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Photochemistry and organic complexation of iron

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Chapter 7

Kinetic study reveals new Fe-binding ligand which affects the solubility of Fe in the Scheldt estuary

Loes J.A. Gerringa, Micha J.A. Rijkenberg, Bert Th. Wolterbeek, Tona G. Verburg, Marie Boye, Hein J.W.de Baar

Abstract

The chemistry of dissolved Fe(III) was studied in the Scheldt estuary (The Netherlands). Two discrete size fractions of the dissolved bulk ($<0.2 \mu\text{m}$ and $<1 \text{ kDa}$) and three salinities ($S=26$, 10 and 0.3) were considered.

Characteristics of dissolved organic Fe-binding ligands were determined by competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-ACSV) and by observing kinetic interactions. The conditional stability constant (K'), of the traditionally recognised dissolved organic Fe-binding ligands varies between $10^{20.4}$ and $10^{21.1}$ over the salinity range $S=0.3$ to $S=26$. For both size fractions the ligand concentrations are slightly lower than the dissolved Fe concentrations. Within the upper estuary where the fresh river water first meets seawater, the dissolved Fe and ligand concentrations decrease steeply with increasing salinity. Further downstream in the middle and lower estuary the decrease continues but far less pronounced. Since Fe is in excess over these ligands the solubility product of Fe(hydr)oxides apparently is exceeded in the whole estuary. The introduction of a new, relatively weak, ligand class and the study of kinetics between the different Fe(III) pools provided an explanation of the existence of high dissolved “labile” concentrations of Fe. We concluded that weak ligands (P) prevented precipitation of Fe(hydr)oxides. Its α value (binding potential = $K' \cdot [P]$) varies between 10^{11} and $10^{11.5}$. The relatively weak ligand is thought to retard direct precipitation of an extra input of Fe. The presence of the weak ligand influences the analysis by CLE-ACSV, masking a salinity dependence in K'_{FeL} . It is the dissociation rate constant of FeL that causes this salinity dependence, but this can only partly be ascribed to the change in ionic strength. Changes in the origin of the organic ligands must take place as well, hence serve as the complementary explanation.

Rate constants of Fe with the competing ligand TAC were also measured in relation to salinity. Both rate constants, formation and dissociation of $\text{Fe}(\text{TAC})_2$ decreased with salinity, in such a way that $K'_{\text{Fe}(\text{TAC})_2}$ remained constant between $S=10$ and $S=26$ with the value known from the literature ($\log\beta'_{\text{Fe}(\text{TAC})_2}=22.4$; Croot and Johansson, 2000). Yet the $\log\beta'_{\text{Fe}(\text{TAC})_2}$ is 26.8 at $S=0.3$, substantially higher than 22.4 .

1. Introduction

Fe is essential for phytoplankton growth and is known to be limiting not only in the open ocean but also in coastal areas (Hutchins et al., 1998). To study the influence of the organic ligands on the solubility and reactivity of Fe may give valuable information to understand the biogeochemical behaviour of Fe. In this study we estimate the influence of dissolved organic ligands on the solubility and reactivity of Fe in the Scheldt estuary. Since the nature of organic ligands changes from terrestrial to marine environments, large solubility changes of the metals can be expected across salinity gradients (Baeyens et al., 1998).

The Scheldt estuary can be characterised as a system with strong hydrodynamic and physico-chemical gradients. The mixing zone of fresh and salt water extends over a distance of 70 to 100 km (Wollast, 1988). The upper part of the estuary has a high organic and nutrient load. Because most particulate matter is negatively charged, mixing of fresh water and seawater neutralises the surface charges resulting in a flocculation zone between $S=1-5$ (Sholkovitz, 1976; Wollast, 1988; Paucot and Wollast, 1997). Due to the large input of biodegradable organic matter during the summer, anoxia occurs (Duinker et al., 1983; Wollast, 1988). During the nineteen-eighties the quality of the estuary has been improved, reducing the nutrient and organic matter load, but still in summer anoxia occurs (Zwolsman and van Eck, 1993; Baeyens et al., 1998).

The residence time of water in the upper estuary is around 3 months during which non-refractory organic matter of terrestrial origin is almost completely mineralised (Wollast, 1988). Decrease in turbidity at salinities higher than 5 in combination with the large supply of nutrients by the river Scheldt produces phytoplankton blooms in spring and summer. During these periods the produced organic matter almost equals the amount of terrestrial organic carbon removed by respiration and sedimentation in the upper part (Wollast and Peters, 1978). Most of this freshly formed organic matter is transported to the North Sea.

Processes influencing metal solubility in the Scheldt estuary have been studied extensively in the past. The concentration of dissolved metals, like Zn, Cd, Mn, Cu and Fe, depends for 100% on the combination of redox conditions and organic ligand content (Chapter 8, Duinker and Nolting, 1978; van den Berg et al., 1987; Regnier and Wollast, 1993; Zwolsman and van Eck, 1993; Gerringa et al., 1996; Paucot and Wollast, 1997). In the upper estuary, where anoxia can occur, the dissolved Fe concentrations are relatively high (Duinker and Nolting, 1978; Zwolsman and van Eck, 1993). Metals like Zn and Cd easily precipitate as sulphides, since the solubility product of these solid sulphides is low (Emerson et al., 1983; Comans and van Dijk, 1988; Gerringa et al., 2001). In contrast Fe sulphides have a relatively high solubility product and their precipitation is unlikely in the Scheldt estuary, since the dissolved sulphide concentrations are not high enough. Concentration of dissolved organic

ligands are thought to determine the concentration of dissolved Fe above the solubility product of solid phases stable at the redox potential of the system (van den Berg et al., 1987).

However, Whitworth et al. (1999) could not explain the dissolved Fe concentrations in the Scheldt estuary with their model considering binding with dissolved organic ligands on the one hand and adsorption on particles on the other hand. The dissolved Fe concentrations were too high according to their model. From other studies it is known that high labile (with respect to the applied method) Fe concentrations occur in seawater which cannot be easily explained (Croot and Johansson, 2000; Boye et al., in press; Powell, personal communication; Chapter 8). The existence of a weak ligand, outside the detection window of the method applied to determine dissolved ligands, may constitute a perfect explanation for both "too high" dissolved Fe concentrations in the Scheldt estuary and relatively high labile Fe concentrations. To examine a possible existence of other ligand groups than the organic ones commonly found with K' between 10^{19} and $10^{23.5}$ (Gledhill and van den Berg, 1994; Croot et al., 2004), the complexation characteristics were studied in the Scheldt estuary in combination with a kinetic study.

We sampled three specific regions: **a.** upstream of the high turbidity zone, where terrestrial organic matter is present in high concentration ($S=0.3$); **b.** the zone where high primary production of plankton blooms generates fresh organic matter ($S=10$); **c.** the zone with predominantly coastal marine organic matter ($S=26$). Characteristics, like the dissolved organic ligand concentration L , the conditional stability constant K' of organic Fe complexes, and its dissociation and formation rate constants were determined in two size fractions ($<0.2 \mu\text{m}$ and $<1 \text{ kDa}$).

2. Methods

2.1. Sampling, filtration and measurement of dissolved Fe

In April 2002, samples were taken during a cruise on the ship *Navicula* (Royal-NIOZ) in the estuary of the river Scheldt in the south of the Netherlands (Figure 1). A torpedo towed alongside the ship was used to pump (Teflon diaphragm pump, Almatec A-15, Germany, driven by a compressor, Jun-Air model 600-4B, Denmark) water from 1 a 2 meter depth via acid-washed braided PVC tubing to an overpressurized class 100 clean container. The samples were filtered inline using 0.2 μm polycarbonate filter-cartridges (Sartorius Sartobran filter capsule 5231207H8). Salinity was measured with a simple TS meter.

Samples to characterise the dissolved organic Fe-binding ligands were collected from three stations in the Scheldt estuary at $S=0.3$, upstream of Antwerp, at $S=10$ near Hansweert and at $S=26$ where the river outlet enters the North Sea (Figure1). Part of these samples was then ultra-filtrated using an acid-cleaned Amicon SP60 cartridge (<1 kDa fraction) and a peristaltic pump of Watson Marlow (604S/R), enabling filtration of large sample volumes. Samples for the determination of dissolved Fe (<0.2 μm and <1 kDa) were immediately acidified to $\text{pH}=2$ with concentrated 3 x quartz-distilled (3xQD) HNO_3 and stored at room temperature. Dissolved Fe in samples at high salinity ($S=26$) was measured with flow injection analysis (FIA) (de Jong et al., 1998). Samples of medium and low salinity (0.3 and 10) were directly measured by graphite furnace atomic absorption spectroscopy GFAAS (Perkin Elmer). Samples taken for the characterisation of the dissolved organic ligands were frozen immediately after sampling.

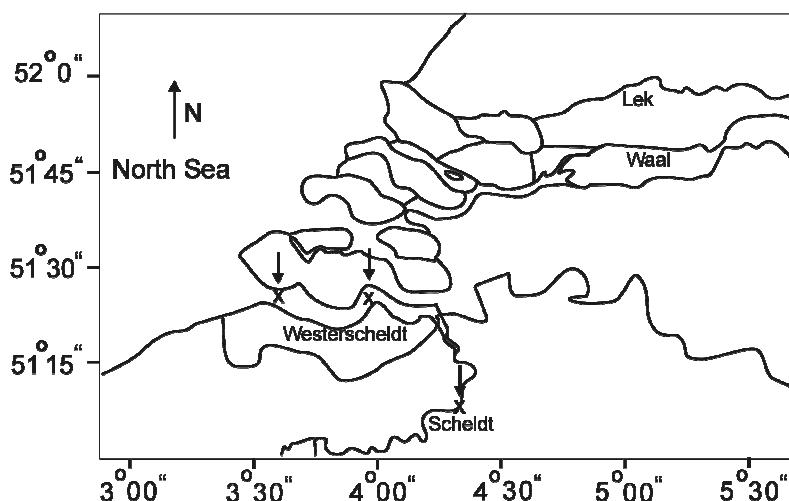


Figure 1. The estuary of the river Scheldt in the southwest of The Netherlands. The three sampling sites are indicated.

2.2 Complexation characterisation

2.2.1 Titration with Fe to estimate [L] and K' after equilibration of the samples

Determination of the organic speciation of iron in seawater water was performed using Competing Ligand Exchange-Absorptive Cathodic Stripping Voltammetry (CLE-ACSV). The 2-(2-Thiazolylazo)-p-cresol (TAC) was used as competing ligand (Croot and Johansson, 2000). All solutions were prepared using 18.2 M Ω nanopure water. The equipment consisted of a μ Autolab voltammeter (Ecochemie, The Netherlands), a static mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl reference electrode with a salt bridge containing 3 M KCl and a counter electrode of glassy carbon. The titration was performed using 0.01 M stock solution of TAC in 3xQD methanol, 1 M boric acid (Suprapur, Merck) in 0.3 M 3x QD ammonia (Suprapur, Merck) (extra cleaning by the addition of TAC after which TAC and Fe(TAC)₂ was removed with a C18 SepPak column) to buffer the samples to a pH of 8.05 (Boye et al., 2003) and a 10⁻⁶ M Fe(III) stock solution acidified with 0.012 M HCl (3xQD). Aliquots of 15 ml were spiked with Fe(III) until final concentrations between 0 and 20 nM and allowed to equilibrate overnight (> 15 hours) with 5 mM borate buffer and 10 μ M TAC. The borate buffer was adapted to samples of differing salinities and pH's.

The concentration Fe(TAC)₂ in the samples was measured using the following procedure: i) removal of oxygen from the samples for 200 seconds with dry nitrogen gas, a fresh Hg drop was formed at the end of the purging step, ii) a deposition potential of -0.40 V was applied for 30-60 seconds according to the sample measured, the solution was stirred to facilitate the adsorption of the Fe(TAC)₂ to the Hg drop, iii) at the end of the adsorption period the stirrer was stopped and the potential was scanned using the differential pulse method from -0.40 to -0.90 V at 19.1 mV s⁻¹ and the stripping current from the adsorbed Fe(TAC)₂ recorded.

The Fe bound by TAC (10 μ M) after equilibration overnight (>15 hours) is defined as Fe labile with respect to TAC.

2.2.2 Kinetic characterisation of the dissolved ligands

To determine dissociation and formation rate constants of the natural organic Fe-complexes a slightly modified method as described by Wu and Luther (1995) was used. Instead of 1-nitroso-2-naphtol (NN) TAC was used as competing ligand (Croot and Johansson, 2000). The Fe or TAC is added to the sample and the changes imposed by these additions are followed with time until equilibrium is reached. All reactions considered are

assumed to go via inorganic Fe (Fe^{\prime}), direct formation of one complex of Fe out of another complex of Fe are ignored, i.e. assumed only to exist via dissociation from one complex followed by the formation of the other complex.

The frozen samples were thawed directly before the kinetic experiments.

To measure the binding and precipitation after an addition of Fe, 20 nM of Fe is added to 250 ml seawater and 15 sub-samples were taken in time. Labile Fe, defined as that pool of Fe complexed with TAC within 120 s after addition of 10 μM TAC, was measured. The TAC was added to the sample after the 200 s nitrogen bubbling (measuring procedure is described in section 2.2.1) to remove oxygen and before an extra period of 30 s of nitrogen bubbling and a collection period of 90 s. This is the shortest time used to measure labile Fe in these samples. The observed decrease with time after Fe addition of labile Fe is explained by the formation of Fe complexes (FeL and FeP) and the precipitation of oxyhydroxides (FeX) (Figure 2 A, B).

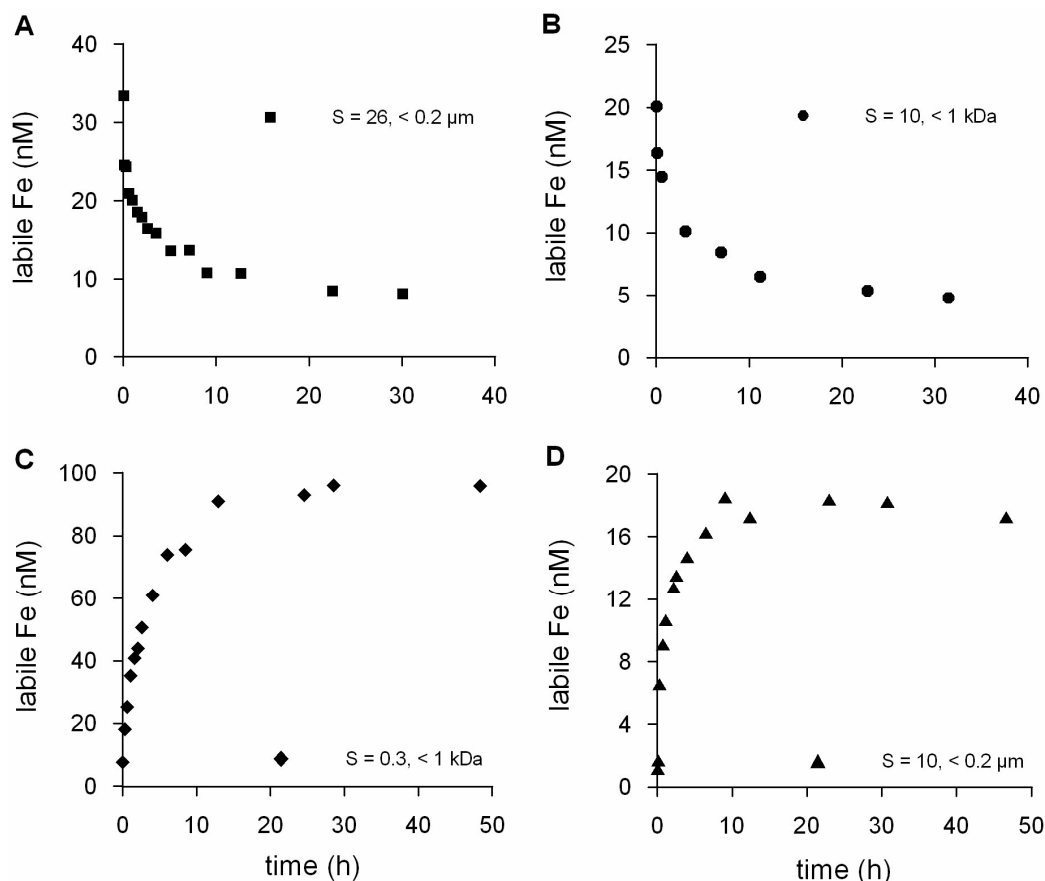


Figure 2. Changing Fe concentrations with time after an addition of Fe (A and B) and TAC (C and D). In figures A and B the presence of TAC was as short as possible (120 s), therefore the measured Fe concentration is called labile. In figures C and D TAC is competing with the natural ligands for Fe and the measured Fe concentration is called labile with respect to TAC ($[\text{TAC}] = 10^{-5} \text{M}$).

The classical titration with Fe followed by an equilibration period (section 2.2.1.) is assumed to represent the equilibration situation with the ligand L ($K' = 10^{19} - 10^{23.5}$). (Obviously in the case that a second ligand exists as well, as here reported, this assumption is not necessarily valid, as discussed below). To measure the effect of addition of a competing ligand with time, 10 μM TAC was added to the seawater forcing the dissociation of dissolved Fe-complexes. The subsequent increase of the concentration $\text{Fe}(\text{TAC})_2$ with time is measured as reflection of the dissociation of natural Fe complexes on the one hand and formation of $\text{Fe}(\text{TAC})_2$ on the other hand (Figure 2 C, D). The formation and dissociation rate constants of $\text{Fe}(\text{TAC})_2$ can be estimated this way and a relation of these rate constants with salinity can be studied.

2.3 Modelling

In order to formulate models for the calculation of rate constants, some general assumptions have to be made. The inorganic speciation of Fe was described by: inorganic $\text{Fe} = \text{Fe}' = 10^{10} * \text{Fe}^{3+}$ (Hudson et al., 1992) and the solubility product of iron-oxyhydroxides FeX is assumed to be 0.2 nM (Byrne and Kester, 1976; Kuma et al., 1996; Liu and Millero, 2002). After filtration the samples were frozen immediately preventing chemical reactions to take place, only during thawing of the samples precipitation could happen. Although this possible precipitation cannot be ruled out, it was assumed that the initial concentration of oxyhydroxides is $[\text{FeX}] = 0$ at $t = 0$. Whether this assumption is valid will be discussed in the discussion section.

2.3.1 Model 1: addition of Fe

Addition of Fe will cause formation of Fe complexes and possibly precipitation of an Fe(hydr)oxide (hereafter called FeX). Titration analyses, used to obtain the ligand characteristics, showed that the Fe-binding ligand sites were saturated with Fe. Thus the assumption is that, upon addition of 20 nM Fe, labile Fe would decrease due to formation of FeX . Since the labile Fe concentration was too high to fit the above mentioned assumptions, the presence of a relative weak ligand called P is assumed. This is discussed extensively in the results section. We do not have any information on the concentration of P. Since P is not detected during the titrations analyses, it must be below the detection window of TAC (10 μM) ($\alpha\text{Fe}(\text{TAC})_2 = \beta * [\text{TAC}]^2 = 10^{22.4} * 10^{-10} = 10^{12.4}$ thus $\alpha\text{FeP} \leq 10^{11.4}$). Here it is assumed that due to the short time TAC is present in the sample, that it does not influence the concentrations of either FeL or FeX .

Furthermore it is implicitly assumed that FeP dissociates within 120 s in the presence of 10 μM TAC. Measured labile Fe would thus contain $[\text{Fe}'] + [\text{FeP}]$, which needs to be

tested. Moreover the FeL, FeP and FeX all have been defined as having the simple 1:1 coordination, where the free concentration of the constituents is annotated with a prime ([L']).

The reactions which take place after addition of Fe can be described by,



Total [L] is known from the titration analysis. Total dissolved [Fe] is known and 20 nM Fe is added at t=0 as Fe'. The k_1 , k_3 , and k_5 are the dissociation rate constants and the k_2 , k_4 and k_6 are the formation rate constant of FeL, FeP and FeX, respectively (Figure. 3). Thus the changes in concentration of all species are defined by the following suite of equations

$$d[Fe']/dt = k_1*[FeL] + k_3*[FeP] + k_5*[FeX] - k_2*[Fe']*[L'] - k_4*[Fe']*[P'] - k_6*[Fe']*[X'] \quad (4)$$

$$d[FeL]/dt = k_2*[Fe']*[L'] - k_1*[FeL] \quad (5)$$

$$d[L']/dt = k_1*[FeL] - k_2*[Fe']*[L'] \quad (6)$$

$$d[FeP]dt = k_4*[Fe']*[P'] - k_3*[FeP] \quad (7)$$

$$d[P]/dt = k_3*[FeP] - k_4*[Fe']*[P'] \quad (8)$$

$$d[FeX]/dt = k_6*[Fe']*[X'] - k_5*[FeX] \quad (9)$$

$$d[X']/dt = k_5*[FeX] - k_6*[Fe']*[X'] \quad (10)$$

Together with the boundary conditions these 7 equations (4-10) can be used to solve the 6 rate constants and 2 species concentrations. Modelling was done using Excel (Microsoft) and Scientific software (Micromath Scientific software) and the calculations were performed using a least squares fit.

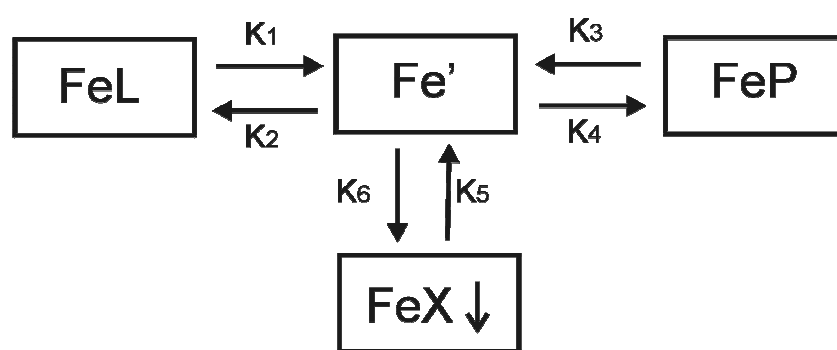


Figure 3. Model 1 representing the reactions taking place after addition of Fe between inorganic Fe (Fe'), the complexes FeL and FeP , and the precipitate FeX . X represents an oxyhydroxide molecule. With k_1 , k_3 and k_5 as dissociation or dissolution rate constants and k_2 , k_4 and k_6 as formation rate constants.

The following boundary conditions were used for the concentrations of X and P :

A). For the results in Table 2 a conditional statement was used, to let FeX only be formed if $[Fe'] > 0.2$ nM. A similar conditional statement of a maximum metal concentration before precipitation can take place was also used by Laan et al. (2004). A concentration for total P was chosen as three times the total dissolved Fe concentration. This choice is rather arbitrary and is based on the results of Nolting et al. (1998), they found in the Southern Ocean that the ligand concentration was larger or equal to three times the dissolved Fe concentration ($[Fe_{diss}]$).

B). For the results in Table 3 it was assumed that total $[X]$ at $t=0$ was so large, that formation of FeX would not change its concentration, implying $[X'] \approx \text{constant}$ and $d[X']/dt \approx 0$, such that equation 10 can be eliminated from the model. At constant pH and assuming a kind of oxyhydroxide to form, a constant $[X']$ is a credible assumption since $[OH^-] \approx 10^{-6} M$. The advantage above the preceding assumption (case A above, where a threshold value is applied before formation of FeX can occur), is the possibility of the formation and dissociation (dissolution in this case) of FeX independently of $[Fe']$. Moreover the term $k_6 * X'$ then can be substituted by k_6' such that a conditional statement in the formation of FeX is not necessary anymore. This changes the equations 4 and 9 into,

$$[Fe'] = k_1 * [FeL] + k_3 * [FeP] + k_5 * [FeX] - k_2 * [Fe'] * [L'] - k_4 * [Fe'] * [P'] - k_6' * [Fe'] \quad (4b)$$

$$[FeX] = k_6' * [Fe'] - k_5 * [FeX] \quad (9b)$$

As second assumption a very high total $[P]$ was chosen, being 10 μM , implying $[P'] \approx \text{constant}$ and $d[P']/dt \approx 0$ resulting in the elimination of equation 8 from the model.

C). As described above (B) the assumptions $[X'] \approx \text{constant}$ and $d[X']/dt \approx 0$ were used for the results given in Table 4. For the total P concentration the assumption described in A was used, $[P] = 3 * [Fe_{\text{diss}}]$.

2.3.2 Model 2: addition of TAC.

Here no Fe was added, only 10 μM TAC as competing ligand. In this research the changes in $[Fe(\text{TAC})_2]$ are followed until equilibrium is reached. An extra reaction has to be taken into account for model 2,



with two new rate constants, changing eq. 4 into 4c and adding eq. 13 and 14 to eq. 4-10 in the model (Figure 4).

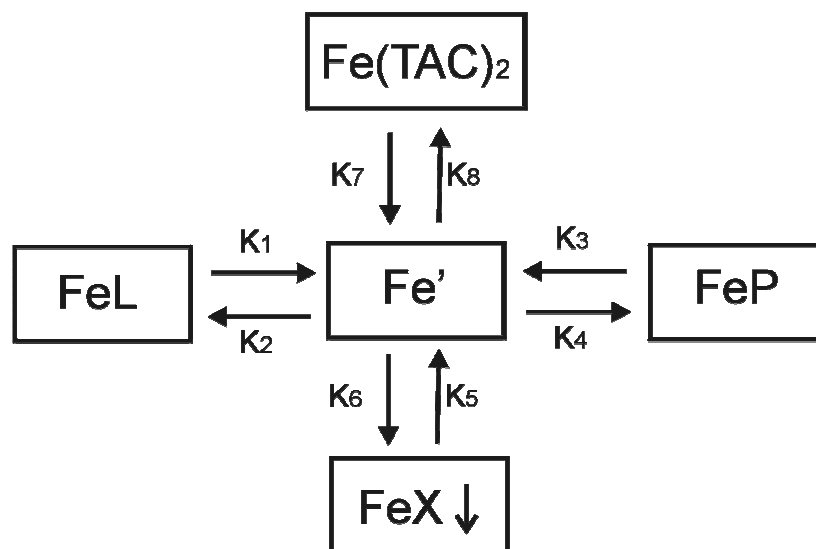


Figure 4. Model 2 representing the reactions taking place after addition of TAC between inorganic Fe (Fe'), the natural Fe complexes FeL, FeP, the added complex $Fe(\text{TAC})_2$ and the precipitate FeX. X represents an oxyhydroxide molecule. With k_1 , k_3 , k_5 and k_7 as dissociation or dissolution rate constants and k_2 , k_4 , k_6 and k_8 as formation rate constants.

$$d[Fe'] / dt = k_1 * [FeL] + k_3 * [FeP] + k_5 * [FeX] + k_7 * [Fe(TAC)_2] - k_2 * [Fe'] * [L'] - k_4 * [Fe'] * [P'] - k_6 * [Fe'] - k_8 * [Fe'] * [TAC']^2 \quad (4c)$$

$$d[Fe(TAC)_2] / dt = k_8 * [Fe'] * [TAC']^2 - k_7 * [Fe(TAC)_2] \quad (13)$$

$$d[TAC'] / dt = k_7 * [Fe(TAC)_2] - k_8 * [Fe'] * [TAC']^2 \quad (14)$$

The program Scientist was used to fit the data. We had chosen the rate constants k_1 - k_6' from Table 3 in order to calculate the rate constants of TAC, k_7 and k_8 . The choice to use these rate constants and not the ones from Table 2 or 5 is explained in the results section.

3. Results

3.1 Dissolved Fe

Data on dissolved Fe in the Scheldt estuary are scarce (van den Berg et al., 1987; Herzl, 1999; Nolting et al., 1999). Otherwise Nolting et al. (1999) presented dissolved Fe concentrations from 1978 which were much higher than the values found in this work. Their Fe concentrations ranged from 50 nM at salinity 30, via 300 nM at $S=10$ to $>1 \mu M$ at $S=1$. This is more than twofold higher than our data (Table 1). Van den Berg et al. (1987) measured comparable Fe concentrations as Duinker et al. (1983) and Nolting et al. (1999), ranging from 400 nM at $S=10$ to 30 nM at $S=27$. For a large part the higher dissolved Fe concentrations are due to a higher organic load during the nineteen seventies-eighties, exemplified by the oxygen depletion reached up to $S=15$ (Duinker et al., 1983). Dissolved and colloidal Fe concentrations from 1993 to 1997 are presented by Herzl (1999). She found mean dissolved Fe concentrations in the upper estuary ($S=0.3$) of 524 nM, near Vlissingen ($S \approx 30$) a mean Fe concentration of 28 nM was observed.

The dissolved Fe concentrations decrease very steeply in the upper estuary due to flocculation and possibly oxidation (Sholkovitz, 1976; Duinker et al., 1983; Zwolsman and van Eck, 1993; Herzl, 1999) followed by a more gradual decrease at higher salinities (Table 1). The soluble Fe fraction <1 kDa is 3 to 6 times smaller than the total dissolved Fe ($<0.2 \mu m$), suggesting a high content of colloidal Fe (>1 kDa - $<0.2 \mu m$) in the estuary. This is in line with results from Galveston Bay where 80% of dissolved Fe was >1 kDa (Wen et al., 1999), similar to the Venice Lagoon (Martin et al., 1995) and other estuaries (Powell et al., 1996). Herzl (1999) filtered Scheldt water over two size fractions $<0.45 \mu m$ and $<0.05 \mu m$. She found that almost all dissolved Fe was $<0.05 \mu m$. Only during high river discharge high concentrations of Fe between 0.45 and $0.05 \mu m$ were measured.

Table 1. The dissolved Fe concentrations (nM), conditional stability constants (K') and the dissolved organic ligand concentrations (nano-equivalents of M Fe = neq of M Fe) of the three salinities in the Scheldt estuary. The 0.2/1kDa denotes the ratio of the concentrations of Fe and L between the two size fractions <0.2 μm and <1 kDa, respectively. Since the ligand sites were saturated with Fe, the estimation of K' and L could not be done with the usual accuracy (see method section) and errors on these calculations could not be estimated. The log K' and the ligand concentrations are mean values of n determinations. The α value is the product of the conditional stability constant and the concentration ($\alpha = K' \cdot [L_t]$).

	[Fe _{diss}] (nM)	Log K'	Ligand (neq M Fe)	[Fe _{diss}]/ [L _t]	Log α
S=26					
<0.2 μm	12.2	20.4 (n=5)	8	1.5	12.3
<1kDa	2	20.5 (n=4)	1.3	1.5	11.6
(0.2/1kDa)	6.1		6.1		
S=10					
<0.2 μm	104.5	21.1 (n=2)	95	1.1	14.1
<1kDa	36	20.6 (n=3)	30	1.2	13.1
(0.2/1kDa)	3		3		
S=0.3					
<0.2 μm	536	21.2 (n=2)	525	1.02	14.9
<1kDa	102	20.8 (n=3)	85	1.2	13.7
(0.2/1kDa)	5.3		6.2		

3.2 Complexation characteristics of Fe

3.2.1 [L] and K' estimated by titration with Fe after equilibration of the samples

The concentrations of organic Fe-binding ligands in size fractions, <0.2 μm and <1 kDa, and at all three salinities (S=26, 10 and 0.3) are slightly lower than the concordant dissolved Fe concentrations (Table 1). Thus, the Fe-binding ligand sites of all samples are saturated with Fe. A titration curve is therefore seldom found, most titrations resulted thus in a straight line and in all cases the concentration of Fe labile with respect to TAC is relatively high. Estimation of the ligand characteristics by nonlinear regression is not possible (Gerringa et al., 1995). Instead the linearization of van den Berg/Ružić is used (van den Berg, 1982), but an error estimation of the parameters is not possible. Reasoning from experience the error of

the estimation of $\log K_{\text{FeL}}'$ must be large, near 1; the error of the estimation of the ligand concentration near 10%. The relatively high error of K' is due to statistical causes since calculation of K_{FeL}' is hardly possible when no curvature is found in the titration curve (Gerringa et al., 1995).

For both size fractions considered, the K_{FeL}' was estimated to be around 10^{21} with respect to Fe^{3+} (Table 1). This value is in concert with other findings in the open ocean and the Mediterranean Sea (Gledhill and van den Berg, 1994; Rue and Bruland, 1995; van den Berg, 1995; Rue and Bruland, 1997; Nolting et al., 1998; Boye et al., 2001; Boye et al., 2003). In this estuary with a gradient in salinity and in the origin and characteristics of the dissolved organic matter the conditional stability constant is surprisingly constant. An excess of dissolved Fe over the ligand concentration is found, at S=26 even 1.5 times more Fe than ligand is present, at the other salinities this ratio is around 1.1 (Table 1). It appears that a colloidal ligand group with the same binding characteristics exists.

It is probable that a weaker ligand group exists in both size fractions, since otherwise the solubility product, 0.2 nM (Liu and Millero, 2002), of solid Fe(oxy)hydroxides is exceeded. However, only one strong ligand group was detected. Thus the conceivable weak ligand group falls outside the detection window, product of concentration and stability constant, of our method. According to Apte et al. (1988) the detection window of CSV is 2 to 3 orders of magnitude wide, thus with a centre of the detection window, of $10^{12.4}$ meaning that ligands with an α value lower than about $10^{10.9}$ cannot be detected. During the analysis of the ligand characteristics K' and the total ligand concentration [L], TAC had more than 15 hours time to compete with the natural organic ligands present. The method of the titration is based on measuring the competition between TAC and the natural ligands until the point in the titration where the ligands are filled with Fe and only TAC is complexing the added excess Fe. Weaker ligands below the detection window of TAC cannot compete, and dissociate within the time of the equilibration period. Ligands within the detection window used do compete with TAC for the added Fe in the titration, but also dissociate to a small extent assuming reversible complexation. Since TAC was selected to compete with these ligands, the centre of the detection window equals more or less with the α value ($K_{\text{FeL}}' \cdot \text{total [L]}$) of the complexing ligands. Therefore even when strong ligands are saturated with Fe, a small horizontal part in the titration curve is possible. This horizontal part represents the filling of the empty spaces L^- as created by dissociation of the natural ligand due to competition with TAC during the overnight equilibration period.

High concentrations of Fe labile with respect to TAC have been reported by Boye et al. (in press) in the open ocean, by Croot and Johansson (2000) in coastal water, by Rijkenberg et al. (Chapter 8) in coastal areas and estuaries and after a dust deposition (Powell, personal communication). High concentration of Fe labile with respect to TAC means that within the equilibration time (overnight) $\text{Fe}(\text{TAC})_2$ is formed to a substantial concentration.

The most logical explanation is that TAC is stronger than the natural ligands. Interpretation becomes problematic when, as in these samples, high concentrations of Fe labile with respect to TAC do occur in samples in which a titration curve was found with a horizontal part steeply curving into the linear part of the titration. The horizontal part reflects the filling of empty ligand sites with Fe, the form of the curving represents the strength of the binding, a smooth curve a relative weak binding, a sharp curve a relatively strong binding strength. A combination of high concentrations of Fe labile with respect to TAC with a relative strong binding strength of the ligands can only be explained when two ligands are present, a relatively weak ligand, causing a high concentration of Fe labile with respect to TAC, falling outside the detection window of the method and a relatively strong ligand reflected by the sharp curvature in the titration graph. For the relatively weak ligand this implies α values must be less than $10^{10.9}$. Assuming concentrations of the weak ligand P three times the dissolved Fe concentration, then for $S=26$, K_{FeP}' has a maximum value of $10^{18.3}$ ($10^{10.9}/3*12.2*10^{-9}$) and for $S=0.3$ K_{FeP}' has a maximum value of $10^{16.7}$ ($10^{10.9}/3*536*10^{-9}$). An alternative assumption being used here is for [P] to be at an even larger overdose here arbitrarily taken to be 1000 nM, this resulting in a maximum K_{FeP}' of $10^{16.9}$.

3.2.2 Kinetic characterisation of the dissolved ligands

Until now the method of Wu and Luther (1995) had been applied to open ocean samples with low concentrations of dissolved Fe (<1-2 nM Fe) and low concentrations of organic ligands (≈ 5 nano-equivalents of M Fe), or been applied to low concentrations of organic model ligands in open ocean samples (Witter et al., 2000). The samples of the Scheldt estuary are different from open ocean samples, since the concentrations of Fe and organic matter are much higher, the salinity is lower and varies ($S=26$, 10 and 0.3 in our samples) and the organic ligands sites of L ($K'=10^{21}$) are saturated with Fe.

Since the dissolved organic ligands L are filled with Fe in the Scheldt samples, addition of Fe should theoretically result in precipitation of Fe-(hydr)oxides. For the ocean it is assumed that the solubility product (SP) of Fe-(hydr)oxides is 0.2 nM (Byrne and Kester, 1976; Kuma et al., 1996; Liu and Millero, 2002). The SP of inorganic Fe(hydr)oxides depends, apart from pH and temperature, also on salinity. According to Liu and Millero (2002) the SP decreases from 0.3 nM Fe' at $S=26$ to 0.16 nM Fe' at $S=10$, after which it increases to 0.4 nM Fe' at $S=0.03$ (pH=8.1 and 25 °C). We used 0.2 nM for all salinities in our calculations. However, as discussed before, the concentration Fe labile with respect to TAC was so high that a relatively weak ligand P is assumed to exist. During the kinetic measurements high labile Fe concentrations were measured (Figure 2 A). Moreover, in the samples from $S=0.3$ and the fraction <0.2 μm , after addition of 20 nM of Fe no decrease with

time of labile Fe was observed. This would mean that this 20 nM of added Fe did not form precipitates as oxy-hydroxides within 48 hours.

3.2.2.1 Model 1: addition of Fe

A decline in labile Fe with time was observed in all samples except for the samples of S=0.3, in the fraction <0.2 μm (Figure 2A, B). The decline in labile Fe cannot be the result of the formation of FeL since these ligand sites are saturated with Fe. On the other hand, the decrease in labile Fe could be due to the formation of FeP complexes, but this effect would not have been detected since FeP is assumed to be labile with respect to 10 μM TAC in 120 s. It is very likely that Fe was precipitating as Fe(hydr)oxides. A precipitation of Fe is expected to be Fe-(hydr)oxides of amorphous colloidal nature. This precipitate must then be so stable that it cannot be dissolved after addition of TAC by formation of $\text{Fe}(\text{TAC})_2$ within 120 s. Rijkenberg et al. (Chapter 5) showed in open ocean Antarctic water that indeed these fresh precipitates were stable with respect to the conditions of the measurement. Unpublished results in estuarine water from the Wadden Sea also showed that fresh Fe-precipitates were stable with respect to the 120 s exposure of TAC. Moreover dissolution by prolonged presence of TAC showed a relatively slow dissolution process. The decrease in labile Fe over time observed in samples from S=26 and S=10 and from the fraction <1 kDa in S=0.3 (Figure 2A, B) is assumed to result from Fe(hydr)oxides precipitation, annotated as FeX.

In the samples of S=0.3, in the fraction <0.2 μm apparently no precipitation took place; the high labile Fe cannot be explained by relatively high concentrations of P. Due to high variance in the data of the samples S=0.3, the <0.2 μm fraction could not be modelled. Initially the measured concentrations of labile Fe increased with time after which it remained constant. An increase with time is not the expected result (see for comparison Figures 2A and B). However, the concentrations were very low with respect to the total dissolved Fe concentration (10 nM compared to 525 nM Fe_{diss}). Moreover at low salinity and high organic matter the chemical analysis is operating at the borders of what is possible. It was decided to discard the data from the samples S=0.3, <0.2 μm .

*A. Formation of FeX if $[\text{Fe}'] > 0.2 \text{ nM}$; $[\text{P}] = 3 * [\text{Fe}_{\text{diss}}]$*

For the results in Table 2 a conditional statement was used, to let FeX only be formed if $[\text{Fe}'] > 0.2 \text{ nM}$ as used by Laan et al. (2004). The theoretical drawback of this model is that

Table 2. Results of model 1, which assume that formation of FeX is only possible if $[\text{Fe}'] > 0.2 \text{ nM}$. The K' values and α values are given with respect to $[\text{Fe}^{3+}]$, the rate constants with respect to $[\text{Fe}']$. The concentrations (P, L, X) are in nM, the k_f in $\text{M}^{-1} \text{s}^{-1}$, the k_d in s^{-1} , and the K' in M^{-1} .

	S=26		S=10		S=0.3	
	0.2 μm	1kDa	0.2 μm	1kDa	0.2 μm	1kDa
k_d1	1.41E-05	3.82E-05	2.85E-07	2.99E-07		8.08E-05
k_f2	3.13E+05	8.32E+05	1.28E+06	1.13E+06		1.14E+06
k_d3	7.47E-05	1.49E-04	6.29E-05	4.54E-05		6.28E-05
k_f4	4.65E+03	1.12E+05	6.24E+03	6.75E+03		4.80E+03
k_d5	4.91E-06	4.68E-06	2.39E-07	2.48E-07		7.02E-06
k_f6	5.52E+04	4.37E+05	1.00E+05	1.05E+05		4.08E+04
init[X]	37	19.3	49.1	36.8		35
[L]	8	2	95	30	525	85
[P]	150	40	400	180		350
$\text{Log}K'_{\text{FeL}}$	20.35	20.34	22.65	22.58		20.15
$\text{Log}K'_{\text{FeP}}$	17.79	18.87	18.00	18.18		17.89
$k6/k5 \text{ FeX}$	20.05	20.97	21.62	21.63		19.77
$\text{Log } \alpha_{\text{FeL}}$	12.25	11.64	15.63	15.05		13.08
$\text{Log } \alpha_{\text{FeP}}$	10.97	11.48	11.60	11.43		11.43
$\text{Log } \alpha_{\text{FeX}}$	12.62	13.26	14.31	14.19		12.31

the assumption that Fe' should be smaller than 0.2 nM is an assumption belonging to the equilibrium conditions domain, yet here used in kinetics. Moreover precipitation and dissolution also occurs when $\text{Fe} < 0.2 \text{ nM}$. The fact that in Table 2 the conditional stability constant of the precipitate FeX can be higher than the conditional stability constant of FeL and FeP and still that not all Fe is precipitated is due to the 'if then' statement used. Latter statement is meaning that FeX can only be formed if $\text{Fe}' > 0.2 \text{ nM}$. Here please notice the fact that the concentration of X is also fitted and if X is related to the pH the concentration of X must remain constant. However, in these calculations $[\text{X}']$ decreases while forming FeX. Table 2 shows that K'_{FeL} of the samples S=10 are substantially higher. This is not found by the equilibrium method (Table 1). The values found for K'_{FeP} are a little higher than expected considering that FeP was expected to fall outside the detection window of TAC. Furthermore, Table 2 shows rate constants of the three different species that are not very specific per species. For all species the k_d varies around values of 10^{-5} s^{-1} , similar values as found by Witter et al. (2000) and Witter et al. (2000). These results suggest that the value of 10^{-5} to 10^{-6} s^{-1} is a mean value for the dissociation rate constant, applicable for all Fe species. The formation rate constant shows more variation, making discrimination between FeL, with $k_f=3 \cdot 10^5 - 10^6$, and FeP, with $k_f=4 \cdot 10^3 - 10^5$, possible.

B. $[X'] \approx \text{constant}$, $d[X']/dt \approx 0$, $[P'] \approx \text{constant}$, $d[P']/dt \approx 0$

The results in Table 3 show K'_{FeL} values that vary negatively with salinity and thus with ionic strength. Such lower K' values with higher ionic strength is in agreement with the thermodynamic theory stating that activity coefficients become lower at higher ionic strength, this effect increases with increasing charge of the species (Stumm and Morgan, 1996). The decrease in K'_{FeL} is caused by the increase of the dissociation rate constant of FeL and a decrease of the formation rate constant with increasing salinity (Table 3). The increase in dissociation rate of FeL can be shown considering the half life times, $t_{1/2} = 0.693/k_d$ (Stone and Morgan, 1990). At $S=26$ the half life time is 48 to 100 s; at $S=10$ the half life varies between 582 and 1155 s (9.7-19 min); at $S=0.3$ (<1 kDa) the half life time is 814 s (13.5 min.). For each salinity the dissociation rate of FeL of the size fraction <1 kDa is smaller than for the <0.2 μm fraction. The formation rate constant of FeL decreases with S with a factor 100 between $S=26$ and $S=0.3$ <1 kDa.

The K'_{FeP} values are substantially lower than K'_{FeL} , the K'_{FeP} and the rate constants of FeP do not show a trend with S , (Table 3). The dissociation half life is between 1.8 and 3.6 h.

A distinct differentiation in dissociation rate constants between the two ligand groups L and P exists, ranging from $10^{-2} - 10^{-4} \text{ s}^{-1}$ for FeL to 10^{-5} s^{-1} for FeP. The dissolution rate

Table 3. Results from model 1, assuming $[X] \approx \text{constant}$ and $[P]=10\mu\text{M}$. The $k_f'6$ is $k_f6*[X']$. The concentrations (P, L) are in nM, the k_f in $\text{M}^{-1} \text{ s}^{-1}$, the k_d in s^{-1} , and the K' in M^{-1} . The K' values and α values are given with respect to $[\text{Fe}^{3+}]$ and the rate constants with respect to $[\text{Fe}']$.

	S=26,		S=10		S=0.3	
	0.2 μm	1kDa	0.2 μm	1kDa	0.2 μm	1kDa
k_d1	1.42E-02	5.94E-03	1.19E-03	5.97E-04		8.51E-04
k_f2	1.13E+07	1.91E+07	7.00E+08	9.15E+07		1.09E+09
k_d3	7.72E-05	2.31E-04	1.19E-04	5.25E-05		6.84E-05
k_f4	7.80E+01	6.81E+02	3.02E+02	1.61E+02		3.61E+02
k_d5	4.84E-05	8.46E-05	9.28E-05	3.18E-05		9.18E-05
$k_f'6$	2.38E-03	7.61E-03	4.53E-03	3.65E-03		5.15E-03
[L]	8	2	95	30	525	85
$\log K'_{\text{FeL}}$	18.9	19.51	21.77	21.19		22.11
$\log K'_{\text{FeP}}$	16.00	16.47	16.40	16.49		16.72
Log α_{FeL}	10.81	10.81	14.75	13.66		15.04
Log α_{FeP}	11.00	11.47	11.40	11.49		11.72
Log α_{FeX}	11.69	11.95	11.69	12.06		11.75

constant of FeX is also in the order of 10^{-5} to 10^{-4} s^{-1} . This value is at least 100 times faster than found by Kuma et al. (1992). Rose and Waite (2003) observed comparable dissolution rates as obtained in this research, they found that the k_d depends on the ageing of the Fe-precipitate, and measured dissolution rates of $2.3 \cdot 10^{-4} \text{ s}^{-1}$ from freshly precipitated iron and $4.6 \cdot 10^{-6} \text{ s}^{-1}$ for precipitates aged for one week. The formation rate constants differ considerably, with fast formation kinetics for FeL and substantially slower formations kinetics for FeP. Note the relatively low α value for FeL at S=26, especially when compared with the α value of FeP. The α values of FeL, FeX and FeP indicate the distribution of Fe over these species when equilibrium is reached.

C. $[X'] \approx \text{constant}$, $d[X']/dt \approx 0$, $[P] = 3 \cdot [Fe_{diss}]$

We know that a negative salinity relationship exists for DOC and for the total concentration of L. If P is of an organic nature it is sensible to assume such a relationship also for P. By assuming [P] to be related to $[Fe_{diss}]$ a relationship between P and S is created in an indirect way. In Table 4 the result are shown. The α values do remain as given in Table 3, but

Table 4. Results from model 1, assuming $[X'] \approx \text{constant}$ and $[P] = 3 \cdot [Fe_{diss}]$. The $k_f'6$ is $k_f6 \cdot [X']$. The K' values and α values are given with respect to $[Fe^{3+}]$, the rate constants with respect to $[Fe']$. The concentrations (P, L) are in nM, the k_f in $M^{-1} \text{ s}^{-1}$, the k_d in s^{-1} and the K' in M^{-1} .

	S=26		S=10		S=0.3	
	0.2 μm	1kDa	0.2 μm	1kDa	0.2 μm	1kDa
k_{d1}	9.08E-03	5.27E-03	1.14E-03	5.70E-04		7.02E-04
k_f2	1.06E+07	1.59E+07	6.51E+08	8.74E+07		9.05E+08
k_{d3}	7.49E-05	2.03E-04	1.18E-04	5.15E-05		7.22E-05
k_f4	5.56E+03	1.55E+05	7.61E+03	8.80E+03		1.01E+04
k_{d5}	4.64E-05	7.44E-05	8.47E-05	3.12E-05		7.51E-05
$k_f'6$	2.19E-03	6.88E-03	4.52E-03	3.58E-03		4.97E-03
[L]	8	2	95	30		85
[P]	150	40	400	180		350
Log K'_{FeL}	19.07	19.48	21.76	21.19		22.11
Log K'_{FeP}	17.87	18.88	17.81	18.23		18.15
Log α FeL	10.97	10.78	14.73	13.66		15.04
Log α FeP	11.05	11.48	11.41	11.49		11.69
Log α FeX	11.67	11.97	11.73	12.06		11.82

the formation rate constant of FeP and thus K'_{FeP} changed with the assumptions of [P]. A relationship between salinity and the total concentration of P does not create a relation between kinetics and salinity.

3.2.2.2 Model 2: addition of TAC.

The addition of TAC to the samples introduces two new rate constants, k_{d7} and k_{f8} (equations 4c, 12 and 13 and Figure 4). The kinetic rate constants obtained applying boundary condition set B ($[X'] \approx \text{constant}$, $d[X']/dt \approx 0$, $[P'] \approx \text{constant}$, $d[P']/dt \approx 0$; results shown in Table 3) were used to calculate the formation and dissociation rate constants of $\text{Fe}(\text{TAC})_2$. As shown before the results using boundary condition set A (Table 2) did not allow a distinction between two ligands (L and P). The choice between the rate constants displayed in Tables 3 and 4 is rather arbitrary. In principle these results do not differ from each other, only the assumed [P] does. Since the results in Table 4 do not give any confirmation that [P] is related to S and/or $[\text{Fe}_{\text{diss}}]$, the results obtained with boundary condition set B (Table 3) were used.

The thus calculated rate constants of $\text{Fe}(\text{TAC})_2$ have a distinct relation with salinity. The formation rate constant of $\text{Fe}(\text{TAC})_2$ increases with decreasing S and varies from $2 \cdot 10^7 \text{ M}^{-2} \text{ s}^{-1}$ at $S=26$ to $8 \cdot 10^8 \text{ M}^{-2} \text{ s}^{-1}$ at $S=10$. The dissociation rate constant of $\text{Fe}(\text{TAC})_2$ increases with decreasing S, and varies from $4 \cdot 10^{-6} \text{ s}^{-1}$ at $S=26$ to $1 \cdot 10^{-4} \text{ s}^{-1}$ at $S=10$. The $\beta'_{\text{Fe}(\text{TAC})_2}$ remains constant at $10^{22.7}$, varying between 10^{23} and 10^{22} , very close to the value given by Croot and Johansson (2000) of $10^{22.4}$. Although only one calculation was done for $S=0.3$ and it is not very secure to draw conclusions, we observed the same trend with salinity continuing to increasing k_f and decreasing k_d . However, at $S=0.3$ the value of $\beta'_{\text{Fe}(\text{TAC})_2}$ also changes, increasing to $10^{26.3}$.

4. Discussion

4.1 The assumptions and the methods

Application of a threshold $[\text{Fe}']$ for precipitation of FeX did not give satisfactory results since a distinction between the different Fe species was not possible (Table 2). The rate constants are more or less the same for the different Fe species and the result of a mean value of the existing Fe species. However these rate constants do coincide with those found for model ligands and natural ligands in the Arabian Sea (Witter et al., 2000; Witter et al., 2000). The assumption of a high and thus more or less constant concentration of X resulted in three distinct sets of rate constants for the three Fe species.

Table 5. The estimated rate constants and stability constant of $\text{Fe}(\text{TAC})_2$ according to model 2. The rate constants k_1 - k_6 from Table 3 were used together with the assumption that $[\text{P}] = 10000 \text{ nM}$. The k_f is in $\text{M}^{-2} \text{ s}^{-1}$, the k_d in s^{-1} , and the K' in M^{-2} . The K' values and α values are given with respect to $[\text{Fe}^{3+}]$ and the rate constants with respect to $[\text{Fe}']$. The $\alpha_{\text{Fe}(\text{TAC})_2}$ is $\beta_{\text{Fe}(\text{TAC})_2} * [\text{TAC}']^2$.

sample	Log $\beta \text{Fe}(\text{TAC})_2$	k_f	k_d	Log $\alpha \text{Fe}(\text{TAC})_2$
S=26, <0.2 μm	22.62	2.71E+07	6.43E-06	12.62
S=26, <0.2 μm	22.69	1.97E+07	4.00E-06	12.69
S=26, <1kDa	23.00	1.36E+07	1.38E-06	13
S=26, <1kDa	22.84	1.47E+07	2.12E-06	12.84
S=10, <0.2 μm	22.98	1.00E+09	1.06E-04	12.98
S=10, <0.2 μm	23.08	8.50E+08	7.11E-05	13.08
S=10, <1kDa	21.98	8.78E+07	9.09E-05	11.98
S=10, <1kDa	22.34	2.90E+08	1.31E-04	12.34
S=0.3, <1kDa	26.28	3.25E+10	1.74E-06	16.28

Table 1 shows the binding characteristics of FeL determined by the titration, although no trend in K'_{FeL} with S is present. However, the α values do show a trend with S as observed by the kinetic experiments (Tables 4 and 5). No distinction could be made between the ligand groups with the equilibration method of titration (Table 1); a mean K' value of more than one ligand group was obtained. This value of K' represents both the ligands L and P. The ligand P is only just outside the detection window of TAC and it will interfere to some extent during the titration method. The technique of ligand equilibration is vulnerable for these interferences.

According to Table 5 the conditional stability constant of $\text{Fe}(\text{TAC})_2$ at S=0.3 is much higher than for the higher salinities, thus the K'_{FeL} values in Table 1 for the samples at S=0.3 are an underestimation since all these samples are calculated with $\beta_{\text{Fe}(\text{TAC})_2} = 10^{22.4}$ given by Croot and Johansson (2000). This underestimation is indeed confirmed by the K'_{FeL} obtained from the kinetic experiments (Tables 3 and 4).

Model 2 produced for S=26 and S=10 the binding characteristics of Fe and TAC that are very close to the values from the literature (Croot and Johansson, 2000). Knowing the rate constants for Fe binding and dissociation with TAC we can discuss the meaning of labile Fe and Fe labile with respect to TAC. The formation rate constants of $\text{Fe}(\text{TAC})_2$ are fast but not fast enough to complex all labile Fe within the time of the measurement. Different collection times will result in different labile Fe concentrations and thus the resulting labile Fe measured as $\text{Fe}(\text{TAC})_2$ is very sensitive to the conditions during the measurement. Thus labile Fe is not necessarily the same as excess Fe with respect to L.

The data from S=0.3, <0.2 μm could not be modelled. No decline in labile Fe after an addition of Fe was observed. This can not be explained by the formation of FeP. The constant

high Fe concentration in this practically fresh water can only be explained by formation of instable colloidal Fe species of unknown composition. The concentration of colloidal Fe is higher than 400 nM here (Table 1).

4.2 Comparison with literature values

The FeL is the traditionally known complexed Fe species with fast formation rates, more or less 10 times faster than the model ligands used by Witter et al. (2000) and the surface samples of Witter and Luther (1998) (Table 6). The k_d values found by Witter and Luther (1998) and Witter et al. (2000) are more comparable to the values we find for the relatively weak ligand P. Since this investigation considers estuarine samples and not open ocean water as was used by Witter and Luther (1998) and Witter et al. (2000) the discrepancy might also be due to the presence of coastal ligands differing from truly marine organic ligands. Another reason for differences in rate constants might be because the method of calculation in this research is an improvement of the method copied from Wu and Luther (1995), the latter also used in the papers of Witter and co-authors. Our improvement made it possible to distinguish between two ligands. We found for the strong ligand L (that also might consist of more than one ligand group as found by Rue and Bruland (1995, 1997) that the k_d values are between $8 \cdot 10^{-4}$ and $1.42 \cdot 10^{-2} \text{ s}^{-1}$ and for the weak ligand P k_d values between $5 \cdot 10^{-5}$ and $2.3 \cdot 10^{-4} \text{ s}^{-1}$. The relatively strong ligand L dissociates faster than the relatively weak ligand P (Table 3). Rose and Waite (2003) studied kinetics of Fe with ligands from terrigenous organic matter from coastal areas. They distinguished two classes, a ligand class with k_d constants between $3.24 \cdot 10^{-4}$ to $3.4 \cdot 10^{-3}$ and a ligand class with k_d between 10^{-6} and 10^{-4} s^{-1} (Table 6). So the k_d of the so-called weak ligand from the data of Rose and Waite (2003) coincides with the k_d of our strong ligand L and the k_d of the so-called strong ligand of Rose and Waite (2003) coincides with k_d values of our weak ligand P.

The k_f for the strong ligand L found in the Scheldt varies between 10^7 to $10^9 \text{ M}^{-1}\text{s}^{-1}$, with the highest value occurring in the almost fresh water part of the estuary (Table 3). Rose and Waite (2003) found formation rate constants between 10^6 to $10^8 \text{ M}^{-1}\text{s}^{-1}$. The most estuarine samples in our research have k_f values close to values found by Rose and Waite (2003) in coastal organic matter and the surface values in the NW Atlantic Ocean of Witter and Luther (1998). Witter et al. (2000) found values in the Arabian Sea which are a little higher (Table 6).

The dissolution rate of FeX is between $3.2 \cdot 10^{-5}$ and $9.3 \cdot 10^{-5} \text{ s}^{-1}$, almost exactly the same value of $3.5 \cdot 3.2 \cdot 10^{-5} \text{ s}^{-1}$ as found by Witter and Luther (1998) for dissolution of added (and then assumed to be precipitated) Fe to UV-oxidized North-western Atlantic Ocean water. The dissolution rate compares less well with the dissolution rate measured by Kuma et al., (1992) at pH=8.06, they found more than 10 times higher values in seawater.

Table 6. Comparison of the rate constants found in this research with other publications. All rate constants are given with respect to $[\text{Fe}^3]$. The data of this research are given for both size fractions; first the values for the fraction $<0.2 \mu\text{m}$ and then the values of $<1 \text{ kDa}$ according to the salinity. The formation rate constant of FeP depends on the assumed concentration for P, therefore they are not given in this table (see Tables 3 and 4).

sample	k_f in $\text{M}^{-1} \text{s}^{-1}$	k_d in s^{-1}	references
<u>Natural marine ligands</u>			
NW Atlantic	$2 \cdot 10^6$	$3 \cdot 10^{-5}$	Wu and Luther, 1995
NW Atlantic 200m	$1.13 \cdot 10^6$	$3.9 \cdot 10^{-5}$	Witter and Luther, 1998
NW Atlantic 2874m	$4.21 \cdot 10^4$	$1 \cdot 10^{-7}$	
<u>Model ligands in</u>			
NW Atlantic water	$1 \cdot 10^5 - 2 \cdot 10^6$	$5 \cdot 10^{-8} - 1.2 \cdot 10^{-5}$	Witter et al., 2000a
Arabian Sea	$5 \cdot 10^5 - 10^4$	$10^{-6} - 2 \cdot 10^{-5}$	Witter et al., 2000b
<u>Terrigenous NOM in</u>			
<u>Offshore Sydney, S=36</u>			
Strong ligand	$2.1 \cdot 10^5 - 9.6 \cdot 10^7$	$1 \cdot 10^{-6} - 1.3 \cdot 10^{-4}$	Rose and Waite, 2003b
Weak ligand		$2 \cdot 10^{-4} - 4 \cdot 10^{-3}$	
<u>Scheldt Estuary S=26</u>			
Strong ligand L	$1.1 - 2 \cdot 10^7$	$1.4 \cdot 10^{-2} - 6 \cdot 10^{-3}$	This research*
Weak ligand P		$7.7 \cdot 10^{-5}$	
<u>Scheldt Estuary S=10</u>			
Strong ligand L	$7 \cdot 10^8 - 9.1 \cdot 10^7$	$1.2 \cdot 10^{-3} - 6 \cdot 10^{-4}$	This research*
Weak ligand P		$1.2 \cdot 10^{-4} - 5.2 \cdot 10^{-5}$	
<u>Scheldt Estuary S=0.3</u>			
Strong ligand L	$1 \cdot 10^9$	$8.5 \cdot 10^{-4}$	This research*
Weak ligand P		$6.8 \cdot 10^{-5}$	

4.4. Salinity

The kinetic rate constants were found to depend on salinity resulting in a salinity-related K' . Yet no variation in K' with salinity was found upon application of the classical titration using CLE-ACSV. This coincides with the results obtained by Witter and Luther (1998) when they observed a relation between K' and depth using the kinetic approach and no relation between K' and depth using the titration method.

The K'_{FeL} values increase from $10^{18.9}$ at $S=26$ to $10^{22.11}$ at $S=0.3$. Whether the change of K'_{FeL} with S is related to changes in ionic strength and the activity coefficients of the reacting species, or that the origin of the organic ligands changes is an interesting question that we want to address now. According to Stone and Morgan (1990) an increase in the ionic strength lowers the reaction rate between a cation and an anion and has little effect on reaction rate when one or both of the reactants are uncharged. Here we assume that Fe is bound in a 1:1 coordination to the ligand in ionic binding. According to Hering and Morel (1990) the formation rate constant of metal chelation is decreasing with increasing strength for charged metals, although this also depends on the type of complex formation and the therefore on the rate-limiting reactions. The dissociation rate constant is related to the formation rate constant through the principle of micro reversibility, hence proportional to the formation rate constant by division through the conditional stability constant. The observed increase in K'_{FeL} in Table 3 can partially be explained by the decrease of the ionic strength. Since the complex is supposed to have no charge and the ionic species are assumed to have a charge of three, the effect on changing activity coefficients is thus higher on the formation rate constant. At the salinities 26, 10 and 0.3 the ionic strengths are 0.47, 0.23, and 0.03 respectively. According to the Davies equation the activity coefficients for ions with a charge of three are 0.0115, 0.056 and 0.21 respectively (Stumm and Morgan, 1996). A difference of factor 324 in formation rate constants between $S=26$ and $S=0.3$ can be accounted for by salinity ($0.21/0.0115=18$, a difference of 18 for Fe^{3+} and for the assumed L^{3-} or P^{3-} and thus 18^2). The actual difference is factor 100 (Table 3) and is thus of the order of magnitude that it can be explained by ionic strength alone. However, a difference exists also between the size fractions per salinity, which apart from ligand concentration is the only difference that can be found with size fraction as discrimination. The presence of colloidal molecules increases the dissociation of the two ligand classes (FeL and FeP) with a factor of 2 (Tables 3 and 4). This is also an indication for a difference in the origin of the organic ligands. Moreover, a faster dissociation with increasing S cannot be explained by means of ionic strength and this probably reflects the changing origin of the organic ligands, which in this estuary should be quite large. At $S=0.3$ the organic material is terrestrial of nature since it is upstream of the area with maximum turbidity, terrestrial organic matter prevails here and the conditions may differ considerably with more saline water downstream where most of the terrestrial organic matter has already been lost due to flocculation. Therefore it is quite possible that we cannot compare data from $S=0.3$ with the other data. Although Rose and Waite (2003) also used organic matter of terrigenous origin, a comparison is difficult since they measured at a constant high salinity in their experiments. It is thus not surprising to find differences in organic ligands, although until now since everybody found the same K' values, a distinction to source of the organic ligands was hardly possible. A faster dissociation of Fe from the organic ligands is favourable for

uptake by phytoplankton, since the Fe concentration can be limited in seawater, so this might be essential for the role of Fe in plankton growth.

4.4. The new ligand P

The ligand P was distinguished and since no information was present about this ligand a concentration had to be assumed. An assumed high concentration of 1000 nM implying no changes in P' worked very well. It has to be remembered however, that the estimated K'_{FeP} must always be considered in combination with the assumed total concentration of P, the α value (product of total ligand concentration and conditional stability constant) remains constant. A change in the concentration of P is reflected by changes in the formation rate constant and so in the value of K' , whereas α remains constant. The influence of salinity cannot be found in the estimated characteristics of this ligand. When constructing the models it was assumed that labile Fe consisted of Fe' and FeP, since the dissociation was assumed to be relatively fast. To verify this hypothesis let us use the time 120 s of the measurement and the rate constants from tables 3 and 5, $[\text{TAC}] = 10^{-5}$ M, $[\text{Fe}'] = 20 \cdot 10^{-9}$ M, $[\text{Fe}(\text{TAC})_2] = 0$ at $t=0$ and for the sake of simplicity let us assume that equal concentrations of FeL and L' and equal concentrations of FeP and P' exist. Then 4.8 and 7800 nM $\text{Fe}(\text{TAC})_2$ can be formed (depending on the salinity), no dissociation occurred, 500 times more FeP is dissociated than formed, and 14 times more FeL is formed than dissociated, using equations 13, 7 and 5 respectively. High labile Fe concentrations can thus be attributed to the dissociation of the relatively weak ligand. The measured $[\text{Fe}(\text{TAC})_2]$ is due to added Fe' (20 nM) and the dissociating FeP. The dissociation of FeP is slow compared to FeL, but the formation is very much slower compared to FeL and slower than FeX. Thus after an Fe addition first FeL would be formed, which is not possible here since the sites were already saturated with Fe, then FeX will precipitate and in a later stage FeP can be formed.

4.5 Consequences for the mobility of Fe in the Scheldt

The α value of FeL is lower at $S=26$ but higher at the other salinities than the other two α values of FeP and FeX, which are of the same order of magnitude. The FeX as species was treated in the same way as the ligands, with a constant concentration of X, which might be close to the truth thinking of OH^- linked species (oxyhydroxides). Thus at high salinity in the estuary, L', P' and X' compete with each other for Fe', and part of the Fe will precipitate as FeX. Upstream in the estuary at lower salinities, L must be saturated before any formation of FeP or precipitation as FeX can occur. There, equal amounts of excess Fe will be precipitated as FeX and exist as FeP according to the more or less equal α values (Tables 3 and 4). Thus half of the excess Fe with respect to L remains in the dissolved phase!

As much as 90% of the Fe complexes had dissociated by competition with TAC at $t \approx \infty$ in the samples S=26 (both fractions) as well as in S=0.3 in the fraction <1 kDa (Figure 2C). Dissociation of organic Fe complexes in samples of S=10 (both fractions) and S=0.3, <0.2 μm occurred only for 20% (Figure 2D). These results are well explained when the α values of FeL, FeP, FeX and Fe(TAC)₂ are considered. At S=26 and S=0.3 <1 kDa the α values of the ligands L and P and of the precipitate FeX (Table 3) are all below those of Fe(TAC)₂ (Table 5) and indeed around 10% of the α value of Fe(TAC)₂. On the other hand for the samples at S=10 the α value of FeL is larger than of Fe(TAC)₂ (Tables 3 and 5). According to equilibrium assumptions and the fact that α of FeL is around 10 times larger than α Fe(TAC)₂, only 10% of total Fe should exist as Fe(TAC)₂ at the end of the experiment, and indeed this varies between 11 and 20% for the samples at S=10 (results not shown).

It is also apparent from the relatively high α values of FeX in Table 3 that some Fe will precipitate after disturbing the equilibrium with the solid phase due to filtration and removal of the colloidal size fraction. The α values of the Fe species FeL, FeP, FeX and Fe(TAC)₂ suggest that Fe will even precipitate in the presence of TAC.

Whitworth et al. (1999) described the binding constants of several metals on suspended particulate matter in the Scheldt estuary. With these binding constants and those of dissolved organic ligands they can model the competition between ligands and particles and predict the dissolved metal concentration. This was impossible for Fe because the conditional stability constants for the adsorption on particles ($\log K' = 15$) differed too much from the conditional stability constant of the dissolved organic ligands ($\log K' = 18-23$). With our results on the ligand P and FeX, we fill this gap.

Ligands like the here called P might be of minor importance in the open ocean where free ligand sites of the strong ligand are still in excess. In coastal areas however, or in the open ocean after a sudden input of Fe these relatively weak ligands can become important. The presence of weak ligands can be indicated by high labile Fe or Fe concentrations in excess over the ligand concentrations (Croot and Johansson, 2000; Boye et al., in press; Powell, personal communication; Chapter 8).

5. Conclusions

Modelling of the kinetic data of Fe in natural estuarine waters gave new insight in the chemistry and mobility of Fe. A distinction could be made in rate constants between three different Fe species. A strong well known ligand, L, with fast formation kinetics, 10 to 100 times faster than the model ligands found by Witter et al. (2000), is accompanied by a relatively weak ligand, P, and a precipitate of Fe, FeX. The existence of this relatively weak ligand P was not detected before. Calculations pointed out that half of excess Fe (with respect

to L) was kept in the dissolved phase by the weak ligand P. The presence of P explained the high concentrations of Fe labile with respect to TAC in the estuary.

The K' , and both rate constants of the strong ligand L are dependent on the salinity. The formation rate constant decreases with salinity, which could for the largest part be explained by ionic strength and related changes in activity coefficients. The positive relation between salinity and the dissociation rate constant indicates a changing origin of the ligands, which in the Scheldt estuary is not surprising.

The formation rate constant of $\text{Fe}(\text{TAC})_2$ is not as fast as expected by its high stability constant. The detection window for 10 μM TAC allows interferences with the relatively weak ligand P. TAC can be used in samples of all salinities. Until $S=10$ the $\beta_{\text{Fe}(\text{TAC})_2}$ value of $10^{22.4}$ from Croot and Johansson (2000) can be used, at lower salinities $\beta_{\text{Fe}(\text{TAC})_2}$ value increases considerably ($10^{26.3}$ at $S=0.3$).

The influence of size fractions was found, except on Fe and ligand concentrations, also on the dissociation rate constant of FeL . Fe from the colloidal fraction dissociates faster than from the truly dissolved phase.

Finally, precipitation cannot be neglected in filtered, non-acidified samples, even in the presence of excess strong ligands.

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