<td>0.92**</td>
<td>0.94**</td>
<td>0.84**</td>
<td>0.98**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

a Asterisks indicate a statistically significant correlation (**p < 0.01).
Table 5. Pearson’s correlation coefficient matrix for the removal ratios obtained with in-line coagulation–MF ($n = 18$).

<table>
<thead>
<tr>
<th></th>
<th>AdV</th>
<th>CV</th>
<th>HAV</th>
<th>MNV</th>
<th>MS2</th>
<th>φX174</th>
<th>PMMoV</th>
</tr>
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<tbody>
<tr>
<td>AdV</td>
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<tr>
<td>CV</td>
<td>0.76**</td>
<td>1.00</td>
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<tr>
<td>HAV</td>
<td>0.94**</td>
<td>0.85**</td>
<td>1.00</td>
<td></td>
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<td></td>
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<tr>
<td>MNV</td>
<td>0.88**</td>
<td>0.92**</td>
<td>0.95**</td>
<td>1.00</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MS2</td>
<td>0.81**</td>
<td>0.93**</td>
<td>0.87**</td>
<td>0.95**</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>φX174</td>
<td>0.51*</td>
<td>0.83**</td>
<td>0.63**</td>
<td>0.71**</td>
<td>0.77**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>PMMoV</td>
<td>0.73**</td>
<td>0.96**</td>
<td>0.84**</td>
<td>0.93**</td>
<td>0.97**</td>
<td>0.83**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Asterisks indicate a statistically significant correlation (*$P < 0.05$, **$P < 0.01$).
Figure 1. Removal of virus particles by means of direct MF with different types of membranes at pH 7. Values are the means of three experiments, and error bars indicate standard deviations. Arrows indicate that the virus concentrations were below the quantification limit.
Figure 2. Removal of virus particles by means of direct UF with membranes with different nominal MWCOs at pH 7. Values are the means of three experiments, and error bars indicate standard deviations. Arrows indicate that the virus concentrations were below the quantification limit.
Figure 3. Change in virus removal ratio with filtration volume during direct MF at pH 7. Filtration volumes were 50–250 mL (a–g) and 200–1000 mL (h–n). Arrows indicate that the virus concentrations were below the quantification limit.
Figure 4. Removal of virus particles by means of in-line coagulation–MF with different aluminum-based coagulants at pH 7 (a) and pH 8 (b). The PVDF-0.1-LP membrane was used. Values are the means of three experiments, and error bars indicate standard deviations. Arrows indicate that the virus concentrations were below the quantification limit.
Figure 5. $^{27}$Al NMR spectra of the coagulants used in the present study.