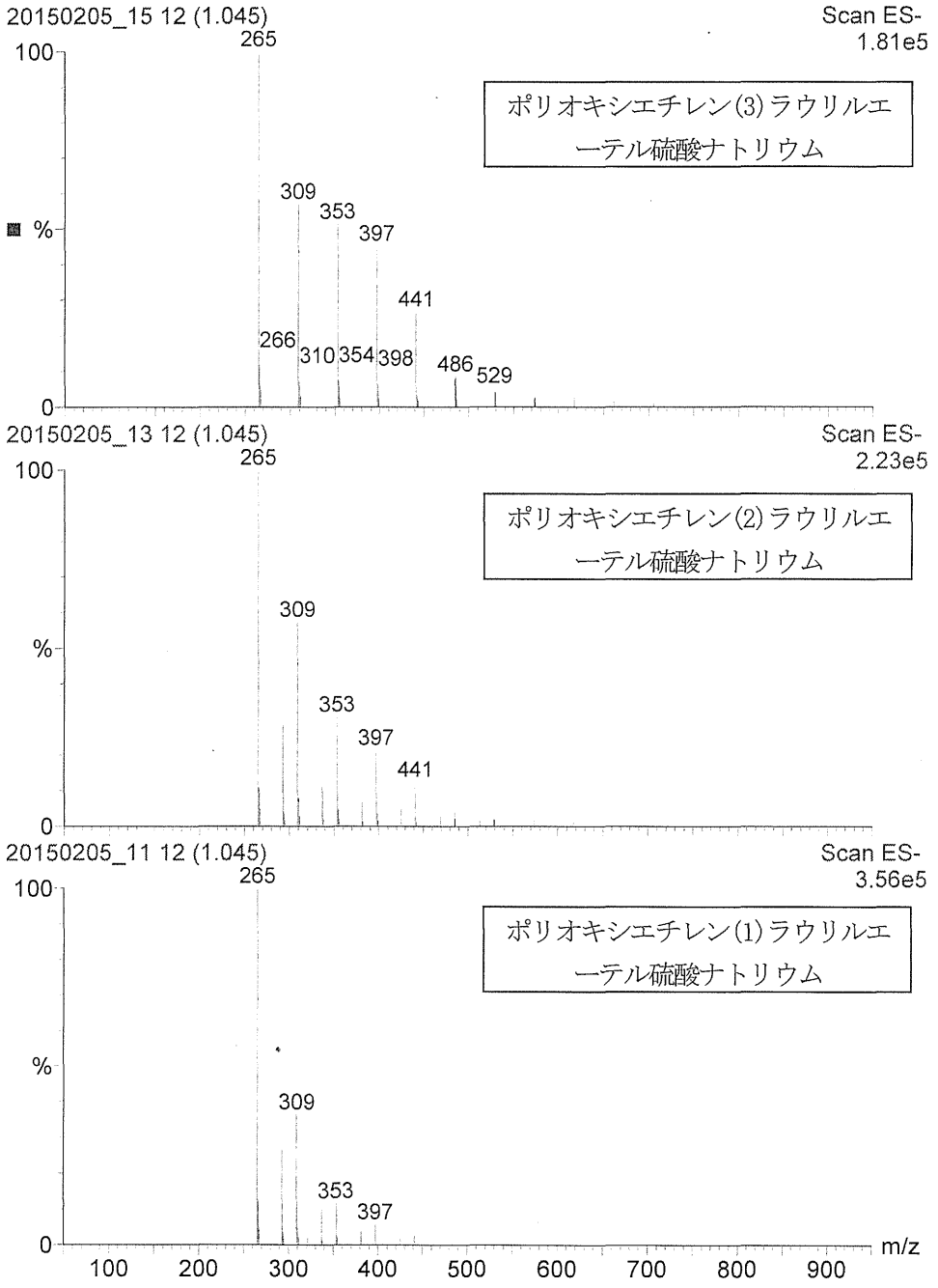

図 **4-3.** ポリオキシエチレンアルキルエーテル硫酸塩（AES，エマール 170J（ポリオキシエチレン(1) ラウリルエーテル硫酸ナトリウム），エマール 270J（ポリオキシエチレン(2) ラウリルエーテル硫酸ナトリウム），エマール 20C（ポリオキシエチレン(3)ラウリルエーテル硫酸ナトリウム））の FIA-MS の ESI スペクトル

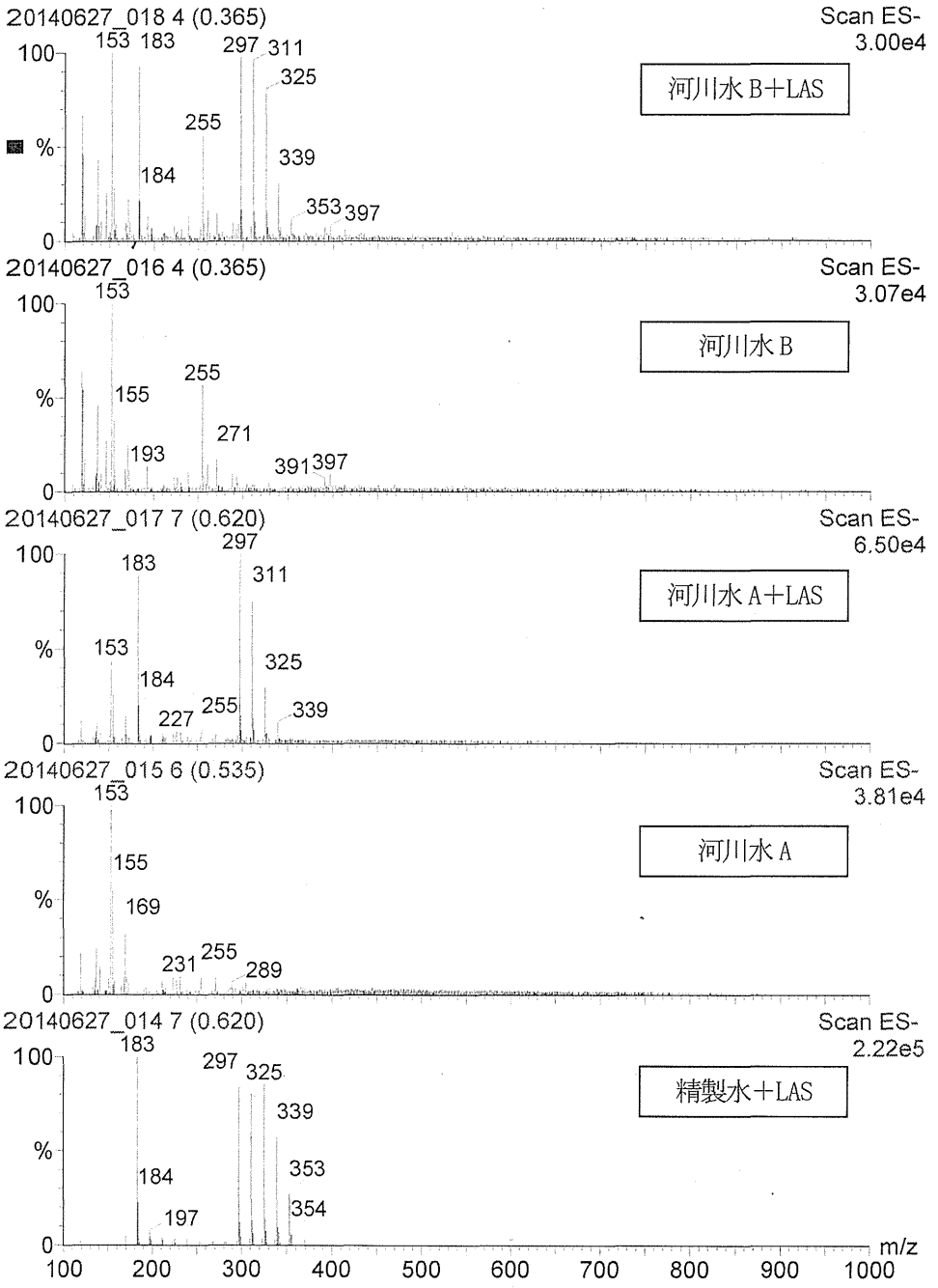

図 4-4. 市販の洗剤を河川水に LAS (C10-C14) 添加した時の FIA-MS の ESI スペクトル
河川水：フレッシュ度 50%, LAS 添加濃度：1 mg/L

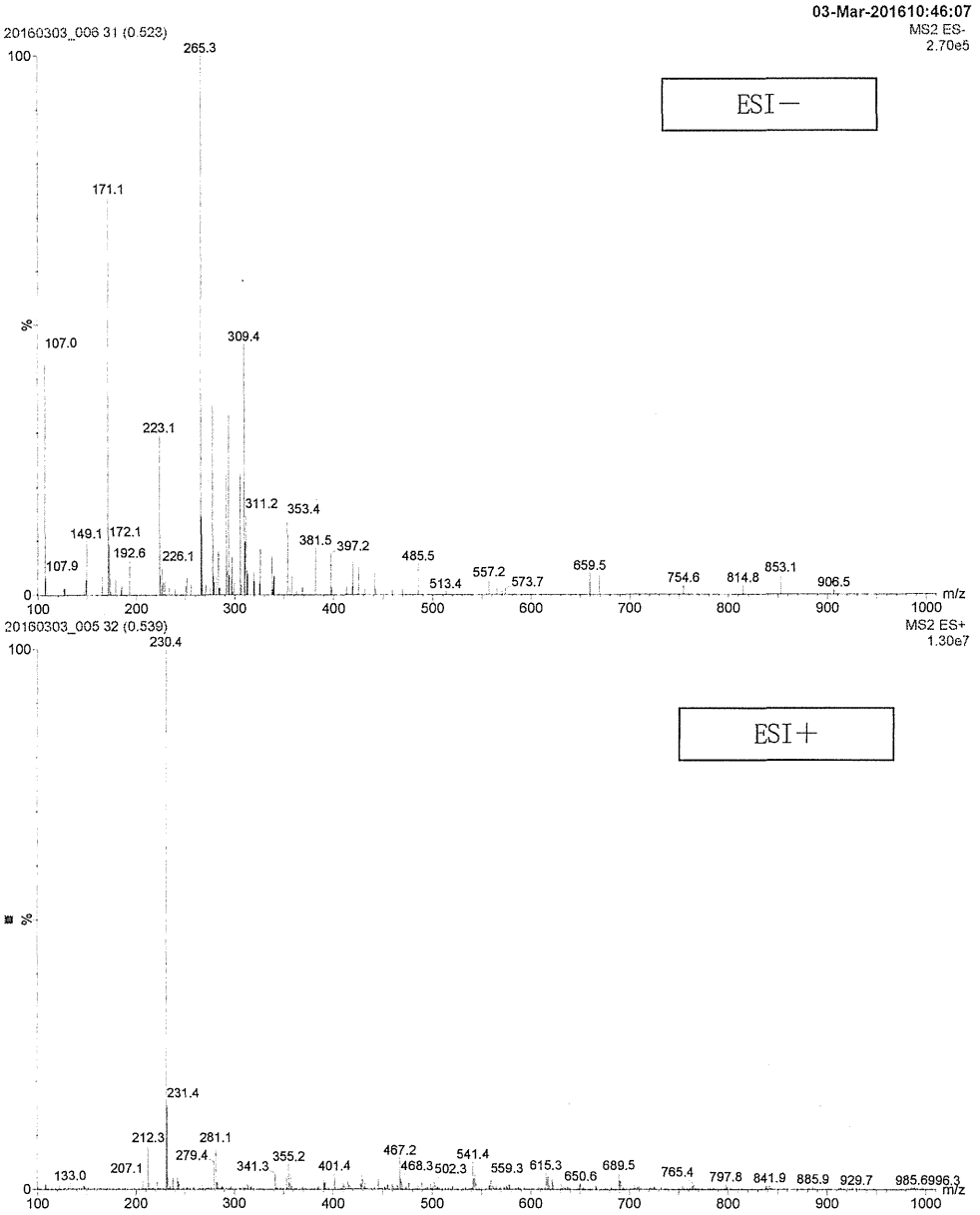

図 4-5. 市販の家庭用洗剤の FIA-MS の ESI スペクトル

家庭用洗剤の濃度：**10 mg/L**，成分表示（**31%**，**AO**，**AES**，**POE**，**AS**）

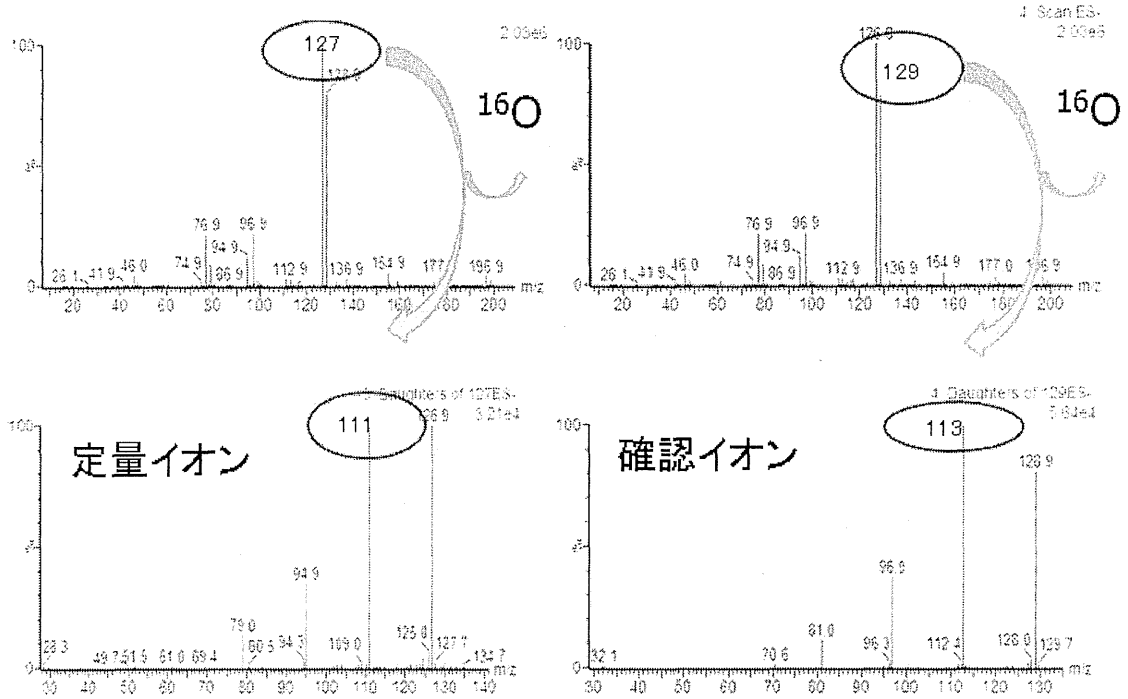

図 5-1　臭素酸イオンの MS/MS スペクトル

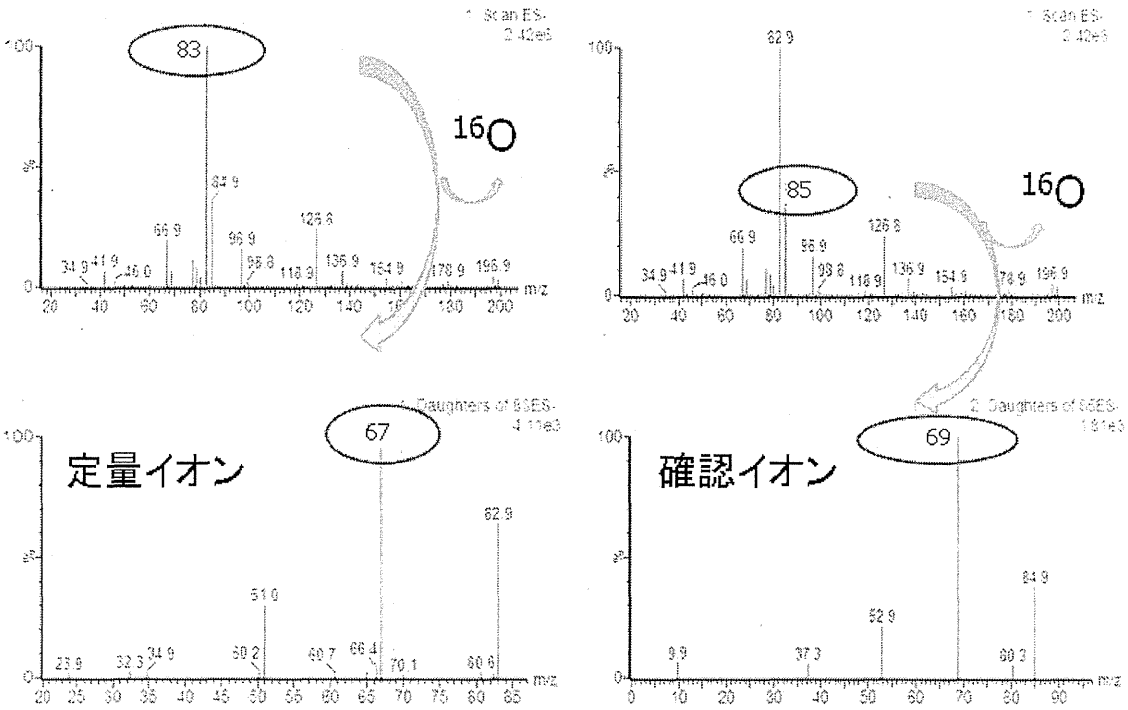

図 5-2　塩素酸イオンの MS/MS スペクトル

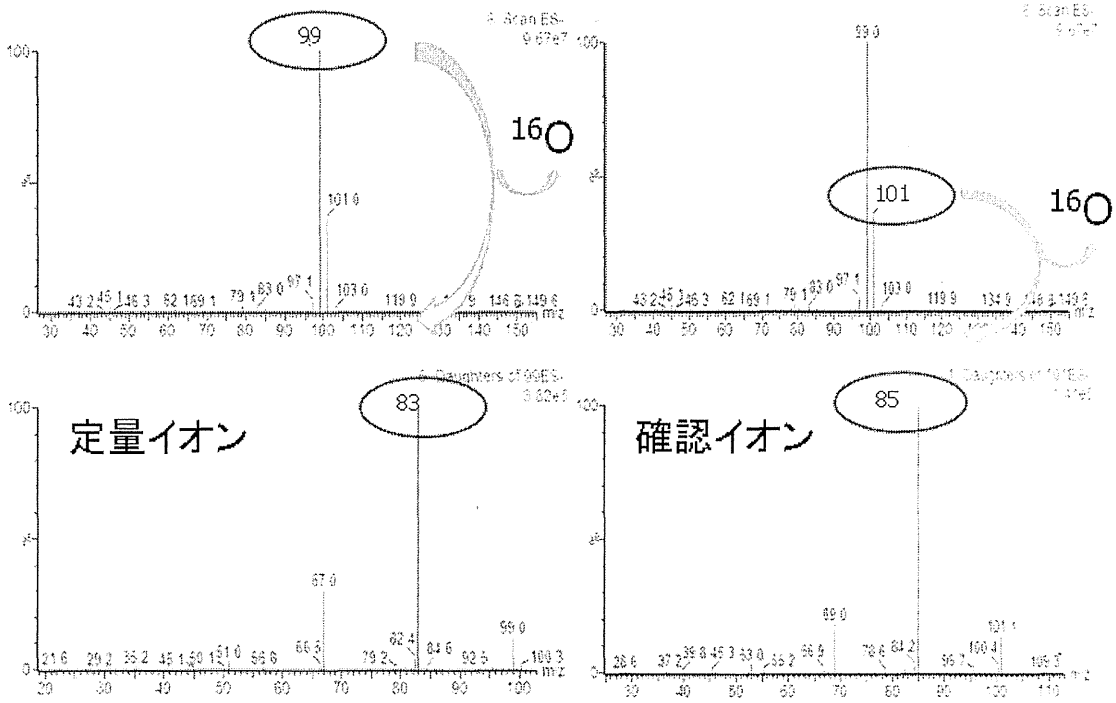

図 5-3　過塩素酸イオンの MS/MS スペクトル

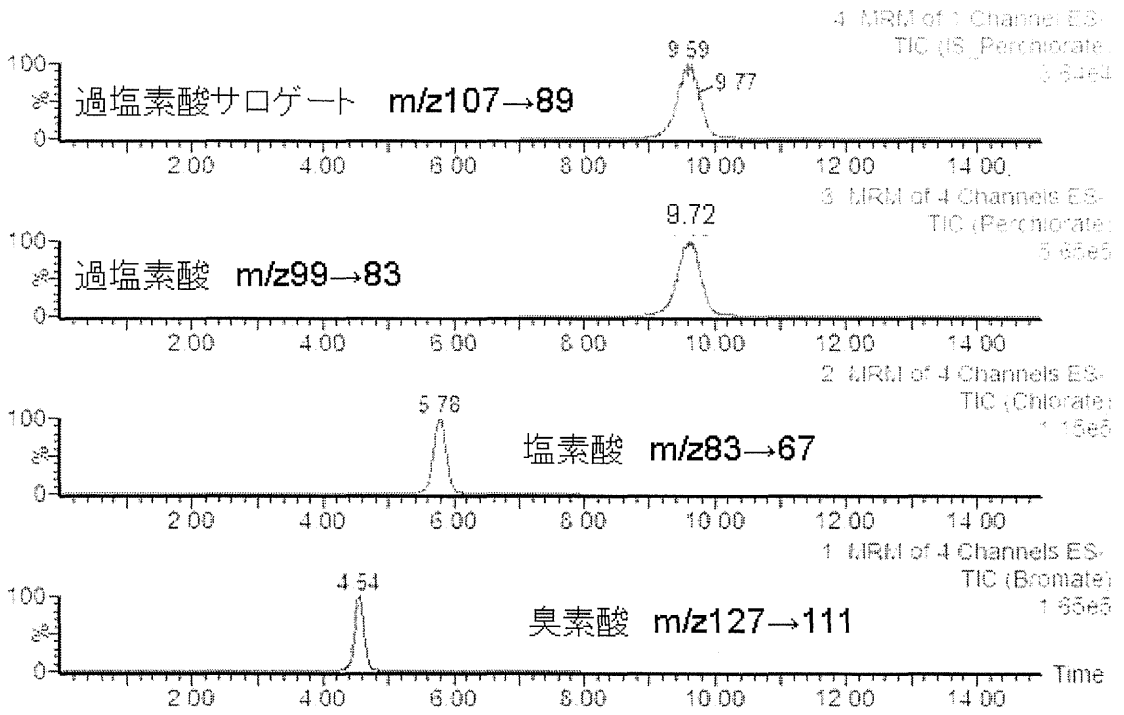

図 5-2　臭素酸，塩素酸および過塩素酸イオンの LC-MS/MS クロマトグラム

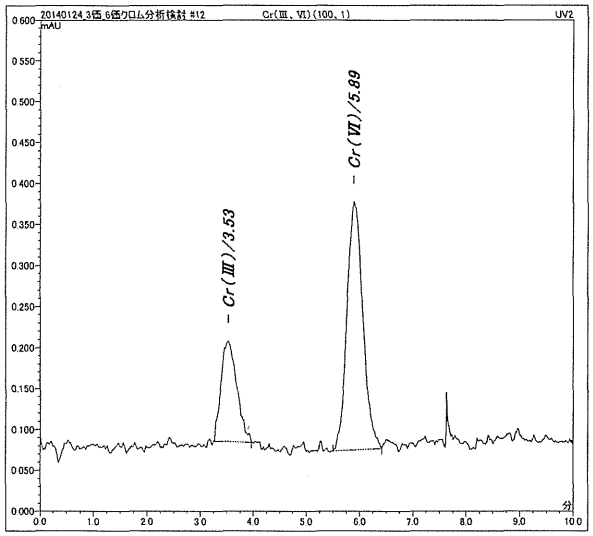

図 6-1.　Cr（Ⅲ）100μg/L、Cr（Ⅵ）1μg/L の混合液のクロマトグラム

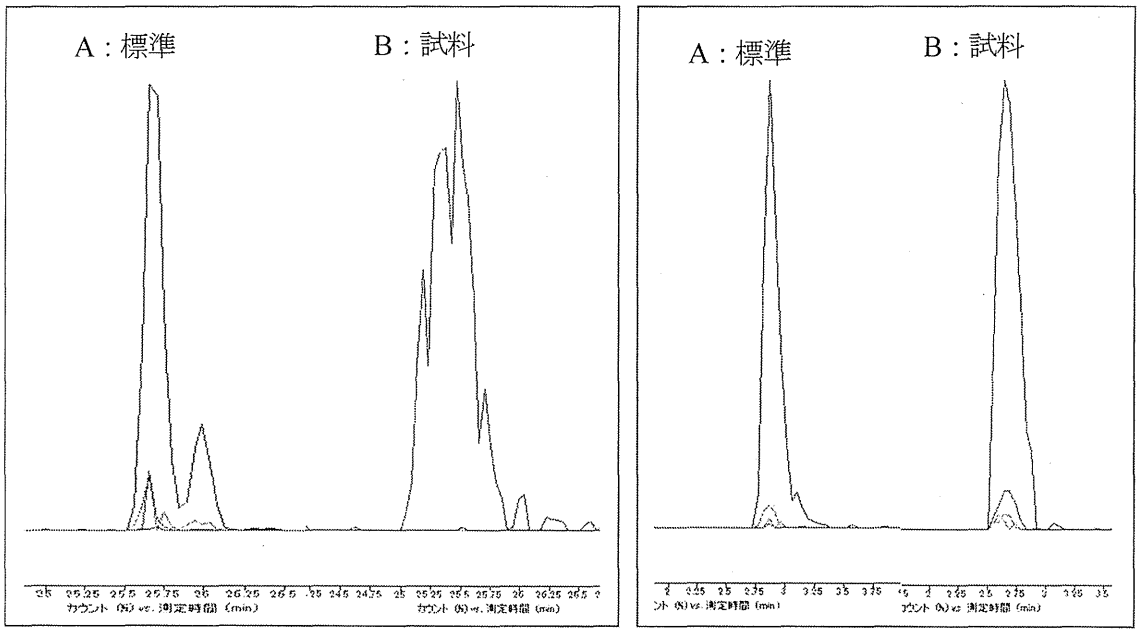

図 8-1. インソースフラグメントイオンによる誤検出の例（Siduron）（左）
図 8-2. インソースフラグメントイオンによる検出の例（Metformin）（右）

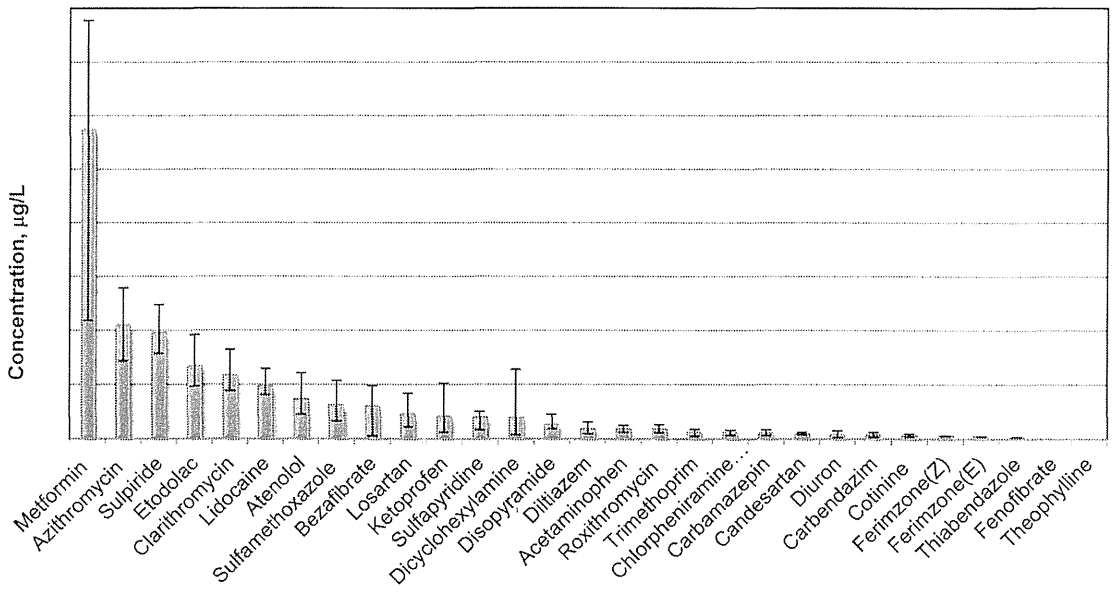

図 **8-3.** 北九州市内の **5** 下水処理場での検出物質

研究成果の刊行に関する一覧表

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研究成果の刊行物・別刷

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Effect of aluminum hydrolyte species on human enterovirus removal from water during the coagulation process

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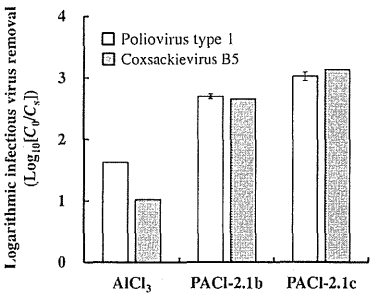
HIGHLIGHTS

- Differences in aluminum species in coagulant affected enterovirus removal.
- High Al_c content PACl with basicity 2.1 effectively removed enteroviruses.
- Al_{30} species in PACl probably played a major role in enterovirus removal.
- PV and CV removal ratios were almost the same during coagulation.
- Viruses were removed mainly by coprecipitation into growing aluminum hydroxide.

GRAPHICAL ABSTRACT



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ABSTRACT

We prepared different types of aluminum-based coagulants, consisting of mainly monomeric aluminum species, polymeric aluminum species, or colloidal aluminum species, to investigate the effect of aluminum hydrolyte species on the removal of two types of human enteroviruses, poliovirus (PV) type 1 and the free-chlorine-resistant virus coxsackievirus (CV) B5, from lake and river water samples during the coagulation process. We found that differences in the distribution of the aluminum hydrolyte species in the coagulant affected the removal of these enteroviruses during coagulation: the removal ratios of PV and CV observed with polyaluminum chloride (PACl) with a high colloidal aluminum content and a basicity of 2.1 (i.e., PACl-2.1c) were larger than those observed with high monomeric aluminum content coagulant (i.e., $AlCl_3$ solution) and with high polymeric aluminum content coagulant PACl (PACl-2.1b). Unlike $AlCl_3$ or PACl-2.1b, PACl-2.1c contains Al_{30} species, indicating that Al_{30} species probably play a major role in the removal of enteroviruses. The PV and CV removal ratios were almost identical, regardless of the coagulant type or viral quantification method used (plaque-forming unit method or real-time polymerase chain reaction method), suggesting that PV and CV behaved similarly during the coagulation process. We also experimentally confirmed that the main mechanism for virus removal was coprecipitation into growing aluminum hydroxide during charge neutralization; virus adsorption onto formed aluminum hydroxide flocs also contributed to virus removal, but played a limited role.

1. Introduction

Coagulation is an important process in water treatment to remove contaminants. Of the various coagulants used for the coagulation process, prepolymerized aluminum coagulants, such as polyaluminum chloride (PACl), have been widely used in drinking

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1385-8947/

water treatment because of their superiority to traditional aluminum coagulants [e.g., aluminum chloride ($AlCl_3$) and alum]; PACls are more efficient, are less dependent on temperature and pH, can be used at a lower dosage, and produce less sludge [1–3].

The hydrolysis reactions of aluminum species are very complicated, depending on water quality and coagulant type, which contribute to the formation of various aluminum hydrolyte species [4,5]. The ferron method has been employed extensively to categorize the aluminum hydrolyte species into the following three fractions on the basis of the kinetic differences between the reactions of the aluminum species and the ferron regent: monomeric species, fast-reacting polymeric species, and slow-reacting colloidal species, denoted as Al_a, Al_b, and Al_c, respectively [6]. Al_a is composed mainly of monomeric species such as Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)_2^+$; dimeric and trimeric species such as $[Al_2(OH)_2]^{4+}$ and $[Al_3(OH)_4]^{5+}$ are also classified as Al_a [1]. Al_b is the intermediate polymeric species, and many researchers have suggested that Al_b could include the Al_{13} species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$, which was identified by using liquid ^{27}Al nuclear magnetic resonance (NMR) spectroscopy [1,4,7]. Al_c is the large polymer or colloidal species, and the Al_{30} species $[Al_{30}O_8(OH)_{56}(H_2O)_{24}]^{18+}$, which was also identified by using liquid ^{27}Al NMR spectroscopy, is classified into Al_c [7].

The distribution of aluminum hydrolyte species in PACls and the formation of specific aluminum hydrolyte species such as Al_{13} and Al_{30} might be controlled by basicity ($[OH^-]/[Al^{3+}]$), aluminum concentration, rate and mode of neutralization, and reaction temperature and time during PACl preparation [7–9]. In addition, the aluminum species originally contained in the PACls or formed *in situ* during the coagulation process play an important role in determining the behavior and efficiency of coagulants [4,5]. For these reasons, many researchers have paid particular attention to the effects of aluminum species on coagulation efficiency. For example, Yan *et al.* [1] reported that the removal efficiencies of turbidity and dissolved organic carbon (DOC) correlate well with the Al_c content and Al_b content in PACls, respectively, whereas both Al_b and Al_c content influence the removal of ultraviolet absorbance at 254 nm (UV254, an indicator of natural organic matter concentration). Our research group [10] and Duan *et al.* [11] reported that the residual aluminum concentration in treated water is closely related to the Al_a content in the PACl: low Al_a content in the PACl yields a low residual aluminum concentration. Moreover, among the identified aluminum hydrolyte species in PACls, Al_{13} and Al_{30} are believed to be effective coagulation species due to their strong charge neutralization capability and high structural stability [12]. In fact, Lin *et al.* [13] showed that DOC removal by PACl with a high Al_{13} content is superior to that by commercially available PACl with a low Al_{13} content at about pH 6, and that a lower dosage of high Al_{13} content PACl than of commercially available PACl is required for optimal DOC removal. Zhang *et al.* [2] reported that compared to $AlCl_3$, PACl with a high Al_{30} content exhibits greater UV254 removal efficiency and leads to a lower residual aluminum concentration across a broad pH range and a wide coagulant dosage range. In addition, Hu *et al.* [4] showed the value of PACl with a high Al_{13} content for the removal of arsenic (As) from As-spiked tap water over a broad pH range, and concluded that the removal efficiencies of As(V) correlated with the amount of Al_{13} species present. Moreover, Mertens *et al.* [14] reported that PACl with a high Al_{30} content contributed to the efficient removal of As(III) and As(V) from As-contaminated groundwater at pH 7–8.

Human enteric viruses, which are frequently present in contaminated drinking water sources and do not settle from suspension by gravity, can also be removed by the coagulation process with PACl [15]. However, the relationship between the removal efficiencies of human enteric viruses and the aluminum hydrolyte species in PACl remains unclear, and the role of Al_{13} and Al_{30} species in the removal of human enteric viruses has not been investigated. We recently reported that laboratory-prepared PACl with a high Al_c content removed bacteriophages (i.e., viruses that infect bacteria) more efficiently than did laboratory-prepared PACl with a high Al_b content or commercially available PACls, suggesting that the Al_{30} species in high Al_c content PACl probably play a major role in bacteriophage removal during the coagulation process [16]. Because the removal ratios of human enteric viruses including poliovirus were different to and smaller than that of bacteriophage MS2 in the coagulation process with aluminum-based coagulant [17], the effect of aluminum hydrolyte species on virus removal, and specifically the role of Al_{13} and Al_{30}, may also differ between human enteric virus and bacteriophage removal. In addition, because human enteric viruses have high resistance to free-chlorine disinfection compared with human enteric bacteria [18], and the increase in free-chlorine dosage needed for sufficient disinfection of human enteric viruses sometimes results in the formation of high levels of toxic disinfection by-products [19], improvements in the coagulation efficiency of virus removal are highly desired. Therefore, the identification of aluminum hydrolyte species in aluminum-based coagulants that efficiently remove human enteric viruses from drinking water would improve coagulation efficiency and support the development of novel aluminum-based coagulants for the prevention and control of waterborne disease caused by exposure to such viruses through drinking water.

Here, we conducted batch coagulation experiments to investigate the effect of aluminum hydrolyte species on the removal of human enteric viruses, specifically human enteroviruses, by comparing five coagulants with different distributions of aluminum hydrolyte species. In addition, we investigated the mechanism of human enterovirus removal during the coagulation processes by examining the coprecipitation of enteroviruses into growing aluminum hydroxide during charge neutralization and the absorption of enteroviruses onto preformed aluminum floc particles. Because poliovirus (PV) type 1 is commonly used as representative of human enteric viruses [17,20], and coxsackievirus (CV) B5 has high resistance to free-chlorine disinfection compared with other types of CVs (e.g., CV B3 and CV B4) and other human enteric viruses including PVs (types 1, 2 and 3), echoviruses (EV types 1 and 11), and adenoviruses (AdV types 40 and 41) [21,22], we chose these two human enteroviruses for use in our study.

2. Materials and methods

2.1. Source water and coagulants

Lake water and river water were sampled from Lake Imbanuma in Chiba, Japan, and the Toyohira River in Sapporo, Japan, on 17 November 2014 (water quality data are shown in Table 1). The source water samples were stored at 4 °C until use, and the temperature was adjusted to 20 °C prior to use.

Five aluminum-based coagulants were used for the coagulation experiments (Table 2). $AlCl_3$ solution was prepared by dilution of reagent-grade aluminum(III) chloride hexahydrate ($AlCl_3 \cdot 6H_2O$, Wako Pure Chemical Industries, Osaka, Japan) dissolved in Milli-Q water (Milli-Q Advantage, Millipore Corp., Billerica, MA, USA).

Table 1
Water quality data for the source water samples.

	Lake water	River water
pH	8.6	7.4
Turbidity (NTU)	40.5	1.5
DOC (mg/L)	2.2	0.9
UV260 (cm^{-1})	0.052	0.024
Alkalinity (mg-$CaCO_3$/L)	84.1	17.5

Table 2
Characteristics of the aluminum-based coagulants used in the present study.

Coagulants	Basicity	Aluminum concentration (g-Al/L)	Relative density at 20 °C	Aluminum species distribution		
				Al_a (%)	Al_b (%)	Al_c (%)
$AlCl_3$	0.0	13.5	1.1	88.6	11.4	0.0
PACl-2.1b	2.1	1.5	1.0	15.5	73.9	10.6
PACl-2.4b	2.4	1.6	1.0	5.3	87.2	7.5
PACl-2.1c	2.1	10.4	1.1	11.8	6.0	82.2
PACl-2.4c	2.4	10.3	1.1	3.6	8.7	87.7

Four nonsulfated high-basicity PACls (PACl-2.1b, PACl-2.4b, PACl-2.1c, and PACl-2.4c, where 2.1 and 2.4 are basicity values, and "b" and "c" indicate high Al_b and Al_c content, respectively, as measured by the ferron method; see below) were prepared by using a base titration method in our laboratory. A certain amount of 0.25 M $AlCl_3$ solution was transferred to a glass beaker, and then the solution was titrated with 0.15 M NaOH at a constant rate (4 mL/min) by use of a peristaltic pump to achieve the targeted basicity value to prepare PACl-2.1b and PACl-2.4b. The solution in the beaker was stirred at 630 rpm and the temperature was maintained at 90–95 °C by a hot plate stirrer during the titration. PACl-2.1c and PACl-2.4c were prepared from 1.5 M $AlCl_3$ solution and 0.9 M NaOH by using the same method just described, and then the solution was continuously stirred at 630 rpm and kept at 90–95 °C for 24 h after the titration. All coagulants were used in batch coagulation experiments immediately after dilution with Milli-Q water.

2.2. Characterization of coagulants

The aluminum hydrolyte species in the coagulants were analyzed by using the ferron method and liquid ^{27}Al NMR spectroscopy after the coagulants were diluted with Milli-Q water to a concentration of 2.7 g-Al/L, that is, 0.1 M-Al (for $AlCl_3$ solution, PACl-2.1c, and PACl-2.4c), or 1.35 g-Al/L, that is, 0.05 M-Al (for PACl-2.1b and PACl-2.4b), depending on the aluminum concentration in the coagulant (Table 2). The positive colloid charges of the coagulants (diluted with Milli-Q water to 1–3 mg-Al/L) were determined by using a colloid titration method. The details of the ferron method, liquid ^{27}Al NMR analysis, and the colloid titration method were described in our previous report [16].

2.3. Human enteroviruses

The Sabin attenuated LSc/2ab strain of PV type 1 was kindly provided by Dr. Hiroyuki Shimizu, National Institute of Infectious Diseases, Tokyo, Japan. The Faulkner strain of CV B5 (ATCC VR-185) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). PV and CV were propagated in buffalo green monkey kidney epithelial cells (BGM cells), kindly supplied by Dr. Daisuke Sano, Hokkaido University, Sapporo, Japan, and were maintained in 1X Eagle's minimum essential medium (EMEM, with phenol red, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Life Technologies, Carlsbad, CA, USA), 2 mM l-glutamine (Life Technologies), 100 units/mL penicillin, 100 μg/mL streptomycin (Pen Strep, Life Technologies), and 1.125 g/L $NaHCO_3$. One milliliter of 10-fold diluted PV or CV stock solution [diluted with Dulbecco's phosphate-buffered saline (without Ca and Mg ions, Nissui Pharmaceutical Co. Ltd.)] was inoculated onto a monolayer of approximately 90% confluent BGM cells in a 75 cm^2 flask. The flask was then incubated in a humidified incubator at 37 °C in 5% CO_2 for 30 min. After this incubation, 20 mL of 1X EMEM was added to the flask, and then the flask was incubated at 37 °C in 5% CO_2 for 2–3 days until 100% cytopathic effects of BGM cells were confirmed, at which point the viruses were harvested. Following incubation, viruses were released from the infected BGM cells by freezing at −83 °C and thawing at 37 °C, three times. The PV or CV culture solution was passed through a membrane filter (nominal pore size 0.2 μm, hydrophilic cellulose acetate; Dismic-25cs, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) to prepare the PV or CV stock solution. The filtrate was further purified by using a tangential-flow filtration cassette (nominal molecular weight cutoff 1,000,000, regenerated cellulose (RC); Pellicon XL, Millipore Corp.), and by using a centrifugal filter device (nominal molecular weight cutoff 100,000, RC; Amicon Ultra-15, Millipore Corp.) twice to ensure that only a minimal amount of DOC components from the culture medium was introduced into the virus-spiked source water (see Section 2.4). The concentrations of the purified PV and CV solutions were approximately 10^6 and 10^7 PFU/mL, respectively.

2.4. Coagulation experiments

Batch coagulation experiments were conducted with 300 mL of virus-spiked source water in square plastic beakers at 20 °C. The purified PV or CV solution (see Section 2.3) was added to the beaker at a concentration of approximately 10^3 PFU/mL (C_0). Because the purified virus solution was diluted during spiking of the source water, virus addition contributed less than 0.2–0.3 mg/L of unintentional DOC carryover. After enough HCl or NaOH was added to the spiked water to bring the final pH to a target value of 6, 7, or 8, coagulant was injected into the water. Because the dosages used in the actual drinking water treatment plants for the treatments of Lake Imba-numa water (Kashiwai drinking water treatment plant, Chiba, Japan) and Toyohira River water (Moiwa drinking water treatment plant, Sapporo, Japan) were 2.70 and 1.08 mg-Al/L, respectively, on the day we sampled the source water, those coagulant dosages were used in the present study. The water was stirred rapidly for 1 min ($G = 200\ s^{-1}$, 94 rpm) and then slowly for 10 min ($G = 20\ s^{-1}$, 20 rpm) with an impeller stirrer. The water was then left at rest for 60 min to allow the generated aluminum floc particles to settle. Supernatants were taken from the beaker after settling for quantification of the PV or CV concentration (C_s).

To investigate the importance of charge neutralization on virus removal during the coagulation process, 300 mL of source water without virus addition was coagulated rapidly for 1 min at pH 7, as described above. Following charge neutralization, the purified PV solution was added to the beaker at approximately 10^3 PFU/mL (C_0). The water was stirred slowly for 10 min and then left at rest for 60 min. Supernatants were taken from the beaker after settling for quantification of the PV concentration (C_s). To assess virus adsorption onto preformed aluminum floc particles, 300 mL of source water without virus addition was coagulated rapidly for 1 min and then slowly for 10 min at pH 7, as described above, to generate aluminum floc particles. Following the formation of the aluminum floc particles, the purified PV solution was added to the beaker at approximately 10^3 PFU/mL (C_0). The water was stirred slowly for 10 min and then left at rest for 60 min. Supernatants were taken from the beaker after settling for quantification of the PV concentration (C_s).

2.5. Enterovirus assay

Infectious PV or CV was quantified by use of a plaque assay. Approximately 90% confluent BGM cells in a 75 cm^2 flask were seeded in 6-well (8.96 cm^2/well) tissue culture plates. One milliliter of 10-fold serially diluted sample (diluted with 1X EMEM supplemented with 2 mM l-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 1.125 g/L $NaHCO_3$) or of 2-fold diluted sample (diluted with 2X EMEM containing 4 mM l-glutamine, 200 units/mL penicillin, 200 μg/mL streptomycin, and

2.25 g/L $NaHCO_3$) was inoculated onto a monolayer of 100% confluent BGM cells in a 6-well plate, which was then incubated in a humidified incubator at 37 °C in 5% CO_2 for 90 min. After this incubation, the inoculum was removed by inverting the plate, and then 3 mL of ager overlay was applied to the monolayer. The overlay was prepared by combining equal volumes of 2.5% (w/v) agarose (Agar-EPI, Nacalai Tesque, Inc., Kyoto, Japan) and 2X EMEM (without phenol red, Nissui Pharmaceutical Co. Ltd.) supplemented with heat-inactivated fetal bovine serum (2% for PV, 20% for CV), 4 mM l-glutamine, 200 units/mL penicillin, 200 μg/mL streptomycin, and 2.25 g/L $NaHCO_3$. After the addition of the ager overlay, the plate was incubated at 37 °C in 5% CO_2 for 2 days. After this incubation, the cell monolayer was stained with 1 mL of 0.15 g/L neutral red at 37 °C in 5% CO_2 for 3 h, and then excess stain was discarded by inverting the plate. Plaques of each well were counted for 1–3 days (1 day for PV, 3 days for CV) after discarding the neutral red until no new plaques appeared. The average plaque count of triplicate wells or twelve wells prepared from one sample was considered as the infectious PV or CV concentration for that sample.

The real-time polymerase chain reaction (PCR) method, which detects all viruses regardless of their infectivity or the presence of aggregates, was used to quantify viral RNA. PV and CV RNA was specifically quantified by using the real-time reverse transcription-PCR (RT-PCR) method. Viral RNA was extracted from 200 μL of sample with a QIAamp MinElute Virus Spin Kit (Qiagen K. K., Tokyo, Japan) to obtain a final volume of 20 μL. The extracted RNA solution was added to a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems Japan, Tokyo, Japan) for the RT reaction, which was conducted at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s, with subsequent cooling to 4 °C in the thermal cycler (Thermal Cycler Dice Model TP600, Takara Bio Inc., Otsu, Japan). The cDNA solution was then amplified with a TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems Japan) with 400 nM primers (HQ-SEQ grade, Takara Bio Inc.) and 250 nM TaqMan probe (Applied Biosystems Japan). The oligonucleotide sequences of the primers and the probe used for the PV and CV quantifications were taken from previous reports [23,24]. Amplification was conducted at 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min in an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems Japan). The standard curve for the real-time PCR method was based on the relationship between the infectious PV or CV concentration of a freshly prepared purified PV or CV solution measured by the plaque assay described above, and the number of cycles (C_t value) of PCR amplification.

2.6. Aluminum species distribution analysis during the coagulation process

The effect of pH on the aluminum hydrolyte species transfer of coagulants during the coagulation process was analyzed mostly by following the method of Yan *et al.* [25]. Batch coagulation experiments were conducted with 1000 mL of prepared Milli-Q water containing 0.5 mM $NaHCO_3$ and $NaNO_3$ in square plastic beakers at 20 °C. After enough HCl or NaOH was added to the prepared Milli-Q water to bring the final pH to a target value of 6, 7, or 8, 2.7 mg-Al/L of coagulant was injected in the water. The water was stirred rapidly for 1 min ($G = 339\ s^{-1}$, 200 rpm), and then the solution was analyzed by using the ferron method.

3. Results and discussion

3.1. Characterization of coagulants

To investigate the influence of the type of aluminum hydrolyte species present in coagulants on virus removal, five aluminum-based coagulants containing different aluminum hydrolyte species were prepared and used in this study. Application of the ferron method showed that the major aluminum species in $AlCl_3$ was a monomeric aluminum species (Al_a) (Table 2); ^{27}Al NMR spectroscopy confirmed that the major aluminum species in $AlCl_3$ was monomeric because only two peaks, (the aluminum monomer peak at 0 ppm and the internal standard substance peak at 80 ppm) were observed in the $AlCl_3$ spectrum (Fig. 1). In contrast, the major aluminum species in PACl-2.1b and PACl-2.4b was polymeric aluminum species (Al_b) (Table 2). Moreover, unlike $AlCl_3$, these PACls contained Al_{13} species as shown by the central tetrahedral Al peak in the Al_{13} species at 63 ppm in the ^{27}Al NMR spectra of PACl-2.1b and PACl-2.4b (Fig. 1). In contrast, PACl-2.1c and PACl-2.4c contained a high proportion of colloidal aluminum species (Al_c) (Table 2), and these PACls contained not only Al_{13} species but also Al_{30} species, which was not the case for PACl-2.1b and PACl-2.4b, because the broad peaks of the octahedral Al of the external shells in the Al_{13} and Al_{30} species at 10–12 ppm and the central tetrahedral Al in the Al_{30} species at 70 ppm were observed in the ^{27}Al NMR spectra of PACl-2.1c and PACl-2.4c, in addition to the peak of the Al_{13} species (Fig. 1). These results concur with those of other researchers who have reported that aluminum monomer can be classified as Al_a, Al_{13} species can be classified as Al_b, and Al_{30} species can be classified as Al_c [1,4,7].

3.2. Effect of aluminum species in coagulants on virus removal

The effect of aluminum species in the coagulants on the infectious PV removal ratio ($\log_{10}[C_0/C_s]$) during the coagulation process was evaluated by using the PFU method after settling (Fig. 2a). Because of the small size of PV, no PV removal (0.1-$\log_{10}$) was observed in the absence of coagulant at approximately pH 7. In contrast, the coagulation process removed infectious PV over the pH range of 6–8 no matter what type of coagulant was used. The addition of the coagulant destabilized the dispersion of the virus particles in the source water. The particles were then entrapped in or adsorbed onto the aluminum floc particles generated during coagulation, and then the floc particles with the entrapped or adsorbed viruses settled from suspension under the influence of gravity

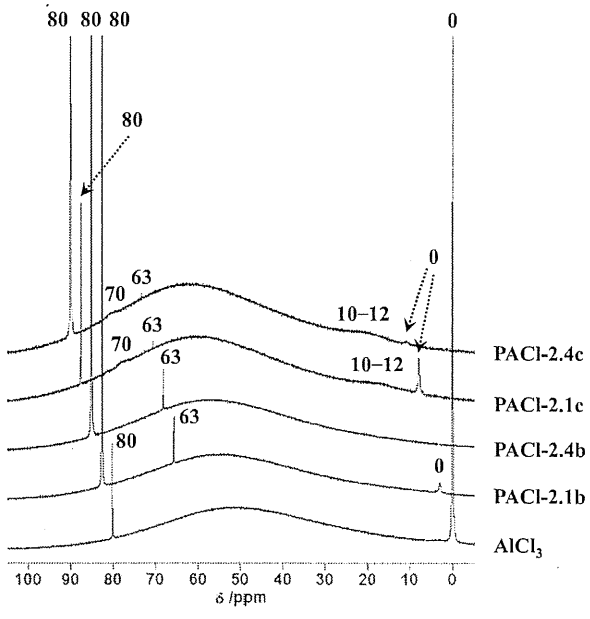

Fig. 1. ^{27}Al NMR spectra of the coagulants used in the present study.

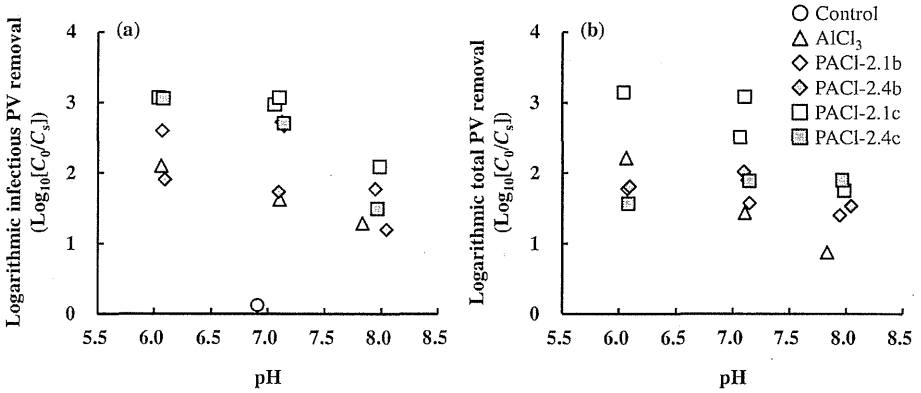

Fig. 2. Effect of aluminum hydrolyte species in coagulants on (a) infectious PV removal as evaluated by using the PFU method and on (b) total PV removal as evaluated by using the PCR method after settling during the coagulation process. Source water, lake water; coagulant dosage, 2.70 mg-Al/L.

during the settling process. The removal ratios of the infectious PV depended on the coagulant type: whereas coagulation with $AlCl_3$ and PACl-2.4b resulted in approximately 2-log_{10} removal over a pH range of 6–7, approximately 3-log_{10} removal was achieved with PACl-2.1b, PACl-2.1c, and PACl-2.4c. In addition, the removal ratios observed with PACl-2.1c were somewhat larger than those observed with the other aluminum-based coagulants, and approximately 2-log_{10} removal was obtained with PACl-2.1c even at pH 8. Taken together, these data indicate that the ability of the coagulants to remove infectious PV from virus-spiked lake water followed the order: PACl-2.1c > PACl-2.4c ≥ PACl-2.1b > PACl-2.4b = $AlCl_3$. The removal ratios of total PV, evaluated by using the PCR method, were also observed to be somewhat larger with PACl-2.1c than those observed with the other aluminum-based coagulants tested (Fig. 2b). The ability of PACl-2.1c to remove infectious PV from virus-spiked river water was also higher than that of the other aluminum-based coagulants, in agreement with the result from the virus-spiked lake water experiments (Fig. 3a), although the removal ratios obtained with the lake water and the river water were different, due to differences in water quality (Table 1). Moreover, the total PV removal ratios were also found to be somewhat larger with PACl-2.1c than those with the other aluminum-based coagulants tested in the river water sample (Fig. 3b). These results indicate that differences in the distribution of the aluminum species in the coagulants affected virus removal performance during coagulation and that PACl-2.1c effectively removed PV, particularly at pH 6–7.

In the present study, similar removal ratios of PV were observed with $AlCl_3$ and PACl-2.4b, even though the original compositions of the aluminum species in these coagulants were quite different (Fig. 4, the analytical pH condition was approximately 4–5, these are the same data as those shown in Table 2). Because further transformation of aluminum species after coagulant dosing is known to occur, and the distribution of aluminum species depends largely on the original composition of the coagulants and the pH of the treated water [4,25,26], we investigated the effect of pH on the transformation of the aluminum species after coagulant dosing across a pH range of 6–8 (Fig. 4). The distribution of the aluminum species of the four PACls during coagulation tended to be maintained (i.e., the original composition of the aluminum species was retained): the Al_b fraction, which represented a large proportion of PACl-2.1b and PACl-2.4b, and the Al_c fraction, which represented a large proportion of PACl-2.1c and PACl-2.4c, were almost unchanged between the pH ranges of 4–5 and 6–8 (Fig. 4), although the Al_c fraction, which represented a small proportion of PACl-2.1b and PACl-2.4b, was smaller when the pH was increased from 4–5 to 6. This result indicates that most of the original Al_b and Al_c content of the PACls was relatively stable over the pH range of 4–8, in agreement with previous studies [4,26]. In contrast, the distribution of the aluminum species of $AlCl_3$ changed markedly during coagulation compared with the original composition: the Al_a fraction, which represented a large proportion of $AlCl_3$ decreased and the Al_b fraction increased when the pH was increased from 4–5 to 6–8 (Fig. 4). This result indicates that a large proportion of the original Al_a content of $AlCl_3$ transformed into Al_b during coagulation, and that the formation of Al_b *in situ* probably led to the similar removal ratios of PV that were observed with PACl-2.4b.

The removal ratios of PV observed with high Al_c content PACls tended to be larger than those observed with high Al_b content PACls (Figs. 2 and 3). Because PACl-2.1c and PACl-2.4c contained not only Al_{13} species but also Al_{30} species, which was not the case for PACl-2.1b and PACl-2.4b, we hypothesize that the Al_{30} species in PACl played a major role in PV removal during the coagulation process. These results, taken together with our previous findings [16], suggest that Al_{30} species in aluminum-based coagulants are the important species to achieve efficient removal of not only bacteriophages but also human enteric viruses during the coagulation process.

When the basicity was increased from 2.1 to 2.4, the Al_c content in the high Al_c content PACls increased slightly and the Al_a content in those PACls decreased slightly (Fig. 4, the analytical pH condition was approximately 4–5). A similar trend was observed for the distribution of the aluminum species of PACls during coagulation (Fig. 4, the analytical pH condition was approximately 6–8). However, the removal ratios of PV observed with PACl-2.4c tended to be lower than those with PACl-2.1c (Figs. 2 and 3). This result suggests that the coagulation efficiency of virus during the coagulation process is not determined simply by the amount of Al_c in the coagulant.

To elucidate why PACl-2.1c effectively removed PV, we used the colloid titration method to determine the positive colloid charges of the coagulants (Fig. 5). The colloid charge densities of $AlCl_3$ were almost zero and constant, regardless of the aluminum concentration. In contrast, the colloid charge densities of the four PACls increased with increasing aluminum concentration. However, we observed no large differences in the colloid charge densities of the PACls, whereas the removal ratios observed with PACl-2.1c were somewhat larger than those observed with the other aluminum-based coagulants, as described above. Therefore, the virus coagulation efficiency was not solely dependent on either the amount of Al_c in the coagulant or the positive colloid charge of the coagulant. Ye *et al.* [12] recently reported that the amount of Al_{30} species in the coagulant increases with increasing basicity

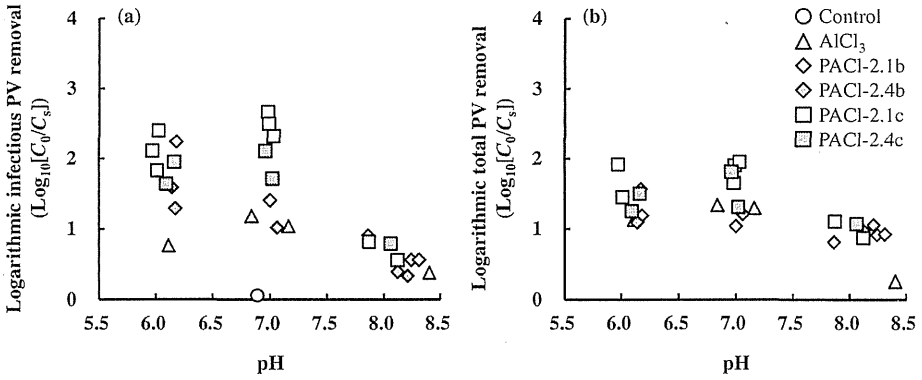

Fig. 3. Effect of aluminum hydrolyte species in coagulants on (a) infectious PV removal as evaluated by using the PFU method and on (b) total PV removal as evaluated by using the PCR method after settling during the coagulation process. Source water, river water; coagulant dosage, 1.08 mg-Al/L.

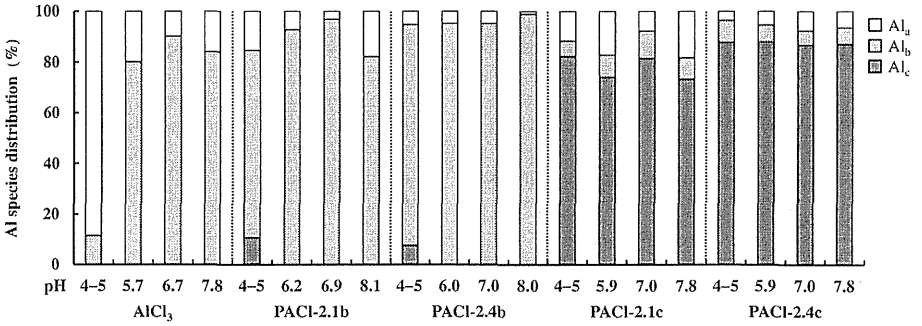

Fig. 4. Effect of pH on the transformation of aluminum species after coagulant dosing, as evaluated by using the ferron method.

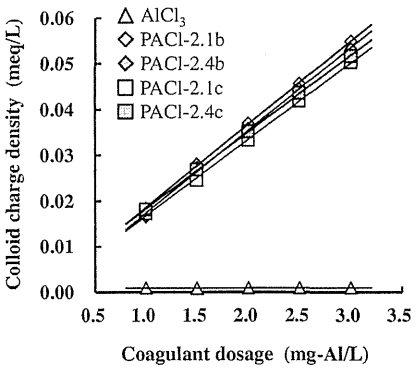

Fig. 5. Positive colloid charges of the coagulants used in the present study, as evaluated by using the colloid titration method.

from 1.0 to 2.0, but decreases when the basicity increases from 2.0 to 2.5. Therefore, the difference in the amount of Al_{30} species between PACl-2.1c and PACl-2.4c due to the difference in basicity probably led to the difference in virus removal performance during coagulation. Further investigation is needed to determine why the removal ratios of PV observed with PACl-2.4c tended to be lower than those with PACl-2.1c, even though the Al_c content of PACl-2.4c was slightly higher than that of PACl-2.1c.

3.3. Comparison of PV and CV removal ratios during coagulation

As described above, PACl-2.1c removed PV more efficiently than did the other aluminum-based coagulants tested, especially at approximately pH 7. To confirm that PACl-2.1c actually removed viruses more effectively than did $AlCl_3$ or PACl-2.1b, we also evaluated the removal ratios of a CV that has high resistance to free-chlorine disinfection relative to other human enteric viruses, including PV [21,22]. We evaluated the CV removal ratios by using the PFU and PCR methods after settling during the coagulation process, and then compared the results with those for PV (Fig. 6). For both enteroviruses, the removal ratios observed with PACl-2.1c were larger than those observed with $AlCl_3$ and PACl-2.1b at about pH 7: approximately 3-log_{10} removals were achieved with PACl-2.1c. This result indicates that coagulation with PACl-2.1c more effectively removes human enteroviruses, including a virus with high resistance to free-chlorine disinfection, relative to coagulation with $AlCl_3$ and PACl-2.1b.

The removal ratios of infectious PV and CV as evaluated by use of the PFU method were almost identical during coagulation regardless of the coagulant type used (Fig. 6). A similar trend was observed for the removal ratios of total PV and CV, as evaluated by using the PCR method. In contrast, Mayer *et al.* [20] reported that the removal ratio of CV B6, evaluated by using the 50% tissue culture infectious dose ($TCID_{50}$) method, was consistently larger than that of PV type 1 evaluated by the same method during enhanced coagulation with $FeCl_3$: for example, the optimal enhanced coagulation conditions of 40 mg/L $FeCl_3$ and a pH of between 5.5 and 6.5 resulted in 1.0–2.5-log_{10} and 1.5–3.0-log_{10} removals of infectious PV and CV, respectively. This result is not consistent with our result, although we did observe similar ranges of removal ratios for infectious PV and CV. In general, the removal ratios of microorganisms including viruses during coagulation are influenced by several factors such as source water quality, coagulant type, coagulant dosage, and coagulation pH [27]. Several of these factors, in particular coagulant type (aluminum-based