Interferences by Natural Organic Matter in the Determination of Iron in Natural Waters by Flow Injection Analysis with 1,10-Phenanthroline

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Abstract

A comparison of three simple reagent injection Flow Injection (FI) manifolds are reported for detection of Iron(Fe) in freshwater systems involving a simple reaction sequence of Fe(II) with 1,10-phenanthroline and photometric detection at 510 nm. The results of this work show that there is a requirement for an adequate amount of acetate buffer to be added in order to avoid interferences by Natural Organic Matter (NOM). Two of the manifolds designed for speciation of Fe encountered problems with detection of field samples believed to be as a consequence of complexation with NOM and to avoid refractive index effects (RIE). Subsequently, a FI manifold was proposed that uses a combination of matrix matching and reagent injection to overcome the effects of complexation with NOM. This approach was capable for detection of only total Fe (TFe) and gave linear responses in the range of 0 - 0.5 mgL^{-1} Fe ($r^2 = 0.9994$), with a detection limit of 0.01 mgL^{-1} Fe and a sample throughput of 144 injections hr^{-1}.

Keywords: Iron, 1,10 phenanthroline, reagent injection, natural organic matter, flow analysis

1. Introduction

Fe in the form of oxides and/or hydroxide coatings on sediments plays an important role in the transport of phosphorus and heavy metals in freshwater and estuarine systems by binding these constituents to colloidal material which is subsequently transported by fluvial processes[1]. Knowledge of the chemical dynamics of Fe in natural waters is therefore crucial for understanding the cycling of Fe and associated elements[2].

A number of methods for measurement of Fe using FIA and spectrophotometric detection have been reported[3-9]. Selected examples of some of the approaches taken for determination of Fe are shown in Table 1.

Table 1 – Spectrophotometric Detection of Fe by FIA

Species Measured	Basis of Method	Detection Method/ Analytical Performance	Notes	Reference
Total Fe (TFe)	Catalytic effect of Fe on the oxidation of N,N-dimethyl-p-phenylelediamine dihydrochloride by hydrogen peroxide	Spectrophotometric at 514 nm LoD – 1.4 ng Fe L^{-1} % rsd – 2.5 (n = 6)	Involves in-line ion-exchange pre-concentration column for determination of Fe in seawater	Measures et al.[3]
Fe(III)-vanadium	Reduction of Fe(III) to Fe(II) by vanadium followed by formation of Fe(II)-phenanthroline complex	Spectrophotometric at 510 nm %rsd – 0.54 sample throughput 60 h^{-1} LoD – 0.06 mg Fe L^{-1}	Simultaneous determinations based on successive merging of reagents, to initiate oxidation/reduction reactions	Teshima et al.[10]
TFe	Reduction of Fe(III) to Fe(II) by ascorbic acid and formation of Fe(II)-phenanthroline complex	Spectrophotometric at 512 nm %rsd - <1 sample throughput 180 h^{-1} LoD – 0.02 mg Fe L^{-1}	Determination of TFe in natural waters and plant digests	Mortatti et al.[11]
Fe(II) and TFe	Formation of Fe(II)-phenanthroline complex. Reduction of Fe(III) with ascorbic acid.	Spectrophotometric at 520 nm %rsd – 0.6 & 1.2 Sample throughput 90 injections h^{-1} LoD – 0.1 and 0.2 mg FeL^{-1}	Based on sandwich injection (chasing zone) technique	Alonso et al.[4]

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Methods described in Table 1 are based on conventional (sample injection) FIA, and are less suitable forcontinuous field monitoring because of the higher rate of reagent consumption. In general, reverse FIA methods are more suitable for field based or on-line monitoring where there is an abundance of sample, and a need to minimise reagent usage.

This paper describes an evaluation of three simple reagent injection manifolds for the determination of Fe(II), Fe(III) and TFe, based on the reaction of Fe(II) with 1,10 phenanthroline and photometric detection of the complex formed at 510 nm and examines the interference of NOM in the detection of Fe using this chemistry. It was found that the interference by NOM necessitated the design and development of two other manifolds to that of the initial FI manifold proposed for Fe detection, in order to obtain adequate recoveries and detection of Fe in field samples.

2. Experimental

2.1 Reagents

All solutions were prepared using analytical grade (AR) reagents, and ultra pure water (UPW) obtained from a water purification system (Continental Water Systems Corp, Modulab® Analytical). All reagents used for these methods were prepared according to references [11][11] and [12][12].

2.2 Standard Reference Material

A standard reference material (SRM) (Analytical Product Group, Inc.) was used for validation. SRM's were provided in a concentrated form, and were accurately diluted in a 1:100 ratio with UPW and 0.5 % (v/v) HNO_3. The certified value of the SRM was 333.02 ± 1.50 µg Fe/L (2σ) in the diluted form.

2.3 Refractive Index Measurements

Refractive index (RI) measurements were performed using an Abbé refractometer. The RI for the mixture of 1% (w/v) ascorbic acid, 0.25 % (w/v) 1, 10-phenathroline and 0.2 M acetate buffer reagent was found to be 1.3350. This injected zone was matched to a carrier solution of 0.5 M acetate buffer with a corresponding RI of 1.3351.

2.4 Sample Collection

Samples were taken from freshwater sites at Dobson's Creek, Sassafras Creek, Scotchman's Creek, and Yarra Hill Cl, Templestowe, Victoria, Australia. Samples were taken at the surface of each site and were filtered on site using a 0.45 micron filter and stored in polypropylene bottles, and were refrigerated pending further analysis.

2.5 Instrumentation

The FIA system used to analyse the Fe standards was constructed in-house. This system comprised of a motor driven Rheodyne, six port rotary injection valve (5041), with two peristaltic pumps (Istamatec CA5E). PTFE tubing of 0.5 mm id was used for all flow lines. An ABI Spectroflow 757 UV-Vis detector with a tapered longitudinal flow cell of 6 mm path length (11 µL internal volume) was used to measure the absorbance of the Fe(II)-phenanthroline complex at 510 nm. Injection valve, pump timing and data acquisition and processing were controlled using FCS software (A-Chem Technologies, Melbourne).

3. Results and Discussion

3.1 rFIA Manifold for Determination of Fe(III)

To perform detection of Fe(III), online reduction of Fe(III) to Fe(II) was achieved by injection of the ascorbic acid reductant. The colorimetric 1,10 phenanthroline reagent was continually merged to give a resultant peak for Fe(III). The optimised manifold gave a linear relationship between Fe(III) concentration and peak response for Fe concentration between 0.05 – 0.5 mg Fe/L (r^2 = 0.9989, % rsd 2.5%; n = 10; 0.5 mg Fe/L), to produce a limit of detection (LoD) of 0.01 mg Fe/L and a sample throughput of 180 injections hr^{-1}.

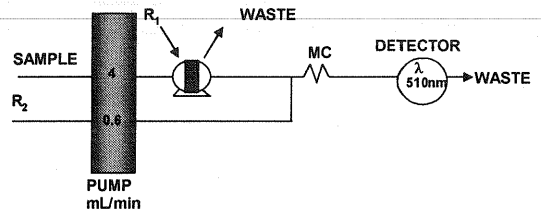

Figure 1 - rFIA manifold used for Fe(III) determination. The reductant is injected into the sample line and merged with the reagents and combined in the mixing coils. R_1= 1% w/v Ascorbic acid, R_2= 0.25 % w/v 1,10-Phenanthroline mixed with Acetate Buffer (2M, pH-3.7) Injection volume of 15 µL and MC = 30 cm knitted mixing coil

Spike recovery tests were used to investigate any matrix interferences, and also the efficiency of ascorbic acid reduction. Three freshwater samples from Dobson's Creek (containing 15 mg/L DOC) were spiked with 0.3 mg Fe(III)/L and recoveries of close to 85 % were achieved for two of the samples. However, sample 1 resulted in a recovery of only 65 % suggesting that a major interference is occurring (see Figure 2).

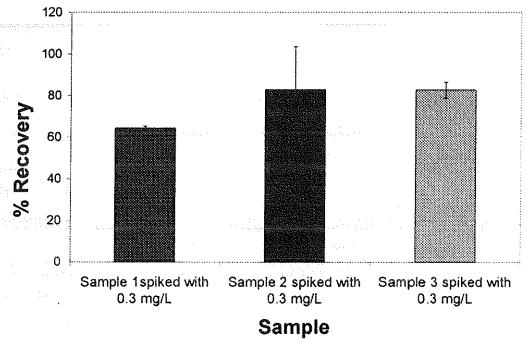

Figure 2 – Percentage recovery from three different samples collected Dobson Creek. These samples were spiked with 0.3 mg Fe(III) /L. Error bars of ± $2\sigma_{n-1}$ are shown.

These interferences are most probably due to complexation of Fe with NOM[11], which in natural waters has the role of controlling Fe behaviour by regulating the adsorption of the metal onto particulate and dissolved organic matter[13,14]. This behaviour is not peculiar for reverse FIA having been reported in sample injection flow analysis where waters containing large excess of organic constituents resulted in heavily suppressed signals for direct determination of TFe (40 – 80 % decrease in signal response)[11]. Other possible interfering substances include strong oxidizing agents, cyanide, nitrite, chromium and zinc concentrations exceeding 10 times or in excess of 5mg/L that of Fe. However, such high concentrations of these substances are unlikely in natural water samples and the reductant used in the reaction sequence eliminates errors caused by excessive concentrations of strong oxidizing agents. In addition the use of a larger excess of phenanthroline could be expected to eliminate the effects of interfering metal ions[12]. However, Fe has been shown to be > 99% complexed by very strong organic ligands of NOM[15]. It was therefore deemed important to investigate in further studies whether Fe was complexed by organic matter and available for detection, where it was a possibility that natural complexes of Fe were not labile. Many of the FIA detection methods for Fe with phenanthroline have been developed for use in freshwaters that contain large amounts of Ca^{2+} and Mg^{2+} and low concentrations of organic matter where concentrations of only a few mg C/L[16] typically encountered. However, this is often not the case for Australian freshwaters that are low in Ca^{2+} and Mg^{2+}, and often contain concentrations of humic substances as high as 50 mg C/L[16,17].

Thus it appeared that dissolved organic carbon (DOC) of even 15 mg/L[18] was still causing problems for detection. An alternative approach was therefore attempted that would enable detection of both Fe(II) and TFe.

3.2 Reagent Sandwich Injection FIA manifold for Fe Speciation

This approach is based on a "sandwich technique", in which, two different zones are introduced and so two different interfaces are formed between the sample and reagent solutions[5].

In this manifold reagent zones of the reductant ascorbic acid and the 1,10 phenanthroline were inserted between zones of sample (Figure 3). Consequently, the method of reagent sandwich injection yields two, separate and distinct peaks, the initial peak representing Fe(II) and the second peak corresponding to both Fe(II) and Fe(III) converted to the Fe(II) form. At the rear of the injected reagent zone any Fe(III) is reduced by ascorbic acid, R_2 (to Fe(II)) and hence the total iron concentration (TFe) is detected. This occurs because there is probably minimal sample penetration and incomplete mixing to the region between the two injected zones as described by Ruzicka and Hansen[19] (see Figure 4). This manifold consequently enables detection of both Fe(II) and TFe. These gave linear responses over the range 0-0.5 mg Fe/L (r^2 = 0.9978), with a LoD of 0.01 mg Fe/L and a relative standard deviation (%rsd) of 2% (n =10; 0.5 mg Fe/L). This approach offers a potential method for monitoring of Fe speciation in natural waters.

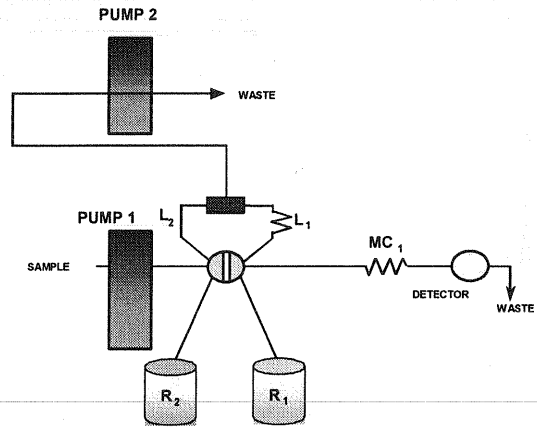

Figure 3 – Reagent Sandwich Injection FIA manifold. R_1= 1,10 Phenanthroline 0.25 % (m/v), R_2= Ascorbic acid (1% m/V),. Detection at 510 nm. L_1 = 500 µL phenanthroline injection loop, L_2 = 30 µL Ascorbic acid injection loop and MC_1 = 30 cm knitted mixing coil.

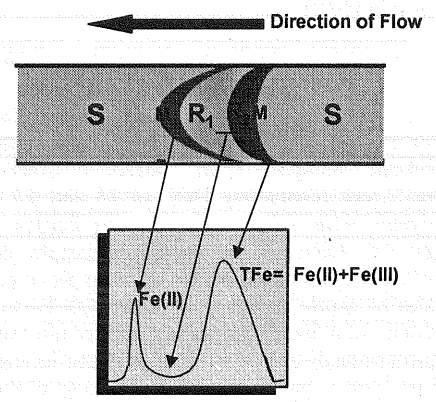

Figure 4 - Schematic diagram depicting signal output from sandwich injection resulting in two peaks representing Fe(II) and Total Iron. R_1 = 1, 10 phenanthroline, R_2 = Ascorbic acid, S = Sample Zone and M =Mixing zone. Sample does not completely penetrate into the injected zone of reagents R_1 andR_2, and this provides at least partial resolution of the Fe(II) and TFe peak.

To ensure that the peak response obtained comprised two distinct peaks of Fe(II) and TFe, and not a doublet as a result of limited mixing, a solution of 0.5 mg Fe(III)/L was used as the sample stream. This should result in only one peak for Total Fe (the second speak), and this was observed, whereas the injection of 0.5 mg Fe(II)/L

solution resulted in formation of a doublet peak (see Figure 5).

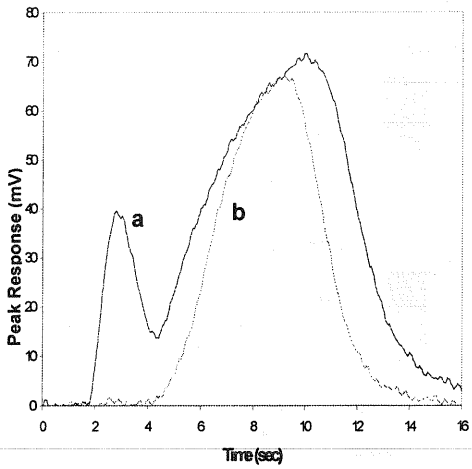

Figure 5 - Validation of Fe Speciation. a – 0.5 mg Fe(II)/L standard is injected and the resultant peak is a doublet as complexation with the phenanthroline is occurring at the leading and trailing edge of the injected zone **b** – When a 0.5 mg Fe(III)/L standard is injected the initial peak is not expected as reduction will only occur at the trailing edge of the injected zone, consequently only a single peak is observed.

3.2.1 Validation of rFIA Sandwich Zone Manifold

Spike recovery was used as a means of validating the detection of Fe(II) and TFe and to tests for other chemical interferences. Samples from Dobson's Creek and Sassafras Creek (containing DOC of 15 and 4.7 mg/L respectively) were spiked with 0.5 mg Fe(II)/L. The concentrations calculated for the spiked samples can be seen in Figure 6. Initial results for both samples produced recoveries of approximately 100 % for both Fe species, but if a delay of a few minutes was permitted after spiking, subsequent re-analysis resulted in significant decreases in recoveries. This is believed to be due to complexation by NOM as discussed in section 3.1. Previous studies have shown the stability constants for the Fe-organic ligand complexes to range from $\log K_{FeL}$ 22.1 to greater than 24[14,15]. However, Fe(II)-phenanthroline complexes in aqueous solutions have somewhat lower stability constants ($\log K_{FePhen}$ 21.3)[20-23] and hence Fe will have a greater tendency to bind preferentially with organic matter.

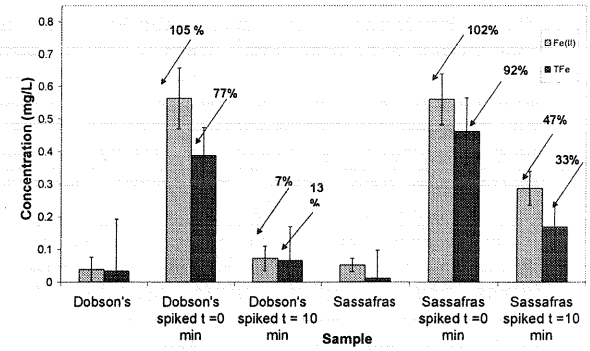

Figure 6 – Effect of Extended Reaction Time. Concentrations and percentage recoveries for spiked samples from Dobson's and Sassafras Creek over time. These samples were spiked with 0.5 mg Fe /L and contained DOC concentrations of 15 and 4.7 mg/L respectively. Error bars of $\pm 2\sigma_{n-1}$ are shown.

3.2.2 Effect of Dilution and Buffering on Recovery

To further test the effect of NOM interactions on the Fe-phen complexation, spike recovery was again performed followed by successive dilution of sample. Results showed that a 1:10 dilution of sample was required to achieve any improvement in recovery, again showing the affinity of NOM to bind to aqueous Fe (Figure 7). Therefore while dilution of NOM concentration can be used as a means of improving recovery, this is not entirely successful.

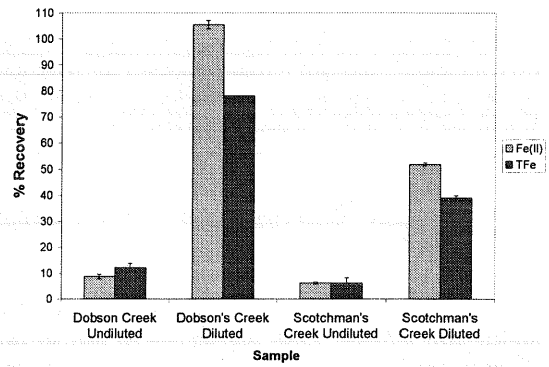

Figure 7 – Effect of Dilution on Recovery Percentage recovery from samples collected from Dobson's and Scotchman's Creek These samples were spiked with 0.5 mg Fe /L and contained DOC concentrations of 15 and 10 mg/L, respectively. Diluted samples were diluted by a factor of 1:10. Error bars of $\pm 2\sigma_{n-1}$ are shown.

The stability constants of metal ion-NOM complexes have been shown to be dependent on pH, ionic strength and the presence of competing ligands. Many of these studies have noted that an increase in pH usually enhances NOM adsorption, where NOM replaces hydroxyl groups on iron oxide surface[24,25]. Therefore, by

altering the pH of the solution phase, the equilibrium of a reaction can be significantly displaced. This effect was investigated by the direct addition of 10% (v/v) acetate buffer (pH 3.7) to the samples. Marked improvements in recoveries were observed and substantial recoveries were stillobserved days later, suggesting that interferences had significantly decreased (see Figure 8). The large recoveries observed after the addition of large amounts of buffer are most likely due to a combination of releasing labile Fe and perhaps also to reducing the stability of Fe-organic complexes. This large recovery for determination of Fe(II) and TFe could only be achieved by direct addition of a substantial amount of buffer, and could not be attained online by reagent sandwich injection with the sample as the carrier. Hence the possibility of having the buffer as the carrier was investigated.

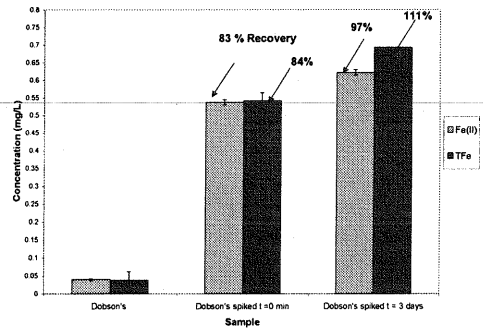

Figure 8 - Addition of 10% (v/v) acetate buffer to the Dobson Creek sample solution to increase recovery. Error bars of $\pm 2\sigma_{n-1}$ are shown

3.3 On-line Suppression of Interferences from Natural Organic Matter

Based on these findings, a third manifold was developed to overcome interference by NOM. As described in the previous section, using a buffer had proven to be the best option for increasing recovery and decreasing interferences by NOM. Consequently, the FI system shown in Figure 9, uses a buffer as the carrier stream, which is matched to the RI of the injected zone. In addition to reducing any RIE, this suppresses any complexation with natural organic matter, and allows Fe to be freely available for detection. TFe detection is subsequently achieved via online reduction of Fe(III) to Fe(II) using ascorbic acid. This approach gave linear responses over the range of 0 - 0.5 mg FeL^{-1} ($r^2 = 0.9994$), with a LoD of 0.01 mg FeL^{-1}, % rsd 2% (n = 10; 0.5 mg Fe/L) and a potential sample throughput of 144 injections hr^{-1}.

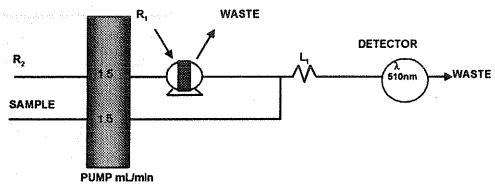

Figure 9 – Developed rFIA manifold used for TFe determination .The mixture of reagents is injected into the continuously flowing buffer line which is matching the RI of the injected zone and merged with the sample and then combined in the mixing coil. Where, R1= a mixture of Ascorbic acid (1% m/v) and 1,10 Phenanthroline (0.25 % (m/v) mixed in with 0.2 M Acetate Buffer R_2= 0.5 M Acetate Buffer, and L_1 = 30 cm knitted coil.

The proposed TFe manifold (Figure 9) was validated using a Standard Reference Material (SRM), . Figure 10a. shows that the observed concentration was well within $2\sigma_{n-1}$ standard deviations of the expected concentration.

Spike recovery of a creek water and tap water sample was also performed to further validate the TFe manifold. Results obtained provided recoveries close to 100 %, and these were maintained with time. This suggests that no major competitive interferences occur using this manifold configuration for the determination of TFe (see Figure 10b).

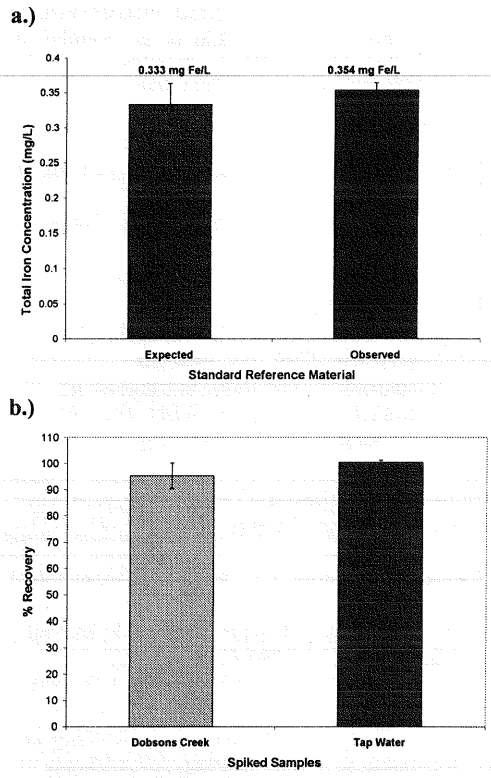

Figure 10 - **a**- Concentration of SRM from the TFe manifold showing the measured concentration and the expected concentration as specified by the EPA. Error bars of $\pm 2\sigma_{n-1}$ are shown. **b.** Percentage recovery from sample collected from Dobson Creek and from the tap. These samples were spiked with 0.5 mg Fe /L. Error bars of $\pm 2\sigma_{n-1}$ are shown.

4.0 Conclusions

Investigation of three reverse flow injection methods for the determination of various Fe species using the colorimetric reagent 1,10-phenanthroline, and spectrophotometric detection, have shown that:

(i) The initial rFIA manifold could effectively detect only Fe(III) in field samples.
(ii) the "Sandwich Zone" manifold could detect both Fe(II) and TFe. However, adequate recovery was not achieved in samples containing NOM without pre-treatment of sample. This was thought to be primarily due to complexation with NOM and an alternative approach was investigated.
(iii) The manifold designed for on-line suppression of interferences by NOM was capable of detecting TFe and used a carrier buffer to match the RI of the injected zone. This manifold provided the most reliable results obtained to date, with a detection limit of 0.01 mg Fe/L and a rapid sample throughput of 120 injections per hour. Recoveries of spiked real samples were in the range of 95-100 %.

The results of this work also raises some questions about the robustness of the 1,10-phenanthroline method which is widely used for Fe(II) and Fe(III) measurement in natural waters, but often with little or no mention of potential interferences by natural organic matter.

5.0 References

[1] Mayer, T. D.; Jarrell, W. M. *Water Research* **1996**, *30*, 1208-1214.
[2] Deng, Y.; Stumm, W. *Applied Geochemistry* **1994**, *9*, 23-36.
[3] Measures, C. I.; Yuan, J.; Resing, J. A. *Marine Chemistry* **1995**, *50*, 3-12.
[4] Alonso, J.; Bartroli, J.; Del Valle, M.; Barber, R. *Analytica Chimica Acta* **1989**, *219*, 345-350.
[5] Alonso, J.; Bartroli, J.; Del Valle, M.; Barber, R.; Escalada, M. *Analytica Chimica Acta* **1987**, *199*, 191-196.
[6] Bubnis, B. P.; Straka, M. R.; Pacey, G. E. *Talanta* **1983**, 30, 841-844.
[7] Carneiro, J. M. T.; Dias, A. C. B.; Zagatto, E. A. G.; Honorato, R. S. *Analytica Chimica Acta* **2002**, *455*, 327-333.
[8] Galhardo, C. X.; Masini, J. C. *Analytica Chimica Acta* **2001**, *438*, 39-48.
[9] Pascual-Reguera, M. I.; Ortega-Carmona, I.; Molina-Diaz, A. *Talanta* **1997**, *44*, 1793-1801.
[10] Teshima, N.; Ayukawa, K.; Kawashima, T. *Talanta* **1996**, *43*, 1755-1760.
[11] Mortatti, J.; Krug, F. J.; Pessenda, L. C. R.; Zaggatto, E. A. G.; Jorgensen, S. S. *Analyst* **1982**, *107*, 659-663.
[12] Clesceri, L. S.; Eaton, A. D.; Greenberg, A. E., Eds. *Standard Methods for the Examination of Water and Wastewater*; 20th ed.; United Book Press Inc.: Baltimore, 1998.
[13] Millero, F. J.; Yao, W.; Aicher, J. *Marine Chemistry* **1995**, *50*, 21-39.
[14] van den Berg, C. M. G. *Marine Chemistry* **1995**, *50*, 139-157.
[15] Witter, A. E.; Hutchins, D. A.; Butler, A.; Luther, G. W. I. *Marine Chemistry* **2000**, *69*, 1-17.
[16] Stumm, W.; Morgan, J. J. *Aquatic Chemistry - Chemical Equilibria and Rates in Natural Waters*; 3rd ed.; John Wiley & Sons, Inc.: Toronto, 1996.
[17] Hart, B. T.; McKelvie, I. D. In *Limnology in Australia*; De Deckker, P., Williams, W., D., Eds.; CSIRO/Dr W. Junk Publishers: Melbourne, 1986, pp 3-31.
[18] Walsh, C. J.; Monash University, *Personal Communication,* Cooperative Research Center for Freshwater Ecology - Urban Project -DOC analysis.
[19] Ruzicka, J.; Hansen, E. L. *Flow Injection Analysis*; 2nd ed.; John Wiley & Sons: Canada, **1988**.
[20] Kolthoff, I. M.; Leussing, D. L.; Lee, T. S. *Journal of the American Chemical Society* **1950**, *72*, 2173-2177.
[21] Lee, T. S.; Kolthoff, I. M.; Leussing, D. L. *Journal of the American Chemical Society* **1948**, *72*, 2348-2352.
[22] Makrlik, E.; Vanura, P. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1990**, *287*, 159-162.
[23] Hudson, R. J. M.; Covault, D. T.; Morel, F. M. M. *Marine Chemistry* **1992**, *38*, 209-235.
[24] Lu, Y.; Allen, H. E. *Water Research* **2002**, *36*, 5083-5101.
[25] Gu, B.; Schmitt, J.; Chen, Z.; Liang, L.; McCarthy, J. F. *Geochimica et Cosmochimica Acta* **1995**, *59*, 219-229.

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