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ARTICLE *in* ANALYTICA CHIMICA ACTA · DECEMBER 2012

Impact Factor: 4.51 · DOI: 10.1016/S0003-2670(99)00758-8

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Determination of Fe(II) and total iron in natural waters with 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT).

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Published in *Analytica Chimica Acta*, vol 406, pp 289-302, 2000

Abstract

Several methods for detecting Fe(II) in natural waters using the chelating agent 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) are reported. PDT is a ferroin type ligand and forms a strongly coloured Fe(II) complex , whose concentration can be measured colorimetrically. PDT is the non-sulfonated precursor to Ferrozine (FZ), a commonly used reagent for Fe(II). Solvent extraction of the $Fe(II)(PDT)_3$ complex into 1,2-dichloroethane with the counter ion Tetrabromophenolphthalein ethyl ester (TBPE) (Tsurubou and Sakai, 1984) was found to be the most sensitive technique for measuring Fe(II) in natural waters. However due to the rapid oxidation of Fe(II) in ambient seawater (pH 7.7-8.2) this method is used for measuring total iron, with the addition of a reducing agent.

Trapping of Fe(II) by Ferrozine loaded C18 Sep-Paks (King *et al*, 1991) was found to be the most sensitive method for the in-situ determination of Fe(II) in oxygenated seawater. Attempts to use the ligand PDT in an analogous fashion to FZ were hindered in seawater by the loss of PDT from the Sep-Pak. However in low ionic strength solutions, PDT was retained on the Sep-Pak and quantitatively trap Fe(II). This method could be utilized for measuring nanomolar concentrations of Fe(II) in lakes and rivers.

1. Introduction

The recent surge of interest in the biogeochemical cycling of iron in natural waters has prompted more research into the development of analytical techniques with which to measure and quantify distinct parts of the iron cycle. In natural waters Fe(III) is strongly hydrolysed resulting in a very low solubility and the formation of various Fe(III) oxyhydroxide phases with differing chemical reactivities. In oxygenated natural waters Fe(III) is the dominant redox species, as while Fe(II) is more soluble than Fe(III) at alkaline pH, Fe(II) is rapidly oxidised by O_2 and H_2O_2 (Millero et al, 1987; Millero, 1989; Moffett and Zika, 1987). However the reduction of Fe(III) to Fe(II), with subsequent reoxidation to Fe(III), by photochemical or other processes is a possible mechanism by which colloidal iron is made more bioavailable to phytoplankton (Finden et al, 1984; Wells et al, 1991).

Direct measurement of Fe(II) in natural waters is complicated by the combination of low concentrations and possible post sampling redox artifacts induced by the methodology used (Shapiro, 1966). For example, lowering of the pH by the addition of a buffer will slow the oxidation rate of Fe(II) but may lead to the formation of Fe(II) by chemical processes enhanced by a decrease in pH. Similarly, the addition of a reducing agent (or strong Fe(II) chelator) will stabilise Fe(II) from oxidation but may also lead to the reduction of Fe(III) oxyhydroxides. Thus for studies into the speciation and cycling of Fe(II) in natural waters, analytical techniques that minimise perturbations to the natural system are desired. In this study we report research into using the Fe(II) chelating ligand agent 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) for the determination of Fe(II) under ambient conditions in natural waters.

1.1 Ferroins

The reaction of Fe(II) with 1,10-phenanthroline to form ferroin [$Fe(phen)_3^{2+}$] discovered by Blau (1898) has found wide application in chemical analysis. Since that time other chromogens of the ferroin type have been synthesised. Ferroin type chromogens are distinguished structurally by the

bidentate chelate functional group =N-C=C-N= (see figure 1). Many derivatives of 1,1-phenanthroline and 2,2'-bipyridine have been synthesised and their coloured complexes with Fe(II) and Cu(I) investigated. Much of this work has focused on obtaining improved colorimetric reagents for iron and copper. Research undertaken by Schilt and Case uncovered a new group of ferroin complexes based on substituted triazines. Case synthesised new ligands by utilising condensation reactions between 1,2-diketones and substituted hydrazines or hydrazones (Case, 1965, 1968, 1970, 1971; Schilt and Case, 1980). Schilt and co-workers then investigated the colorimetric properties of the Fe(II) and Cu(I) complexes of the new chromogens (Schilt, 1966; Schilt and Kluge, 1968; Schilt et al, 1970; Schilt et al, 1974; Schilt et al, 1977; Schilt and Case, 1980).

Schilt (1966) reported a series of promising chromogens for Fe(II) based on substituted 1,2,4-triazines. Several of the new ligands formed stable Fe(II) complexes with high absorption characteristics and were extractable into isoamyl alcohol. Schilt and Taylor (1970) further investigated the properties of one of these chromogens 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT). They developed a colorimetric solvent exchange method for determining ppb levels of iron and copper (as the Cu(I) complex). Chriswell and Schilt (1974) similarly reported a method for the spectrophotometric determination of iron in acids and acidic solutions by extraction-formation of tris [PDT] iron (II) thiocyanate.

Schilt (1966) first reported the Fe(II) chelating properties of PDT in his study of 24 of compounds containing the ferroin group. Schilt found that PDT formed a tris complex with Fe(II), $[Fe(PDT)_3]^{2+}$. The iron complex was magenta coloured (maximum absorbance at 555 nm), while weaker brown complexes were formed with Cu(I) and Co(II). Since this initial investigation many other studies have utilised PDT complexation of Fe(II) with subsequent spectrophotometric analysis.

The structure and properties of $[Fe(PDT)_3]^{2+}$ were investigated by Hage et al (1990a) using NMR spectroscopy, differential pulse polarography (DPP) and cyclic voltammetry (CV). They found

(from NMR) that the PDT ligand binds bidentately via N2 of the triazine ring and the pyridyl-nitrogen atom to Fe(II) to form a low-spin complex. Due to the asymmetrical nature of the PDT molecule, two geometrical isomers were observed; *facial (fac)* and *meridional (mer)*. In the *fac* isomer, the three ligands point to one site (AAA), but for the *mer* isomer three non-equivalent possibilities are now present to point one of the three ligands to the other site (AAB, ABA and BAA). $[Fe(PDT)_3]^{2+}$ was isolated as a mixture of the isomers with a ratio of *fac*:*mer* of approximately 3:1 (determined by NMR). The oxidation potential of $[Fe(PDT)_3]^{2+}$ was measured by CV at 1.29 V (in CH_3CN, V vs SCE). Reduction peaks from DPP analysis were observed at -0.91 V, -1.12 V and -1.36 V (in CH_3CN, V vs SCE). Hage *et al* (1990b) also reported the properties and structure of a ruthenium PDT complex.

1.2 Pre-concentration strategies

There are several possible strategies for enhancing the overall senstivity for metal determination when using a metal-chelate technique. In an effort to develop a sensitive preconcentration technique for the measurement of Fe(II) and total iron in natural waters, investigations were conducted into the use of PDT as a preconcentrating agent and chromopore for iron.

1.2.1 Solvent extraction

Schilt and Taylor (1970) developed a protocol for measuring Fe(II) and Cu(I) simultaneously by PDT complexation with extraction into iso-amyl alcohol. The absorbance of the iso-amyl alcohol was measured both before and after the addition of sodium cyanide. The cyanide was added to convert the PDT-Cu(I) complex into a cyanide complex, the PDT-Fe(II) is unaffected by the addition of cyanide. Later Chriswell and Schilt (1974) determined iron in acids and acidic solutions by extraction with PDT. Thiocyanate was used a counter ion to help extract the Fe(II) PDT complex into chloroform.

Thiocyanate and chloride are known to form extractable iron complexes, and these ions can act as carriers to transfer iron to the chloroform phase more efficiently. Tests showed that thiocyanate was capable of reducing the iron by itself, but ascorbic acid can be used as an auxiliary reductant to prevent oxidation of the complex in the chloroform. Once in the chloroform phase the reduced iron is complexed by PDT. The molar absorptivity of the complex with thiocyanate in chloroform was found to be 24,500 L cm^{-1} mol^{-1} at 555 nm. Studies using other solvents and anions showed a slight dependence for the type of solvent or anion, on molar absorptivity.

Kotsuji *et al* (1977) developed a method for determining trace levels (100 nmol/kg level) of iron in seawater by using the solvent sublation of $[Fe(PDT)_3]^{2+}$ cations with an anionic surfactant, sodium lauryl sulphate (SLS) as a collector. An aliquot of acidified seawater was buffered with acetic acid and the iron reduced using hydroxylammonium sulphate. This sample was then transferred to a floatation cell with PDT and SLS added. Iso-amyl alcohol as foam breaker and extractant, is then added to the surface of the aqueous solution. Nitrogen is then bubbled through the aqueous phase. After a suitable collection time, the organic phase containing the PDT-Fe(II) chelate SLS ion pair is removed, diluted and analysed spectrophotometrically.

1.2.2 Counter ions

Enhancing the absorption sensitivity of colorimetric reagents can be accomplished by increasing the delocalisation of electrons (greater degree of conjugation) for the complex, this leads to a more intense visible absorption and a shift for the maximum absorbance to longer wavelengths (Schilt, 1966). This increased delocalistion of electrons can be accomplished by synthesising new ligands with more phenyl or pyridyl groups, however it is balanced by sensitivity losses due to steric problems (Hinderance of iron complexation by bulky side chains and loss of coplanarity of the π bonding groups leading to decreased delocalisation). Alternatively, highly absorbing counter ions may

be used to increase the sensitivity of colorimetric reagents by similarly increasing the delocalistion of electrons.

Using a similar methodology, Knizek and Musilova (1968) utilised sulfonated azo dyes as counter ions in their experiments to improve the colorimetric sensitivity of 1,10-phenanthroline for Fe(II) when extracted into 1,2-dichloroethane. They found that only mono-sulfonated dyes were suitable and they could only be used under weakly acidic or weakly alkaline conditions. The dye Orange III (C.I. 13025) was found to increase the iron sensitivity of 1,10-phenanthroline by approximately six fold.

Tsurubou and Sakai (1984) developed a highly sensitive extraction-spectrophotometric method for the determination of iron in water, using PDT and tetrabromophenolphthalein ethyl ester (TBPE) as the counter ion, with subsequent extraction of the iron-PDT-TBPE complex into 1,2-dichloroethane. They reported an apparent molar absorptivity of 190000 l cm^{-1} mol^{-1} for the Fe(II)-PDT-TBPE ion association complex.

1.2.3 Adsorption of metal-chelate complex on columns

Lundgren and Schilt (1977) adsorbed PDT onto Amberlite XAD-2 (a styrene-divinylbenzene copolymer) in the course of a study on immobilization of ferroin chromogens. A solution of PDT in methanol was passed through a column of XAD-2, the column was washed with distilled water to remove any remaining methanol. From the PDT content of the water and methanol washes, a PDT content of 120 ± 10 µmol/kg of XAD-2 was measured. The Fe(II)-PDT complex was similarly adsorbed to the XAD-2 column. The retention of Fe(II) by PDT coated columns was considerably influenced by anion type and concentrations. Large, easily polarised monovalent anions enhance retention, while organic ions (oxalate, tartrate) reduced retention by competing with PDT for complexation of Fe(II). The trapped Fe(II)-PDT complex were removed from the column by Soxhlet extraction in methanol, trace metals were analysed by atomic absorbance analysis of the methanol

extract; iron(II) content was determined by the measuring the absorbance at 555 nm of the methanol extract. Cu(I) was found to have a higher retention capacity then other trace metals, presumably due to it being retained predominately as a monochelate with PDT. This method was applied to the determination of iron in synthetic seawater and the purification of analytical reagents. A similar study was undertaken by Hutchinson and Schilt (1983) in which the adsorption of ferroin-type ligands with various activated carbons was investigated. PDT-Fe(II) and Ferrozine-Fe(II) complexes were found to be rapidly destroyed on contact with the activated carbon Norit A. No free ligand but all of the Fe(II) was detectable in the supernatant liquid.

The sulfonated derivative of PDT, 3-(2-pyridyl)-5,6,-bis(4-phenylsulfonic acid)-1,2,4-triazine, commonly known as Ferrozine (FZ), has found wide use as a reagent for iron analysis of iron in natural waters since it's introduction by Stookey (1970). Ditzler et al (1986) used Ferrozine on Dowex 1X8 anion exchange resin in an approach to preconcentrating Fe(II). However they found that immobilized Ferrozine became more selective for copper than for iron. This selectivity was proposed to arise from the inability of the $Fe(II)(FZ)_3$ complex to form due to restrictions imposed by reagent-substrate interactions. Using a similar approach King et al (1991) preconcentrated Fe(II) using Ferrozine adsorbed to C18 Sep-Pak cartridges. Seawater samples were passed through the Sep-Pak with Fe(II) being retained as the coloured $Fe(II)(FZ)_3$ complex, the complex was then eluted with methanol and the absorbance measured. A further adaption of this method was made by Zhen et al (1992), by analysing the methanol eluate using HPLC, which allowed the separation of the absorbance due to the uncomplexed Ferrozine from the $Fe(II)(FZ)_3$ complex. This method increases the sensitivity of the analysis by removing interferences that absorb at similar wavelengths to the $Fe(II)(FZ)_3$ complex. The authors used this technique to measure the Fe(II) content of marine aerosols, rainwater and seawater and stated a detection limit of 0.1 nmol/kg.

2. Experimental Section

2.1. *Synthesis of PDT*

PDT was initially prepared following the method of Case (1965) as modified by Gibbs (1976). Early attempts at synthesis using this method were hampered by low yields of the intermediate amidrazone product due to the formation of the orange coupled product 3,6-di(2-pyridyl)-1,2-dihydro-1,2,4,5-tetrazine (identified by IR and CHN analysis). Adapting the methods of Hage et al (1990a) resulted in improved yields and less tetrazine produced. This was accomplished by using the following protocol:

> Equimolar amounts of 2-cyano-pyridine (Aldrich) and hydrazine hydrate were mixed together, after which a small amount of ethanol was added until a clear solution was visible. Upon standing overnight at room temperature (15° C), the white crystals of 2-pyridyl-amidrazone were filtered off and washed with a small amount of ether and air dried. The crystals were further purified by recrystallation from benzene. Equimolar (approx 0.1mol) amounts of benzil and the amidrazone were then refluxed in ethanol for 1 hour. After cooling to room temperature, a yellow precipitate was isolated.

The orange tetrazine was also synthesised directly in an effort to compare its iron chelating properties to PDT. The tetrazine was formed by reacting 2-cyano-pyridine and hydrazine hydrate using the method of Geldard and Lions (1965).

The melting point of PDT was determined to be 187 ± 3 °C which compares well to values found by other investigators, 189-190 °C by Case (1965) and 185-187 °C by Hage et al (1990a). Infrared spectral analysis of the reaction products were performed to provide an indication of purity. The absence of any signal from N-H stretching modes in the recrystallised PDT was used to determine that no unreacted amidrazone was present. The results of the elemental analysis (CHN) of the isolated products from the synthesis is presented in Table 1.

^{1}H and ^{13}C Nuclear Magnetic Resonance (N.M.R.) analysis were performed on a sample of PDT dissolved in d-chloroform using a Varian 300 MHz NMR spectrophotometer. The proton chemical shifts are displayed in Table 2. Using 2-D COSY NMR spectroscopy, the pyridyl proton signals could be assigned (Table 2). These assignments agreed with those made by Gibbs (1976) and Hage et al (1990a,b). ^{13}C proton decoupled NMR of PDT showed the presence of 16 different carbon peaks, all the peaks had chemical shifts associated with multiply bonded C atoms. The observed spectrum was similar to that recorded by El Jammal et al (1986) for PDT in their study of the electrochemical reduction products of PDT. The ^{13}C spectrum was used as a further check for confirming the purity of the isolated PDT. PDT is insoluble in water, but soluble in chloroform, 1,2-dichloroethane, iso-amyl alcohol, ethanol and methanol.

2.2. *Reagents*

Wherever practical, reagent preparation was carried out in a class-100 laminar flow bench to reduce contamination. All plasticware and glassware were acid-cleaned prior to use.

Seawater/Riverwater

For this study seawater was collected from Taiaroa Head, New Zealand using ultraclean techniques (Croot and Hunter, 1996). The seawater (S= 34.4) was filtered through a Nuclepore 0.4μm filter and stored in acid-cleaned polyethylene bottles at 4 °C in the dark. Riverwater was collected from the Water of Leith, adajcent to the Chemistry Department, University of Otago.

Ultrapure water

Milli-Q (18 MΩ) ultrapure water was used throughtout this study - for convenience it is abbreviated to MQ.

Acids

Hydrochloric acid and acetic acid were prepared by double-distillation of analytical grade reagents in a quartz sub-boiling still (Kuehnen et al, 1972); in this study they will be identified by the prefix Q (i.e. Q-HCl).

Reducing agents

A 1% (w/v) solution of ascorbic acid (Analar grade) was freshly prepared each day, immediately prior to analysis. Ascorbic acid was used in this study as it was found to give a lower absorbance blank in organic solvents than hydroxylamine hydrochloride.

Tetrabromophenolphthalein ethyl ester (TBPE)

TBPE was obtained as the sodium salt from the Aldrich Chemical company, no attempt was made to purify this compund further.

pH Buffers

Control of pH was achieved by using acetate buffers (acetic acid/ammonium acetate) for pH values 4-7 and the buffer TRIS (tris(hydroxymethyl)aminomethane) (Aristar grade) for the pH range 7-9. The solution pH was measured by an Orion model EA 920 pH meter and electrodes. NBS buffers (phosphate pH = 7.413 at 25 °C, borate pH = 9.22 at 25 °C) were used to standardise the pH electrodes and determine the pH in experiments with MQ water. In seawater however these buffers do not yield reproducible results due to liquid junctions (Millero, 1986). TRIS seawater buffers were used to calibrate the electrode system and determine the pH_F (free hydrogen ion scale) of seawater solutions using the equations of Millero (1986). Using this method, pH could be determined to a precision of ± 0.02.

Pure solid ammonium acetate was prepared by bubbling ammonia gas through quartz distilled acetic acid. The solid was stored in a freezer to prevent loss of ammonia. The purified buffer was stored in an acid-cleaned Teflon bottle.

Organic Solvents

1,2-dichloroethane and ethanol were purified by sub-boiling distillation in a quartz still. Chloroform used for extractions was doubly-distilled (normal distillation) in a Pyrex apparatus and extracted 4 times with 20% ultrapure HCl in a Teflon separating funnel. Clean chloroform was stored in a acid-cleaned brown-glass volumetric dispenser.

2.3. *Reccomended Procedures*

2.3.1 Proposed Method for Analysis of Total Iron in Seawater using PDT

The following method was developed for the analysis of total iron in seawater using PDT with solvent extraction into chloroform.

1: A 200 g aliquot of acidified seawater (1 mL Q-HCl per litre of sample) is transferred to a 250 mL Teflon separatory funnel and buffered to pH 4.5 with a ammonium acetate/acetic acid buffer.

2: 1 mL of 1% (w/v) ascorbic acid (freshly prepared) is added, along with 1 mL of 5 mmol/kg PDT in Q-ethanol.

3: 6 mL of chloroform is added and the contents of the Teflon funnel shaken vigorously for at least 3 minutes (for complete extraction).

4: After allowing 5 minutes for the phases to settle, the chloroform layer is drained into a clean quartz glass vial with Teflon stopper.

5: The absorbance of the PDT-Fe(II) complex in chloroform is then measured at 555 nm using a 1 cm cuvette.

The concentration of iron in the seawater can then be determined by standard additions analysis. Alternatively the iron concentration can be obtained from direct comparison with iron standards and subtraction of a system blank (turbidity blank for each seawater sample plus a reagent blank, measured in MQ water).

2.3.2 Proposed Method for Analysis of Total Iron (or Fe(II)) in Seawater using PDT with TBPE.

The following method was developed for the analysis of iron in seawater using PDT with TBPE as the counter ion for extraction into 1,2-dichloroethane.

1: A 180 g aliquot of acidified seawater is transferred to a 250 mL Teflon separatory funnel. The sample is buffered with 5 mL of an acetate buffer (2M sodium acetate adjusted to pH 6.0 with acetic acid).

2: 1 mL of 1% (w/v) Ascorbic acid (prepared fresh) is added as a reducing agent. After standing for 5 minutes, 1 mL of 5 mmol/kg PDT (dissolved in Q-ethanol) is added along with 0.5 mL of 3 mmol/kg TBPE (dissolved in Q-ethanol).

3: The solution is then shaken with 6 mL of Q-1,2-dichloroethane for 5 minutes. After a further 5 minutes to allow separation of the phases, the 1,2-dichloroethane is drained into a clean glass vial.

4: The absorbance of the extract is then measured at 610 nm and 750 nm (background) against Q-1,2-dichloroethane as a reference.

The method is best applied with the technique of standard additions. If Fe(II) is to be measured, the addition of the reducing agent should be omitted from the above procedure.

2.3.3 Proposed Method for Analysis of Fe(II) in freshwaters using PDT adsorbed to C18 Sep-Pak cartridges.

The method used here was adapted for PDT from the Ferrozine method of King et al (1991).

1: The Sep-Pak cartridge (Waters - C18 Sep-Pak Classic) was precleaned by passing 20 mL of Q-methanol, followed by 10 mL of MQ-water through the cartridge using a peristaltic pump (silicon tubing, flow rate 30 mL/min).

2: 1 mL of 5 mmol/kg PDT (dissolved in Q-ethanol) was combined with 1 mL of MQ and passed through the Sep-Pak to load the cartridge. The Sep-Pak was then stored in an acid-cleaned black polyethylene container until required for use.

3: For Fe(II) measurement, water samples (40-400 mL) were drawn through the Sep-Pak using the peristaltic pump (typical flow rate 40 mL/min). No MQ-washing of the Sep-Pak was required after sample filtration.

4. The PDT-Fe(II) complex is eluted from the Sep-Pak with 4 mL of Q-methanol. The absorbance of the methanol effluent was measured at 555 nm (Peak absorbance of $Fe(PDT)_3$ complex). All absorbances are normalised to the baseline at 700nm to reduce errors in absorbance measurements due to baseline shifts.

In the present study the absorbance of the PDT-Fe(II) complex was measured using either a 1 cm, 5 cm (total volume 4 mL) or 10 cm pathlength cuvette on a Shimadzu model UV 240 spectrophotometer.

3. Results and Discussion

3.1 Direct analysis of Fe(II) and Total iron in water by PDT

The absorbances of the samples were measured using 1 cm cells on a Perkin Elmer spectrophotometer. The molar absorptivity varied little over the pH range 4-8 using Fe(III) with either ascorbic acid or hydroxylamine hydrochloride as the reducing agent. However recovery was reduced using Fe(II) at pH > 6, due to oxidation of Fe(II). There was no detectable difference using MQ water or filtered seawater on the molar absorptivity (Table 3). Blank values were higher for filtered seawater

than for MQ water, this was probably due to colloidal iron not removed by filtration and dissolved organic material present in the seawater. The Fe(II)-PDT complex was found to obey the Beer-Lambert law up to approximately 30 µmol/kg Fe(II). Using the protocol above a detection limit (3σ) for iron in seawater using a 1 cm cuvette of 10 nmol/kg is obtained. The sensitivity of the analysis may be enhanced by using longer pathlength cuvettes. However longer cuvettes normally require a larger volume of chloroform, which will decrease the preconcentration factor unless the seawater sample size is enlarged with subsequent longer shaking times. Copper (if present as Cu(I)) may interfere in this analysis (Schilt and Taylor, 1970), but at the concentrations typically found in open ocean and unpolluted coastal seawater (Total Cu: 1 - 5 nmol/kg: Sunda, 1989; < 1 nmol/kg for Cu(I), Moffett and Zika, 1983) the effects should be minimal. Dissolved organic compounds that complex iron may also interfere by limiting iron complexation by PDT. The visible absorption spectrum of the Fe(II)-PDT complex is displayed in figure 2. This method was found to be not suitable for direct measurement of Fe(II) in oxygenated seawater because of it's lack of sensitivity and pH requirements. However total iron concentrations in seawater may be measured using PDT with an appropriate reducing agent.

3.1.1 Measurement of Extraction Efficiency

The efficiency of extraction for this method was measured by using radiolabelled iron. Aliquots of ^{55}Fe (obtained from Amersham, halflife 2.7 years) standards of a known activity and known iron concentration were added to water samples in polyethylene separatory funnels. Buffer, reducing agent and PDT (dissolved in ethanol) were added to the sample, followed by chloroform. The amount of PDT used in this experiment was always in excess of the amount of iron added. The sample was shaken vigorously for 2 minutes and the phases allowed to settle. The chloroform was drawn into a beaker and an aliquot (500 µL) of the remaining aqueous phase was pipetted into a glass scintillation vial containing 10 mL of PCS^{TM} liquid scintillation cocktail (Amersham).

The ^{55}Fe activity of the vial was then counted using the Packard Tri-Carb 1500 Liquid Scintillation Counter at Portobello Marine Laboratory. Background counts of a vial containing 10 mL PCS^{TM} and 500 µL of water sample with no added ^{55}Fe were also measured. The activity of the ^{55}Fe in the water sample prior to chloroform addition was used as a calibration standard. The counting efficiency for ^{55}Fe in PCS^{TM} under these conditions was determined to be 56 ± 3 % by addition of ^{55}Fe standards of known activity. The overall extraction efficiency using PDT was found to be 96 ± 3 % (1σ , n = 6).

Attempts to measure the ^{55}Fe content of the chloroform were hampered due to quenching of the scintillant by the chloroform. Standards of ^{55}Fe in chloroform were prepared to produce a quenched standard curve (using the internal software of the Tri Carb 1500). Application of this standard curve to extracted chloroform samples however were subject to large errors and subsequently of little use.

3.2. *Solvent Extraction of Fe(II) by TBPE/PDT complex*

The formation and extraction of Fe(II)-PDT complexes with TBPE as a counter ion were investigated in MQ water at pH 6.0 (sodium acetate/acetic acid buffer) and at pH 7.0 (Tris buffer). Standard solutions of Fe(II) (Ferrous Ammonium Sulphate) were buffered and then allowed to react with PDT and TBPE. The complexed iron was then extracted into 1,2-dichloroethane and its visible wavelength (400 - 800 nm) absorbance spectrum obtained (figure 3)

Extraction of Fe(II) standard solutions from MQ water into 1,2-dichloroethane at pH 6.0 were found to be highly reproducible and complete as illustrated in figure 4. Problems were encountered with the loss of Fe(II) by oxidation when analysed at pH 7.0 or higher. Although the complexation of Fe(II) by PDT appeared to be very rapid, higher concentrations of Fe(II) had a lower extraction efficiency (probably due to oxidation) as evidenced by the decline in the relative molar absorptivity

(see Table 4). Molar absorptivities were calculated using observed absorbances and the expected iron concentration (typically 30 times preconcentration) in 1,2-dichloroethane (assuming 100% extraction). Attempts to lessen the effects of oxidation by increasing the concentration of PDT had little effect, except to increase the absorbance of the reagent blank.

Blanks and Limit of Detection

All absorbances were measured versus 1,2-dichloroethane (Quartz distilled). Using a 1 cm cell (as used throughout these experiments) a reagent blank absorbance of 0.030 ± 0.004 (1) was obtained, this is equivalent to an iron concentration of 160 ± 20 (1σ) nmol/kg (using a molar absorptivity of 190000 l cm^{-1} mol^{-1}). The detection limit (calculated by 3σ of the blank) for Fe(II) (using a preconcentration factor of 30) in water samples is then 2.0 nmol/kg. The precision of this analysis for 20 nmol/kg Fe(II) MQ water samples was found to be 6.3 % (n = 6).

To measure Fe(II) in natural waters it is often impractical to perform sample manipulation in a clean manner *in situ*, so samples have to be collected and then transported to the laboratory. In seawater samples this may lead to the oxidation of all Fe(II) before laboratory analysis can be performed. Samples for Fe(II) in seawater were obtained from Otago Harbour on June 30 , 1993 (samples collected around noon under bright sunshine) as part of a study into the concentration and reactivity of iron species in this harbour (Croot and Hunter, in preparation). To combat oxidation samples had PDT added immediately on collection, in an effort to complex Fe(II) rapidly. No Fe(II) was detectable in any of the seawater samples obtained by this method, nor was there any Fe(II) detectable in situ, using the Ferrozine Fe(II) trapping technique of King et al (1991). Background absorbances were increased for seawater samples compared to MQ water samples. This increased background was probably because of co-extraction of organic material from the seawater into the 1,2-dichloroethane. Standard additions of Fe(II) to seawater collected from Otago Harbour during this

study showed almost 100% recovery, indicating that there was no interference by organic material for the analysis of Fe(II) by PDT/TBPE.

Determination of Total Iron

The analysis of total iron in seawater by PDT-TBPE solvent extraction was investigated. Water samples were acidified (Q-HCl) and aliquots of standard iron (Ferric chloride) added. Prior to analysis the pH was adjusted using either acetate (pH 6.0) or Tris buffers (pH 7.0). Buffers were purified by repeated extraction with PDT and chloroform prior to use. PDT (dissolved in Q-ethanol), TBPE (dissolved in Q-ethanol) and a reducing agent (ascorbic acid or hydroxylamine hydrochloride) was added to the sample and allowed to react for several minutes before extracting (by shaking) the iron-PDT-TBPE complex with 1,2-dichloroethane. The visible wavelength (400 - 800 nm) absorbance spectrum was then recorded. Figure 5 shows the results of an experiment into the recovery of standard additions of iron from various waters (FSW - filtered, acidifed seawater from Otago harbour: CPSW - acidified seawater from the Tasman Sea).

The ion association complex appears greeny-blue in 1,2-dichloroethane, while TBPE and PDT (in the absence of Fe(II)) both appear yellow when extracted into 1,2-dichloroethane solutions (TBPE is an intense blue colour in ethanol). Typically the water samples would appear blue upon addition of TBPE and gradually a green colour would develop in the organic layer as the ion association complex was transferred to the 1,2-dichloroethane. However on many occasions this green colour turned to yellow upon elution of the organic solvent from the teflon separatory funnel. The cause of this colour loss was probably due to contamination by the rubber o-rings used in the teflon tap of the funnel. Replacement of the o-rings with similar polyethylene rings eliminated the occurrence of colour loss.

It was important to remove any water from the 1,2-dichloroethane as solutions often become opaque when placed in the light path of the spectrophotometer if water was present. Also on some occasions, samples changed colour when placed in the spectrophotometer, changing from their original

blue or green to yellow. When this colour change occurred, absorbance peaks would shift from 610 nm (ion association complex) to 555 nm (PDT-Fe complex), indicating the loss of the counter ion (TBPE anion). Removal from the light path would see the sample gradually recover its original colour over time (typically 10 - 20 minutes). The 1,2-dichloroethane was then further purified by sub-boiling distillation in a Quartz still, specially designed for the distillation of organic solvents. Sub-boiling distillation appeared to reduce the incidence of colour change or loss, possibly by removing some interfering organic substances, and reduce the overall iron blank.

Polyethylene vials were not suitable for this method as samples often became decolourised over time. Glass (quartz) vials with teflon stoppers were found acceptable, after acid cleaning and rinsing in MQ water to remove any trace of acid.

Experiments were conducted into determining the minimum shaking time for complete extraction of the ion pair into 1,2-dichloroethane. A shaking time of 3 minutes was found to be best for MQ water samples (Figure 6). Longer shaking times may be needed however for seawater samples, as slower iron complexation kinetics may result from interfering ions. The molar absorptivity of the complex was calculated from standard additions experiments on different water samples (Table 5 and Figure 5).

Blanks and Limits of Detection

Measurements were performed in an identical manner to that for Fe(II) determination. System blanks were obtained from extraction of MQ water and previously extracted seawater (Table 5 and figure 5).

The absorbance blank signal was converted into an equivalent iron signal using a molar absorptivity of 190000 l cm^{-1} mol^{-1}. Detection Limits were calculated assuming a 30 times preconcentration factor. The precision of analysis at the 25 nmol/kg level was calculated at 8.9 % from

replicate (n = 4) analysis. Most of this blank signal appeared to be absorbance from the free TBPE ion or an impurity associated with it.

Application to Seawater samples

This method was applied to samples collected from Otago harbour as part of a study into the reactivity of iron species in the harbour (Croot and Hunter, in preparation). The results for total (acidified and reducing agent added) and dissolved (0.4 μm Nuclepore filter) iron as measured by the PDT-TBPE solvent extraction method agreed within experimental error with those obtained directly by using Ferrozine (method of Gibbs, 1976) on replicate samples (data not shown). Two coastal seawater samples were taken from the Otago Shelf (45 57.24 S, 170 59.43 E) using the rosette sampler onboard the *R.V. Munida*, in June 1992. Samples were obtained at 100 and 300 metres depth. Iron concentrations were corrected for reagent blanks (n = 4) and analysed in triplicate (Table 6). Total iron concentrations measured at a nearby station (using Graphite Furnace atomic absorption spectrophotometry, after dithiocarbamate extraction) in May and November 1993 were found to range between 5 - 14 nmol/kg throughout the upper 100 m of the water column (Croot and Hunter, 1996).

The measured sample blank was due primarily to the absorbance of the TBPE anion and small amounts of iron contamination in the reagents (even after repeated extractions). This high blank value must be considered a major disadvantage of this method for determining typical open ocean iron concentrations.

3.3. *Pre-concentration of Fe(II) on PDT loaded C18 columns*

3.3.1 Seawater

Fe(II) standard solutions in filtered seawater, buffered to pH 6.8 with Pipes, were passed through a PDT loaded Sep-Pak. The Fe(II) trapped as $Fe(PDT)_3$ on the cartridge was eluted with Q-methanol. The absorbance at 555 nm of the effluent was measured and converted to an equivalent

Fe(II) concentration using a molar absorptivity of 24500 ± 300 (n=6) for the $Fe(PDT)_3$ complex measured in Q-methanol. Recoveries of Fe(II) using this method were poor and not reproducible. The recovery of Fe(II) appeared to be dependent on the volume of seawater passed through it. PDT was rapidly removed from the Sep-Pak by the seawater.

Trials with Ferrozine adsorbed to the C18 cartridges as described in King et al (1991), showed good recoveries of Fe(II) at low flow rates (90% or better at flow rates < 50 mL/min) and at low sample volumes (< 500 mL seawater). This was is in good agreement with the early work by King et al (1991). We also observed some potential artifacts in measuring Fe(II), on several ocassions elevated iron levels in sample blanks and stored samples, when the Ferrozine loaded Sep-Paks were exposed to prolonged direct sunlight (possible photoreduction of column bound Fe(III) and also when exposed to S^{2-} fumes (reduction of Fe(III) in Sep-Pak).

Freshwater

However a small scale study of using PDT loaded Sep-Paks to trap Fe(II) in aqueous solutions of low-ionic strength (MQ-water), found high recoveries and good reproducibility. The results of this study are presented in figure 7. The recovery of Fe(II) decreased with increasing flow rate (Figure 7). This is probably due to the shorter contact time at high flow rates for the Fe(II) with the complexing agent PDT (also observed for Ferrozine trapping). The reagent absorbance blank was found to be 0.007 ± 0.001 (n = 3) using a 1 cm cuvette. The MQ-water is assumed to have a negligible contribution to the absorbance blank. Using a preconcentration factor of 50 and a 1 cm cuvette a detection limit of 1.6 nmol/kg is obtained (obviously a higher value would be obtained for natural samples). Although the possibility has not been extensively explored, this method should be suitable for the analysis of Fe(II) in lakes and rivers.

In an attempt to further increase the sensitivity of this method, TBPE was added to the methanol effluent and extracting the $[Fe(PDT)_3](TBPE)_2$ ion association complex into the Q-1,2-dichloroethane was tentatively explored. Buffered MQ water was required to help the extraction of the complex from the methanol to the 1,2-dichloroethane. As methanol by itself is soluble in 1,2-dichloroethane, mixing leads to a higher polarity solution, in which the complex does not form. The complex was able to be extracted quantatively into the 1,2-dichloroethane from the methanol, but the amount of water required to accomplish this largely diminshed any sensitivity gains.

Alternatively the methanol solution can be analysed for iron by Graphite Furnace Atomic Absorption Spectrophotometry. Using this technique it was found that lower concentrations of iron could be measured than by using the absorption spectra of the Fe(II)-PDT complex, however this method measured both Fe(II)-PDT and Fe(III) (probably colloidal) that adsorbed to the Sep-Pak.

4. Conclusions

Several methods for the determination of Fe(II) in seawater by colorimetric analysis have been reported. In general methods involving solvent extraction have limited appeal to the in situ determination of Fe(II) in natural waters, due to the degree of sample manipulation required. Solvent extraction methods appear to be best suited for the determination of total iron in seawater, in particular the combination of PDT and TBPE showed great promise as a relatively uncomplicated technique to measure low concentrations of total iron in seawater.

Fe(II) trapping by FZ loaded Sep-Paks (King *et al*, 1991) was found to be the most reliable method for the in-situ determination of Fe(II) in oxygenated seawater, however some potential artifacts were discovered. Attempts to use the ligand PDT in an analogous fashion to FZ were hindered in seawater by the loss of PDT from the Sep-Pak. However in low ionic strength solutions such as MQ water, PDT was retained on the Sep-Pak and appeared to quantitatively retain Fe(II), this method may provide the potential for measuring low level concentrations of Fe(II) in freshwater solutions.

Literature cited:

Blau, F. (1898). Monatshefte fuer Chemie. 19: 647

Case, F.H. (1965). The Preparation of Hydrazidines and as-Triazines Related to Substituted 2-Cyanopyridines. Journal of Organic Chemistry. 30: 931-933.

Case, F.H. (1968). The Preparation of Diazine Derivatives Containing the FerroinGroup(1). Journal of Heterocyclic Chemistry. 5: 223-226.

Case, F.H. (1970). The Preparation of 1,2,4-Triazines and 1,2,4-Triazolines from Substituted Carboxamide Hydrazones. Journal of Heterocyclic Chemistry. 7: 1001-1005.

Case, F.H. (1971). The Preparation of 2,4 and 2,6-Bis-Triazinyl and Triazolinyl Derivatives of Pyridine. Journal of Heterocyclic Chemistry. 8: 1043-1046.

Chriswell, C.D. and Schilt, A.A. (1974). Spectrophotometric determination of iron in acids and acidic solutions by an extraction-formation reaction involving 2-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine as the chromogenic-extraction reagent. Analytical Chemistry. 46: 992-996.

Croot, P.L. and Hunter, K.A. (1996). "Trace Metal and Nutrient Distributions across the Continental Shelf near Otago Peninsula, New Zealand". (submitted to Marine Chemistry).

Ditzler, M.A., Pierre-Jacques, H. and Harrington S.A. (1986). Immobilization as a Mechanism for improving the inherent selectivity of photometric reagents. Analytical Chemistry. 58: 195-200.

El Jammal, A., Graf, E. and Gross, M. (1986). Reduction Electrochmique de la (pyridyle-2)-3-diphenyle-5,6-triazine-1,2,4. Electrochimica Acta. 31: 1457-1465.

Finden, D.A.S., Tipping, E., Jaworski, G.H.M. and Reynolds, C.S. (1984). Light-induced reduction of natural iron(III) oxide and its relevance to phytoplankton. Nature. 309: 783-784.

Geldard, J.F. and Lions, F. (1965).The organic chemistry of a new weak filed tridentate chelating agent. 3,5-di(2-pyridyl)-1,2,4-triazole. Journal of Organic Chemistry. 30:318-319.

Gibbs, C.R. (1976). Characterization and Application of FerroZine Iron Reagent as a Ferrous Iron Indicator. Analytical Chemistry. 48: 1197-1201.

Hage, R., Haasnoot, J.G. and Reedijk, J. (1990a). Structure and properties of new pyridyltriazine low-spin Fe(II) complexes. Inorganica Chimica Acta. 172: 19-23.

Hage, R., van Diemen, J.H., Ehlrich, G., Haasnoot, J.G., Stufkens, D.J. Snoeck, T.L., Vos J.G., and Reedijk, J. (1990b). Ruthenium compounds containing pyridyltriazines with low-lying π^* orbitals. Inorganic Chemistry. 29: 988-993.

Hutchinson, D.J. and Schilt, A.A. (1983). Investigation of the adsorption of ferroin-type ligands and metal chelates on activated carbons for application in reagent purification. Analytica Chimica Acta. 154: 159-171.

King, D.W., Lin, J. and Kester, D.R. (1991). Spectophotometric determination of iron(II) in seawater at nanomolar concentrations. Analytica Chimica Acta. 247: 125-132.

Knizek, M. and Musilova, M. (1968). Improvement of iron determination by use of ferroin-organic dye ion-association complexes. Talanta. 15: 479-481.

Kotsuji, K. Kameyama, Y. Arikawa, M. and Hayash, S. (1977). Spectrophotometric determination of iron using solvent sublation method. Bunseki Kagaku. 26: 475-479.

Kuehnen, E.C., Alvarez, R., Paulson, P.J. and Murphy, T.J. (1972). Production and analysis of special high purity acids purified by sub-boiling distillation. Analytical Chemistry. 44: 2050-2056.

Lundgren, J.L. and Schilt, A.A. (1977). Analytical studies and applications of ferroin type chromogens immobilized by adsorption on a styrene-divinylbenzene copolymer. Analytical Chemistry. 49: 974-980.

Millero, F.J. (1986). The pH of estuarine waters. Limnology and Oceanography. 31: 839-847.

Millero, F.J., Sotolongo, S. and Izaguirre, M. (1987). The oxidation kinetics of Fe(II) in seawater. Geochimica et Cosmochimica Acta. 51: 793-801.

Millero, F.J. (1989). Effect of ionic interactions on the oxidation of Fe(II) and Cu(I) in natural waters. Marine Chemistry. 28: 1-18.

Moffett, J.W. and Zika, R.G. (1983). Oxidation kinetics of Cu(I) in seawater: Implications for its existence in the marine environment. Marine Chemistry. 13: 239-251.

Moffett, J.W. and Zika, R.G. (1987). Reaction kinetics of hydrogen peroxide with copper and iron in seawater. Environmental Science and Technology. 21: 804-810.

Schilt, A.A. (1966). Some New Chromogens for Iron, Cobalt and Copper. Substituted Hydrazidines and 1,2,4-triazines containing the Ferroin group. Talanta. 13: 895-902.

Schilt, A.A. and Kluge, K.R. (1968). New chromogens of the ferroin type - I. Substituted triazines related to 6-cyano-2,2'-bipyridine. Talanta. 15: 475-478.

Schilt, A.A. and Dunbar, W.E. (1969). New chromogens of the ferroin type - V. Pyridylpyrimidines, bidiazines and other substituted derivatives of diazines. Talanta. 16: 519-522.

Schilt, A.A. and Taylor, P.J.. (1970). Simultaneous determination of Iron and Copper by a new spectrophotometric method. Analytical Chemistry. 42: 220-224.

Schilt, A.A., Dunbar, W.E.,Gandrud, B.W. and Warren, S.E. (1970). New chromogens of the ferroin type - VI. Some derivatives of 1- and 3- Cyanoisoquinoline and substituted 2-cyanopyridines. Talanta. 17: 649-653.

Schilt, A.A., Chriswell, C.D. and Tsailing, A.Y. (1974). New chromogens of the ferroin type - VII. Some 3-substituted 1,2,4-triazines, 3,5-disubstituted-1,2,4-triazolines and triazolines , and 2,4- and 2,6-bis triazinyl nad triazolinyl substituted pyridines. Talanta. 21: 831-836.

Schilt, A.A., Tsailing, A.Y., Wu, J.F. and Nitzki, D.M. (1977). New chromogens of the ferroin type - VIII. Some di- and trisubstituted 1,2,4-triazines, 3-substituted-9H-indeno[1,2-e]-1,2,4-triazin-9-ones, and di- and trisubstituted 1,2,4-triazolines. Talanta. 24: 685-687.

Schilt, A.A. and Case F.H. (1980). New chromogens of the ferroin type - X. Synthesis and Chelation properties of some 2-Thiazolyl and 2-Pyrimidinylhydrazones. Talanta. 27: 55-58.

Shapiro, J.(1966). On the measurement of ferrous iron in natural waters. Limnology and Oceanography. 11: 293-298.

Stookey, L.L. (1970). Ferrozine - A New Spectrophotometric Reagent for Iron. Analytical Chemistry. 42: 779-781.

Sunda, W.G. (1989). Trace Metal Interactions with Marine Phytoplankton. Biological Oceanography. 6:411-442.

Tsurubou, S. and Sakai, T. (1984). High-sensitivity extraction-spectrophotometric determination of iron with 2-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine and tetrabromophenolphthalein ethyl ester. Analyst. 109:1397-1399.

Wells, M.L., Mayer, L.M., Donard, O.F.X., de Souza Sierra, M.M. and Ackelson, S.G. (1991). The photolysis of colloidal iron in the oceans. Nature. 353: 248-250.

Table 1: Elemental Analysis - PDT synthesis

Sample	Carbon %	Hydrogen %	Nitrogen %
PDT	**77.58 (77.40)**	**4.47 (4.55)**	**18.12 (18.06)**
Amidrazone	**52.64 (52.92)**	**5.63 (5.92)**	**40.85 (41.16)**
Tetrazine	**60.40 (60.49)**	**4.02 (4.23)**	**35.28 (35.28)**

( ) denoted expected value

PDT: 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine $C_{20}H_{14}N_4$ M.W. = 310.352

Amidrazone: 2-pyridyl-hydrazidine $C_6H_8N_4$ M.W. = 136.164

Tetrazine:3,6-di(2-pyridyl)-1,2-dihydro-1,2,4,5-tetrazine $C_{12}H_{10}N_6$ M.W. = 238.26

Table 2: ^{1}H NMR Analysis - PDT synthesis

Proton	ppm (TMS)	
H3 Pyridyl	**8.7**	**doublet**
H4 Pyridyl	**8.1**	**triplet**
H5 Pyridyl	**7.5**	**triplet**
H6 Pyridyl	**8.9**	**doublet**
R (phenyl group)	**7.2-7.6**	**multiplet**

Table 3: Calculated Molar Absorptivity for Fe(II)$(PDT)_3$ complex

Solvent	Molar absorptivity at 555nm ($l\ cm^{-1}\ mol^{-1}$)
ethanol/water	24270 ± 165 (n=25)
chloroform	24300 ± 400 (n=16)

Table 4: Calculated Molar Absorptivities of TBPE-PDT-Fe(II) complex

[Fe(II)] range (1,2-dichloroethane)	Molar Absorptivity ($l\ cm^{-1}\ mol^{-1}$)	Buffer
0 - 0.6 µmol/kg	191000 ± 1100	Acetate (n = 4)
0 - 1.5 µmol/kg	144700 ± 4100	Acetate (n = 10)
0 - 0.6 µmol/kg	187600 ± 2200	Tris (n = 4)
0 - 1.5 µmol/kg	142000 ± 6500	Tris (n = 10)

Note: Both buffers at pH 7.0

Table 5: Calculated Molar Absorptivity of TBPE-PDT-Fe(II) with reducing agent

[Fe(II)] (1,2-dichloroethane)	Molar Absorptivity (l cm^{-1} mol^{-1})	Sample Matrix
0 - 1.75 µmol/kg	191600 ± 2300	MQ (n=12)
0 - 1.34 µmol/kg	189800 ± 8300	MQ (n=10)
0 - 1.34 µmol/kg	192900 ± 8000	FSW (n=10)
0 - 1.34 µmol/kg	195400 ± 14100	CPSW (n=10)

Buffer: 2 M Sodium Acetate adjusted to pH 6.0 with Q-acetic acid.

MQ - MQ water

FSW - Filtered (0.4 µmol/kg) Seawater, collected from Taiaroa Head.

CPSW - Unfiltered acidified (aged) seawater, collected from Tasman Sea.

Table 6: Total Iron Otago Shelf - TBPE-PDT-1,2-dichloroethane extraction

Depth (m)	[Fe] nmol/kg
100	6.0 ± 1.9
300	8.3 ± 0.3
Blank (n = 4)	8.1 ± 0.5

Figure Legends

Figure 1. Chemical structure of PDT: 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine.

Figure 2. Visible absorbance spectrum of the $[Fe^{II}(PDT)_3]$ complex.

Figure 3. Visible absorbance spectrum of the $[Fe^{II}(PDT)_3(TBPE)_2]$ complex.

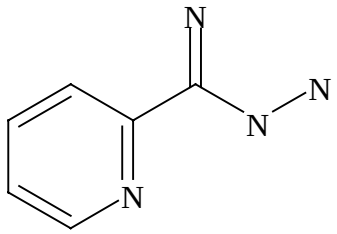
Figure 4. Plot of absorbance at 610 nm of $[Fe^{II}(PDT)_3(TBPE)_2]$ complex against Fe(II) concentration.

Figure 5. Absorbance at 610 nm of $[Fe^{II}(PDT)_3(TBPE)_2]$ against total iron concentration for three water sources.

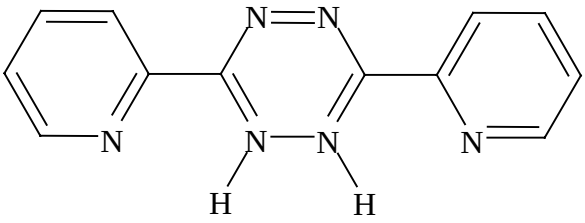
Figure 6. Percentage extraction of $[Fe^{II}(PDT)_3(TBPE)_2]$ into 1,2-dichloroethane as a function of shaking time.

Figure 7. Percentage recovery of Fe(II) as a function of the MQ water flow rate for Fe(II) trapping by a PDT loaded Sep-Pak.

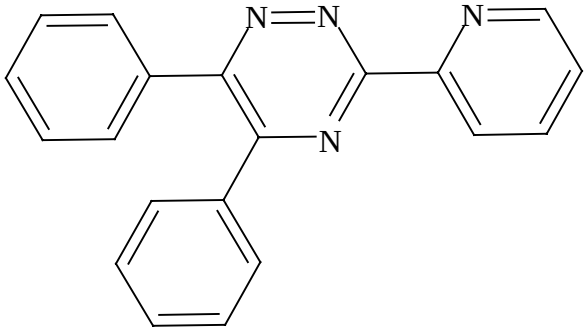
Figure 1:



2-pyridyl-hydrazidine

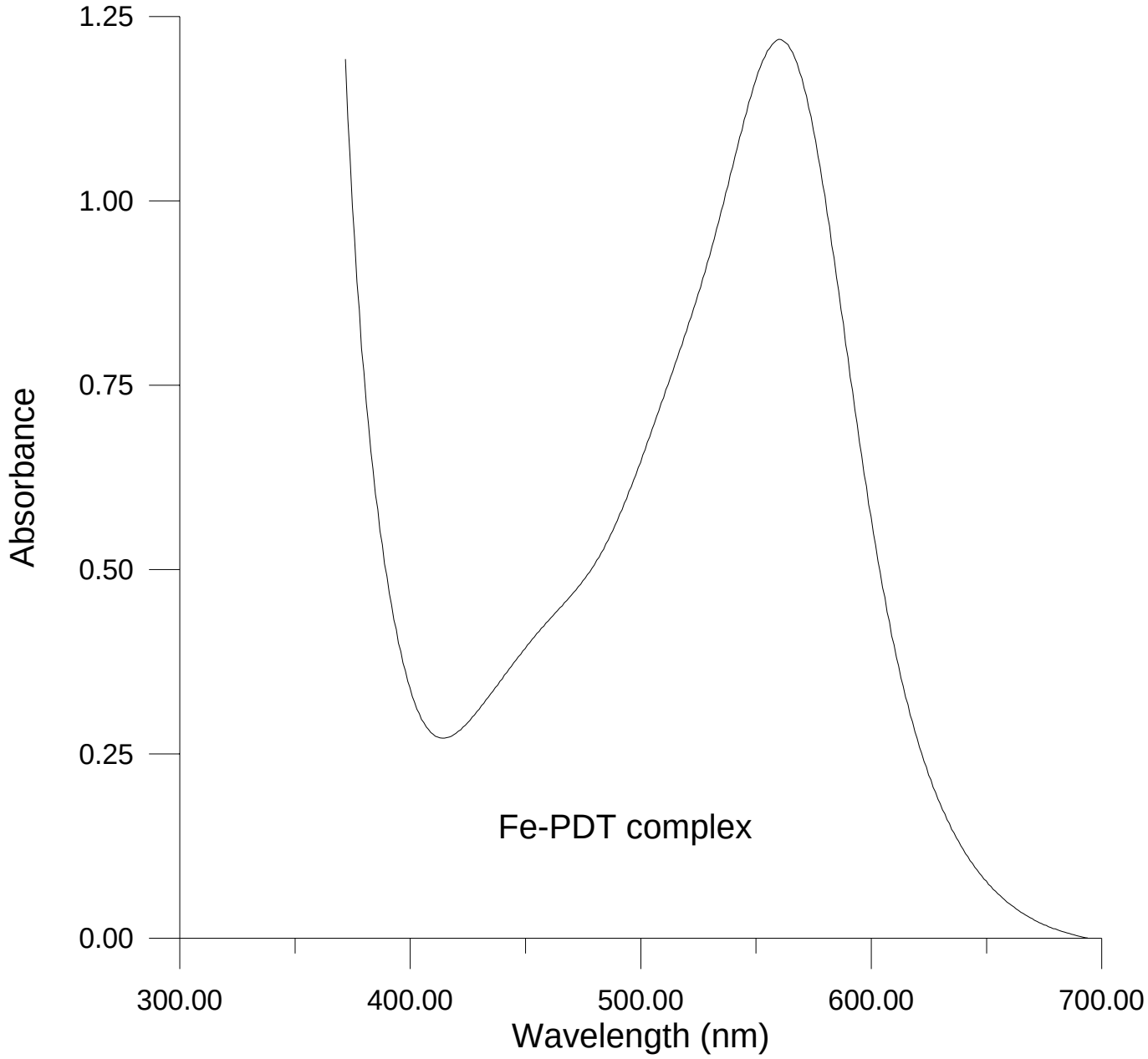


3,6-di(2-pyridyl)-1,2-dihydro-1,2,4,5-tetrazine



3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine

Figure 2: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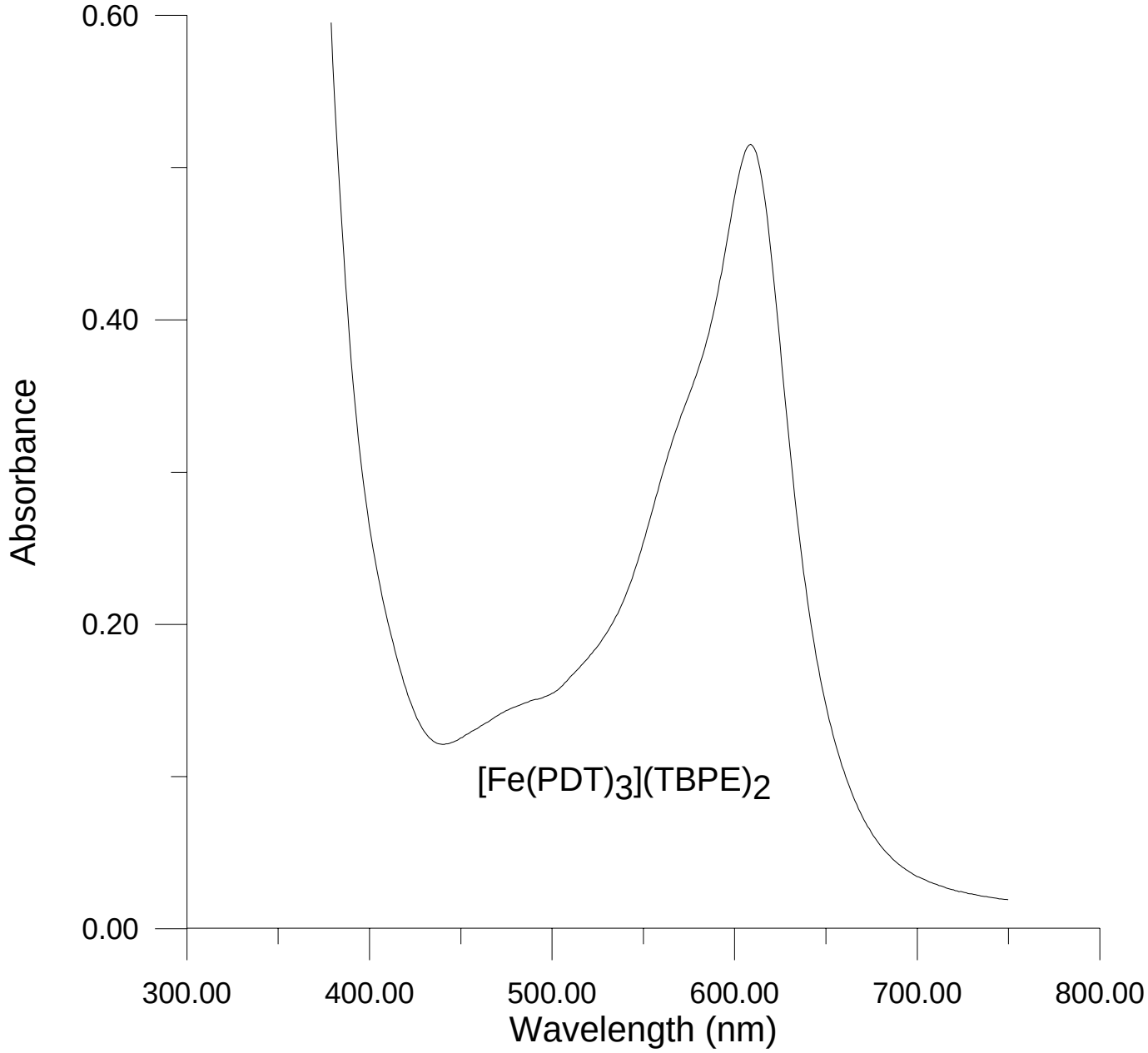
1.00
0.75
0.50
0.25
0.00
Absorbance
Fe-PDT complex
300.00
400.00
500.00
600.00
700.00
Wavelength (nm)

Figure 3: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0.40
0.20
0.00
Absorbance
[Fe(PDT)3](TBPE)2
300.00
400.00
500.00
600.00
700.00
800.00
Wavelength (nm)

Figure 4:

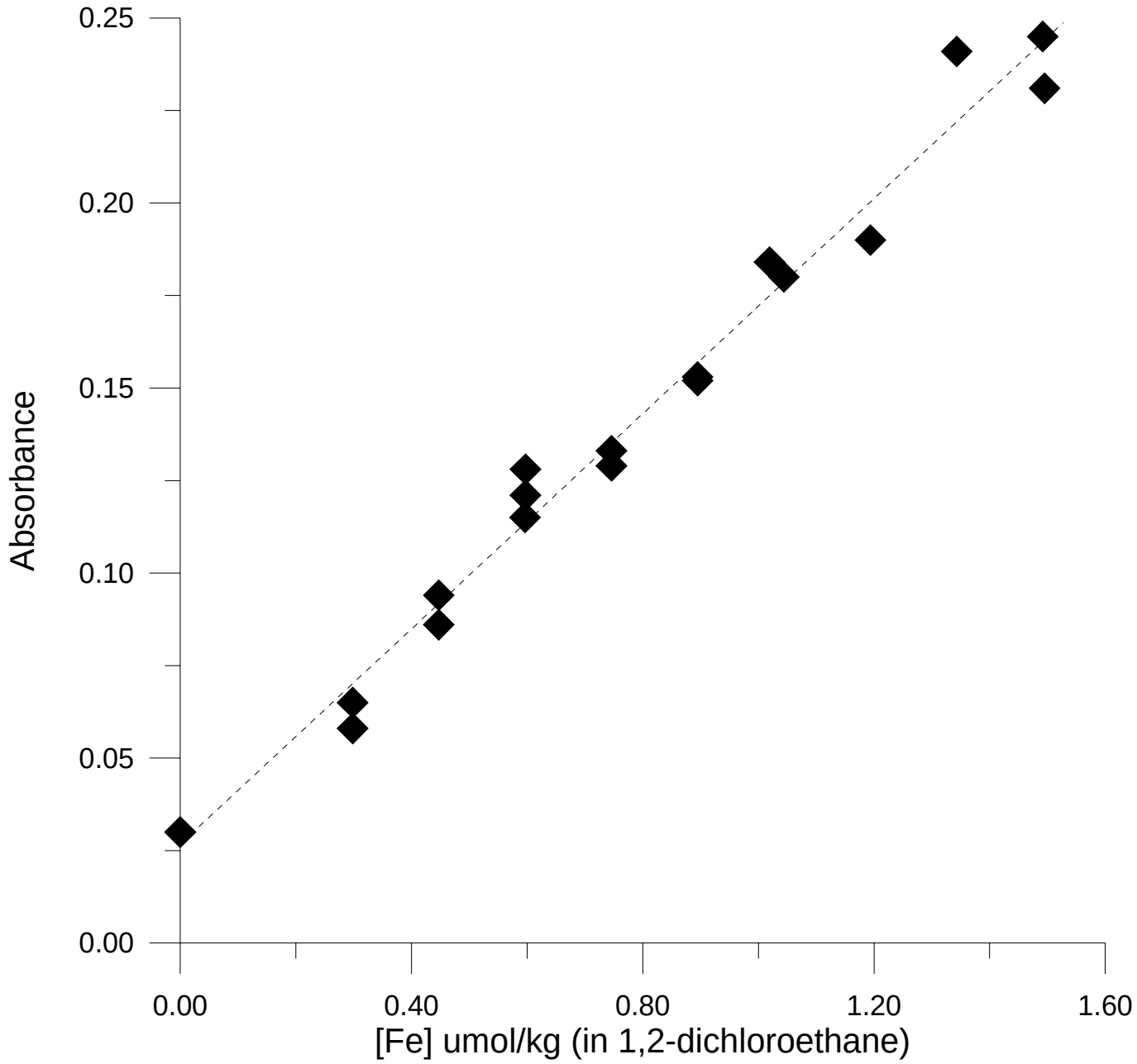
0.25
0.20
0.15
0.10
0.05
0.00
Absorbance
0.00
0.40
0.80
1.20
1.60
[Fe] umol/kg (in 1,2-dichloroethane)

Figure 5:

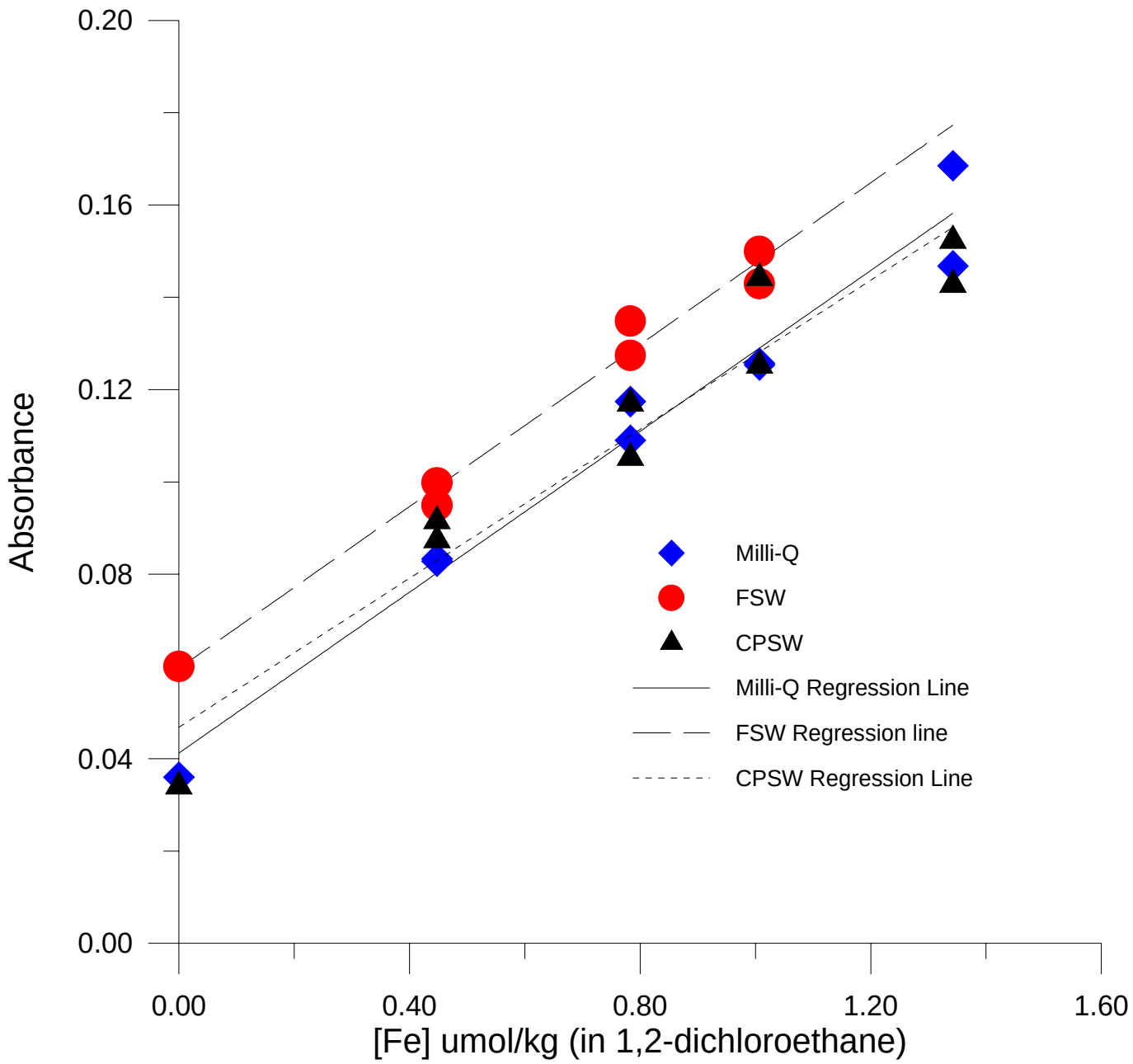
0.20
0.16
0.12
0.08
0.04
0.00
Absorbance
0.00
0.40
0.80
1.20
1.60
[Fe] umol/kg (in 1,2-dichloroethane)
Milli-Q
FSW
CPSW
Milli-Q Regression Line
FSW Regression line
CPSW Regression Line

Figure 6:

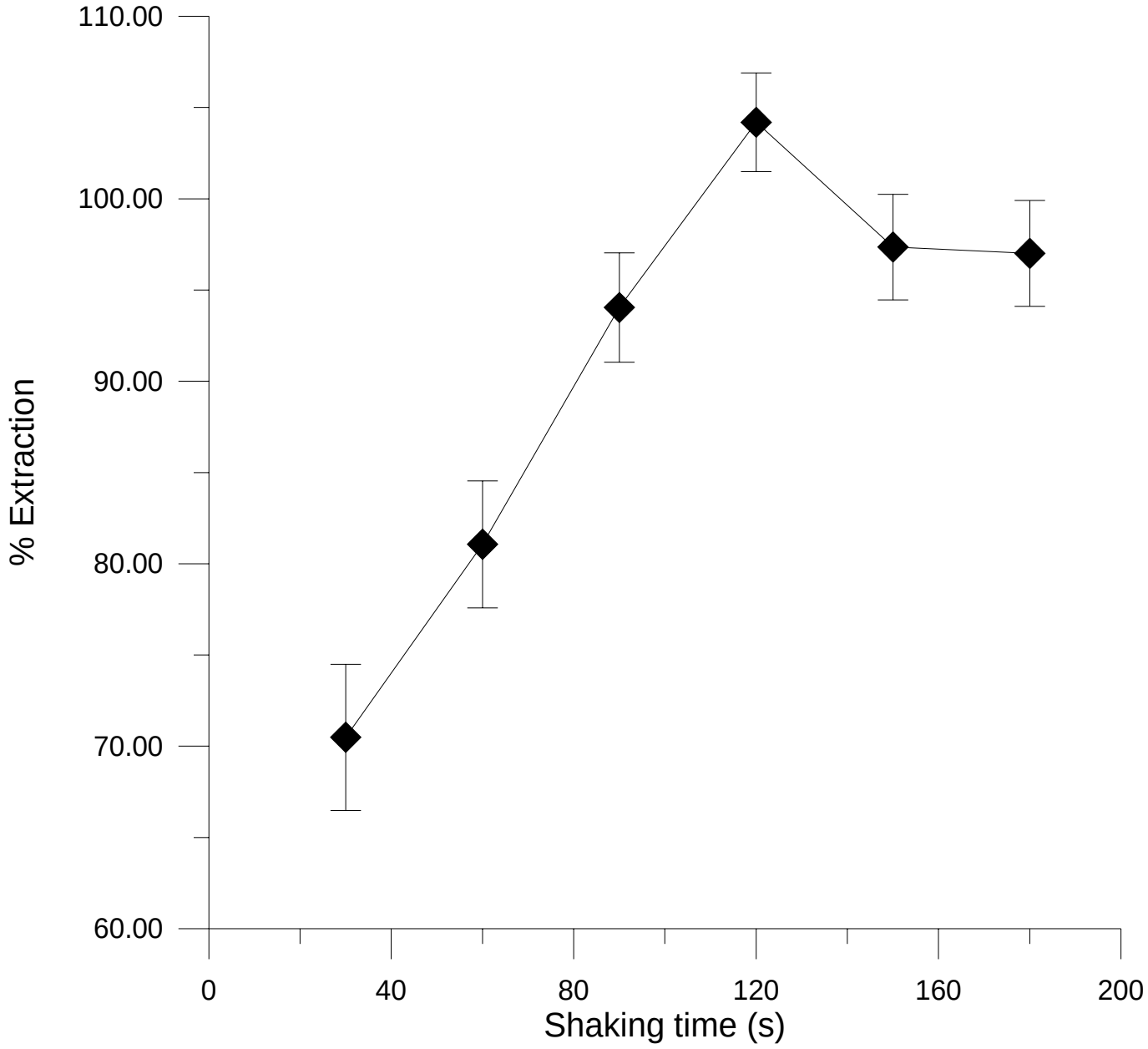
110.00
100.00
90.00
80.00
70.00
60.00
% Extraction
0
40
80
120
160
200
Shaking time (s)

Figure 7: