Plus, Shimadzu, Kyoto, Japan) and GC-MS/MS selected reaction monitoring (SRM) (TSQ Quantum XLS, Thermo Fisher Scientific, Yokohama, Japan)). Measurement conditions for both instruments are described by Duong et al. (2014b). Total ion chromatograms from the GC-MS Scan were processed by an identification and quantification system in a GC-MS database (AIQS-DB) (Kadokami et al. 2005) that was able to determine the concentrations of the 940 OMPs. Substances targeted by SIM and SRM were quantified by the internal standard method as reported by Duong et al. (2014b). If samples were measured by multiple methods (Scan, SIM, and/or SRM), we preferentially used the results from SRM, followed by SIM, and finally, Scan. The method detection limits (MDLs) of PAHs, OCPs, sterols, and PCBs measured by SIM were 1, 2, 8–320, and 0.4–1.6 ng L^{-1}, respectively, while the MDL of OCPs and PCBs measured by SRM ranged from 0.1 to 0.4 ng L^{-1}. The MDL of the remaining compounds measured by TIM were between 5 and 500 ng L^{-1}.

Quality control

Quality controls consisted of blank analysis, duplicate analysis, and recovery tests of 13 surrogates (deuterium-labeled internal standards) that were spiked prior to extraction. These surrogates had a similar range of physicochemical properties as were found among the OMPs measured. Good recoveries (69–115 %) were obtained for 10 out of the 13 surrogate compounds, except for highly polar and/or volatile compounds such as 2-fluorophenol. Tris(2-ethylhexyl)phosphate-d_{51} showed high recovery rates (140 %), probably due to the matrix effect (Kadokami et al. 2012). Relative standard deviations of surrogates were mostly less than or equal to 20 % (Table S2), confirming that sample analyses were acceptably precise. Blank samples were processed regularly for every set of five samples using 1 L of purified water previously passed through SPE disks. When reporting data, blank concentrations were subtracted from sample concentrations. Duplicate analyses were performed on the sample from well HN14 (Table S3), and the relative average deviations for 10 of the 13 detected substances were below 20 %, indicating that the analyses gave results with good reproducibility and that the precision was sufficient for environmental surveys.

Results and discussion

We have mainly based our discussion on the results from HN because the number of samples and sampling sites were limited in HCM, where samples were collected once at four sites, compared with HN, where samples were collected twice at 22 sites. These initial findings about OMPS in groundwater in HCM will serve as reference values and will be the basis of further intensive studies on the occurrence of OMPs in groundwater in HCM.

Occurrence of organic micro-pollutants

Of the 940 analytes, 74 that represent a variety of uses and origins were detected at least once (Table 1, Table S3). The maximum and the median number of compounds detected per well were 43 and 16, respectively. None of the samples were free of analytes; however, five of the samples contained less than 10 compounds. Of the 43 samples examined, 20 had total detectable concentrations that were less than 3.0 μg L^{-1}, while 17 had values between 3 and 7 μg L^{-1}. OMPs occurred more frequently in groundwater samples collected in the first sampling round (62 compounds, Sep 2013) than in the second sampling round (37 compounds, Aug 2014), with seven (Sep 2013) and three compounds (Aug 2014) detected in 100 % wells, respectively. The total concentrations in the first sampling round were highest at HN1 and HN14 (8.0 and 16 μg L^{-1}, respectively), while the total concentrations in the second round were highest at HN6 (15 μg L^{-1}) and HN16 (10 μg L^{-1}). The OMPs with the highest concentrations (greater than or equal to 0.7 μg L^{-1}) were not necessarily among the most frequently detected compounds (Fig. 2). For example, although several compounds, such as di-n-butyl phthalate (DBP) and 4-cymene, were detected infrequently, their maximum concentrations exceeded 0.7 μg L^{-1} (Fig. 2, Table 1). Previous studies (Kolpin et al. 2002; Loos et al. 2010) have also demonstrated that the compounds that occur most frequently are not always those with the highest concentrations. The measured concentrations of individual chemicals were generally low, and 89 % of concentrations were less than or equal to 0.5 μg L^{-1}. Bis(2-ethylhexyl)phthalate (DEHP), benzyl alcohol, cholesterol, DBP, and stigmasterol (Fig. 2) were detected in the samples 27, 19, 9, 5, and 3 times, respectively, at concentrations exceeding 0.5 μg L^{-1}.

Sterols and caffeine

The samples were screened for 10 sterols, of which campesterol, cholestanol, cholesterol, beta-sitosterol, stigmasterol, and ergosterol were detected. Cholesterol was observed in 100 % of wells, followed by beta-sitosterol (21 %) and stigmasterol (16 %), while cholestanol, ergosterol, and campesterol were observed in only one, one, and two wells, respectively (Table 1). Sterol appearances and concentrations in groundwater were highly variable across wells. Out of 43 samples investigated (both rounds), 33 were contaminated with only one sterol at detectable concentrations of less than 0.62 μg L^{-1} except for HCM3 (1.0 μg L^{-1}), while two, seven, and one samples were polluted with two, three, and five sterols, respectively (Table S3). The highest total concentrations of sterols were observed in HN2 (2.1 μg L^{-1}), HN1

Table 1 Summary of analytical results of groundwater wells sampled for 940 OMPs in Hanoi and Ho Chi Minh City

No.	Name	CAS number	LOD ($\mu g\ L^{-1}$)	Number of detections		Maximum concentration ($\mu g\ L^{-1}$)		Drinking water standards and health advisories ($\mu g\ L^{-1}$)	Origin/source	Detector
				Sep 2013 (n=22)	Aug 2014 (n=21)	Sep 2013 (n=22)	Aug 2014 (n=21)			
	Sterol									
1	Campesterol	474-62-4	0.01	0	2	0	0.16	–	Phytosterol	GC-MS/SIM
2	Cholestanol	80-97-7	0.01	0	1	0	0.35	–	Animal sterol	GC-MS/SIM
3	Cholesterol	57-88-5	0.01	22	21	1.0	0.88	–	Animal sterol	GC-MS/SIM
4	beta-Sitosterol	83-46-5	0.01	7	2	0.62	0.53	–	Phytosterol	GC-MS/SIM
5	Stigmasterol	83-48-7	0.01	7	0	5.8	0	–	Phytosterol	GC-MS/SIM
6	Ergosterol	57-87-4	0.01	0	1	0	0.34	–	Phytosterol	GC-MS/SIM
	Pharmaceuticals and Personal Care Products (PPCPs)									
7	L-Menthol	2216-51-5	0.01	18	4	0.06	0.02	–	PPCPs	GC-MS/TIM
8	Squalane	111-01-3	0.01	14	0	0.14	0	–	PPCPs	GC-MS/TIM
9	Diethyltoluamide	84-66-2	0.01	3	4	0.04	0.40	–	PPCPs	GC-MS/TIM
	Lifestyle compounds									
10	Caffeine	58-08-2	0.01	2	0	2.7	0	–	PPCPs	GC-MS/TIM
	Phthalates									
11	Diethyl phthalate (DEP)	84-66-2	0.01	22	15	0.09	0.71	[3]0.8; [4]30,000	Plasticizer	GC-MS/TIM
12	Diisobutyl phthalate (DIBP)	84-69-5	0.01	22	0	0.80	0	–	Plasticizer	GC-MS/TIM
13	Dimethyl phthalate (DMP)	131-11-3	0.01	4	3	0.02	0.04	–	Plasticizer	GC-MS/TIM
14	Di-n-butyl phthalate (DBP)	84-74-2	0.01	22	16	0.39	10	[3]0.1 [4]4000	Plasticizer	GC-MS/TIM
15	Di(2-ethylhexyl)phthalate (DEHP)	117-81-7	0.01	22	8	3.6	6.2	[1]6.0; [3]0.02; [4]700; [5]300; [7]8.0	Plasticizer	GC-MS/TIM
16	Butyl benzyl phtalate	85-68-7	0.01	18	9	0.10	0.11	[3]0.2; [4]7,000	Plasticizer	GC-MS/TIM
	Other OMP compounds									
17	3- and 4-tert-Butylphenol	585-34-2 and 98-54-4	0.01	1	0	0.04	0	–	Antioxidant	GC-MS/TIM
18	Acetophenone	98-86-2	0.01	17	7	0.12	0.07	[3]0.1	Cosmetics/fragrance	GC-MS/TIM
19	alpha-Terpineol	10482-56-1	0.01	1	0	0.65	0	–	Cosmetics/fragrance	GC-MS/TIM
20	Benzyl alcohol	100-51-6	0.01	15	21	3.6	0	–	Cosmetics/fragrance	GC-MS/TIM
21	Phenylethyl alcohol	60-12-8	0.01	0	3	0	0.02	–	Cosmetics/fragrance	GC-MS/TIM
22	Methyl palmitoleate	1120-25-8	0.01	0	1	0	0.13	–	Fatty acid methyl ester	GC-MS/TIM
23	Tributyl phosphate	126-73-8	0.01	11	1	0.50	1.4	–	Fire retardant	GC-MS/TIM
24	4-Chloro-3-methylphenol	59-50-7	0.01	0	1	0	0.30	–	Fungicide, paint	GC-MS/TIM
25	e-Caprolactam	105-60-2	0.01	0	19	0	0.11	[3]0.5	Intermediate for fiber	GC-MS/TIM
26	Bisphenol A	80-05-7	0.01	5	0	0.04	0	[3]0.05	Intermediate for resin	GC-MS/TIM
27	4-Chloro-2-nitroaniline	89-63-4	0.01	18	0	1.9	0	–	Intermediate in organic synthesis	GC-MS/TIM
28	3,5-Dimethylphenol	108-68-9	0.01	0	21	0	0.38	–	Intermediate in organic synthesis	GC-MS/TIM
29	Diphenylamine	122-39-4	0.01	0	5	0	0.07	[3]0.025		GC-MS/TIM

Table 1 (continued)

No.	Name	CAS number	LOD (μg L^{-1})	Number of detections		Maximum concentration (μg L^{-1})		Drinking water standards and health advisories (μg L^{-1})	Origin/source	Detector
				Sep 2013 (n=22)	Aug 2014 (n=21)	Sep 2013 (n=22)	Aug 2014 (n=21)			
									Intermediate in organic synthesis	
30	Nitrobenzene	98-95-3	0.01	0	1	0	0.10	[3]0.002	Intermediate in organic synthesis	GC-MS/TIM
31	Safrole	94-59-7	0.01	1	0	0.69	0	–	Intermediate in organic synthesis/preservative	GC-MS/TIM
32	2(3H)-Benzothiazolone	934-34-9	0.01	1	1	0	0.39	–	Leaching from tire	GC-MS/TIM
33	2-(Methylthio)-benzothiazol	615-22-5	0.01	5	1	0.11	0.09	–	Leaching from tire	GC-MS/TIM
34	Benzothiazole	95-16-9	0.01	12	4	0.04	0.13	–	Leaching from tire	GC-MS/TIM
35	Ethanol, 2-phenoxy-	122-99-6	0.01	22	5	0.30	0.04	–	Leaching from tire	GC-MS/TIM
36	4-tert-Octylphenol	140-66-9	0.01	2	0	0.06	0	–	Nonionic detergent metabolite	GC-MS/TIM
37	Longifolene	475-20-7	0.01	4	0	0.09	0	–	Other	GC-MS/TIM
38	2-Ethyl-1-hexanol	104-76-7	0.01	21	0	0.32	0	–	Plasticizer	GC-MS/TIM
39	Bis(2-ethylhexyl) sebacate	122-62-3	0.01	2	1	0.02	0.13	–	Plasticizer	GC-MS/TIM
40	Di(2-ethylhexyl)adipate	103-23-1	0.01	22	17	0.1	0.95	[1]400; [2]400; [3]0.6; [4]20,000; [5]3,000	Plasticizer	GC-MS/TIM
41	4-Cymene	99-87-6	0.01	5	0	6.8	0	–	Solvent	GC-MS/TIM
42	trans-Decahydronaphthalene	493-02-7	0.01	21	0	0.34	0	–	Solvent	GC-MS/TIM
43	Isophorone	78-59-1	0.01	5	8	0.02	0.20	[2]100; [3]0.2; [4]7,000; [5]4,000	Solvent/paint	GC-MS/TIM
	Organochlorine pesticides[a]									
44	trans-Chlordane	5103-74-2	0.4	1	0	3.0	0	–	Insecticide	GC-MS-MS/SRM
45	cis-Chlordane	5103-71-9	0.4	1	0	1.7	0	–	Insecticide	GC-MS-MS/SRM
	Σchlordane							[1]2.0; [2]4.0; [3]0.0005; [4]20; [5]10; [7]0.2		
46	trans-Nonachlor	5103-73-1	0.4	1	0	0.72	0	–	Insecticide	GC-MS-MS/SRM
47	Endrin	72-20-8	0.4	4	7	1.3	1.7	[1]2.0; [2]2.0; [3]0.0003; [4]10	Insecticide	GC-MS-MS/SRM
48	Dieldrin	60-57-1	0.4	2	0	2.8	0	[3]0.00005; [4]2.0; [5]0.2; [6]0.03	Insecticide	GC-MS-MS/SRM
49	Hexachlorobenzene	118-74-1	0.1	2	0	0.35	0	[1]1.0; [3]0.0008; [4]30; [5]2.0	By-product	GC-MS-MS/SRM
50	Heptachlor epoxide (B)	1024-57-3	0.4	0	8	0	5.9	[1]0.2; [3]0.00001; [4]0.4; [5]0.4; [6]0.03	Insecticide	GC-MS-MS/SRM
51	a-HCH	319-84-6	0.4	1	0	1.9	0	–	Insecticide	GC-MS-MS/SRM
52	g-HCH	58-89-9	0.4	1	0	1.3	0	[1]0.2; [3]0.005; [4]200	Insecticide	GC-MS-MS/SRM
53	p,p′-DDT	50-29-3	0.1	6	11	3.6	12	[3]0.0005; [7]1.0[b]	Insecticide	GC-MS-MS/SRM
54	p,p′-DDE	72-55-9	0.1	1	1	0.11	0.33	–	Insecticide	GC-MS-MS/SRM
55	p,p′-DDD	72-54-8	0.1	2	4	0.46	0.55	–	Insecticide	GC-MS-MS/SRM
56	o,p′-DDT	789-02-6	0.2	2	2	0.38	1.3	–	Insecticide	GC-MS-MS/SRM

Table 1 (continued)

No.	Name	CAS number	LOD ($\mu g\ L^{-1}$)	Number of detections		Maximum concentration ($\mu g\ L^{-1}$)		Drinking water standards and health advisories ($\mu g\ L^{-1}$)	Origin/source	Detector
				Sep 2013 (n=22)	Aug 2014 (n=21)	Sep 2013 (n=22)	Aug 2014 (n=21)			
57	o,p′-DDD	53-19-0	0.1	16	8	2.7	6.4	–	Insecticide	GC-MS-MS/SRM
	Polychlorinated biphenyls[a]									
58	PCB #8	34883-43-7	0.1	3	0	0.35	0	–	PCB	GC-MS-MS/SRM
59	PCB #74	32690-93-0	0.1	2	0	0.13	0	–	PCB	GC-MS-MS/SRM
60	PCB #70	32598-11-1	0.1	2	0	0.11	0	–	PCB	GC-MS-MS/SRM
61	PCB #66	32598-10-0	0.1	2	0	0.12	0	–	PCB	GC-MS-MS/SRM
62	PCB #60	33025-41-1	0.1	5	7	1.1	0.33	–	PCB	GC-MS-MS/SRM
63	PCB #52	35693-99-3	0.1	2	0	0.46	0	–	PCB	GC-MS-MS/SRM
64	PCB #44	41464-39-5	0.1	2	0	0.25	0	–	PCB	GC-MS-MS/SRM
65	PCB #41	52663-59-9	0.1	2	0	0.34	0	–	PCB	GC-MS-MS/SRM
66	PCB #4 and 10	13029-08-8 and 33146-45-1	0.1	2	0	0.29	0	–	PCB	GC-MS-MS/SRM
67	PCB #37	38444-90-5	0.2	1	0	0.20	0	–	PCB	GC-MS-MS/SRM
68	PCB #33	38444-86-9	0.1	3	0	0.40	0	–	PCB	GC-MS-MS/SRM
69	PCB #28	7012-37-5	0.1	4	0	1.8	0	–	PCB	GC-MS-MS/SRM
70	PCB #22	38444-85-8	0.2	2	0	0.30	0	–	PCB	GC-MS-MS/SRM
71	PCB #19	38444-73-4	0.1	1	0	0.15	0	–	PCB	GC-MS-MS/SRM
72	PCB #18	37680-65-2	0.1	4	0	0.78	0	–	PCB	GC-MS-MS/SRM
73	PCB #15	2050-68-2	0.1	2	0	0.19	0	–	PCB	GC-MS-MS/SRM
74	PCB #1	2051-60-7	0.1	1	0	0.11	0	–	PCB	GC-MS-MS/SRM
	ΣPCBs							[1]0.5; [5]10		GC-MS-MS/SRM

LOD limit of detection, – not available

[a] Compounds presented concentrations in ng L^{-1}

[b] Total DDT and metabolites concentration ($\mu g\ L^{-1}$)

Drinking Water Standards and Health Advisories:

[1] US EPA Maximum Contaminant Levels (MCL) ($\mu g\ L^{-1}$)

[2] US EPA Lifetime Health Advisory ($\mu g\ L^{-1}$)

[3] US EPA Reference Dose (RfD) (mg kg^{-1} day^{-1})

[4] US EPA Drinking Water Equivalent Level (DWEL) ($\mu g\ L^{-1}$)

[5] US EPA 10^{-4} Cancer Risk ($\mu g\ L^{-1}$)

[6] European Health-Based Chemical Standards ($\mu g\ L^{-1}$). http://www.doeni.gov.uk/niea/european_and_national_drinking_water_quality_standards_-_october_2011.pdf.

[7] WHO Guidelines for Drinking-water Quality ($\mu g\ L^{-1}$)

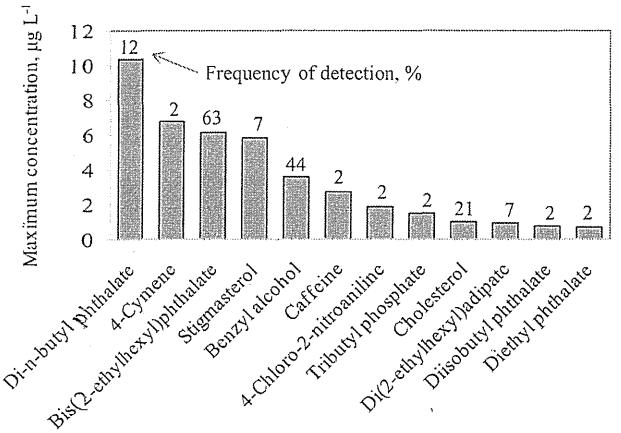

Fig. 2 Maximum concentrations and detection frequencies of compounds that were detected at concentrations greater than 0.7 μg L^{-1}

(6.6 μg L^{-1}), and HN9 (1.1 μg L^{-1}) in the first sampling round, while HN6 was contaminated with high sterol concentrations in both rounds (Table S3). Stigmasterol and cholesterol were the only two sterols detected at concentrations greater than 1 μg L^{-1} (Table 1), whereas the other four remaining sterols were identified at concentrations that were lower than 0.88 μg L^{-1}. These results may indicate a high degree of heterogeneity in loadings from local sources and/or spatial differences in hydraulic conductivity and biogeochemical conditions, as mentioned by Schaider et al. (2014).

Six sterols that were detected in groundwater were also abundant in river waters and sediments collected in the same study areas (HN and HCM) in previous studies (Duong et al. 2014a, b). However, a fecal sterol (coprostanol) that was detected most often and at extremely high concentrations in river waters and sediments due to sewage contamination (Duong et al. 2014a, b) was not found in groundwater samples.

Caffeine was one of the most frequently detected compounds in previous groundwater studies (Barnes et al. 2008; Focazio et al. 2008) and was detected in nearly 100 % of river waters in HN and HCM (Duong et al. 2014a). In this study however, it was observed in only two (HN14 and HCM3) of the 43 groundwater samples. The maximum concentration that was detected in this study (2.7 μg L^{-1}) is about 20 times higher than the maximum detected in groundwater in the USA (0.13 μg L^{-1}, Barnes et al. 2008), 14 times higher than the maximum concentration detected in groundwater in Europe (0.19 μg L^{-1}, Loos et al. 2010), and 10 times higher than the maximum value in untreated drinking water sources in the USA (0.27 μg L^{-1}, Focazio et al. 2008). The presence of caffeine in shallow drinking water wells has been suggested as an indicator of wastewater impacts (Seiler et al. 1999), and it is thought that it is attenuated in oxic subsurface conditions (Schaider et al. 2014). Moreover, it persists in anaerobic conditions in groundwater (Ying et al. 2003). Therefore, the presence of caffeine in two wells probably reflects direct entry of leachate from surface water, leaks in sewage canals, or underground septic tanks.

Emerging chemicals

Of the 14 PPCPs analyzed, N,N-diethyl-m-toluamide (DEET), L-menthol, and squalane were detected in groundwater samples at maximum concentrations of 0.40, 0.06, and 0.14 μg L^{-1}, respectively (Table 1, Table S3). L-menthol and DEET, which are well-known molecular markers of sewage contamination (Nakada et al. 2008), were detected in 22 and 7 groundwater samples (in both sampling rounds), respectively, while squalane, which is used in numerous vaccine and drug delivery emulsions (Fox 2009), was only detected in 14 out of 22 groundwater samples in the first sampling round (Table 1, Table S3). DEET was the most frequently detected compound in groundwater in the USA at maximum concentrations of 13.5 μg L^{-1} (Barnes et al. 2008), 34 times higher than those of this study. However, the maximum levels of DEET in this study were six times higher than those (0.07 μg L^{-1}) in groundwater from the metropolitan area of Tokyo (Nakada et al. 2008).

The three detected PPCPs in groundwater samples were the most abundant in surface water of HN and HCM that was contaminated with sewage (Duong et al. 2014a), and two of them (L-menthol and squalane) were detected in sediments at the same study areas, which demonstrates the ubiquitous use of these PPCPs; the fact that they exist in the water environment in Vietnam reflects the lack of adequate wastewater treatment facilities. Nakada et al. (2008) reported that DEET and other PPCPs were also detected in groundwater in Tokyo as the consequence of sewage leakage from decrepit sewers. Therefore, the presence of PPCPs in groundwater in this study is probably the result of a combination of leaking underground septic tanks, infiltration of untreated wastewater through old sewer canal systems, and urban storm water recharge/runoff. Furthermore, because a large number of PPCPs are known to be found in domestic wastewater, it is thought that groundwater is polluted by many PPCPs. However, the number of PPCPs (14) registered in the AIQS database is very limited, so a more detailed survey on PPCPs should be carried out after new PPCPs are registered in the database.

Two phenolic EDCs, BPA and OP, deserve particular attention because of their estrogenic activity and widespread application and ubiquity in the environment (Kuch and Ballschmiter 2001; Meesters and Schroder 2002). There is evidence that BPA has estrogenic potential even at low concentrations (Jin et al. 2004) and OP can cause estrogenic effects in fish and other aquatic organisms (Jobling et al. 1996). BPA was detected in 5, while OP was detected in 2 of the 22 wells sampled in the first round at concentrations that ranged from 0.02 to 0.04 μg L^{-1} and 0.02 to 0.06 μg L^{-1}, respectively (Table S3). The maximum BPA concentrations were 64 and

57 times lower than those in groundwater in the USA (2.55 μg L^{-1}, Barnes et al. 2008) and in Europe (2.3 μg L^{-1}, Loos et al. 2010), respectively, and the maximum OP concentration was six times lower than those reported in Oxford, England (0.83 μg L^{-1}, Stuart et al. 2014) and 1.5 times higher than those in European groundwater (0.04 μg L^{-1}, Loos et al. 2010). The concentrations of BPA detected in this study are much lower than the human health-based guideline values (100 μg L^{-1}) for exposure to BPA in groundwater proposed by the Minnesota Department of Health (MDH 2013).

Of the six phthalate compounds detected, diethyl phthalate (DEP), DBP, and DEHP were detected in over 70 % of the sampled wells with maximum concentrations of 0.71, 10, and 6.2 μg L^{-1}, respectively (Table 1, Table S3). These compounds have been detected in groundwater in England; however, the concentrations in this study were more than 2.5 times lower than those reported in Boxford but more than 1.5 times higher than those reported in Oxford (Stuart et al. 2014). Dimethyl phthalate (DMP) was found in scattered wells in this study at trace concentrations of less than 0.04 μg L^{-1} and was detected only once in groundwater from Maresme, Spain, at a concentration of 0.12 μg L^{-1} (Sánchez-Avila et al. 2009). The highest concentration of DEHP (6.2 μg L^{-1}), an EDC (Akingbemi et al. 2004), was comparable with the concentration detected in the Llobregat River aquifers, Spain (5.67 μg L^{-1}, López-Roldán et al. 2004).

Organochlorine pesticides and polychlorinated biphenyls

Pollution from OCPs and PCBs in surface waters and sediments has been extensively researched in Vietnam (Nhan et al. 1998, 2001; Minh et al. 2007; Hoai et al. 2010). However, they are of little concern as groundwater contaminants because they are relatively insoluble in water and are retained strongly by soil. Our previous studies (Duong et al. 2014a, b) showed that PCBs and OCPs still remain in the aquatic environment and most of the banned OCPs can still be found in high concentrations in the environment throughout Vietnam (e.g., Kishida et al. 2007; Minh et al. 2007; Hoai et al. 2010). In addition, Belfroid et al. (1998) demonstrated that many pesticides entered the aquatic environment via soil percolation, air drift, or surface runoff and eventually ended up in groundwater, where their transformation products could remain for years.

In this study, 14 OCPs and 17 PCBs were detected, and the detection frequency was higher in the first sampling round than in the second round (Table 1, Table S3). Of the 43 samples, 17 and 33 contained at least one of the OCP and PCB congeners, respectively. o,p′-DDD, p,p′-DDT, and endrin were the most frequently detected OCPs with maximum concentrations of 6.4, 12, and 1.7 ng L^{-1}, respectively. trans-Chlordane was detected in one well, and dieldrin was detected in two wells, and their concentrations reached around 3 ng L^{-1} (Table 1, Table S3). As was observed for PCBs, none of the PCBs were detected in the groundwater samples from the second sampling round except for PCB #60, which was the most abundant PCB congener and had the highest occurrence in both rounds. The highest concentrations of individual PCB congeners were generally less than 0.5 ng L^{-1} except for PCB #28 (1.8 ng L^{-1}), followed by PCB #60 (1.1 ng L^{-1}) and PCB #18 (0.78 ng L^{-1}) (Table S3). Well HCM2 was seriously polluted by PCBs and OCPs with a total concentration of 20 ng L^{-1}, while HN3A, HN4, and HN5 were heavily polluted by OCPs and had total detected concentrations of 16, 14, and 9.4 ng L^{-1}, respectively (Table S3). This is not surprising, as the surface waters and sediments collected at these sites were also extremely polluted by elevated levels of OCPs and PCBs (Duong et al. 2014a, b). OCPs such as α-, β-, γ-, δ-hexachlorocyclohexane (HCH), aldrin, dieldrin, heptachlor, and DDT and its metabolites have been detected in groundwater in India (Sankararamakrishnan et al. 2005; Shukla et al. 2006; Lari et al. 2014); Syria (Jamal 2011); and in Beijing, China (He et al. 2011). However, the pathways by which these contaminants can make their way into the groundwater are still unclear. For example, Jabbar et al. (1993) demonstrated that the principle mechanism by which OCPs are transported from soil to groundwater is via downward percolation of water containing dissolved pesticides, while Cerejeira et al. (2003) indicated that because of drift, runoff, drainage, and their leaching potential, OCPs caused contamination of surface and groundwater. However, more detailed studies need to be carried out to gain an improved understanding of the leaching behavior of OCPs and PCBs in groundwater.

Organic micro-pollutant compound groups

The 74 OMPs (or semi-volatile organic compounds, SVOCs) can be divided into 18 groups based on general use category or type of compound (Fig. 3). n-Alkanes were excluded from the pollution profile because of their widespread existence in environmental samples. However, it should be noted that the uses of any given compound can vary widely; therefore, the tabulated use categories are presented for illustrative purposes and may not be all-inclusive. The frequency of detection was calculated as the ratio of the measured detection to the total measurement. Although these groupings are composed of unequal numbers of compounds, the detection frequency of the compound group is not controlled by the number of compounds in the group. The phthalate group, consisting of six compounds, had the greatest detection frequency and accounted for the highest percentage of the total concentrations in the first round, while the fiber group contained only ε-caprolactam, which was detected in 19 out of 21 samples in the second sampling round at relatively low concentrations. A total of 9 out of 16 groups in the first sampling round had a detection frequency exceeding 40 %, and two groups (phthalates and sterols) accounted for over 60 % of the total

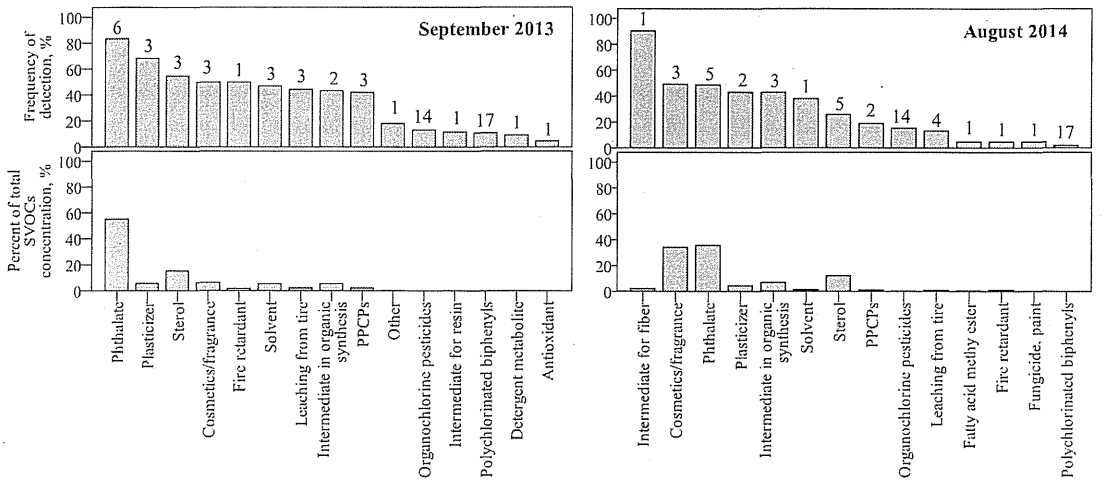

Fig. 3 (1) Detection frequency of SVOCs by general use category and (2) percent of total concentration of SVOCs by general use category in the first round (Sep 2013) and the second round (Aug 2014). Number of compounds in each category are shown above the bar

concentrations. While detection frequencies that exceeded 40 % were reported for five groups in the second sampling round, three groups (cosmetics/fragrance, phthalate, sterol) accounted for 80 % of the total concentrations (Fig. 3).

General comparison with the surface water study

Data collected for groundwater in this study can be qualitatively compared with data collected for the surface waters in our previous study (Duong et al. 2014a). This is a valid comparison because the same analytical method was used for this study and the previous surface water study, although fewer surface water samples were collected (26 surface water samples compared with 43 groundwater samples). Because a large number of chemicals that originated from domestic wastewater were detected in surface waters (Duong et al. 2014a), it was thought that groundwater would also be contaminated with many chemicals. Therefore, the groundwater sampling sites were close to the surface water sampling sites. Overall, fewer contaminants were detected in groundwater, and only 74 of the 940 analytes were detected in groundwater, while 235 were detected in surface waters (Duong et al. 2014a). All of the compounds that were detected in groundwater samples were also detected in the surface water samples. Although similar compounds were detected in the groundwater, the number of compounds having a detection frequency exceeding 70 % in groundwater (six compounds) was much smaller compared with surface water (42 compounds). The concentrations of pollutants in surface water were higher than those in groundwater. Total concentrations of the detected pollutants in 12 out of the 43 groundwater wells exceeded 5 $\mu g\ L^{-1}$, with three wells having total concentrations higher than 10 $\mu g\ L^{-1}$. The total concentrations in 50 % of the surface waters were greater than 120 $\mu g\ L^{-1}$ (from 120 to 440 $\mu g\ L^{-1}$) while the levels were below 10 $\mu g\ L^{-1}$ in only six samples. Coprostanol and coprostanone were detected at extremely high concentrations in all surface water samples in HN and HCM but were not detected in groundwater samples. While surface waters in HN and HCM were heavily contaminated by permethrin and carbamate insecticide (fenobucarb), these compounds were not detected in any of the groundwater wells. This is most likely because of their hydrophobic properties (K_{ow} log P 6.1 and 2.79, respectively) and their tendency to adsorb onto organic matter and sediment, meaning it is difficult for them to reach the aquifer. In addition, Sharom and Solomon (1981) and Kim et al. (2014) demonstrated that permethrin and fenobucarb are biodegraded in aqueous systems and soils, so they were not present in aquifers.

Identification of potential sources of contamination

Information about the aquifer conditions, such as hydrogeological processes, aquifer characteristics (gravel, chalk, hyporheic zone), kinetics, groundwater flow velocity, and information on the well depth, was not available for all the wells sampled. Therefore, an evaluation of the characteristics of chemicals detected in groundwater in this study may provide a useful insight into the factors that control the presence of organic contamination in the aquifers of HN and HCM. The sampled groundwater wells were close to the surface water and sediment sampling sites. As such, heavily polluted surface waters may influence aquifers via numerous processes, including concentrations at the source, dilution, adsorption, and degradation (Jurado et al. 2012). The largest number of contaminants (27 compounds) and the highest total concentration (18 $\mu g\ L^{-1}$) were detected at HN14. In addition, 4-cymene, caffeine, and DEHP were the main contributors to pollution at this well. Out of these, caffeine, an indicator of wastewater impacts in shallow drinking water wells (Seiler et al. 1999), was only detected in 2 out of 43 wells. Therefore, it is thought that this well may be impacted by

point sources, such as direct influences from the surface or leaks from underground septic systems. Three other wells (HN13, HN16, and HN17), located close to the most polluted canals in HN, had elevated total concentrations of 9.0, 9.7, and 8.3 $\mu g\ L^{-1}$, respectively, and a similar range of chemicals contributed to the high total concentrations at each of these sites. The leakage of surface water contaminated by sewage from the decrepit sewer canals to the aquifers is probably the cause of the pollution at these wells. The total concentrations in the remaining wells were less than 6 $\mu g\ L^{-1}$. At 20 of the sites, the total concentrations were lower than 3 $\mu g\ L^{-1}$ and fewer than 20 compounds were detected; these sites were possibly influenced by non-point source pollution. There are multiple possible sources of non-point pollution to aquifers, such as storm water and urban runoff, leakages from urban sewerage systems, diffuse aerial deposition (Nakada et al. 2008; Buerge et al. 2011; Vulliet et al. 2008), or intentional and unintentional recharge of wastewater sources (Drewes 2009). However, the possibility of contaminant leakages from septic systems is not excluded because it was reported that about 32 % of HN's population is served by septic tanks (World Bank 1996). In addition, several case studies in the USA and Canada have reported a range of emerging organic contaminants in groundwater impacted by septic tanks (Carrara et al. 2008; Swartz et al. 2006). Hence, further research is needed to clarify the major source of detected contaminants as well as to determine the primary fate, degradation, transformation, and transport processes in impacted aquifers.

Risk assessment

Well water sampled in this study is not used for drinking purposes; however, more than half of the population of HN get their tap water from groundwater sources. Hence, the presence of contaminants in HN and HCM's groundwater raises human health concerns even though most of the detected contaminants are not currently regulated in drinking water or were present at low levels. Therefore, in this context, we were not able to evaluate the potential health implications of all the detected contaminants because of a lack of toxicity information for many organic pollutants (Stephenson 2009) and limited testing requirements (Schaider et al. 2014). Further, while this study included a diverse list of 940 organic pollutants, there are about 80,000 chemicals currently in use (Schaider et al. 2014), suggesting that other organic pollutants, as well as their metabolites, were also present in our samples. Consequently, we have assessed the health risk for 19 out of the 74 detected contaminants, for which health-based guidelines are available, by comparing the measured concentrations with the drinking water standards or health advisories (Table 1) proposed by the US Environmental Protection Agency (USEPA), European Commission (EU), and the WHO. None of the detected concentrations exceeded the maximum contaminant levels, drinking water equivalent levels or lifetime health advisory proposed by the USEPA (Table 1), or the health-based chemical standards and guideline values for drinking water issued by EU and WHO, respectively. A non-carcinogenic assessment of some chemicals was carried out based on the exposure of a 50 kg adult consuming 2 L of water per day by dividing the reference dose (USEPA). The risk quotients for the 18 investigated pollutants were much smaller than 1, which demonstrates that there is no risk to humans from these detected contaminants.

Conclusions

This is the first comprehensive study of OMPs in groundwater in Vietnam, and as such, this study provides new baseline knowledge about the occurrence and levels of organic contaminants in groundwater. Key findings are as follows: (1) Out of 940 analytes, 74 were detected, which represented about one third of the number of contaminants that were detected in surface waters. (2) Cholesterol, di(2-ethylhexyl)adipate, DBP, DEP, DEHP, and benzyl alcohol were the most frequently detected compounds and were found in over 80 % of samples. The total concentrations in 75 % of the groundwater samples were less than 5.0 $\mu g\ L^{-1}$. (3) Many of the wells and aquifers are probably influenced by non-point source pollution, such as leaks from the urban sewerage system or recharge by wastewater. (4) There is concern for human health related to the presence of pollutants in groundwater in HN and HCM, even though most detected contaminants are not currently regulated in drinking water or were present at low levels. A health risk assessment for 19 detected contaminants showed that there were no risks to humans from these contaminants.

This study was carried out over a limited time period (Sep 2013 and Aug 2014) with a limited number of sampling events. The number of sampling sites was particularly limited in HCM, which means that we only have limited information on the occurrence of OMPs in groundwater in HCM. In spite of the limited information, these initial results can be used as baseline data and will form the basis for further intensive studies on the occurrence of OMPs in groundwater in HCM and on the primary fate, degradation, and transport processes of OMPs in impacted aquifers. A full evaluation of the potential risks of detected contaminants should also be carried out.

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Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants

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ABSTRACT

We investigated the effects of basicity, sulfate content, and aluminum hydrolyte species on the ability of polyaluminum chloride (PACl) coagulants to remove F-specific RNA bacteriophages from river water at a pH range of 6–8. An increase in PACl basicity from 1.5 to 2.1 and the absence of sulfate led to a reduction of the amount of monomeric aluminum species (i.e., an increase of the total amount of polymeric aluminum and colloidal aluminum species) in the PACl, to an increase in the colloid charge density of the PACl, or to both and, as a result, to high virus removal efficiency. The efficiency of virus removal at around pH 8 observed with PACl-2.1c, a nonsulfated high-basicity PACl (basicity 2.1–2.2) with a high colloidal aluminum content, was larger than that observed with PACl-2.1b, a nonsulfated high-basicity PACl (basicity 2.1–2.2) with a high polymeric aluminum content. In contrast, although extremely high basicity PACls (e.g., PACl-2.7ns, basicity 2.7) effectively removed turbidity and UV260-absorbing natural organic matter and resulted in a very low residual aluminum concentration, the virus removal ratio with PACl-2.7ns was smaller than the ratio with PACl-2.1c at around pH 8, possibly as a result of a reduction of the colloid charge density of the PACl as the basicity was increased from 2.1 to 2.7. Liquid ^{27}Al NMR analysis revealed that PACl-2.1c contained Al_{30} species, which was not the case for PACl-2.1b or PACl-2.7ns. This result suggests that Al_{30} species probably played a major role in virus removal during the coagulation process. In summary, PACl-2.1c, which has high colloidal aluminum content, contains Al_{30} species, and has a high colloid charge density, removed viruses more efficiently (>4 $\log_{10}$ for infectious viruses) than the other aluminum-based coagulants—including commercially available PACls (basicity 1.5–1.8), alum, and PACl-2.7ns—over the entire tested pH (6–8) and coagulant dosage (0.54–5.4 mg-Al/L) ranges.

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1. Introduction

Aluminum-based coagulants such as polyaluminum chloride (PACl) and alum are commonly used in coagulation processes to destabilize suspended and dissolved materials in water and combine them into large flocs that are easily separated from the water by subsequent sedimentation or filtration. Waterborne enteric viruses, which do not settle from suspension under the influence of gravity, can also be removed with aluminum-based coagulants. For example, Nasser et al. (1995) reported that 88.4% and 47% of hepatitis A virus and poliovirus, respectively, can be removed by coagulation with 30 mg/L of alum. We have reported that a coagulation process with PACl or alum effectively removes bacteriophages, which are viruses that infect bacteria and may be indicators for waterborne enteric viruses (Matsushita et al., 2011).

The efficiency of microorganism removal by coagulation processes is strongly influenced by several factors, including the nature and dosages of the coagulant used, pH, temperature, and mixing method (Hijnen and Medema, 2010). In particular, pH control during the coagulation process is essential for optimal coagulation (Bratby, 2006). Guo and Hu (2011) reported that coagulation with alum at pH 8 does not result in significant virus removal, whereas coagulation at pH 6 and 7 does. Worldwide, the pH of various surface drinking water sources is changing from neutral to alkaline because of the excessive growth of algae (Hu et al., 2006; Matsukawa et al., 2006), and this change can be expected to reduce coagulation efficiency and thus virus removal performance if commercially available PACl or alum is used without pH adjustment. Reducing the pH of drinking water sources with acid or increasing the coagulant dosage is sometimes required to improve coagulation efficiency (Hu et al., 2006; Yan et al., 2008a). However, both of these methods have some disadvantages, such as increasing the residual aluminum concentration in treated water (Matsukawa et al., 2006) and increasing the treatment cost (Yan et al., 2008a). Therefore, the development of new coagulation processes that effectively remove suspended and dissolved materials, including viruses, from both neutral and alkaline drinking water sources without the need for pH optimization is highly desired.

For the improvement of coagulation efficiency, PACl coagulants with various aluminum hydrolysis ratios (basicity = $[OH^-]/[Al^{3+}]$) have been produced, and the influence of PACl basicity on coagulation processes has been investigated (Wang et al., 2002; Yan et al., 2008a,b; Yang et al., 2011; Zhang et al., 2008). For example, Wang et al. (2002) reported that turbidity removal at alkaline pH is improved by increases in PACl basicity. Zhang et al. (2008) reported that the coagulation efficiency of PACl increases with increasing basicity: specifically, PACl with a basicity of 2.4 exhibits higher humic acid removal efficiency and lower residual aluminum concentration at a broader pH range and a wider PACl dosage range compared to PACls with basicities of 1.2 and 1.8. In previous work, we compared PACls with basicities of 2.1 and 1.5 in the pH range of 6.8–7.8 and found that the former, which contains a smaller percentage of monomeric aluminum species and a larger percentage of colloidal aluminum species than the latter, removes dissolved organic carbon more efficiently and with a lower residual aluminum concentration (Kimura et al., 2013). Moreover, PACls with basicities of >2.6 yield a very low residual aluminum concentration (<0.02 mg/L), even at a wide pH range (6.5–8.5; Kimura et al., 2013). High-basicity PACls are expected to effectively remove viruses not only at neutral pH but also at weakly alkaline pH; however, virus removal during coagulation processes with high-basicity PACls has not been fully investigated. In addition, little information is available about how the small amount of sulfate (e.g. 3% w/w) that is present in commercially available PACls to improve flocculation and sedimentation efficiency (Pernitsky and Edzwald, 2003) affects virus removal during coagulation processes.

Here, we conducted batch coagulation experiments to investigate the effect of PACl basicity on virus removal by comparing a wide variety of PACls with different basicities, including commercially available PACls (basicity 1.5–1.8) and extremely high basicity PACls (basicity 2.7). In addition, we investigated the effect of sulfate in the PACls on virus removal by comparing sulfated and nonsulfated PACls. Moreover, we experimentally evaluated the aluminum species distributions and colloid charge densities of the tested coagulants to determine what caused the differences in virus removal performance.

2. Materials and methods

2.1. Source water and coagulants

River water was sampled from the Toyohira River in Sapporo, Japan, on 1 October 2010, 24 June 2011, and 4 December 2012 (water quality data are shown in Table S1, Supplementary Information).

We conducted three sets of coagulation experiments on the river water samples. For the first set of experiments, we used five aluminum-based coagulants (Table S2). Two commercially available PACls (PACl-1.5s and PACl-1.8s, where 1.5 and 1.8 are the basicity values, and "s" stands for "sulfated"; Taki Chemical Co., Kakogawa, Japan). A trial high-basicity PACl (PACl-2.1s, which is now commercially available) was also supplied by the same company. For comparison with the commercially available PACls, we evaluated an $AlCl_3$ solution prepared by dilution of reagent-grade aluminum(III) chloride hexahydrate ($AlCl_3 \cdot 6H_2O$, Wako Pure Chemical Industries, Osaka, Japan) in Milli-Q water (Milli-Q Advantage, Millipore Corp., Billerica, MA, USA), and we also evaluated a commercially available alum (Taki Chemical Co.).

After the first set of experiments was completed, we conducted a second set of experiments with eight aluminum-based coagulants (Table S2). In addition to two of the sulfated PACls described above, we evaluated a trial nonsulfated PACl (PACl-1.5ns, where "ns" stands for "nonsulfated"), a high-basicity nonsulfated PACl (PACl-2.1ns), and an extremely high basicity nonsulfated PACl (PACl-2.7ns), all provided by Taki Chemical Co., to further investigate the effects of basicity and sulfate on virus removal. We also evaluated three PACls (PACl-2.1b, PACl-2.1c, and PACl-2.7, where "b" and "c" indicate high Al_b and Al_c content, as measured by a ferron method, described below) prepared by a base titration

method in our laboratory, as described previously (Kimura et al., 2013).

Finally, we conducted a third set of experiments with eight aluminum-based coagulants (Table S2), which were provided by Taki Chemical Co. or prepared in our laboratory by the base titration method.

All the laboratory-made PACls (PACl-0.9, PACl-1.5, PACl-2.1b, PACl-2.1c, and PACl-2.7) were nonsulfated, and they are distinguished from the company-made PACls in that "ns" is not included in the name.

All the coagulants were used in batch coagulation experiments immediately after dilution with Milli-Q water.

2.2. Characterization of coagulants

2.2.1. Ferron method

The aluminum hydrolyte species in the coagulants were analyzed by means of a ferron method (Wang et al., 2004) after dilution with Milli-Q water to a concentration of 2.7 g-Al/L, i.e., 0.1 M-Al (analytical pH condition was approximately 4–5). On the basis of the kinetic differences between the reactions of the aluminum species and the ferron reagent (8-hydroxy-7-iodoquinoline-5-sulfonic acid, Wako Pure Chemical Industries), aluminum hydrolyte species were categorized as monomeric species, fast-reacting polymeric species, or slow-reacting colloidal species, denoted as Al_a, Al_b, and Al_c, respectively (Wang et al., 2004). After addition of the ferron reagent to the diluted coagulant, the mixture was immediately stirred magnetically for 10 s at 400 rpm, and then the absorbance at 366 nm was measured with a UV-1700 Pharma Spec spectrophotometer (Shimadzu Corp., Kyoto, Japan) at predetermined reaction times. The aluminum hydrolyte species were operationally divided into the three categories as follows: Al_a, species that reacted with ferron within 30 s; Al_b, species that reacted with ferron within 120 min (absorbance at 120 s minus the absorbance due to Al_a); and Al_c, species that did not react with ferron ($Al_c = Al_t - [Al_a + Al_b]$, where Al_t = total Al). To obtain Al_t, we adjusted the pH of the diluted coagulant to approximately 0.5 with ultrapure nitric acid (Kanto Chemical Co., Tokyo, Japan), heated it for 3 h at 85 °C in a muffle furnace, cooled it to room temperature, and then analyzed it by the ferron method as described for Al_a.

2.2.2. Liquid ^{27}Al nuclear magnetic resonance analysis

In addition to the ferron method, ^{27}Al nuclear magnetic resonance (NMR) spectrometry was also used to characterize the aluminum hydrolyte species in the coagulants after dilution with Milli-Q water to a concentration of 2.7 g-Al/L, i.e., 0.1 M-Al (analytical pH condition was approximately 4–5). On the basis of chemical shift differences, aluminum hydrolyte species were categorized into four groups: monomeric species (Al_m), dimeric and trimeric species, tridecameric species (Al_{13}), and Al_{30} species (Chen et al., 2006, 2007; Gao et al., 2005). After addition of deuterium oxide (75% v/v, Wako Pure Chemical Industries) to the diluted coagulant, the solution was placed in a 5-mm NMR tube. A 3-mm coaxial capillary filled with diluted sodium aluminate (Wako Pure Chemical Industries) solution, which was diluted with Milli-Q water to 0.01 M-Al and then added the deuterium oxide (75% v/v). The coaxial capillary was used as an internal standard for Al content and as the deuterium lock (Chen et al., 2007; Gao et al., 2005). The NMR spectra were measured with a JEOL JNM-ECA 600 spectrometer (JEOL, Tokyo, Japan) by means of a single-pulse method (field strength 14.09 T, resonance frequency 156.39 MHz, pulse width 5.0 μs, repetition time 1.13 s, scans 8000, X-sweep 78.25 kHz). The reference chemical shift (0 ppm) was adjusted with $AlCl_3$ solution prepared by the procedure described above.

2.2.3. Colloid titration analysis

The positive colloid charges of the coagulants were determined by colloid titration with a COM-555 Potentiometric Titrator (Hiranuma Sangyo Co., Mito, Japan). Each coagulant was diluted with Milli-Q water to 1–2 mg-Al/L (analytical pH condition was approximately 4–5), and then 150 mL of diluted coagulant was transferred to a titration vessel. After addition of 0.3 mL of toluidine blue indicator (Wako Pure Chemical Industries) to the vessel, the solution was titrated by means of a pump with 0.001 N potassium polyvinyl sulfate (a standard negative colloid, Wako Pure Chemical Industries) at a constant rate of 10 mL/min. The vessel contents were magnetically stirred during the titration, and the absorbance at 630 nm was recorded continuously until little change in the absorbance (i.e., subtle change in the color of the indicator from light blue to bluish-purple) was observed. The positive colloid charge was determined from the volume of potassium polyvinyl sulfate that corresponded to the half height of the descending slope of the recorded absorbance curve.

2.3. Bacteriophages

F-specific RNA bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (Kisarazu, Japan). Qβ (Boudaud et al., 2012; Matsui et al., 2003; Matsushita et al., 2011; Shirasaki et al., 2009a,b) and MS2 (Boudaud et al., 2012; Fiksdal and Leiknes, 2006; Guo and Hu, 2011; Matsushita et al., 2011; Nasser et al., 1995; Shirasaki et al., 2009a,b; Zhu et al., 2005) are widely used as surrogates for waterborne enteric viruses in coagulation processes because these bacteriophages are morphologically similar to hepatitis A viruses and polioviruses, removal of which during drinking water treatment is important. Qβ is the prototype member of the genus *Allolevivirus* in the virus family Leviviridae, and MS2 is the prototype member of the genus *Levivirus* in the Leviviridae family. The genomes of these two bacteriophages contain a single molecule of linear, positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm (Fauquet et al., 2005). Each bacteriophage was propagated and purified prior to the preparation of a bacteriophage stock solution as described in our previous report (Shirasaki et al., 2010).

2.4. Coagulation experiments with bacteriophage-spiked river water

Batch coagulation experiments were conducted with 1000 mL of bacteriophage-spiked river water in square plastic beakers at 20 °C. The bacteriophage stock solution (see Section 2.3) was added to the river water in a beaker at approximately 10^8

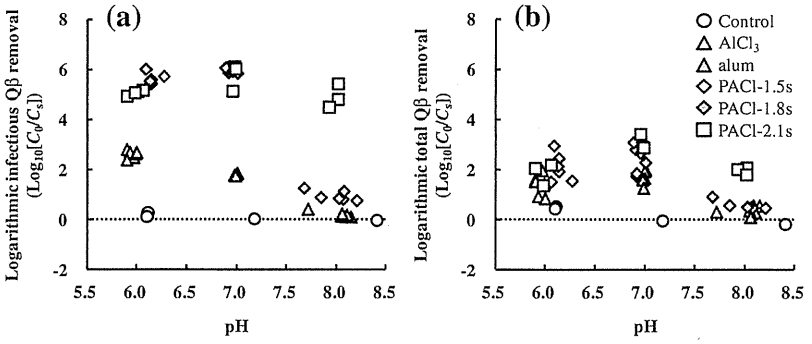

Fig. 1 – Effect of coagulant type on removal of infectious Qβ as evaluated by the PFU method (a) and on total Qβ removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 1, and the coagulant dosage was 2.16 mg-Al/L.

plaque forming unit (PFU)/mL (C_0), and the spiked water was mixed with an impeller stirrer. After enough HCl or NaOH was added to the water to bring the final pH to a target value of at 6, 7, or 8, coagulant was injected into the water at a dosage of 0.54, 1.08, 1.89, 2.16, or 5.4 mg-Al/L. The water was stirred rapidly for 1 min ($G = 200\ s^{-1}$, 136 rpm) and then slowly for 10 min ($G = 20\ s^{-1}$, 29 rpm). The water was left at rest for 60 min to allow the generated aluminum floc particles to settle. Then the supernatant was sampled from the beaker for quantification of the bacteriophage concentrations (C_s) and turbidity. A portion of each supernatant was filtered through a membrane filter (first and second sets of experiments, nominal pore size 0.4 μm, polycarbonate, Isopore, Millipore; third set of experiments, nominal pore size 0.45 μm, polytetrafluoroethylene, Dismic-25HP, Toyo Roshi Kaisha, Tokyo, Japan) for quantification of the ultraviolet absorbance at 260 nm (an indication of natural organic matter [NOM] concentration) and for measurement of the aluminum concentration. Turbidity and UV260-absorbing NOM were quantified with a 2100AN turbidity meter (Hach Company, Loveland, CO, USA) and a UV-1700 Pharma Spec spectrophotometer, respectively. After ultrapure nitric acid (1% v/v, Kanto Chemical Co.) was added to the membrane permeate, the aluminum concentration was determined by means of inductively coupled plasma–mass spectrometry (Agilent 7700 series, Agilent Technologies, Inc., Santa Clara, CA, USA).

2.5. *Bacteriophage assay*

The infectious bacteriophages were quantified by determination of the number of PFUs according to the double-layer method (Adams, 1959) with *Escherichia coli* (NITE Biological Research Center 13965) as the bacterial host. The average of the plaque counts of triplicate plates prepared from one sample was considered as the infectious bacteriophage concentration for that sample.

Bacteriophage RNA was quantified by a real-time reverse transcription-polymerase chain reaction (RT-PCR) method, which detects all bacteriophages regardless of their infectivity and the existence of aggregates. The details of the real-time RT-PCR method are described in Supplementary Information.

3. Results and discussion

3.1. *First set of experiments*

3.1.1. *Effect of coagulant type on bacteriophage removal*

The effect of coagulant type on the infectious Qβ removal ratio ($\log_{10}[C_0/C_s]$) during the coagulation process was evaluated by the PFU method after settling (Fig. 1a). Because Qβ is small and was stably dispersed in the river water (because of electrical repulsion), no removal (<0.3-$\log_{10}$) of infectious Qβ was observed in the absence of coagulant at any pH. In contrast, the coagulation process removed infectious Qβ at a pH range of 6–7 no matter what type of coagulant was used. This result indicates that the Qβ stably monodispersed in the river water was destabilized by the addition of coagulant and became adsorbed on or entrapped in the aluminum floc particles generated during the coagulation process and that the aluminum floc particles along with the destabilized Qβ then settled out from the suspension under the influence of gravity during the settling process. The efficiency of infectious Qβ removal depended on coagulant type: whereas coagulation with $AlCl_3$ and alum resulted in approximately 2-$\log_{10}$ removal at a pH range of 6–7, approximately 6-$\log_{10}$ removal was achieved with all the PACls, regardless of their basicity. Matsushita et al. (2011) also reported that the infectious Qβ removal ratio during the coagulation process with PACl is larger than that with alum at neutral pH. Moreover, we previously reported that PACl is more effective than alum for removing norovirus particles (Shirasaki et al., 2010).

The virus removal performances of $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s markedly decreased when the pH of the treated water was increased from 7 to 8 (Fig. 1a). Hu et al. (2006) reported that the aluminum species distributions of $AlCl_3$ and commercially available PACl during coagulation process were greatly changed depending on the pH: although the aluminum species distributions of those coagulants were almost same in the pH range from 6 to 7, monomeric aluminum species were increased while polymeric and colloidal aluminum species were decreased when the pH of the treated water was increased from 7 to 8. Therefore, difference in the aluminum species distributions of the $AlCl_3$, alum, PACl-1.5s and PACl-

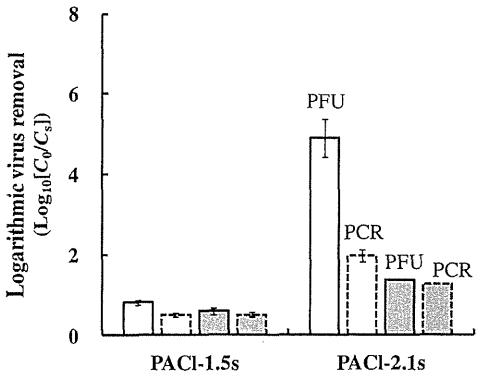

Fig. 2 – Comparison of Qβ (white) and MS2 (gray) removal ratios from treated water at around pH 8 after settling during the coagulation process. The source water was river water 1, and the coagulant dosage was 2.16 mg-Al/L. Values are means (n = 2–3), and the error bars indicate standard deviations.

1.8s probably contribute to the difference in the virus removal performances between pH range from 6 to 7 and pH 8. In contrast, PACl-2.1s retained its high virus removal performance (~5-log_{10} removal) even at weakly alkaline pH. This result indicates that PACl basicity affected virus removal performance during the coagulation process and that a high-basicity PACl (PACl-2.1s) effectively removed the virus not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH. The total Qβ removal ratios evaluated by the PCR method were also observed to be somewhat larger with PACl-2.1s than the ratios with $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s, especially at around pH 8 (Fig. 1b). In addition, the coagulation process with PACl-2.1s removed turbidity and UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s, especially at weakly alkaline pH (Fig. S1).

The Qβ removal ratios determined by the PFU and PCR methods differed markedly: the infectious Qβ removal ratios (Fig. 1a) were larger than the total Qβ removal ratios (Fig. 1b). This difference between the PFU and PCR methods could be explained by the formation of aggregates consisting of several infectious Qβ particles, the inactivation of Qβ during the coagulation process, or both. Matsushita et al. (2011) reported that Qβ loses its infectivity after being mixed with aluminum hydrolyte species during the coagulation process with PACl-1.5s, as indicated by a combination of filtration and particle size measurements at neutral pH. This result suggests that the virucidal activity of the aluminum-based coagulants contributed to the efficiency of infectious Qβ removal during the coagulation process.

3.1.2. *Comparison of Qβ and MS2 removal ratios during the coagulation process*

As described above, PACl-2.1s removed Qβ more efficiently than did other aluminum-based coagulants used in the present study, especially at weakly alkaline pH. To confirm that PACl-2.1s actually removed viruses more effectively than PACl-1.5s, we also evaluated the MS2 removal ratio, because MS2 is less sensitive than Qβ to the virucidal activity of PACl (Matsushita et al., 2011; Shirasaki et al., 2009a). We evaluated the MS2 removal efficiency by means of the PFU and PCR methods after settling during the coagulation process, and then compared the results with those for Qβ (Fig. 2). For both bacteriophages, the removal ratios observed with PACl-2.1s were larger than those with PACl-1.5s at around pH 8. This result means that compared to coagulation with PACl-1.5s, coagulation with PACl-2.1s more effectively removed not only a virus that is highly sensitive to the virucidal activity of the aluminum-based coagulants but also a virus that is less sensitive.

The infectious Qβ removal ratio of PACl-2.1s was approximately 3-log_{10} larger than the infectious MS2 removal ratio, partly because of the different sensitivities of Qβ and MS2 to the virucidal activity of PACl-2.1s. Because Qβ is more sensitive than MS2, the infectious Qβ concentration after settling during the coagulation process may have been less than the quantification limit of the PFU method when the other high-basicity PACl was applied. Therefore, we used MS2 in our second and third sets of experiments.

3.2. *Second set of experiments*

3.2.1. *Effects of coagulant basicity and sulfate content on bacteriophage removal*

To further investigate the effective virus removal observed with PACl-2.1s, we conducted batch coagulation experiments

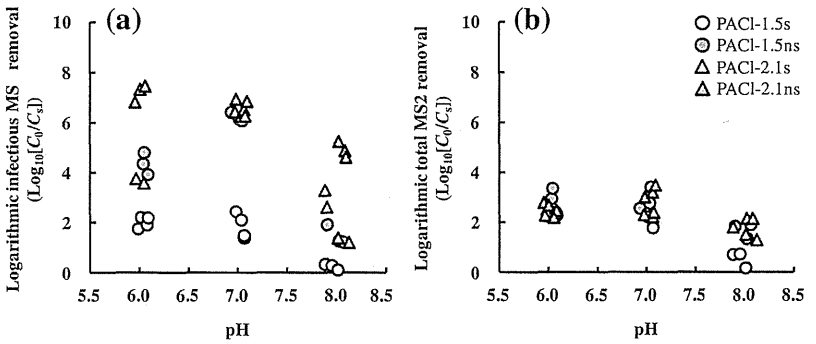

Fig. 3 – Effects of coagulant basicity and sulfate content on infectious MS2 removal as evaluated by the PFU method (a) and on total MS2 removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L.

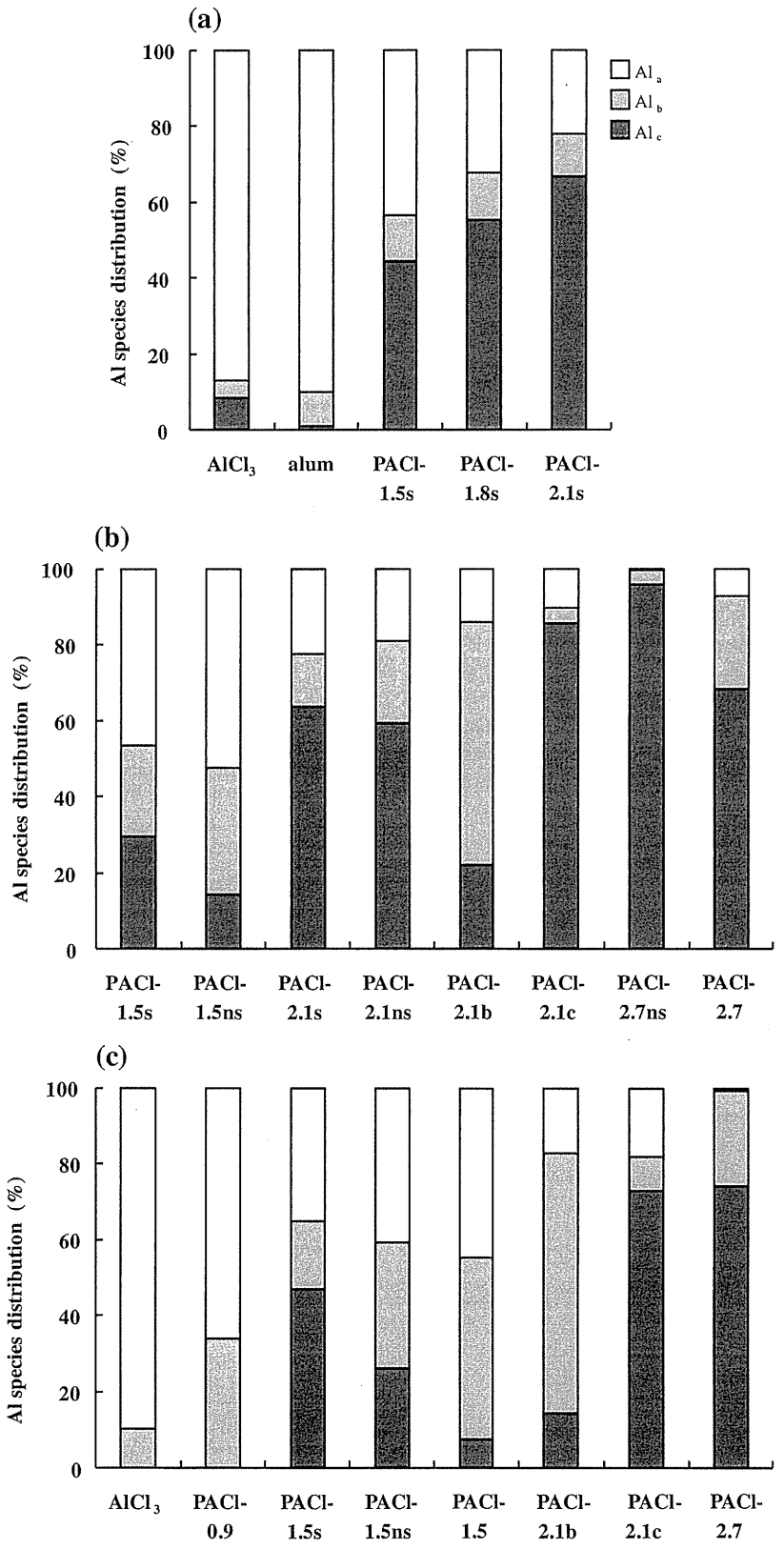

Fig. 4 – Distribution of aluminum species in the coagulants used in the first (a), second (b), and third (c) sets of experiments, as evaluated by the ferron method.

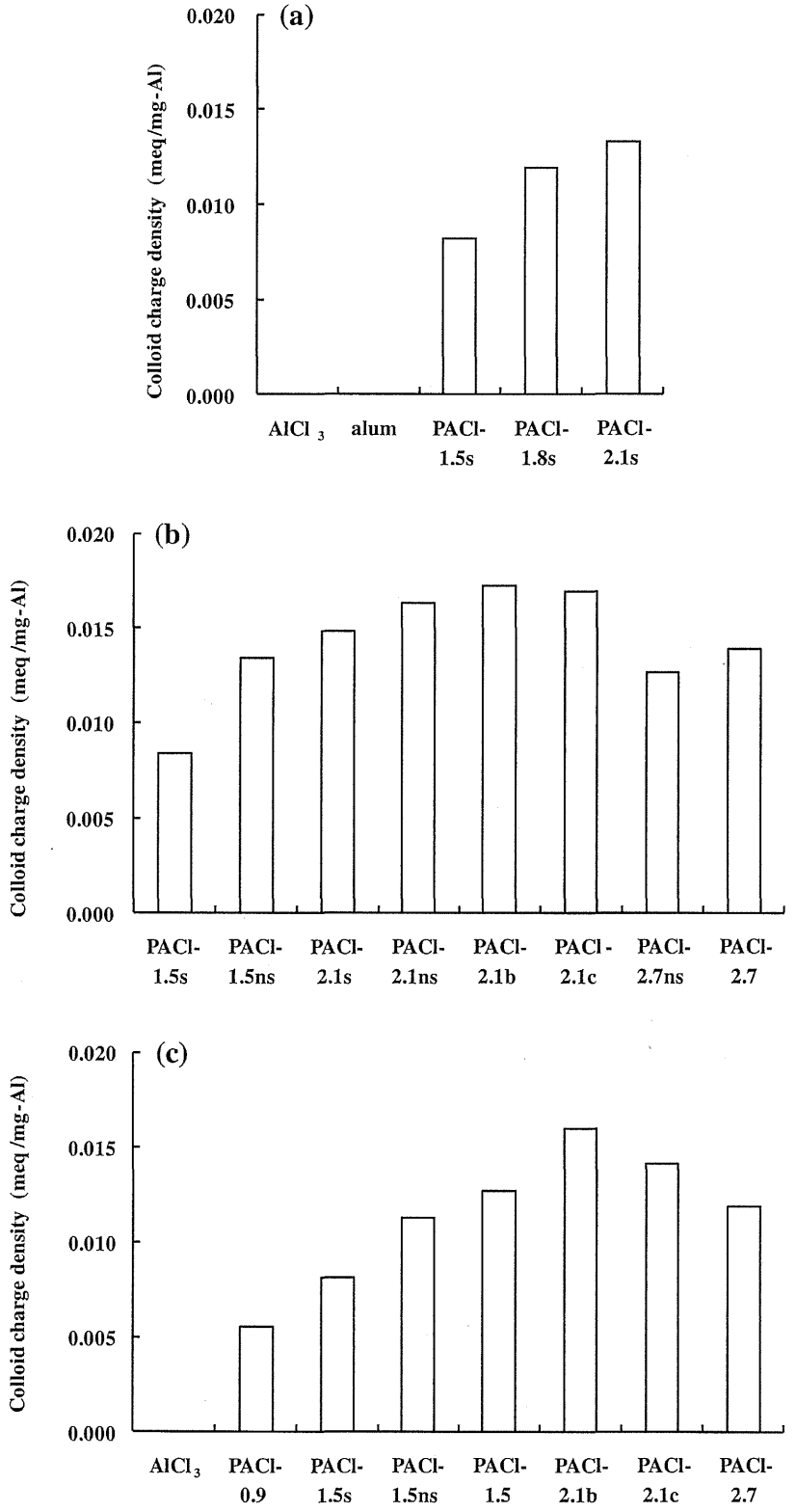

Fig. 5 — Colloid charges densities of the coagulants used in the first (a), second (b), and third (c) sets of experiments, as evaluated by a colloid titration technique.

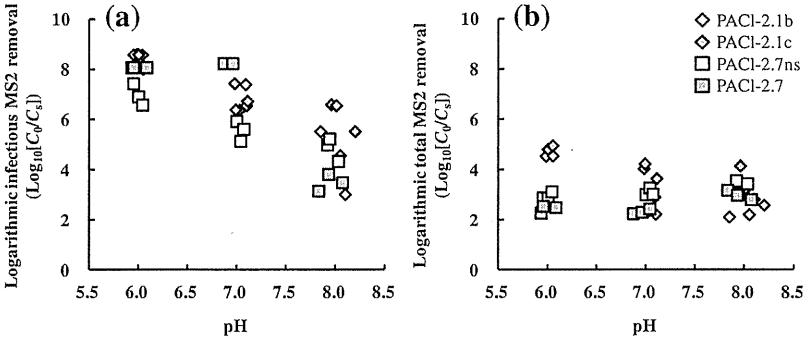

Fig. 6 – Effect of the aluminum hydrolyte species in the coagulants on infectious MS2 removal as evaluated by the PFU method (a) and on total MS2 removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L.

with various sulfated and nonsulfated PACls and evaluated the infectious MS2 removal ratios by means of the PFU method after settling (Fig. 3a). Although no removal of infectious MS2 was observed in the absence of coagulant at any pH, as was the case for Qβ (data not shown), the coagulation process with PACl did remove infectious MS2, and the removal efficiency increased with increasing PACl basicity under all pH conditions. In addition, nonsulfated PACls removed infectious MS2 more efficiently than did sulfated PACls, regardless of their basicity: the infectious MS2 removal ratios during the coagulation process with PACl-1.5ns and PACl-2.1ns were approximately 1–4-log_{10} larger than the ratios with PACl-1.5s and PACl-2.1s, although the removal ratios observed with PACl-2.1s and PACl-2.1ns were almost same at around pH 7. The total MS2 removal ratios evaluated by the PCR method were also observed to be somewhat larger with nonsulfated PACls than the ratios with sulfated PACls, especially at around pH 8 (Fig. 3b). These results indicate that the sulfate in the PACls affected virus removal performance and that a nonsulfated high-basicity PACl (PACl-2.1ns) removed the virus more effectively than PACl-1.5s, PACl-1.5ns, and PACl-2.1s, not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH.

To determine why PACl-2.1ns effectively removed viruses, we used the ferron method to investigate the distribution of aluminum species in the coagulants (Fig. 4). Whereas the major aluminum species in $AlCl_3$ and alum was monomeric aluminum species (Al_a), colloidal aluminum species (Al_c) were present in high proportions in the PACls (Fig. 4a). In addition, the Al_c content in the PACls increased and the Al_a content decreased with increasing basicity, whereas the content of polymeric aluminum species (Al_b) remained almost constant (Fig. 4a). The Al_{13} species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$ is generally believed to be the most effective aluminum species for coagulation processes, because of its strong charge neutralization capability and structural stability (Chen et al., 2006); and the amount of Al_{13} species in a coagulant is almost equivalent to the amount of Al_b measured by the ferron method (Chen et al., 2007). In the present study, the virus removal performances of PACl-1.5s, PACl-1.5ns, PACl-2.1s, and PACl-2.1ns differed markedly, especially at weakly alkaline pH, even though their Al_b contents were not substantially different (Fig. 4b). Therefore, Al_b, including Al_{13} species, may not have been the dominant species responsible for controlling virus removal performance during the coagulation process.

The Al_{30} species $[Al_{30}O_8(OH)_{56}(H_2O)_{24}]^{18+}$ is known to be an effective aluminum species for coagulation processes, and some researchers have demonstrated that PACls with a high Al_{30} content remove more turbidity and more humic acid than PACls with a high Al_{13} content (Chen et al., 2006; Zhang et al., 2008). Because Al_{30} species do not react with the ferron reagent within 120 min, they are categorized as Al_c by the ferron method (Chen et al., 2007). We found that PACl-2.1s and PACl-2.1ns had higher Al_c contents and lower Al_a contents than $AlCl_3$, alum, PACl-1.5s, PACl-1.5ns, and PACl-1.8s (Fig. 4a,b). Therefore, Al_c, including Al_{30} species, may have been the dominant species controlling virus removal performance during the coagulation process. Our investigation of the effects of the Al_b and Al_c contents in the coagulants on virus removal is discussed in Section 3.2.2.

We observed no large differences between the distributions of aluminum species in the sulfated and nonsulfated PACls. These results suggest that PACl basicity affected aluminum species distributions but that the presence of sulfate in the PACls did not.

We also determined the positive colloid charge densities of the coagulants by using a colloid titration technique (Fig. 5). The colloid charge densities of $AlCl_3$ and alum were very small and almost zero; those of the PACls increased with increasing basicity, and PACl-2.1s and PACl-2.1ns showed higher colloid charge densities than $AlCl_3$, alum, PACl-1.5s, PACl-1.5ns, and PACl-1.8s (Fig. 5a,b). In addition, the colloid charge densities of the nonsulfated PACls were higher than those of the sulfated PACls. Wang et al. (2002) reported that the presence of sulfate during the coagulation process reduces the charge neutralization capability of coagulants; this reduction is due to the moderate interaction of sulfate with aluminum hydrolyte species and aluminum hydroxide. Nevertheless, sulfate is often added to aluminum-based coagulants to broaden the pH range of optimum destabilization (i.e., acceleration of floc formation) to the acidic side (Hanna and Rubin, 1970). Therefore, the high Al_c content and the absence of sulfate in PACl-2.1ns probably led to the increased colloid charge density, which gave this coagulant its high

capability to neutralize the negative charge on the viruses during the coagulation process.

3.2.2. *Effect of aluminum species in coagulants on bacteriophage removal*

To investigate the effect of the nature of the aluminum species in the coagulants on virus removal, we compared the MS2 removal efficiencies of nonsulfated high-basicity PACl-2.1b and PACl-2.1c, whose predominant aluminum hydrolyte species are Al_b and Al_c, respectively. PACl-2.1b and PACl-2.1c removed infectious MS2 at a pH range of 6–7 with nearly identical removal efficiencies (~6–7-log_{10} removal), as evaluated by means of the PFU method (Fig. 6a). In contrast, at around pH 8, the infectious MS2 removal ratio observed with PACl-2.1c was approximately 2-log_{10} larger than that with PACl-2.1b. The total MS2 removal ratios evaluated by means of the PCR method were also observed to be somewhat larger with PACl-2.1c than the ratios with PACl-2.1b at around pH 7 and 8 (Fig. 6b). These results indicate that the distribution of aluminum species in the PACls affected virus removal performance during the coagulation process and that Al_c-dominant PACl (i.e., PACl-2.1c) was particularly effective at removing the virus at weakly alkaline pH. Our hypothesis that coagulants with high Al_c content effectively removed viruses during the coagulation process is supported by these results.

To identify the aluminum hydrolyte species in PACl-2.1b and PACl-2.1c, we analyzed the coagulants by ^{27}Al NMR in addition to the ferron method. In the ^{27}Al NMR spectra of all the coagulants, two or three signals were observed (Fig. 7): the signals at 0, 63, and 80 ppm were attributed to monomeric species (Al_m), the central tetrahedral Al in Al_{13} species, and the internal standard (that is, to the formation of $[Al(OH)_4]^-$), respectively (Chen et al., 2006, 2007; Gao et al., 2005; Liu et al., 2009). Whereas no signal or only a weak signal for Al_{13} species was confirmed in the spectra of $AlCl_3$ and PACl-1.5s, a strong signal for this species was observed in the spectrum of PACl-2.1b (Fig. 7). Because the MS2 removal ratios observed with PACl-2.1b were markedly larger than those with PACl-1.5s (Figs. 3 and 6), we suggest that Al_{13} species in PACl are among the important species controlling virus removal performance during the coagulation process. Although a signal for Al_{13} species was also observed for PACl-2.1c, the intensity of the signal was lower than that for PACl-2.1b. This result suggests that the Al_{13} content in PACl-2.1b was higher than that in PACl-2.1c (Fig. 7). This observation is in accord with the results obtained by the ferron method, which indicate that the predominant aluminum hydrolyte species in PACl-2.1b was Al_b and that the Al_{13} species in coagulant are almost equivalent to that of Al_b, as described above.

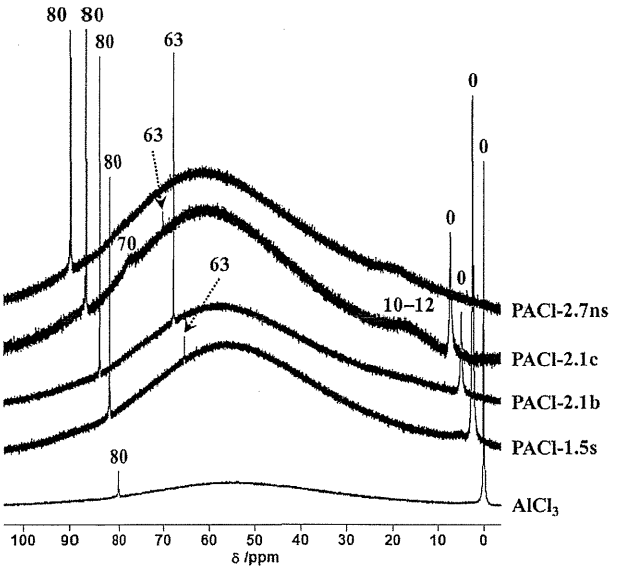

Fig. 7 – ^{27}Al NMR spectra of coagulants used in the second set of experiments.

In addition to the signals at 0, 63, and 80 ppm, the spectrum of PACl-2.1c showed broad signals at 10–12 and 70 ppm, which were attributed to the octahedral Al of external shells in Al_{13} and Al_{30} species and the central tetrahedral Al in Al_{30} species, respectively (Chen et al., 2007). This result indicates that PACl-2.1c contained not only Al_{13} species but also Al_{30} species, which was not the case for PACl-2.1b. In addition, Al_{30} species played the major role in virus removal, as indicated by the fact that the efficiency of MS2 removal with PACl-2.1c was somewhat larger than that with PACl-2.1b (Fig. 6), even though the Al_{13} content in PACl-2.1c was smaller than that in PACl-2.1b (Fig. 7). Although the Al_m (Al_a) species contributed to virus removal—as indicated by the fact that high-Al_m-content coagulants ($AlCl_3$ and PACl-1.5s) removed some virus at a pH range of 6–7, probably because of the formation of Al_{13} (Al_b) species in situ (Hu et al., 2006; Yan et al., 2008a)—conversion of Al_m species in the coagulant to Al_{13} species and further transformation of Al_{13} species into Al_{30} species effectively improved virus removal performance during the coagulation process.

We further investigated the effect of the aluminum species in the coagulants on virus removal by evaluating two extremely high basicity PACls (i.e., PACl-2.7ns and PACl-2.7). The efficiencies of infectious MS2 removal at a pH range of 6–7, as evaluated by the PFU method after settling, were 6–8-log_{10} (Fig. 6a). These removal ratios were similar to the ratio obtained with PACl-2.1c. In contrast, the ratios observed with PACl-2.7ns and PACl-2.7 were approximately 1–3-log_{10} smaller than the ratio with PACl-2.1c at around pH 8. The total MS2 removal efficiencies, as evaluated by the PCR method, during the coagulation process with extremely high basicity PACls were also similar to or somewhat smaller than the ratio observed with PACl-2.1c (Fig. 6b). The effective removal of turbidity and UV260-absorbing NOM, and the very low residual aluminum concentration, observed in the coagulation process with PACl-2.7ns and PACl-2.7 were attained not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH compared with other aluminum-based coagulants used in the present study, including PACl-2.1c (Fig. S2). However, increasing the PACl basicity from 2.1 to 2.7 was not effective for virus removal, even though the Al_c content in PACl-2.7ns was larger than that in PACl-2.1c (Fig. 4b). Moreover, we observed a reduction of the colloid charge densities of the PACls when the basicity was increased from 2.1 to 2.7 (Fig. 5b,c), and no signal for Al_{30} species was observed in the ^{27}Al NMR spectrum of PACl-2.7ns (Fig. 7). The reason why the colloid charge densities of the coagulants were reduced by the increase in basicity is not clear, but these

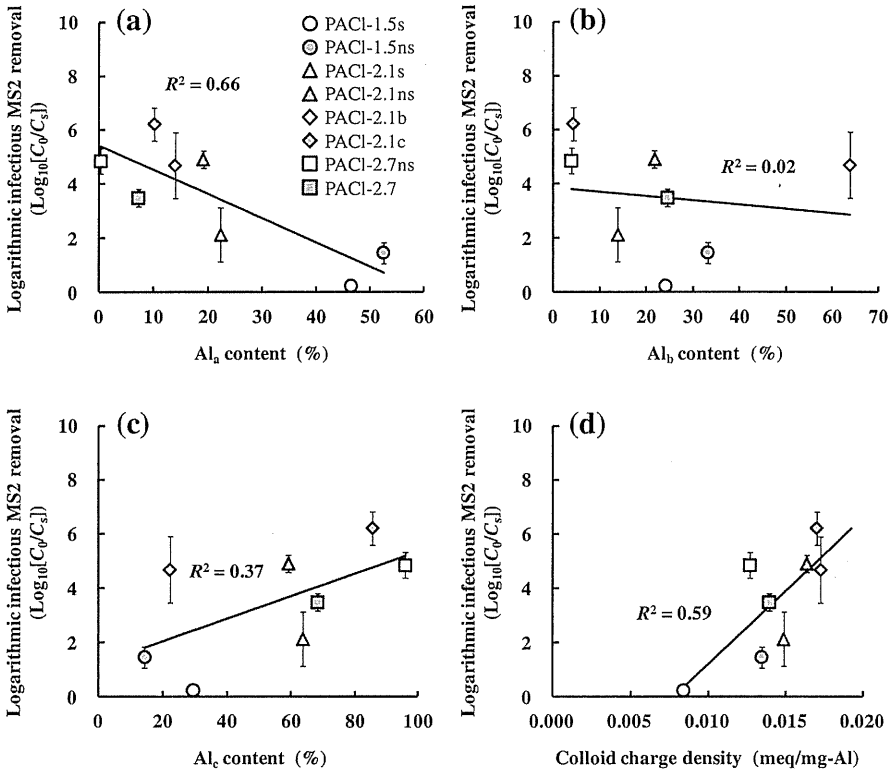

Fig. 8 – Relationship between infectious MS2 removal ratios and Ala (a), Alb (b), Alc (c), and colloid charge density (d). The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L. The pH of the treated water was approximately 8. Values are means (n = 3–4), and the error bars indicate standard deviations.

results suggest that virus removal efficiency during the coagulation process with PACls was not determined simply by the amount of Al_c in the coagulants.

3.2.3. *Relationship between bacteriophage removal, aluminum species and colloid charge density*

In our previous study, we found that the amount of Al_a in PACls, rather than their basicity, was a better indicator to use for minimizing residual aluminum concentration after settling at weakly alkaline pH (Kimura et al., 2013). To investigate whether the Al_a, Al_b, or Al_c content or the colloid charge density of the coagulants could be used as an indicator for the effectiveness of virus removal during the coagulation process, we determined the relationships between the MS2 removal ratio at around pH 8 and the Al_a, Al_b, and Al_c contents and the colloid charge density (Fig. 8). There was no correlation between the efficiency of infectious MS2 removal and the Al_b and Al_c contents; whereas the Al_a content, that is, [100% – (Al_b + Al_c)], and the colloid charge density were weakly correlated with the infectious MS2 removal ratios

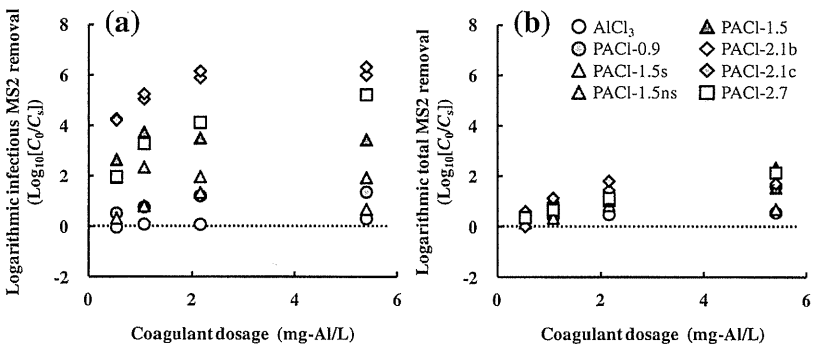

Fig. 9 – Effect of coagulant dosage on infectious MS2 removal as evaluated by the PFU method (a) and on total Qβ removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 3, and the pH of the treated water was approximately 8.