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Compositions and Constituents of Freshwater Dissolved Organic Matter Isolated by Reverse Osmosis

Yulong Zhang

Wen Huang

Jingdong Mao
Old Dominion University, jmao@odu.edu

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Yulong Zhang [a], Wen Huang [a,b], Yong Ran [a,*], Jingdong Mao [c]

[a] State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529, United States

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ABSTRACT

Dissolved organic matter (DOM) from riverine and lacustrine water was isolated using a reverse osmosis (RO) system. Solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) was used to quantitatively evaluate the compositions and constituents of DOM, which are compared with previous investigations on marine DOM. Results indicated that concentration factor (CF) was a key metric controlling yield and sorption of DOM on the RO system. The sorption was likely non-selective, based on the ^{13}C NMR and δ^{13}C analyses. Carbohydrates and lipids accounted for 25.0–41.5% and 30.2–46.3% of the identifiable DOM, followed by proteins (18.2–19.8%) and lignin (7.17–12.8%). The freshwater DOM contained much higher alkyl and aromatic C but lower alkoxyl and carboxyl C than marine DOM. The structural difference was not completely accounted for by using structure of high molecular weight (HMW) DOM, suggesting a size change involved in transformations of DOM during the transport from rivers to oceans.

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1. Introduction

Dissolved organic matter (DOM) is one of the most important active carbon pools and plays an important role in the global carbon cycle (Amon and Benner, 1996; Hedges and Oades, 1997). DOM significantly influences the bioavailability, transport, and fate of many metals and contaminants, such as iron (Benner, 2011), copper (Ran et al., 2000), tetrachlorobiphenyl (Gunnarsson and Rosenberg, 1996) and endocrine-distributing chemicals (Gong et al., 2009). Therefore, understanding the nature of DOM in aquatic environment is important. Due to the extremely low abundance (ca. 5 mg/L) and complex composition of DOM in natural water (Gjessing et al., 1998), its biogeochemical properties and cycling are poorly constrained (Mopper et al., 2007).

In order to obtain representative DOM with low salt content and little chemical alteration, various approaches to isolation and fractionation have been developed in the past four decades. Among them, reverse osmosis (RO) is the most efficient one, with typical dissolved organic carbon (DOC) recovery of ∼90% (Mopper et al., 2007; Sun et al., 1995). In addition, large amounts of water samples can be rapidly processed, and DOM is not exposed to harsh chemical conditions (Serkiz and Perdue, 1990; Sun et al., 1995).

However, dissolved inorganic components are substantially co-concentrated in the RO process, resulting in high salt in the isolated DOM. To solve this problem, cation exchange resin (CER) and electro-dialysis (ED) have been applied to remove dissolved ions from the DOM retentates. So far, the RO approach combined with CER and/or ED has successfully isolated DOM from ground, riverine and marine water (Koprivnjak et al., 2006; Serkiz and Perdue, 1990; Sun et al., 1995; Vetter et al., 2007).

In the isolation, a fraction of DOM is not recovered via permeation through and sorption onto the RO system. The DOM loss in the former and latter processes commonly account for 1% and 10–20% of total DOC, respectively (Ouellet et al., 2008). Like ultra-filtration (Benner et al., 1992; Guo and Santschi, 2007), the permeate fraction passing through RO membrane (nominal pore size of 15 nm) is probably composed of low molecular weight (LMW) compounds. The sorption has a potential to fractionate DOM and consequently reduce the representativeness of the RO-isolated DOM. Hence, understanding what factors control the sorption during RO isolation is very important. Concentration factor (CF = sample volume/retentate volume) was suspected to be a metric for affecting the recovery of RO systems and possibly DOM sorption (Sun et al., 1995).

^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy has been widely applied to characterize DOM (e.g. Abdulla et al., 2013; Koprivnjak et al., 2009; Mao et al., 2012; Sannigrahi et al., 2005). Most of the previous work has focused on specific fractions

of freshwater DOM, such as dissolved humic substances (XAD resin), and high molecular weight (HMW) DOM (>1000 Da, tangential-flow ultrafiltration) (see references in Perdue and Ritchie, 2003). In contrast, RO-isolated DOM would provide a more representative picture of total DOM due to its high yield. The RO DOM in freshwater has been previously investigated using liquid-state ^{13}C NMR (Maurice et al., 2002). However, the liquid-state ^{13}C NMR spectra appeared to differ significantly from those of solid-state ^{13}C NMR for RO and HMW DOM (e.g. Jaffé et al., 2012; this study), due in part to different solubilities of DOM constituents in solvent (Mopper et al., 2007). Solid-state ^{13}C NMR experiment can analyze samples non-destructively irrespective of solubility, providing representative DOM structural information (Mao et al., 2012; Zhang et al., 2013, 2014). Solid-state ^{13}C NMR spectrum has been extensively used for characterizing DOM from fresh and marine water (Benner et al., 1992; Bianchi et al., 2004; Engelhaupt and Bianchi, 2001; Jaffé et al., 2012; Koprivnjak et al., 2006; Maie et al., 2005; Mao et al., 2012). However, to the best of our knowledge, this technique has not been used for characterizing RO DOM from freshwater. Little information is available on solid-state ^{13}C NMR of RO DOM, which was only investigated in the marine environment (Koprivnjak et al., 2009; Mao et al., 2012).

In the present study, a large range of CFs was adopted for water samples to evaluate its role in DOM sorption and yield on a RO system. ^{13}C NMR spectra were used to provide structural features of freshwater DOM isolated by RO. More importantly, we compared our investigation with previous work regarding marine DOM by using ^{13}C NMR. This investigation could supply new data on the compositions of freshwater DOM as well as the transformation of DOM during transport from rivers to oceans.

2. Materials and methods

2.1. Sample collection and isolation

Eight water samples were collected from November 2008 to July 2010 in two reservoirs (JK and WLH) and four river sites (BL, LHS, SW and YZ) from the Pearl River Delta, Guangdong province, China (Fig. S1). The surface water (0.5–1 m) was pumped into plastic carboys (25 L, HDPE) precleaned with diluted HCl and carried back to the laboratory. Suspended particulate matter in the water samples was removed using continuous flow centrifugation (10,700 g, 25 °C). A 0.45 μm cartridge filter was used subsequently to remove suspended particles completely. The particulate-free raw water was pumped through H$^+$ cation exchange resin (CER, strong acid type, Product catalogue 001×7) and collected in a sample reservoir (~90 L). The water volume in the reservoir decreased as the RO proceeded. The reservoir was periodically replenished with the raw water, and 1 mL of the subsamples were collected at the time of feeding.

A laboratory RO system was used to concentrate DOM from water samples. The RO system was equipped with a commercial RO membrane (TFC-1812-50, Chanitex, Shanghai) and a diaphragm pump (maximum pressure = 135 psi). The RO system produced about 6–7 L/h of permeate, and the permeate was subsampled and then discarded at the time when a plastic carboy (25 L) was filled up. The RO concentration process was detailed by Serkiz and Perdue (1990) and Sun et al. (1995). The DOM retentate was transferred to storage bottles. The RO system was sequentially rinsed with an aliquot of permeate water and 2 L of 0.025 M NaOH. All the fractions produced in the RO process were subsampled for DOC analyses. The subsamples of retentate and alkaline rinsing fractions were neutralized and/or diluted 5–10-fold with MilliQ water. The raw and permeate subsamples were respectively combined for DOC analyses. All the subsamples were poisoned with

NaN$_3$ and stored in 4 °C until DOC analyses. Several method blanks (20 L distilled water) of the RO equipment were run and the final DOC results were calibrated based on the blanks. The pH of the raw and retentate samples was monitored before and after the RO treatment. The raw water generally had higher pH (6–8) than the retentate water (pH = 3–5) did. Before and after processing samples in the RO system, NaOH (0.2%), HCl (1%), detergent (0.1%) and EDTA-2Na (0.8%) were separately used to clean up possible residual contamination.

The DOC yields were calculated by the following equation:

$$\text{Yield} = \frac{V_{con.} \cdot C_{con.}}{V_{raw} \cdot C_{raw}} \times 100\%$$

where V and C are the volumes and DOC concentrations of retentate and raw water, respectively.

2.2. Desalination

Dissolved cation concentrations in the water samples were repeatedly reduced using CER before and during RO process. The application of CER in RO system was previously described (Serkiz and Perdue, 1990; Sun et al., 1995).

At the end of RO process, the retentate fraction was transferred into the sample reservoir of an electro-dialysis (ED) instrument (40 cell pairs of sulfonic and quaternary ammonium membranes). Concentrated NaOH solution was added to the samples to adjust the pH within 9.2–9.6. The ED instrument was set in constant electric current/variable voltage mode. The limiting current and voltage were 5A and 40 V, respectively. A certain volume of tap water was used as ion accepter and was replaced every 30 min with new tap water. When the conductivity of DOM sample decreased to 1 mS/cm, NaCl solution was added to the sample reservoir to avoid the DOM loss (Koprivnjak et al., 2006). The ED process was commonly performed for 2–4 h and was stopped when the final conductivity of DOM sample was 1 mS/cm.

2.3. Instrumental analyses

DOC concentrations were measured using a Shimadzu TOC-VCPH analyzer. Prior to analyses, the subsamples were acidified to pH < 2 with HCl to remove inorganic carbon. A mean value was adopted based on triplicate analyses. Milli-Q water was analyzed every 12th sample to test the accuracy of the measurements. TOC and TN concentrations of the freeze-dried DOM samples were determined by a Vario EL III Elementar (Germany). DOM retentate samples were acidic due to the H$^+$ exchange, indicating that carbonate should be completely eliminated. Therefore, the dried DOM samples were directly analyzed without any extra acidification. Duplicate samples were analyzed and the mean values were calculated. The relative errors for five tests of acetanilide (a standard reference) were <2% for TOC and <3% for TN. The analysis precisions of TOC and TN were <0.05% and <0.02%, respectively. The stable carbon isotopic compositions of the DOM samples were measured using a Finnigan Delta Plus XL. Black carbon (GBW04408, China) was used as a standard and δ^{13}C values (‰) were converted relative to ^{13}C/^{12}C of Peedee belemnite. The precision of δ^{13}C based on duplicate analyses was <0.3‰. Four cations, Ca^{2+}, Mg^{2+}, Na$^+$ and K$^+$, were analyzed using an ion chromatograph (ICS 900, Dionex) or an inductively coupled plasma/atomic emission spectrometry (ICP-AES, Varian Vista-Pro). The cation separation was achieved in a CS2A column with a analytical precision better than 5% in the ICS 900. Each sample was measured eight times in the ICP-AES, and the analytical precision was better than 1%. A Bruker DSX400 spectrometer was used for ^{13}C NMR analyses. The NMR experiments were performed using Cross Polarization/

Total Sideband Suppression (CP/TOSS) at 100 MHz in 4 mm rotors. More details have been described elsewhere (Mao et al., 2012).

3. Results and discussion

3.1. The role of concentration factor in DOM isolation

The DOC concentrations of raw water ranged from 1.6 to 7.4 mg/L, with the highest concentration observed in the JK reservoir. The DOC concentrations of the retentates ranged between 49.2 and 345.9 mg/L, and the volumes of the samples decreased from 388–1067 L to 3–27 L. The DOM yields ranged from 36% to 107%, with a mean value of 74% (Table 1). The yields, except for LHS1, were significantly higher than that of ultrafiltration-isolated DOM (35%) from rivers (Bianchi et al., 2004; Engelhaupt and Bianchi, 2001; Jaffé et al., 2012; Maie et al., 2005). High yields are indicative of the representativeness of the isolated DOM sample. The yields >100% in a few samples could be attributed to the analysis errors of the DOC instrument. Analytical uncertainties were evident in low DOC concentrations (Vetter et al., 2007). The contamination from residual DOM in the RO system is another possibility for high yields (Vetter et al., 2007), which, however, could be impossible due to the thorough rinsing method in this investigation.

The CF was suspected to be a key metric for the yield of RO system (Sun et al., 1995). However, relatively constant CF values (26.7 ± 9.4, $n = 47$) were previously applied in the DOM isolation to minimize the biases derived from the RO process (e.g. Koprivnjak et al., 2006, 2009; Ouellet et al., 2008). Here a wide range of CF (17–221) was adopted to test the effect of the CF in DOC yields of the RO system. Fig. 1 shows that the yields are negatively related to CF ($R^2 = 0.89$, $n = 7$), indicating that CF can control and adjust DOM yields. If the DOM yields higher than 80% are sufficient, CF should be lower than 60 based on the equation of the regression curve.

The low yields of DOC were attributed to high DOM loss via either penetrating through or being sorbed onto the RO membrane. The proportions of permeate DOC were not correlated with the concentration factors. In contrast, the permeate DOC appeared to be highly dependent on the total DOC in the raw samples (Fig. 2). This phenomenon indicated that the low yields of DOC were due to the increase in DOM sorption in the RO system, rather than permeating through the RO membrane. The sum DOC from the rinsing and alkaline rinsing fractions only account d for ~6% of total DOC, lower than the simulated percentages of sorbed DOC. This implied that some unrecoverable DOM components could be tightly sorbed in the RO system. As this sorbed fraction could lead to the potential cross-contamination among the samples, the rinsing steps for the RO system with NaOH, HCl, detergent, and EDTA before and after the sample processing is necessary.
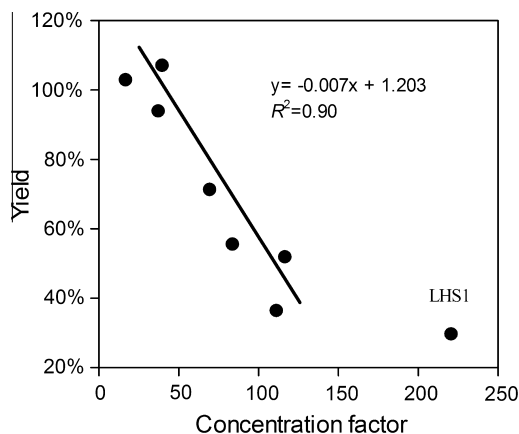

Fig. 1. Concentration factors and yields of DOM samples. Water sample at the LHS1 station was treated with a different process.
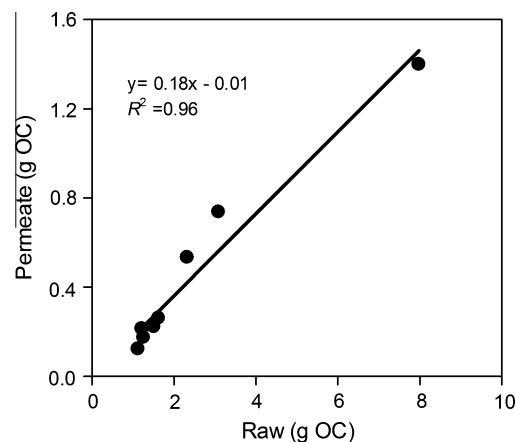
Fig. 2. Dissolved organic carbon in the raw water and the permeate fraction of eight samples.

The proportions of permeate in total DOC varied from 11% to 24% (Table 1). The permeate fractions were much higher than ~1%, a typical percentage as observed in other RO systems (Ouellet et al., 2008). The high ratios of DOC loss in this investigation could be due to the material of the RO membrane (Schutte and Belfort, 1987). However, the DOC yields using the RO system were still higher than those using other isolation approaches (Mopper et al., 2007).

3.2. Desalination by cation exchange resins and electrodialysis

The high content of inorganic salts in DOM isolated by RO approach is a challenge for some instrumental measurements.

Table 1
Concentration factors, yields of DOM isolation, and atomic C/N ratios and stable carbon isotopic compositions of dried DOM.

| Sample	Raw (mg OC)	Permeate (mg OC)	Retentate (mg OC)	Rinse (mg OC)	Alkali rinse (mg OC)	CF[a]	Yield (%)	C/N	$\delta^{13}C$ (‰)
BL	3087	736	1119	23.4	169	111	36	14.6	−26.5
JK	7978	1397	4415	283	303	84	55	10.9	−26.7
LHS1	2326	531	685	27.7	29.1	221	29[b]	10.6	−25.1
LHS2	1511	221	1615	2.33	69.8	40	107	9.48	−26.0
SW	1624	259	1523	39.9	62.1	37	94	9.65	−26.2
WLH	1255	172	649	55.2	n.d.	117	52	5.94	−26.4
YZ1	1124	120	1155	44.6	n.d.	17	103	2.58[c]	−25.2
YZ2	1217	212	866	146	n.d.	70	71	3.97[c]	−25.4

[a] CF = concentration factor.
[b] The extremely low yield of LHS1 is due to a significant loss of DOC in ED treatment.
[c] C/N ratios could be underestimated due to nitrate.

For instance, solid-state ^{13}C NMR experiment typically requires sufficient OC (5–500 mg OC) due to low natural abundance and gyromagnetic ratio of ^{13}C (Mopper et al., 2007). Additionally, the removal of some paramagnetic materials such as iron is necessary for ^{13}C NMR experiment to gain reliable spectra. Thus, eliminating or lowering salts in DOM is vital prior to ^{13}C NMR characterization.

Replacing cations with H$^+$ is a key process for not only avoiding the contamination of RO membrane but also removing many mineral anions such as NO_3^-, HCO_3^- and Cl$^-$ (Serkiz and Perdue, 1990; Sun et al., 1995). A 10 L of tap water was repeatedly treated with 0.8 L CER. Results showed that removal efficiencies were 95% for Mg^{2+}, 98% for Ca^{2+}, 100% for K$^+$, and 52% for Na$^+$ (Fig. S2). The high removal efficiencies of Mg^{2+} and Ca^{2+} were due to the preferential exchange of polyvalent cations by H$^+$-saturated CER (Amjad et al., 1993). K$^+$ was substantially eliminated because of its low abundance. Relatively low removal efficiency of Na$^+$ was due to the similar affinity of Na$^+$ and H$^+$ to the resin matrix (Amjad et al., 1993). 90% of total iron was removed (data not shown), indicating the CER efficiently removed the iron. Besides the tap water, a river sample (YZ1) was used to test the removal efficiencies of cations by CER in the RO system. The whole treatment removed >90% of the four cations (Fig. S2). Most of the remaining cations in the retentate were Na$^+$ (<10%), consistent with the tap water test. The above tests indicated that the CER can efficiently remove mono/polyvalence cations. It was noted that the remaining cations may form salts, which prevent the removal of mineral anions such as NO_3^-. This could be a reason for the low C/N ratios (2.6 and 4.0) for the YZ1 and YZ2 DOM without the ED treatment (Table 1). Soluble Fe concentrations were not determined here. However, Fe^{3+} and Fe^{2+} are expectedly eliminated with CER, due to their polyvalence and low abundance (<0.6 mg/L) in the Pearl River (Ho and Hui, 2001).

The water sample at LHS1 was collected in the dry season when seawater invaded the lower Pearl River. The water sample was initially desalted using ED and then was alternately treated with RO and ED. The DOC and conductivity were monitored at each treatment to elucidate the mechanisms of DOC retention and inorganic material elimination (Fig. S3). In the first ED phase, the conductivity declined sharply from 14.3 to 1.0 mS/cm, while the DOC concentration increased slightly from 3.1 to 3.2 mg/L. The slight enrichment in DOC concentration could be due to the analytical errors of DOC measurement. Water (~92%) was largely removed in the first RO phase with a considerable loss (41%) of DOC. The whole permeate fraction only accounted for 23% of total DOC. Thus, approximately half of the lost DOC was presumably sorbed in the RO system. In the meantime, ion concentrations increased as evidenced by the increased conductivity (8.8 mS/cm). In the following ED phase, another loss of DOC (25%) occurred. This phenomenon may be explained by the competition between inorganic anions and organic compounds for anion exchange sites. The abundant anions such as Cl$^-$ in the raw water preferentially penetrated through the anion exchange membrane relative to organic compounds (Gurtler et al., 2008; Kim et al., 2003), resulting in the retention of DOC in the first ED phase. Because the DOC was considerably concentrated after the first RO phase, the molar ratio of DOC to inorganic ions increased greatly. The remaining anions cannot block the sorption or/and permeation of organic compounds at the second ED phase, although competition was still presented as expected. An alternative possibility is that these remaining anions such as SO_4^{2-} could have weaker abilities to compete with the sites in anion exchange membrane relative to Cl$^-$ and organic compounds (Mopper et al., 2007). The above observations are consistent with previous studies (Koprivnjak et al., 2006; Vetter et al., 2007; Gurtler et al., 2008). In the following RO and ED procedure, no significant loss of DOC was observed. It was noted that in the third phase of ED the conductivity decreased from 6.7 to 0.9 mS/cm and only 3% of total DOC was lost. This DOC loss was much

lower than in the second ED phase, implying that these remaining DOM may be primarily comprised of uncharged species, which did not move in the electrical field.

3.3. ^{13}C NMR and δ^{13}C characterization for RO-isolated DOM

Fig. 3 shows the CP/TOSS ^{13}C NMR spectra of the DOM samples from JK, BL and LHS2 sites. All the spectra were divided into eight ranges of 0–45 ppm (alkyl C), 45–60 ppm (methoxyl C), 60–90 ppm (O–Alkyl C), 90–109 ppm (di-O-alkyl C), 109–145 ppm (aromatic C), 145–162 ppm (O-aromatic C), 162–187 ppm (carboxyl C), and 187–220 ppm (ketone and aldehyde C). Based on the integral ranges of the chemical shifts, the relative abundances (%) of the corresponding functional groups are shown in Table 2. The DOM samples had δ^{13}C narrowly ranging from −26.5‰ to −25.1‰ (Table 1).

Two riverine DOM samples (LHS2 and BL) showed quite similar carbon distribution of ^{13}C NMR (Fig. 3; Table 2). The variations were larger for small peaks (e.g. 109–154 ppm and 162–187 ppm) than major peaks, probably due to the fluctuations of baseline. Besides, the two DOM samples had similar isotopic compositions (δ^{13}C = −26.0‰, −26.5‰). The DOM yield of BL (36%) was much lower than that (107%) of LHS2. This reflects that a considerable proportion of BL DOM was sorbed in the RO system, whereas the sorption of LHS2 DOM was rare. The above phenomenon implies that sorption of DOM during the RO process was not selective, and the ^{13}C NMR spectra for the two samples with different recovery of DOM still represent the whole DOM structure.

Some differences in the carbon distribution between the riverine (LHS2 and BL) and reservoir (JK) DOM were observed (Table 2; Fig. 4B). The reservoir DOM comprised much more alkyl C but much less O–alkyl C than the riverine DOM. Leenheer et al. (2003) and Lam et al. (2007) found that the hydrophobic fractions (ca. 60% of DOC) of DOM in lake water and landfill leachate were enriched in alkyl C and depleted in O–alkyl C. Given that a landfill is near the JK site, it is not surprising to observe the enrichment in alkyl C and depletion in O–alkyl C.

The carbon distribution of the DOM differed significantly from that of aqueous humic substance, including humic and fulvic acids. Dissolved humic acids contained more aromatic and carboxyl carbons relative to RO DOM (Perdue and Ritchie, 2003). Humic substances generally accounted for 54 ± 14% of DOM in freshwater (Perdue and Ritchie, 2003). Relative to other components in DOM, humic substances were more hydrophobic. However, the hydrophilic components mainly consisted of hydrocarbon, amino acids, and fatty acids (Wershaw et al., 1982). In comparison with other investigations, it was found that these hydrophilic compounds could be considerably recovered in the RO DOM as evidenced by the significant signals of alkyl and O–alkyl C in ^{13}C NMR spectra, and the low aromatic carbon (Fig. 3).

Carbohydrates, proteins, lignin, and lipids are the primary identifiable constituents of freshwater DOM. The contribution of these biomolecules to DOM can be estimated based on the ^{13}C NMR

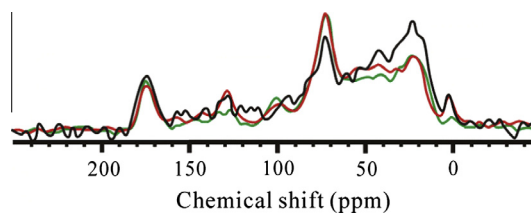**Fig. 3.** CP/TOSS ^{13}C NMR spectra of DOM samples at sites of JK (black), LHS2 (red) and BL (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Integration results of chemical shift (ppm) based on CP/TOSS ¹³C NMR.

Sample	0–45 Alkyl C (%)	45–60 OCH₃/NCH (%)	60–90 O–Alkyl (%)	90–109 O–Alkyl–O (%)	109–145 Arom.C (%)	145–162 Arom.C–O (%)	162–187 COO/NC=O (%)	187–220 Ketone/Aldehyde (%)
JK	45.1	11.7	19.8	4.1	8.3	2.6	8.3	0.0
BL	33.3	11.9	29.9	6.9	6.8	2.0	8.3	0.9
LHS2	34.1	13.1	28.4	5.6	9.5	2.3	7.1	0.0
HMW DOM (n = 21)[a]	20.1	17.2	24.7	6.9	14.7		12.5	3.8
Lake (n = 1)[b]	45.1	11.7	19.8	4.1	8.3	2.6	8.3	0.0
River (n = 2)[b]	33.7	12.5	29.1	6.2	8.1	2.1	7.7	0.5
Coast (n = 2)[c]	24.5	14.5	27.0	7.5	9.2	1.8	12.5	3.0
Marine (n = 3)[c]	26.1	12.5	34.9	6.8	5.7	0.0	12.3	1.7

[a] References (ultrafiltration-isolated DOM from rivers): Bianchi et al. (2004); Engelhaupt and Bianchi (2001); Jaffé et al. (2012); Maie et al. (2005).
[b] This study.
[c] References (RO/ED): Mao et al. (2012); Koprivnjak et al. (2009). The C distribution (from 1 ppm to 162 ppm) of DOM samples in Koprivnjak et al. (2009) was recalculated assuming that the distribution ratios of alkyl/OCH₃, O–alkyl/O–alkyl–O, and aromatic/aromatic O were similar to those reported by Mao et al. (2012).
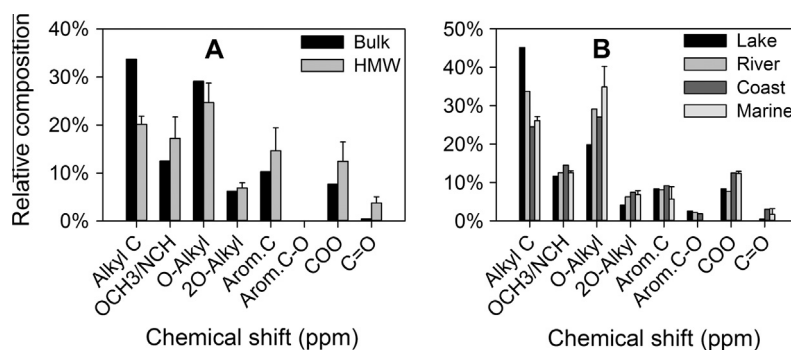
Fig. 4. Carbon distributions of RO-isolated (bulk) and ultrafiltration-isolated (HMW) DOM from river water (A); Carbon distributions of RO-isolated DOM samples from lake, river, coast, and marine water (B). The carbon distributions of HMW DOM and RO-isolated DOM from coast and marine water was adopted from other studies (see Table 2).

Table 3
Relative contributions of four model components of DOM. The approach for this calculation is described in supplementary data.

Sample	Carbohydrate (%)	Protein (%)	Lignin (%)	Lipid (%)
BL	41.5	19.5	7.17	31.8
JK	25.0	18.2	10.5	46.3
LHS2	37.1	19.8	12.8	30.2
River (n = 2)	39.2	19.7	10.1	31.0
Coast (n = 2)	44.4	36.6	7.88	11.2
Marine (n = 3)	51.0	29.6	4.50	14.9

spectra (Tables 3 and S1; Nelson and Baldock, 2005). Carbohydrates and lipids accounted for 25.0–41.5% and 30.2–46.3% of the identifiable DOM samples, followed by proteins (18.2–19.8%) and lignin (7.17–12.8%). The molecular-level analyses revealed that carbohydrates, lipids, proteins, and lignin accounted for 23–35%, 0.2–0.5%, 13–17%, and <1% of freshwater DOC, and a substantial fraction (48–64%) remained unidentified (Loh et al., 2006). The discrepancies suggest that the use of ¹³C NMR spectra to evaluate carbohydrates and proteins could be feasible. For lipids and lignin, additional end members are needed to provide more constraints on their estimations, which will be explored in the future investigation.

3.4. Comparison between the RO DOM and HMW DOM

The chemical structure of the RO DOM differed significantly from that of tangential-flow ultrafiltration-isolated DOM (HMW DOM) in rivers (Table 2; Fig. 4A). The former contained much higher alkyl and alkoxyl C, and lower aromatic and carboxyl C (33.7%, 29.1%, 10.2%, and 7.7%, respectively) than the latter

(20.1%, 24.7%, 14.7%, and 12.5%, respectively) (Table 2). It was noted that the RO DOM included the LMW and HMW DOM, but the ultrafiltration only isolated HMW DOM (Benner et al., 1992; Koprivnjak et al., 2009). Thus, the above comparison provided an insight into the functional groups of LMW DOM by difference. The alkyl C contents suggested that a fraction of lipid-like compounds is present in the LMW DOM. The elevated aromatic and methoxyl C contents suggested a noticeable proportion of dissolved lignin in HMW DOM. This observation was consistent with higher lignin phenol contents in HMW DOM than in LMW DOM from the Mississippi River by using molecular-level analysis (Hernes and Benner, 2003). The simulated compositions of LMW DOM by difference between the RO DOM and HMW DOM were involved in the following discussions to elucidate the transformation mechanisms of DOM during estuarine transition.

We compared the solid-state ¹³C NMR carbon distribution between riverine and marine DOM in order to reveal the transformation of DOM during transport to oceans. To avoid the inconsistency caused by different isolation approaches, coastal and marine DOM samples separated by RO (Koprivnjak et al., 2009; Mao et al., 2012) were selected to compare with our freshwater DOM samples (Fig. 4B; Table 2). Although the sample set is not large, it provides an initial comparison of the total DOM from various aqueous environments. Furthermore, this comparison, combined with the compositional changes of HMW DOM (Abdulla et al., 2010; Maie et al., 2005), exerts an important constraint on size and chemical transformation of DOM. The freshwater DOM (river and reservoir DOM) contained much more alkyl C than the seawater DOM (coastal and marine DOM; Fig. 4B), which was also consistent with the other investigations on the alkyl C of HMW DOM in the Chesapeake Bay and the Florida Bay (Abdulla et al., 2010; Maie et al., 2005). The most abundant alkyl C was observed in the reservoir

DOM, which was in part attributed to a landfill leachate enriched in alkyl C. The elevated alkyl C contents in freshwater DOM could be due to the input of soil OM enriched in recalcitrant lipid-like compounds. Soil DOM indeed was one of the key sources for river and reservoir DOM. In contrast, "fresh" DOM released from primary production appeared to be a predominant origin for marine surface DOM (Sannigrahi et al., 2005).

The peak area at 45–60 ppm was quite similar in the four DOM samples, with the strongest signal in the coastal DOM. This peak in the freshwater DOM was due in part to methoxyl C of lignin (Mao et al., 2012). In contrast, the peak in the seawater DOM should largely correspond to NCH, such as the bands in peptides or amino sugars (Mao et al., 2012).

Alkoxyl C was primarily abundant in carbohydrates, which was a major component in both riverine and marine DOM (Benner et al., 1992; Mao et al., 2012). The riverine DOM in this investigation contained much less alkoxyl C (60–109 ppm) than the marine DOM, which was consistent with the comparison of HMW DOM (Abdulla et al., 2010; Maie et al., 2005). The dissimilarity between riverine and marine DOM was likely related to the DOM sources. The marine HMW DOM from surface water in the open ocean had an overwhelming source from primary production (Sannigrahi et al., 2005). Thus, relatively "fresh" DOM substantially contained labile compounds such as carbohydrates in surface water. In contrast, the sources of riverine DOM were complex. For example, degraded humic substances accounted for $54 \pm 14\%$ of DOC and contained significantly lower alkoxyl C (Mopper et al., 2007). Plant-derived components such as lignin and tannin, which were enriched in aromatic C, could dilute alkoxyl C.

Aromatic C (109–162 ppm) was usually attributed to lignin, which was exclusively present in terrestrial plants. Molecular-level analyses showed that the dissolved lignin concentration in river was two orders of magnitude higher than in ocean (Benner et al., 2005). Thus, it is not surprising that the river and reservoir DOM contained higher aromatic C than marine DOM did. This was also observed for HMW DOM (Abdulla et al., 2010; Maie et al., 2005). It is noted that the peak at 45–60 ppm for the marine DOM was largely attributed to NCH (Mao et al., 2012), rather than methoxyl C of lignin. The aromatic C of marine HMW DOM was selectively accumulated during sedimentation in oceans (Benner et al., 1992; Sannigrahi et al., 2005), which was consistent with the degradation resistance of lignin. It could be argued that aromatic amino acids contributed to the aromatic C of marine DOM. However, this possibility could be ruled out because phenylalanine and tyrosine appeared not to be accumulated during sedimentation due to their high bioavailability (Cowie and Hedges, 1992).

Freshwater DOM contained lower carboxyl C than seawater DOM (Fig. 4B). In contrast, the carboxyl C contents of HMW DOM in freshwater were higher than in seawater (Abdulla et al., 2010; Maie et al., 2005). This observation suggested that a significant fraction of carboxyl C may be present in LMW DOM in oceans. High carboxyl C was commonly observed in humic substances of freshwater as well as in HMW DOM of deep sea water (Hertkorn et al., 2006; Perdue and Ritchie, 2003). Hence, carboxyl C was considered to be a key component of refractory DOM, such as carboxyl-rich alicyclic molecules (CRAM) (Hertkorn et al., 2006; Lam et al., 2007). This suggested that LMW DOM in ocean surface was highly reworked.

The coastal DOM had the highest carbonyl C, while the signals of carbonyl C were weak in the freshwater DOM spectra (Fig. 4B). The photolysis of seawater DOM can release LMW carbonyl compounds such as formaldehyde, acetone, and glyoxyal (Mopper and Stahovec, 1986), and was extensively present in terrigenous DOM-dominated regions (Kieber and Mopper, 1987). However, their percentage is inconsistent with the percentage of carbonyl C (3–5%) in the coastal HMW DOM (Bianchi et al., 2004). More

studies would be required regarding natural source, production, and consumption of carbonyl C compounds.

The coastal DOM constantly showed different contents of the functional groups from the riverine and marine DOM (Fig. 4B). This may indicate a non-conservative mixing behavior (i.e., a simple mixing combined with source and biogeochemical alteration) of DOM during the transport to oceans. The behavior for HMW DOM along estuary was also investigated (Abdulla et al., 2010; Hernes and Benner, 2003). However, some compositional changes of DOM differed from those of HMW DOM. A striking increase in alkoxyl C (60–109 ppm) of HMW DOM was observed (Abdulla et al., 2010), while the alkoxyl C of DOM decreased slightly. Two possibilities could explain this: (1) LMW carbohydrates were much more degradable than those of HMW; (2) HMW DOM had an input of alkoxyl C-enriched source. The second possibility was supported by a finding (Abdulla et al., 2013) that HMW DOM had a new autochthonous source in the higher salinity region of an estuary. The aromatic C (109–162 ppm) of the HMW DOM decreased along a salinity gradient (Abdulla et al., 2010), whereas that of DOM increased slightly. This suggests that a fraction of dissolved lignin in HMW DOM may be degraded to LMW lignin, rather than remineralization, which is consistent with a finding based on lignin biomarkers in HMW and LMW DOM (Hernes and Benner, 2003). It also supports the hypothesis that alteration of HMW DOM is an important process in the estuarine mixing zone, in addition to the mixing of river and marine sources (Guo et al., 2009). In a word, the differences in carbon distribution among river, coastal and marine water DOM provide a powerful tool to elucidate the mechanism for the transformation of DOM from river to ocean. Moreover, the comparisons between DOM and HMW DOM further reveal the underlying size changes of organic components.

4. Conclusions

In the present study, we verified that the concentration factor is important for controlling the DOM sorption in the RO system. High concentration factors led to elevated sorption and low yields of DOM. Non-selective sorption of DOM in the RO system was suggested based on ^{13}C NMR spectra and δ^{13}C. Although a portion of DOM was sorbed, the retentate was still representative. However, sorbed DOM should be rinsed from the RO system, especially when in situ sampling is carried out. The carbon distribution of freshwater DOM differed distinctly from those of HMW DOM isolated from the marine water, due in part to different source and biogeochemical alteration. The analysis of DOM by using ^{13}C NMR is important to understanding compositions of riverine, coastal, and marine DOM as well as their transformation process during the transport from river to open ocean.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.06.022.

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