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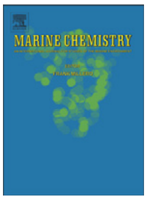

Chemical and isotopic composition of high-molecular-weight dissolved organic matter from the Mississippi River plume

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ABSTRACT

In order to examine the source and transformation of high-molecular-weight dissolved organic matter (HMW-DOM) in the mixing zone of the Mississippi River plume, HMW-DOM with sizes between 1 kDa and 0.2 µm was collected along a salinity gradient using cross-flow ultrafiltration. Isolated OM samples were desalted, freeze-dried and characterized for elemental (C and N) and biochemical composition (proteins, carbohydrates and uronic acids), stable isotopic (δ^{13}C and δ^{15}N) and radiocarbon (Δ^{14}C) signatures, and relative molecular composition using pyrolysis-gas chromatography/mass spectrometry. The organic carbon content of HMW-DOM samples ranged from 31 to 36 wt.%, indicating that isolated colloids are mostly organic in nature. Contents of N ranged from 1.9% to 3.3%, resulting in a C/N ratio of 19–20 at lower salinity stations with a strong influence by terrestrial DOM and 12–15 at higher salinity stations with more freshly photosynthesized marine DOM. While OC-normalized protein contents decreased with increasing salinity, both carbohydrate and uronic acid contents increased with increasing salinity. Variations of pyrograms demonstrated that the proportion of furfural (an indicator of polysaccharides) in HMW-DOM also increased with increasing salinity, while the proportion of phenols decreased with increasing salinity. Changes in carbohydrate, (acid) polysaccharide and phenol contents of HMW-DOM samples reflect the variation in DOM sources along the salinity gradient, with higher phenol and low polysaccharide contents in lower salinity areas but higher polysaccharide and low phenol contents in coastal waters. Values of δ^{13}C increased from −25.24‰ at the Mississippi River fresh water end-member station to −21.86‰ at an offshore station in the Gulf of Mexico. Changes in stable isotope composition resemble the changes in molecular composition from freshwater to coastal waters. Values of δ^{15}N, on the other hand, varied little, from 3.5‰ to 4.9‰ without a consistent trend, indicating that δ^{15}N is a less sensitive source tracer. Measured radiocarbon signatures (Δ^{14}C) expressed as apparent ^{14}C ages ranged from > modern at lower salinity stations to 400–800 y BP at coastal stations. Results of isotopic mass balance revealed that, in addition to end-member organic matter from river and marine sources, at least 10–25% of the HMW-DOM could derive from reworked or regenerated DOM in the Mississippi River plume, most likely through sediment–water interactions and lateral transport. Thus, reworking processes are important in governing the chemical and isotopic composition of DOM in the estuarine mixing zone.

1. Introduction

The riverine export of dissolved organic matter (DOM) to the ocean represents a major component of the global carbon cycles (Meybeck, 2003), affecting carbon inventory and biogeochemical processes, especially in the river-dominated ocean margins such as the Mississippi River plume (McKee, 2003). It has been shown that riverine and estuarine DOM is mostly partitioned and transported in the high-molecular-weight (HMW) or colloidal phase (Hedges et al., 1994; Guo and Santschi, 1997; Benner and Opsahl, 2001; Guo and Macdonald, 2006). Colloidal and HMW-DOM seem to have higher biological and chemical reactivities (Amon and Benner, 1994; Santschi et al., 1995) and are the most effective carriers for trace metals and organic pollutants, affecting their mobility, bioavailability and physicochemical behavior (e.g., Sigleo and Means, 1990; Wang and Guo, 2000; Lead and Wilkinson, 2006). Applications of sampling techniques for aquatic colloids, together with chemical and isotopic characterization have advanced our knowledge of the chemical composition of HMW-DOM in estuarine systems (Guo and Santschi, 2007 and references therein). However, chemical, molecular and isotopic composition of HMW-DOM and its sources and transformation processes in the estuarine mixing zone remain poorly understood.

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Sigleo and her colleagues first characterized the organic and stable isotopic composition of estuarine HMW-DOM isolated using stirred cell ultrafiltration (Sigleo et al., 1982; Sigleo and Macko, 1985). Mitra et al. (2000) investigated the dispersal and terrestrial origin of lignin phenols from the Chesapeake Bay throughout the Middle Atlantic Bight. van Heemst et al. (2000) examined the isotopic and molecular composition of HMW-DOM (1 kDa–0.2 μm) from the Ems–Dollart estuary using both ^{13}C NMR and analytical pyrolysis. While they found a slight increase in δ^{13}C values, the ^{14}C activity in the HMW-DOM remained constant for all four samples. Mannino and Harvey (2000a,b) characterized two size fractions of organic matter (1–30 kDa and 30 kDa–0.2 μm) from the Delaware Estuary for C/N ratios, stable C isotopic composition, and biomolecules including fatty acids and lignin. Using size exclusion chromatography and direct temperature-resolved mass spectrometry, Minor et al. (2002) characterized estuarine HMW-DOM samples from both the lower Chesapeake Bay and Oosterschelde estuary. They reported an enrichment of aminosugars, deoxysugars, and methylated sugars in the larger size classes and hexose sugars in the smaller size classes of DOM samples. Recently, Zou et al. (2004) reported the compound specific isotopic composition of estuarine HMW-DOM samples. Overall, different sampling devices with different membrane cutoffs were used in collecting estuarine HMW-DOM samples, which were then characterized for different biomolecules using different techniques. In addition, estuarine HMW-DOM seems highly heterogeneous in terms of size, chemical composition and isotope signatures, which could be estuary specific.

In the Mississippi River plume, Benner et al. (1992) isolated HMW-DOM (>1 kDa) and measured the stable isotope composition and its relationship to bacterial activities. Benner and Opsahl (2001) also reported the contents of combined neutral sugars and lignin-derived phenols in the HMW-DOM. They found that combined neutral sugar content was elevated in the mid salinity regions but lignin contents elevated in the lower salinity waters. However, very little data is available on radiocarbon composition of HMW-DOM in the Mississippi River plume.

It is not clear if DOM regeneration can occur on time scales of 2–20 days in the estuarine mixing zone of the Mississippi River (Moore and Krest, 2004). Thus, our hypothesis is that HMW-DOM in the estuarine mixing zone is more a “reworked” organic component with a mixed radiocarbon signature reflecting the fraction of freshly photosynthesized organic matter and the extent of diagenesis/reworking or additional inputs of marine and/or terrestrial organic matter. During two sampling cruises, HMW-DOM (1 kDa–0.2 μm) was isolated using ultrafiltration at stations along a salinity gradient in the Mississippi River plume. Desalted DOM samples were chemically characterized in detail. Our objective was to use multiple characterization techniques to determine sources, transport and transformation of HMW-DOM in the Mississippi River plume.

2. Methods

2.1. Sampling

Surface seawater was collected for ultrafiltration along a salinity gradient from the Mississippi River plume in the northern Gulf of Mexico aboard the R/V *Pelican* in August 1997 and March 1998. A freshwater end-member sample was also collected from the Mississippi River near New Orleans after the Mississippi River plume sampling. Detailed sampling locations and hydrographic parameters are given in Table 1.

Large volumes of seawater were filtered in situ through an in-line filtration setup with a 0.2 μm cartridge filter (Osmotic) to remove suspended particles as described in Guo et al. (1996). The 0.2 μm filtrate was pumped directly into the reservoirs of the ultrafiltration system (Amicon DC-10). HMW-DOM was isolated using a 1 kDa ultrafiltration membrane (Amicon S10Y1). Therefore, the HMW-DOM is operationally defined here as the fraction with size or molecular weight ranges between 1 kDa and 0.2 μm. The ultrafiltration cartridge was checked for integrity using standard macromolecules with known MW (such as vitamin B_{12}) and thoroughly cleaned before sampling using 1% Micro detergent, 0.05 M NaOH, 0.02 M HCl, and large volumes of Nanopure water (with a DOC concentration of ~2 μM). Details of the cleaning and calibration procedures are described in Guo and Santschi (1996) and Guo et al. (2000). Before ultrafiltration, the ultrafiltration system was preconditioned using 10 l of prefiltered water. An optimum cross-flow ratio of >20 (ratio of rejected to permeate flux) was used for all samples. Our main purpose was to isolate sufficient amounts of HMW-DOM from large volumes of seawater for chemical and isotope characterization. Thus, a high concentration factor of 50–100 (ratio of initial seawater volume to final retentate volume) was used for ultrafiltration. HMW-DOC recovery was estimated based on DOC concentration difference between initial solution and integrated permeate. For quantitative determination of LMW and colloidal concentrations, which is not the focus of this work, time series sampling from permeate and the use of an ultrafiltration permeation model are required (Guo and Santschi, 1996, 2007).

Table 1
Sampling locations, water salinity, temperature, and DOC concentrations from the Mississippi River plume.

Station	Location	Water depth (m)	Salinity	Temp (°C)	DOC (μM)	HMW-DOC (%)[a]
01A	28°39.97′N; 90°04.12′W	90	30	30.9	149	–
02A	28°15.33′N; 89°39.22″W	820	28.2	31.2	162	42
03A	28°45.01′N; 89°50.13′W	71	28.4	31.1	171	44
06A	28°53.45′N; 89°27.65′W	25	20	29.4	196	48
08A	28°54.08′N; 89°26.14′W	8	7	29.5	278	49
MRA	29°59.86′N; 90°25.80′W	–	0.02	30	304	62 (52)
01B	28°22.78′N; 90°34.69′W	50	32.65	18.1	98	–
02B	27°40.80′N; 91°31.76′W	675	35.97	20.5	89	35
04B	28°42.12′N; 89°58.01′W	55	30.96	17.7	110	42
06B	28°48.79′N; 89°35.96′W	70	22.52	16.4	191	46
07B	28°50.75′N; 89°28.55′W	–	12.5	15.7	229	48
MRB	29°59.86′N; 90°25.80′W	–	0.02	–	316	59 (50)

“–”: no available. Station-A samples were collected in August 1997, while station-B samples were collected during March 1998.

[a] Numbers inside the parenthesis are the percentage of HMW-DOC after diafiltration and all other numbers are those before diafiltration and calculated based on DOC concentration difference between initial solution and integrated permeate.

To obtain powdered HMW-DOM samples low in sea salt, the final retentate solution (about 2–3 l) was further diafiltered to remove sea salts using 20 l of Nanopure water (Guo and Santschi, 1996). The purified HMW-DOM concentrate was then freeze-dried to yield a powdered sample for further elemental, isotopic and molecular characterization, and stored in the freezer (<−20 °C) until analysis.

2.2. Measurements of elemental (C and N), carbohydrates, uronic acids and protein content

The freeze-dried DOM samples were measured for content of organic carbon (OC), total nitrogen (TN), total carbohydrates, uronic acids and proteins. Powdered DOM samples were treated with HCl fumes before OC and N analysis. Total OC and N concentrations were measured on a Perkin-Elmer Series II CHNS/O Analyzer (2400). Acetanilide (71.09% C and 10.36% N) was used as an analytical standard. Replicate measurements gave a relative standard deviation of 1% for C and ≤3% for N.

For the measurements of total carbohydrates, uronic acids and proteins, freeze-dried powdered DOM samples were re-dissolved in Nanopure water and aliquots of samples were taken for the analyses based on samples' organic carbon contents.

Total carbohydrates (TCHO) were measured by the TPTZ method (Myklestad et al., 1997); proteins by the bicinchoninic acid assay

Table 2
List of major organic compounds with the highest abundance index from pyrolysis-GCMC results.

Major compound	Category	Class
Furfural, Methyl furfural	Furfurals (Furf)	Sugar
Dimethyl benzene	Alkyl-benzene (Alkben)	Aromatic
Phenol	Phenol (Phe)	Aromatic
Methyl cyclopentenone	Cyclopentenone (CycP)	Sugar
Indene	Indene (Ind)	–
Naphthalene	Naphthalene (Naph)	Aromatic
Methyl pyridine	Pyridine (Pyr)	–

(Smith et al., 1985); uronic acids (URA) by the sulfamate/m-hydroxydiphenyl assay (Filisetti-Cozzi and Carpita, 1991), modified by Hung and Santschi (2001). Contents of uronic acids were measured according to Walters and Hedges (1988). All components were normalized to total organic carbon (TOC).

2.3. Measurements of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon composition

The freeze-dried DOM samples were treated with HCl fumes before measurements of stable carbon and nitrogen isotopic (δ^{13}C and δ^{15}N), and radiocarbon (Δ^{14}C) composition (Guo and Santschi, 1997). Both δ^{13}C and δ^{15}N were measured by continuous flow isotope ratio mass spectrometry. Stable C and N isotope ratios were calculated in terms of δ^{13}C and δ^{15}N, with

$$\delta = \left(R_{\text{sample}} / R_{\text{standard}} - 1\right) \times 1000$$

where R is the ratio of ^{13}C/^{12}C, or ^{15}N/^{14}N, in samples or standard (PDB for carbon and atmospheric N_2 for nitrogen). The precision and accuracy of δ^{13}C and δ^{15}N analysis were ±0.1‰ and ±0.2‰, respectively, as determined by replicate analysis of standards and samples (Guo et al., 2003).

Radiocarbon (Δ^{14}C) in HMW-DOM samples was determined using accelerator mass spectrometry (AMS) at the National Ocean Science AMS Facility at Woods Hole Oceanographic Institution. Acid (HCl) treated DOM samples were combusted to CO_2 and converted to graphite for radiocarbon analysis. Values of Δ^{14}C and apparent ^{14}C ages are reported following the convention outlined by Stuiver and Polach (1977) and Stuiver (1980) using a half-life of 5568 years for radiocarbon. Reported errors were the internal error calculated from the number of counts measured for each sample and the error of the standard.

2.4. Sample characterization using pyrolysis-GC/MS

Aliquots of HMW-DOM samples were characterized by pyrolysis-gas chromatography/mass spectrometry (py-GC/MS) techniques (White and Beyer, 1999; White et al., 2004). Briefly, pyrolysis was conducted with a CDS Model 2500 pyrolyzer and an auto-sampler in tandem with a gas chromatograph/mass spectrometer. During pyrolysis the sample was heated from a starting temperature of 235 °C to 700 °C in 0.1 s and held at a constant 700 °C for 9.9 s. The pyrolysis reactor was mounted on an HP 5890 Series II GC, with a Supelco SPB 35 (35% Ph Me silicon) column, 60 m × 0.25 mm × 0.25 µm. The GC interface temperature was set at 235 °C. The GC temperature program was 45 °C for 5 min, 2 °C/min to 240 °C and held for 25 min using helium as a carrier gas at a flow rate of 0.5 cm^3/min. The GC was plumbed directly to an HP 5971A Series mass selective detector on electron impact mode. The MS scanned mass units from 45 to 650. All mass spectra were compared to the NBS54K spectral library.

Following pyrolysis, approximately 100 of the most abundant compounds in each pyrogram were identified using a mass spectral library. The major compounds were the same in each pyrogram, but occurred in different proportions. An index set of eight of the most abundant compounds was selected for comparison between HMW-DOM samples. The compounds were then assigned categories based on the origin and nature of the pyrolysis products (Table 2). An "abundance index" for each category was calculated as a percentage by summing the total ion chromatogram areas of compounds in that category divided by the sum of the areas of all index compounds (White and Beyer, 1999; Coban-Yildiz et al., 2000). The samples were further differentiated by comparing categories that are generally believed to be derived from polysaccharides (i.e., sugars) to those containing aromatic rings. The approach was used to compare samples. The abundance index is not directly related to the actual abundance of sugars or aromatics in the sample. However, the technique did allow us to look at a suite of compounds that are abundant in the samples, and compare the samples respectively.

3. Results and discussion

3.1. Abundance of organic carbon and nitrogen in isolated HMW-DOM

The content of TOC and total nitrogen (TN) in the isolated HMW-DOM samples is listed in Table 3. The TOC content in the desalted, freeze-dried HMW-DOM samples varied from 31% to 36.5% with an average of 33.9 ± 1.8%. High TOC content indicates that estuarine and marine colloidal materials are mostly organic in nature with the remainder being residual sea salts and water. Total nitrogen content ranged from 1.86% to 3.32%, with an average of 2.42 ± 0.41% (Table 3).

Table 3
Contents of total organic carbon (TOC), total nitrogen (TN), C/N molar ratio, proteins, carbohydrates (TCHO) and uronic acids (URA) in HMW-DOM from surface waters of the Mississippi River plume.

Sample	TOC	TN	C/N	Protein	TCHO	URA	URA/TCHO
ID	(%)	(%)	Ratio	(% OC)	(% OC)	(% OC)	(%)
01A	32.5 ± 0.8	2.59 ± 0.17	14.6	7.79 ± 0.17	19.7 ± 1.8	2.24	11.4
02A	33.2 ± 0.5	2.34 ± 0.34	16.5	7.53 ± 0.11	19.5 ± 0.9	2.44	12.5
03A	33.5 ± 0.3	2.64 ± 1.34	14.8	7.75 ± 0.24	16.7 ± 0.7	1.95	11.7
06A	35.2 ± 0.4	2.42 ± 0.59	16.9	13.2 ± 0.6	16.6 ± 1.6	1.53	9.2
08A	35.6 ± 0.1	2.13 ± 0.14	19.5	16.8 ± 0.5	11.6 ± 1.0	0.59	5.1
MRA	36.5 ± 0.4	1.86 ± 0.05	22.9	16.8 ± 0.6	12.3 ± 1.0	0.36	2.9
Average ± std	34.4 ± 1.6	2.33 ± 0.29	17.5 ± 3.2	11.6 ± 4.5	16.1 ± 3.4	1.5 ± 0.9	8.8 ± 3.9
01B	33.6	2.59	15.1	9.67 ± 0.10	13.9 ± 0.47	1.45	10.4
02B	33.2	2.80	13.8	7.19 ± 0.39	11.9 ± 0.1	1.78	14.9
04B	31.0	2.35	15.4	12.3 ± 0.3	13.8 ± 0.3	2.27	16.5
06B	35.4	3.32	12.4	17.9 ± 0.6	12.3 ± 0.1	0.49	4.0
07B	31.1	1.89	19.2	22.7 ± 0.3	14.7 ± 0.5	0.87	5.9
MRB	36.0	2.06	20.4	29.8 ± 0.3	15.0 ± 0.7	0.4	2.7
Average ± std	33.4 ± 2.1	2.50 ± 0.52	18.1 ± 3.1	16.59 ± 8.58	13.61 ± 1.26	1.21 ± 0.75	9.1 ± 5.8

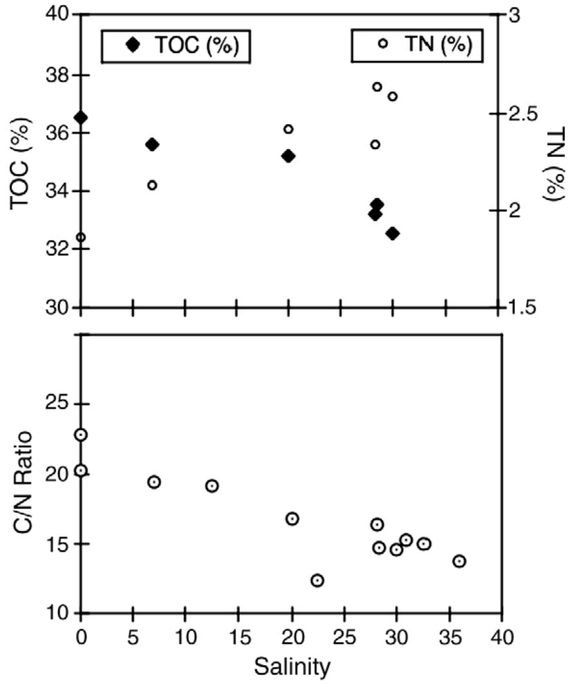

Fig. 1. Variations of total organic carbon (TOC), total nitrogen (TN), and C/N molar ratio of HMW-DOM with salinity in the Mississippi River plume.

These TOC and TN contents result in a C/N molar ratio ranging from 12 to 23, with an average of 17±3, similar to values measured for the HMW-DOM in the Mississippi River plume (Benner and Opsahl, 2001) and marine DOM.

In general, TOC content (in wt.%) in HMW-DOM samples decreased with increasing salinity while TN content increased with increasing salinity, especially for samples collected during the August cruise (Fig. 1, upper panel). Higher TOC but lower TN content in freshwater and lower salinity waters indicate the dominance of terrestrial organic matter containing higher humic substances with higher TOC and higher C/N ratios. In contrast, lower TOC but higher TN contents in the higher salinity coastal waters point to HMW-DOM containing more diagenetically fresh marine organic matter components. In general, the C/N ratio of HMW-DOM decreased with increasing salinity (Fig. 1), suggesting again a transition in DOM chemical composition from largely terrestrial organic matter with a higher C/N ratio in river and low salinity waters to mostly diagenetically younger marine DOM with a lower C/N ratio in coastal waters. These results resemble the variations of C/N ratios with salinity previously reported for Galveston Bay waters (Guo and Santschi, 1997) and the Mississippi River plume (Benner and Opsahl, 2001).

3.2. Variations in biopolymer composition

The OC-normalized concentrations of proteins, total carbohydrates (TCHO) and uronic acids (URA) are given in Table 3. Concentrations of proteins normalized to TOC varied from 7.5% OC to 29.8% OC with an average of 14.1 ± 7.0%. TOC-normalized protein content was highest in the freshwater end-member, the Mississippi River, and decreased with increasing salinity in the Mississippi River plume (Fig. 2). This trend observed for HMW-DOM proteins might, at first, look somewhat surprising and seemingly contradictory to the variations of C/N ratios. For example, the C/N ratio of HMW-DOM decreased with increasing salinity (Fig. 1), suggesting that the N-containing organic fraction should increase with increasing salinity in the HMW-DOM pool, as supported by the data of TN content. If proteins are a major DON fraction, one would expect that marine HMW-DOM should contain a higher protein content compared to terrestrial organic matter. However, because amino sugars and amides are the major fraction of DON in the ocean (McCarthy et al., 1997; Aluwihare et al., 2005), and proteins only a minor fraction, opposite trends are quite compatible. Assuming that proteins contain 44% C, and 16% N (e.g., Thurman, 1985; Villanueva and Norman, 2008), our data showed that in most of the samples from a salinity >30, proteins contributed less than 50% to the N content. In agreement with our findings, protein concentrations in HMW-DOM fractions across the Delaware estuary reported by Mannino and Harvey (2000b) also showed a decreasing trend across the salinity gradient.

Contents of TCHO in the HMW-DOM samples (normalized to OC) ranged from 11.6% to 19.7%, with an average of 14.8 ± 2.8% (Table 3). Uronic acids are a major acid polysaccharide species in the TCHO pool. The percent of URA in the TOC varied from 0.36 to 2.4, with an average of 1.36 ± 0.79%. Within the TCHO pool, URA comprised 2.7–16.5% with an average of 8.9 ± 4.7%. The lowest URA/TCHO ratio was observed at the freshwater station and higher ratios was seen for samples at offshore stations. Acid polysaccharides are biopolymers with strong aggregating and thorium-complexing properties and thus have a short residence time (e.g., Guo et al., 2002). High URA concentrations and high URA/TCHO ratios in HMW-DOM samples (up to 0.15 or 15%) from the high salinity region are consistent with sources from marine organisms (Hung et al., 2003; Santschi et al., 2003). However, the highest URA/TCHO ratio was not observed at the highest salinity station although URA/TCHO ratios show a general increase with increasing salinity (Fig. 2). This indicates that the content of acid polysaccharides in the

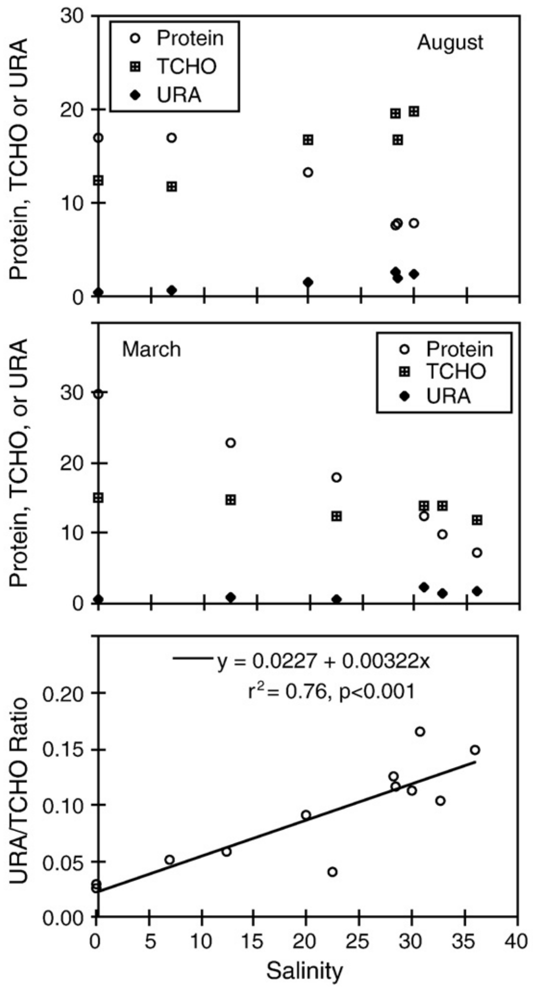

Fig. 2. Contents of OC-normalized proteins, carbohydrates and uronic acids (all in % OC) of HMW-DOM and their variations with salinity in the Mississippi River plume.

Table 4
Stable isotopic composition (δ^{13}C and δ^{15}N) and radiocarbon signatures (Δ^{14}C) of HMW-DOM from the Mississippi River plume.

Station	δ^{13}C (‰)	δ^{15}N (‰)	Δ^{14}C (‰)	^{14}C age (yr BP)	Terrestrial fraction	Difference in terrestrial fraction
01A	−23.08	4.92	–	–	0.32	–
02A	−23.62	5.21	–	–	0.43	–
03A	−23.14	4.41	–	–	0.33	–
06A	−24.78	4.75	–	–	0.68	–
08A	−26.06	3.92	–	–	0.96	–
MRA	−26.26	3.99	–	–	1.0	–
Avg ± st. dev.	−23.5 ± 1.5	4.15 ± 0.45				
01B	−21.86	4.03	−101 ± 0.5	800 ± 45	0.07 (0.22)	0.15
02B	−22.09	4.27	−51 ± 0.3	370 ± 40	0.13 (0.37)	0.24
04B	−22.56	3.86	−56 ± 0.4	415 ± 60	0.26 (0.36)	0.09
06B	−24.00	4.92	3.4 ± 0.1	>Modern	0.66 (0.53)	−0.13
07B	−25.00	3.59	58 ± 0.2	>Modern	0.93 (0.69)	−0.24
MRB	−25.24	4.20	162 ± 0.8	>Modern	1.0	—
Avg ± st. dev.	−24.5 ± 1.4	4.53 ± 0.52	–	–	–	

The fraction of terrestrial organic matter was estimated based on isotopic mass balance calculation (values inside the parenthesis were derived from Δ^{14}C data). Differences in the terrestrial fraction were calculated from values of the terrestrial fraction derived from both δ^{13}C and Δ^{14}C.
"–": not measured.

HMW-DOM pool could be affected by other factors such as nutrient status, primary production, microbial biomass, and coagulation processes (Santschi et al., 2003).

Compared with protein content, carbohydrate concentrations were less variable between freshwater and seawater HMW-DOM samples within a wide range of salinity, ranging from 0 to 36. However, carbohydrate content in HMW-DOM seemed to increase with increasing salinity especially for samples collected in August 1997 although this trend was not as clear in the samples collected in March 1998 (Fig. 2). The increase in TCHO content with increasing salinity was consistent with our data from pyrolysis-GC/MS analyses (see next section) and with that observed for TCHO/DOC ratios in Galveston Bay and the Gulf of Mexico (Hung et al., 2003), indicating that polysaccharides are mostly produced in marine environments resulting in a higher URA and polysaccharide content at higher salinity stations.

While the carbohydrate contents of HMW-DOM had a small variability, both the content of URA and the fractions in the TCHO demonstrated a larger variability between freshwater and seawater end-member stations (~3% vs. 16%). Therefore, acid polysaccharides seem to be a more sensitive biopolymer as a proxy (or biomarker) for estuarine and marine biogeochemical cycling compared to the total carbohydrate concentration. Concurrent measurements of both acid polysaccharides and neutral sugars in seawater are needed in future studies.

3.3. Stable isotopic (δ^{13}C and δ^{15}N) composition

Values of δ^{13}C in the HMW-DOM samples ranged from −25.2‰ in the Mississippi River to −21.86‰ in the Gulf of Mexico, with an average of −23.5 ± 1.5‰, for samples collected in March (Table 4). For samples collected in August, values of δ^{13}C varied from −26.26‰ in the freshwater sample to −23.08‰ in HMW-DOM from higher salinity waters, with an average of −24.5 ± 1.4‰ (Table 4). The average δ^{13}C value during August was relatively lower than that during March, due to salinity differences and likely seasonal changes in river water discharge and terrestrial organic matter export. As shown in Fig. 3, the δ^{13}C values increased in general with increasing salinity in the Mississippi River plume, supporting changes in DOM sources from terrestrially dominated HMW-DOM in the lower salinity area to mostly marine derived HMW-DOM in higher salinity waters in the Gulf of Mexico. The non-linear (concave) distribution of the δ^{13}C vs. salinity (Fig. 3), which suggests that there is more terrestrial C in higher salinity waters than expected from physical mixing alone, has also been observed for Galveston Bay using δ^{13}C (Guo and Santschi, 1997; Guo et al., 2003) and ^{129}I/^{127}I ratios (Schwehr et al., 2005). Both tracer distributions can be explained by sediment-resuspension and colloid saltation processes. This result is consistent with conclusions derived from variations in TOC and TN concentrations and the composition of biopolymers.

Values of δ^{15}N in HMW-DOM samples ranged from 3.59‰ to 4.27‰, with an average of 4.15 ± 0.45‰ for samples collected in March; and ranged from 3.94‰ to 5.52‰ with an average of 4.53 ± 0.52‰ for samples collected in August. Interestingly, the average δ^{15}N values had a smaller difference (0.4‰) between March and August samples, while the difference in δ^{13}C values was up to 1‰. In addition, there

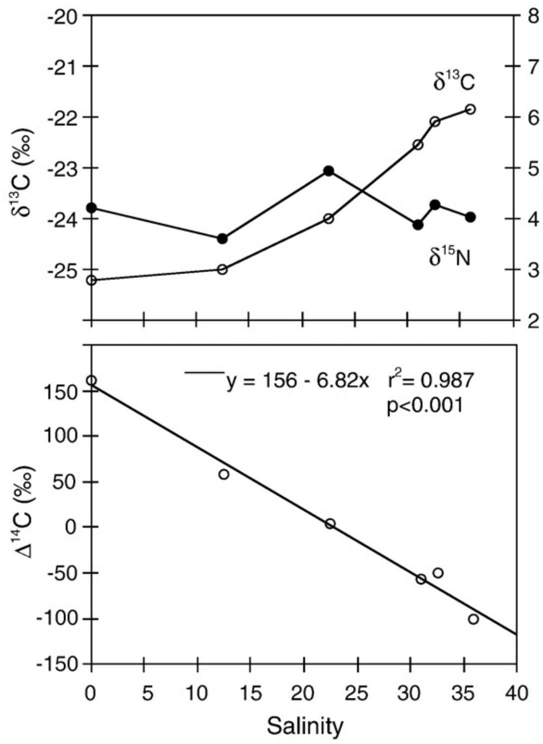

Fig. 3. Variations of stable isotope (δ^{13}C and δ^{15}N) and radiocarbon composition of HMW-DOM in the Mississippi River plume.

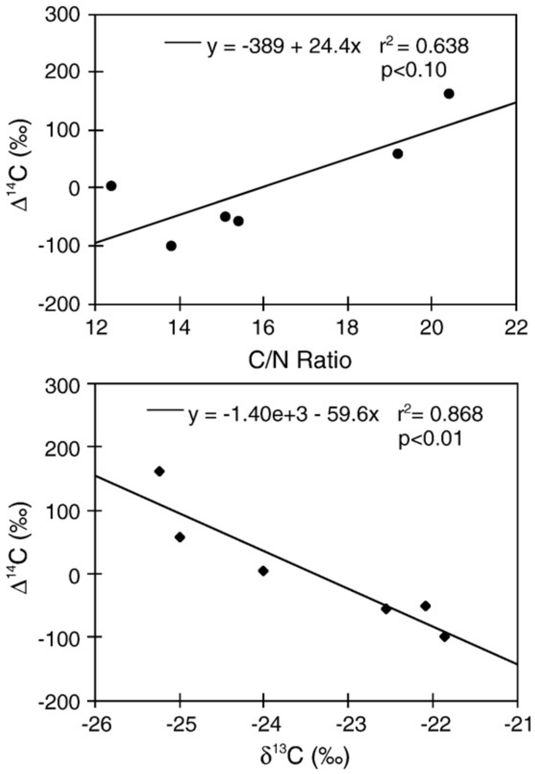

Fig. 4. Relationship between $\Delta^{14}C$ (‰) and $\delta^{13}C$ (or C/N ratio) of HMW-DOM in the Mississippi River plume.

were no significant differences in $\delta^{15}N$ values between HMW-DOM samples (Fig. 3). In contrast to stable C isotope composition, the $\delta^{15}N$ values changed little and did not show a consistent variation pattern along the salinity gradient in the Mississippi River plume, a pattern that is similar to that reported by Benner et al. (1992). While stable C isotopes are a quantitative tracer for organic carbon sources in estuarine environments, N isotopic composition appears to be less sensitive in elucidating sources of organic matter although nitrogen isotopes have been shown to be an excellent indicator in marine trophic transfer and the diagenetic status of organic matter resulting from microbial degradation (e.g., Schell et al., 1998). Little difference in $\delta^{15}N$ values between HMW-DOM samples between the Mississippi River and Gulf of Mexico end-member stations was likely related to the fact that DOM exported from the Mississippi River could contain more reprocessed or microbially altered organic matter than other natural rivers due to the damming effect and other human impacts from the upper stream (e.g., Duan et al., 2007).

3.4. Radiocarbon (^{14}C) composition

Results of radiocarbon composition, in terms of $\Delta^{14}C$ (‰) values and ^{14}C ages, are given in Table 4. Values of $\Delta^{14}C$ varied from $+162 \pm 1$‰ from the Mississippi River to -101 ± 1‰ at the offshore station in the plume. In general, values of $\Delta^{14}C$ in HMW-DOM decreased with increasing salinity in surface waters (Fig. 3), with relatively higher values at lower salinity stations and lower $\Delta^{14}C$ values at higher salinity stations, a pattern that is similar to that for other estuaries (Guo and Santschi, 1997; Guo et al., 2003). In addition, values of $\Delta^{14}C$ are correlated negatively with $\delta^{13}C$ values, but positively with C/N ratios (Fig. 4). This result indicates that HMW-DOM samples with higher $\Delta^{14}C$ values were accompanied by lower $\delta^{13}C$ values but higher C/N ratios at lower salinity stations. As salinity increased, HMW-DOM samples with lower $\Delta^{14}C$ values were accompanied by higher $\delta^{13}C$ values but lower C/N ratios in the Gulf of Mexico.

These $\Delta^{14}C$ values corresponded to a ^{14}C age ranging from contemporary (>modern) in the Mississippi River to 800 ± 45 years BP in the Gulf of Mexico. Contemporary ^{14}C age in HMW-DOM from Mississippi River and low salinity stations was consistent with that measured for the Trinity River, Texas (Guo and Santschi, 1997) and other rivers (Benner et al., 2004; Guo and Macdonald, 2006) and reflected the influence of terrestrial organic matter at lower salinity stations. However, the ^{14}C ages in HMW-DOM at higher salinity waters were up to several hundreds of years (370–800 years), which are much older than contemporary dissolved inorganic carbon (DIC) in surface oceanic waters (Druffel et al., 1996). One limitation of using radiocarbon comes from the long decay half-life of ^{14}C (5730 ± 40 years) and the resolution of varying time scales in DOM biogeochemical cycling in surface waters. Thus, these ^{14}C ages are indicators of mixing of organic carbon with different sources and ages rather than residence times of the DOM pool in the Mississippi River plume.

Based on elemental and molecular composition, HMW-DOM from surface waters at offshore stations contained more diagenetically younger organic materials such as carbohydrates and uronic acids (Fig. 2). The relatively older ^{14}C ages observed for offshore station surface water samples seemed inconsistent with the younger diagenetic status derived from chemical composition. This indicates that the HMW-DOM (>1 kDa) was a mixture of different size fractions of biopolymers with different radiocarbon signatures (Santschi et al., 1995; Guo and Santschi, 1997) and macromolecular biopolymers. Since ^{14}C ages of oceanic DIC in surface waters are contemporary (Druffel et al., 1996), older HMW-DOM than DIC points to a more reworked DOM component compared to freshly photosynthesized organic matter. This confirms our hypothesis that reworking (i.e., selective regeneration and recycling) of HMW-DOM is an important process in the estuarine mixing zone, in addition to mixing of end-member organic matter from river and marine sources, resulting from intensive transformation reactions concurrently occurring within both particulate and HMW-DOM fractions, resulting in an older than expected apparent ^{14}C age of the mixture of compounds in HMW-DOM.

To further test our hypothesis, an isotope mass balance approach was applied to the data to determine the fractional contribution of freshly photosynthesized organic matter and the extent of recycling or reworking of marine organic matter. The relative contributions of terrestrial (f_T) and marine organic matter (f_M) at a given station (or HMW-DOM sample) can be estimated using two end-member isotope mass balance equations, i.e.,

$$f_T + f_M = 1$$

and

$$\delta^{13}C = f_T \times \delta^{13}C_T + f_M \times \delta^{13}C_M$$

or

$$\Delta^{14}C = f_T \times \Delta^{14}C_T + f_M \times \Delta^{14}C_M.$$

Values of $\delta^{13}C$ and $\Delta^{14}C$ from the Mississippi River sample (Table 4) were used as the river end-member, while $\delta^{13}C$ and $\Delta^{14}C$ values (−21.60‰ and −177‰, respectively) from open Gulf of Mexico surface waters (Guo et al., 1996) were used as marine end-members. As shown in Table 4, the terrestrial contribution to HMW-DOM varied from 7% to 93% for March samples and 32% to 96% for August samples based on stable C isotope mass balance. However, terrestrial contributions estimated from the $\Delta^{14}C$ mass balance (numbers inside the parenthesis in Table 4) were significantly different from those derived from the $\delta^{13}C$ mass balance. Deviations between $\delta^{13}C$ and $\Delta^{14}C$ mass balance calculations likely reflect the selective and extensive reworking and

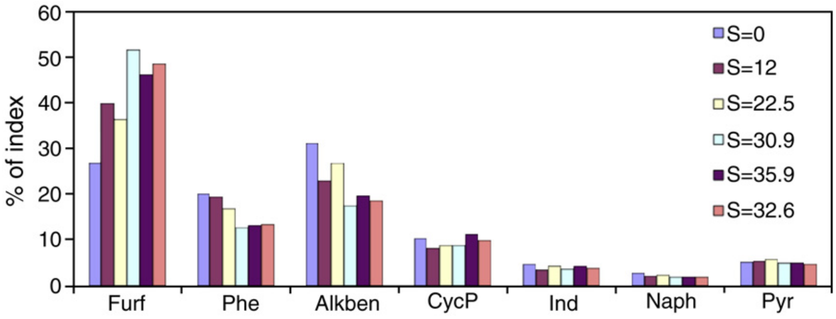

Fig. 5. Variations in the relative abundance (% in the total index) of major organic compounds in HMW-DOM as determined by pyrolysis-GCMS (Furf = Furfurals and methyl furfurals, Alkben = Alkyl-benzene, Phe = phones, CycP = Methyl cyclopentenone, Ind = Indene, Napth = Naphthalene, Pyr = Methyl pyridine).

transformation processes in the water column affected by sediment–water interactions.

Results of the isotope mass balance revealed that, in addition to sources from river and marine end-member organic matter, 10–25% of HMW-DOM could derive from reworked or regenerated DOM in the Mississippi River plume. Thus, colloidal or HMW-DOM are not simply derived from contemporary phytoplankton and terrestrial organic matter and reworking in the water column and/or sediment–water exchange processes could play an important role in affecting the isotope and chemical composition of HMW-DOM in estuarine and coastal environments. This effect is particularly noticeable at higher salinity waters, where $\Delta^{14}C$ values would predict a 20% higher terrestrial C contribution for HMW-DOM. Higher terrestrial OC contributions than by mixing processes alone are, however, possible by benthic resuspension and particle or colloid saltation processes.

3.5. Sample comparison based on py-GCMS analysis

Results of py-GC/MS are summarized in Fig. 5. Among all seven compound categories, furfurals, a pyrolysis product of polysaccharides (Bracewell et al., 1989), had the greatest abundance of index, ranging from ~27% in Mississippi River waters to ~50% at offshore stations in the Gulf of Mexico (Fig. 5). These values were derived from an index that is not intended to represent an actual percentage of the compounds in DOM. A lower abundance index for the furfurals in the river DOM sample agrees well with its terrestrial origins (van Heemst et al., 2000), while the greater abundance index for furfurals in DOM from the high salinity samples suggest marine-dominated DOM sources. These results are consistent with the high abundance of polysaccharides reported for HMW-DOM (e.g., Santschi et al., 1998).

The second highest abundance index was for the alkylbenzenes (Alkben), which comprised 17–22% of the total index, with a decreasing trend with increasing salinity. The initial organic compounds that produce alkylbenzenes during pyrolysis are mostly refractory organic components (e.g., van Heemst et al., 2000). Therefore, the decrease in the abundance index for alkylbenzenes with increasing salinity is consistent with the refractory and terrestrial nature of alkylbenzenes.

The third highest abundance index was for phenols, comprising from 13% to 20% in the Mississippi River plume (Fig. 5). The relative abundance of phenols also decreased with increasing salinity, indicating that phenols are also mostly derived from terrestrial sources. Phenols, including alklyphenols, are pyrolysis products from diverse sources including lignins, proteins, algal-derived polyphenols, and hydrolyzed polysaccharide/protein mixtures (van Heemst et al., 1999). Their abundance in terrestrial materials (Bracewell et al., 1989; van Heemst et al., 1999) seems consistent with the trends shown here.

Cyclopentenones are common in the pyrolyzates of aquatic organic matter. Bracewell et al. (1989) identified 2-cyclopenten-1-one as a product of polycarboxylic acids. Coban-Yildiz et al. (2000) lump cyclopentenones with furans as carbohydrate indicators; similarly, van Heemst et al. (1999) refer to cyclopentenones and furans as polysaccharide products. While the relative abundance index for furfurals increased with increasing salinity, the relative abundance of both alkylbenzenes and phenols decreased with increasing salinity. However, the relative abundance of cyclopentenones did not show a consistent pattern between HMW-DOM samples, suggesting that cyclopentenones also have other sources compared to furfurals, alkylbenzenes, and phenols in the Mississippi River plume (Fig. 5).

The relative abundance index for other organic compounds, including indene, naphthalene, and pyridine, was in general lower than 5% of the total index. Similar to cyclopentenones, the relative abundance of both indene and pyridine did not vary significantly between samples from different stations, although the relative abundance of naphthalene seemed to decrease with increasing salinity (Fig. 5).

Considering the relative abundance of the two major classes defined as aromatic compounds and sugars, one can see a consistent decrease in aromatic components from low salinity to high salinity stations (Fig. 6). Accompanying this decrease was an increase in the relative abundance of sugars from near-shore to offshore stations. Interestingly, there existed a significant negative correlation between % aromatic and $\delta^{13}C$ ($r^2 = 0.96$, $p < 0.001$; figure not shown) in the HMW-DOM. These results are consistent with those observed for uronic acid content and with conclusions derived from isotope composition and radiocarbon signatures (see previous section).

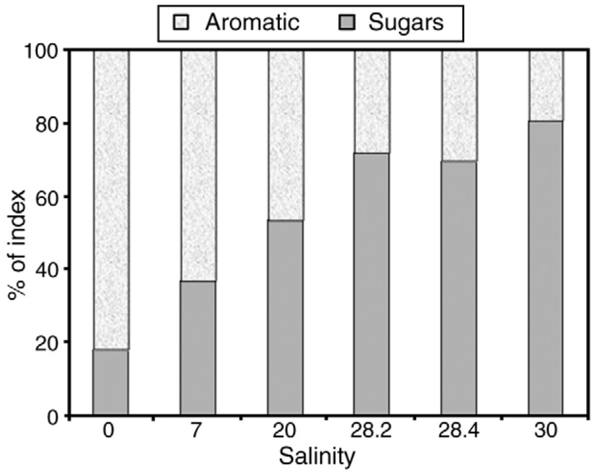

Fig. 6. Changes in the relative percentage of aromatic compounds and polysaccharides of HMW-DOM in the Mississippi River plume.

4. Summary and conclusions

Isolated HMW-DOM or colloids with sizes between 1 kDa and 0.2 µm were mostly organic in nature with total organic carbon and nitrogen content of up to 31–36% in weight for C and 1.8–3.3% for N. Yields of total carbohydrates, uronic acids, and total nitrogen increased with increasing salinity, whereas the content of TOC, C/N ratio and proteins decreased with increasing salinity. Changes in chemical composition of HMW-DOM suggest a predominant marine source for polysaccharides, with acid polysaccharides such as uronic acids being a more sensitive organic source tracer.

Values of δ^{13}C gradually increased with increasing salinity from −26.2‰ in river samples to −21.8‰ in coastal seawater samples, while values of Δ^{14}C linearly decreased with increasing salinity and N isotopic composition (δ^{15}N) changed little. Both stable C isotope and radiocarbon composition could be a quantitative organic source tracer in estuarine environments. However, isotopic mass balance calculations using δ^{13}C and Δ^{14}C gave different end-member source fractions. This deviation may result from intensive recycling of DOM in estuarine and coastal environments, especially through sediment–water interactions.

Consistent with DOM source variations in estuarine environments, the abundance index for furfurals (an indicative of polysaccharides and thus of OM freshness) increased with increasing salinity, whereas the percentage of index for phenols and the relative aromaticity decreased with increasing salinity. Py-GCMS results support our hypothesis that polysaccharides have predominantly marine sources. Combining chemical and isotopic characterization of HMW-DOM could improve our understanding of the biogeochemical cycling of estuarine and marine organic matter.

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