Monitoring free chlorine and free bromine in aquarium seawater treated by ozone

Zhimin Qiang,^{*a} Yanjun Jiang,^a Weiwei Ben,^a Craig Adams^b and Huiyu Dong^a

Received 25th July 2012, Accepted 5th September 2012

DOI: 10.1039/c2ay25806d

Free chlorine (FC) and free bromine (FB) produced during ozonation treatment of aquarium seawater pose a serious threat to the health of ornamental aquatic animals, but little is known about the concentration levels of these chemicals in aquarium facilities. In this study, a highly selective and sensitive analytical method based on the derivatization of FC and FB to corresponding 4-halo-2,6-dimethylphenols by 2,6-dimethylphenol was developed. The formed halo-derivatives were extracted by hexane and subsequently quantified by gas chromatography-mass spectrometry. The calibration curves showed good linearity ($r^2 > 0.999$) in a broad concentration range of 2.1–4200 $\mu\text{g L}^{-1}$ for FC and 3.3–1700 $\mu\text{g L}^{-1}$ for FB. The method detection limits ($S/N = 3$) were determined to be 62.7 and 71.6 ng L^{-1} for FC and FB, respectively. The developed method exhibited good recovery, selectivity, precision and was successfully applied to monitor the formation and decay of FC and FB in ozonated seawater. During the ozonation process, the formation of FB dominated, while much less chloride was oxidized to FC; FB decayed much faster than FC. The concentrations of FC and FB in various animal tanks of Beijing Aquarium were also monitored. The results show that 30.1–56.4 $\mu\text{g L}^{-1}$ FC and 26.5–730.7 $\mu\text{g L}^{-1}$ FB were present in aquarium tanks, revealing that the health of aquatic animals (particularly fish, jellyfish, and coral) was under severe threat.

1. Introduction

Ozone has been commonly applied in closed artificial seawater (ASW) systems such as aquarium and mariculture facilities to inactivate bacteria, decompose dissolved organic matter and control algae, color, odor and taste.¹ The ASW has a high salinity of about 3.5%, containing approximately 19 g L^{-1} chloride and 67 mg L^{-1} bromide.² During the ozonation process, chloride and bromide can be readily oxidized to free chlorine (FC, HOCl/OCl^-), free bromine (FB, HOBr/OBr^-) and bromate ions (BrO_3^-) by ozone.^{3,4} Hypohalites in seawater not only bring about respiratory and osmoregulatory dysfunctions in fish,⁵ but also react with other inorganic or organic materials to produce harmful byproducts (e.g., trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetonitriles (HANs)).⁶ Investigation of the formation and decay of FC and FB during seawater ozonation process could indicate the toxicity variation related to these species in seawater. Thus, it is imperative to determine the concentrations and stabilities of FC and FB in ozonated aquarium seawater to assess relevant risks.

Various analytical methods utilizing spectrophotometry,^{7–9} potentiometry,^{10,11} amperometry,¹² resonance scattering,¹³ electrochemical,¹⁴ and chemiluminescent¹⁵ techniques have been developed to determine halogen species in aqueous samples. However, these methods are mostly based on the redox properties of target analytes and thus cannot differentiate FC from FB because of the similar properties of the two oxidants. Besides, previous research regarding the formation and decay of FC and FB were either conducted in separate synthetic systems,¹⁶ or expressed by their total concentration^{17,18} due to a lack of suitable detection methods. Conversion of halogen species into organic derivatives by appropriate derivatization agents provides one way to resolve this problem, and pre-column derivatization followed by chromatographic quantification has been used to detect chlorine,^{19,20} bromate and bromide,^{21,22} iodine, iodate and iodide^{21,23} in various matrixes. One method adopting pre-column derivatization and liquid chromatography (LC) quantification has recently been established to simultaneously analyze FC and FB in ballast water.²⁴ However, no study has addressed the concentration levels of FC and FB in aquarium seawater so far. Moreover, the high-salinity of seawater may quickly clog the pipelines and the LC column, which renders LC unfeasible for long-term operations. Gas chromatography-mass spectrometry (GC-MS) detection, with organic extraction prior to chromatographic analysis, tends to be an effective solution since the salts stay in the aqueous phase while the target analytes are transferred to the organic phase.

^aState Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: qiangz@rcees.ac.cn; Fax: +86 10 628 496 59; Tel: +86 10 628 496 32

^bDepartment of Civil and Environmental Engineering, Utah State University, Logan, UT 84322, USA

In this work, a highly selective and sensitive analytical method based on the derivatization of FC and FB to 4-halo-2,6-dimethylphenols (4-halo-2,6-DMPs) was successfully developed, with the derivatization, organic extraction and GC-MS instrumental parameters all optimized. The linearity of calibration curves, method detection limit (MDL), and method recovery and precision were systematically assessed. Thereafter, the developed method was applied to monitor the formation and decay of FC and FB in ozonated seawater as well as the concentration levels of FC and FB in various animal tanks of the Beijing Aquarium.

2. Experimental

2.1 Chemicals and standard solutions

The chemicals 2,6-dimethylphenol (2,6-DMP), 4-chloro-2,6-dimethylphenol (4-Cl-2,6-DMP), 4-bromo-2,6-dimethylphenol (4-Br-2,6-DMP), and 2,4,6-trichlorophenol (2,4,6-TCP) were obtained from Alfa Aesar (Haverhill, MA, USA). Cyclohexane and hexane (HPLC grade) were purchased from Fisher Scientific (Houston, TX, USA). Guaranteed reagent grade potassium bromide and sodium chloride, and other chemicals of at least analytical grade were supplied by Beijing Chemical Reagents Company (Beijing, China). All aqueous solutions were prepared with ultrapure water produced by a Milli-Q system (Advantage A10, Millipore, Billerica, MA).

The stock solution of hypochlorite was prepared by diluting 0.5 mL of analytical grade sodium hypochlorite solution (containing >10% available chlorine) to 50 mL with ultrapure water, and then its concentration was standardized by spectrophotometry (Hach 8021 DPD Method). This solution was sequentially diluted to a series of concentrations to establish the calibration curve of hypochlorite.

The stock solution of hypobromite was prepared by adding a desired amount of ozone solution (*ca.* 15 mg L⁻¹) to a potassium bromide (KBr) solution at pH 4.0 (10 mM phosphate buffer) to reach a molar ratio of bromide to ozone of 4 : 5.⁹ After reacting for 24 h, the residual ozone was removed by nitrogen gas purging. The hypobromite solution (*ca.* 18 mg L⁻¹ as Br₂) was standardized by direct photometric determination of the hypobromite (OBr⁻) concentration at 329 nm ($\epsilon = 332 \text{ mol}^{-1} \text{ L cm}^{-1}$) after adjusting the solution pH to 11.0 by NaOH.²⁵ The prepared solution could maintain stable for several days when stored at 4 °C.

A prepared solution of 2,6-DMP (5% in methanol) was used as derivatization agent. The stock solution of internal standard (IS) was prepared by dissolving 50 mg of 2,4,6-TCP in 50 mL of methanol. This solution was further diluted to obtain 5 mg L⁻¹ working solutions.

2.2 Aquarium seawater treatment and characteristics

Beijing Aquarium is a typical closed ASW system with a total water volume of 18 million liters. Fresh aquarium seawater is prepared in a separate tank, with all the major ions of seawater externally added to maintain a salinity of 3.0–3.5% and simulate natural seawater ion ratios. The prepared seawater is disinfected by ozone before being supplied to each tank to make up for the volume loss during treatment and recycling.

Beijing Aquarium mainly adopts a decentralized treatment strategy for each animal tank, which generally includes sand

filtration, foam fractionation, and chemical disinfection. In general, fish (*e.g.*, big fish, small fish) tanks and jellyfish tank use ozone for disinfection, while mammal (*e.g.*, white whale, sea lion, dolphin) tanks use both ozone and chlorine dioxide as disinfectants. Ozone is injected on-line and contacts for about 5 min; chlorine dioxide in mammal tanks is maintained at about 0.04 ppm. Coral tank is only disinfected by ultraviolet (UV) irradiation. Since the fresh aquarium seawater is disinfected by ozone and then supplemented to each tank, FC and FB can also exist in the coral tank. The seawater in each tank is totally recirculated about every 0.5–3 h.

Seawater samples were collected from seven representative tanks of this aquarium, including the big fish (*e.g.*, ray, rockfish), small fish (*e.g.*, clown fish, sea horse), jellyfish, coral, white whale, sea lion and dolphin tanks. The major chemical characteristics of seawater in each tank were measured, as shown in Table 1. The results show that the pH of different tanks varied only in a small range of 8.0–8.3. The TOC, TN, and TP concentrations of mammal tanks were significantly higher than those of fish and jellyfish tanks because mammals have more intake of food and more excretion of feces and urine. The concentrations of free ammonia (NH₃-N) in all tanks were below its limit of detection (LOD, 0.4 mg L⁻¹) due to an oxidizing stress.

Free ammonia (NH₃-N), total nitrogen (TN), total phosphorus (TP) were determined by Hach methods 10031, 10072, and 10127 with a DR5000 spectrophotometer (Hach, Loveland, CO), respectively. The total organic carbon (TOC) was analyzed with a Total Organic Carbon Analyzer (Phoenix 8000, Tekmar-Dohrmann Co., Manson, OH) after filtering the samples through 0.45 µm glass fiber filters.

2.3 Sample analysis

To determine FC and FB concentrations in seawater, an aliquot of sample (9 mL) was added to a 15 mL vial containing 0.25 mL of sulfuric acid (2.0 M), 100 µL of 2,6-DMP (5% in methanol) and 200 µL of 2,4,6-TCP (5 mg L⁻¹). The mixture was vigorously shaken for 1 min on a vortexer (VX-III, Beijing Targin Technology Co., Beijing) to allow derivatization reactions to complete, and then added with 200 µL of hexane and further shaken for 1 min for extraction. The vial was kept undisturbed to separate the organic and aqueous layers, and a portion of the hexane layer was withdrawn for GC-MS analysis. For aquarium seawater samples, the FC and FB were derivatized (*i.e.*, fixed) on site immediately after sampling, and then the samples were transferred to our laboratory, extracted with hexane, and analyzed by GC-MS.

The Agilent GC-MS system consisted of a gas chromatograph (Model 7890A), a quadrupole mass spectrometer (Model 5975C, VL MSD), an auto-sampler (Model 7683B), and an HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium (99.999%) was used as carrier gas at a flow rate of 1 mL min⁻¹. The GC oven temperature was held at 40 °C for 2 min, raised to 300 °C at 40 °C min⁻¹ and maintained for 5 min. The temperatures of the injector and GC-MS transfer line were maintained at 300 and 280 °C, respectively. For each analysis, 1 µL of sample was injected in splitless mode with electron impact ionization (70 eV). The MS was operated in total ion

Table 1 Characteristics of seawater samples collected from seven different tanks of the Beijing Aquarium^a (sampling date: 25 April 2012)

Parameters	Big fish tank	Small fish tank	Jellyfish tank	Coral tank	White whale tank	Sea lion tank	Dolphin tank
pH	8.2	8.0	8.3	8.2	8.3	8.1	8.2
TOC (mg L ⁻¹)	2.8	2.7	2.9	2.0	15.4	18.2	18.4
TN (mg L ⁻¹)	48	18	7.0	3.0	235	250	245
TP (mg L ⁻¹)	1.8	0.9	<LOD ^b	<LOD	10.7	11.8	11.0

^a Concentrations of chloride and bromide were 19.4 g L⁻¹ and 66 mg L⁻¹, respectively. ^b The LOD of TP was 0.3 mg L⁻¹.

chromatogram (TIC) mode from *m/z* 45 to 450 for qualitative analysis and in selected ion monitoring (SIM) mode for quantitative analysis. Quantification and confirmation ion pairs (*m/z*) selected were 156/121 for 4-Cl-2,6-DMP, 196/97 for 2,4,6-TCP (IS), and 200/121 for 4-Br-2,6-DMP.

2.4 Calibration curve and method detection limit

A twelve-point calibration curve was established for FC, and a nine-point calibration curve for FB. The stock solution of hypochlorite or hypobromite was diluted with ultrapure water to a series of concentration levels to prepare the standard samples (10 mL each). After the standard samples were derivatized and extracted as described above, the concentrations of FC and FB were determined by GC-MS.

The calibration curves were established according to the peak area ratios of 4-halo-2,6-DMP to 2,4,6-TCP (IS) at various concentration levels, and the MDL was defined as the analyte concentration corresponding to a signal to noise ratio of 3 : 1. In this study, the concentrations of FC and FB were expressed in terms of the mass concentrations of Cl₂ and Br₂, respectively.

2.5 Method recovery and precision

ASW was prepared in laboratory according to Kester *et al.*² To evaluate the method recovery and precision, both ASW and aquarium seawater samples (collected from Beijing Aquarium) were pre-ozonated for about 15 min in the laboratory to fully oxidize potential reducing materials. Thereafter, FC and FB with three concentration levels were spiked inside, and for each concentration level, five replicate samples were analyzed with the relative standard deviations (RSD) calculated to assess the intra-day precision. In addition, the inward concentrations of FC and FB right after pre-ozonation treatment were measured and then subtracted from the total concentrations in the spiked samples to determine the method recovery. The inter-day precision was assessed by repeating the recovery experiments (in pre-ozonated ASW spiked with certain concentrations of FC and FB) once every other day for six continuous days.

3. Results and discussion

3.1 Derivatization chemistry and method optimization

A desirable halogen scavenger should easily utilize halogens and form only one isomer for each halo-derivative that can be readily extracted and sensitively detected. The chemical 2,6-DMP was selected because it has only the *para*-position available for electrophilic substitution when present in excess, and no

polyhalogenated products are formed. Furthermore, the weakly electron-donating methyl groups of 2,6-DMP can enhance the electrophilic substitution, and the halo-derivatives formed have relatively high extractability from an acid medium.^{20,22,26} Under acidic conditions, FC and FB can undergo derivatization reaction with 2,6-DMP according to eqn (1), in which one mole of the halogen species is proportionally converted to one mole of 4-halo-2,6-DMP,

where X represents either Cl or Br.

The suitable derivatization time was examined by detection of target derivatives, 4-halo-2,6-DMPs, at reaction times of 1, 3, 5, and 7 min, respectively. Results show that FC and FB could undergo rapid electrophilic substitution reactions with 2,6-DMP and 1 min was sufficient for complete derivatization at ambient temperature. The temperature program and other operational parameters of GC-MS were optimized as described in Section 2.3. Fig. 1 shows the TIC and mass spectra of the halo-derivatives and 2,4,6-TCP (*i.e.*, IS). The peaks of the derivatized FC and FB were confirmed by comparison of the retention times and mass spectra with those of authentic standards (*i.e.*, 4-Cl-2,6-DMP and 4-Br-2,6-DMP). No side-chain halogenated products were formed and an adequate separation of the analytes was achieved; the mass spectra contained the molecular ions as base peaks (the highest peak in a mass spectrum) along with the diagnostic fragment ions. The fragment ions of *m/z* 156 and 200, which represent the molecular ions of 4-Cl-2,6-DMP and 4-Br-2,6-DMP, respectively, were selected as the quantification ions. The fragment ion of *m/z* 121, which results from the cleavage of Cl or Br from the 4-halo-2,6-DMPs, was selected as the confirmation ion. The fragment ions of *m/z* 196 (molecular ion) and 97 were selected as the quantification and confirmation ions for 2,4,6-TCP, respectively.

The optimum pH for derivatization was also tested. Fig. 2a compares the SIM chromatograms of 4-halo-2,6-DMP derivatives produced at different pH values (*i.e.*, pH = 1.0, 3.0, 5.0, and 7.0). The strongest signals of both 4-Cl-2,6-DMP and 4-Br-2,6-DMP appeared at pH 1.0, so this pH was considered as optimal for derivatization. It was reported that HOCl could be converted to more reactive chlorinating agents (*e.g.*, ClO–Cl, Cl–Cl, and possibly H₂OCl⁺) in the presence of acid.²⁷ Thus, a low pH favors the formation of target halogenated derivatives.

The extraction procedure was optimized by comparing the extraction efficiencies of different extractant types (hexane, cyclohexane), extractant volumes (200, 600, 1000 μL), and extraction times (1, 3, 5, 7 min). Fig. 2b shows that hexane

Fig. 1 Total ion chromatograms (TIC) of 0.46 mg L⁻¹ FC derivatized to 4-Cl-2,6-DMP (RT = 6.37 min), 0.52 mg L⁻¹ FB derivatized to 4-Br-2,6-DMP (RT = 6.72 min), and 0.50 mg L⁻¹ 2,4,6-TCP (RT = 6.52 min) used as internal standard (a), and the mass spectra of chromatographic peaks of 4-Cl-2,6-DMP (b), 2,4,6-TCP (c), and 4-Br-2,6-DMP (d).

yielded higher extraction efficiencies for all the three analytes than cyclohexane. At an initial concentration of 5 mg L⁻¹, the extraction recoveries of 4-Cl-2,6-DMP, 2,4,6-TCP and 4-Br-2,6-DMP with 200 μ L of hexane reached 74.2%, 86.7% and 85.9%, respectively. Our additional experiments reveal that 200 μ L was a suitable extractant volume on the basis of comprehensive consideration of extraction recovery, detection sensitivity, and extractant consumption. The extraction was completed quickly with vortex shaking for 1 min (data not shown). Thus, the optimized procedure was extraction with 200 μ L of hexane by vortex shaking for 1 min.

3.2 Method validation

Rectilinear calibration curves were established for 2.1–4200 μ g L⁻¹ FC (at 12 concentration levels) and 3.3–1700 μ g L⁻¹ FB (at 9

Fig. 2 Selected ion monitoring (SIM) chromatograms of 1.05 mg L⁻¹ FC and 0.48 mg L⁻¹ FB derivatized at different pHs (a), and 0.5 mg L⁻¹ 4-Cl-2,6-DMP, 2,4,6-TCP and 4-Br-2,6-DMP extracted by 200 μ L of hexane or cyclohexane (b).

concentration levels) in ultrapure water. The calibration data and MDLs ($S/N = 3$) are given in Table 2. The correlation coefficients (r^2) of linear regression were above 0.999, indicating good linearity of the two calibration curves. The MDL was determined to be 62.7 ng L⁻¹ for FC and 71.6 ng L⁻¹ for FB, which are sensitive enough for detecting FC and FB in aquarium facilities.

The method recovery and intra-day precision were measured in ozonated ASW and aquarium seawater samples (collected from the big fish tank and the sea lion tank of Beijing Aquarium) at three spiked concentration levels. Table 3 shows that the recoveries of FC ranged from 96.5% to 104.5% (RSD = 0.7–4.8%), and those of FB ranged from 96.9% to 104.4% (RSD = 0.1–5.2%). The inter-day precision was evaluated in ozonated ASW and the RSDs for spiked FC (22.8 μ g L⁻¹) and FB (39.2 μ g L⁻¹) were determined to be 4.9% and 2.7%, respectively. The results demonstrate that the established

Table 2 Calibration data and method detection limits (MDLs) for FC and FB in ultrapure water

Analytes	Linear range (μ g L ⁻¹)	Slope	y-Intercept	r^2	MDL (ng L ⁻¹)
FC	2.1–4200	80.34	32.59	0.9996	62.7
FB	3.3–1700	48.71	10.01	0.9996	71.6

method had a high recovery and precision in seawater samples, thus was applicable to determine the concentrations of FC and FB produced during ozonation treatment of aquarium seawater.

3.3 Formation and decay of FC and FB in ozonated seawater

The experiments below investigated the formation and decay of FC and FB when seawater was ozonated. To simulate the practical operation conditions of Beijing Aquarium, 500 mL each of laboratory-prepared ASW (pH adjusted to 8.0) and aquarium seawater samples (from the big fish tank and sea lion tank) were continuously ozonated for 2.5 min. Timing was started when ozone was introduced into the working solutions, and the aqueous ozone concentration reached about 1.5–2.0 mg L⁻¹ at 2.5 min when the ozone generator was turned off. Derivatization was conducted instantly after samples were taken. Fig. 3 shows that FC and FB were formed rapidly by ozonation within 2.5 min, and thereafter, the build-up of FC and FB continued due to the existence of residual ozone. At about 10 min, FC and FB reached their maximum concentrations and started to decay. At 180 min, the FC in the ASW, sea lion tank and big fish tank samples decayed to 67.1%, 68.1%, and 60.8% of its peak concentrations, respectively; and the FB decayed to 70.8%, 55.9%, and 43.6% of its peak concentrations, respectively. It is seen that without addition of external quenching agents, FC and FB would not decay completely before the start of next ozonation cycle (the seawater recirculates about every 0.5–3 h in Beijing Aquarium). Therefore, FC and FB would increase to a certain concentration level in the aquarium tanks, thus causing harmful effects to aquatic animals. In addition, the molar concentration ratios of FC/(FC + FB) were mostly below 10% throughout the reaction course (data not shown). Therefore, during ozonation treatment of seawater, the formation of FB dominated, while much less chloride was oxidized to FC.

The natural decay of FC and FB in aquarium seawater samples was also examined. The initial concentrations (C_0) of FC and FB were fixed by on-site derivatization immediately after sampling. After the samples were transferred to our laboratory, the concentrations of FC and FB in the samples of the big fish and sea lion tanks at various times (*i.e.*, 3.0, 5.0, 7.0, 9.0, 11.0, and 13.0 h) were analyzed (C_t). Note that 3 h was the sample-transferring time from Beijing Aquarium to our laboratory. The initial concentrations of FC in the samples of the big fish and sea lion tanks were determined to be 41.7 and 56.4 $\mu\text{g L}^{-1}$, respectively;

Fig. 3 Formation and decay of FC and FB in seawater with ozonation treatment. (a) ASW; (b) sea lion tank; (c) big fish tank.

and those of FB were determined to be 252.2 and 730.7 $\mu\text{g L}^{-1}$, correspondingly. The decay (C_t/C_0) of FC and FB as a function of time is shown in Fig. 4. The results show that FC in the samples of the big fish and sea lion tanks decayed by 22.7% and 20.6% at 3 h after sampling, respectively, and those of FB decayed by 72.8% and 44.9%, respectively. It is seen that FB decayed much faster than FC. Afterwards, the concentrations of FC and FB reached nearly steady-state. According to previous research, the decay rate of total residual oxidants (including both FC and FB) are dependent on various variables such as salinity, light condition and organic content, and the half-lives of FC and FB ranged from less than 1 h to more than 12 h.^{17,28} Therefore, to accurately determine the concentrations of FC and FB in aquarium seawater, on-site derivatization with 2,6-DMP is

Table 3 Recovery of FC and FB spiked in ozonated ASW and aquarium seawater samples ($n = 5$)

Samples	Spiked FC ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)	Spiked FB ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)
ASW	28.4	102.5	1.8	18.1	100.6	2.7
	56.8	102.3	1.7	36.1	96.9	0.1
	113.6	103.3	0.7	72.2	100.9	2.1
Big fish tank	25.6	103.9	4.1	18.9	101.0	1.5
	51.2	99.1	2.8	36.8	104.4	3.6
	102.4	102.7	2.1	56.7	102.7	4.1
Sea lion tank	27.8	96.5	4.8	249.1	100.9	1.5
	55.6	104.5	4.2	498.2	99.9	5.2
	111.2	100.5	4.2	747.3	98.1	1.2

Fig. 4 Natural decay of FC and FB concentrations in aquarium seawater samples.

recommended due to their notable decay within the initial hours after sampling.

3.4 Monitoring FC and FB in Beijing Aquarium

The developed method was applied to determine the concentrations of FC and FB in seawater samples collected from the big fish, small fish, jellyfish, coral, white whale, sea lion, and dolphin tanks of Beijing Aquarium. Triplicate samples were analyzed for each tank, and the mean concentrations of FC and FB are listed in Table 4. The results indicate that FC was present in the range of 30.1–56.4 $\mu\text{g L}^{-1}$ and FB in the range of 26.5–730.7 $\mu\text{g L}^{-1}$. As mentioned above, much less chloride was oxidized by ozone than bromide, so there was no significant difference among the FC concentrations in different tanks. Moreover, the concentrations of FC and FB decreased from the mammal tanks (*i.e.*, white whale, sea lion, and dolphin tanks), the big fish tank, to the other three tanks (*i.e.*, small fish, jellyfish, and coral tanks), which coincides with a corresponding decrease of the ozone dose (as informed by the operation staff of Beijing Aquarium). It is noted that the coral tank, disinfected by UV irradiation, had the lowest FC and FB concentrations. The FC and FB in this tank mainly came from the supplementary fresh aquarium seawater, which was disinfected by ozone after preparation and then supplied to each tank. The acute toxicity of chlorine-produced oxidants, measured as 48 h LC_{50} for certain fishes, was reported to be less than 1–46 μM ,^{5,29,30} while bromine-produced oxidants are two to five times more toxic than chlorine analogues.³¹ It is seen that the health of aquatic animals (particularly fish, jellyfish, and coral) was under a severe threat.

Table 4 FC and FB concentrations in various animal tanks of Beijing Aquarium ($n = 3$)

Samples	FC ($\mu\text{g L}^{-1}$)	FB ($\mu\text{g L}^{-1}$)
Big fish tank	41.7 $\pm$ 0.1	252.2 $\pm$ 2.8
Small fish tank	31.3 $\pm$ 0.1	48.4 $\pm$ 0.7
Jellyfish tank	31.7 $\pm$ 0.1	58.4 $\pm$ 0.4
Coral tank	30.1 $\pm$ 0.1	26.5 $\pm$ 0.7
White whale tank	44.6 $\pm$ 0.4	387.3 $\pm$ 8.5
Sea lion tank	56.4 $\pm$ 0.7	730.7 $\pm$ 13.2
Dolphin tank	44.0 $\pm$ 0.4	381.6 $\pm$ 5.3

4. Conclusions

This work developed a highly selective and sensitive analytical method to simultaneously determine the concentrations of FC and FB in aquarium seawater with GC-MS. By use of this method, it was observed that during the seawater ozonation process, the formation of FB dominated, while much less chloride was oxidized to FC; FB decayed much faster than FC. Therefore, to accurately determine the concentrations of FC and FB in aquarium seawater, on-site derivatization of FC and FB was recommended. This method was also applied to monitor the concentrations of FC and FB in various animal tanks of Beijing Aquarium. The existence of considerable amounts of FC and FB raised a severe concern regarding the harmful effects of ozonation treatment of aquarium seawater. The established method can also be applied to determine the mixed or individual FC and FB species in disinfected drinking water that contains a high level of bromide.

Our further research will focus on the development of feasible treatment technologies (*e.g.*, ozone followed by biological activated carbon filtration) to reduce the concentrations of FC and FB in aquarium seawater as well as to minimize the formation of other disinfection byproducts (*e.g.*, THMs, HAAs, and HANs).

Acknowledgements

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (Project no. 50921064), the Ministry of Housing and Urban-Rural Development of China (Project no. 2012ZX07403-002), and the Special Co-construction Project of Beijing Municipal Commission of Education.

References

- 1 A. A. Goncalves and G. A. Gagnon, *Ozone: Sci. Eng.*, 2011, **33**, 345–367.
- 2 D. R. Kester, I. W. Duedall, D. N. Connors and R. M. Pytkowicz, *Limnol. Oceanogr.*, 1967, **12**, 176–179.
- 3 E. A. Creclius, *J. Fish. Res. Board Can.*, 1979, **36**, 1006–1008.
- 4 P. M. Williams, R. J. Baldwin and K. J. Robertson, *Water Res.*, 1978, **12**, 385–388.
- 5 D. P. Middaugh, L. E. Burnett and J. A. Couch, *Estuaries*, 1980, **3**, 132–141.
- 6 H. A. Jenner, C. J. L. Taylor, M. van Donk and M. Khalanski, *Mar. Environ. Res.*, 1997, **43**, 279–293.
- 7 J. G. March, M. Gual and B. M. Simonet, *Talanta*, 2002, **58**, 995–1001.
- 8 L. Moberg and B. Karlberg, *Anal. Chim. Acta*, 2000, **407**, 127–133.
- 9 U. von Gunten, U. Pinkernell, B. Nowack and H. Gallard, *Water Res.*, 2000, **34**, 4343–4350.
- 10 M. Trojanowicz, W. Matuszewski and A. Hulanicki, *Anal. Chim. Acta*, 1982, **136**, 85–92.
- 11 D. Midgley, *Talanta*, 1983, **30**, 547–554.
- 12 W. Matuszewski and M. Trojanowicz, *Anal. Chim. Acta*, 1988, **207**, 59–65.
- 13 C. Y. Kang, D. L. Xi, S. M. Zhou and Z. L. Jiang, *Talanta*, 2006, **68**, 974–978.
- 14 H. Iketake and A. Yamada, *Bunseki Kagaku*, 1999, **48**, 1123–1127.
- 15 J. B. Claver, M. C. V. Miron and L. F. Capitan-Vallvey, *Anal. Chim. Acta*, 2004, **522**, 267–273.
- 16 W. J. Cooper, A. C. Jones, R. F. Whitehead and R. G. Zika, *Environ. Sci. Technol.*, 2007, **41**, 3728–3733.
- 17 J. C. Perrins, W. J. Cooper, J. H. van Leeuwen and R. P. Herwig, *Mar. Pollut. Bull.*, 2006, **52**, 1023–1033.

- 18 G. Grguric, J. H. Trefry and J. J. Keaffaber, *Water Res.*, 1994, **28**, 1087–1094.
- 19 J. M. Cheplen, C. Barrow and E. L. White, *Anal. Chem.*, 1984, **56**, 1194–1196.
- 20 J. Ellis and P. L. Brown, *Anal. Chim. Acta*, 1981, **124**, 431–436.
- 21 K. Reddy-Noone, A. Jain and K. K. Verma, *J. Chromatogr., A*, 2007, **1148**, 145–151.
- 22 S. Mishra, S. Gosain, A. Jain and K. K. Verma, *Anal. Chim. Acta*, 2001, **439**, 115–123.
- 23 S. Mishra, V. Singh, A. Jain and K. K. Verma, *Analyst*, 2000, **125**, 459–464.
- 24 H. S. Shin, *Chromatographia*, 2010, **71**, 647–651.
- 25 R. C. Troy and D. W. Margerum, *Inorg. Chem.*, 1991, **30**, 3538–3543.
- 26 K. K. Verma, A. Jain and A. Verma, *Anal. Chem.*, 1992, **64**, 1484–1489.
- 27 C. G. Swain and D. R. Crist, *J. Am. Chem. Soc.*, 1972, **94**, 3195–3200.
- 28 L. B. Richardson, D. T. Burton, G. R. Helz and J. C. Rhoderick, *Water Res.*, 1981, **15**, 1067–1074.
- 29 A. Soivio, E. Nikunen and H. Tuurala, *Aquat. Toxicol.*, 1988, **13**, 77–87.
- 30 Q. J. Stober and C. H. Hanson, *Trans. Am. Fish. Soc.*, 1974, **103**, 569–576.
- 31 D. J. Fisher, D. T. Burton, L. T. Yonkos, S. D. Turley and G. P. Ziegler, *Water Res.*, 1999, **33**, 760–768.