

Separation of dissolved iron from the aqueous system with excess ligand

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1 | **Separation of Dissolved Iron from the Aqueous System with Excess Ligand**

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23 **Abstract**

24 A new technique for the separation and preconcentration of dissolved Fe(III) from the ligand-
25 rich aqueous system is proposed. A solid phase extraction (SPE) system with an immobilized
26 macrocyclic material, commonly known as molecular recognition technology (MRT) gel and
27 available commercially, was used. Synthetic Fe(III) solution in aqueous matrices spiked with a
28 100-fold concentration of EDTA was used. Dissolved iron that was 'captured' by the MRT gel
29 was eluted using hydrochloric acid and subsequently determined by graphite furnace atomic
30 absorption spectrometry. The effect of different variables, such as pH, reagent concentration,
31 flow rate and interfering ions, on the recovery of analyte was investigated. Quantitative
32 maximum separation (~100%) of the dissolved Fe(III) from synthetic aqueous solutions at a
33 natural pH range was observed at a flow rate of 0.2 mL min⁻¹. The extraction efficiency of the
34 MRT gel is largely unaltered by the coexisting ions commonly found in natural water. When
35 compared with different SPE materials, the separation performance of MRT gel is also much
36 higher.

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38 **Keywords**

39 Dissolved iron; preconcentration; excess ligand in aqueous solution; Molecular Recognition
40 Technology; atomic absorption spectrometry

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46 **1.0 Introduction**

47 The accurate determination of iron in dissolved and particulate forms or size fractions is
48 necessary for describing the iron chemistry within a given body of water (Bruland and Rue,
49 2001). The iron content in an aqueous system is important for environmental protection,
50 hydrogeology and some chemical processes, such as photosynthesis and phytoplankton
51 production in the open oceans (Riley and Chester, 1971; Martin and Fitzwater, 1988; Martin,
52 1990). Recent studies suggest that Fe(III) solubility in oceanic water is controlled by
53 complexation with natural organic ligands (Kuma et al., 1996; Waite, 2001; Liu and Millero,
54 2002), and these complexation equilibria subsequently regulate the concentrations of dissolved
55 iron in the oceanic system (Johnson et al., 1997; Kuma et al., 2003). Thus, the majority of
56 dissolved iron in the oceans appears to be chelated (as FeL_i) with organic ligands (L_i) (Wu and
57 Luther, 1995; Rue and Bruland, 1997; Boye et al., 2001).

58 Sensitive analytical methods, such as graphite furnace atomic absorption spectrometry (GF-
59 AAS), have led the way in providing reliable profiles for iron in oceanic waters over the past
60 three decades (Johnson et al., 1997; Bruland and Rue, 2001). However, precise determination of
61 iron is very difficult due to the risk of sample contamination (Blain and Treguer, 1995; de Jong
62 et al., 2008). Reports have shown that there are discrepancies between the estimated
63 concentrations of dominant hydroxo-complex species of Fe(III), such as $\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})_3^0$ and
64 $\text{Fe}(\text{OH})_4^-$, and values (~0.1–10 nM) for the thermodynamic solubility of Fe(III) hydroxide in
65 seawater (Byrne and Kester, 1976; Kuma et al., 1996; Liu and Millero, 2002). These low
66 concentrations also make it difficult to measure iron in ocean waters (de Jong et al., 2008). For
67 reliable analysis, method development is important to overcome limitations caused by the high
68 salt matrix of oceanic water samples that could cause interferences during measurements. One of

69 the popular analysis methods is to separate and preconcentrate the desired metal ion(s) out of the
70 sample matrix for precise determination (Hosten and Welz, 1999). Various methods, such as co-
71 precipitation, liquid-liquid extraction, solid-liquid extraction, and ion exchange resins, have
72 been developed for the separation of trace metals from natural samples (Filik et al., 1997;
73 Pourreza and Mousavi, 2004; Ghaedi et al., 2005; Ghaedi et al., 2007; Ghaedi et al., 2008).
74 However, the solid phase extraction (SPE) approach has gained rapid acceptance since the 1980s
75 because it is one of the fastest, most economical and cleanest methods for separating and
76 concentrating trace metals from aqueous samples (Hosten and Welz, 1999; Firdaus et al., 2007).

77 In SPE, appropriate materials are immobilized onto support matrices to prepare solid phases
78 with a 'capturing capability' for separation and preconcentration (Masi and Olsina, 1993). Some
79 of the sorbent materials have the ability to interact with various metal ions while others are fairly
80 specific for a particular ion within complex matrices (Carbonell et al., 1992; Nickson et al.,
81 1995; Ghaedi et al., 2006; Ghaedi et al., 2008; Ghaedi et al., 2009). One group of SPE materials
82 includes those with macrocyclic ligands, such as crown ethers, immobilized on a silica or
83 polymer support; this type of SPE has been reportedly used for ion-selective separation and
84 preconcentration of metal ions, and the technique is commonly known as molecular recognition
85 technology (MRT) (Izatt et al., 1995; Hasegawa et al., 2010; Rahman et al., 2010). Reports on
86 the separation/preconcentration of dissolved iron using SPE from an aqueous system in which
87 Fe-organic ligand complexation is prevalent is still very limited (Wells and Bruland, 1998). The
88 extraction efficiency of SPE materials significantly decreases in aqueous systems containing
89 excess ligand because ligands often compete with SPE materials for metal ions. This limitation
90 can be minimized with the MRT-SPE systems, which can provide non-destructive separation of
91 metal ions from ligand-rich aqueous systems (Hasegawa et al., 2010).

92 In this work, we propose a simple method using a MRT-SPE system for the separation of
93 dissolved iron from aqueous system of Fe-ligand complexes containing an excess amount of
94 ligand in solution. The SPE sorbent is a proprietary polymeric organic material comprised of ion-
95 selective sequestering agents based on molecular recognition and macrocyclic chemistry.
96 Synthetic, ligand-rich aqueous Fe-ligand matrices were used as the samples. Hydrochloric acid
97 was used as the eluent with subsequent determination of iron using ~~graphite furnace atomic~~
98 ~~absorption spectrophotometry (GF-AAS)~~GF-AAS.

99 **2.0 Experimental**

100 **2.1 Materials**

101 *2.1.1 Reagents*

102 Analytical grade commercial products were used as received. Stock solutions of Fe(III) and
103 Fe(II) were prepared by dissolving FeCl₃·6H₂O and FeCl₂·4H₂O (Wako Pure Chemical
104 Industries, Japan), respectively, in 1 M HCl. A Perkin-Elmer (Norwalk, CT, USA) Model ICP
105 Optima 3000 inductively coupled plasma emission spectrometer was used to standardize the iron
106 solutions.

107 Nitriiotriacetic acid (NTA), O,O'-bis(2-aminoethyl)ethyleneglycol-N,N',N'-tetraacetic
108 acid (GEDTA), ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA) and diethylenetriamine-
109 N,N,N',N',N''-pentaacetic acid (DTPA) were purchased from Dojindo Laboratories, Japan;
110 desferrioxamine B (DFB) was supplied by Sigma-Aldrich, St. Louis, MO. Each of these reagents
111 was dissolved in 0.1 M sodium hydroxide to prepare stock solutions of the chelating ligands.

112 Working standards of metal and ligand solutions in the μM to mM range were prepared by
113 dilution on a weight basis. The experimental pH range was 4 to 8, and solution pH was adjusted

114 using either 1 M HCl or 1 M NaOH. The pH was maintained using the following buffer
115 solutions: 0.1 M CH₃COONa/CH₃COOH (pH 4–5) and 0.1 M NaH₂PO₄/Na₂HPO₄ (pH 6–8).

116 Aqueous solutions of 10 mM chelating ligands in the appropriate buffer were spiked with 0.1
117 mM Fe(III) or Fe(II) in 1.0 M HCl, and allowed to stand for 20 min, and these solutions were
118 then added to the sample solutions. For Fe(II) sample solutions, 0.2% hydroxyl ammonium
119 chloride was added to prevent oxidation.

120 To study the effect of coexisting ions, NaCl, KCl, CaCl₂ and MgCl₂ were used as sources of
121 cations, and NaCl, NaNO₃, CH₃COONa, Na₃PO₄, Na₂SO₄, NaClO₄ were used as sources of
122 anions; all salts were purchased from Nacali Tesque, Japan. Working solutions at a concentration
123 of 10 mM were prepared in a H₂O matrix, and the pH was adjusted to 8.0. The final solutions
124 were allowed to equilibrate for 24 h before analysis. Considering the competitive behavior of
125 Ca²⁺ and Mg²⁺ ions in seawater in terms of ligand capturing, the metal-to-ligand ratio was
126 maintained at 1:100.

127 Deionized water obtained from a Barnstead 4 Housing E-Pure system was used to prepare all
128 solutions and is hereafter referred to as EPW.

129 2.1.2 SPE materials

130 Different SPE materials, as listed in Table 1, were used. MRT gel and AnaLig TE-01 were
131 purchased from GL Sciences, Japan. Other SPE materials were purchased from Bio-Rad
132 Laboratories (Chelex-100), GL Sciences (MetaSEP ME-1, ME-2 and ME-3) and Hitachi High-
133 Technologies Corporation (NOBIAS Chelate PA-1, PB-1, Ion SC-1 and SA-1).

134 2.2 Cleaning

135 Low-density polyethylene and laboratory equipment (Nalge, USA) were used to store the
136 solutions and to hold solutions during the experiments. Before use, bottles and laboratory

137 equipment were soaked in an alkaline detergent (Scat 20X-PF, Nacali Tesque, Japan) overnight
138 and rinsed with EPW; they were then soaked in 4 M HCl overnight and rinsed with EPW.
139 PerFluoroAlkoxy tubes and micropipette tips (Nichiryo, Japan) were cleaned according to the
140 procedure described by Sohrin et al. (1998).

141 **2.3 Column separation procedure**

142 *2.3.1 Column cleaning and conditioning*

143 SPE materials packed in 3-mL columns were used in this experiment. Column cleaning was
144 conducted with HNO₃ (8 mL) and EPW (6 mL). The appropriate buffer solution, consisting of 5
145 mL of 0.1 M CH₃COONa/CH₃COOH (pH 4–5) or 5 mL of 0.1 M NaH₂PO₄/Na₂HPO₄ (pH 6–8),
146 was allowed to flow through the column to ensure the desired pH conditions (4–8).

147 *2.3.2 Treatment of samples*

148 Sample solution (5 mL) with ligand (10 mM) and spiked with Fe(III) or Fe(II) (0.1 mM),
149 which was pH adjusted with 0.1 M solution of ~~CH₃COONa/CH₃COOH (pH 4–5) or~~
150 ~~or NaH₂PO₄/Na₂HPO₄ (pH 6–8)~~ buffer, was passed through the SPE column at the pre-set flow
151 rate of 0.2 mL min⁻¹. The column effluent was collected. The analyte concentration in the
152 column effluent represents the unrestrained concentration of analyte in the SPE system. The
153 second and final step was the elution of analyte from the SPE system using HCl (1 and 6 M). The
154 analyte concentrations from the sample solution, in the effluent, and in the eluent were measured
155 with GF-AAS. The recovery efficiency was calculated as follows:

$$156 \quad \text{Recovery (\%)} = \frac{\text{number of mol of Fe recovered in all fractions}}{\text{number of mol of Fe applied to the column}} 100 \quad (1)$$

157 Three replicate measurements per sample were made in all instances. The peak height of the
158 reported signal was proportional to the concentration of the respective iron species and was used
159 for all measurements.

160 **2.4 Instruments**

161 A Hitachi Model Z-8100 GF-AAS (Hitachi, Japan) operating at the 248.3 nm wavelength
162 with a slit width of 0.2 nm and 15.0 mA lamp current was used for analyzing iron concentrations.
163 A temperature program was performed with the following steps: drying at 100 °C, ashing at 1000
164 °C, atomization at 2700 °C and cleaning at 3000 °C with holding times of 60, 60, 10 and 4 s,
165 respectively. Argon was used as the inert gas at a flow rate of 200 mL min⁻¹ except during the
166 atomization step (30 mL min⁻¹). After a calibration step, 20 µL of sample and 10 µL of matrix
167 modifier (NiSO₄) were introduced into the graphite furnace for three replicates of each
168 measurement. The pH of the sample solutions was measured with a Navi F-52 pH meter (Horiba
169 Instruments, Japan) and a combination electrode.

170 **3.0 Results and Discussion**

171 **3.1 Comparative study with different SPE materials**

172 The separation efficiencies of Fe(III) from aqueous solutions containing excess ligand with
173 AnaLig TE-01 and different commercially available SPE products were investigated in a non-
174 competitive environment (Fig. 1). Thermodynamic equilibrium calculations suggest that 99.9%
175 of the ambient, dissolved Fe(III) exists as Fe(III) chelates when the ligand concentrations are ~25
176 times higher in the aqueous system (Bruland and Rue, 2001).

177 Therefore, a maximal amount of Fe(III) is assumed to be present as the Fe-EDTA complex in
178 the EDTA-spiked sample solutions containing excess EDTA. As shown in Fig. 1, it is apparent

179 that AnaLig TE-01 ensures quantitative extraction of the total amount of Fe(III) from an aqueous
180 solution containing a 100-fold concentration of EDTA spiked with Fe(III). However, the
181 extraction efficiencies of other SPE products are limited to 10–50% for the same sample
182 solutions. Consequently, AnaLig TE-01 is a more suitable SPE-type system to separate the iron
183 dissolved as the metal-ligand complex in a ligand-rich aqueous environment.

184 3.2 Effect of pH

185 The retention of dissolved iron on the AnaLig TE-01 column containing immobilized
186 macrocyclic material was studied as a function of pH. The pH of the aqueous system with Fe(III)
187 and Fe(II) in excess EDTA was maintained in the range of 4–8 with $\text{CH}_3\text{COONa}/\text{CH}_3\text{COOH}$
188 (pH 4–5) and $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 6–8) buffer solutions at 0.1 M concentration. This study
189 was restricted to the pH range 4–8 because EDTA is not very water soluble at very low pH
190 (Ueno et al., 1992), and the increasing solubility of silica gel with pH (Vogelsberger et al., 1992)
191 may dissolve the silica gel base support of AnaLig TE-01 column.

192 As shown in Fig. 2, quantitative retention of the Fe(III) and Fe(II) complexes from the
193 ligand-rich medium in the pH range 4–8 were at a maximum on the AnaLig TE-01 column. The
194 variation in the pH of the sample solution and the column effluent were insignificant. Therefore,
195 we can conclude that the MRT gel has significant affinity for dissolved iron in a ligand-rich
196 matrix or in iron-ligand complexes, and the iron-ligand complex is stable in the studied pH range.
197 In view of the fact that oceanic water is limited to the pH range of 7 to 9, the majority of the
198 dissolved iron (Fe(III)) is chelated with organic ligands of biological origin (Bruland and Rue,
199 2001), and further experiments were carried out at pH 8.0 ± 0.2 .

200 **3.3 Effect of sample loading flow-rate**

201 The retention of analytes in the SPE system depends upon the flow rate of the metal-fortified
202 sample solution (Bag et al., 1998). The effect of sample loading flow rate on the recovery
203 percentage was examined under optimum conditions. The solution was passed through the SPE
204 column with the flow rates that were adjusted in the range of 0.2–4.0 mL min⁻¹. As shown in Fig.
205 3a, retention of Fe(III) on the MRT gel column was quantitative up to a flow rate of 0.25 mL
206 min⁻¹. A gradual decrease in retention with increasing flow rate was observed in the range of
207 0.3–1.0 mL min⁻¹, and retention decreased to about 60% at higher flow rates (2.0–4.0 mL min⁻¹).
208 This behavior indicates the constant retaining capability of the MRT gel at the initial loading
209 period. Because quantitative, maximum extraction of the analyte is desirable, we applied a flow
210 rate of 0.2 mL min⁻¹ to further experiments to ensure selective pre-concentration of the sample
211 solution for analytical determination.

212 **3.4 Effect of eluent concentration**

213 A satisfactory eluent should effectively elute the extracted analytes using a small volume,
214 which is desired for a high enrichment factor of the analyte, but the eluent should not affect the
215 accurate determination of analytes (Chen et al., 2009). The effect of eluent concentration on the
216 elution of analyte from the MRT gel column was studied by first adding 2 mL of the Fe(III)-
217 spiked, aqueous EDTA solution at pH 8.0 onto the column, and the analyte extracted by the
218 MRT gel column was then eluted using 5 mL of eluent (0.1–6.0 M HCl). The recovery of the
219 analyte was determined with GF-AAS. The Fe(III) recovery percentage was found to increase as
220 the concentration of HCl increased up to a concentration of 0.5 M, at which point the recovery
221 level remained constant (Fig. 3b). This behavior indicates that a HCl concentration equal to 0.5
222 M is sufficient for quantitative elution of the bound ions in the TE-01 MRT gel column.

223 However, acid concentrations greater than or equal to 5.0 M were recommended as eluent for
224 TE-01 (IBC Advanced Technologies, 2007). Thus, a combination of 1 M HCl (4 mL) and 6 M
225 HCl (1 mL) was selected as eluent for subsequent experiments to ensure quantitative elution of
226 the analyte.

227 *3.5 Effect of metal-ligand stability constants*

228 Ligands form water-soluble metal complexes of high thermodynamic stability (Lim et al.,
229 2005). The high stability of the metal-ligand complexes may influence the extraction
230 performance of the SPE materials. Various investigators have suggested that dissolved iron
231 content in the open oceans exists primarily as Fe-ligand species (Bruland and Rue, 2001), and
232 two classes of Fe(III)-binding natural organic ligands were observed with the following
233 conditional stability constants K'_{ML} : $5 \times 10^{12} \text{ M}^{-1}$ ($\text{p}K'_{ML} = 12.7$) and $6 \times 10^{11} \text{ M}^{-1}$ ($\text{p}K'_{ML} =$
234 11.78) (Rue and Bruland, 1997).

236 The effect of the metal-ligand complexes' K'_{ML} values on the performance of the MRT-SPE
237 system was evaluated for the extraction of Fe(III) from the ligand-rich, Fe(III)-spiked aqueous
238 system. Solutions of ligands in 0.1 M HCl with varying iron complex stability constants (K_{ML}),
239 such as NTA, GEDTA, EDTA, DTPA and DFB (Table 2), were added to the iron-spiked
240 solution to prepare each sample solution. The Fe(III)-ligand complex stability at pH 8 was
241 considered during the selection of the chelating ligands. AnaLig TE-01 demonstrated better
242 separation efficiency than the other selected SPE materials (Chelex-100, InterSEP ME-1 and
243 NOBIAS Chelate PA-1) for the metal-ligand complexes of NTA, GEDTA and EDTA (Fig. 4). A
244 significant decrease in the AnaLig TE-01 separation performance was observed for metal-ligand
245 complexes with high K'_{ML} values, e.g., metal-DTPA and metal-DFB. In ocean waters, the $\text{p}K'_{ML}$

246 values for Fe(III)-binding organic ligand classes are limited to a pK of 12.7 (Rue and Bruland,
247 1997). However, quantitative separation performance for ligands with pK'_{ML} values of up to 22.8
248 was observed with the proposed MRT-SPE system. Therefore, the thermodynamic stability of
249 the commonly observed Fe(III)-ligand complexes in ocean waters has a negligible effect on the
250 iron separation performance of the AnaLig TE-01.

251 **3.6 Effect of coexisting ions**

252 The interference of other coexisting ions on the separation and preconcentration of dissolved
253 iron was examined under optimal conditions. Various cations, including Na^+ , K^+ , Ca^{2+} and Mg^{2+} ,
254 and anion species, including Cl^- , NO_3^- , CH_3COO^- , PO_4^{2-} , SO_4^{2-} and ClO_4^- , were added
255 individually to the ligand-rich Fe(III) sample solutions, which were then allowed to equilibrate
256 for 24 hours. The studies were carried out in a non-competitive environment by applying 5 mL
257 of the ion-fortified sample at the optimized flow rate with subsequent collection using the
258 appropriate eluent. Variation in pH was negligible between the fortified samples and column
259 effluents. Quantitative recovery of Fe(III) was observed in the presence of the selected cations
260 and anions (Fig. 5). Therefore, it can be concluded that the developed method is reasonably free
261 from interference resulting from coexisting ions that are commonly found in open aqueous
262 systems.

263 **3.7 Extraction capacity**

264 Extraction capacity is an important factor that must be evaluated because it determines how
265 much MRT gel is required for quantitative separation of Fe(III) from a solution. Batch method
266 experiments were used for a capacity study, and the experiments were conducted as follows: 0.5
267 g of the MRT gel was added to 20 mL of Fe-EDTA solution (Fe(III) – 0.1 mM, ligand: EDTA –

268 10 mM, matrix: H₂O, pH: 8.0), and the mixture was continuously shaken for one hour. Iron
269 concentrations in the filtrate were determined by GF-AAS. The extraction capacity for Fe(III)
270 under ligand-rich condition was 0.115 ± 0.002 mmol g⁻¹ of AnaLig TE-01 (mean \pm SD, $n = 3$).
271 This result was in good agreement with the certified value 0.1–0.3 mmol g⁻¹ of AnaLig TE-01
272 (IBC Advanced Technologies, 2007) for the simultaneous complexation of Ag(I), Au(III), Cd(II),
273 Co(II), Cu(II), Fe(II), Hg(II), Ni(II), Pb(II), Pd(II) and Zn(II) from aqueous solutions to
274 concentrate the ions or remove interfering matrices.

275 **3.8 Regeneration and reusability**

276 The regeneration ability and stability of the MRT gel were investigated because regeneration
277 is one of the key factors in evaluating the performance of an extraction material. The
278 experimental results indicated that AnaLig TE-01 is stable during operation of the column, and
279 this material could be regenerated for more than 100 loading and elution cycles without loss of
280 analytical performance. Systems with macrocycles attached onto solid supports (Bradshaw et al.,
281 1988; Izatt, 1997), which is used for the AnaLig TE-01, allow non-destructive separation of
282 metals from the matrix (ligand-rich aqueous solution) and also facilitate the repeated use of the
283 macrocycles because the material can be regenerated after each use. Thus, the initial high cost of
284 MRT gel synthesis is amortized over time.

285 **3.9 Analytical characteristics**

286 GF-AAS was used to measure the iron concentrations in the MRT gel treated, fortified
287 aqueous samples. Under optimum conditions, the linear range was found to be 0.01–0.11 $\mu\text{g mL}^{-1}$
288 ¹. The method detection limits, as calculated from three times the standard deviation ($n = 15$) of

289 the blank, was $0.02 \mu\text{g L}^{-1}$. Based on the relative standard deviation, the precision of the method
290 was calculated to be 1.4% from 10 replicate measurements at a concentration of $0.4 \mu\text{M Fe(III)}$.

291 **3.10 Recovery test with artificial seawater**

292 Artificial seawater was prepared according to Lyman and Fleming (1940) and used to
293 evaluate the ion-recovery performance of the MRT-SPE column in the presence of multiple
294 interfering ions. Artificial seawater samples spiked with 0.1 mM Fe(III) and 10 mM EDTA were
295 passed through the MRT-SPE system at pH 8. The recovery of Fe(III) was observed to be $96 \pm$
296 3% ($n = 3$).

297 **4.0 Conclusions**

298 MRT gel, a column-packed, immobilized macrocyclic material, was used for the
299 separation/preconcentration of dissolved iron from an aqueous system containing excess ligand,
300 and GF-AAS was used to determine the concentrations of eluted iron. Quantitative collection of
301 the dissolved iron was obtained under the following optimized conditions: pH range: 4–8; flow
302 rate: 0.2 mL min^{-1} ; eluent: 1 M and 6 M HCl.

303 Compared with previously tested SPE materials, MRT gel possesses several advantages.
304 MRT gel is chemically stable with excellent separation properties and extraction capacity. It can
305 be used repeatedly with little loss of performance. Separation of the dissolved iron is rapid,
306 reproducible, and efficient. In addition, the presence of large concentrations of other coexisting
307 ions had no affect on the separation process.

308 In natural oceanic systems, dissolved Fe(III) primarily exists as organically-bound Fe-ligand
309 species, and the total organic ligand concentrations are ~ 25 times higher than total iron
310 concentrations. In freshwater systems, Fe(III) often forms complexes with humic substances.

311 Conventional SPE systems may not be applicable for the quantitative determination of dissolved
312 iron in natural bodies of water due to competition with strong Fe(III)-binding organic ligands
313 that limits their accuracy. This limitation is minimized with the proposed MRT-gel separation
314 technique, and it is expected that the total dissolved iron content can be accurately measured with
315 this technique in the presence of an excess of strong Fe(III)-binding organic ligands. Importantly,
316 the quantitative separation performance of the proposed MRT-SPE system was achieved for
317 metal-ligand complexes with a maximum pK'_{ML} of 22.8. Current work is underway to determine
318 ultra-trace amounts of iron in an aqueous system containing excess organic ligands using the
319 MRT gels; the goal of this work is to develop a technique for practical
320 separation/preconcentration and subsequent determination of dissolved iron in oceanic water
321 samples.

322

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474 Table 1: Different SPE materials used in this study

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SPE material type	Commercial Name	Base Support	Functional group
MRT Gel	AnaLig TE-01	Silica gel	Crown ether
Ion-exchange resin	NOBIAS Ion SC-1	Hydrophilic methacrylate	Sulfonic acid
	NOBIAS Ion SA-1	Hydrophilic methacrylate	Quaternized amine
Chelate resin	Chelex-100	Styrene divinylbenzene	Iminodiacetic acid
	MetaSEP ME-1	Methacrylate polymer	Iminodiacetic acid
	MetaSEP ME-2	Methacrylate polymer	Iminodiacetic acid +a
	MetaSEP ME-3	Methacrylate polymer	Iminodiacetic acid +b
	NOBIAS Chelate PA-1	Hydrophilic methacrylate	Polyamino- polycarboxylic acid
	NOBIAS Chelate PB-1	Divinylbenzene/ methacrylate polymer	Polyamino- polycarboxylic acid

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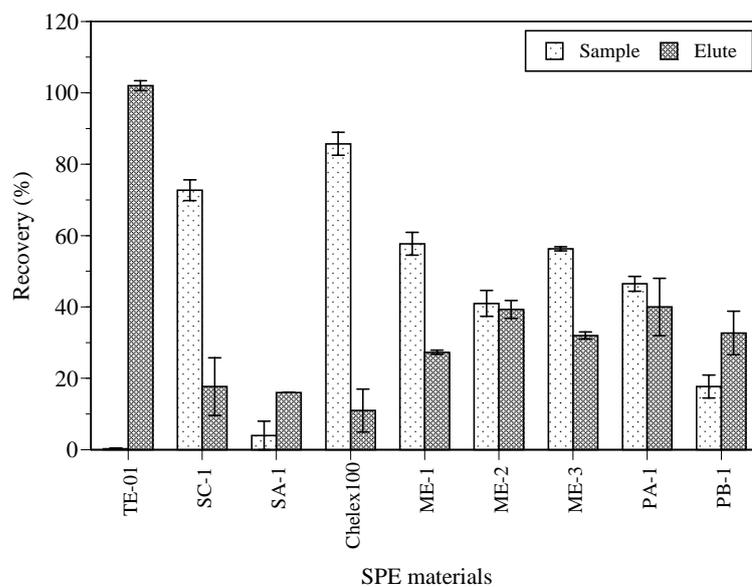
Table 2: Acid dissociation constants (pK_a), stability constants (K_{ML}) and conditional stability constants (K'_{ML}) of Fe(III)-ligand complexes at 25 °C

<i>Ligand</i>	K_{ML}	pK_a	K'_{ML} (at pH 8)
NTA	15.9 ^a	1.89, 2.49, 9.73	14.1
GEDTA	20.5 ^a	2.00, 2.68, 8.85, 9.46	18.1
EDTA	25.0 ^a	1.99, 2.67, 6.16, 10.26	22.8
DTPA	28.6 ^b	2.14, 2.38, 4.26, 8.60, 10.53	25.4
DFB	31.9 ^c	8.39, 9.03, 9.70	29.0

490 ^aMartell and Smith (1974), ^bSillen and Martell (1964; 1993), ^cMorel and Hering (1993)

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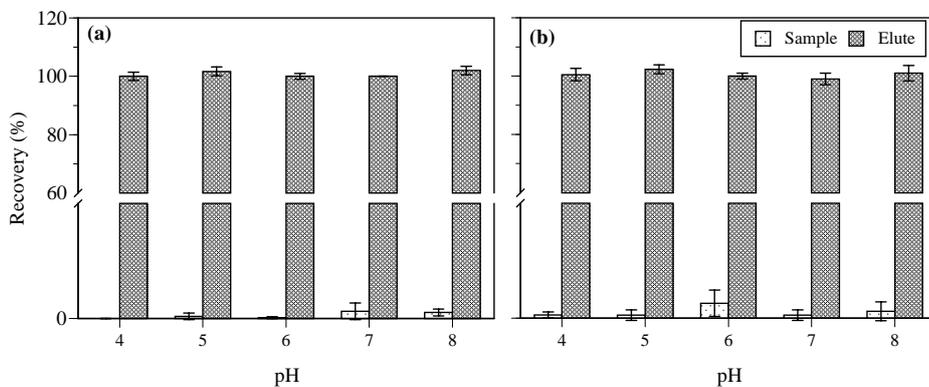
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511 Figure 1: Comparative performance of AnaLig TE-01 with different commercial SPE materials
512 for aqueous metal solution containing excess ligand ($n = 3$). Sample solution: Fe(III) – 0.1 mM,
513 ligand: EDTA – 10 mM, matrix: H₂O, pH: 8.0, sample volume: 5 mL, flow rate: 0.2 mL min⁻¹,
514 eluent: 1 M HCl (4 mL) + 6 M HCl (1 mL). ‘Sample’ denotes the analyte concentration in the
515 column effluent collected after passing the sample solution through the MRT-SPE system;
516 ‘elute’ denotes the analyte concentration in the collected eluent.

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524 Figure 2: Effect of pH on the recovery percentage ($n = 3$). Sample solution: (a) Fe(III) – 0.1 mM

525 and (b) Fe(II) – 0.1 mM, ligand: EDTA – 10 mM, matrix: H₂O, pH: 4.0–8.0, sample volume: 5

526 mL, flow rate: 0.2 mL min⁻¹, eluent: 1 M HCl (4 mL) + 6 M HCl (1 mL). ‘Sample’ denotes the

527 analyte concentration in the column effluent collected after passing the sample solution through

528 the MRT-SPE system; ‘elute’ denotes the analyte concentration in the collected eluent.

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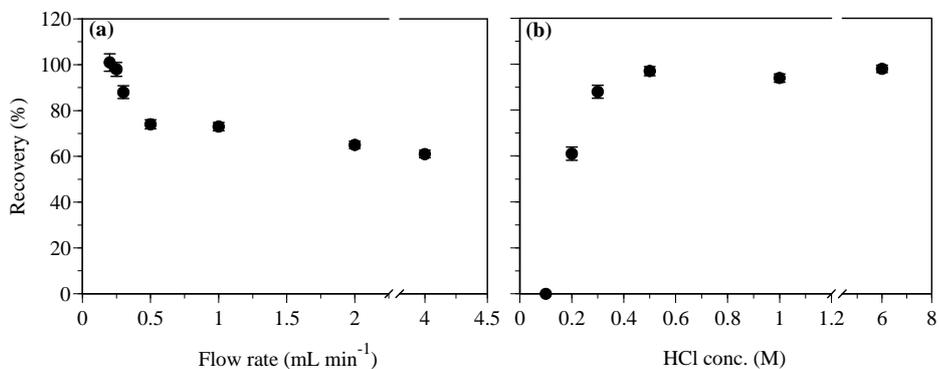
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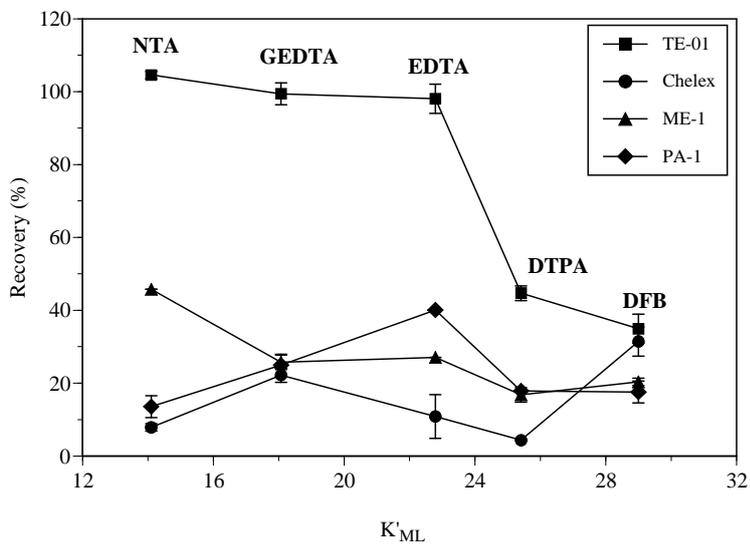
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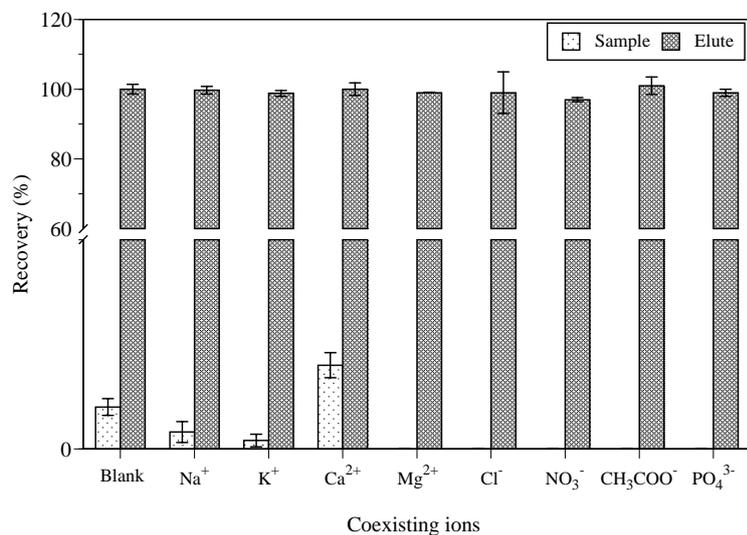
546 Figure 3: Effect of (a) sample loading flow rate and (b) eluent concentration on the recovery
547 percentage of analyte ($n = 3$). Sample solution: Fe(III) – 0.1 mM, ligand: EDTA – 10 mM,
548 matrix: H₂O, pH: 8.0, sample volume: 5 mL, flow rate: (a) 0.2–4.0 mL min⁻¹ and (b) 0.2 mL min⁻¹,
549 eluent: (a) 1 M HCl (4 mL) + 6 M HCl (1 mL) and (b) HCl (0.1–6.0 M) – 5 mL.

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 567 Figure 4: Effect of metal-ligand stability constants on the performance of SPE materials ($n = 3$).
 568 Sample solution: Fe(III) – 0.1 mM, ligand: NTA, GEDTA, EDTA, DTPA, DFB – 10 mM,
 569 matrix: H₂O, pH: 8.0, sample volume: 5 mL, flow rate: 0.2 mL min⁻¹, eluent: 1 M HCl (4 mL) +
 570 6 M HCl (1 mL).

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586 Figure 5: Effect of coexisting ions on the performance of AnaLig TE-01 ($n = 3$). Sample
 587 solution: Fe(III) – 0.1 mM, ligand: EDTA – 10 mM, ion concentration – 10 mM, matrix: H₂O,
 588 pH: 8.0, sample volume: 5 mL, flow rate: 0.2 mL min⁻¹, eluent: 1 M HCl (4 mL) + 6 M HCl (1
 589 mL). ‘Sample’ denotes the analyte concentration in the column effluent collected after passing
 590 the sample solution through the MRT-SPE system; ‘elute’ denotes the analyte concentration in
 591 the collected eluent.

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