

Phase partitioning and solubility of iron in natural seawater controlled by dissolved organic matter

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[1] The phase partitioning and solubility of Fe as well as its relationship with marine dissolved organic matter (DOM) in different natural seawater and phytoplankton cultures were examined using radiotracer and ultrafiltration techniques to better understand Fe biogeochemical cycling and its biological availability in the ocean. Fe solubility in seawaters was related to the filter's cutoff, with the Fe solubility in the $<3 \times 10^3$ amu fraction being about one third of that in the $<10 \times 10^3$ amu filtrate. The Fe solubility decreased from estuarine to coastal to oceanic seawater and then to DOM-free seawater. There was a significant linear relationship between Fe solubility or [FeL] concentration and the dissolved organic carbon concentration for the seawater of different origins tested, suggesting that Fe solubility was largely controlled by the amount of dissolved organic matter. In addition, Fe solubility was significantly enhanced by the presence of fresh phytoplankton exudates, indicating that the nature of organic ligands also plays an important role in controlling the Fe solubility in seawater. Most of the Fe-bound organic ligands were in the size fraction $<10 \times 10^3$ amu and decreased from the estuarine to the coastal and then to the oceanic seawater. Among the standard macromolecular organic compounds examined, siderophores (deferrioxamine mesylate and ferrichrome) showed the highest binding capacity for Fe, and carrageenan (a high molecular weight-sulfated acid polysaccharide) also slightly increased Fe solubility. Complexation of organic ligands with Fe appeared to be Fe-specific or Fe preferential. Our results highlight quantitatively the importance of DOM in controlling Fe solubility in seawater. Further studies are needed to elucidate the interrelationship between the biogeochemical cycles of Fe and the chemistry of DOM in the ocean. *INDEX TERMS*: 4805 Oceanography: Biological and Chemical: Biogeochemical cycles (1615); 4807 Oceanography: Biological and Chemical: Chemical speciation and complexation; 4809 Oceanography: Biological and Chemical: Colloids; 4835 Oceanography: Biological and Chemical: Inorganic marine chemistry; *KEYWORDS*: dissolved organic carbon, iron solubility, phase partitioning, iron partitioning, organic ligands, Fe solubility

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1. Introduction

[2] Fe is the fourth most abundant element in the Earth's crust, but the solubility of Fe and its bioavailable concentrations in marine environments are very low because of its highly hydrolysis nature even though Fe complexes strongly with natural organic matter [Stumm and Morgan,

1981; van den Berg, 1995]. Fe can limit primary production not only in the high-nitrate low-chlorophyll (HNLC) regions of the open ocean [Martin *et al.*, 1994; Coale *et al.*, 1996; Boyd *et al.*, 2000] but also in the coastal environments [Hutchins and Bruland, 1998; Bruland *et al.*, 2001; Hutchins *et al.*, 2002]. Knowledge of Fe phase and chemical speciation in seawater is indispensable for a better understanding of its geochemical cycling in the ocean and its biological availability to marine organisms. Fe exists in seawater in a continuous size spectrum, ranging from truly dissolved to low molecular weight ligand-complexed, high molecular weight ligand-complexed, and colloidal and particulate Fe. The size distri-

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bution of Fe and its relationship to biological availability and the fate and transport of Fe in the ocean remain poorly understood, mainly because of its extremely low concentration in seawater. Recent studies have demonstrated that colloidal or high molecular weight-complexed Fe, which represents the majority of the filterable Fe in seawater, is much less available to phytoplankton than its low molecular weight counterparts [Chen and Wang, 2001; Chen et al., 2003; Wang and Dei, 2003]. However, how marine dissolved organic matter (DOM) affects the size distribution and the apparent solubility of Fe and thus its bioavailability is still unclear.

[3] Thermodynamic calculations indicate that Fe occurs predominantly as Fe(III) in organic-free and oxygenated seawater, which can strongly hydrolyze to form $\text{Fe}(\text{OH})_3$ and $\text{Fe}(\text{OH})_2^+$ at pH 8 [Millero, 1998; Byrne et al., 2000]. The solubility of the Fe oxyhydroxide $\text{Fe}(\text{OH})_3$ in seawater largely depends on the formation of $\text{Fe}(\text{OH})_3$ and the filter cutoffs. Higher solubility (~ 0.08 nM [Wu et al., 2001]) was found for freshly formed amorphous $\text{Fe}(\text{OH})_3$ as compared to aged $\text{Fe}(\text{OH})_3$ using a 0.02 μm filter (~ 0.01 nM [Liu and Millero, 2002]). It is also likely that the different Fe solubilities measured in various studies may have resulted from differences in the actual pore size of the 0.02 μm filters used (M. Chen et al., unpublished data, 2003). Currently, the definition of “dissolved” Fe is highly operational. For example, the use of a 0.02 μm filter in the determination corresponds to a molecular weight cutoff of over 2000×10^3 amu and thus can contain a considerable fraction of colloidal material. On the other hand, a 10×10^3 or a 1×10^3 amu filter cutoff may give rise to a much lower Fe solubility than those measured with a <0.02 μm filtrate.

[4] Because the concentrations of soluble Fe hydrolysis species in seawater are only <2 pM [Gledhill and van den Berg, 1994; Rue and Bruland, 1995; van den Berg, 1995], Fe solubility in seawater is largely the result of its complexation with organic ligands. Evidence suggests that $>90\%$ of the dissolved Fe in seawater is complexed with organic ligands of unknown origin in the North Sea [Gledhill and van den Berg, 1994], the western Mediterranean [van den Berg, 1995], the North Pacific [Rue and Bruland, 1995], the northwest Atlantic [Wu and Luther, 1994], and the equatorial Pacific [Rue and Bruland, 1997]. These ligands are present at very low concentrations (nM levels) in seawater but can effectively form metal-specific complexes with very high conditional stability constants. It has been proposed that low molecular weight siderophores ($<1 \times 10^3$ amu) are one of the strongest Fe-binding ligands in seawater [Wilhelm et al., 1996; Rue and Bruland, 1997; Martinez et al., 2001]. However, the immediate sources of strong Fe-complexing ligands in seawater are not well understood. Marine prokaryotes are known to release siderophore molecules but only under certain conditions [Reid et al., 1993]. Nakabayashi et al. [2002] determined the Fe solubility (<0.025 μm) and dissolved Fe concentration (<0.2 μm) inside and outside a highly productive patch in the northwestern North Pacific Ocean and concluded that natural organic Fe(III) chelators may be released by dominant phytoplankton or bacteria species. There is a

consensus that natural organic matter regulates Fe solubility in seawater and thus Fe bioavailability and carbon sequestration in the ocean. However, the interactions of Fe with different classes of natural organic compounds and the mechanisms that control the solubility and transformation of Fe species are not well understood.

[5] The objectives of this study were to determine the Fe solubility and its phase partitioning in seawater using ^{59}Fe radiotracers and in controlled laboratory experiments and to examine the complexation of Fe with organic compounds with different molecular weights and functionalities, including natural marine organic matter, phytoplankton exudates, and model organic compounds. We considered seawaters from different representative areas, including estuarine, coastal, and oceanic environments.

2. Materials and Methods

2.1. Collection of Seawaters

[6] The surface seawaters to be used in subsequent laboratory experiments were collected from different regions representing estuarine, coastal, and oceanic environments. Yuen Long seawater from Hong Kong (YL, salinity of 20) with a heavy influence from the Pearl River Estuary plume can be considered representative of estuarine waters. Tolo Harbor (TH) and Clearwater Bay (CWB), also from Hong Kong, with a salinity of 32 and 33, respectively, were considered representative of coastal environments. Oceanic seawater (PW) from the Pacific Ocean was collected from the equatorial Pacific, $00^{\circ}00'36''\text{S}$ and $145^{\circ}00'0''\text{E}$. The seawater was drawn into acid-cleaned polyethylene bottles in the field and passed through a 0.22 μm polycarbonate membrane in a class 100 clean bench as soon as the samples arrived in the laboratory. It took 1–2 hours to transport the estuarine and coastal water samples from the field to the laboratory. The oceanic sample was prefiltered onboard ship upon sampling and passed through the 0.22 μm polycarbonate membrane again before the experiments.

2.2. Phase Speciation of Fe in Seawater

[7] Chelex 100 resin and UV irradiation were used to remove background Fe and dissolved organic carbon (DOC) to produce Fe- and DOC-free seawater for the laboratory experiments. Fe removal efficiency was quantified by passing the radiotracer ^{59}Fe through a Chelex 100 resin column. Dissolved organic carbon, including any organic ligands bound with Fe, was destroyed by UV irradiation. The concentration of DOC was measured during the UV oxidation to ensure that most of the background DOC was destroyed before the experiments.

[8] Four treatments were designed to evaluate the influences of organic matter on Fe solubility and phase partitioning: (1) prefiltered seawater (PSW, <0.22 μm), (2) prefiltered seawater with UV oxidation (UV-PSW), (3) prefiltered seawater passed through a Chelex 100 column (Chelex-PSW), and (4) prefiltered seawater sequentially treated with Chelex 100 resin and UV irradiation (Chelex-UV-PSW) (Table 1). For the preparation of the UV-PSW, 500 mL of prefiltered seawater was placed in a 1 L quartz

Table 1. Salinity and Concentrations of Dissolved Organic Carbon (DOC) Used in the Experiments^a

Experiment	Source of Seawater	Treatments	Salinity	DOC, μM
1	Yuen Long (estuarine)	PSW	20	443
		UV-PSW	20	133
		Chelex-PSW	20	416
		Chelex-UV-PSW	20	133
2	Tolo Harbor (coastal)	PSW	32	158
		UV-PSW	32	15
		Chelex-PSW	32	162
		Chelex-UV-PSW	32	25
3	Clearwater Bay (coastal)	PSW	33	99
		UV-PSW	33	<2
		Chelex-PSW	33	92
		Chelex-UV-PSW	33	3
4	Pacific (oceanic)	PSW	36	79
		UV-PSW	36	9
		Chelex-PSW	36	82
		Chelex-UV-PSW	36	9
5	Algal exudates (biogenic)	Chelex-3H	33	61
		Chelex-UV-3H	33	<2
		Chelex-Syn	33	77
		Chelex-UV-Syn	33	7
6	Clearwater Bay (coastal)	Chelex-PSW	33	94
		Chelex-PSW plus Cu	33	94
		Chelex-PSW plus Zn	33	94

^aPSW is prefiltered seawater (<0.22 μm), UV-PSW is prefiltered seawater with UV oxidization, Chelex-PSW is prefiltered seawater after passing through a Chelex 100 column, Chelex-UV-PSW is prefiltered seawater sequentially treated with Chelex 100 resin and UV irradiation, 3H is diatom *Thalassiosira pseudonana*, and Syn is cyanobacteria *Synechococcus* sp. Seawater was irradiated with UV for 24 hours.

reactor and irradiated for 24 hours (450 W, ACE UV lamp, Model 7480). For the Chelex-PSW treatment, 1 L of prefiltered seawater (<0.22 μm) was passed through a Chelex 100 resin column (3 cm diameter, 10 cm height, at $\sim 3 \text{ mL min}^{-1}$) to remove any background Fe in the seawater. The first 100 mL of effluents was discarded to ensure a consistent quality of treated seawater for laboratory experiments. DOC concentrations before and after passing through the resin were also monitored to evaluate the possible DOC change resulting from column chromatographic effects. After the Chelex 100 resin treatment, aliquots of the Fe-free seawater were placed in the quartz reactor for further UV irradiation to remove any residual DOC (Chelex-UV-PSW treatment).

[9] The dissolved concentrations of ⁵⁹Fe(III) hydroxide in the PSW, UV-PSW, Chelex-PSW and Chelex-UV-PSW were determined by filtration and γ -activity counting. Briefly, 0.4 μCi of radioactive ferric ⁵⁹Fe (corresponding to 0.44 nmol Fe, New England Nuclear) solution was spiked with a predetermined amount of stable ferric Fe and added to acid-cleaned 250 mL Teflon beakers containing 100 mL of the various treated seawater samples. A predetermined amount of 0.1 N NaOH was added to keep the pH constant. The final Fe concentration after the Fe addition was 10 nM. For each treatment a 3 mL sample was taken for the determination of the initial total ⁵⁹Fe radioactivity. All treatments were then kept in the dark at room temperature. At 2, 6, 12, and 24 hours a 3 mL sample aliquot was filtered through either a 0.2 μm filter (Whatman), a 10×10^3 amu centrifugal membrane, or a 3×10^3 amu centrifugal membrane (Millipore). In addition, another 3 mL sample was taken for total ⁵⁹Fe measurement at each time interval. The samples were acidified with

0.5 mL of 1 N HCl to prevent possible adsorption of Fe(III) on the collecting vials. ⁵⁹Fe radioactivity in the total sample and the filtrates was measured by gamma spectrometry at 1092 keV.

[10] A 0.02 μm filter was not used in the Fe size fractionation experiments because the actual cutoff of the 0.02 μm Anotop syringe filter (Whatman) was considerably different from its rated pore size of 0.02 μm or $\sim 2000 \times 10^3$ amu (M. Chen et al., unpublished data, 2003). This manufacture-rated 0.02 μm membrane was measured to have an actual cutoff of $\sim 3 \times 10^3$ amu when using fluorescein tagged macromolecular compounds for cutoff calibrations [Guo et al., 2000].

2.3. Solubility Experiments Using Phytoplankton Exudates

[11] The diatom *Thalassiosira pseudonana* and the cyanobacterium *Synechococcus* sp. were obtained from the Provasoli-Guillard Phytoplankton Collection Center, Maine, United States, and maintained in axenic culture in an *f/2* medium [Guillard and Ryther, 1962]. Cells in the exponential growth phase were filtered through a 3 or 1 μm polycarbonate membrane, rinsed with prefiltered UV-irradiated seawater, and resuspended in DOC-free CWB prefiltered seawater for further incubation. After the cells had reached the stationary phase at 18°C under light illumination of 70 $\mu\text{mole photons m}^{-2} \text{ s}^{-1}$, they were filtered through a 0.22 μm polycarbonate membrane. The filtrates were passed through Chelex 100 resin to remove any background Fe. A portion of the Chelex-treated medium was further placed in the quartz reaction cell for UV irradiation. The DOC concentrations in the filtrate and the pretreated seawater were then measured. The partitioning of

Table 2. Standard Macromolecular Compounds Used to Examine the Fe Complexation With Organic Ligands of Different Functionalities

Chemical Compounds	Type	Molecular Weight, amu	Catalog Number
Deferoxamine mesylate	siderophore	656.8	Sigma, D9533
Ferrichrome	siderophore	687.7	Sigma, F-8014
Protoporphyrin	phorphyrin	562.7	Sigma, P-8293
Carrageenan	acid polysaccharides	—	Sigma, C-4014
L-leucine	leucine	131.2	Sigma, L-8912
D-glucose	glucose	180.2	Gibco BRL, 50-99-7

Fe in the Chelex-treated and Chelex-UV-treated algal exudates was then determined using ^{59}Fe tracers as described in section 2.2.

2.4. Influences of Model Organic Compounds on Fe Solubility and Phase Partitioning

[12] The model organic compounds considered in this study included deferoxamine mesylate, ferrichrome, protoporphyrin, carrageenan, L-leucine, and D-glucose. The molecular weight of each compound and other relevant information is shown in Table 2. The concentration of each organic compound was 1 mg L^{-1} . A stock solution (100 mg L^{-1}) of each chemical compound was prepared in Fe- and DOC-free seawater, which was obtained from natural seawater collected from Clearwater Bay ($S = 33$) by filtering through a $0.22 \mu\text{m}$ polycarbonate membrane and further ultrafiltering through a 1×10^3 amu stirred cell membrane (YM1, Millipore). The permeate solution was then passed through Chelex 100 resin to remove the background Fe, followed by UV irradiation. The DOC concentration was $<2 \mu\text{M}$ after the UV irradiation.

2.5. Specificity of Fe-Complexing Organic Ligands Against Cu and Zn Complexation

[13] To test the specificity of the organic ligands for Fe complexation against other trace metals such as Cu and Zn, we compared the Fe solubility in samples prespiked with Cu and Zn with samples without Cu and Zn. CuCl_2 and ZnCl_2 stock solutions (1 mM) were prepared first with DOC-free CWB prefiltered seawater. CWB prefiltered seawater pretreated with Chelex 100 resin was used as the solvent. These seawater samples were saturated with Cu or Zn for 12 hours to give a final concentration of 10 nM . On the basis of our data (see section 3) the total ligand binding with Fe was $\sim 1.5 \text{ nM}$, thus the amount added (10 nM) substantially exceeded the ligand concentrations. Fe concentrations were compared to a control treatment with only CWB prefiltered seawater pretreated with Chelex 100 resin.

2.6. Radioactivity and Organic Carbon Measurements

[14] At the end of the experiments any ^{59}Fe adsorbed on the Teflon beakers was removed by rinsing with 1 N HCl and then gamma counted to quantify the wall sorption during the experiments. All experiments were performed in duplicate inside a class 100 clean bench. The DOC concentration was measured using a total organic carbon analyzer (Shimadzu TOC Vcph). Radioactivity of ^{59}Fe was measured using a Wallac gamma counter. The counting time

was adjusted to ensure that the statistical errors in counting were lower than 10%.

3. Results

3.1. DOC and Fe Removal Efficiency by UV Irradiation and Chelex 100 Resin

[15] To assess the destruction efficiency of DOC by UV irradiation, we monitored the change of DOC concentration in the CWB prefiltered seawater. Following UV irradiation, the concentration of DOC decreased exponentially initially but decreased slowly after ~ 10 hours and reached the detection limit after 24 hours (data not shown). The results showed that the DOC destruction efficiency of UV irradiation varied in different seawaters with different organic chemical composition (Table 1). For example, after 24 hours of irradiation the DOC destruction efficiency was almost 100% for the Clearwater Bay seawater and algae exudates, whereas it was 89, 91, and 70% for the Pacific, Tolo Harbor, and Yuen Long seawaters, respectively (Table 1). The estuarine seawater exhibited the highest resistance to UV oxidation. These results strongly suggest the importance of quantifying the DOC concentration after UV irradiation.

[16] Using ^{59}Fe as a tracer, the Fe removal efficiency of Chelex 100 resin was determined by measuring the radioactivity before and after passing through the resin column. Phytoplankton exudates were used in this experiment since the concentrations of organic ligands were higher than the natural seawater (see section 3.2). The equilibration time for the adding ^{59}Fe with organic ligands was 2 hours. In this experiment, $91 \pm 1\%$ of the spiked ^{59}Fe in the seawater was removed by the Chelex 100 resin, suggesting that most Fe, including the Fe-ligand complexes, are labile enough to be extracted by the Chelex 100 resin. In contrast, the DOC concentration (Table 1) did not vary significantly after passing through the resin, indicating that Chelex 100 resin did not noticeably remove organic matter from the seawater.

[17] A mass balance for the Fe in each experiment was calculated based on the recovery of spiked Fe from the experimental system. The recovery percentage of Fe ranged from 88 to 105% (averaging 96%), except in the Chelex-PSW treatment with CWB seawater, in which the recovery percentage was only 77%. The overall good mass balance indicated that adsorption of Fe onto beakers and membranes during the experimental procedures was minimal.

3.2. Phase Partitioning of Fe in Different Seawaters

[18] The size-fractionated species of Fe was quantified using the percent of Fe in different pore-size filtrates, since

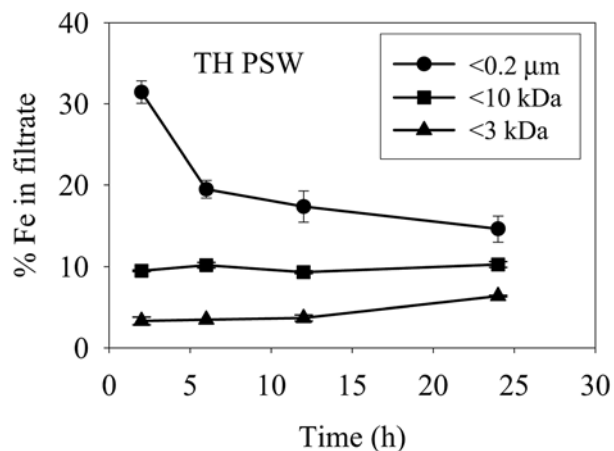


Figure 1. Variations of the percent of Fe in the filtrates of different sized membranes in seawater from Tolo Harbor (TH PSW) over time. Data are mean plus or minus semirange ($n = 2$).

the added total Fe concentrations were 10 nM for all treatments. The percent of Fe in the filtrates was compared at different equilibrium times (2, 6, 12, and 24 hours) to evaluate the variations in Fe phase partitioning. Because the trends in all treatments and experiments over time were the same, only the time series data from Tolo Harbor PSW are given (Figure 1). After Fe was added to the seawater, the percent of Fe in the $<10 \times 10^3$ and $<3 \times 10^3$ amu filtrates was relatively constant, whereas the concentrations in the $<0.2 \mu\text{m}$ filtrate decreased rapidly within the first 6 hours and then started to level off. The decrease of Fe in the $0.2 \mu\text{m}$ filter-passing fraction over time may have resulted largely from the coagulation/precipitation and other physicochemical processes. Since the percent of Fe in both the 10×10^3 and 3×10^3 amu membrane-passing fractions was relatively constant, a reaction time of 2 hours was chosen in subsequent experiments to avoid possible artifacts associated with long experimental periods.

[19] The percentages of Fe in the filtrates decreased with decreasing filter pore size for all treatments (Figure 2), indicating that Fe solubility depended on the filter pore size. When comparing the percent of Fe in the filtrates with the same pore size from different seawater, it was evident that the percent of Fe in the filtrates decreased from the estuarine to the coastal and then to the oceanic seawater. Thus the concentration of Fe in the filtrate increased with increasing DOC concentration. For example, for the Chelex-treated prefiltered seawater, the percent of Fe in the $<3 \times 10^3$ amu filtrates decreased from 15% in Yuen Long water to 4.8% in Tolo Harbor water, 4.5% in Clearwater Bay water, and then to 2.3% in Pacific water.

[20] Differences in Fe solubility between the UV-treated and the non-UV-treated samples were also observed (Figure 2). In most cases the percentage of Fe in the filtrates after UV irradiation was lower than in those without UV irradiation, regardless of the size fraction. For example, the percents of Fe in the $<10 \times 10^3$ amu filtrates from Yuen Long seawater were 27 and 19% for the PSW and UV-PSW

treatments and 49 and 29% for Chelex-PSW and Chelex-UV-PSW treatments, respectively. Again, UV-treated seawater had a lower Fe solubility or percentage in all filter-passing fractions. The only exception occurred with

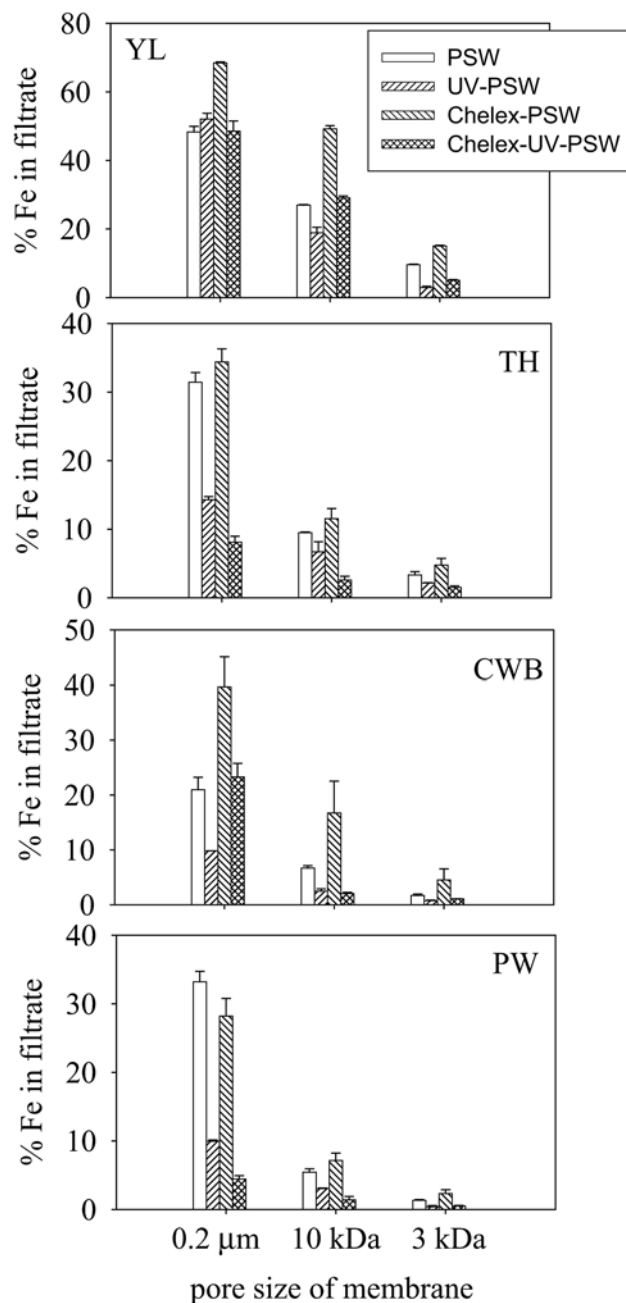


Figure 2. Fe partitioning (percent of total) in different filter-passing fractions of seawater: YL, Yuen Long; TH, Tolo Harbor; CWB, Clearwater Bay; PW, oceanic seawater from the Pacific Ocean; PSW, prefiltered seawater ($<0.22 \mu\text{m}$); UV-PSW, prefiltered seawater with UV oxidation; Chelex-PSW, prefiltered seawater passed through a Chelex 100 column; and Chelex-UV-PSW, prefiltered seawater sequentially treated with Chelex 100 resin and UV irradiation. Data are mean plus or minus semirange ($n = 2$).

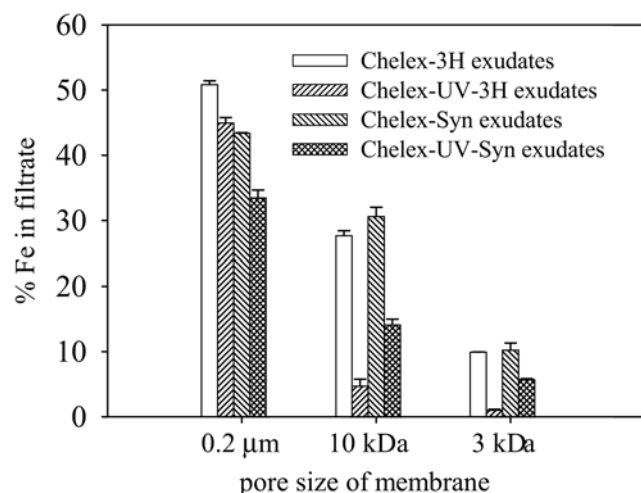


Figure 3. Fe partitioning (percent of total) in different filter-passing fractions in treatments containing exudates of the diatom *Thalassiosira pseudonana* (3H) and cyanobacteria *Synechococcus* (Syn). Data are mean plus or minus semirange ($n = 2$).

the Yuen Long seawater, which had similar percentages in the 0.2 μm filtrates after PSW and UV-PSW treatment. It seems that the UV treatment did not efficiently destroy the Fe-binding ligands in this sample.

[21] For all seawaters the Fe solubility (or the percentages of Fe in the filtrates) was higher in samples pretreated with Chelex 100 resin (Chelex-PSW) (Figure 2), indicating that background Fe decreased the solubility of added Fe, especially in estuarine and coastal seawater that contained relatively high background Fe concentrations. Although we did not directly measure the background Fe concentrations, it is reasonable to assume that the Fe concentrations in the nearshore waters were higher than those in the open ocean. For Pacific seawater the difference in the percent of Fe in the filtrates between the PSW and the Chelex-PSW treatments was small as a result of the low Fe concentration in the initial seawater. Although there were different Fe background concentrations among the original seawaters, the percent of Fe in the Chelex-PSW treatment filtrates can be compared because the background Fe had been removed by the Chelex 100 resin. Regardless of the pore size of the

membrane the percent of Fe in the filtrate decreased from estuarine (YL) to coastal (TH and CWB) and then to PW (Figure 2), consistent with the decreasing DOC concentration from YL to TH and CWB and to PW (Table 1).

[22] The DOC concentrations produced by the marine diatom *Thalassiosira pseudonana* and the cyanobacteria *Synechococcus* during the log growth phase were 61 and 77 μM, respectively. Although the DOC concentrations in the algal cultures were lower than those in natural seawater (Table 1), the percent of Fe in the filtrates was higher than in coastal or oceanic seawater and was comparable to that in estuarine seawater (Figure 3). There was no difference in the dissolved Fe concentrations between these two exudate experiments. The Fe partitioning in the $<10 \times 10^3$ and $<3 \times 10^3$ amu filtrates was significantly lower in samples which had been exposed to UV irradiation. For the diatom exudates, there was a small difference in the percent of Fe in the $<0.2 \mu\text{m}$ filtrates with and without UV irradiation.

[23] On the basis of the Fe partitioning between the different size fractions in the presence of different DOC and Fe concentrations, the Fe(III) solubility in Chelex-treated and non-Chelex-treated seawater can be calculated using equation (1):

$$\text{Fe(III) solubility (nM)} = \frac{\text{cpm}_t}{\text{cpm}_0} \times \text{added Fe (nM)}, \quad (1)$$

where cpm_t is the ^{59}Fe radioactivity in the $<10 \times 10^3$ or $<3 \times 10^3$ amu filtrates at $t = 2$ hours, and cpm_0 is the ^{59}Fe radioactivity in the initial solution. The calculated Fe solubilities in the $<10 \times 10^3$ and the $<3 \times 10^3$ amu fractions from all treatments are shown in Table 3. Fe solubilities ranged from 0.65 to 4.60 nM for the $<10 \times 10^3$ amu fraction and from 0.21 to 1.40 nM in the $<3 \times 10^3$ amu fraction in the non-UV-irradiated seawaters with different DOC concentrations. For the algal exudate treatments, Fe solubilities were ~ 2.8 nM in the $<10 \times 10^3$ amu and 1 nM in the $<3 \times 10^3$ amu phases, which is within the range measured for natural seawater. Exudates from both phytoplankton species had similar Fe solubilities.

3.3. Complexation of Fe With Model Macromolecular Compounds

[24] Phase partitioning of Fe in seawater in the presence of different model organic compounds is shown in Figure 4. Interestingly, almost all added Fe was bound with side-

Table 3. Fe Solubilities in Chelex-Treated and Non-Chelex-Treated Seawaters^a

Type of Seawater and Algal Exudates	Fe Solubilities in Chelex-Treated Seawaters, nM				Fe Solubilities in Non-Chelex-Treated Seawaters, nM			
	$<10 \times 10^3$ amu		$<3 \times 10^3$ amu		$<10 \times 10^3$ amu		$<3 \times 10^3$ amu	
	Non-UV-Irradiated	UV-Irradiated	Non-UV-Irradiated	UV-Irradiated	Non-UV-Irradiated	UV-Irradiated	Non-UV-Irradiated	UV-Irradiated
North Pacific	0.65 ± 0.11	0.13 ± 0.04	0.21 ± 0.06	0.05 ± 0.01	0.51 ± 0.04	0.29 ± 0.00 ^b	0.12 ± 0.01	0.04 ± 0.02
Clearwater Bay	1.07 ± 0.11	0.17 ± 0.02	0.36 ± 0.13	0.09 ± 0.00 ^b	0.60 ± 0.04	0.24 ± 0.04	0.15 ± 0.02	0.07 ± 0.01
Tolo Harbor	1.01 ± 0.13	0.22 ± 0.04	0.42 ± 0.09	0.13 ± 0.02	0.84 ± 0.02	0.65 ± 0.15	0.29 ± 0.03	0.21 ± 0.00 ^b
Yuen Long	4.60 ± 0.10	2.71 ± 0.05	1.40 ± 0.02	0.47 ± 0.02	2.51 ± 0.01	1.76 ± 0.15	0.89 ± 0.01	0.27 ± 0.04
Diatom exudates	2.63 ± 0.02	0.44 ± 0.09	0.94 ± 0.02	0.09 ± 0.01	N.D. ^c	N.D. ^c	N.D. ^c	N.D. ^c
Cyanobacteria exudates	2.94 ± 0.12	1.41 ± 0.18	0.98 ± 0.10	0.56 ± 0.02	N.D. ^c	N.D. ^c	N.D. ^c	N.D. ^c

^aData are mean plus or minus semirange ($n = 2$).

^bTwo replicates for this measurement were the same.

^cN.D. is not determined.

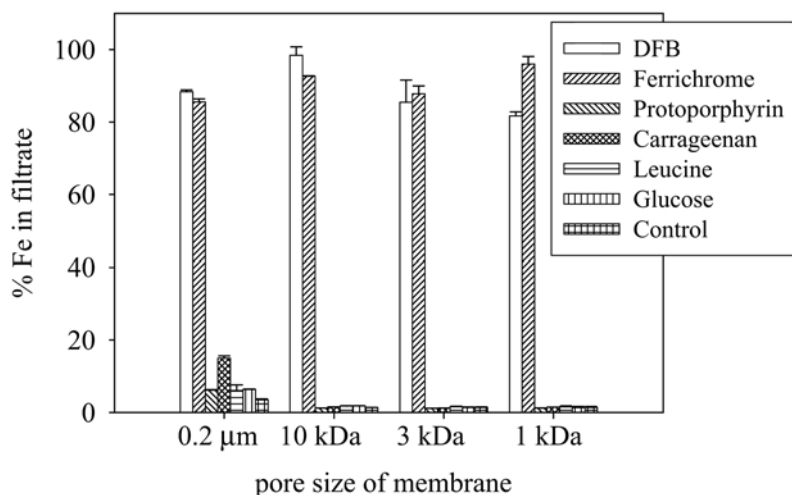


Figure 4. Fe partitioning in different filter-passing fractions of seawater containing different model macromolecular compounds. Data are mean plus or minus semirange ($n = 2$). DFB is desferrioxamine B.

rophores (85–98%), including desferrioxamine mesylate and ferrichrome, and passed through the 1×10^3 amu membrane. Compared with the control treatment, other organic compounds, such as protoporphyrin, carrageenan, leucine, and glucose, only slightly increased or had little effect on Fe partitioning in the filtrates. Among all the chemical compounds examined, carrageenan showed the third highest affinity for Fe.

3.4. Specificity of Fe-Complexing Organic Ligands

[25] Cu and Zn were used as competitive metals for organic ligand binding to test the specificity of organic ligands in binding with Fe. Although the spiked Cu and Zn were allowed to bind with the organic ligands for 12 hours before the addition of Fe, there was no significant difference in the Fe partitioning in different filtrates with and without Cu and Zn additions (Figure 5).

4. Discussion

4.1. Variation in Fe Solubilities

[26] The Fe solubilities measured in the present study were consistent with those reported by *Kuma et al.* [1996, 1998], *Sunda* [1989], and *Nakabayashi et al.* [2002] and were slightly higher than those obtained by *Liu and Millero* [2002] and *Byrne and Kester* [1976] but lower than those obtained by *Kuma et al.* [1992] and *Kuma and Matsunaga* [1995] (Table 4). In addition to the differences in sampling locations and DOC concentrations, two additional factors may also account for the inconsistent Fe solubilities reported in the literature. One is the pore size of the membranes from different manufactures used in different studies. As stated in section 2.2, the manufacturer's rated pore size can be significantly different from the actual pore size of a membrane (M. Chen et al., unpublished data, 2003). The other factor is the equilibration time of Fe hydroxides with seawater. In our study, Fe solubility was calculated after 2 hours of equilibration, which may represent fresh Fe hydroxides solubility. Most previous work on

Fe solubility used a 7 day equilibration time, which may allow for the aging of the Fe hydroxides.

[27] Fe solubilities decreased from estuarine to coastal and then to oceanic seawaters. The higher Fe solubility measured for the nearshore seawaters is probably related to higher concentrations of organic ligands released by marine biota and from riverine inputs. For comparison, *Kuma et al.* [1996] determined the solubility of Fe(III) hydroxide ($<0.025 \mu\text{m}$) in coastal and oceanic waters and found that Fe solubility in oceanic water was about one order of magnitude lower than that in coastal water. *de Baar and de Jong* [2001] summarized the data on dissolved Fe (<0.2 or $0.4 \mu\text{m}$) concentrations and pointed out that dissolved Fe concentration decreased from 1–100 nM in coastal waters to 0.03–0.5 nM in the surface waters of the open ocean. The higher Fe solubility in coastal seawater highlights the importance of dissolved organic matter and

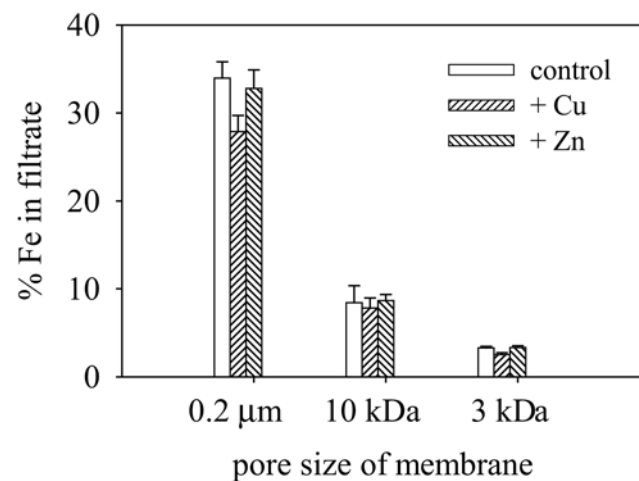


Figure 5. Fe partitioning in different filter-passing fractions of seawater prespiked with Cu and Zn. Data are mean plus or minus semirange ($n = 2$).

Table 4. Comparisons of Fe Solubility in Seawater From Different Regions

Locations	Salinity	Solubility Definition	Fe Solubility, nM	References
Coastal region near Hokkaido, Japan	—	<0.025 μm	2.8–2.9	<i>Kuma et al.</i> [1996]
Eastern Indian	34.9–35.4	<0.025 μm	0.3–0.6	<i>Kuma et al.</i> [1996]
Northern North Pacific	32.8–34.5	<0.025 μm	0.6–3.6	<i>Kuma et al.</i> [1998]
Western North Pacific	33.0–34.6	<0.025 μm	0.3–0.5	<i>Kuma et al.</i> [1996]
Funka Bay, Japan	~33	< 1×10^3 amu, <0.025 μm	~10	<i>Kuma et al.</i> [1992] <i>Kuma and Matsunaga</i> [1995]
North Pacific	HP: 32.9 ^a LP: 33–33.1 ^b	<0.025 μm	HP: 2–4 ^a LP: 0.3–0.9 ^b 1.5	<i>Nakabayashi et al.</i> [2002] <i>Sunda</i> [1989]
Gulf Stream Miami coast	36	<0.02 μm	0.50	<i>Liu and Millero</i> [2002]
Sargasso Sea	36.2	<0.05 μm	0.4 \pm 0.3	<i>Byrne and Kester</i> [1976]
Estuarine Yuen Long	20	< 10×10^3 amu	4.60 \pm 0.10	this study
Coastal Tolo Harbor	32	< 10×10^3 amu	1.01 \pm 0.13	this study
Coastal Clearwater Bay	33	< 10×10^3 amu	1.07 \pm 0.11	this study
Pacific	36	< 10×10^3 amu	0.65 \pm 0.11	this study

^aHP is high-production patch area.

^bLP is low-production patch area.

horizontal input of coastal Fe to the open ocean via water advection.

[28] Fe solubility also changed with the filter pore sizes. In this study the Fe solubilities in the $<3 \times 10^3$ amu phase were only about one third of those in the $<10 \times 10^3$ amu phase (Table 3). Partitioning of the dissolved Fe in the different physical phases may be largely related to the size distribution of organic ligands and the inorganic form. Since the measured Fe solubility depends on the operational definition of a dissolved phase, data from different studies should be compared with caution.

[29] The Fe solubilities in the UV-treated seawaters were lower than those in the non-UV-treated seawaters, further indicating that DOM can significantly increase Fe solubility. However, Fe solubilities in UV-irradiated seawater were higher than those in NaCl solution or artificial seawater (0.01 nM [*Liu and Millero*, 2002]). Such difference may be related to the presence of residual organic matter in the UV-irradiated seawater, as reported by *Kuma et al.* [1996] and *Liu and Millero* [2002]. It should be noted that most previous studies have adopted a short-time UV irradiation protocol and did not monitor the change of DOC concentration during the exposure [*Kuma et al.*, 1996; *Liu and Millero*, 2002; *Boye et al.*, 2003]. Our data suggest that the DOC removal rate was greatly different for different seawaters under the same UV irradiation conditions.

[30] When all samples with or without UV irradiation are considered, there was a strong positive linear relationship between the Fe solubilities and the DOC concentration (Figure 6). Such relationship provides strong evidence that Fe solubility was controlled by the organic ligands in natural seawater. Although it is now generally believed that Fe is complexed with organic ligands, these data present the first evidence of a direct linear relationship between DOC concentration and Fe solubility. Such a strong linear relationship seems strange because the concentration of organic ligands was far lower than those of DOC (see section 4.2). On the basis of the regression equation (i.e., intercept) one can estimate the Fe solubilities in DOC-free seawater as 0.07 and 0.02 nM for the $<10 \times 10^3$ and $<3 \times 10^3$ amu phases, respectively. These estimates are comparable to the reported Fe solubility in NaCl solution and artificial seawater.

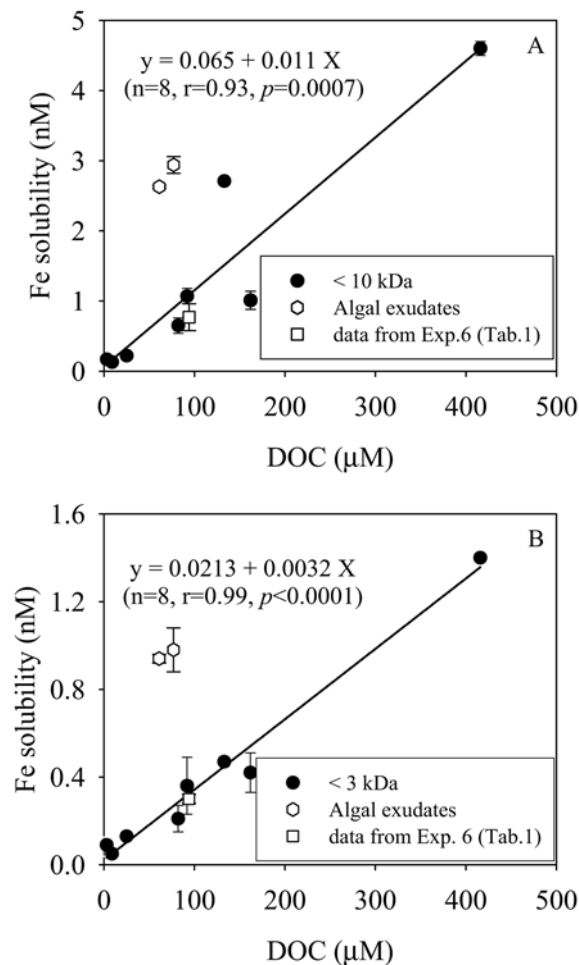


Figure 6. Relationship between the dissolved organic carbon (DOC) concentration and the Fe solubility in the (a) $<10 \times 10^3$ amu fraction and (b) $<3 \times 10^3$ amu fraction in natural seawaters. Data are mean plus or minus semirange ($n = 2$). Data shown above the regression line are those from algal exudate and ligand specificity experiments (Table 1).

Table 5. Calculated Concentrations of Size-Fractionated Organic Ligands in Different Seawaters

Region	Size	Total Ligands $[L]_T$, nM	Excess Ligands $[L']$, nM	$[FeL]$, nM
Pacific Ocean	$<3 \times 10^3$ amu	0.16 ± 0.06	0.08 ± 0.02	0.08 ± 0.06
	$3-10 \times 10^3$ amu	0.36 ± 0.13	0.14 ± 0.04	0.22 ± 0.14
Clearwater Bay	10×10^3 amu to $0.2 \mu\text{m}$	1.73 ± 0.29	1.92 ± 0.12	-0.19 ± 0.31
	$<3 \times 10^3$ amu	0.27 ± 0.13	0.08 ± 0.02	0.19 ± 0.13
Tolo Harbor	$3-10 \times 10^3$ amu	0.63 ± 0.17	0.28 ± 0.06	0.35 ± 0.18
	10×10^3 amu to $0.2 \mu\text{m}$	0.73 ± 0.30	0.68 ± 0.21	0.05 ± 0.37
Yuen Long	$<3 \times 10^3$ amu	0.29 ± 0.09	0.08 ± 0.03	0.21 ± 0.09
	$3-10 \times 10^3$ amu	0.50 ± 0.17	0.11 ± 0.15	0.39 ± 0.23
Yuen Long	10×10^3 amu to $0.2 \mu\text{m}$	1.54 ± 0.21	1.22 ± 0.27	0.32 ± 0.34
	$<3 \times 10^3$ amu	0.93 ± 0.28	0.62 ± 0.04	0.31 ± 0.28
	$3-10 \times 10^3$ amu	0.96 ± 0.30	0.13 ± 0.16	0.83 ± 0.34
	10×10^3 amu to $0.2 \mu\text{m}$	-0.04 ± 0.30	-1.10 ± 0.28	1.06 ± 0.41

ter (0.01 [Liu and Millero, 2002] and 0.08 nM [Wu et al., 2001]).

[31] Because the DOC concentrations in seawater exceed those of Fe solubility by several orders of magnitude, most of the functional sites of the organic matter may be occupied by major ions in seawater (e.g., Mg^{2+} and Ca^{2+}) and/or other trace metals. In addition, organic ligands with a high conditional stability constant for Fe complexation appear to represent only a small fraction of the marine DOC pool.

[32] The Fe solubilities in algal exudates were well above the regression line for natural seawater, suggesting that freshly released biogenic organic matter had higher complexing capacity than the DOM in natural seawater. The ratios of Fe solubility (nM) to DOC (μM) in the exudate treatments were 3 times higher than those in the natural seawater treatments, likely due to the freshness of organic matter and the presence of uncharacterized active organic ligands. This provides strong evidence that marine phytoplankton are the major sources of Fe-binding organic ligands. Boye and van den Berg [2000] quantified the organic ligands released by the coccolithophore *Emiliania huxleyi* after Fe addition and suggested that the production of Fe-complexing ligands was a common feature of this alga. Rue and Bruland [1997] observed that Fe-binding ligands were biologically produced when Fe was added to Fe-depleted water for the equatorial Pacific in the second IRONEX experiment. Thus marine phytoplankton may produce organic matter to facilitate Fe solubility and, presumably, Fe bioavailability in the ocean.

4.2. Organic Ligand Concentrations in Different-Size Fractions

[33] Assuming that relative partitioning of inorganic Fe is not related to the DOC concentration and all of the organic ligands are decomposed by 24 hours of UV irradiation, the total ligands and excess ligands in the seawater can be calculated. The concentration of total ligands corresponds to the difference in dissolved Fe concentration between the Chelex-PSW and Chelex-UV-PSW treatments (equation (2)):

$$\text{total ligand concentration (nM)} = (f_{\text{ch}} - f_{\text{ch-uv}}) \times [Fe]_{\text{added}}, \quad (2)$$

where f_{ch} and $f_{\text{ch-uv}}$ represent the percentage of dissolved Fe in the Chelex-PSW and Chelex-UV-PSW treatments,

respectively. $[Fe]_{\text{added}}$ is the added Fe concentration (10 nM). Similarly, the concentration of excess ligands corresponds to the difference of dissolved Fe concentration between the PSW and UV-PSW treatments (equation (3)):

$$\text{excess ligand concentration (nM)} = (f_{\text{psw}} - f_{\text{uv}}) \times [Fe]_{\text{added}}, \quad (3)$$

where f_{psw} and f_{uv} represent the percentage of dissolved Fe in the PSW and UV-PSW treatments, respectively.

[34] The concentration of the organic ligands complexed with Fe can be calculated from the difference between the total organic ligands and the excess ligands. Since the DOM was not fully destroyed by UV irradiation (Table 1), such a calculation likely underestimates the concentration of organic ligands. The calculated concentrations of organic ligands in different size fractions are shown in Table 5. The concentrations of total organic ligands ($[L]_T$) were 0.16–0.93 and 0.36–0.96 nM for the $<3 \times 10^3$ and the 3×10^3 to 10×10^3 amu fractions, respectively, and decreased from the estuarine to the coastal and then to the oceanic seawaters. Concentrations of excess organic ligands ($[L']$) were 0.08–0.62 and 0.11–0.28 nM for the $<3 \times 10^3$ and the 3×10^3 to 10×10^3 amu fractions, respectively. Unlike total organic ligands, there was no consistent trend for the excess organic ligands from nearshore to offshore waters. Boye et al. [2003] measured the total organic ligand concentration along a transect across the eastern North Atlantic and found that the concentration of organic-complexing ligands increased toward coastal waters along with the Fe concentration.

[35] It is interesting that most organic ligands bound with Fe ($[FeL]$) were found in the size fraction of $<10 \times 10^3$ amu. Within the $<10 \times 10^3$ amu organic ligand pool, ~27, 35, 35, and 27% were in the $<3 \times 10^3$ amu size fraction for the Pacific, Clearwater Bay, Tolo Harbor, and Yuen Long seawaters, respectively. Although the concentration of colloidal organic carbon (COC) in the 3×10^3 amu to $\sim 0.2 \mu\text{m}$ size fraction was significantly lower than that of low molecular weight fractions (LMW, $<3 \times 10^3$ amu) [Guo et al., 1995], the 3×10^3 to 10×10^3 amu COC fraction appears to contain stronger ligands for Fe complexation as compared to the $<3 \times 10^3$ amu LMW organic matter. This is to be expected given that the COC is the most dynamic DOM fraction in the ocean [Santschi et al., 1995; Guo et al., 1996]. However, Macrellis et al. [2001] suggested that 63% of the compounds extracted by Biobeads SM-2 and Amber-

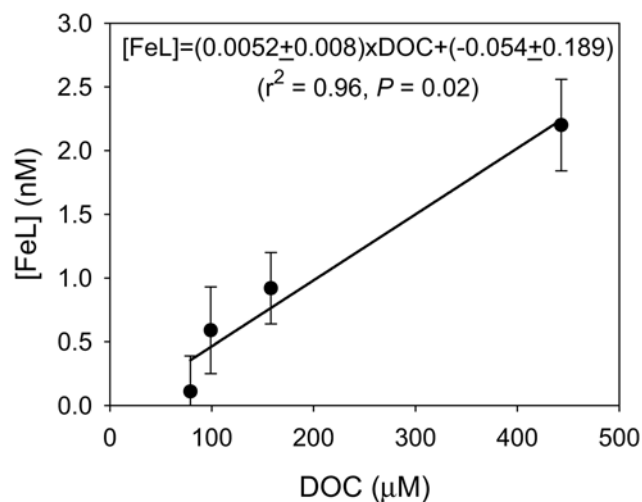


Figure 7. Relationship between the dissolved organic carbon (DOC) concentration and the [FeL] concentration in the $<0.2 \mu\text{m}$ fraction for YL, TH, CWB, and PW seawaters. Data are mean plus or minus 1 standard deviation ($n = 2$).

lite XAD-16 resins fall within the defined size range of siderophores (0.3×10^3 to 1×10^3 amu). It seems that the quality of DOM is also an important factor in controlling Fe solubility in seawater.

[36] Concentrations of total organic ligands in the 10×10^3 amu to $0.2 \mu\text{m}$ fraction were close to those of excess organic ligands (Table 5), suggesting that most Fe in this fraction should be in inorganic form, such as colloidal Fe hydroxides. For comparison, *Rue and Bruland* [1995] found two classes of Fe-binding ligands at station ALOHA in central North Pacific, a strong ligand class (L_1) with an average surface water concentration of 0.44 nM , and a weaker ligand class (L_2) with an average concentration of 1.5 nM . There was an excess of $\sim 0.2 \text{ nM}$ of strong ligands and 1.5 nM of weaker ligands above ambient dissolved Fe in the surface water. On the basis of the conditional stability constant and the difference of Fe solubility in seawater and in 0.7 M NaCl , *Liu and Millero* [2002] estimated that the total and excess ligand concentrations were $0.4\text{--}0.7$ and $0.1\text{--}0.2 \text{ nM}$ in Gulf Stream seawater, respectively. *Gobler et al.* [2002] found that colloidal Fe ($>10 \times 10^3$ amu), organically complexed Fe (FeL), and Fe binding ligands (L) were highly correlated with each other in West Neck Bay, New York, and suggested that much of the ligand pool may be colloidal in origin. However, our results suggest that the molecular weights of the Fe-complexed organic ligands in natural seawater were mostly in the $<10 \times 10^3$ amu fraction.

[37] Since most Fe is complexed by organic ligands in natural seawater, it is necessary to consider whether there is any relationship between DOC and [FeL]. We found that there was a good linear relationship between the DOC concentrations and the calculated [FeL] values in the YL, TH, CWB, and PW seawaters (Figure 7), further suggesting the important role of DOM in controlling the dissolved Fe concentration in marine environments. Covariation of DOC

and Fe concentrations in the deep ocean is also evident from available field data. Deep ocean DOC concentrations decrease from $48 \mu\text{M}$ in the North Atlantic to $\sim 41 \mu\text{M}$ in the Southern Ocean and $\sim 43 \mu\text{M}$ in the equatorial Pacific and $\sim 34 \mu\text{M}$ in the North Pacific [*Hansell and Carlson*, 1998]. Similarly, dissolved Fe concentration decreases from $\sim 0.7 \text{ nM}$ in the North Atlantic Ocean [*Wu and Luther*, 1994; *Wu et al.*, 2001] to $\sim 0.35 \text{ nM}$ in the Atlantic sector of the Southern Ocean [*de Jong et al.*, 1999] and then to $\sim 0.25 \text{ nM}$ in the Pacific and Indian sector of the Southern Ocean [*Coale et al.*, 1999; *Obata et al.*, 1997] and increases to $\sim 1 \text{ nM}$ in the equatorial Pacific [*Landing and Bruland*, 1987], then further decreases to $\sim 0.5 \text{ nM}$ in the North Pacific [*Bruland et al.*, 1994; *Rue and Bruland*, 1995; *Wu et al.*, 2001]. These trends imply that the deep ocean circulation may concomitantly control the distribution of DOC and dissolved Fe concentrations, although many more measurements are needed before a definite conclusion can be drawn. Interestingly, the total Fe concentration in fresh water systems is also significantly correlated with the DOC concentration [*Maranger and Pullin*, 2003], suggesting that the link between DOC and Fe was strong in the aquatic system, including both seawater and freshwater, although the controlling mechanisms may be different.

4.3. Functionality of Fe-Binding Organic Ligands

[38] Our results showed that almost all added Fe are complexed with siderophores, suggesting that siderophores (desferrioxamine B (DFB) and ferrichrome) can significantly increase the soluble Fe concentration in seawater. Given the strong binding ability of natural organic ligands, it is reasonable to speculate that one kind of the organic ligands bound with Fe in the ocean may be similar to siderophores in molecular weights and chemical properties. The high affinity of siderophores for Fe observed in these experiments is consistent with the results reported in the literature [e.g., *Wilhelm et al.*, 1996; *Rue and Bruland*, 1997; *Martinez et al.*, 2001]. *Macrellis et al.* [2001] used a simple, effective solid-phase technique to extract and concentrate dissolved natural Fe-binding ligands from seawater and confirmed that a significant fraction of the Fe-binding organic compounds are biologically produced siderophores. Besides siderophores, carrageenan, a type of acid polysaccharide, also slightly increased the Fe solubilities in seawater. Since polysaccharides are a major component of marine colloids [*Benner et al.*, 1992; *Santschi et al.*, 1998], the occurrence of marine colloids and polysaccharides should also increase Fe solubility in the ocean [*Quigley et al.*, 2002].

[39] The high affinity of siderophores for Fe does not conflict with our laboratory experiments, indicating that the majority of the Fe-binding ligands in seawater saturated with respect to Fe(II) oxyhydroxides are in 3×10^3 to 10×10^3 amu size range. There are other organic ligands that can bind with Fe in natural seawater. For example, the complexed forms of Fe are primarily $<1 \times 10^3$ amu, with only a small fraction existing as colloidal size in the HNLC regions [*Rue and Bruland*, 1997]. However, in coastal seawater with high Fe concentration, most of the organically complexed dissolved Fe exists in the colloidal Fe fraction, with only a small fraction as $<1 \times 10^3$ amu [*Bruland and Rue*, 2001].

Powell *et al.* [1996] showed that in the high-Fe, low-salinity regions of the Ochlockonee estuary the vast majority of Fe was in the colloidal phase ($>10 \times 10^3$ amu) but this component was only a minimal fraction in the higher-salinity regions. Thus the chemical forms of dissolved Fe are different among the different marine systems.

[40] Cu and Zn are reported to mostly complex with the organic ligands in natural seawater. Thus metals may compete for organic ligands if their bindings are not metal-specific. Our results showed that the Fe solubility remained constant with the additions of Cu and Zn, suggesting that these organic ligands were Fe-specific or a rapid equilibration was established between CuL and ZnL and the added Fe(III), forming FeL and freeing up the Cu and Zn.

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