

Rainwater as a source of Fe(II)-stabilizing ligands to seawater

Joan D. Willey,¹ Robert J. Kieber Pamela J. Seaton, and Carrie Miller

Department of Chemistry and Biochemistry, University of North Carolina Wilmington, Wilmington, North Carolina 28403-5932

Abstract

Rainwater hydrophobic extractable dissolved organic matter (EDOM) contains ligand(s) that completely prevent Fe(II) oxidation for at least 4 h after rain is mixed with seawater. The EDOM Fe(II) complex is at least of comparable strength to the ferrozine complex, indicating that this rainwater Fe(II) ligand is among the strongest Fe(II) ligands ever observed in natural waters. In addition to the strong class of ligands that prevent oxidation, rainwater EDOM also contains weaker Fe(II) ligands that slow oxidation of Fe(II) in seawater. Rainwater EDOM is not a single molecule but rather a complex mixture of relatively hydrophobic compounds, even in marine rain with minimal continental influence. When EDOM from the nearby Cape Fear River was extracted using the same method as for rainwater, the river EDOM could not prevent or even slow the oxidation of Fe(II) on mixing with seawater. Therefore, rainwater EDOM is fundamentally different than surface-water EDOM with respect to the strength of Fe(II) ligands. The stability of EDOM complexed Fe(II) most likely affects the bioavailability of rainwater-derived Fe in the surface ocean because the length of time atmospherically deposited Fe remains dissolved in seawater is critical to its role as a phytoplankton nutrient.

There is tremendous interest in the distribution and cycling of iron in seawater because of its role as a limiting nutrient in certain regions of the world's oceans. The speciation of iron in seawater is of particular interest because speciation determines stability and residence time and hence affects bioavailability. Unusually high and unexplained concentrations of Fe(II) are occasionally observed in seawater even though many experiments and calculations indicate that Fe(II) should be quickly oxidized by hydrogen peroxide to Fe(III) at the pH of seawater (Moffett and Zika 1987; Millero and Sotolongo 1989). Nannomolar concentrations of Fe(II) were observed during spring plankton blooms several years in a row in oxic surface waters near Japan, perhaps produced by photochemical reduction of Fe(III) transported from sediments (Kuma et al. 1996). Fe(II) was also observed in bottom waters near Peru (Hong and Kester 1986), near the chlorophyll maximum in the Equatorial Pacific (O'Sullivan et al. 1991), in the surface waters of the North Sea (Gledhill et al. 1995), in surface water in the Boston Harbor (Zhuang et al. 1995), and in coastal seawater around Okinawa Island (Okada et al. 2005). Fe(II) can be photochemically produced from Fe(III); however, not all these studies were in surface seawater.

Unexpectedly high concentrations of Fe(II) have also been observed during iron enrichment experiments. Persis-

tent (5 d) nmol L⁻¹ concentrations of Fe(II) after a fourth iron addition in the Southern Ocean Iron Release Experiment in February 1999 were reported, perhaps resulting from photochemical production from Fe(III), slow oxidation (cold temperatures 3°C), low concentrations of H₂O₂ (10 nmol L⁻¹), and possible organic complexation of Fe(II) (Croot et al. 2001). During another Southern Ocean iron addition experiment in the summer of 2000 (EisenEx), high levels of Fe(II) (tenths of nmol L⁻¹) were observed up to 8 d after iron additions (Croot et al. 2005). This was attributed to cold temperatures, limited vertical mixing, and reduction of Fe(III) by superoxide. The authors also suggested that the high Fe(II) concentrations might result from intense rainfall during the latter part of this experiment. They were able to collect one rain sample using trace metal clean conditions in which they determined that total dissolved iron (TFe) was 260 ± 20 nmol L⁻¹. This high concentration was explained by back-trajectory calculations that indicated that the air mass traveled from southern Patagonia and hence probably contained iron-rich dust. They reported a minimum rainwater concentration of 40 ± 20 nmol L⁻¹ Fe(II). The rain event collected by Croot et al. (2005) during the EisenEx Experiment was obtained in early summer (27 November 2000) between 15:30 h and 18:00 h. The iron concentration in this precipitation is consistent with data generated in the summer of 1999 for rainwater collected on the South Island of New Zealand (Kieber et al. 2001a). For rains collected between noon and 18:00 h, Kieber et al. (2001a) found a volume-weighted average concentration of 240 nmol L⁻¹ for total iron and 62 nmol L⁻¹ for Fe(II), indicating that the sample collected by Croot et al. (2005) 1 yr later during EisenEx was representative of rain over the Southern Ocean during this season and time of day.

Fe(II) concentrations that are higher than expected have therefore been observed in several marine environments by investigators working in different laboratories using various analytical techniques. Although analysis for Fe(II)

¹ Corresponding author (willeyj@uncw.edu).

Acknowledgments

The Marine and Atmospheric Chemistry Research Laboratory at the University of North Carolina Wilmington assisted with sampling and analyses. Joshua Dixon, Kelly Gordon, Andrew McGinnis, Lorri Resetar, and Jason Williams helped with the extractions and experiments. The authors would like to thank two anonymous reviewers who made constructive and insightful comments on an earlier version of this work.

This work was supported by National Science Foundation grants ATM-0342420 and ATM-0646153.

in seawater at low concentrations is difficult, it seems unlikely all these observations could be attributed to analytical artifacts. A recent intercalibration study of dissolved Fe (DFe) in seawater (not Fe[II]) with analysts from 19 laboratories gave encouraging analytical results for seawater for most but not all analytical methods used. Analysis of a seawater sample with approximately 0.9 nmol L⁻¹ yielded almost all results in the 0.9 ± 0.2 nmol L⁻¹ range, and even a 0.1 nmol L⁻¹ DFe had results ± 0.05 nmol L⁻¹ (Johnson et al. 2007), although several analysts reported concentrations twice the assigned concentration.

Our previous research indicated that when rainwater is mixed with seawater, with resulting elevated pH values, the concentration of rainwater Fe(II) remains unchanged even after 4 h of storage (Kieber et al. 2001b). This stability with respect to oxidation is destroyed by exposure to high-intensity ultraviolet (UV) light, suggesting that rainwater Fe(II) is protected against oxidation by complexation with an organic ligand (Kieber et al. 2001b). Similar results were obtained when rainwater collected from the Bermuda Atlantic Time Series Station (BATS) was mixed with seawater from the same site, suggesting that the stability of rainwater-deposited Fe(II) in seawater is a widespread phenomenon occurring even at remote marine locations (Kieber et al. 2003b).

Despite the profound significance of an Fe(II)-stabilizing ligand in rainwater to the biogeochemistry of seawater, virtually nothing is known regarding the nature of the ligand(s). Our objective in this study was to investigate and characterize a series of potential Fe(II)-stabilizing ligands present in rainwater as a source of stable Fe(II) to seawater. We specifically address the role of hydrophobic extractable dissolved organic matter (EDOM), which is a significant fraction (12–75%) of the dissolved organic carbon (DOC) in precipitation (Gordon, 2006), as a source of ligands that can stabilize rainwater Fe(II) on mixing with seawater. This fraction was chosen because it excludes many organic compounds present in high concentrations in rainwater that are not good ligands for Fe(II), such as small organic acids and aldehydes (Willey et al. 2000a). Understanding the mechanisms that contribute to the long lifetime of rainwater Fe(II) in surface seawater is critical to understanding the relative importance of rainwater Fe(II) to the stimulation of primary productivity in the open ocean.

Methods

Sampling—Rainwater samples were collected on an event basis at the University of North Carolina Wilmington, a coastal location in southeastern North Carolina (34°13.9'N, 77°52.7'W). Four Aerochem-Metrics (ACM) Model 301 Automatic Sensing Wet/Dry Precipitation Collectors were used to collect event rain samples. All glassware used for sampling, filtration, storage, extraction, and experiments was baked at 550°C for 6 h prior to use to remove organics. One of these ACM contained a 4-liter Pyrex glass beaker from which samples for dissolved organic carbon were collected. The remaining three ACM collectors were set up using trace metal-clean procedures

and consisted of a high-density polyethylene (HDPE) funnel connected by Tygon® FEP-lined tubing to a 2.2-liter Teflon bottle (Kieber et al. 2006). Rain samples were filtered under low vacuum through 0.2-μm, acid-washed Gelman Supor® polysulfonone filters enclosed in a glass filtration apparatus immediately on collection.

Extraction—Hydrophobic dissolved organic material was extracted from 50- or 500-mL rainwater samples or 500-mL deionized water blank (MilliQ Plus Ultra-pure water system, >18.2 MΩ, indicated as DI) using C₁₈ cartridges (WAT020515, Waters Chromatography) (Kieber et al. 2006). C₁₈ cartridges were conditioned prior to use by washing twice with 5 mL methanol followed by twice with 5 mL DI. Samples for extraction were then loaded onto C₁₈ cartridges. The cartridges were washed twice with 5 mL of DI water to remove residual salts, and the bound constituents were eluted two times with 3 mL of methanol. Samples were pulled through C₁₈ cartridges by house vacuum, eluted into 25-mL round-bottom flasks, and concentrated to dryness under reduced pressure (Buchi Rotavapor, Model RE 111). This method was chosen because earlier studies have demonstrated that C₁₈ extraction is better able to retain the UV-visible and fluorescence characteristics of isolated chromophoric organic material relative to XAD (Amador et al. 1990). When the column-retained material was eluted and reconstituted in synthetic rainwater (SRW; pH 4.5 H₂SO₄ made by diluting 5 mM H₂SO₄ with DI), followed by 30 s of sonication to ensure dissolution, the patterns of absorbance and 3D fluorescence were identical to the original rain, indicating that the optical properties did not change on extraction. Fluorescence and absorbance blanks comprised of DI were negligible. The dissolved organic material (DOM) extracted and recovered by this method is called extractable DOM, or EDOM. The fraction of this that is fluorescent is called chromophoric EDOM. The fluorescent DOM in whole, unextracted rain is referred to as chromophoric DOM, or CDOM, consistent with earlier studies.

Fluorescence—CDOM and chromophoric EDOM optical properties and abundance were determined by excitation-emission matrix (EEM) fluorescence spectroscopy measured on a Jobin Yvon SPEX Fluoromax-3 scanning fluorometer equipped with a 150-W Xe arc lamp and a R928P detector as described in Kieber et al. (2006). EEMS were constructed every 4 nm from 250 to 550 nm using excitation wavelengths from 250 to 500 nm (Del Castillo and Coble 1999). The abundance of CDOM in rainwater was quantified by integration of the fluorescence in the excitation emission spectrum (total integrated fluorescence) (Kieber et al. 2006). Replicate scans were within 5% agreement in terms of intensity and within band-pass resolution in terms of peak location (Kieber et al. 2006). Fluorescence intensity was measured relative to a quinine bisulfate standard and is expressed in quinine sulfate equivalent units (QSE).

NMR—Liquid-phase ¹H-NMR spectra were obtained on a Bruker Avance 400-MHz NMR spectrometer using an

inverse gradient probe. NMR preparations involved adding 0.60 mL of D₂O to a completely dried EDOM extract in a 25-mL round-bottom flask followed by sonication for several minutes to ensure complete dissolution of EDOM. An internal standard (20 μ L of 187 μ mol L⁻¹ 3-[trimethylsilyl]-1-propanesulfonic acid-d₆, sodium salt [DSS]) was then added to the sample in the NMR tube and the sample sonicated for 5–10 s. The trimethylsilyl peak of DSS was calibrated to 0 ppm, integrated to 1.0, and set to a height of 10 cm for comparison of samples. Blanks prepared with deionized water in place of the rain event demonstrated that there was very little signal due to the extraction method or sample preparation. More analytical details can be found in Kieber et al. (2006). Analysis of ¹H-NMR spectra provided information regarding hydrogen distributions and major functional groups present in EDOM (Leenheer et al. 1997; Decesari et al. 2000, 2005).

Fe(II) analyses—Fe(II) was determined by a modification of the spectrophotometric ferrozine method (Stookey 1970; Kieber et al. 2003a). Ferrozine (10 mmol L⁻¹), a reagent that reacts with Fe(II) to form a colored complex, was added and thoroughly mixed with the sample followed by addition of pH 5.5 ammonium acetate buffer to adjust pH. Absorbances of the iron ferrozine complex were read at 562 nm using a 1-m (Ocean Optics) or 5-m (World Precision Instruments) liquid wave-guide capillary cell attached to an Ocean Optics SD2000 spectrophotometer and an Analytical Instrument Systems Model DT 1000 CE UV/Vis Light Source. Absorbance measurements were referenced to a nonabsorbing wavelength (700 nm) in order to compensate for turbidity and instrument drift. The relative standard deviation for absorbance readings for a 20 nmol L⁻¹ standard measured 10 times on different days during a 1-month time period was <1% for Fe(II). A conservative detection limit based on three times the signal-to-noise ratio for Fe(II) is 0.4 nmol L⁻¹. Deionized water run through the entire collection and filtration system had undetectable Fe (Willey et al. 2005).

Fe(II) addition/mixing studies—In all experiments Fe(II) was added as FeCl₂ from a secondary Fe(II) stock (10 μ mol L⁻¹; pH = 4, prepared by diluting 10 mmol L⁻¹ FeCl₂·4H₂O). Samples of rainwater were spiked to a final concentration of 250 nmol L⁻¹ Fe(II) by addition to 0.2- μ m-filtered rainwater. There was no measurable change in pH of samples after Fe addition, indicating that any changes observed were not due to differences in hydrogen ion concentrations. Samples were allowed to incubate for 1 h in the presence of the Fe(II) spike to allow for complete complexation. The fluorescence of Fe(II) spiked samples was then compared to analogous unaltered samples in order to determine the effect of Fe(II) on rainwater fluorescence.

Seawater mixing experiments were conducted by mixing equal volumes of seawater and various types of rainwater and then monitoring Fe(II) concentrations for 4-h storage at room temperature and in the dark. The 4- time period clearly showed the difference in reactivity between rainwater additions and controls, so it was an appropriate time period for these experiments. Gulf Stream or outer continental shelf

seawater was collected off southeastern North Carolina for these experiments. The salinity was between 30 and 36 with a pH of 8.2 and DOC between 90 and 100 μ mol L⁻¹. After mixing with rainwater, the salinity was between 15 and 18, the pH was approximately 8.1, and the DOC varied depending on the initial concentration in the rainwater. Stability experiments were conducted using EDOM reconstituted in synthetic rain with no added Fe(II). Reconstituted samples were spiked with H₂O₂ to a final concentration of 20 μ mol L⁻¹ and then mixed with seawater. This was done to see if the Fe(II)-stabilizing ligands are a component of the EDOM fraction of rainwater. One whole unaltered rain sample was run for comparison with the reconstituted EDOM. The control for these experiments was SRW with 40 nmol L⁻¹ Fe(II) and 20 μ mol L⁻¹ H₂O₂ with no added EDOM. Additional experiments were performed to see if ferrozine could act as a model ligand to stabilize rainwater Fe(II) after mixing with seawater. Synthetic rain with 100 nmol L⁻¹ Fe(II) and ferrozine (5, 20, or 200 μ mol L⁻¹) was spiked to contain 20 μ mol L⁻¹ H₂O₂ and then mixed with seawater in these experiments.

Supporting data—Hydrogen peroxide analyses were done using a scopoletin-induced fluorescence decay technique (Kieber and Helz 1986; Kieber et al. 2001a). Inorganic anions were determined by ion suppressed chromatography. Dissolved organic carbon was analyzed using a Shimadzu TOC 5000 (Willey et al. 2000a). Absorbance scans were made from 240 to 800 nm using 10-cm Suprasil cuvettes on a Varian Cary 1E dual-beam spectrophotometer (2-nm slit width) (Kieber et al. 2006). Back-trajectory analysis of storm path was done using the Hysplit method described elsewhere (Kieber et al. 2005).

Results and discussion

Rainwater composition—Dissolved organic carbon in rainwater is composed of more than 300 different compounds (Seitzinger et al. 2003). Organic acids (acetic, formic, lactic, oxalic, pyruvic, malonic, maleic, succinic, methyl sulfonic, and C 6, 9, and 10 oxoacids) contribute 40% of the DOC in rainwater in this region with small aldehydes and alcohols contributing another 10% (Willey et al. 2000a; Magolan 2005). None of these compounds are good ligands for Fe(II), especially in the presence of nanomolar concentrations of Fe(III) and micromolar calcium and magnesium. Theis and Singer (1974) reported stabilization of Fe(II) with glutamate and tannic acid in laboratory studies mimicking surface water; however, stability studies using rainwater conditions indicate that glutamate, methyl sulfonic acid, thiourea, oxalate, and tannic acid cannot prevent oxidation of Fe(II) in solutions with pH and hydrogen peroxide concentrations typical of rain received in Wilmington, North Carolina (Jacobs 2000; Smith 2003). Seawater-mixing experiments demonstrate that the amino acids glycine, cysteine, serine, and histidine at 1 μ mol L⁻¹ also cannot stabilize 100 nmol L⁻¹ Fe(II) against oxidation when mixed with seawater (Smith 2003). The question remains as to what is the nature of the rainwater Fe(II) ligand.

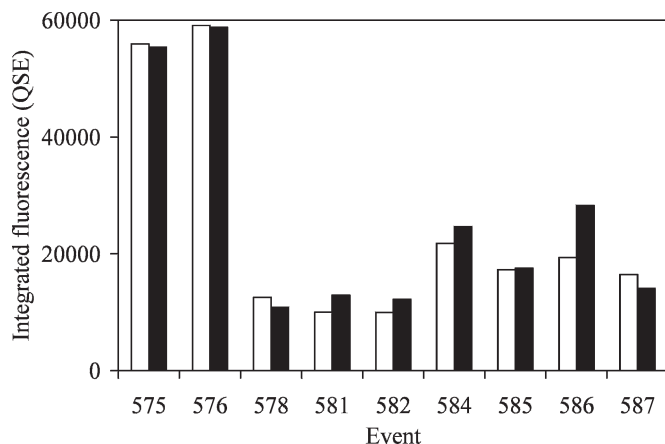


Fig. 1. Total integrated fluorescence (QSE) for nine rain samples (white bars) and after addition of 250 nmol L⁻¹ Fe(II) added as FeCl₂ (black bars).

Earlier studies have demonstrated that significant quantities of hydrophobic and chromophoric organic material exist in rainwater, aerosols, fog, and cloud water, and these compounds have some structural similarity to aquatic humic and fulvic compounds in surface waters but in other respects are very different (Graber and Rudich 2006; Kieber et al. 2006; Duarte et al. 2007). A rainwater composition modeling study suggested that humic-like substances may be able to complex with both Fe(II) and Fe(III) in rain; however, results were very dependent on the values for formation constants entered into the model (Willey et al. 2000b). Inclusion of humic like substances as a rainwater component was necessary to explain observed apparent quantum yields for photoproduction of Fe(II) from Fe(III) (Kieber et al. 2003a), again suggesting the importance of hydrophobic and chromophoric organic compounds as metal ligands in rain. Despite the documented importance of humic material, when EDOM from the nearby Cape Fear River was extracted using the same method as for rainwater, the river EDOM could not prevent or even slow the oxidation of Fe(II) on mixing with seawater (Jacobs 2000). Therefore, rainwater EDOM is fundamentally different than surface-water EDOM with respect to the strength of Fe(II) ligands in this geographical region.

Fe(II) reaction with CDOM—Comparison of total integrated fluorescence values before and after addition of 250 nmol L⁻¹ Fe(II) to nine rainwater samples (whole, not extracted) was used to determine whether complexation between Fe(II) and rainwater CDOM occurred. This concentration was chosen because it is approximately 10 times higher than ambient Fe(II) concentrations and would essentially normalize Fe(II) concentrations in all samples. When inorganic Fe(II) was added to rainwater, the total integrated fluorescence increased in four samples, decreased in two, and did not change in three others (Fig. 1). The change in fluorescence greater than the analytical uncertainty of 5% indicates that complexation between Fe(II) and excess CDOM ligand sites occurred. A decrease in fluorescence would be expected if Fe(II) acted as a weak fluorescence quencher. A fluorescence increase would occur if the

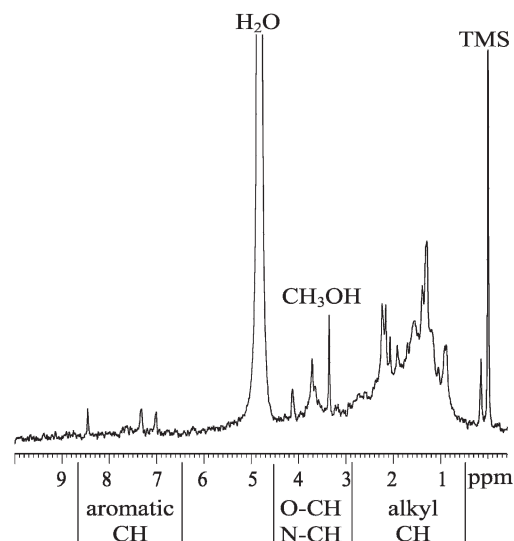


Fig. 2. ¹H-NMR spectra of EDOM in a marine rain. The peaks from residual water and solvent methanol, and the trimethylsilyl peak (TMS) from the standard DSS are shown.

relatively high concentrations of Fe(II) displaced a stronger fluorescence quencher present in lower concentrations such as Cu(II) (Willey 1984; Witt et al. 2007). The absence of a fluorescence change suggests that the Fe(II) ligand sites in these rains were already saturated with ambient Fe(II), allowing no additional complexation with added iron. The fluorescence changes observed in Fig. 1 demonstrate that at least some of the ligands important for binding Fe(II) in rainwater are in the CDOM fraction of DOC.

NMR of extracted EDOM—The ¹H-NMR spectra of EDOM from a marine rain (event 659, 05 September 2006, 12 mm of rain) appears as a relatively continuous and broad distribution of signals, suggesting the presence of complex mixtures of compounds, with the majority of the signal coming from alkyl protons (Fig. 2). There are also several small peaks in the aromatic region, which is important because structures that play a role in complexing Fe(II) would most likely have protons that would absorb in these regions. Compounds such as polyphenols (Theis and Singer 1974) and phthalic acid residues (Santana-Casiano et al. 2004), which complex and stabilize Fe(II) in solution, would give aromatic signals similar to the small peaks between 6.9 and 8.5 ppm in this rain EDOM NMR spectrum. Nitrogen containing heterocycles, such as the strong Fe(II)-chelating phenanthrolines, would also give peaks in the aromatic region. Additionally, there are signals in the region (3.5–4.5 ppm) of protons bound to carbons that bear electron withdrawing atoms, such as oxygen or nitrogen (O-CH, N-CH). Compounds such as the siderophores, glutamine, and aminocarboxylates (Theis and Singer 1974; Santana-Casiano et al. 2000), which stabilize Fe(II) against oxidation in certain solutions, would also show signals in this region. The NMR spectrum of rainwater EDOM presented in Fig. 2 is important because it suggests the presence of structures capable of binding and stabilizing Fe(II) in marine rain.

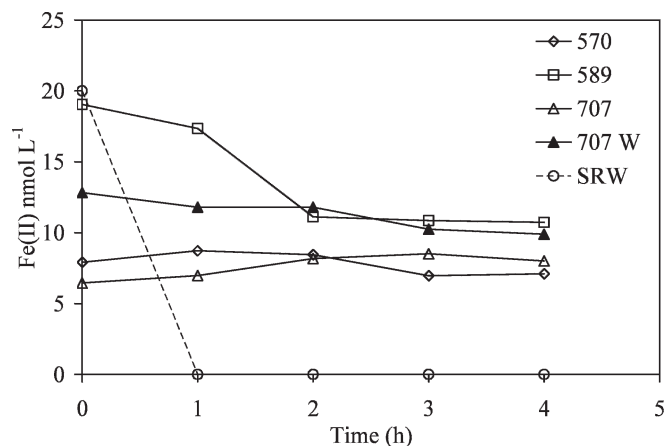


Fig. 3. Concentration of Fe(II) (nmol L^{-1}) as a function of time (h) after mixing coastal NC seawater in a 1:1 ratio (equal volumes of synthetic rainwater and seawater) for three reconstituted EDOM samples (570, 589, and 707) and one whole rain (707 W). Synthetic rainwater (SRW) with 40 nmol L^{-1} added Fe(II) and $20 \text{ }\mu\text{mol L}^{-1}$ H_2O_2 but no added EDOM (open circles and dashed line) was mixed with seawater for use as a control.

The presence of potential Fe(II)-stabilizing compounds in this rain sample is noteworthy because this was a marine rain with a very low DOC ($22 \text{ }\mu\text{mol C L}^{-1}$). A previous extensive study of DOC in marine rain found that $23 \text{ }\mu\text{mol C L}^{-1}$ was the background concentration for remote marine rain, including rain collected from the southwest coast of New Zealand that originated over the Southern Ocean (Willey et al. 2000a). The abundance of NMR peaks in EDOM from this very low DOC marine rain suggests that this would be a minimum for what would be expected for terrestrial, coastal, or mixed rain events.

Rainwater and seawater mixing—Previous experiments suggest that rainwater Fe(II) can be stabilized in seawater by an undefined strong organic complex that can be destroyed by high-energy UV oxidation (Kieber et al. 2001b). This is particularly important to oceanographic iron studies because the significance of atmospherically deposited iron to seawater depends in part on its residence time after deposition. Earlier studies with marine systems have demonstrated that Fe(II) oxidation can be slowed by organic complexation (Santana-Casiano et al. 2000; Rose and Waite 2003; Santana-Casiano et al. 2004); however, the rainwater ligand(s) is unique because it completely stops oxidation for at least 4 h.

A set of experiments were performed in which EDOM from three different rain events, 570, 589 and 707, was reconstituted in SRW to their preextraction volumes, H_2O_2 was added, and then the SRW was mixed with seawater in order to determine if EDOM could provide stabilizing ligands for Fe(II) (Fig. 3). Whole, unextracted rain sample 707 was used for comparison with EDOM 707 (Fig. 3). SRW with added Fe(II) and H_2O_2 but no added EDOM was mixed with seawater and used as a control (Fig. 3). The whole-rain 707 and the EDOM from 707 and 570 had no change in Fe(II) over the 4-h storage period. The SRW control had undetectable Fe(II) before the first measure-

ment at 1 h. The Fe(II) concentration in the 589 EDOM decreased during the first 2 h but then remained at the same concentration for the subsequent 2 h (Fig. 3).

All Fe(II) in the control with no added EDOM oxidized within the first hour after mixing, indicating that the ligand protecting Fe(II) against oxidation was in EDOM, not in seawater. The pattern presented in Fig. 3 with reconstituted EDOM is identical to previous experiments in which whole (unextracted) rain was mixed with Gulf Stream seawater, coastal seawater, estuarine water obtained off the southeast coast of North Carolina (Kieber et al. 2001b), and seawater from the Bermuda Atlantic Time Series Station (Kieber et al. 2003b), suggesting that the ligands protecting Fe(II) from oxidation in previous studies were contained in the EDOM fraction of rainwater.

The degree of Fe(II) stabilization depended to some extent on the relative abundance of rainwater CDOM and initial Fe(II) concentration. The total integrated fluorescence of the EDOM SRW solution prior to mixing with seawater was 33,700 QSE in event 570 and 24,800 in event 707 but was only 10,000 in event 589. The lower abundance of chromophoric EDOM in event 589 in combination with a higher initial Fe(II) concentration resulted in oxidation of approximately half the initial iron in this rain event. Once this fraction of Fe(II) in event 589 oxidized, there was sufficient ligand available to complex the remaining Fe(II), stabilizing it against further oxidation.

The slow oxidation rate of Fe(II) in event 589 during the first 2 h (Fig. 3) suggests that rainwater EDOM contains weaker Fe(II) ligands that slow oxidation in addition to the strong class of ligands that prevent oxidation. Two classes of copper-complexing ligands were observed in rain from this location in a previous study (Witt et al. 2007). This is also similar to the conclusion reached in a recent study exploring dissolved Fe-binding ligands in the Scheldt estuary in the Netherlands, where the authors concluded that in addition to strong Fe-binding organic ligands ($\log K' = 19\text{--}22$), weaker Fe-binding ligands also existed ($\log K'$ varying between 11 and 12) that altered the kinetics of iron precipitation in this system (Gerringa et al. 2007).

Ferrozine as a model ligand—Experiments were conducted using ferrozine (Fz), a known complexing agent for Fe(II) used in the spectrophotometric analysis of iron, to see if ferrozine could serve as a model ligand for EDOM ligand(s) with respect to providing stability against oxidation of Fe(II) when mixed with seawater. If Fe(II) remained uncomplexed in this experiment, it would rapidly oxidize to Fe(III) on mixing with seawater; however, if Fe(II) was bound to ferrozine, it should be stable. The $5 \text{ }\mu\text{mol L}^{-1}$ ferrozine concentration used in this experiment was chosen to reflect the carbon content in EDOM from a typical rainwater sample containing 100 nmol L^{-1} Fe(II). A rainwater sample with 100 nmol L^{-1} Fe(II) usually has between 100 and $200 \text{ }\mu\text{mol C L}^{-1}$ as DOC (Willey et al. 2005). Assuming a rainwater DOC of $200 \text{ }\mu\text{mol C L}^{-1}$, the concentration of EDOM would be approximately $100 \text{ }\mu\text{mol C L}^{-1}$. Ferrozine contains 20 carbon atoms, so this concentration of DOC would be equivalent to $5 \text{ }\mu\text{mol L}^{-1}$ as ferrozine. When synthetic rainwater prepared

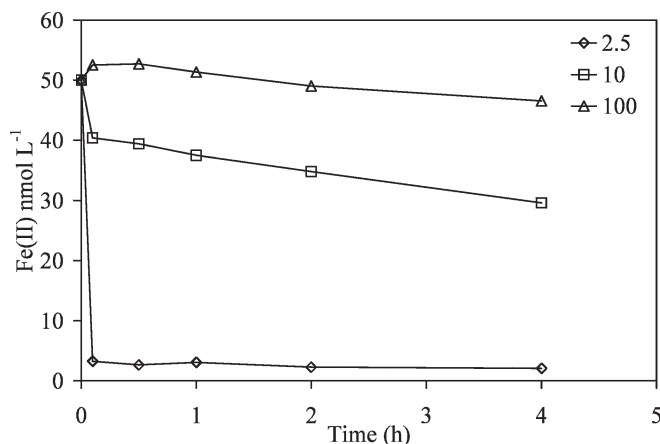


Fig. 4. Concentration of Fe(II) (nmol L⁻¹) as a function of time (h) after mixing Gulf Stream seawater from offshore NC with synthetic rain in a 1:1 ratio (equal volumes of synthetic rainwater and seawater). The synthetic rain and seawater mixture composition immediately after mixing was 50 nmol L⁻¹ Fe(II), ferrozine concentrations as indicated (μmol L⁻¹), and 10 μmol L⁻¹ H₂O₂. The lowest ferrozine concentration (2.5 μM) did not prevent oxidation of Fe(II); higher ferrozine concentrations were required to stabilize Fe(II) at pH 8.1 after mixing with seawater.

containing 5 μmol L⁻¹ ferrozine, 100 nmol L⁻¹ Fe(II), and 20 μM H₂O₂ was mixed with seawater, the Fe(II) concentration decreased rapidly to less than 5 nmol L⁻¹ from an initial concentration of approximately 50 nmol L⁻¹ Fe(II) (Fig. 4). This concentration of ferrozine was not sufficient to stabilize Fe(II) in the same way as the EDOM ligand(s). When the ferrozine concentration was increased to 200 μmol L⁻¹, the Fe(II) remained in the seawater mixture for 4 h; however, there was a small decrease in Fe(II) over time (Table 1).

The FeFz₃ complex has a formation constant of 10^{+15.53} (Gibbs 1976). This experiment suggests that the EDOM ligand(s) form a complex with Fe(II) of at least similar strength to ferrozine. Precise quantitative comparison of the formation constant for the EDOM complex with the FeFz₃ complex cannot be made because the stoichiometry and concentration of the EDOM complex are not known. The presence of a strong ligand(s) in EDOM is consistent with previous rainwater research at this location that has shown that approximately 25% of the total dissolved iron (Fe(II) plus Fe(III)) in rain cannot be detected by analysis with ferrozine until organic ligands are destroyed by oxidation with intense UV light (Kieber et al. 2005), which indicates the presence of very strong iron ligands in rainwater. In addition, previous rainwater research at this location has shown the presence of Cu-binding ligands with binding constants in the range 10¹³–10¹⁶, similar to FeFz₃ (Witt et al. 2007).

When ferrozine was added as a ligand to the chemical speciation program Mineql+ (Version 4.5, 1998) and the ionic strength set at half that of seawater (0.35 eq L⁻¹) as appropriate for these experiments after mixing, the speciation results obtained were consistent with those observed in Fig. 4 (Table 1). In the 5 μmol L⁻¹ ferrozine solution, the free Fe²⁺ ion dominated (Table 1) and the Fe(II) oxidized (Fig. 4), but at higher ferrozine concentrations, most or all

the Fe(II) was present as Fe(Fz)₃, and little oxidation was predicted or observed (Table 1; Fig. 4). The presence of the Fe(Fz)₃ complex results in the stabilization of Fe(II) observed in Fig. 4. Results of this model also demonstrate that the interactions between ferrozine and Fe(II) can explain results observed in Fig. 4, suggesting that competition with other seawater ions for ferrozine and competition for Fe(II) by seawater ligands were negligible.

Ferrozine	Model prediction	Experimental results, 2 h	Experimental results, 4 h
2.5	5	5	4
10	77	70	60
100	100	98	93

Summary and future work—This study demonstrates for the first time that the organic ligand(s) responsible for stabilization of Fe(II) deposited by rainwater to seawater is contained within the C₁₈-extractable hydrophobic class of compounds in rainwater referred to as EDOM (Fig. 3). Seawater with no added rain ligands does not contain a sufficient ligand concentration to stabilize rainwater Fe(II). Humic substances extracted from the a local river using the same extraction procedure as for rain could not stabilize Fe(II); hence, rainwater EDOM is fundamentally different than surface-water EDOM or seawater with respect to Fe(II)-binding capability. The inability of ferrozine at a realistic carbon concentration in rain to stabilize Fe(II) on mixing with seawater (Fig. 4) suggests that the EDOM Fe(II) complex is at least of comparable strength to ferrozine and specific for Fe(II) relative to seawater ions. Rainwater EDOM hence contains one of the strongest Fe(II) ligand(s) reported in any natural water to date. The variable Fe(II) stability observed for EDOM from rain event 589 in Fig. 3 indicates that there is more than one class of Fe(II) ligands in EDOM. Future work will focus on elucidation of the chemical composition, sources and photoreactivity of rainwater ligands that form strong complexes with Fe(II) and other trace metals.

References

- AMADOR, J. A., P. J. MILNE, C. A. MOORE, AND R. G. ZIKA. 1990. Extraction of chromophoric humic substances from seawater. *Mar. Chem.* **29**: 1–17.
- CROOT, P. L., AND OTHERS. 2001. Retention of dissolved iron and Fe(II) in an iron induced Southern Ocean phytoplankton bloom. *Geophys. Res. Lett.* **28**: 3425–3428.
- , AND OTHERS. 2005. Spatial and temporal distribution of Fe(II) and H₂O₂ during EisenEx, an open ocean mesoscale iron enrichment. *Mar. Chem.* **95**: 65–88.

- DECESARI, S., M. C. FACCHINI, AND S. FUZZI. 2000. Characterization of water soluble organic compounds in atmospheric aerosols: A new approach. *J. Geophys. Res.* **105**: 1481–1489.
- , ———, G. B. MCFIGGANS, H. COE, AND K. N. BOWER. 2005. The water soluble organic component of size segregated aerosol, cloud water and wet deposition from Jeju Island during ACE Asia. *Atmos. Environ.* **39**: 211–222.
- DEL CASTILLO, C. E., AND P. G. COBLE. 1999. Analysis of the optical properties of the Orinoco River Plume by absorption and fluorescence spectroscopy. *Mar. Chem.* **66**: 35–51.
- DUARTE, R. M. B. O., E. B. H. SANTOS, C. A. PIO, AND A. C. DUARTE. 2007. Comparison of structural features of water-soluble organic matter from atmospheric aerosols with those of aquatic humic substances. *Atmos. Environ.* **41**: 8100–8113.
- GERRINGA, L. J. A., M. J. A. RIJKENBERG, H. T. WOLTERBEEK, T. G. VERBURG, M. BOYE, AND H. J. W. DE BAAR. 2007. Kinetic study reveals weak Fe binding ligand which affects the solubility of Fe in the Scheldt estuary. *Mar. Chem.* **103**: 30–45.
- GIBBS, C. R. 1976. Characterization and application of FerroZine iron reagent as a ferrous iron indicator. *Anal. Chem.* **48**: 1197–1201.
- GLEDHILL, M., M. G. CONSTANT, AND C. M. G. VANDEN BERG. 1995. Measurement of the redox speciation of iron in seawater by catalytic cathodic stripping voltammetry. *Mar. Chem.* **50**: 51–61.
- GORDON, K. 2006. Composition and sources of chromophoric dissolved organic matter in rainwater. M.S. thesis, Univ. of North Carolina Wilmington.
- GRABER, E. R., AND Y. RUDICH. 2006. Atmospheric HULIS: How humic-like are they? A comprehensive and critical review. *Atmos. Chem. Phys.* **6**: 729–753.
- HONG, H., AND D. R. KESTER. 1986. Redox state of iron in the offshore waters of Peru. *Limnol. Oceanogr.* **31**: 512–524.
- JACOBS, B. A. 2000. Factors affecting iron speciation in remote marine rain. M.S. thesis, Univ. of North Carolina Wilmington.
- JOHNSON, K. S., AND OTHERS. 2007. Developing standards for dissolved iron in seawater. *EOS* **88**: 131–132.
- KIEBER, R. J., D. R. HARDISON, R. F. WHITEHEAD, AND J. D. WILLEY. 2003a. Photochemical production of Fe(II) in rainwater. *Environ. Sci. Technol.* **37**: 4610–4616.
- , AND R. G. HELZ. 1986. Two method verification of hydrogen peroxide determinations in natural waters. *Anal. Chem.* **58**: 2312–2315.
- , M. S. LONG, AND J. D. WILLEY. 2005. Factors influencing nitrogen speciation in coastal rainwater. *J. Atmos. Chem.* **52**: 81–99.
- , B. PEAKE, J. D. WILLEY, AND B. JACOBS. 2001a. Iron speciation and hydrogen peroxide concentrations in New Zealand rainwater. *Atmos. Environ.* **35**: 6041–6048.
- , R. F. WHITEHEAD, J. D. WILLEY, S. REID, AND P. J. SEATON. 2006. Chromophoric dissolved organic matter (CDOM) in rainwater collected in southeastern North Carolina, USA. *J. Atmos. Chem.* **54**: 21–41.
- , J. D. WILLEY, AND G. B. AVERY. 2003b. Temporal variability of rainwater iron speciation at the Bermuda Atlantic Times Series Station. *J. Geophys. Res.* **108**: 33-1–33-7, doi:10.1029/2001JC001031.
- , K. H. WILLIAMS, J. D. WILLEY, S. A. SKRABAL, AND G. B. AVERY. 2001b. Iron speciation in coastal rainwater: Concentration and deposition to seawater. *Mar. Chem.* **73**: 83–95.
- KUMA, K., J. NISHIOKA, AND K. MATSUNAGA. 1996. Controls on iron(III) hydroxide solubility in seawater: The influence of pH and natural organic chelators. *Limnol. Oceanogr.* **41**: 396–407.
- LEENHEER, J. A., T. I. NOYES, AND R. L. WERSHAW. 1997. Acquisition and interpretation of liquid state ¹H NMR spectra of humic and fulvic acids, p. 295–303. *In* M. A. Nanny, R. A. Minear and J. A. Leenheer [eds.], Nuclear magnetic resonance spectroscopy in environmental chemistry. Oxford University Press.
- MAGOLAN, K. M. 2005. Analysis of methanol, ethanol and propanol in aqueous environmental matrices. M.S. thesis, Univ. of North Carolina Wilmington.
- MILLERO, F., AND S. SOTOLONGO. 1989. The oxidation of Fe(II) with H₂O₂ in seawater. *Geochim. Cosmochim. Acta* **53**: 1867–1873.
- MOFFETT, J. W., AND R. G. ZIKA. 1987. Reaction kinetics of hydrogen peroxide with copper and iron in seawater. *Environ. Sci. Technol.* **21**: 804–810.
- OKADA, K., H. NAKAJIMA, H. FUJIMURA, T. ARAKAKI, A. TANAHARA, AND T. OOMORI. 2005. Photochemical behavior of Fe(II) in coastal seawater around Okinawa Island by HPLC with a ferrozine reagent. *Bunseki Kagaku* **54**: 861–867.
- O'SULLIVAN, D. W., A. K. HANSON, W. L. MILLER, AND D. R. KESTER. 1991. Measurement of iron(II) in surface water of the equatorial Pacific. *Limnol. Oceanogr.* **36**: 1727–1741.
- ROSE, A. L., AND T. D. WAITE. 2003. Kinetics of iron complexation by dissolved natural organic matter in coastal waters. *Mar. Chem.* **84**: 85–103.
- SANTANA-CASIANO, J. M., M. GONZALEZ-DAVILA, M. J. RODRIGUEZ, AND F. MILLERO. 2000. The effect of organic compounds in the oxidation kinetics of Fe(II). *Mar. Chem.* **70**: 211–222.
- , ———, AND ———. 2004. The oxidation of F(II) in NaCl-HCO₃⁻ and seawater solutions in the presence of phthalate and salicylate ions: a kinetic model. *Mar. Chem.* **27**: 27–40.
- SEITZINGER, S. P., R. M. STYLES, R. LAUCK, AND M. M. MAZUREK. 2003. Atmospheric pressure mass spectrometry: A new analytical chemical characterization method for dissolved organic matter in rainwater. *Environ. Sci. Technol.* **37**: 131–137.
- SMITH, B. J. 2003. Iron speciation in coastal rainwater: Oxidation kinetics and organic complexation. M.S. thesis, Univ. of North Carolina Wilmington.
- STOOKEY, L. C. 1970. Ferrozine—a new spectrophotometric reagent for iron. *Anal. Chem.* **42**: 779–781.
- THEIS, T. L., AND P. C. SINGER. 1974. Complexation of iron(II) by organic matter and its effect on iron(II) oxygenation. *Environ. Sci. Technol.* **8**: 569–573.
- WILLEY, J. D. 1984. The effect of seawater magnesium on natural fluorescence during estuarine mixing and implications for tracer applications. *Mar. Chem.* **15**: 19–45.
- , R. J. KIEBER, M. S. EYMAN, AND G. B. AVERY. 2000a. Rainwater dissolved organic carbon: Concentrations and global flux. *Global Biogeochem. Cycles* **14**: 139–148.
- , R. J. KIEBER, K. H. WILLIAMS, J. S. CROZIER, S. A. SKRABAL, AND G. B. AVERY. 2000b. Temporal variability of iron speciation in coastal rainwater. *J. Atmos. Chem.* **37**: 185–205.
- , R. F. WHITEHEAD, R. J. KIEBER, AND D. R. HARDISON. 2005. Oxidation of Fe(II) in rainwater. *Environ. Sci. Technol.* **39**: 2579–2585.
- WITT, M., S. A. SKRABAL, R. J. KIEBER, AND J. D. WILLEY. 2007. Copper complexation in coastal rainwater in southeastern USA. *Atmos. Environ.* **41**: 3619–3630.
- ZHUANG, G., Z. YI, AND G. T. WALLACE. 1995. Iron (II) in rainwater, snow, and surface seawater from a coastal environment. *Mar. Chem.* **50**: 41–50.

Received: 11 June 2007

Accepted: 13 February 2008

Amended: 31 March 2008