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## Utilizing Radioisotopes for Trace Metal Speciation Measurements in Seawater

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### 1. Introduction

The chemical speciation of trace metals in seawater is of critical importance to studies in marine biogeochemistry; as such information is essential for interpreting and understanding the chemical reactivity of trace metals in the environment. Foremost in this respect are studies into the role that chemical speciation plays in determining the biological availability (bioavailability) or toxicity of metals to organisms. Research on this topic over the last 30 years has clearly shown that open ocean productivity can be directly limited by iron. Other studies have revealed more subtle effects, such as co-limitation or limitation/toxicity affecting only some phytoplankton species, can occur with other trace metals and lead to controls on the composition of the phytoplankton community. Thus studies addressing chemical speciation in seawater are of relevance to the entire marine ecosystem.

Work on chemical speciation draws on skills and expertise from a diverse range of fields including; analytical chemistry, environmental chemistry, toxicology, geochemistry, genomics, proteomics, biological oceanography, physical oceanography and chemical oceanography. A tool common to all of these fields is the use of radioisotopes to examine the transfer or exchange between chemical species at environmentally relevant concentrations, which would be impossible with conventional analytical techniques. In this role radiotracers have been invaluable in the development of several key discoveries in Chemical and Biological Oceanography:

- <sup>14</sup>C measurements of primary productivity
- The development of the Free Ion Association Model (FIAM) and Biotic Ligand Model (BLM) for metal uptake kinetics by phytoplankton
- Iron limitation in the ocean and its impact on primary productivity
- The biological utilization of Cadmium by phytoplankton
- Quantification of the exchange kinetics between different metal species in solution

Oceanographic field research requires the ability to work on a moving ship in the ocean and if this was not difficult enough, work on trace metals necessitates the use of ultraclean techniques to avoid the ubiquitous contamination from the ship itself. Combining this with the normal precautions and safe working environment needed for using radioisotopes can present researchers with a formidable challenge. However despite these problems radiotracers have always been a useful tool for marine scientists, both on land

and sea, as they allow the direct quantification of rates or fluxes and the identification of transformation pathways and mechanisms related to biogeochemical processes in the ocean. In recent years the development of extremely sensitive analytical techniques to determine the concentration of stable elements in seawater (ICP-MS) and phytoplankton (e.g. Nanosims, Synchrotron XRF), coupled with the problems of using radioisotopes, has seen a general decline in their use compared to the genesis of trace metal marine biogeochemistry in the 1970's and 1980's. However in recent years a number of new questions have emerged where radioisotopes once again can provide crucial data and this has seen a mini-renaissance in their use.

The aim of this article is to provide a short overview on the previous use of radioisotopes in marine biogeochemistry (Section 2), where they have been applied directly in studies where chemical speciation is directly addressed. For the purposes of this article we only consider studies that utilize radioactive isotopes to directly assess the chemical speciation of trace metals in seawater or the use of chemical speciation techniques to determine the kinetics of exchange between different chemical species and their uptake by phytoplankton.

Research in marine biogeochemistry continues to develop rapidly and radioisotopes will continue to have a role as tools for enhancing our understanding of key processes involving trace metals in the ocean. New areas of research include the impacts of global warming, ocean acidification and ocean deoxygenation, all of which will impact on trace metal speciation and bioavailability. For this work radiotracers still have an important role to play and will continue to be utilised in major ocean-going international programs such as GEOTRACES and SOLAS and in laboratory work. As despite recent developments in analytical techniques, some important processes are still more easily followed by the use of radiotracers. In this context, here we also provide data from new applications (Section 3) of radioisotopes to current problems in this growing field.

### 1.1 Trace metals in seawater

In the following section we provide a short overview of trace metal chemistry in seawater and for more information we refer the reader to other review articles on this topic (Bruland and Lohan, 2003; Donat and Bruland, 1995). Other review articles examine the role of trace metals in biological cycles in the ocean (Bruland et al., 1991; Hunter et al., 1997; Morel et al., 2003).

**Note on the units used in this section:** It is common practice for oceanographers to report concentrations on both the molality (moles per kg of seawater) and molarity (moles per liter of seawater) scales, conversion between the two scales is easily accomplished when the density of the seawater is known (easily calculated from the salinity and temperature). In this text we report concentrations on the molarity scale (symbol M, units of mol L<sup>-1</sup>) using the following standard SI abbreviations:  $\mu\text{M}$  ( $1 \times 10^{-6}$  M), nM ( $1 \times 10^{-9}$  M), pM ( $1 \times 10^{-12}$  M), fM ( $1 \times 10^{-15}$  M) and aM ( $1 \times 10^{-18}$  M). Radiochemical activities are given in Becquerels (Bq).

#### 1.1.1 Concentrations and distributions of trace metals in seawater

Trace metals are found in seawater over a wide range of concentrations stretching from  $\mu\text{mol kg}^{-1}$  to  $\text{amol kg}^{-1}$  (Bruland and Lohan, 2003) and can exist in a variety of physical and chemical forms (Section 1.2). Table 1 (below) lists the typical concentrations in seawater found for the bio-active trace metals considered in this work.

Element	Concentration	Inorganic Speciation	Distribution
Al	0.3 – 40 nM	Al(OH) <sub>4</sub> <sup>-</sup> , Al(OH) <sub>3</sub>	Scavenged
Ti	6 – 250 pM	TiO(OH) <sub>2</sub>	Scavenged
V	30 – 36 nM	HVO <sub>4</sub> <sup>-</sup>	Conservative
Cr	3 – 5 nM	CrO <sub>4</sub> <sup>2-</sup> , Cr <sup>3+</sup>	Nutrient
Mn	0.08 – 5 nM	Mn <sup>2+</sup>	Scavenged
Fe	0.02 – 2 nM	Fe(OH) <sub>2</sub> <sup>+</sup> , Fe(OH) <sub>3</sub>	Nutrient
Co	4 – 300 pM	Co <sup>2+</sup> , Co <sup>3+</sup>	Nutrient
Ni	2 – 12 nM	Ni <sup>2+</sup>	Nutrient
Cu	0.5 – 4.5 nM	Cu <sup>2+</sup>	Nutrient
Zn	0.05 – 9 nM	Zn <sup>2+</sup>	Nutrient
Se	0.5 – 2.3 nM	SeO <sub>4</sub> <sup>2-</sup> , SeO <sub>3</sub> <sup>2-</sup>	Nutrient
Mo	105 nM	MoO <sub>4</sub> <sup>2-</sup>	Conservative
Cd	1 – 1000 pM	CdCl <sub>2</sub>	Nutrient

Table 1. Dissolved concentrations of bio-active trace metals in seawater (Bruland and Lohan, 2003; Donat and Bruland, 1995; Nozaki, 1997).

Traditionally chemical oceanographers have made a simple distinction between particulate and dissolved forms by separation via filtration (0.2 µm or 0.4 µm). More recently with the application of ultrafiltration techniques the dissolved fraction has been further divided into soluble (passes through a 1-200 kDa ultrafilter) and colloidal (difference between dissolved and soluble). Particulate forms include metals located intracellularly, or adsorbed extracellularly to biogenic particles or metals that form the matrix of minerals or are adsorbed to them.

An important concept in the development of the field of chemical oceanography was that of “Oceanographic consistency” (Boyle and Edmond, 1975), by which data for dissolved metals had to meet the following criteria:

1. Form smooth vertical profiles.
2. Have correlations with other elements that share the same controlling mechanisms.

Application of this approach has resulted in a reliable test for analytical data and led to the determination of vertical profiles for all the natural elements of the periodic table (Nozaki, 1997). Based on the shape of the vertical profile of each trace metal they can be grouped into three distinct groups reflecting their chemical behaviour in seawater:

1. *Conservative type distribution* - Metals showing this behaviour have concentrations that maintain a relatively constant ratio to salinity, have long oceanic residence times (> 10<sup>5</sup> years).
2. *Nutrient type distribution* - Concentrations are lowest in surface waters and increase with depth and are often strongly correlated to the distribution of the macronutrients (N, P and Si), indicating that these metals are assimilated by plankton in the euphotic zone and remineralized at depth.
3. *Scavenged type distribution* - Typical of trace metals that are adsorbed to particles (scavenged) and have oceanic residence times (~100-1000 y) less than the mixing time of the ocean. Highest concentrations are found nearest the sources of these elements to the ocean.

## 1.2 Trace metal speciation in seawater

Studies on trace metal speciation in seawater are concerned with determining the concentrations and processes affecting individual chemical species. Operationally this typically requires the application of specific techniques for the determination of analytically distinguishable chemical species or the application of thermodynamic or kinetic models to predict the behaviour of different species in seawater. Over the years the term 'speciation' began to be used for a number of different uses so to avoid confusion the International Union for Pure and Applied Chemistry (IUPAC) has published guidelines or recommendations for the definition of speciation analysis (Templeton et al., 2000):

- i. *Chemical species*. Chemical elements: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure
- ii. *Speciation analysis*. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample
- iii. *Speciation of an element; speciation*. Distribution of an element amongst defined chemical species in a system
- iv. *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

### 1.2.1 Inorganic speciation

The inorganic speciation of trace metals in seawater (Table 1) is reasonably well described due to the extensive work performed by physical chemists in simple salt solutions. For the more complex media that is seawater, the use of Pitzer equations (Pitzer, 1973) is required but for many species in seawater this data is still missing. The reader is referred to a number of review chapters that cover the inorganic speciation of trace metals in more detail (Byrne et al., 1988; Turner et al., 1981). In particular recent reviews (Byrne, 2010; Millero et al., 2009) have focused on those elements whose inorganic speciation is dominated by hydroxide and/or carbonate species which are sensitive to decreases in pH and increasing CO<sub>2</sub> concentrations due to anthropogenic inputs.

### 1.2.2 Organic speciation

Many trace metals have been found to be strongly complexed by organic ligands in seawater, most notably iron (Gledhill and van den Berg, 1994) and copper (Coale and Bruland, 1988). However very little is known about these metal-organic complexes though it appears that they are produced by organisms in response to metal stress (Croot et al., 2000). Only a few of these ligands have been isolated and the chemical structures determined; iron complexing siderophores (Martinez et al., 2001) and heavy metal sequestering thiol complexes such as phytochelatins (Ahner et al., 1994). For a general overview of organic speciation in seawater see Hirose (2006). A recent paper by Vraspir and Butler (2009) provides a summary of the current information on trace metal binding ligands that have been isolated and identified in seawater.

### 1.2.3 Redox speciation – Importance of kinetics

For many trace metals there are major differences in the reactivity, bioavailability and toxicity between redox species. A critical factor here is the role of kinetics and/or oxygen concentrations in maintaining thermodynamically unstable redox species in solution where

rapid reduction rates and slower oxidation rates leads to significant concentrations of the lower oxidation states of some metals in ambient seawater. For more on this and the impact of sulfide on metal speciation see general reviews on this subject (Cutter, 1992; Emerson and Huested, 1991; Morse et al., 1987).

1.3 Commonly used radioisotopes for trace metal seawater speciation studies

There are a number of trace metal radioisotopes that are commonly used for speciation work and they are listed in Table2 below.

Isotope	Half-life	Mode of decay	Detection Method (keV)
<sup>48</sup> V	15.98 d	EC to <sup>48</sup> Ti	γ (983, 1312)
<sup>51</sup> Cr	27.7 d	EC to <sup>51</sup> V	γ (320)
<sup>54</sup> Mn	312.2 d	EC to <sup>54</sup> Cr β <sup>-</sup> to <sup>54</sup> Fe	γ (835)
<sup>55</sup> Fe	2.73 y	EC to <sup>55</sup> Mn	LSC (5.9)
<sup>57</sup> Co	271.8 d	EC to <sup>57</sup> Fe	γ (122.1)
<sup>58</sup> Co	70.88 d	EC to <sup>58</sup> Fe	γ (810.8)
<sup>59</sup> Fe	44.51 d	β <sup>-</sup> to <sup>59</sup> Co	γ (1099,1292)
<sup>60</sup> Co	5.271 y	β <sup>-</sup> to <sup>60</sup> Ni	β(318.7), γ (1173, 1332)
<sup>63</sup> Ni	100 y	β <sup>-</sup> to <sup>63</sup> Cu	LSC (66.9)
<sup>64</sup> Cu	12.7 h	EC to <sup>64</sup> Ni β <sup>-</sup> to <sup>64</sup> Zn	γ (511)
<sup>65</sup> Zn	243.8 d	EC to <sup>65</sup> Cu	γ (1115)
<sup>67</sup> Cu	2.58 d	β <sup>-</sup> to <sup>67</sup> Zn	γ (185)
<sup>75</sup> Se	119.78 d	EC to <sup>75</sup> As	γ (136,265)
<sup>99</sup> Mo	2.7476 d	β <sup>-</sup> to <sup>99m</sup> Tc	LSC (739, 778)
<sup>109</sup> Cd	462 d	EC to <sup>109</sup> Ag	γ (22)

Table 2. Commonly used radioisotopes for trace metal speciation work in seawater (Data complied from sources mentioned in the text). Abbreviations used: d – days, h – hours, y – years, EC – electron capture, β<sup>-</sup> beta decay, γ - gamma counting and LSC – liquid scintillation counting.

Most of the radioisotopes listed above are routinely available commercially and many can be obtained as high specific activity carrier free solutions. See the later sections for more details regarding experiments involving the individual metals.

1.4 Typical applications of radioisotopes to trace metal speciation in seawater

Typically speciation work in marine biogeochemistry has utilized radioisotopes for two types of experiment: (i) Biological uptake under conditions of chemical equilibrium. (ii) Kinetics of transformation of a known species in seawater.

1.4.1 Uptake of trace metals by phytoplankton

Radioisotopes have been extremely important in improving our understanding of the links between chemical speciation and bioavailability of trace metals to phytoplankton and bacteria in the ocean. The genesis of this field began with the application of trace metal



buffers utilizing aminocarboxylate ligands; Nitrilotriacetic acid (NTA), Ethylenediaminetetraacetic acid (EDTA) and Diethylenetriaminepentaacetic acid (DTPA). A well characterized seawater media, AQUIL, was developed for use in trace metal uptake experiments (Price et al., 1989). New analytical tools were also required to determine the intracellular metal content from that simply adsorbed (Hudson and Morel, 1989) and this allowed the determination of metal quotas for cells (metal to carbon ratio, or metal per cell). Theoretical developments occurred simultaneously with new important paradigms and hypotheses that could be tested based on thermodynamic equilibrium between species; The Free ion association model (FIAM), see review by Campbell (1995) and later the Biotic Ligand Model (BLM) (Di Toro et al., 2001). The recognition that for some metals the system is not in equilibrium, due to slow exchange reactions (Hering and Morel, 1989; Hering and Morel, 1990), saw the use of pulse-chase experiments where a radio-isotope is added as a known species and its uptake followed over time. These new approaches led to important concepts with regard to the kinetic limitations (Hudson and Morel, 1993) on uptake by phytoplankton and how this can impact phytoplankton physiology (e.g. cell size, number of transport ligands).

Applications of the FIAM and BLM to experiments with natural seawater and phytoplankton communities are more complex as the chemical species which are bioavailable are mostly unknown. However if the added radio-isotope is in isotopic equilibrium natural uptake rates and metal quotas can be determined.

#### 1.4.2 Kinetics of exchange between trace metal species

Experiments investigating the kinetics of exchange between chemical species in seawater have also been applied using radioisotopes. This has typically been done in a pulse-chase fashion utilizing an analytical detection method that was capable of determining the chemical species of interest. These experiments have not always been at the lab bench scale, as past experiments have been performed under controlled conditions in mesocosms, including sediments, and using multiple tracers (Li et al., 1984; Santschi et al., 1987; Santschi et al., 1980).

## 2. Present state of the art

In the following sections we review the current and previous use of radioisotopes in seawater speciation studies for bio relevant trace metals.

### 2.1 Iron (Fe)

Our understanding of the marine biogeochemistry of iron (Fe) has developed rapidly over the last 30 years. The thermodynamically favoured redox form of Fe in seawater, Fe(III), is only weakly soluble in seawater (Millero, 1998). The reduced form, Fe(II), is found in oxic waters as a transient species, primarily generated by photochemical processes (Croot et al., 2008), and existing at extremely low concentrations (picomolar or less) because of rapid oxidation by  $O_2$  and  $H_2O_2$  in warm surface waters. The oxidation of Fe(II) to the less soluble Fe(III) species, leads to the formation of colloidal oxyhydroxide (Kuma et al., 1996) species which coagulate and form particulate iron (Johnson et al., 1997). Dissolved iron is strongly organically complexed throughout the water column (Boye et al., 2001). Iron is an essential element for all life and is a limiting nutrient in many parts of the global ocean as has been so clearly demonstrated in the mesoscale iron enrichment experiments (de Baar et al., 2005).

Work on iron biogeochemistry has greatly benefited from the easy availability of both  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  for tracer studies and no other trace metal has been so widely studied.

### 2.1.1 Solubility of iron in seawater

The solubility of iron in seawater is a controlling factor in its distribution in the ocean and information on this topic has been achieved predominantly through the use of radioisotope experiments. Initial work (Kuma et al., 1992) focused on the determination of the rate of dissolution as a function of pH, as measured using a dialysis tube (1kDa), of amorphous ferric oxides formed upon addition of  $^{59}\text{Fe}(\text{III})$  to seawater. This approach was then adapted to determine solubility directly in seawater samples by simple syringe filtration with a 0.025  $\mu\text{m}$  filter (Millipore MF) of a seawater solution that had been amended with 100 nM of radiolabelled Fe (Kuma et al., 1996). This technique has subsequently been applied to a range of oceanic environments; coastal Japan (Kuma et al., 1998b), the Pacific Ocean (Kuma et al., 1998a) and the Indian Ocean (Kuma et al., 1996). Liu and Millero (2002) used the same approach but employed a 0.02  $\mu\text{m}$  Anotop filter to measure iron solubility in UV irradiated seawater as a function of temperature and salinity. Field studies using the Anotop filter and  $^{55}\text{Fe}$  have been reported from the Mauritanian upwelling (Schlosser and Croot, 2009). Ultrafiltration (Vivaflow 50) has also been applied to studies of the effects of different ligands on iron solubility in seawater (Schlosser and Croot, 2008). The kinetics of iron hydroxide formation was determined using  $^{55}\text{Fe}$  and ion-pair solvent extraction of chelated iron (Pham et al., 2006).

### 2.1.2 Kinetics of exchange between different iron species

Iron radioisotopes have proven extremely useful for examining the exchange kinetics between different iron species in seawater. Hudson et al. (1992) utilised  $^{59}\text{Fe}$  in combination with ion-pair solvent extraction of iron chelated by sulfoxine (8-hydroxyquinoline-5-sulfonate). Using this approach they measured the rate at which the inorganic  $\text{Fe}(\text{III})$  hydroxide species at seawater pH (referred to as  $\text{Fe}'$ ) are complexed by EDTA and the natural terrestrial siderophore desferrioxamine B (DFO-B). Another approach to measuring  $\text{Fe}'$  in UV irradiated seawater was developed by Sunda and Huntsman (2003) using solid phase extraction with EDTA on C18 Sep-Paks, by where the  $\text{Fe}'$  was retained on the column. The phenomena of colloidal pumping, where iron initially in the colloidal size range is transformed into particles has also been investigated in seawater using  $^{59}\text{Fe}$  (Honeyman and Santschi, 1991).

### 2.1.3 Iron uptake by phytoplankton and regeneration by zooplankton grazing

The use of radioisotopes to determine the rate of iron uptake by phytoplankton in trace metal buffered media is the best example there is for the advantages that this approach has over stable isotopes. The literature abounds with several key studies from Morel's group that shaped the direction of marine research on iron; the availability of  $\text{Fe}(\text{II})$  and  $\text{Fe}(\text{III})$  to diatoms (Anderson and Morel, 1980), iron colloids (Rich and Morel, 1990), the ability to separate intracellular from extracellular iron (Hudson and Morel, 1989) and the importance of kinetics (Hudson and Morel, 1990). Later work by Sunda and colleagues showed the differences in iron requirements between coastal and oceanic species (Sunda and Huntsman, 1995; Sunda et al., 1991) and the relationship between iron, light and cell size (Sunda and Huntsman, 1997).



There have also been a number of studies examining the role of zooplankton grazing in transforming iron contained in phytoplankton back into the dissolved phase (Hutchins and Bruland, 1994). A dual tracer ( $^{55}\text{Fe}$  and  $^{59}\text{Fe}$ ) approach has also been used to study the fate of intracellular and extracellular iron in diatoms when grazed by copepods (Hutchins et al., 1999). The direct remineralisation of colloidal iron by protozoan grazers has also been observed (Barbeau et al., 1996). Other trophic transfer mechanisms investigated include the transfer of bacterial iron to the dissolved phase by ciliates (Vogel and Fisher, 2009) and remineralisation via viral lysis (Poore et al., 2004).

#### **2.1.4 Iron redox speciation**

Somewhat surprisingly there have been very few studies examining iron redox processes in seawater using radioisotopes. Though in part this is most likely due to the short life-time of this species in ambient seawater and the application of chemiluminescence techniques to detect pM Fe(II) (Croot and Laan, 2002). The photoreduction of  $^{59}\text{Fe}$ -EDTA has been used as a model system by both Hudson et al. (1992) and Sunda and Huntsman (2003). Photoreduction of natural iron complexes in the Southern Ocean has been shown to be strongly related to UV-B (Rijkenberg et al., 2005). The biological reduction of iron by phytoplankton has also been investigated by Shaked et al. (2004) who used Ferrozine as an Fe(II) chelator and then retained the complex on C18 Sep-Paks.

### **2.2 Manganese (Mn)**

Manganese (Mn) is a redox sensitive element which is important to phytoplankton due to its involvement in photosynthesis through photosystem II in converting  $\text{H}_2\text{O}$  to  $\text{O}_2$  (Falkowski and Raven, 1997). Mn is also utilized in superoxide dismutases (Peers and Price, 2004). Mn has a scavenged type profile (Landing and Bruland, 1987) and a secondary Mn maximum occurs in the oxygen minimum zone (Johnson et al., 1996). Mn(IV) is the thermodynamically favoured form in seawater but is strongly hydrolysed forming particulate  $\text{MnO}_2$ . Mn(II) is weakly hydrolysed in seawater and does not form strong organic complexes in seawater and is slowly oxidized to particulate Mn(III) and Mn(IV) under seawater conditions (von Langen et al., 1997).

#### **2.2.1 Mn uptake by phytoplankton**

The uptake of Mn by phytoplankton has been investigated for a number of species by using  $^{54}\text{Mn}$ . In a series of now classical laboratory studies by Sunda and Huntsman (1983; 1996) the interactive effects between Mn and Cu, Zn and Cd in phytoplankton were investigated and showed clearly the competition for uptake by these elements for the same transport ligands in the diatom species tested. Other work has shown that the  $^{54}\text{Mn}$  taken up by phytoplankton can be recycled back into the dissolved phase through the action of zooplankton grazing by copepods (Hutchins and Bruland, 1994).

#### **2.2.2 Mn oxidation**

The oxidation of dissolved Mn(II) in seawater to particulate manganese oxides has been studied extensively in the field via the use of  $^{54}\text{Mn}$  (Emerson et al., 1982) and taking advantage of the differences in the solubilities of the different Mn redox states. Initial studies focused on the role of oxygen in the bacterially mediated oxidation of Mn(II) in sub-oxic zones (Tebo and Emerson, 1985). Work in oxygenated surface waters by Sunda and

Huntsman (1988) found that in the Sargasso Sea that Mn oxidation was inhibited by sunlight, consistent with photoinhibition of manganese oxidizing bacteria. Moffett (1997) confirmed this for the Sargasso Sea but found in the Equatorial Pacific that phytoplankton uptake of Mn may be more important. Similar Mn oxidation studies have been performed in the Eastern Caribbean (Waite and Szymczak, 1993) and in hydrothermal plumes (Mandernack and Tebo, 1993). A number of field studies by Moffett and co-workers have sought to link bacterial Mn oxidation to the oxidation of Co (Moffett and Ho, 1996) and Ce (Moffett, 1994).

### 2.2.3 Mn photoreduction

The dissolution of  $^{54}\text{MnO}_2$  in seawater has been extensively investigated and found to be strongly related to the presence of  $\text{H}_2\text{O}_2$  formed by the photoreduction of  $\text{O}_2$  by dissolved organic matter (Sunda et al., 1983). Photoreduction of  $\text{MnO}_2$  in shallow sediments has also been observed (McCubbin and Leonard, 1996). Laboratory studies have also investigated the impact of humic acids on the photoreduction of  $\text{MnO}_2$  (Spokes and Liss, 1995).

### 2.3 Copper (Cu)

The speciation of Copper (Cu) in seawater is dominated by organic complexation (Coale and Bruland, 1988) by ligands which are believed to be produced by phytoplankton in response to Cu stress (Croot et al., 2000). While Cu(II) is the thermodynamically favoured redox state in oxygenated seawater there is growing evidence that Cu(I) may also be significant. Radiotracer studies into Cu chemistry however are limited by the short half-lives of the two isotopes available  $^{64}\text{Cu}$  ( $t_{1/2} = 12.7$  hours) and  $^{67}\text{Cu}$  ( $t_{1/2} = 2.58$  days).

Initial studies on Cu uptake by phytoplankton used  $^{64}\text{Cu}$  and were focused on pulse chase experiments with NTA buffers and lipophilic  $^{64}\text{Cu}$  complexes that could pass directly through the phytoplankton cell wall (Croot et al., 1999). Later work showed the existence of an efflux system for Cu from the Cu stressed cells of the cyanobacteria *Synechococcus* (Croot et al., 2003). Recent works on the uptake of Cu by phytoplankton have utilized the longer lived isotope  $^{67}\text{Cu}$  to obtain important information on the uptake kinetics of Cu by diatoms (Guo et al., 2010), determined cellular Cu quotas for different phytoplankton types (Quigg et al., 2006) and showed the dependence of Cu on Fe uptake (Maldonado et al., 2006) and in turn the role of Fe in determining the cellular quota for Cu (Annett et al., 2008). However the most exciting application so far has been the first reported use of  $^{67}\text{Cu}$  for work performed using natural phytoplankton assemblages from the North Pacific (Semeniuk et al., 2009).

### 2.4 Zinc (Zn)

Zinc (Zn) is a required metal for bacteria and phytoplankton in the ocean as it serves as a metal cofactor for many important processes (Vallee and Auld, 1993). Most notably Zn is utilized for both nucleic acid transcription and repair proteins (Anton et al., 2007) in the enzyme alkaline phosphatase (Shaked et al., 2006) and for the uptake of  $\text{CO}_2$  via the enzyme Carbonic Anhydrase (CA) (Morel et al., 1994). The strong requirement for Zn by phytoplankton results in low concentrations in surface waters and a nutrient like profile in the ocean (Table 1). In most surface waters Zn is strongly organically complexed (Bruland, 1989), however in deep waters and in surface waters of the Southern Ocean inorganic complexes can dominate (Baars and Croot, 2011).

The use of  $^{65}\text{Zn}$  was central to the first speciation studies of Zn uptake by phytoplankton performed on cyanobacteria (Fisher, 1985) and diatoms (Sunda and Huntsman, 1992).

Studies into  $^{65}\text{Zn}$  uptake by bacteria (Vogel and Fisher, 2010) found a much lower uptake of Zn than Cd.  $^{65}\text{Zn}$  has also been used in assessing the release of Zn from phytoplankton (Hutchins and Bruland, 1994) and bacteria (Vogel and Fisher, 2009) during zooplankton grazing.

## 2.5 Cobalt (Co)

Cobalt (Co) is present in seawater at very low concentrations ( $< 100 \text{ pM}$ ) and can exist as either inert Co(III) complexes or more labile Co(II) organic species (Saito and Moffett, 2002). Despite the range of Co isotopes available (Table 2) there have been relatively few studies examining the seawater speciation of Co. Work by Nolan et al. (1992) utilised a dual tracer approach where the uptake of  $^{57}\text{Co}$ -cobalamine was compared to that of  $^{60}\text{Co}$ -Co(II) and found that the cobalamine was taken up significantly faster and retained for longer in phytoplankton.  $^{57}\text{Co}$  was also used to show that Co could replace Zn in the enzyme carbonic anhydrase in some phytoplankton (Yee and Morel, 1996). An early finding with  $^{57}\text{Co}$  was that the oxidation of Co(II) to Co(III) in solution (Lee and Fisher, 1993) may be mediated by the same bacteria responsible for Mn oxidation (see section 2.3.2). Though new data (Murray et al., 2007) suggests no Co(II) oxidation occurs in the complete absence of Mn(II) and that the mechanism by which bacteria oxidize Co(II) is through the production of the reactive nano-particulate Mn oxide. Co has also been found to be released back to the dissolved phase from grazed and decomposing phytoplankton (Lee and Fisher, 1994).

## 2.6 Speciation studies with other trace metals

Studies into the biogeochemical cycles of other elements in seawater that are strongly hydrolysed and thus analogous to iron are limited by the lack of suitable radiotracers. There are no Aluminium (Al) radioisotopes suitable for use in trace metal speciation studies, as the majority of them have half-lives shorter than 10 minutes. The long lived isotope  $^{26}\text{Al}$  (710,000 y) has found application in paleo applications (Lal et al., 2006). Similarly there are also no seawater studies on Titanium (Ti) biogeochemistry with radioisotopes due to the short half-lives of  $^{45}\text{Ti}$  (3.08 h),  $^{51}\text{Ti}$  (5.76 min) and  $^{52}\text{Ti}$  (1.7 min). The longest lived Ti isotope,  $^{44}\text{Ti}$  (43.96 y) is difficult to produce and it is not yet available commercially.

Vanadium (V) exists in oxygenated seawater as the inorganic vanadate ( $\text{VO}_4^{3-}$ ) species and while a useable radio-isotope exists,  $^{48}\text{V}$  (Table 2), it has so far been only applied to a few studies in marine systems, most notably examining the uptake of vanadate by ascidians who accumulate high concentrations of vanadium in their blood (Michibata et al., 1991). Chromium (Cr) exists in seawater in two redox states as either chromate ( $\text{CrO}_4^{2-}$ ) or the reduced form Cr(III). There have only been a limited number of studies using either  $^{48}\text{Cr}$  or  $^{51}\text{Cr}$  (Table 2) and most have focused on the uptake of  $\text{CrO}_4^{2-}$  (Wang and Dei, 2001b) or colloidal Cr (Wang and Guo, 2000) by phytoplankton.

### 2.6.1 Nickel (Ni)

In seawater Nickel (Ni) shows a nutrient like behaviour and is present in surface waters at nM concentrations (Table 1). While some studies have shown organic complexation of Ni in seawater such work is complicated by the slow exchange kinetics for Ni(II) in seawater. A number of important advances in our understanding of Ni biogeochemistry in the ocean have come about through the use of  $^{63}\text{Ni}$ . Firstly Price and Morel (1991) observed that Ni was required for growth on urea, a Ni containing enzyme, by the diatom *Thalassiosira*

*weissflogii*. Later Dupont and co-workers investigated the uptake of  $^{63}\text{Ni}$  by the globally important cyanobacteria *Synechococcus* in a laboratory study (Dupont et al., 2008) and made field measurements of  $^{63}\text{Ni}$  uptake by natural phytoplankton assemblages (Dupont et al., 2010). They showed that Ni was a required element for many strains of *Synechococcus* and by comparison to the available genomic data most likely all strains of *Prochlorococcus*. This was due to the use of a Ni containing superoxide dismutase and in the enzyme for urease uptake. Importantly they also observed that isotopic equilibrium was not established between the added radiotracer and the natural pools of Ni within 24 hours indicating the slow exchange kinetics of Ni in seawater (Hudson and Morel, 1993).

### 2.6.2 Selenium (Se)

Selenium (Se) is found in very low concentrations in seawater ( $< 1 \text{ nM}$ ) and its chemistry is under kinetic redox control (Cutter, 1992) with the oxyanions Selenate ( $\text{SeO}_4^{2-}$ ) (thermodynamically favoured in oxygenated seawater and selenite ( $\text{SeO}_3^{2-}$ ), both showing nutrient like profiles in the ocean. In surface waters enhanced concentrations of organic selenide (operationally defined) is typically present (Cutter and Bruland, 1984).

The radioisotope  $^{75}\text{Se}$  has been used in a number of studies to elucidate the biogeochemistry of Se in phytoplankton cells. A key early finding was the identification of the pathway of uptake of selenite into the diatom *Thalassiosira pseudonana* and its conversion into the Se containing enzyme glutathione peroxidase (Price and Harrison, 1988). Later studies using the coccolithophorid *Emiliania huxleyi* as a model organism (Obata et al., 2004), have shown in more detail the steps involved into the uptake of selenite by the cells, and identified a pool of low molecular weight compounds which are used to store Se before incorporation into specific seleno-proteins in the cell. The interspecies differences in selenite uptake and accumulation have also been assessed (Vandermeulen and Foda, 1988), and the release of Se contained in phytoplankton cells by zooplankton grazing or phytoplankton decomposition (Lee and Fisher, 1992).

### 2.6.3 Molybdenum (Mo)

Molybdenum (Mo) is almost conservative (105 nM) in oxygenated seawater (Collier, 1985) where it is present as the oxyanion molybdate ( $\text{MoO}_4^{2-}$ ), under reducing conditions Mo is reduced to form Mo-sulfides (Erickson and Helz, 2000) and is rapidly precipitated from the water column (Helz et al., 2004). Molybdenum is an essential element for phytoplankton which is utilized as a co-factor in a number of different enzymes (Kisker et al., 1997) and in particular in nitrogenase and nitrate reductase which catalyze the reduction of  $\text{N}_2$  and nitrate to bioavailable N in the ocean (Mendel, 2005). While Mo has been shown to be a limiting nutrient for freshwater organisms the evidence of Mo limitation in marine organisms is unclear. The use of  $^{99}\text{Mo}$  in seawater studies requires special sample handling so that the daughter product  $^{99}\text{Tc}$  does not interfere for this reason it has been almost exclusively in nitrogen fixation studies where the focus has been on the potential for sulfate inhibition of Mo uptake leading to Mo limitation of nitrogen fixation (Marino et al., 2003).

### 2.6.4 Cadmium (Cd)

The marine biogeochemistry of Cadmium (Cd) has already yielded a number of surprises as it was long thought to be simply only toxic to organisms making its nutrient like profile in seawater and tight coupling with phosphate difficult to understand. However the



finding that Cd could replace Zn in the enzyme carbonic anhydrase showed for the first time a biological function for Cd (Lane and Morel, 2000) and changed biogeochemists view of this element. Cd appears to be weakly complexed by organic ligands in surface waters with inorganic species dominating in deeper waters (Bruland, 1992). The radioisotope  $^{109}\text{Cd}$  is frequently used for studies investigating the uptake of Cd by phytoplankton (Sunda and Huntsman, 1996). A particular focus has been with regard to bio-dilution effects and the role that growth limitation may have on their Cd content, either through iron limitation (Sunda and Huntsman, 2000), macronutrient limitation (Wang and Dei, 2001a) or temperature and irradiance uptake (Miao and Wang, 2004). Other studies have investigated the release of Cd from phytoplankton by cellular efflux mechanisms (Lee et al., 1995) or from zooplankton grazing/phytoplankton decomposition (Xu et al., 2001). The uptake of  $^{109}\text{Cd}$  has also been followed in natural phytoplankton communities in the English Channel (Dixon et al., 2006).

### 3. Potential new applications for iron speciation in seawater

In this section we outline some new applications using  $^{55}\text{Fe}$  for determining thermodynamic and kinetic information on iron speciation in seawater. Each of the outlined methods has been evaluated during shipboard trials at sea.

#### 3.1 Organic speciation of iron in seawater

Iron organic species are important to the biogeochemical cycling of iron in the ocean as they may determine the bioavailability of iron to phytoplankton and increase the solubility of iron in seawater. Currently most techniques to determine Fe speciation in seawater use voltammetry (Croot and Johansson, 2000; Gledhill and van den Berg, 1994; Rue and Bruland, 1995). In the following we have adapted the chemistry of an existing voltammetric method (Croot and Johansson, 2000) for use with a radiotracer.

##### 3.1.1 Theory: Competitive Ligand Exchange (CLE) – $^{55}\text{Fe}$ TAC

The theory behind the CLE approach was introduced by Ruzic (1982), van den Berg (1982). A brief outline of the theory for determining dissolved iron speciation is presented below, a fully treatment can be found in Croot and Johansson (2000).

For dissolved iron in ambient seawater, a mass balance can be constructed:

$$[\text{Fe}_T] = [\text{Fe}'] + [\text{FeL}_i] \quad (1)$$

where  $[\text{Fe}']$  represents the sum of all the inorganic species (predominantly  $\text{Fe}(\text{OH})_{x(3-x)+}$ ) and  $[\text{FeL}_i]$  represents the organically bound iron with  $L_i$  being classes of natural organic ligands. The speciation of Fe(II) is not considered, as in most cases the long equilibration times used in these experiments should have seen the oxidation of any Fe(II) present. Reactions between one class of the natural ligands and  $\text{Fe}'$  can be expressed as:



where  $L'$  is the Fe-binding ligand not already bound to Fe(III). The equilibrium expression is then:



$$K'_{\text{Fe'L}} = \frac{[\text{FeL}]}{[\text{Fe}'][\text{L}']} \quad (3)$$

where  $K'_{\text{Fe'L}}$  is the conditional stability constant with respect to  $\text{Fe}'$  under these specific conditions (in this case pH 8.0 seawater). To convert  $K'_{\text{Fe'L}}$  to  $K'_{\text{FeL}}$ , the conditional stability constant for FeL with respect to free  $\text{Fe}^{3+}$ , the relationship between  $\text{Fe}'$  and  $\text{Fe}^{3+}$ ,  $\alpha_{\text{Fe}'} = [\text{Fe}']/[\text{Fe}^{3+}]$ , can be used (e.g.  $K'_{\text{FeL}} = \alpha_{\text{Fe}'} K'_{\text{Fe'L}}$ ).

Upon addition of the competing ligand TAC, a new equilibrium is established between TAC, the natural organic ligands and iron:

$$[\text{Fe}_T] = [\text{Fe}'] + [\text{FeL}_i] + [\text{Fe}(\text{TAC})_2] \quad (4)$$

The complexation of  $\text{Fe}'$  by TAC can be described as:

$$\beta_{\text{Fe}(\text{TAC})_2} = \frac{[\text{Fe}(\text{TAC})_2]}{[\text{Fe}'][\text{TAC}]^2} \quad (5)$$

The side reaction coefficient for  $\text{Fe}(\text{TAC})_2$  with respect to  $\text{Fe}'$  is then denoted by:

$$\alpha_{\text{Fe}'(\text{TAC})_2} = \frac{[\text{Fe}(\text{TAC})_2]}{[\text{Fe}']} = \beta'_{\text{Fe}(\text{TAC})_2} [\text{TAC}]^2 \quad (6)$$

As  $[\text{TAC}'] \gg [\text{Fe}_T]$  for this method, the assumption  $[\text{TAC}'] = [\text{TAC}_T]$  can be used.

Titration performed using CLE-ACSV yield the fraction of Fe complexed by TAC at different Fe concentrations. This fraction is related to the side reaction coefficient by the following relationship (all relative to  $\text{Fe}'$ ):

$$\frac{[\text{Fe}(\text{TAC})_2]}{[\text{Fe}_T]} = \frac{\beta'_2 [\text{TAC}]^2}{1 + \sum K'_i L_i + \beta'_2 [\text{TAC}]^2} = \frac{\alpha_{\text{Fe}'(\text{TAC})_2}}{1 + \alpha_o + \alpha_{\text{Fe}'(\text{TAC})_2}} \quad (7)$$

$K_i$  is the conditional stability constant,  $L_i$  the concentration of the  $i$ th natural ligand,  $\alpha_o$  is the side-reaction coefficient for the naturally occurring ligands, and  $\beta'_2 [\text{TAC}]^2$  the side-reaction coefficient for TAC complexes, which was determined previously (Croot and Johansson, 2000). The side-reaction coefficient of Fe ( $\alpha_{\text{Fe}}$ ) for all naturally occurring ligands (including inorganic ligands) is related to the concentration of  $[\text{Fe}']$  by the relationship:

$$\frac{[\text{Fe}']}{[\text{Fe}_T] - [\text{Fe}(\text{TAC})_2]} = \frac{1}{1 + \sum K_i L'_i} \quad (8)$$

Data in this study were analyzed with a single ligand model that was a nonlinear fit to a Langmuir adsorption isotherm (Gerringa et al., 1995). The single ligand model is derived from equation 3, where  $[\text{L}_T] = [\text{L}'] + [\text{FeL}]$ . Rearranging Eq. 3, 4 and 8 yields a reciprocal Langmuir isotherm:

$$\frac{[\text{FeL}]}{[\text{Fe}']} = \frac{K[\text{L}_T]}{1 + K[\text{Fe}']} \quad (9)$$

We solved Eq. 9 for  $K$  and  $[\text{L}]$  by nonlinear regression analysis (Levenberg, 1944; Marquardt, 1963) with  $\text{Fe}'$  as the independent variable and  $[\text{FeL}]/[\text{Fe}']$  as the dependent variable using a purpose built program running Labview™. For the case of multiple ligands a more correct form of the equation is:

$$\frac{\Sigma[\text{FeL}_i]}{[\text{Fe}']} = \Sigma K'_i L_{i(i>1)} + \frac{[L_1]K'_1}{1 + K'_1[\text{Fe}']} \quad (10)$$

$\Sigma K_i L_{i(i>1)}$  is the side-reaction coefficient for the weaker ligands, and  $K_1$  and  $L_1$  represent  $K$  and  $L$  in Eq. 7.

### 3.1.2 Methodology: Competitive Ligand Exchange (CLE) – $^{55}\text{Fe}$ TAC

The seawater samples analyzed here were collected by the snorkel sampling system on the Polarstern (Schüßler and Kremling, 1993) from the Atlantic sector of the Southern Ocean during Polarstern expedition ANTXXIII-9 ((1) March 9, 2007 at 61° 55.67' S, 72° 43.23' E (2) March 19, 2007 at 61° 58.51' S, 82° 50.00' E).

A 0.01 M stock solution of TAC 2-(2-Thiazolylazo)-p-cresol; Aldrich was prepared in HPLC grade methanol, when not in use the stock solution is kept refrigerated. A 1.0 M stock buffer of EPPS (N-(2-hydroxyethyl)piperazine-N'-2-propanesulfonic acid; pKa 8.00; SigmaUltra) was prepared in 1 M  $\text{NH}_4\text{OH}$  (Fluka, TraceSelect). Ultrapure ( $R > 18 \text{ M}\Omega \text{ cm}^{-1}$ ) deionized water (denoted UP water) was produced using a combined systems consisting of a Millipore Elix 3 and Synergy point of use system. All equipment used was trace metal clean and performed under a class 100 laminar airflow bench (AirClean Ssystems). Waters C18 Sep-Pak cartridges (holdup volume 1 mL) were pre-cleaned using 5 mL of quartz-distilled methanol (Q-MeOH) and 5 mL of UP water.

In this work the  $^{55}\text{Fe}$  (Hartmann Analytics, Braunschweig, Germany) 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. The  $^{55}\text{Fe}$  stock solution was diluted to form working stock solutions with UP water and acidified with quartz-distilled HCl (Q-HCl) to a pH < 2 to prevent precipitation of the iron.

Subsamples (20 mL) of seawater were pipetted into a series of 12 Teflon bottles (60 mL) and 100  $\mu\text{L}$  of 1M EPPS added. Iron ( $^{55}\text{Fe}$ ) was added to all but two of the bottles, yielding concentrations from 0 to 12 nM. The added Fe was allowed to equilibrate with the natural ligands for one hour at laboratory temperature. At the end of this equilibration period, 20  $\mu\text{L}$  of 10 mM TAC was added and then left to equilibrate for 24 hours. At the end of this time the complete sample was pumped through the C18 column, followed by 5 mL of UP water and the filtrate (including UP water rinse) collected for counting. The  $^{55}\text{Fe}$  TAC complex retained on the column was recovered by a 5 mL rinse with Q-MeOH.

The activity of the samples was quantified using a liquid scintillation counter (Packard Tri-Carb 2900TR) with the scintillation cocktail Lumagel Plus (Lumac LSC). The efficiency of the instrument was obtained by quench curve calibration measurements. Separate quench curves were obtained for samples with seawater or methanol/TAC.

### 3.1.3 Example: Competitive Ligand Exchange (CLE) – $^{55}\text{Fe}$ TAC

The results of the ligand titration are shown in Figure 1 below and are analogous to similar titrations using electrochemical detection (Croot and Johansson, 2000). The  $^{55}\text{Fe}$ -TAC complex is efficiently retained by the C18 column (Baliza et al., 2009). Comparison of the FeL concentration determined by difference between the measured  $^{55}\text{Fe}$ -TAC concentration and that which directly passed through the C18 column indicates that either an appreciable amount of the FeL was hydrophobic and retained on the C18 column after the methanol rinse (see section 3.3). Non-linear fitting of the data (Figure 2) to equation 9 gave  $\log K = 21.23 \pm 0.08$  and  $L_T = 1.50 \pm 0.07$ , values consistent with other data from the Southern Ocean

using electrochemical techniques (Croot et al., 2004). The data shown here provides initial confirmation that this approach can be applied to measuring iron speciation in seawater and could potentially be less labour intensive and time consuming than the current electrochemical method.

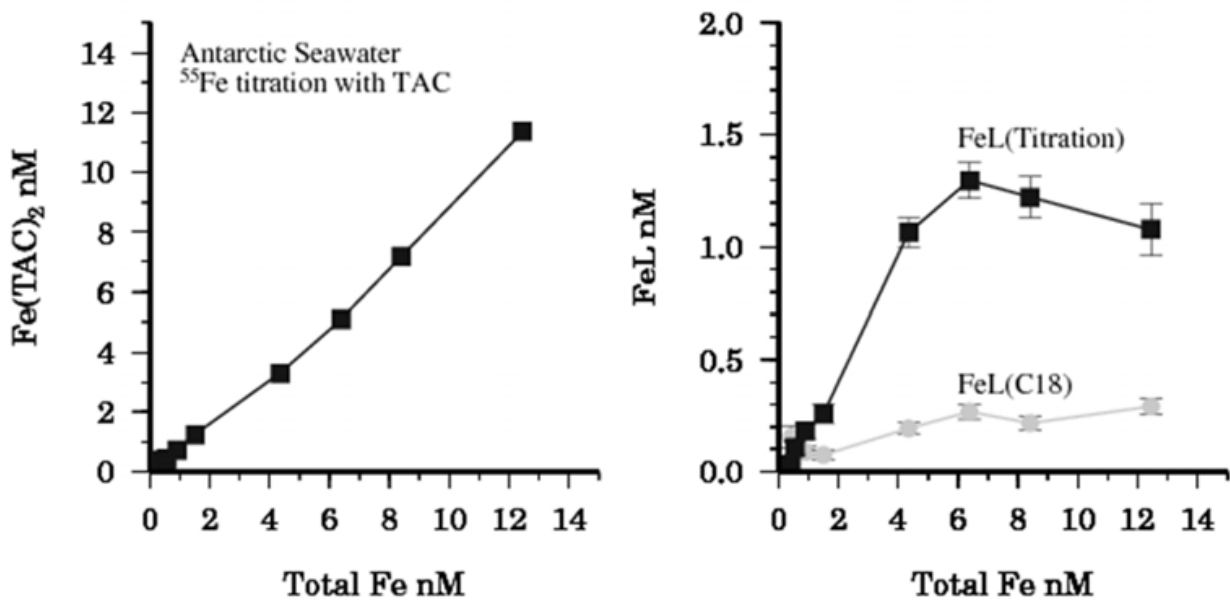


Fig. 1. (left) Recovery of  $\text{Fe(TAC)}_2$  as a function of the total iron in solution (natural iron plus added radiotracer). (right) The concentration of organic iron (FeL) measured in the samples as a function of the total iron in solution (natural iron plus added radiotracer). FeL(Titration) refers to the FeL determined by difference from the measured  $^{55}\text{Fe}$ -TAC concentrations and FeL(C18) is the directly measured concentration of the seawater filtrate that passed through the C18 Sep-Pak.

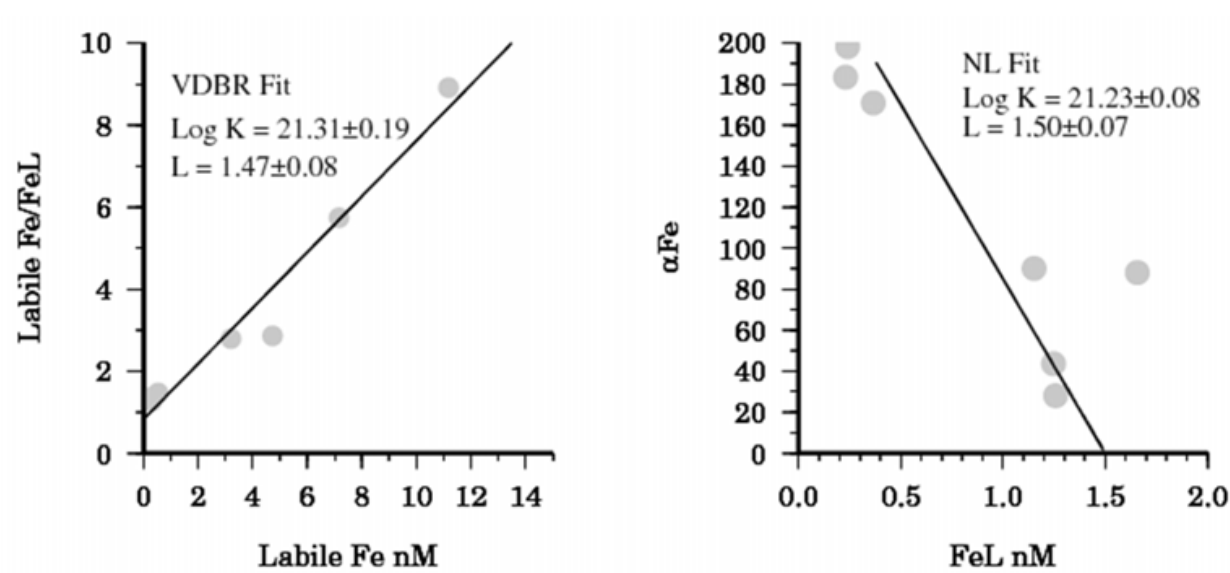


Fig. 2. (left) Van den berg/Ruzic fit to the data shown in Figure 1. (right) Non-linear fit (equation 9) to the data shown in Figure 1.

### 3.1.4 Issues: Competitive Ligand Exchange (CLE) – $^{55}\text{Fe}$ TAC

If this method is to be used more routinely there are a number of issues that would need to be addressed further. A critical factor in the interpretation of the data is whether the C18 column also retains hydrophobic organic Fe in addition to the  $^{55}\text{Fe}$  TAC complex as this could result in the retention of  $^{55}\text{Fe}$  not bound to TAC and lead to an underestimation of  $L_T$  if these complexes are removed by the MeOH rinse used to elute the  $^{55}\text{Fe}$  TAC complex. We examine the issue of natural Fe hydrophobic organic complexes in more detail in section 3.3. Additionally this method relies on an accurate measurement of the dissolved iron concentration in the seawater and this needs to be taken into account for the addition of  $^{55}\text{Fe}$ , as the ratio of  $^{55}\text{Fe}$  to stable iron increases with each subsequent addition of  $^{55}\text{Fe}$ . This variation in the overall specific activity of the solution could have an impact on the time required to establish isotopic equilibrium between radiotracer and ambient iron. In the present case it is assumed that the 24 hour equilibration time used was sufficient given that TAC most likely reacts with natural iron ligands via an adjunctive mechanism (Hering and Morel, 1990), though this is not yet confirmed. Finally the use of a high specific activity  $^{55}\text{Fe}$  source is essential if low level ( $< \text{nM}$ ) work is performed.

### 3.2 Dissociation kinetics of weak iron binding complexes

The earlier work on iron solubility in seawater by Kuma and co-workers (1992, 1993) assumed that the decrease in the concentration of soluble iron with time was due to the aging of meta-stable iron colloids and reduction in their solubility. However an alternative explanation is also possible as subsequent research has suggested that much of the added iron is initially complexed by weaker iron binding ligands (Gerringa et al., 2007) that slowly dissociate over time resulting in the loss of soluble iron from solution. In the following we adapt an existing radiotracer protocol for iron solubility measurements to determine the kinetics of the dissociation of the weak iron binding complexes in seawater.

#### 3.2.1 Filtration and size exclusion

Recently we became aware of a potential problem when comparing between different filters. In comparing an ultrafiltration system (Vivaflow 50) and the Anotop (Whatman) syringe filters (Figure 3) we found that the Anotop retained far more  $^{55}\text{Fe}$  than the ultrafilters. Further measurements comparing the  $0.02 \mu\text{m}$  Anotop filters with another type of ultrafiltration membranes (5, 10, 30, and 100 kDa) also found that the Anotop filters have a much smaller molecular weight cut off ( $< 5 \text{ kDa}$ ) than  $20 \text{ nm}$  (C. Schlosser, unpublished data). It seems likely then that the aluminium oxide matrix of the Anotop filter may also interact and adsorb some inorganic and organically complexed Fe species. Our finding agrees with an earlier study by Chen et al. (2004) which reported that they had observed that the Anotop filters were considerably different from its rated pore size of  $0.02 \mu\text{m}$  (or  $\sim 2000 \text{ kDa}$ ) as they found by using fluorescein tagged macromolecular compounds that it had an actual cutoff of  $\sim 3 \text{ kDa}$ . Our own initial work with  $0.025 \mu\text{m}$  Millipore MF filters suggest that these have a cutoff more in keeping with their stated poresize based on comparison with ultrafiltration. These results contrast with an ICP-MS study reporting on the existence of colloidal Fe in the ocean (Wu et al., 2001) which found apparently good agreement between the Millipore MF  $0.025 \mu\text{m}$  filters and the Anotop  $0.02 \mu\text{m}$  syringe filters. More work is needed urgently to address and understand the differences between ultrafiltration systems and how this effects our interpretation of the natural system being investigated.

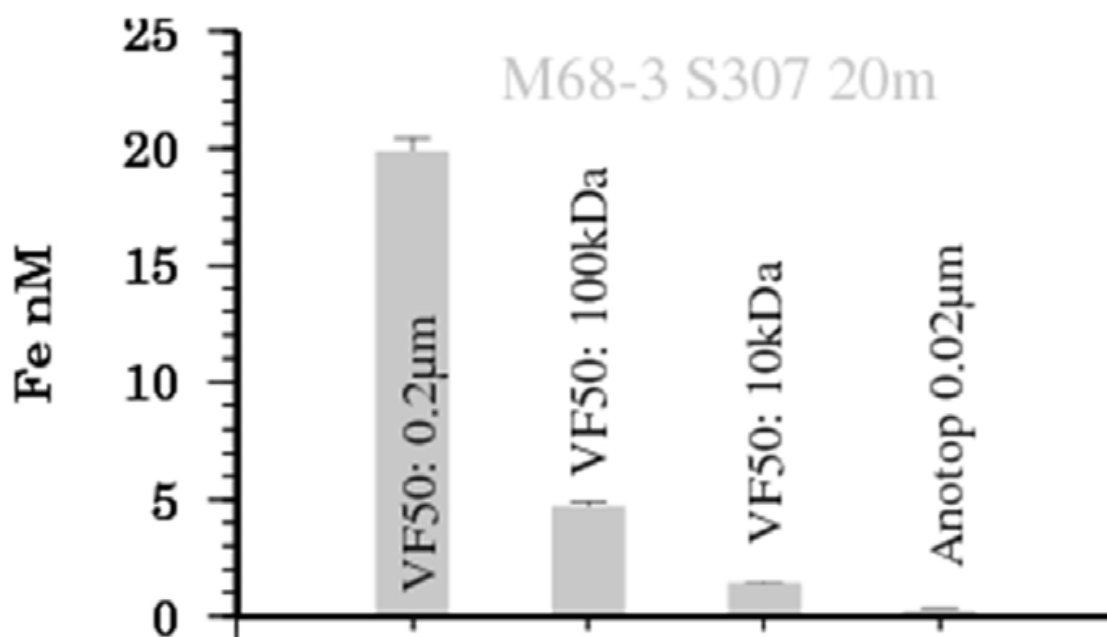


Fig. 3. Comparison of ultrafiltration methods using water collected by GO-FLO and amended with 20 nM  $^{55}\text{Fe}$

### 3.2.2 Dissociation kinetics of weak complexes

The following approach is based on the assumption that the observed decrease in soluble iron with time is due to the exchange of Fe between the weak organic ligands and the colloidal phase which does not pass through the filter. Support for this assumption lies partly in the findings that inorganic iron colloids formed from oversaturation of the solution will be formed very rapidly (Nowostawska et al., 2008) and be considerably larger (Hove et al., 2007) than the cutoff of the Millipore MF filter (25 nm) or Anotop (20 nm – though see 3.2.1 above). An earlier study by Okumura confirms that in the absence of a strong chelator over 95% of the Fe is found in the  $> 0.025 \mu\text{m}$  fraction (Okumura et al., 2004).

Thus the formation and dissociation of Fe complexes can be described by equations 2a and 2b from section 3.1.1. We now further assume that the ligands can be divided into two groups; a strong ligand ( $L_S$ ) that is practically inert to dissociation and a weaker ligand ( $L_W$ ) that at equilibrium is not able to keep iron in solution. Thus the soluble Fe concentration can be described by the following equation as a function of time, assuming that the formation of both weak and strong complexes is equally rapid.

$$\text{Fe}_{\text{sol}} = \text{Fe}L_S + \text{Fe}L_W(e^{-kt}) \quad (11)$$

where  $\text{Fe}_{\text{sol}}$  is the measured solubility of iron,  $\text{Fe}L_S$  is the concentration of the strong ligand and  $\text{Fe}L_W$  is the concentration of the weaker ligands which at thermodynamic equilibrium do not prevent the precipitation of iron from solution,  $k$  is the observed dissociation rate of the weaker iron organic complexes.

### 3.2.3 Methodology – Dissociation kinetics of weak iron-organic complexes

Filtered (0.2  $\mu\text{m}$ ) seawater samples were obtained from throughout the water column using GO-FLO sampling bottles on a trace metal clean line at two stations in the Tropical Atlantic



during the Polarstern expedition ANTXXVI-4. All sample handling was performed in a clean room container. In this work the  $^{55}\text{Fe}$  (Perkin Elmer) had a specific activity of 1985.42 MBq/mg Fe, a total activity of 75 MBq and a concentration of 1466.79 MBq/mL. The  $^{55}\text{Fe}$  stock solution was diluted as described in section 3.1.2.

Seawater (200 mL) from different depths was transferred into Teflon FEP bottles (1 L) and an aliquot of  $^{55}\text{Fe}$  was added to the bottles to give an addition of 21 nM. Subsamples (20 mL) for filtration were taken after 3, 6, 24 and 48 hours and were filtered through 47 mm 0.025  $\mu\text{m}$  Millipore MF filters using an all Teflon filtration unit (Savillex), the filtrate was collected in a Teflon vial. All experiments were performed at 23° C. The activity of the samples was quantified as described in section 3.1.2.

### 3.2.4 Example – Dissociation kinetics of weak iron-organic complexes

Samples for the kinetic experiments were obtained from vertical profiles at two stations in the Tropical Atlantic; (i) S283 - April 28, 2010 at 01° 46.62' N, 23° 00.18' W in the Equatorial Atlantic and (ii) S287 - May 4, 2010 at 17° 34.98' N, 24° 15.18' W, the TENATSO time series site (for more information on TENATSO see Heller and Croot (2011)). Figure 4 below shows example results for the time dependent decrease in soluble iron from two different depths from the TENATSO site.

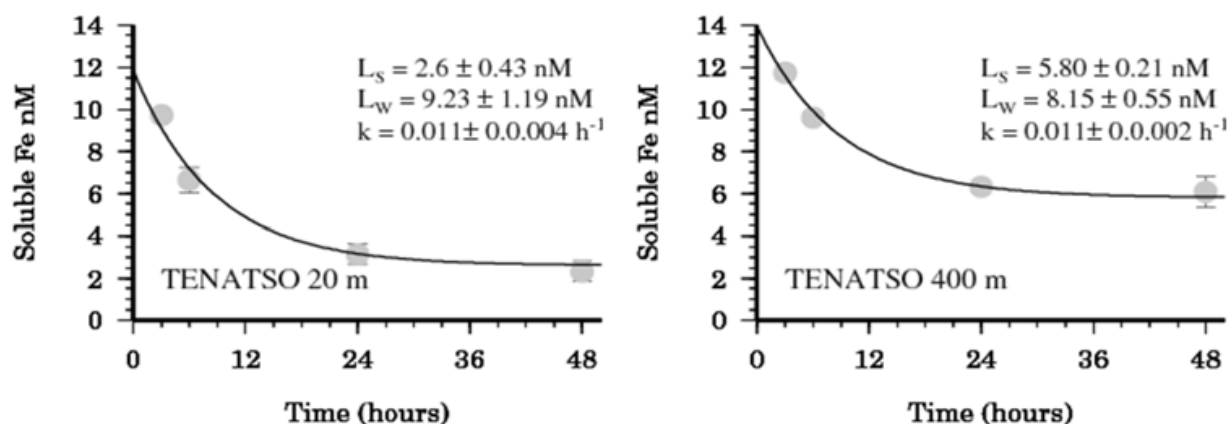


Fig. 4. Change in the concentration of Soluble Fe (0.025  $\mu\text{m}$  Millipore MF) over 48 hours after the addition of 21 nM  $^{55}\text{Fe}$  to water samples from the TENATSO station in the Eastern Tropical Atlantic. (left) 20 m depth. (right) 400 m depth. Error estimates are the result of duplicate measurements and correspond to the 95% confidence interval. The least squares fit to equation 11 are also shown (solid line).

In all samples soluble Fe was initially high (10-13 nM) and declined rapidly over the first 24 hours with only small or no changes in the concentration over a further 24 hours. This indicates that the equilibrium value for  $L_S$  was reached typically within 24 hours and that the weaker  $L_W$  ligands had dissociated within the same timeframe. Previous studies have indicated that this equilibrium is established over timescales ranging from 1-2 weeks for studies using 0.45  $\mu\text{m}$  pre-filtration (Kuma et al., 1996; Liu and Millero, 2002) to less than 24 hours when 0.2  $\mu\text{m}$  pre-filtration was used (Chen et al., 2004). This highlights the role of colloidal matter/ligands in the time it takes to reach equilibrium. All data was fit to equation 11 using least squares regression.

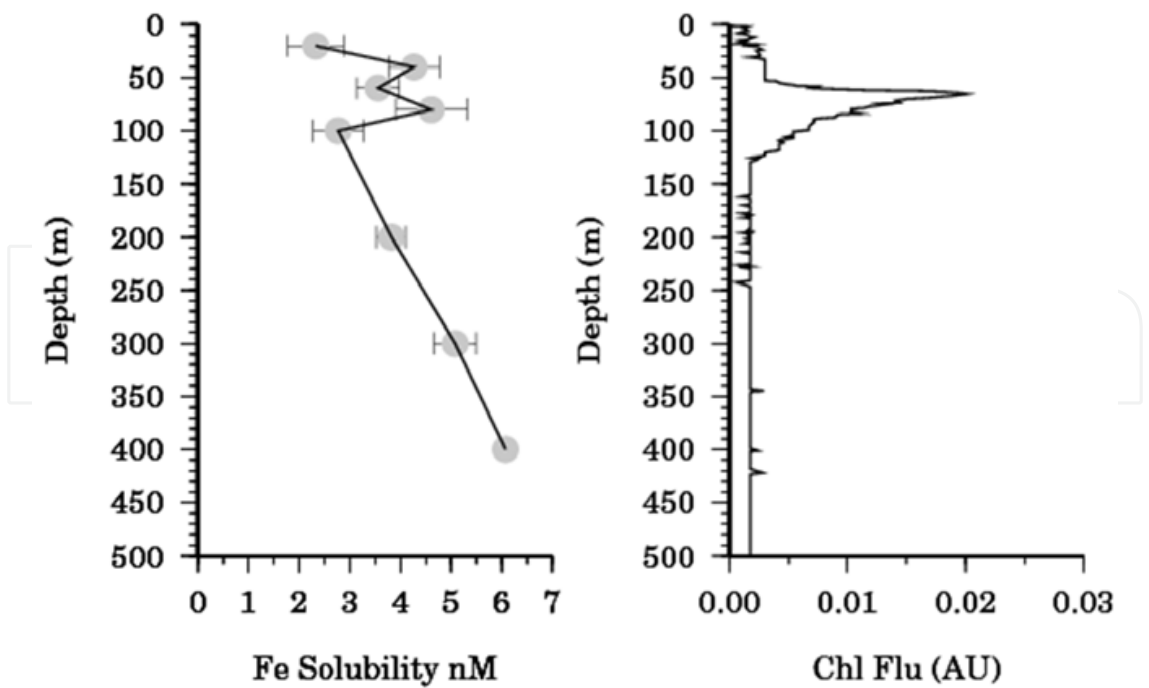


Fig. 5. (left) Vertical profile of iron solubility after 24 hours at TENATSO (ANTXXVI-4, S287). (right) Chlorophyll fluorescence (arbitrary units) at TENATSO.

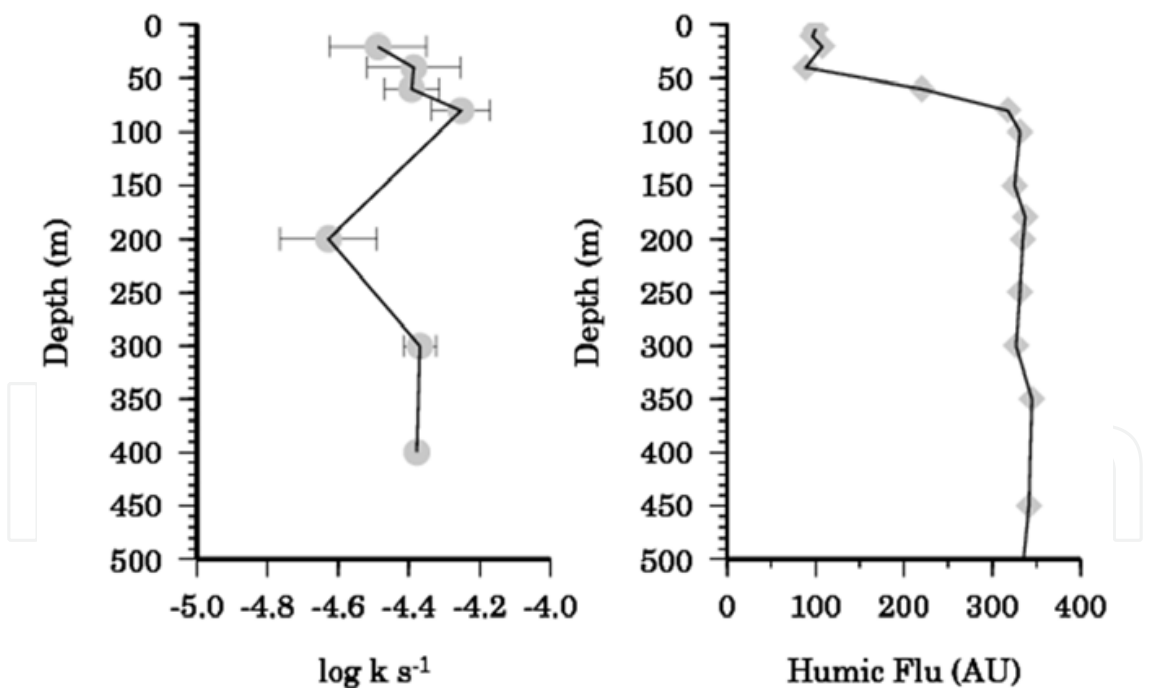


Fig. 6. (left) Vertical profile of dissociation rate ( $k$ ) for FeL<sub>W</sub> at TENATSO. (right) Vertical profile of marine humic fluorescence (arbitrary units, 320 nm excitation, 420 nm emission).

In earlier studies in the Pacific, iron solubility in intermediate and deep waters has been found to be highly correlated to the fluorescence of marine humic substances (Tani et al., 2003). The humic fluorescence profile at the TENATSO station is shown in figure 6 and is clearly poorly correlated with L<sub>S</sub> at this location. However at station (S283) in the Equatorial

Atlantic (Figure 7) we did observe a strong correlation between  $L_S$  and humic fluorescence (Figure 8).

The vertical distribution of iron solubility after 24 hours at TENATSO is shown in figure 5 and shows a generally increasing trend with depth with a small local maximum in the surface waters in the vicinity of the chlorophyll maximum consistent with a biological source for  $L_S$ . Values for  $L_W$  showed no systematic variation in the water column and ranged from 8-10 nM. The estimated dissociation rate,  $k$ , for  $L_W$  ranged from  $2 \times 10^{-5}$  to  $8 \times 10^{-5} \text{ s}^{-1}$  similar to voltammetric observations of weak iron binding ligands (Gerringa et al., 2007).

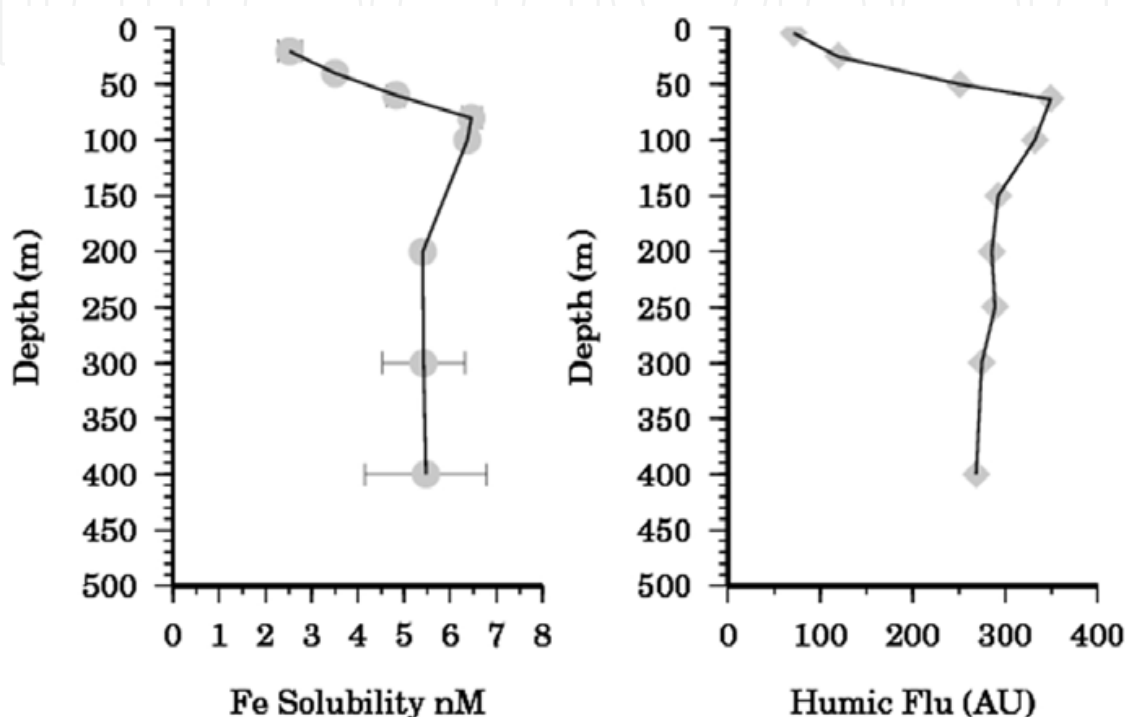


Fig. 7. (left) Vertical profile of Iron solubility after 24 hours at S283 in the Tropical Atlantic (ANTXXVI-4). (right) Marine Humic fluorescence (arbitrary units, 320 nm excitation, 420 nm emission).at the same location.

At S283  $L_W$  showed no consistent pattern over the depth range examined with values from 6-10 nM. Estimated rates for the dissociation of the iron complexes from the weak ligands,  $k$ , ranged from  $0.4 - 4.4 \times 10^{-5}$  and also showed no discernable pattern with depth. The correlation between humic fluorescence and  $L_S$  suggests that in this case the ligands were mostly derived by the same process inferred for the production of marine humics, the remineralisation of organic matter by microbial action. It furthermore suggests the photochemical destruction of the ligands in near surface waters at both S283 and TENATSO. The differences in the profiles between S283 and TENATSO may be related to a greater production of iron binding ligands by phytoplankton or bacteria at TENATSO. This may be in response to the greater dust flux this site receives as it lies directly under the path of the Saharan dust plume (Heller and Croot, 2011).

Our approach here clearly provides important information with regard to the kinetics of processes relevant to dust deposition to the ocean (Baker and Croot, 2010) and highlights the role that weaker ligands may play in solubilising iron from aerosols and allowing phytoplankton a critical few extra hours were it is still soluble and potentially bioavailable.

More work is clearly needed on this subject and the method outlined here should be a key contribution to this.

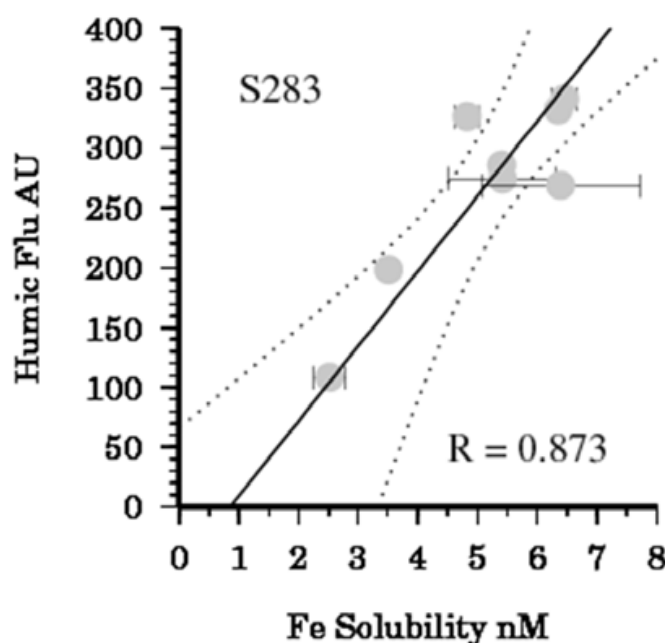


Fig. 8. Correlation between humic fluorescence and Fe solubility (after 24 hours) for samples from Station 283 in the Tropical Atlantic (Polarstern ANTXXVI-4).

### 3.3 Hydrophobic organic Fe complexes

As noted early in section 3.1 information on hydrophobic Fe complexes is important for the interpretation of methods using C18 columns to recovery the Fe from solution. Such information is also useful for assessing the scavenging behaviour of iron organic complexes to particles in seawater. Hydrophobic Fe complexes are known to exist as many siderophores possess a hydrophobic tail which facilitates the uptake of iron by the phytoplankton (Martinez et al., 2000). A number of siderophore complexes are quantitatively retained by C18 columns including the terrestrial siderophore desferrioxamine B and its Fe chelate, ferrioxamine B (Gower et al., 1989). This has lead to the development of extraction techniques for siderophores from seawater using C18 solid phase extraction (Freeman and Boyer, 1992). Other dissolved organic matter is also retained by this approach (Mopper et al., 2007) including marine humic complexes, though recoveries are highest when the sample is acidified (Amador et al., 1990).

There have been a number of studies that have utilized C18 or similar substrates to trap organic complexes using solid phase extraction techniques (Mackey, 1983). Previous work combining C18 solid phase extraction with radioisotopes in seawater has utilized  $^{64}\text{Cu}$ , finding that there is a significant but variable concentration of hydrophobic Cu complexes (Croot et al., 2003).

#### 3.3.1 Methodology – Hydrophobic organic Fe complexes

The description of the seawater sampling, sample handling and  $^{55}\text{Fe}$  standard preparation are the same as described in section 3.2.3. In these experiments, 20 mL of the seawater samples described in section 3.2.3 were pumped through Waters C18 Sep-Paks (cleaned as

described in section 3.1.2), rinsed with 5 mL UP water and then the  $^{55}\text{Fe}$  retained on the C18 column was eluted with 5 mL Q-MeOH. The activity of the MeOH samples was quantified as described in section 3.1.2. Samples were taken after 3, 6, 24 and 48 hours after the addition of the  $^{55}\text{Fe}$ .

### 3.3.2 Example – Hydrophobic organic Fe complexes

Seawater for this experiment was obtained from 4 depths at S283 (see description in section 3.2.4) and run as described above. The results from two of the kinetic runs are shown in Figure 9 below. In the sample from 40 m there was a clear decrease with time in the concentration of the hydrophobic Fe trapped by the C18 column. This was similar to the decrease in Fe solubility for the same sample (data not shown) suggesting that for this sample a significant portion of the weak organic ligands (section 3.2) were hydrophobic in nature. Contrastingly samples from deeper in the water column showed little variation with time (Figure 9) indicating that the bulk of the hydrophobic component here were stronger iron binding ligands.

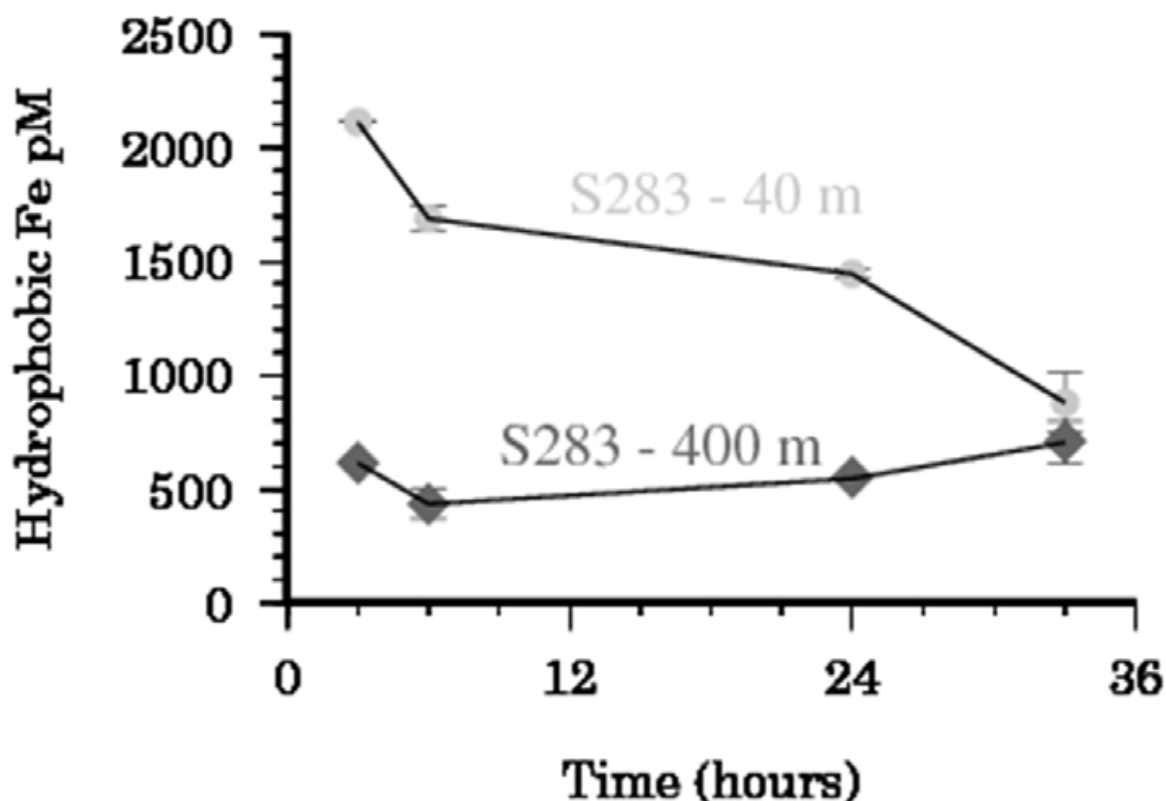


Fig. 9. Hydrophobic organic Fe complexes at S283 in the Equatorial Atlantic. Samples were obtained from 40 m depth (circles) and 400 m depth (triangles). The 95% confidence intervals for the data are represented as error bars.

The vertical distribution of hydrophobic Fe complexes is shown in Figure 10 and indicates a maximum in near surface waters with lower concentrations in deep waters suggesting a biological source. Comparison with the iron solubility data from section 3.2 indicates that the percentage of hydrophobic Fe that was in the soluble phase was high in surface waters and decreased rapidly to be only ~10 % below 200 m (Figure 10).



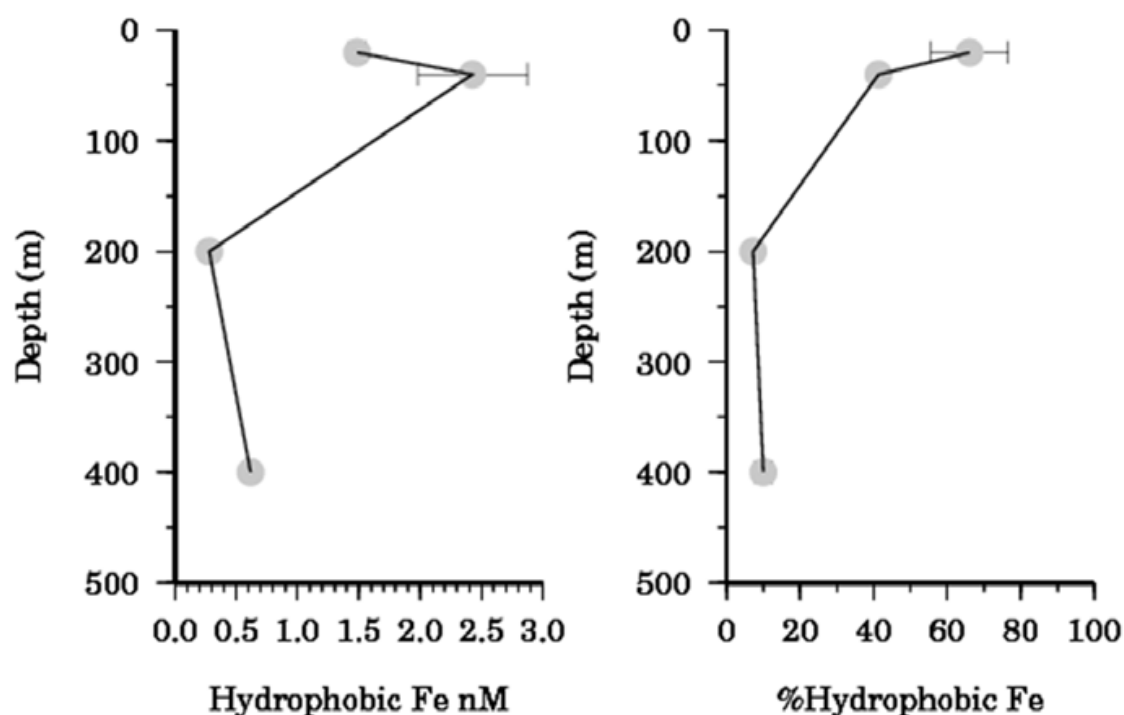


Fig. 10. Data from Station 283 (Polarstern expedition ANTXXVI-4. (left) Hydrophobic iron recovered by C18 Sep-Pak ( $t=24$  hours). (right) percentage of iron passing through  $0.025\ \mu\text{m}$  filter that is hydrophobic.

Thus at S283 it suggests that in waters below 200 m the Fe complexation was dominated by hydrophilic humic complexes while nearer to the surface complexation was by ligands that were more hydrophobic in nature. This is the first data on iron complexation in the ocean to show information on the hydrophobic nature of complexes in the water column. This data also points to the role that hydrophobic Fe complexes may play in the biogeochemical cycling of iron in the ocean. The chemistry of these complexes is a rapidly developing field as shown in a recent review article on metallosurfactants (Owen and Butler, 2011) and their role in bioinorganic processes.

### 3.3.3 Issues: Hydrophobic organic Fe complexes

The data we present above is for Fe retained on a C18 column and eluted with methanol. Previous work has shown that the retention of the Fe complex under similar conditions to those employed here varies between different iron species and that the methanol elution may not remove all the Fe that was trapped (Freeman and Boyer, 1992). Other studies have used nitric acid instead of methanol to recover the Fe retained on the C18 column (Abbas et al., 2002). In the present study we did not attempt a nitric acid rinse due to potential problems with this acid in the scintillation cocktail. Further work is needed to develop a more complete protocol that includes a complete mass balance.

Similarly colloidal inorganic iron has been found to be almost quantitatively trapped on C18 Sep-Paks (Sunda and Huntsman, 2003), in the present case it is unlikely that there is significant concentrations of this form of iron as outlined early in section 3.2. It would be even more unlikely that colloidal inorganic iron was formed in the  $^{55}\text{Fe}$  TAC experiment as this solution would be under saturated with respect to iron precipitation and the TAC is likely to dissolve any amorphous iron colloids quite rapidly (Croot and Johansson, 2000).

#### 4. Conclusions and future prospects

Radioisotopes are a vital tool for trace metal marine biogeochemists as they allow pulse chase experiments for rapid assessment of the kinetics of processes under natural conditions. The applications that radioisotopes can be applied to in seawater speciation studies is strongly linked to, and drives, new analytical developments in techniques to distinguish between individual chemical species. In the present work we have outlined new approaches for examining Fe organic speciation in seawater, but these methods could easily be applied to other trace metal radioisotopes also. New research is needed urgently for assessing the current status and the potential for change in oceanic systems due to global warming, ocean acidification and ocean deoxygenation. In this context we encourage future research into the use of trace metal radioisotopes for determining the changes in speciation and kinetic reactivity for oceanic redox processes in oxygen minimum zones. As this is a critical area of research that needs to be developed over the next decade in order to better assess the impact ocean deoxygenation may play on trace metal redox cycles in the ocean.

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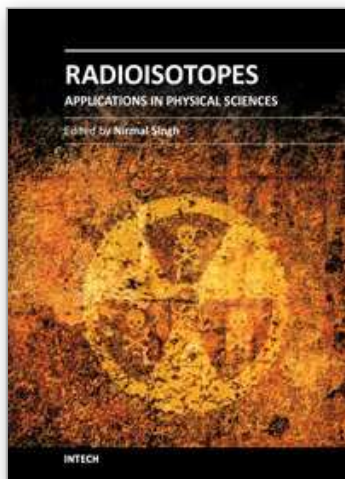
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