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Application of cross-flow filtration for determining the solubility of iron species in open ocean seawater

*Christian Schlosser and Peter L. Croot**
FB2: Marine Biogeochemistry, IFM-GEOMAR and CAU Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Abstract

Measurements of soluble iron species (organic and inorganic) are important for understanding the transport of iron within the ocean and its bioavailability. Recent developments in ultrafiltration equipment and analytical detection techniques for low level Fe determination has turned the spotlight on obtaining data on soluble iron species. However there have, until now, been few studies that have characterized the performance of an ultrafiltration system with respect to well described soluble iron complexes. In the present work, we describe a methodological study characterizing the behavior of soluble and colloidal iron species in seawater by combining a cross-flow ultrafiltration system (Vivaflow 50^{TM}) with a radioisotope (^{55}Fe). During this study, we were able to maintain excellent mass balances by including all components: not only the solution phases (retentate and permeate) but wall-adsorbed and filter-adsorbed iron, which were recovered by an acid-rinsing step. Wall and filter adsorption were unavoidable when solutions were saturated with respect to Fe'. However in undersaturated solutions, such as with an excess of desferrioxamine B, wall and filter adsorption were minimized, indicating that these effects should be slight for natural samples where iron-binding ligands are in excess. Our results have important implications for the use of ultrafiltration membranes for open ocean iron biogeochemical studies.

Introduction

It is now widely accepted that in large parts of the ocean, principally the high nutrient, low chlorophyll (HNLC) regions, phytoplankton growth is limited by the low availability of iron (Boyd et al. 2007; Martin et al. 1994). The main controlling factor on iron (Fe) concentrations in the ocean is its solubility, as in ambient oxygenated seawater the thermodynamically favored redox state, Fe(III) is poorly soluble (20-500 pmol L^{-1}) (Liu and Millero 1999). Measurements of the physical speciation of Fe in seawater are traditionally operationally defined by the filtration system used by the researchers: dissolved (<0.2 or 0.4 μm) and particulate (>0.2 or 0.4 μm) phases. More recent work has further divided the dissolved phases into soluble (<10 kDa or <200 kDa) and colloidal (10-200 kDa to 0.2 μm) components (Bergquist et al. 2007; Cullen et al. 2006; Nishioka et al. 2005; Wu et al. 2001). The colloidal phase in the productive zones of the open ocean is dominated by organic colloidal aggregates (5-200 nm), which may provide numerous binding sites for trace metals such as Fe (Wells and Goldberg 1993, 1994). It is also likely that considerable differences exist in the bioavailability of soluble versus colloidal Fe (Wang and Dei 2003), and so to truly understand Fe as a limiting nutrient in the ocean, the relationship between dissolved Fe and Fe solubility needs to be better understood.

Both soluble and colloidal Fe in the oceans are derived from the impact of terrigenous materials to the global ocean via aeolian deposition (Jickells et al. 2005), riverine inputs (Bergquist and Boyle 2006; Buck et al. 2007; Gaiero et al. 2003), or resuspension of material from continental shelf sediments (Eldridge et al. 2004; Johnson et al. 1999). The amount of dissolvable Fe in aeolian transported materials varies greatly, ranging anywhere from 2% to 20% for direct measurements (Baker and Jickells 2006; Baker et al. 2006) whereas indirect estimates for the Pacfic are higher at ~40% (Boyle et al. 2005). The amount of dissolved Fe (Allard et al. 2004; de Baar and de Jong 2001) carried by rivers varies strongly and is influenced by pH, catchment area, and vegetation cover. Much of the river transported soluble Fe in estuaries, however, is quickly converted, at higher salinity values, into the biologically unavailable colloidal and particulate phase (Boyle et al. 1977; Guieu et al. 1996).

*Corresponding author: E-mail: pcroot@ifm-geomar.de

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As shown in numerous works more than 99% of the dissolved Fe (<0.2 μm) is bound by organic ligands throughout the world oceans (Croot and Johansson 2000; Rue and Bruland 1997; Van Den Berg 1995; Witter and Luther 1998; Wu and Luther 1995). Hutchins et al. (1999b) concluded that the organic complexation of Fe increases the amount of dissolved Fe species and consequently its biological availability. However, the bioavailable differences of organically complexed and colloidal Fe is not well understood (Chen and Wang 2001; Hutchins et al. 1999a; Kuma et al. 2000). The presence of siderophores and other Fe complexing ligands produced, or released via grazing or viral lysis, from phytoplankton, and bacteria may stabilize soluble Fe, increasing both the residence time and total pool size of bioavailable Fe in the surface ocean (Barbeau et al. 2001, 2003). Recent studies have shown that a significant fraction of the dissolved Fe pool exists as colloidal Fe species (Bergquist et al. 2007; Nishioka et al. 2001; Wu et al. 2001) with dissolved Fe concentrations in the euphotic zone being dominated by the variability of the colloidal Fe fraction. The colloidal Fe variability in the NW Atlantic was suggested to be seasonally dependent with higher concentrations in the winter, than in the summer (Bergquist et al. 2007).

Some fundamental issues that remain to be answered in the marine biogeochemistry of Fe are the extent to which organic binding agents increase Fe solubility and how those ligands prevent the formation of colloidal Fe in seawater. Until now only studies from Fe enrichment experiments have examined Fe ligand complexation and the formation of colloidal Fe (Boye et al. 2005; Wells 2003) with little attention paid to the overall solubility of Fe. Here we present a study that through the careful use of cross-flow ultrafiltration technique and theory outlines the impact of different ligands on Fe speciation and solubility.

Materials and procedures

Reagents—The impact of various different organic ligands on the speciation of Fe in seawater was carried out via cross-flow filtration using the radioisotope, ^{55}Fe (Hartmann Analytics). The ^{55}Fe had a specific activity of 157.6 MBq/mg Fe, a total activity of 75 MBq, and was dissolved in 0.51 mL of 0.1 M HCl. ^{55}Fe dilutions were produced with 18 MΩ deionized, ultrapure water and were acidified with quartz-distilled HCl (QD-HCl) to a pH below 2. The 7 ligands tested; desferrioxamine B (DFB), ethylenediaminetetraacetic acid (EDTA), 2-(2-thiazolylazo)-p-cresol (TAC), phytic acid (IP_6), protoporphyrin IX (PP IX), phytagel, and 2-keto-D-gluconic acid (2kDG) were obtained from Sigma-Aldrich. Ligand solutions were made up in 0.2 μm prefiltered Antarctic seawater (sampled during Eifex 2004 under trace metal clean conditions; total dissolved Fe concentration [Fe_d = 0.2 nM]). Prior to use, this seawater was irradiated with UV light (UV-Digester 705 from Metrohm) for 75 min to destroy any organic compounds present. All labware used was soaked in 10M HCl for at least 7 d and then rinsed with ultrapure water prior to use.

Ultrafiltration setup and cleaning procedure—The ultrafiltration of Fe and ligand-containing solutions was carried out using a Masterfex(r) L/S(tm) system with a Vivaflow 50 membrane (10 kDa) constructed of PES (polyethersulfone) with an active membrane area of 50 cm^2. The recirculation rate was set to approximately 300 mL/min, which typically gave a permeate flow rate of 5 mL/min. All ultrafiltration work was carried out using acid-cleaned polycarbonate (PC) container and polyethylene (PE) tubing. The Vivaflow 50 was precleaned by sequential rinsing with 100 mL Ultrapure water, 100 mL of a 1% solution of 6 M Q-HCl, a 100 mL EDTA wash solution (10 mM), and then finally a last rinse with 100 mL Ultrapure water to remove trace metal contamination. The ultrafilter could be reused several times following this procedure.

^{55}Fe measurements—^{55}Fe in the various ultrafiltration fractions was quantified using a liquid scintillation counter (Tri-Carb 2900TR) from Packard and the cocktail Lumagel Plus (Lumac LSC). The efficiency (55%-60%) of the instrument was obtained by several quench curve calibration measurements. The lowest measurable Fe concentration (detection limit) was at 8.1 ± 1.5 pM, equivalent to 1 count per minute (CPM). The ultrafiltration setup (PC container, PE tubing, and 10 kDa membrane filter) was cleaned prior to each experiment with a sequence of two short washes (0.1 M Q-HCl followed by 10 mM EDTA) and a rinse with Ultrapure water.

Theory: cross-flow filtration—The theory for cross-flow filtration of solutes has been described in detail previously by other researchers in this field: Reitmeyer et al. (1996), Guo et al. (2001), and Hassellöv et al. (2007). In general, the separation of Fe species by ultrafiltration can be represented as a simple mass balance in a closed system with a fixed total solution volume:

$$c_i V_i = c_R V_R + c_P V_P \tag{1}$$

where c is the concentration of total Fe in the initial sample (i), the permeate (P) and the retentate (R), and V is the associated liquid volume. Since the colloidal fraction of Fe is too large to pass through the 10 kDa membrane filter, the difference between the concentrations of Fe in the retentate and permeate is the concentration of Fe that is colloidal:

$$c_{Col} V_i = c_R V_R - c_P V_R \tag{2}$$

The extent to which the feed solution has passed across the membrane filter may be described in terms of a concentration factor (CF), the volume ratio between the initial and retentate solution (Logan and Qing 1990; Reitmeyer et al. 1996):

$$CF = \frac{V_i}{V_i - V_P} = \frac{V_i}{V_R}. \tag{3}$$

The colloid Fe concentration (c_{Col}) is then determined from the concentration of Fe in the permeate (c_P) and in the retentate (c_R) and CF:

$$c_{Col} = \frac{c_R - c_P}{CF} \tag{4}$$

At any given time of filtration, the relationship between the

concentration of a given chemical species in the instantaneous permeate (ic_p) outflow and the CF can be described by the following equation (Guo et al. 2001; Logan and Qing 1990):

$$\ln ic_P = \ln(P_c * c_i) + (1 - P_c) * \ln CF \quad (5)$$

where P_c is the permeation coefficient, defined as the ratio of ic_p to c_R at any given time during the filtration, where c_i is the initial concentration of the permeable species in solution. Under constant permeation behavior a plot of ln ic_p versus ln CF will be linear with slope $(1 - P_c)$. When $P_c = 1$, the solute is not retained by the ultrafiltration membrane and $ic_P = c_R$ at all values for the CF Lower values of P_c indicate discrimination by the membrane of the filter either by size exclusion or by polarization effects. Note that this equation holds only for the instantaneous permeate concentration (ic_p) flowing out of the ultra filter and not the concentration (c_p) in the bottle collecting the permeate (see also Appendix 1).

Colloidal fouling of cross-flow filtration membranes—Iron colloids are well known to cause fouling of ultrafiltration membranes (Soffer et al. 2004, 2002; Waite et al. 1999) leading to a reduction of permeate flow. In studies of Fe solubility, this also presents a problem as colloidal Fe will be adsorbed on the filter membrane and not in the retentate, resulting in an apparent loss of Fe from the system when considering a simple mass balance. Thus it is crucial that a mass balance consider all aspects of the cross-flow filtration procedure.

Fouling occurs initially by the advection and deposition of colloids onto the membrane causing pore blockage and is dependent on the permeate flux, colloid size, and zeta potential (Soffer et al. 2004). The rate at which the colloid is deposited on the membrane can be written as (adapted from Soffer et al. 2004):

$$\frac{\partial n_{uf}}{\partial t} = A(J - J_{cr})c_{Col} \quad (6)$$

where n_{uf} is the number of moles of Fe deposited on the membrane, A is the area of the membrane, J is the permeate flux (L m^{-2} h^{-1}), and J_{cr} is the critical permeate flux (L m^{-2} h^{-1}) below which no deposition can occur and is dependent on surface interactions.

Integration over time of Eq. 6 and assuming J is constant leads to a relationship between Fe accumulation on the filter and the permeate volume:

$$n_{uf} = \beta V_p c_{Col} + n_{uf}^0 \quad (7)$$

where $\beta = (1 - J_{cr}/J)$ is a unitless constant term (valid only for $J > J_{cr}$) relating the fraction of colloidal Fe lost to the filter, n_{uf}°, is the amount of Fe deposited on the membrane prior to t = 0. The value of n_{uf} is experimentally determined from the mass balance between permeate and retentate ($n_{uf} = c_i V_i - c_R V_R - c_P V_P$).

While it would be beneficial to keep the Fe adsorption on the filter to a minimum in practice, this may not be possible as the value of J_{cr} will vary between samples, depending on the colloid content and composition and the required permeate flow may be too low for adequate sample throughput. Thus for our experimental work, we focused on evaluating this loss term and its impact on the mass balance for Fe in the system and not on the permeate flux rate that minimized this loss term.

Based on the theory outlined above for the present work we constructed a simple two species model, including fouling of the membrane, to analyze our results. The first Fe complex was allowed to pass through the filtration membrane whereas the second component was purely colloidal and retained. A full description of the model can be found in Appendix 1.

Conditional stability constants of iron-organic species estimated by solubility—For the experiments with well-characterized chelators, conditional stability constants for the complexation with Fe, in seawater, can be calculated using the following assumptions:

$$Fe + L \Leftrightarrow FeL \quad (8)$$

$$[L]_T = [L] + [FeL] \quad (9)$$

$$K'_{Fe'L} = \frac{[FeL]}{[Fe'][L]} \quad (10)$$

where [Fe'] is the sum of the inorganic Fe(III) (Fe not complexed with L), and [L] is the concentration of the ligand not complexed with Fe in seawater (this may include ligands bound to Ca^{2+} and Mg^{2+}). The solubility of Fe(III) in seawater is given by the following equation:

$$[Fe(III)]_{SW} = [Fe'] + [FeL] \quad (11)$$

The concentration of FeL in seawater can be calculated from the measured value by correcting for the contribution from inorganic Fe(III) species:

$$[FeL] = [Fe(III)]_{SW} - [Fe(III)]_{NaCl} \quad (12)$$

Thus in the absence of organic chelators we have the following relationship:

$$[Fe'] = [Fe(III)]_{NaCl} \quad (13)$$

For our experimental solutions where colloidal Fe dominates, we can assume that the solution is saturated for Fe', and it is in turn controlled by the solubility of inorganic Fe formed under these conditions. Under the experimental conditions employed during the present work we find $[Fe'] = [Fe(III)]_{NaCl}$ ~150 pmol L^{-1} (Liu and Millero 1999) for this case.

Under saturated conditions, combining L and the permeate concentration ($[Fe(III)]_{SW}$), we can estimate $K'_{Fe'L}$ for each ligand tested (adapted from Liu and Millero [2002]).

$$K'_{Fe'L} = \frac{[Fe(III)]_{SW} - [Fe(III)_{NaCl}}{[Fe(III)]_{NaCl}[L]} \quad (14)$$

which rearranges to the following equation derived from measured quantities:

$$K'_{Fe'L} = \frac{[Fe(III)]_{SW} - [Fe(III)]_{NaCl}}{[Fe(III)]_{NaCl}([L]_T - [Fe(III)]_{SW})} \quad (15)$$

In the case of an excess of strong Fe binding ligands, the

system will be undersaturated with respect to the formation of colloidal iron, and Fe' will be less than the inorganic solubility term $[Fe(III)]_{NaCl}$. In this situation, only a lower bound for $K'_{Fe'L}$ can be estimated.

In the case of saturated natural seawater samples where the concentration of L is not known, it is not possible to calculate $K'_{Fe'L}$. However, we can compare ultrafiltration data with published Fe speciation data for $K'_{Fe'L}$ and L via the maximum permitted soluble Fe (derived from rearranging Eq. 15):

$$[Fe(III)]_{SW} = \frac{[Fe(III)]_{NaCl}\left(1 + K'_{FeL}[L]_T\right)}{\left(1 + K'_{FeL}[Fe(III)]_{NaCl}\right)} \quad (16)$$

using this approach the maximum soluble Fe can be determined by inputting the observed values of K'_{FeL} and L, along with the value of $[Fe(III)]_{NaCl}$ for a saturated Fe solution under the appropriate experimental conditions.

Side reaction coefficient ($\alpha_{Fe'}$) for Fe' in seawater—The value of the side reaction coefficient for Fe' relative to Fe(III) is critical for calculating conditional stability constants for Fe organic complexes and for determining the solubility of iron in seawater under different environmental conditions. Recently, there has been a consensus toward using $\alpha_{Fe'} = 10^{10.0}$ (pH = 8.02, salinity 35) based on the earlier solubility work by Kuma et al. (1996) and Millero (1998), complementing reactivity measurements by Hudson et al. (1992). More recently, an extremely good data set covering a range of environmental conditions has been made using EDTA (Sunda and Huntsman 2003) and solubility measurements via filtration (Liu and Millero 1999, 2002). What differences there are between experiments appear to be related to the age of the Fe colloids employed (Kuma et al. 1996; Sunda and Huntsman 2003).

Experimental procedure—Experiments were performed (Table 1) using 7 different Fe-binding ligands in UV-treated, 0.2 μm filtered Antarctic seawater (Collected during EIFeX). A further set of experiments was performed using 0.2 μm filtered non-UV irradiated Antarctic seawater and coastal seawater (fjord seawater; Bergen, Norway). Initial volumes of 200 mL seawater were used throughout. In the ligand experiments, ligand concentrations in the solution were ~100 nM. Ligand solutions were allowed to equilibrate with seawater for 4 h before addition of ^{55}Fe (c_{tot} = 60 nM in all, except for PP IX c_{tot} = 40 nM). The Fe concentration was checked immediately after Fe addition (t = 0) and then again after the equilibration period of 24 h. Ultrafiltration of the sample was commenced at 24 h after the Fe addition.

Ultrafiltration of the seawater sample was performed under constant pressure and flow rate conditions. Samples were always collected from both the permeate and the retentate at each sampling period. Samples were taken based on the collected permeate volume, starting at 5 mL, 20 mL, and then every further 20 mL until 180 mL (corresponding to the CF range 1.03-10). For each of the permeate samples, the last 2 mL passing through the membrane filter was collected into 60 mL PTFE bottles and 20 μL of HCl was added to prevent the sorption of Fe onto the bottle walls. Triplicate 400 μL sub-samples of the retentate and permeate were transferred into 6 mL scintillation counting vials. The vials were then filled with 4.5 mL of cocktail and the ^{55}Fe activity measured by liquid scintillation counting.

Table 1. Data at the start and the end of the ultrafiltration

Sample	c_W nmol L^{-1} (24 h)	c_P nmol L^{-1} CF = 1.3	c_R nmol L^{-1} CF = 1.3	c_P nmol L^{-1} CF = 10	c_R nmol L^{-1} CF = 10	$c`_{col}$ nmol L^{-1} CF = 10[a]	P_c	α'_{FeL}	log $K_{Fe'L}$[b] CF = 10	log $K_{Fe'L}$[c]
UV-ANT SW	20.7	0.15	40.49	0.19	91.84	59.67	0.58	0[d]		
ANT SW	21.0	5.40	31.49	5.92	66.53	53.57	0.92	33		11.8 – 12.4 [1–3]
CO SW	19.5	6.94	34.00	6.96	79.11	52.75	0.96	42		10.8 – 11.1 [2]
DFB	0	47.41	50.79	53.27	60.00	5.08	0.97	> 340	> 9.8	> 13[4], 16.5 [5]
EDTA	15.0	11.92	43.80	11.96	54.21	48.04	1.00	82	9.0	7.8[5–6]
TAC	11.8	15.74	43.52	16.09	78.59	43.75	0.99	104	9.1	Log ß$_2$ = 12.4 [2]
Phytic acid	17.7	5.31	32.28	5.66	66.03	54.16	0.97	34	8.6	12.3[7], < 0[8]
PP IX[9]	7.80	2.30	39.71	2.62	90.85	37.06	0.89	14	8.2	12.0[4], 12.4[7]
2keto-D-Glu	15.0	1.15	50.54	1.42	105.42	58.39	0.88	7	7.8	Log ß$_2$ = 11.1[10]
Phytagel	24.2	9.85	36.48	9.54	57.55	51.78	1.16	62	8.8	-

The data have been recovered from the linear part of the double log diagram, CF versus concentration (c_p and c_R), to exclude membrane filter loading effects during the first cycles of ultrafiltration. Notes: [a]The colloidal concentration is calculated using equation 18 (see text). [b]$K_{Fe'L}$ is the conditional stability constant calculated using equation 15 (see text). [c]$K_{Fe'L}$ determined in experimental studies in seawater or estimated using thermodynamic data for salinity 35 and 20°C. [d]This value is 0 by definition, as it is assumed that all organic ligands were destroyed by the UV irradiation.

Reference sources: [1]Atlantic Sector of the Southern Ocean (Boye et al. 2001), [2]Atlantic sector of the Southern Ocean and a Coastal Fjord in Sweden (Croot and Johansson 2000). [3]Atlantic sector of the Southern Ocean (Croot et al. 2004). [4]Laboratory Studies - Rue and Bruland (1995). [5]Laboratory Studies - Hudson et al. (1992). [6]Laboratory Studies - Sunda and Huntsman (2003). [7]Laboratory Studies - Witter et al. (2000). [8] Calculated value for seawater based on laboratory studies (Torres et al. 2005) for Phytic acid complexed with Fe^{3+},Ca^{2+} and Mg^{2+}. [9]PPIX in recent laboratory studies (Rijkenberg et al. 2006) did not appear to significantly complex Fe(III). [10]Laboratory studies (Essington et al. 2005) for the dominant $Fe(OH)_3(2kG)_2$ species. There is currently no published data for Fe binding by Phytagel.

At the end of each ultrafiltration experiment, 40 mL ultrapure water acidified with a 1% solution of 6 M Q-HCl were flushed through the ultrafiltration system to desorb any Fe from the walls of the PC container, the PE tubing, and the membrane filter. The Fe concentrations in the permeate and in the retentate samples taken at 5 and 20 mL were then used for mass balance calculations.

A SenTix 81 combination pH electrode and WTW model 720 pH meter were used to determine the pH. The electrode was calibrated on the free hydrogen scale (pH_F) using TRIS seawater buffers (Millero 1986). All pH data reported here is based on the free hydrogen ion concentration scale and data from other pH scales was converted using the appropriate algorithms (Dickson and Goyet 1994).

Assessment

Iron loss to the walls of the sample container—Initial measurements of the ^{55}Fe activity ion solution prior to commencement of the ultrafiltration are shown in Fig. 1. It can be clearly seen that in most cases there was a considerable loss of ^{55}Fe to the walls on the polycarbonate sample container 24 h after addition of the ^{55}Fe and organic complexing agent. The equivalent loss to wall (c_W) sorption effects (Table 1) was similar to that recently observed by Fischer et al. (2007) and in earlier work by Robertson (1968). Only with the strong complexing agent DFB was no wall sorption effect observed. All other Fe ligands tested and natural seawater solutions resulted in a strong decrease (20%– 40%) of ^{55}Fe in solution. Mass balance considerations (see below) indicate that the wall adsorbed ^{55}Fe was not remobilized during the ultrafiltration procedure. Indeed only a strong acid solution (e.g., HCl) was able to return the wall adsorbed ^{55}Fe to the solution phase.

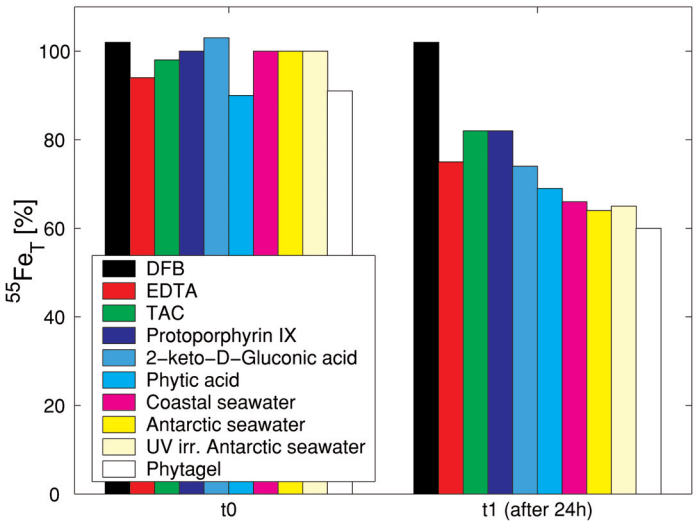

Fig. 1. Measured Fe concentration in the feed solution at hour 0 and 24. For saturated solutions approximately 20%-40% of the Fe was lost to the walls of the sample container. Only for the undersaturated solution containing DFB was there no apparent wall adsorption effect.

Thus wall sorption appears to be unavoidable with solutions saturated for Fe' and needs to be considered carefully in the experimental procedure. For undersaturated solutions, such as with DFB in the present work and in the case of some natural seawaters, this wall adsorption affect may be not significant. This result also has implications for voltammetric studies in which high concentrations of added ligand concentrations (e.g., 10 μM TAC: Croot and Johansson 2000) should minimize wall adsorption for iron speciation studies.

Permeation coefficients for soluble Fe species—The permeation behavior of selected soluble Fe species are shown in Fig. 2. A frequent observation was that initially the permeation behavior varied at low CF values (Fig. 2). However at CF > 1.2 (ln CF > 0.2), constant permeation behavior was observed in all experiments. The initial variation in permeation behavior differed between natural seawater samples (slightly higher initial permeation) and ligand-amended UV seawater (reduced initial permeation) and was possibly related to adsorption of the permeable Fe species and/or permeable ligands to the outlet tubing. Calculated values of P_c for the different Fe species, excepting Phytagel (Table 1), ranged from 0.58 for UV-irradiated Antarctic seawater to 1 for Fe-EDTA, with most in the range 0.88-1.0, indicating that soluble organic Fe species were only weakly retained by the 10 kDa filter used in this study. In contrast soluble inorganic Fe species were strongly retained by the 10 kDa filter (P_c = 0.58) possibly by interaction with weak binding sites on the filter itself. Data for Phytagel (not shown) indicated a decrease in permeation of the soluble Fe species with increasing CF leading to an estimated $P_c > 1$, this was probably due to blocking of the ultrafiltration membrane by the high molecular weight Phytagel as evidenced by increases in the observed pressure across the membrane.

Colloidal iron loss on the membrane—Colloidal Fe concentrations (Fig. 3) calculated using Eq. 4 exhibited significant decreases with increasing CF. This result indicates that there is an appreciable loss of the colloidal Fe during the process of ultrafiltration as has been observed for other ultrafiltration systems (Wells 2003). Consideration of the mass balance for the ultrafiltration procedure indicated that this loss of colloidal Fe is due to adsorption to the filter membrane (Fig. 4), which was also confirmed by later recovery of ^{55}Fe into the permeate by acidification (see below). For samples that were saturated with respect to Fe' formation, at CF = 10, approximately 50% (equivalent to 6 nmol) of total Fe was adsorbed on the filter. The overall loss of colloidal Fe (n_{Fil} [mol]) adsorbed on the membrane filter was linearly dependent on the volume of permeate (Fig. 4) that passed through the filter as expected for both saturated and unsaturated solutions (Eq. 7 above). Comparison of the Fe adsorbed to the filter (for V_p = 180 mL) and the initial colloidal Fe concentration (Fig. 5) were highly correlated (n = 10, R = 0.91) as expected from Eq. 7. Calculated values for β ranged from 0.49-0.88 (mean: 0.70 ± 0.13), this in turn implies that under our constant permeate flow (60 L m^{-2} h^{-1}), we can calculate a critical permeate flux for iron colloids

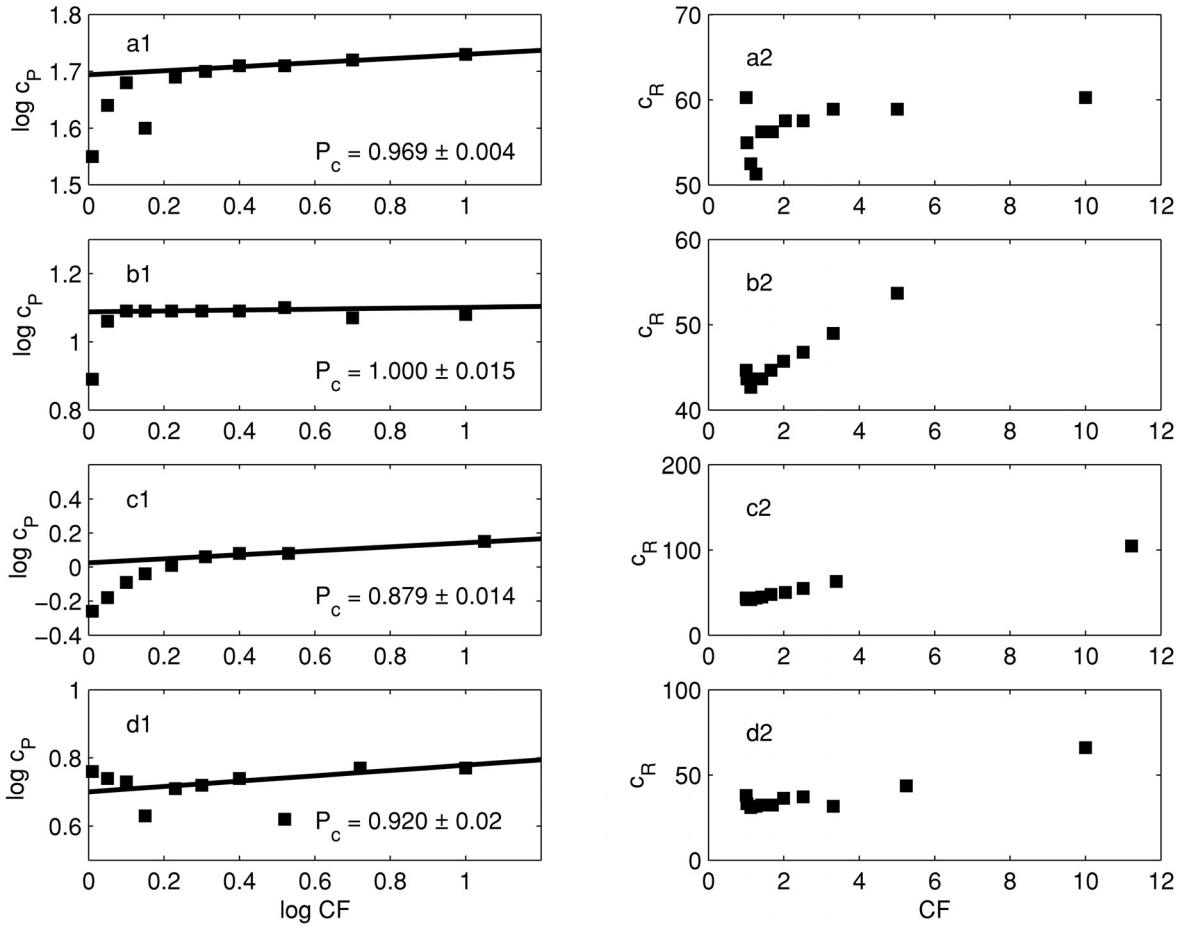

Fig. 2. Show the double log diagram, CF versus c_p (1) and the normal diagram CF versus c_R (2), of (a) DFB, (b) EDTA, (c) 2kDG, and (d) Antarctic seawater. Furthermore, the regression line and the calculated P_c value are inserted in the CF versus c_p diagrams. Outliers and filter loading effects were not respected for the calculation of the regression line and P_c value.

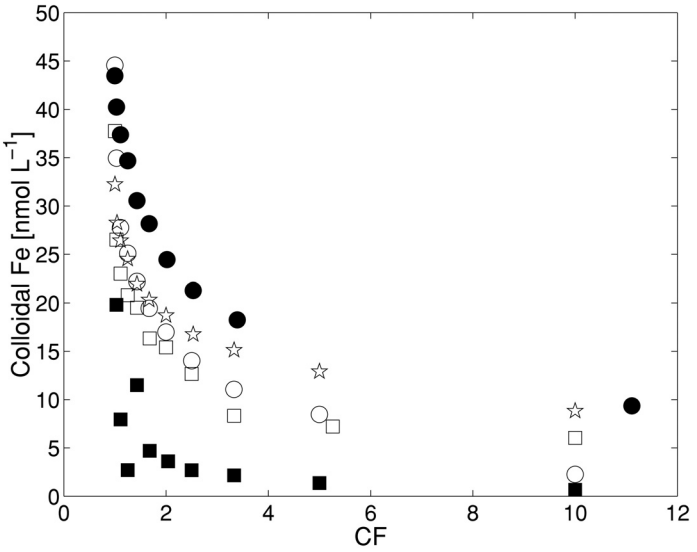

Fig. 3. Estimated colloidal Fe concentration (See Eq. 4 in the text) of Antarctic seawater (□), DFB (■), EDTA (○), 2kDG (●), PP IX (*) as a function of CF.

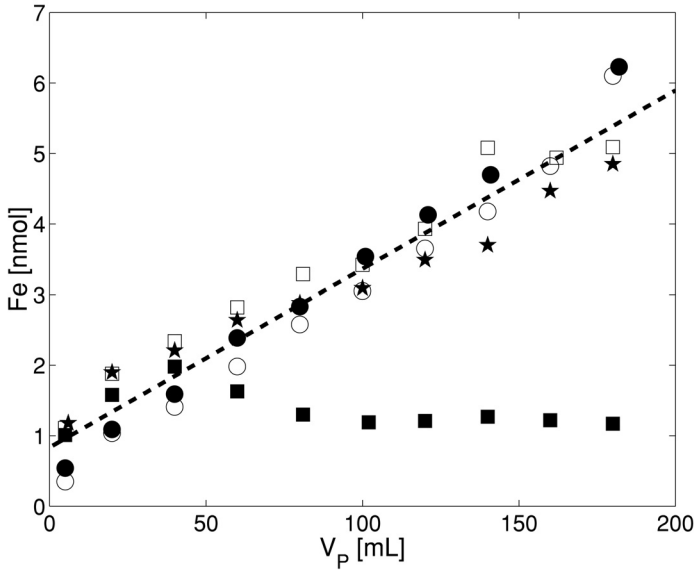

Fig. 4. Filter-loading Fe capacity with increasing V_p of Antarctic seawater (□), DFB (■), EDTA (○), 2kDG (●), and coastal seawater (filled *).

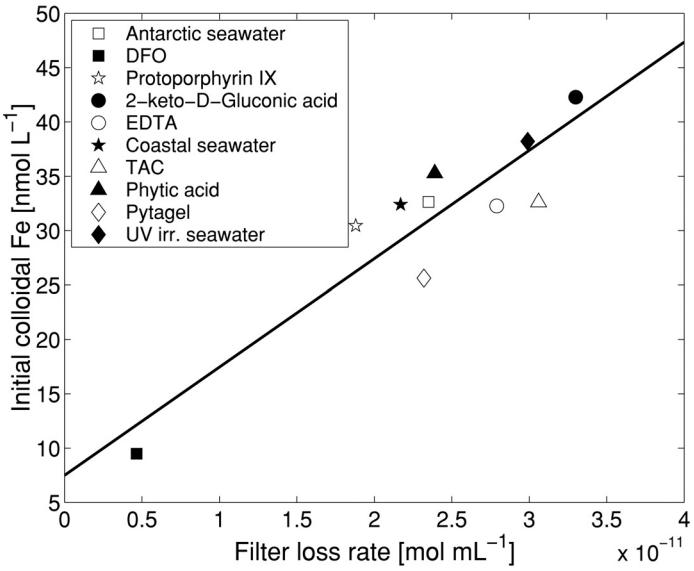

Fig. 5. Loss of ^{55}Fe (moles per milliliter of permeate) to the filter (estimated from mass balance considerations) versus initial colloidal Fe concentrations during cross-flow filtration of natural seawater or seawater amended with different Fe chelators.

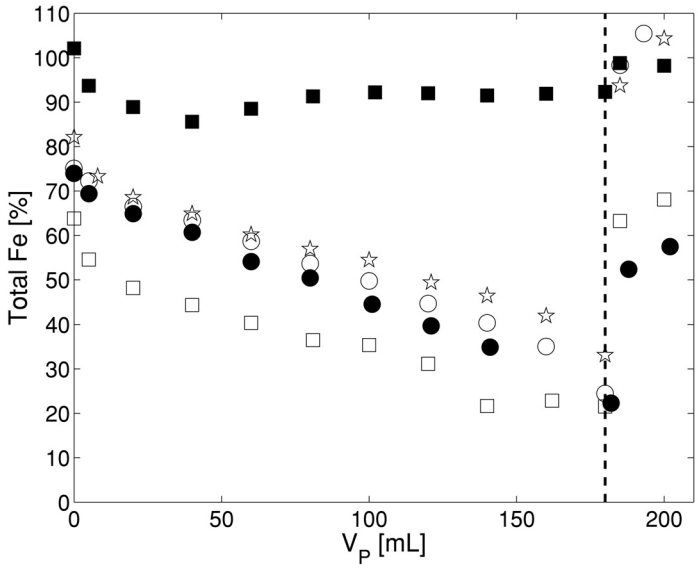

Fig. 6. Mass balance data for ^{55}Fe in the permeate and retentate for selected experiments. 20 mL QD-HCl rinse: Antarctic seawater (□), 2kDG (●), EDTA (○). 40 mL QD-HCl rinse: DFB (■) and PP IX (*). The dashed line indicates the initial point of flushing the system with MQ and QD-HCl (6 M, 10 μL per mL MQ water).

in our system; J_{cr} = 18.1 ± 7.8 L m^{-2} h^{-1}. This result implies that with a slower permeate flux value than used in the present work, we would expect less retention of colloidal iron on the filter. However, this finding ignores the link between recirculation rate and the permeate flux, and in our preliminary work, we found that slowing the recirculation rate to reduce the permeate flux did not enhance recovery. We had also anticipated that the linear relationship predicted from Eq. 7 should break down at higher CF as c_R increases due to the retention of colloidal Fe, but for the range of CF surveyed here, we did not observe this phenomenon.

Overall mass balance and recovery of adsorbed iron—To complete mass balance calculations and to ensure complete recovery of the ^{55}Fe tracer, the ultrafiltration system was rinsed with a dilute QD-HCl rinse with subsequent analysis of the permeate and retentate. Typically, we observed that after addition of the acid solution, initial measurements showed $\acute{\imath}c_p > c_R$, indicating there had been some Fe adsorbed to materials downstream of the filter membrane, presumably on the tubing walls. This may be partly the reason for the lower $\acute{\imath}c_p$ values encountered at low CF with unacidified seawater solutions. However, for all later measurements $\acute{\imath}c_p = c_R$ indicating that all the Fe was solubilized at this low pH. Calculated overall mass balances, incorporating a 40 mL dilute QD-HCl rinse (Fig. 6) showed complete recovery (98 ± 4%, n = 3) of the added ^{55}Fe indicating that both the wall-adsorbed and filter-adsorbed ^{55}Fe was recovered. Using only 20 mL of the dilute QD-HCl rinse we observed, in general, only the recovery of the filter-adsorbed ^{55}Fe, indicating this volume was insufficient to completely remove Fe adsorbed on the walls of the polycarbonate container to the solution phase. In all cases, a further cleaning rinse of either dilute QD-HCl or the EDTA rinse solution used for pre-cleaning resulted in the remaining ^{55}Fe being recovered and subsequent filter blanks returning to below detection limits (<7 pmol L^{-1}).

Discussion

Calculation of "true" colloidal iron concentrations—Implicit in the calculation of c_{Col} (Eq. 4) are the following assumptions: (i) that there is no retention of the permeable species (P_c = 1) and (ii) that no Fe is lost from the solution phase within the ultrafiltration system. As detailed above, both of these assumptions are not always valid for the ultrafiltration of soluble Fe species. However the true colloidal Fe concentration ($c`_{col}$) can be calculated by completing the mass balance throughout the sampling procedure:

$$c`_{col} V_i = c_R V_R - \frac{c_P}{P_c} V_R + n_{uf} \quad (17a)$$

or alternatively in terms of CF

$$c`_{col} = \frac{c_R - \frac{c_P}{P_c}}{CF} + \frac{n_{uf}}{V_i} \quad (17b)$$

As the n_{uf} is derived from mass balance considerations, Eq. 17b can be converted into a form that is dependent only on directly measured quantities.

$$c`_{col} = c_i - \frac{c_P}{V_i}\left(\frac{V_R}{P_c} + V_P\right) \quad (18)$$

Values of $c`_{col}$ calculated using Eq.18 (Table 1 and Fig. 7) show that after CF ≥ 2 a constant value for almost all samples. 2kDG and UV-irradiated Antarctic seawater presented the

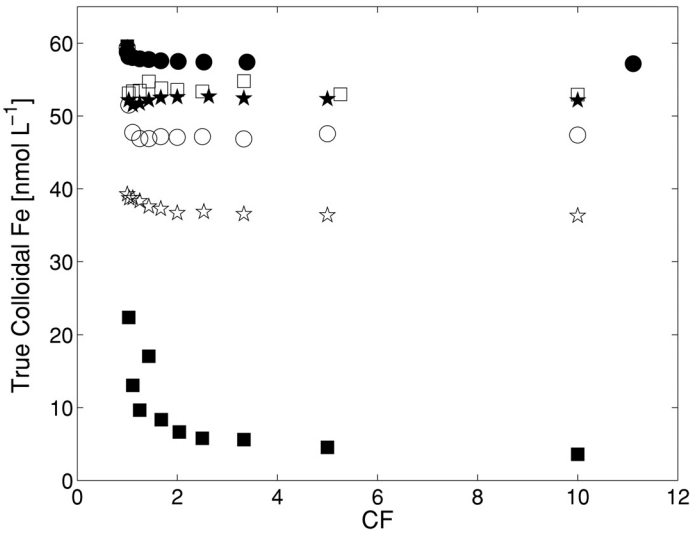

Fig. 7. Ambient 'true' colloidal Fe concentration (Eq. 18) with increasing CF, of Antarctic seawater (□), DFB (■), EDTA (○), 2kDG (●), PP IX (*), and coastal seawater (filled *).

highest amounts of colloidal Fe concentration ($c`_{col}$ = 59 nM) at CF = 10. DFB formed the highest amounts of organically complexed Fe ($c_p$ = 53 nM) and, in a similar manner, a very low colloidal Fe concentration ($c`_{col}$ = 5 nM). All other samples, except for DFB, EDTA ($c`_{col}$ = 48 nM), and TAC ($c`_{col}$ = 44 nM), showed similar values for $c`_{col}$ (51–54 nM).

Solubility of iron-complexes in seawater—Iron was poorly soluble in UV-irradiated Antarctic seawater ($ic_p \approx 0.15$ nM) in good agreement with other recent solubility studies performed with conventional filtration systems in UV-irradiated seawater (Kuma et al. 1998a, 1992b, 1996; Liu and Millero 2002) and in NaCl solutions (Liu and Millero 1999). Interestingly, ic_p increased with increasing CF (P_c = 0.58), which may indicate that one or more of the inorganic hydrolyzed Fe species is discriminated against by interaction with the filtration membrane, possibly via polarization/charge effects.

Natural seawater (Antarctic and Coastal seawater) had significantly more soluble Fe ($ic_p \approx 6$ nM) than UVSW and in both samples ic_p increased with increasing CF (P_c = 0.92 and 0.96 respectively) indicating small but significant retention effects on the soluble Fe species in these samples. This is an important finding because it indicates that accurate determination of the soluble Fe concentration in a sample is dependent on making multiple measurements of ic_p as a function of CF as a single measurement does not provide any information on P_c. The natural seawater Fe solubility values measured here are comparable to earlier studies (Kuma et al. 1998a, 1998b, 1996) in which nM levels were found in coastal and open ocean seawaters. Additionally, the apparent ligand concentrations measured in the Antarctic seawater (5.4 nM) were higher than maximal solubility (Eq. 16) values (1–3 nM) calculated from published speciation parameters in the same region by voltammetric methods (Boye et al. 2005, 2001; Croot et al. 2004; Croot and Johansson 2000). This difference between actual solubility and the predicted values is probably in part related to the detection window used for voltammetric analysis, which would preclude weaker Fe-binding ligands (Croot and Johansson 2000). It is clear that more detailed work using both chemical and physical methods on the same samples is still required.

DFB is a hydroxamate siderophore, which forms strong complexes with Fe(III) (Schwarzenbach and Schwarzenbach 1963) even under ambient seawater conditions (Hudson et al. 1992). Due to its high-binding strength, DFB is frequently used to limit the bioavailability of Fe to phytoplankton in experimental studies (Hutchins et al. 1999a; Wells 1999). In the present work, DFB strongly enhanced the Fe solubility (c_p = 53 nM) and limited the formation of colloidal Fe ($c`_{col}$ = 5 nM). For DFB, P_c = 0.97, indicating that there was some small discrimination by the filter membrane for this soluble species. In an earlier study, it was found that only 50% of the FeHDFB+ (MW = 803) species was found to pass through a 1 kDa membrane at pH 4.8 (Batinic-Haberle et al. 1994). Furthermore these authors found that FeHDFB+ could form higher MW complexes with micelles at concentrations above the critical micelle concentration. While the colloidal concentration would have been expected to be near zero with DFB, as the solution was undersaturated with respect to Fe', it does appear that some of the ^{55}Fe was retained in the colloidal phase, though this colloidal Fe may still have been associated with DFB. As the DFB solution was undersaturated, it was only possible to estimate a lower bound for log K for this complex in seawater (Table 1).

The speciation of Fe-EDTA complexes in seawater is well described (Gerringa et al. 2000; Hudson et al. 1992; Sunda and Huntsman 2003) and indicates that EDTA forms three relatively weak Fe binding ligands in seawater at pH_F 8.10, ~68% $Fe(OH)(EDTA)^{2-}$ (log $K_{Fe'L}'$ = 7.7), ~24% $Fe(EDTA)^{-}$ (log $K_{Fe'L}'$ = 7.3), and ~7% $Fe(OH)_2(EDTA)^{3-}$ (log $K_{Fe'L}'$ = 6.7), due to strong side reactions with Ca^{2+} and Mg^{2+} (Table 1). In the present work we found that 100 nM EDTA maintained ~12 nM in the soluble phase and that there was effectively no retention effect on these soluble complexes (P_c = 1.00). Estimates of the conditional stability constant (Table 1) for the soluble Fe-EDTA complexes (log $K_{Fe'L}'$ = 9.0) were significantly higher than predicted values (overall log $K_{Fe'L}'$ = 7.9). An earlier study by Sunda and Huntsman (2003), using a solid phase extraction (SPE) technique to determine soluble Fe-EDTA species, found good agreement with values predicted by Hudson et al. (1992). As equilibration times were the same in both studies, the reasons for the apparent discrepancy between our results and those of Sunda and Huntsman (2003) may be due to the experimental setups. This could indicate that the presence of EDTA in solution has some effect on the size range of the Fe colloids formed (see also phytic acid below) by adsorbing to the surface and preventing further aggregation (Nowack and Sigg 1996). Such colloids may have been retained by the SPE technique employed by Sunda

and Huntsman but passed through our ultrafiltration system. The photolysis of Fe-EDTA complexes to form Fe(II) (Kari et al. 1995; Sunda and Huntsman 2003) may also play role here in maintaining a fresh source of small Fe colloids that passed through the ultrafilter. Further studies on the solubility of Fe-EDTA complexes are currently being pursued.

TAC is from the family of thiazolylazo compounds (Hovind 1975) that are known to form relatively strong Fe complexes. TAC is used as a reagent in voltammetric analysis for Fe speciation in seawater (Croot and Johansson 2000). In the solubility experiments that we conducted with 100 nM TAC we found ~16 nM in the soluble phase (P_c = 0.99). This result is slightly higher than we had anticipated given the published estimated conditional stability constant for seawater: log $\beta_{Fe'(TAC)2}$ = 12.4 (Croot and Johansson 2000). However, this high solubility may indicate the presence of a strong 1:1 Fe-TAC species that was not anticipated in Croot and Johansson (2000).

PP IX has previously been found to be a strong Fe chelator by voltammetric studies (Rue and Bruland 1995; Witter and Luther 1998) though more recently this has been bought into question by photochemical (Rijkenberg et al. 2006) and bioavailability studies (Sato et al. 2007). In the present work, we find little evidence supporting PP IX as a strong Fe chelator in seawater as 100 nM of this ligand was only able to maintain ~2.5 nM in solution. The apparent discrepancy between the earlier voltammetric measurements and later measurements may be due to either an insufficient equilibration time (Rue and Bruland 1995), as suggested by van Leeuwen and Town (2005), or the lower pH (6.9) used in the Witter et al. (2000) study.

Phytic acid (*myo*-insitol hexakisphosphate) is a major component of eukaryotic cells (Turner et al. 2002) and has been suggested to be capable of forming strong Fe complexes in seawater (Witter et al. 2000). In the present study, phytic acid was found to be a relatively weak Fe ligand complexing only ~6 nM Fe under the conditions employed here. Recently published thermodynamic data (Torres et al. 2005) for phytic acid complexes with Ca^{2+}, Mg^{2+}, H^{+}, and Fe^{+++} indicate that under seawater conditions employed here, there should be no appreciable Fe^{+++} complexes formed. However, a recent study using Field Flow Fractionation (FFF) showed that solutions of Fe and phytic acid at pH 7 formed complexes/colloids in the size range 1-500 kDa in size (Purawatt et al. 2007). Higher concentration ratios of phytic acid to Fe shifted the mean colloid size to lower molecular weights (Purawatt et al. 2007). This behavior is probably related to the ability of the phosphate groups present in phytic acid to act as bridging groups between Fe colloids (Mali et al. 2006). The case of phytic acid then adds a new twist to the Fe solubility story as it apparently has an important role to play in stabilizing colloidal Fe but does not directly form soluble Fe.

The ligand 2kDG is a natural product of glucose oxidation and has been suggested as an important component of trace metal binding in soils (Nelson and Essington 2005). Related compounds to 2kDG have been implicated in the production of Fe(II) in seawater through photochemical reduction of the Fe-carboxylic complex (Kuma et al. 1995, 1992a; Öztürk et al. 2004). In this study, we found only a small enhancement in soluble Fe with 2kDG ($c_p \approx$ 2nM). Analysis of recent complexation data for 2kDG (Essington et al. 2005) indicate that it would not be expected to retain Fe in solution under seawater conditions due to the strong interactions with Ca^{2+}.

Phytagel (Gellan Gum; Miyoshi et al. 1996; Nishinari 1999) is a sugar-like macromolecule with a mean molecular weight of ~490 kDa (Milas et al. 1990) and has been found previously to bind Th(IV) (Quigley et al. 2002) in seawater. Thus we anticipated only a trace amount of ^{55}Fe would pass through the membrane, due to the high molar mass of the ligand, however we surprisingly measured a high concentration (c_p = 9.54 nM). This apparent discrepancy may be due to the ability of some of the long chained molecules being able to pass through the membrane as coils (Nakajima et al. 1996) and transport Fe with it. However clogging of the membrane filter by the large phytagel molecules was also an issue here, and this greatly reduced the permeate flow rate and concentration with time.

Implications for ultrafiltration of natural seawater solutions—The results found here have strong implications for the ultrafiltration of natural seawater solutions for the determination of soluble and colloidal Fe without the use of radio-isotopes. The analysis of the retentate and permeate fluxes can be achieved with other methods for stable Fe; such as chemiluminescence (Croot and Laan 2002; Obata et al. 1993) or ICP-MS techniques (Saito and Schneider 2006; Wu 2007). It is clear that in seawater samples with considerable colloidal concentrations there will be retention of these species on the filter membrane and walls. These species are recoverable with a later acid or combined dilute acid/DFB rinse and can be corrected for in the overall mass balance. A potential problem here is the contribution of Fe from the filter apparatus itself, from our preliminary studies on this, we have found that an EDTA, DTPA, or DFB solution in ultra-pure water passed through the system before the sample reduced the blank to very low levels (<30 pM – our detection limit with FIA-chemiluminscence [Obata et al. 1993]). The use of strong acids should be avoided as much as possible as in our experience this apparently leaches Fe out of the plastic walls.

If the solution is undersaturated, as most natural samples from the open ocean would be expected to be, the problems of wall adsorption should be minimized greatly simplifying the analytical procedures. However, it is critical that measurements (for mass balance purposes) be made as a function of the CF in both the retentate and permeate and that a recovery rinse be employed. We have recently employed a combined Vivaflow-50 and FIA-chemiluminscence system on several cruises in the Atlantic with reasonably good success (Croot et al. in prep.). The only major problem we have encountered so far has been from insufficient cleaning of ultrafilters overloaded with colloidal material (e.g., Saharan dust, Colloidal Fe from iron enrichment experiments).

Overall performance of cross-flow filtration system—The overall performance of this cross-flow filtration system for Fe measurements was particularly good as sample processing times were relatively short compared with other cross-flow filtration systems currently available, and the system was easily manageable for a single operator. The problem of colloidal Fe loss on the filter and walls of the system is common to all such ultrafiltration methods but good mass balances can be obtained by employing a recovery rinse as used in this study and by monitoring both the retentate and permeate flow over time. Our results strongly suggest that ultrafilters should not be used in a single pass mode, but instead only be used in the normal recirculation mode, as critical information would otherwise be lost. While this does significantly increase the processing time compared with conventional filtration systems, it does provide valuable checks and balances on the behavior of the system being investigated. We also suggest that these filters can be extensively reused provided attention is paid to the use of recovery and cleaning solutions.

Conclusions and recommendations

Our work has shown that good mass balances for Fe can be obtained using a cross-flow filtration system when employing a final rinse to recover Fe adsorbed to the filter membrane. It is also very important to monitor both the retentate and permeate fluxes as a function of CF to determine P_c for the solution as soluble Fe may also be partially retained by the filter. We conclude that cross-flow filtration systems such as the one employed in our study can provide valuable information on colloidal and soluble Fe in the ocean. Furthermore, we recommend future studies should concentrate on examining the relationship between solubility and iron speciation in the colloidal and soluble phases through the careful use of ultrafiltration techniques such as we describe here.

References

Allard, T., and others. 2004. Revealing forms of iron in river-borne material from major tropical rivers of the Amazon Basin (Brazil). Geochim. Cosmochim. Acta 68:3079-3094.

Baker, A. R., and T. D. Jickells. 2006. Mineral particle size as a control on aerosol iron solubility. Geophys. Res. Lett. 33:1-4.

———, T. D. Jickells, M. Witt, and K. L. Linge. 2006. Trends in the solubility of iron, aluminium, manganese and phosphorus in aerosol collected over the Atlantic Ocean. Mar. Chem. 98:43-58.

Barbeau, K., E. L. Rue, K. W. Bruland, and A. Butler. 2001. Photochemical cycling of iron in the surface ocean mediated by microbial iron(III)-binding ligands. Nature 413:409-413.

———, E. L. Rue, C. G. Trick, K. W. Bruland, and A. Butler. 2003. Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe(III) binding groups. Limnol. Oceanogr. 48:1069-1078.

Batinic-Haberle, I., I. Spasojevic, and A. L. Crumbliss. 1994. Hydrolysis of Ferrioxamine-B in aqueous micellar solution. Inorg. Chem. 33:3151-3158.

Bergquist, B. A., and E. A. Boyle. 2006. Iron isotopes in the Amazon River system: Weathering and transport signatures. Earth Planet. Sci. Lett. 248:54-68.

———, J. Wu, and E. A. Boyle. 2007. Variability in oceanic dissolved iron is dominated by the colloidal fraction. Geochim. Cosmochim. Acta 71:2960-2974.

Boyd, P. W., and others. 2007. Mesoscale iron enrichment experiments 1993-2005: synthesis and future directions. Science 315:612-617.

Boye, M., J. Nishioka, P. L. Croot, P. Laan, K. R. Timmermans, and H. J. W. De Baar. 2005. Major deviations of iron complexation during 22 days of a mesoscale iron enrichment in the open Southern Ocean. Mar. Chem. 96:257-271.

———, C. M. G. Van Den Berg, J. T. M. De Jong, H. Leach, P. L. Croot, and H. J. W. De Baar. 2001. Organic complexation of iron in the Southern Ocean. Deep Sea Res. 48:1477-1497.

Boyle, E. A., J. M. Edmond, and E. R. Sholkovitz. 1977. The mechanism of iron removal in estuaries. Geochim. Cosmochim. Acta 41:1313-1324.

Buck, K. N., M. C. Lohan, C. J. M. Berger, and K. W. Bruland. 2007. Dissolved iron speciation in two distinct river plumes and an estuary: Implications for riverine iron supply. Limnol. Oceanogr. 52:843-855.

Chen, M., and W.-X. Wang. 2001. Bioavailability of natural colloid-bound iron to marine plankton: Influences of colloidal size and aging. Limnol. Oceanogr. 46:1956-1967.

Croot, P. L., K. Andersson, M. Öztürk, and D. Turner. 2004. The distribution and speciation of iron along 6° E, in the Southern Ocean. Deep-Sea Res. II 51:2857-2879.

——— and M. Johansson. 2000. Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC). Electroanalysis 12(8):565-576.

——— and P. Laan. 2002. Continuous shipboard determination of Fe(II) in Polar waters using flow injection analysis with chemiluminescence detection. Anal. Chim. Acta 466:261-273.

Cullen, J. T., B. A. Bergquist, and J. W. Moffett. 2006. Thermodynamic characterization of the partitioning of iron between soluble and colloidal species in the Atlantic Ocean. Mar. Chem. 98:295-303.

de Baar, H.J.W., de Jong, J.T.M., 2001. Distributions, Sources and Sinks of Iron in Seawater. In: Turner, D., Hunter, K. (Eds.), The Biogeochemistry of Iron in Seawater, IUPAC Book Series on Analytical and Physical Chemistry of Environmental Systems, vol. 7. John Wiley & Sons Ltd, Chichester, pp. 123–253.

Dickson, A. G. and C. Goyet (1994): DOE: Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water, ver. 2. ORNL/CDIAC-74.

Eldridge, M. L., and others. 2004. Phytoplankton community response to a manipulation of bioavailable iron in HNLC

waters of the subtropical Pacific Ocean. Aquat. Microb. Ecol. 35:79-91.

Essington, M. E., J. B. Nelson, and W. L. Holden. 2005. Gibbsite and goethite solubility: The influence of 2-ketogluconate and citrate. Soil Sci. Soc. Amer. J. 69:996-1008.

Fischer, A. C., J. J. Kroon, T. G. Verburg, T. Teunissen, and H. T. Wolterbeek. 2007. On the relevance of iron adsorption to container materials in small-volume experiments on iron marine chemistry: 55Fe-aided assessment of capacity, affinity and kinetics. Mar. Chem. 107:533-546.

Gaiero, D. M., J.-L. Probst, P. J. Depetris, S. M. Bidart, and L. Leleyter. 2003. Iron and other transition metals in Patagonian riverborne and windborne materials: Geochemical control and transport to the southern South Atlantic Ocean. Geochim. Cosmochim. Acta 67:3603-3623.

Gerringa, L. J. A., H. J. W. De Baar, and K. R. Timmermans. 2000. A comparison of iron limitation of phytoplankton in natural oceanic waters and laboratory media conditioned with EDTA. Mar. Chem. 68:335-346.

Guieu, C., W. W. Huang, J. M. Martin, and Y. Y. Yong. 1996. Outflow of trace metals into the Laptev Sea by the Lena River. Mar. Chem. 53:255-267.

Guo, L., B. J. Hunt, and P. H. Santschi. 2001. Ultrafiltration behavior of major ions (Na, Ca, Mg, F, Cl, AND SO4) in natural waters. Water Res. 35:1500-1508.

Hassellöv, M., K. O. Buesseler, S. M. Pike, and M. Dai. 2007. Application of cross-flow ultrafiltration for the determination of colloidal abudances in suboxic ferrous-rich ground waters. Sci. Total Environ. 72:636-644.

Hovind, H. R. 1975. Thiazolylazo dyes and their applications in analytical chemistry. Analyst 100:769-796.

Hudson, R. J. M., D. T. Covault, and F. M. M. Morel. 1992. Investigations of iron coordination and redox reactions in seawater using 59Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes. Mar. Chem. 38:209-235.

Hutchins, D. A., F. M. A. Brzezinski, and K. W. Bruland. 1999a. Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. Limnol. Oceanogr. 44:1009-1018.

———, A. E. Witter, A. Butler, and G. W. Luther Iii. 1999b. Competition among marine phytoplankton for different chelated iron species. Nature 400:858-861.

Jickells, T. D., and others. 2005. Global iron connections between desert dust, ocean biogeochemistry, and climate. Science 308:67-71.

Johnson, K. S., F. P. Chavez, and G. E. Friederich. 1999. Continental-shelf sediments as a primary source of iron for coastal phytoplankton. Nature 398:697-700.

Kari, F. G., S. Hilger, and S. Canonica. 1995. Determination of the reaction quantum yield of the photochemical degradation of Fe(III)-EDTA: Implications for the environmental fate of EDTA in surface waters. Environ. Sci. Technol. 29:1008-1017.

Kuma, K., A. Katsumoto, H. Kawakami, F. Takatori, and K. Matsunaga. 1998a. Spatial variability of Fe(III) hydroxide solubility in the water column of the northern North Pacific Ocean. Deep-Sea Res. 45:91-113.

———, A. Katsumoto, J. Nishioka, and K. Matsunaga. 1998b. Size-fractionated iron concentrations and Fe(III) hydroxide solubilities in various coastal waters. Estuar. Coast. Shelf Sci. 47:275-283.

———, A. Katsumoto, N. Shiga, T. Sawabe, and K. Matsunaga. 2000. Variation of size-fractionated Fe concentrations and Fe(III) hydroxide solubilities during a spring phytoplankton bloom in Funka Bay (Japan). Mar. Chem. 71:111-123.

———, S. Nakabayashi, and K. Matsunaga. 1995. Photoreduction of Fe(III) by hydroxycarboxylic acids in seawater. Water Res. 29:1559-1569.

———, S. Nakabayashi, Y. Suzuki, I. Kudo, and K. Matsunaga. 1992a. Photo-reduction of Fe(III) by dissolved organic substances and existence of Fe(II) in seawater during spring blooms. Mar. Chem. 37:15-27.

———, S. Nakabayashi, Y. Suzuki, and K. Matsunaga. 1992b. Dissolution rate and solubility of colloidal hydrous ferric oxide in seawater. Mar. Chem. 38:133-143.

———, 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.

Liu, X., and F. J. Millero. 1999. The solubility of iron hydroxide in sodium chloride solutions. Geochem. Cosmochim. Acta 63:3487-3497.

——— and ———. 2002. The solubility of iron in seawater. Mar. Chem. 77:43-54.

Logan, B. E., and J. Qing. 1990. Molecular-size distributions of dissolved organic-matter. J. Environ. Eng.116:1046-1062.

Mali, G., M. Sala, I. Arcon, V. Kaucic, and J. Kolar. 2006. Insight into the short-range structure of amorphous iron inositol hexaphosphate as provided by ^{31}P NMR and Fe X-ray absorption spectroscopy. J. Phys. Chem. B 110:23060-23067.

Martin, J. H., and others. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 371:123-129.

Milas, M., X. Shi, and M. Rinaudo. 1990. On the physicochemical properties of gellan gum. Biopolymers 30:451-464.

Millero, F. J. 1986. The pH of estuarine waters. Limnol. Oceanogr. 31:839-847.

———. 1998. Solubility of Fe(III) in seawater. Earth Planet. Sci. Lett. 154:323-329.

Miyoshi, E., T. Takaya, P. A. Williams, and K. Nishinari. 1996. Effects of sodium chloride and calcium chloride on the interaction between gellan gum and konjac glucomannan. J. Agric. Food Chem. 44:2486-2495.

Nakajima, K., T. Ikehara, and T. Nishi. 1996. Observation of gellan gum by scanning tunneling microscopy. Carbohydr. Polym. 30:77-81.

Nelson, J. B., and M. E. Essington. 2005. The association constants of H+ and Ca2+ with 2-keto-D-gluconate in aqueous solutions. J. Sol. Chem. 34:789-800.

Nishinari, K. 1999. Physical chemistry and industrial application of gellan gum. Springer.

Nishioka, J., and others. 2005. Changes in the concentration of iron in different size fractions during an iron enrichment experiment in the open Southern Ocean. Mar. Chem. 95:51-63.

———, S. Takeda, C. S. Wong, and W. K. Johnson. 2001. Size-fractionated iron concentrations in the northeast Pacific Ocean: distribution of soluble and small colloidal iron. Mar. Chem. 74:157-179.

Nowack, B., and L. Sigg. 1996. Adsorption of EDTA and Metal-EDTA complexes onto goethite. J. Colloid Interface Sci. 177:106-121.

Obata, H., H. Karatani, and E. Nakayama. 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. Anal. Chem. 65:1524-1528.

Öztürk, M., and others. 2004. Iron enrichment and photoreduction of iron under PAR and UV in the presence of hydrocarboxylic acid: Implications for phytoplankton growth in the Southern Ocean. Deep-Sea Res. II 51(22-24): 2841-2856.

Purawatt, S., A. Siripinyanond, and J. Shiowatana. 2007. Flow field-flow fractionation-inductively coupled optical emission spectrometric investigation of the size-based distribution of iron complexed to phytic and tannic acids in a food suspension: implications for iron availability. Anal. Bioanal. Chem. 389:733-742.

Quigley, M. S., P. H. Santschi, C.-C. Hung, L. Guo, and B. D. Honeyman. 2002. Importance of acid polysaccharides for 234Th complexation to marine organic matter. Limnol. Oceanogr. 47:367-377.

Reitmeyer, R., R. T. Powell, W. M. Landing, and C. I. Measures. 1996. Colloidal aluminum and iron in seawater: An intercomparison between various cross-flow ultrafiltration systems. Mar. Chem. 55:75-91.

Rijkenberg, M. J. A., L. J. A. Gerringa, V. E. Carolus, I. Velzeboer, and H. J. W. De Baar. 2006. Enhancement and inhibition of iron photoreduction by individual ligands in open ocean seawater. Geochim. Cosmochimica Acta 70:2790-2805.

Robertson, D. E. 1968. The adsorption of trace elements in sea water on various container surfaces. Anal. Chim. Acta 42:533-536.

Rue, E. L., and K. W. Bruland. 1995. Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar. Chem. 50:117-138.

——— and ———. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnol. Oceanogr. 42:901-910.

Saito, M. A., and D. L. Schneider. 2006. Examination of precipitation chemistry and improvements in precision using the Mg(OH)2 preconcentration inductively coupled plasma mass spectrometry (ICP-MS) method for high-throughput analysis of open-ocean Fe and Mn in seawater. Anal. Chim. Acta 565:222-233.

Sato, M., S. Takeda, and K. Furuya. 2007. Iron regeneration and organic iron(III)-binding ligand production during in situ zooplankton grazing experiment. Mar. Chem. 106:471-488.

Schwarzenbach, G., and K. Schwarzenbach. 1963. Hydroxamatkomplexe I. Die Stabilitat der Eisen(III)-Komplexe einfacher Hydroxamsauren und des Ferrioxamins B. Helv. Chim. Acta 46:1390-1401.

Soffer, Y., A. Adin, and J. Gilron. 2004. Threshold flux in fouling of UF membranes by colloidal iron. Desalination 161:207-221.

———, J. Gilron, and A. Adin. 2002. Streaming potential and SEM-EDX study of OF membranes fouled by colloidal iron. Desalination 146:115-121.

Sunda, W., and S. Huntsman. 2003. Effect of pH, light, and temperature on Fe-EDTA chelation and Fe hydrolysis in seawater. Mar. Chem. 84:35-47.

Torres, J., and others. 2005. Solution behaviour of myo-inositol hexakisphosphate in the presence of multivalent cations. Prediction of a neutral pentamagnesium species under cytosolic/nuclear conditions. J. Inorg. Biochem. 99:828-840.

Turner, B. L., M. J. Paphazy, P. M. Haygarth, and I. D. Mckelvie. 2002. Inositol phosphates in the environment. Phil. Trans. Royal Soc. London B-Biological Sciences 357:449-469.

Van Den Berg, C. M. G. 1995. Evidence for organic complexation of iron in seawater. Mar. Chem. 50:139-157.

Van Leeuwen, H. P., and R. M. Town. 2005. Kinetic limitations in measuring stabilities of metal complexes by competitive ligand exchange-adsorptive stripping voltammetry (CLE-AdSV). Environ. Sci. Technol. 39:7217-7225.

Waite, T. D., A. I. Schafer, A. G. Fane, and A. Heuer. 1999. Colloidal fouling of ultrafiltration membranes: Impact of aggregate structure and size. J. Colloid Interface Sci. 212:264-274.

Wang, W., and R. C. H. Dei. 2003. Bioavailability of iron complexed with organic colloids to the cyanobacteria Synechococcus and Trichodesmium. Aquat. Microb. Ecol. 33:247-259.

Wells, M. L. 1999. Manipulating iron availability in nearshore waters. Limnol. Oceanogr. 44:1002-1008.

———. 2003. The level of iron enrichment required to initiate diatom blooms in HNLC waters. Mar. Chem. 82:101-114.

——— and E. D. Goldberg. 1993. Colloid aggregation in seawater. Mar. Chem. 41:353-358.

——— and ———. 1994. The distribution of colloids in the North-Atlantic and Southern Oceans. Limnol. Oceanogr. 39:286-302.

Witter, A., and G. W. Luther. 1998. Variation in Fe-organic complexation with depth in the Northwestwern Atlantic Ocean as determined using a kinetic approach. Mar. Chem. 62:241-258.

Witter, A. E., D. A. Hutchins, A. Butler, and G. W. Luther. 2000. Determination of conditional stability constants and kinetic constants for strong model Fe-binding ligands in seawater. Mar. Chem. 69:1-17.

Wu, J., E. Boyle, W. Sunda, and L. Wen. 2001. Soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific. Science 293:847-849.

——— and G. W. Luther. 1995. Complexation of Fe(III) by natural organic ligands in the Northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach. Mar. Chem. 50:159-177.

———. 2007. Determination of picomolar iron in seawater by double Mg(OH)(2) precipitation isotope dilution high-resolution ICPMS. Mar. Chem. 103:370-381.

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