Physical and Chemical Speciation of Iron in the Polar Oceans

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Chapter 2

Materials and methods

1. Seawater fractions studied for the speciation of Fe

Analyses of Fe concentrations, ligand concentrations and their characteristics were done in unfiltered samples and in two size fractions:

1. Unfiltered samples (UNF), containing the total dissolvable fraction of Fe, which consist of the particulate fraction (>0.2 μm) and the dissolved fraction (<0.2 μm);
2. The dissolved fraction, by convention defined as <0.2 μm;
3. The fraction smaller than 1000 kDa (<1000 kDa), containing the truly soluble and the small colloidal fraction.

Note that the fraction comprised between 1000 kDa and 0.2 μm is called larger colloidal fraction in this thesis. In chapters 4 and 7, only the dissolved fraction was analysed and discussed.

2. Parameters commonly used

The following parameters were used to describe and interpret the results obtained during this thesis:

- Iron concentrations [Fe]: Fe in the unfiltered fraction is called Total Dissolvable Fe **[TDFe]** and represents the concentration of the Fe that has been dissolved after acidification and one year storage of the sample at pH 1.8. Iron in the dissolved fraction is denoted as [**DFe]** and is analysed in a sample that after the filtration was acidified to pH 1.8 and next analysed within 24 hours. The Fe in the fraction smaller than 1000 kDa as [$\mathbf{Fe_{<1000\ kDa}}$] was also acidified to pH 1.8 and analysed within 24 hours.
- Note that **particulate Fe** (>0.2 μm) is sometimes mentioned, and corresponds to [TDFe]-[DFe]. Similarly, **larger colloidal Fe** (between 1000 kDa and 0.2 μm) corresponds to [DFe]-[$Fe_{<1000\ kDa}$].
- Total ligand concentration (per fraction) is **[Lt]**: $\mathbf{[Lt]_{<0.2\ \mu m}}$ in the dissolved fraction, and $\mathbf{[Lt]_{<1000\ kDa}}$ in the fraction <1000 kDa.

- Excess ligand concentration (**[Excess L]** or **[L']**): Excess L corresponds to the empty ligand sites and thus expresses the binding potential of the ligands and is calculated by [Lt]-[Fe] for each size fraction.
- The conditional stability constant **K'** (**log K'** or **$\log_{10}$ K'** in its logarithmic form) reflects the binding strength of the natural ligands with Fe.
- The ratio **[Excess L]/[Fe]** (Chapter 3, 5 and 6) or **[Lt]/[Fe]** (Chapter 4 and 7) represent the relative saturation state of the ligands with Fe (per fraction). Note that the ratio [Excess L]/[Fe] is always >0 and the ratio [Lt]/[Fe] is always >1 as the ligands are in excess of Fe, otherwise Fe would immediately precipitate. A low ratio (close to 0 for [Excess L]/[Fe] or close to 1 for [Lt]/[Fe]) corresponds to ligands saturated with Fe and indicates a low capacity of the ligands to bind and buffer additional Fe input. Higher is the ratio, lesser the ligands become saturated with Fe, thus buffering Fe inputs, hence increasing the solubility of Fe.
- Alpha **α** (**log α** or **$\log_{10}$ α** in its logarithm form) is the product of K' and Excess L. Alpha expresses the reactivity of the ligands.
- The concentration of Fe^{3+} expressed by pFe, the negative logarithm of [Fe^{3+}]. A low pFe value corresponds to a high concentration of free Fe, and reversely.

3. Cleaning procedures

All sample bottles (Nalgene, Low-Density Polyethylene, LDPE) were cleaned according to the following procedure: first the bottles were rinsed with demineralised water, then filled with a detergent solution (5% concentrated, Micro-90, International Products Corporation). Next, the bottles were soaked for 24 h in a hot bath (60-70ºC). After this, each bottle was rinsed with demineralised water to remove the soap and rinsed 2 times with MQ water (Millipore Milli-Q deionised water, R >18.2 MΩ cm^{-1}). Subsequently the bottles were filled with 6 M HCl (diluted from 37% HCl, reagent grade, J.T. Baker) and soaked during 24 h in a 60-70ºC bath. Next the bottles were rinsed 3 timed with MQ water. This acid-wash procedure was repeated, but then using a 3 M nitric acid (diluted from 65%, reagent grade, J.T. Baker). Finally, the bottles were stored filled with 0.2 M 3QD-

HNO3 (from 65% reagent grade, J.T. Baker) and each packed in two LDPE plastic bags. All the filling/emptying and rinsing steps were done in a clean room (class 100).

4. Sampling systems

Onboard R.V. *Pelagia* (Chapter 3) and R.V. *Polarstern* (Chapters 4, 5 and 6) the seawater samples were taken from the surface to the bottom using the Titan Mk. II frame which was connected to a Kevlar hydrowire (De Baar *et al.*, 2008a). On the frame were attached 24 internally Teflon-coated PVC 12 L GO-FLO samplers (General Oceanics Inc.). Immediately upon recovery the frame was placed inside a clean container for a direct sub-sampling from each GO-FLO sampler: "In fact this is within the more stringent criteria of an ISO Class 6 clean room (formerly US FED STD 209E Class 1000)", in De Baar *et al.*, 2008a.

Onboard R.V. *Nathaniel B. Palmer* (Chapter 7) in the Amundsen Sea (Southern Ocean) samples were taken in the upper 300 meters of the water column (typically at 10, 25, 50, 100, 200, 300 m) using the same type of modified Teflon coated GO-FLO samplers as used above on the frame, but here attached to a non-metal 6 mm diameter Dyneema wire. The samplers were closed using messengers.

5. Sampling procedures and filtrations

All samples were collected in acid-cleaned LDPE bottles after 5 times rinsing of the bottles with the sample itself (UNF, dissolved or <1000 kDa samples).

Unfiltered samples were taken first (Figure 1, step 1), then the rest of seawater was directly filtered in-line (0.2 μm pore size, Sartorius Sartobran-300) from the GO-FLO using slight nitrogen gas overpressure of 1.5 atm (Figure 1, step 2). Ultra-filtration of the 0.2 μm filtered water (Figure 1, step 3) was performed immediately after the first filtration in a laminary flow bench (class 0). Onboard R.V. *Nathaniel B. Palmer* (Chapter 7), the GO-FLO samplers were brought one by one to a trace metal clean container and linked to a tubing extension for sub-

sampling under a clean laminar down flow bench. Samples were filtered (0.2 µm pore size, Sartorius Sartobran-300) using N_2 overpressure (1.5 atm).

Ultrafiltration was performed using hollow-fibre-filters (Sterapore, Mitsubishi-rayon Co., Ltd.) with a size cut-off of 1000 kDa (Nishioka *et al.*, 2001). A 12-channels peristaltic pump (ISM 937, Ismatec, IPC-N) with Tygon® LFL (Long Flex Life) tubing was used for the ultra-filtration with a flow rate of 5-7 $ml.min^{-1}$.

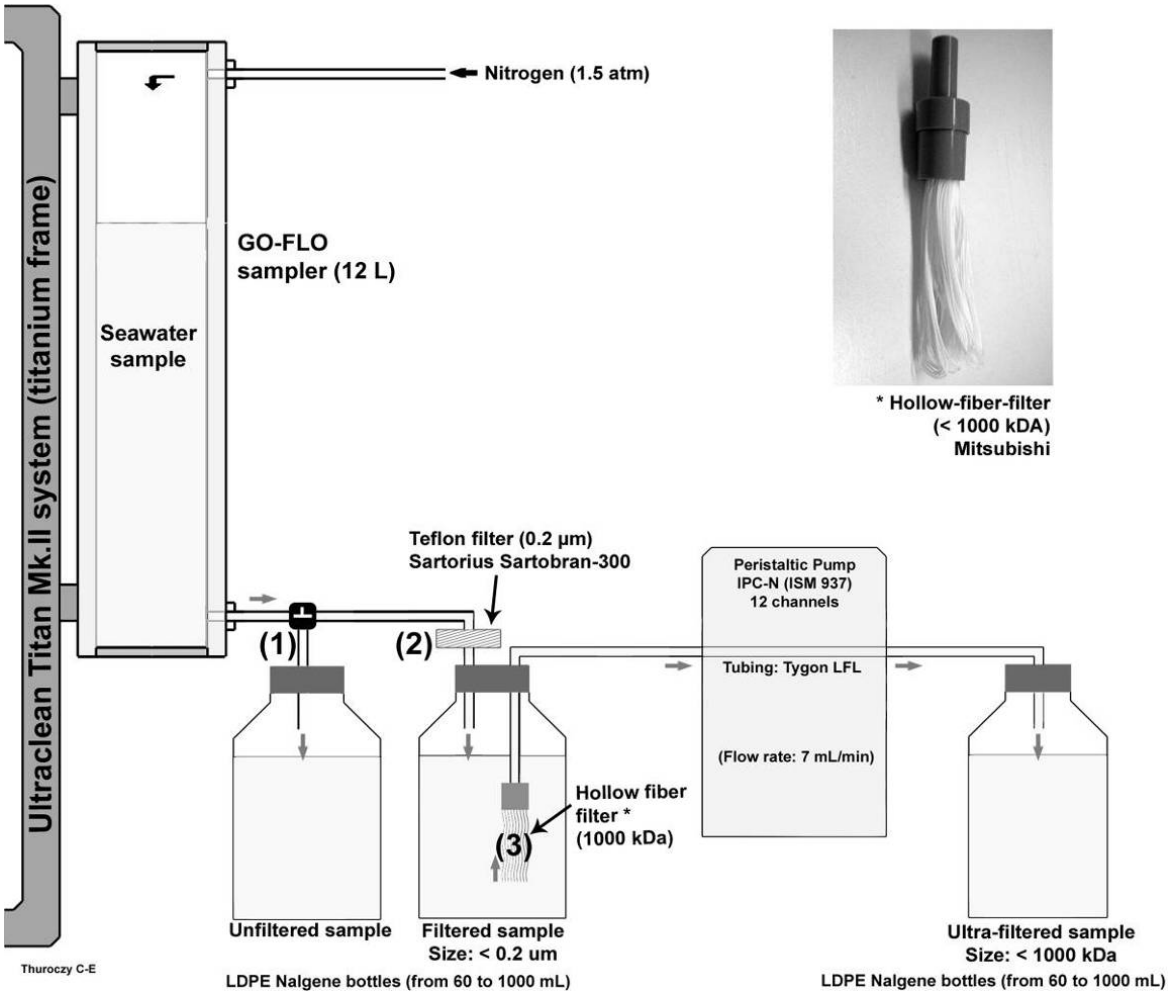

Figure 1: *Sampling using the Titan Mk. II frame and size fractionation of seawater:* ***1:*** *Unfiltered sampling.* ***2:*** *Filtration of seawater sample over 0.2 µm pore size filter.* ***3:*** *Ultra-filtration of the seawater sample over 1000 kDa pore size filter. Steps 1 and 2 were done in the titanium-frame clean air container. Step 3 was performed in a laminary flow bench (class 0) in another clean room.*

The polyethylene hollow-fibre-filters were activated and cleaned in the home laboratory before use on board according to the following protocol adapted from Nishioka *et al.* (2001).

The filters were activated by pumping 15 ml of three times quartz distilled (3QD-) methanol (flow rate of 5 $ml.min^{-1}$), then rinsed with 30 ml MQ water. They were left soaking for 3 days in an HCl bath (1 M, Suprapur, Merck) during which each day 25 ml of HCl 1 M was pumped through the filter (5 $ml.min^{-1}$). Then, they were rinsed with MQ water (140 ml, 7 $ml.min^{-1}$) and stored in acid-cleaned polypropylene tubes closed by caps and filled with acidified MQ water (0.02 M HCl, Suprapur, Merck). Before use on board, the filters were rinsed by pumping MQ water (300 ml, 7 $ml.min^{-1}$) and the sample itself (200 ml, 7 $ml.min^{-1}$).

A mass balance verification for Fe as well as for the ligands was done with 4 samples from the Southern Ocean (cruise ANTXXIV/3). Two samples, one from the surface layer, containing 0.162 nM Fe, and one deep sample near the sediment containing 0.994 nM Fe, were used. The sum of the larger colloidal (here assumed to be between 1000 kDa and 0.2 µm) and <1000 kDa fractions was compared with the dissolved fraction (<0.2 µm). This resulted in a perfect mass balance for Fe concentrations in both samples. Regarding the ligand concentrations, in surface samples a gain of Excess L concentration of 0.10 Eq of nM Fe was measured whereas in the deep samples a loss of Excess L of 0.05 Eq of nM Fe. These results were of the same order of magnitude as the detection limit (0.04 Eq of nM Fe) as mentioned in paragraph 7.1. Moreover, no Fe contamination was detected in the filtrate and in the retentate. Therefore the filters were considered to function properly.

6. Samples conservation and storage

Samples taken for Fe analysis by FIA were immediately acidified to pH 1.8 using 12 M ultraclean HCl (Baseline® Hydrochloric Acid, Seastar Chemicals Inc.). The dissolved and <1000 kDa filtered samples were left acidified for at least 12 h

before analysing. The unfiltered samples were acidified in the same way, but they were stored one year before being analysed in the home laboratory.

Samples taken for the analysis of the Fe speciation were stored at 4ºC when their analysis could be performed within 3 days; otherwise they were immediately frozen at -20ºC in the dark. Unfiltered samples were kept in the dark at 4ºC and measured within 3 days to avoid any influence of biological activity. They were discarded beyond 3 days storage in case that the analysis could not be performed.

7. Iron analyses

Iron analyses were done by an in-line flow injection analysis (FIA) system using chemiluminescence as a detection method (De Jong *et al.*, 1998) and is described by Klunder *et al.* (2011). Samples were acidified to pH 1.8 using 12 M ultraclean HCl (Baseline® Hydrochloric Acid, Seastar Chemicals Inc.). The filtered samples (<0.2 μm and <1000 kDa fractions) were measured directly onboard and left acidified for at least 12 h before analysing. The unfiltered samples were acidified in the same way, but they were stored one year before being analysed in the home laboratory using the same system and procedure in a class 100 clean-room.

The samples for Fe analysis by FIA and Fe speciation were taken from the same station, cast, GO-FLO sampler and using the same filter cartridge, but in different sub-sampling bottle (*i.e.* duplicate bottles).

The method analyses Fe(III), therefore hydrogen peroxide (Merck suprapur 30%) solution was added (60 μl of a 1‰) at least one hour before analysis to ensure oxidation any Fe(II) present (Lohan *et al.*, 2005). The acidified samples were pre-concentrated over a Toyopearl AFChelate 650M (TesoHaas Germany) column during 120 s. Subsequently the column was rinsed with MQ for 60 s, after which Fe was eluted with 0.4 M HCl (Suprapur, Merck) for 120 s and injected in the photon counter (Hamamatsu HC 135). The system was controlled by an interface developed in LabView. The standard deviation of the duplicate measurements (dissolved fraction) or triplicate measurements (<1000 kDa fraction and unfiltered samples) of one sample was lower than 5%. The blank, *i.e.* the background value of Fe in the MQ water and chemicals, is defined as the

calculated amount of photons measured at 0 s loading time. Blank values varied slightly between the different days, but did not exceed 80 pM. The lowest detection limit, defined as three times the standard deviation of the blank (De Jong *et al.*, 1998), was 11, 1 and 8 pM for the measurements of the unfiltered, dissolved and <1000 kDa fractions, respectively.

For the validation of the measurements and for the long term consistency, a certified SAFe reference water was regularly measured. The measured Fe concentrations (Table 1) were in accordance with the community consensus values (Johnson *et al.*, 2007).

Table 1: Validation of the measurements using certified SAFe water for each cruise. Concentrations of Fe measured in SAFe water are in nM ± Standard Deviation (S.D.); n is the number of measurements.

	S1	**D2**
Published value (Johnson *et al.*, 2007)	0.097 ± 0.043; n=140	0.91 ± 0.17; n=168
Arctic Ocean (ARK XXII/2)		0.92 ± 0.06; n=24
Southern Ocean (ANT XXIV/3)	0,101 ± 0,034; n=34	0,97 ± 0,07 ; n=20
Southern Ocean (NBP09-01)	0.078 ± 0.012; n=10	0.942 ± 0.043; n=13

8. Determination of iron speciation

8.1. Voltammetric procedure and sample treatment

Organic complexation of iron was determined by competing ligand exchange – adsorptive stripping voltammetry (CLE-AdSV) using 2-(2-Thiazolylazo)-p-cresol (TAC) as a competing ligand (Croot and Johansson, 2000). The voltammetric equipment consisted of a μAutolab potentiostat (Type II, Ecochemie, The Netherlands), a mercury drop electrode (model VA 663 from Metrohm). The mercury drop size was approximately 0.25 mm^2. The reference electrode was double-junction, Ag/AgCl, 3 M KCl, with a salt bridge filled with 3 M KCl and a glassy carbon counter-electrode. Samples were stirred with a PTFE Teflon stirrer (3000 rpm). A current filter (Fortress 750, Best Power) to which the equipment was linked was used to prevent electrical noise.

The seawater sample was buffered to pH 8.05 by adding a mixed NH_3/NH_4OH borate buffer (final concentration 5 mM). The buffer stock was 1 M boric acid, (Suprapur, Merck) in 0.25 M ammonia (Suprapur, Merck) cleaned through a

SepPak C18 column with 20 µM TAC (2-(2-Thiazolylazo)-p-cresol). A stock of 0.02 M TAC was prepared in 3QD-Methanol for a final concentration of 10 µM in the seawater sample (Croot and Johansson, 2000).

Additions of Fe(III) standard (0, 0.33, 0.5, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8 nM, Chapter 2, 3, 4, 5 and 6; and 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 nM, Chapter 7) were done with a stock of 1 µM Fe(III) (prepared in 0.03 M HCl, Seastar chemicals Inc.) in a series of Teflon PFA vials (Savillex, 30 ml volume) including 2 blanks (0 nM Fe addition). The seawater sample was poured into the Teflon PFA vials (15 ml per vial). The titration series was left overnight to equilibrate before measuring.

Before preparing the first titration series (true sample), all Teflon vials were conditioned two times beforehand by preparing a titration with iron additions using filtered (<0.2 µm or <1000 kDa) seawater containing the lowest concentration of Fe possible.

Before each titration series, the voltammetric Teflon cell was cleaned using a blank solution. The chemical blank was below the detection limit of the method, being 0.027 nM [$Fe(TAC)_2$] obtained by calculating three times the noise.

Each equilibrated aliquot was transferred into the voltammetric Teflon cell and purged with nitrogen for 180 s. The differential pulse method was used. The deposition potential of -0.4 V was applied during 140 to 240 s (depending on the sample and on the sensitivity of the equipment) and the sample was stirred to allow a better adsorption of Fe-$(TAC)_2$ on the mercury drop. An equilibration time of 5 s without stirring was done before scanning between -0.4 to -0.7 V at 1.95 $mV.s^{-1}$ (modulation amplitude was 25.05 mV). Modulation time was 0.01 s and interval time was 0.1 s. The visible peak due to the dissociation of the Fe-complex was found between -0.460 V and -0.500 V. Each measurement was done at least twice.

The detection limit of the method was determined as 3 times the standard deviation of several blank measurements and was 0.040 Eq of nM Fe (n = 11). The chemical blank was below this detection limit.

8.2. Calculation of iron speciation

Ligand concentrations [Lt] (in Equivalent of nano Molar of Fe, Eq of nM Fe), conditional stability constants K' and their respective standard deviations were calculated using the Langmuir model (Eq.1, non-linear regression of the Langmuir isotherm, Gerringa *et al.*, 1995). By using the Langmuir model (Gledhill and Van Den Berg, 1994) it is assumed that equilibrium between all Fe(III) species exists, all binding sites between Fe and the unknown ligand Lt are equal and binding is reversible, as follows:

$$[FeL] = \frac{K' \times [Fe^{3+}] \times [Lt]}{1 + K' \times [Fe^{3+}]} \qquad \text{(Eq.1)}$$

Here [FeL] is the concentration of natural iron-ligand complexes assuming the existence of one organic ligand, and a one to one coordination, $[Fe^{3+}]$ is the ionic iron concentration, K' and [Lt] are two unknown parameters that need to be determined.

K' is the conditional stability constant of Fe with the natural ligand:

$$K' = [FeL] / ([Fe^{3+}] \times [L']) \qquad \text{(Eq.2)}$$

With [L'] being the concentration of empty ligand sites (excess ligand concentration); and assuming equilibrium as follows:

$$Fe^{3+} + L' \leftrightarrow FeL \qquad \text{(Eq.3)}$$

And [Lt] represents the total ligand concentration assuming equilibrium as follows:

$$[Lt] = [FeL] + [L'] \qquad \text{(Eq.4)}$$

Equation 1 is obtained using equations 2 and 4 as follows:

$$\text{From Eq. 4, } [L'] = [Lt] - [FeL] \qquad \text{(Eq.5)}$$

$$\text{So, Eq.2 becomes } K' = \frac{[FeL]}{[Fe^{3+}] \times ([Lt] - [FeL])} \qquad \text{(Eq.6)}$$

When Eq.6 is writen as function of [FeL], it becomes Eq.1.

In order to solve Equation 1 which has two unknowns, K' and [Lt], a titration of the empty ligand sites with different standard Fe additions is needed to obtain a series of $[Fe^{3+}]$ and [FeL]. To do so, the competing ligand TAC is used. Like the natural ligand, TAC is in equilibrium with Fe^{3+}:

$$Fe^{3+} + 2\ TAC' \leftrightarrow Fe(TAC)_2 \qquad \text{(Eq.7)}$$

$$\text{And } \beta_{Fe(TAC)2} = [Fe(TAC)_2] / ([Fe^{3+}] \times [TAC]^2) \qquad \text{(Eq.8)}$$

With $\beta_{Fe(TAC)2}$ being the conditional stability constant of Fe with TAC assuming equilibrium and TAC' being free TAC defined here as the concentration of TAC that is not bound to Fe.
It is assumed that [TAC'] = [TAC] (total) since TAC is largely in excess:
[TAC] = 10 µM ($\beta_{Fe(TAC)2} = 10^{22.4}$, Croot and Johansson, 2000).

$$\text{Using Eq.8:} \qquad [Fe^{3+}] = [Fe(TAC)_2] / (\alpha_{Fe(TAC)2}) \qquad \text{(Eq.9)}$$

$$\text{With } \alpha_{Fe(TAC)2} = \beta_{Fe(TAC)2} \times [TAC]^2 = 10^{12.4} \qquad \text{(Eq.10)}$$

According to the mass balance of Fe,

$$[FeL] = [Fe_{fraction}] + [Fe_{added}] - [Fe(TAC)_2] \qquad \text{(Eq.11)}$$

Where $[Fe_{fraction}]$ is the iron concentration measured by FIA in either the unfiltered (TDFe), orthe dissolved (DFe) or the <1000 kDa fractions ($Fe_{<1000\ kDa}$); $[Fe_{added}]$ is the concentration of iron added for the titration and $[Fe(TAC)_2]$ represents the concentration of iron bound to TAC. The latter $[Fe(TAC)_2]$ is calculated for every Fe addition by dividing the peak height (nA) by the slope (S = sensitivity) of the straight part of the titration curve. The sensitivity S (in $Amper.mol^{-1}$) of the method is influenced by ligand sites not yet saturated with Fe as explained by Turoczy and Sherwood (1997) and Hudson *et al.* (2003). This is accounted for by an algebraic solution of the equilibrium equations including the Langmuir

isotherm, in which S is determined together with Lt and K'. The estimated parameters are given with standard deviation from the fit of the model to the data.

For the calculation of pFe, the negative logarithm of $[Fe^{3+}]$, the sum of the measured alpha of the natural organic ligands (product of the concentration of excess L and K' = [L'] x K') and that of the alpha of the inorganic ligands ($\alpha_{inorg} = 10^{10}$ after Millero, 1998) were used as follows:

$$pFe = -\log [Fe^{3+}] = -\log\{[Fe_{fraction}] / (\alpha_{org}+\alpha_{inorg})\} \quad \text{(Eq.12)}$$

8.3. Estimation of the ligand characteristics in unfiltered samples

The ligand concentration [Lt] and stability constant K' are variables which depend on the Fe concentration used in the calculations. However under natural conditions (seawater pH ~8), part of Fe in unfiltered (UNF) samples is irreversibly bound in colloids or into mineral particles which are refractory (not dissolvable). This refractory Fe (unknown percentage of TDFe) does not participate in the speciation of Fe determined here. Moreover, phytoplankton cells and micro-organisms contain Fe which is released in seawater after acidification, thus over-estimating the Fe concentration in the sample. Therefore, for UNF samples, the concentrations of Lt and Excess L were estimated in two ways: an upper limit using [TDFe] and a lower limit using [DFe] in the calculations (Paragraph 7.2. above).

Note that the ligand concentration [Lt] and stability constant K' are artificially increased when using [TDFe] (assumed to be exchangeable for the calculations). However, the concentration of Excess L ([Lt]-[Fe]), is hardly influenced by the Fe concentration (Thuróczy *et al.*, 2010b, Chapter 3 and 2011a).