Chiang Mai J. Sci. 2003; 30(3) : 153-164
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Field-Based Analytical Techniques for Aquatic Environmental Monitoring

Paul Worsfold* [a], Eric Achterberg [a], Andrew Bowie [b], Paulo Gardolinski [a], Martha Gledhill [a], Grady Hanrahan [a], Ian McKelvie [c] and Simon Ussher [a]

[a] School of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Drake Circus, Plymouth, Devon, England

[b] ACROSS and Antarctic CRC, University of Tasmania, Hobart, Tasmania 7001, Australia

[c] Water Studies Centre, School of Chemistry, Monash University, Clayton, Victoria, 3800, Australia

*Author for correspondence, e-mail:pworsfold@plymouth.ac.uk

Received : 30 March 2003
Accepted : 2 May 2003

ABSTRACT

There is a growing need for rapid and reliable but relatively low cost techniques that can be remotely deployed to provide high quality environmental data. This paper describes the use of flow injection (FI) based instrumentation for aquatic environmental monitoring. FI techniques now impact on a wide cross section of analytical chemistry activities, providing imaginative and practical solutions to challenging analytical problems and contributing to the improvement of data quality. Two specific applications are described. The first is the use of flow injection with spectrophotometric detection (FI-SPEC) for the determination of nitrogen and phosphorus species in catchments, estuaries and sediments in order to investigate the impact of nutrients on water quality and provide decision support systems for catchment management. The second is the use of flow injection with chemiluminescence detection (FI-CL) for the determination of micronutrients (particularly iron) in remote, open ocean environments. As a rate limiting nutrient, iron plays a key role in ocean productivity and climate change. The importance of 'clean' analytical protocols in order to provide high quality environmental data are also considered.

1. ENVIRONMENTAL MONITORING

It is essential that any environmental monitoring programme provides high quality analytical data (e.g. good accuracy and precision) at reasonable cost in order to achieve particular objectives. These need to be clearly specified before designing a suitable sampling and analysis programme. Examples of general objectives include:

- Elucidation of environmental processes and biogeochemical cycles.
- Monitoring compliance with legislation.
- Archiving data and providing baseline surveys, e.g. environmental impact assessments.
- Studying chemical fluxes, pathways and fates in environmental systems.

A major constraint on the design of any environmental monitoring programme is the fact that sampling is expensive and time consuming and, from a data quality perspective, sample integrity may be lost during the process of collection and storage. For example, the stability of natural water samples collected and stored for the determination of macronutrients (nitrate and phosphate) are subject to biological, chemical and physical affects that can significantly alter the measured nutrient concentrations. A standard protocol for storage is therefore desirable but this is

not possible due to the contrasting physico-chemical and biological characteristics of different natural waters, e.g. salinity, bacterial content, hardness. However the following principles are recommended as general guidelines:

- All labware used for sample collection, storage and analysis should be rigorously cleaned, e.g. a 12 h soak in Neutracon, followed by a water rinse (x3), 24 h soak in 10% HCl, and final water rinse (x3).
- All sample containers should be made of inert material, e.g. HDPE and cleaned as stated above.
- All samples should be filtered (0.2 um cellulose acetate) at the time of sampling with a low pressure gradient to avoid cell lysis.
- All samples should be analysed as quickly as possible (ideally < 8 h).
- Samples should not be stored at 4 °C without chemical treatment.
- Calcium rich samples (e.g. from chalk catchments) should not be frozen in order to avoid phosphate precipitation.
- Samples rich in organic matter should not be chemically treated in order to avoid release of cellular enzymes.

In view of the problems associated with discrete sample collection and subsequent laboratory analysis it is desirable to be able to monitor chemical species in the aquatic environment *in situ*. Portable instrumentation has the potential to acquire high quality data with excellent temporal and spatial resolution for environmental process studies and chemical mapping of the aquatic environment. However such instrumentation needs to meet several challenging objectives in addition to the normal analytical requirements of a laboratory technique. These include:

- Rugged, portable, automated instrumentation.
- Provision of a contamination free environment for trace analysis, e.g. reagents, containers, sampling apparatus, ship.
- Sensitive and selective detection
- Removal of matrix interferences e.g. sea salts for marine waters.
- Long term stability of all components, e.g. reagents, standards, pumps and detector.
- Ability to perform in-line filtration and minimise the affect of biofouling.
- Ability to perform remote calibration and validation using on-board standards.
- Minimal maintenance requirements.

One technique that can meet these objectives for specific chemical parameters in aquatic environments is flow injection analysis and the principles are discussed below.

2. Flow Injection Analysis

Flow injection (FI) analysis has become established as an important tool for sample presentation and on-line treatment in the laboratory environment. It is now being increasingly considered for deployment outside of the laboratory, in both process and environmental locations [1].

FI has been described as an unsegmented flow technique in which a volume of liquid sample is inserted into a moving liquid carrier stream, whereupon it undergoes physical dispersion as it is transported to a flow-through detector for measurement [2]. The transient response is usually in the form of a peak, with a sharp rising edge and a more gradual decay, the shape being due to axial dispersion and radial diffusion of the sample zone as travels through the FI manifold. The height and area of the peak are usually directly related to analyte concentration but for convenience peak height is usually the measured parameter. The degree of sample dispersion is controlled by factors such as sample volume, carrier flow rate, length and diameter of the manifold tubing and manifold geometry and under most conditions is highly reproducible (relative standard deviations are typically less than 5 %). The technique is now widely used in analytical laboratories for the automation of wet chemical methods and has considerable potential for use in catchments

[3], on board ship [4] and in submersible analysers [5].

A block diagram of a simple, single channel FI manifold is shown in Figure 1 and typically consists of a means of propulsion (*e.g.* a peristaltic pump), a rotary injection valve for sample introduction (similar to HPLC valves but low pressure) and a flow-through detector (*e.g.* a spectrophotometer). In this manifold the carrier stream transports the sample to the detector. PTFE tubing (typically 0.8 mm i.d.) is used throughout the manifold for sample and reagent transport, with tightly wound coils often included to enhance mixing. If the method requires more than one reagent, additional streams can be merged with the carrier stream at suitable points in the manifold.

Similarly if in-line physical treatment of the sample is required the necessary components can easily be incorporated. These include:

- Solid phase chelating microcolumns for matrix removal and analyte preconcentration, e.g. 8-hydroxyquinoline for the removal of the major seawater ions and simultaneous preconcentration of trace metals.
- Gas dialysis for the diffusion of a gaseous analyte from a carrier (donor) stream through a microporous membrane into a reagent (acceptor) stream, e.g. for the selective extraction of ammonia from seawater.
- Solid phase reaction columns, in which the injected sample reacts with a solid material, e.g. an immobilised enzyme, packed in a column.

Reagent consumption is generally low in FI systems (an important factor for shipboard and submersible applications), and can be reduced still further by using a reagent injection manifold, whereby a discrete volume of reagent is injected into a continuously flowing sample stream. This option is suitable for applications in which the sample is in abundant supply (as in many marine situations), and is particularly beneficial when expensive reagents are required. Simultaneous FI determinations can be performed by designing manifolds in which the sample is injected into more than one flow channel, undergoing different reaction chemistries in each. FI systems are easily automated using off the shelf components and a notebook PC to control the operation of the valves and pumps and data acquisition and processing. A schematic of an automated FI manifold including the facility for on-board calibration is shown in Figure 2.

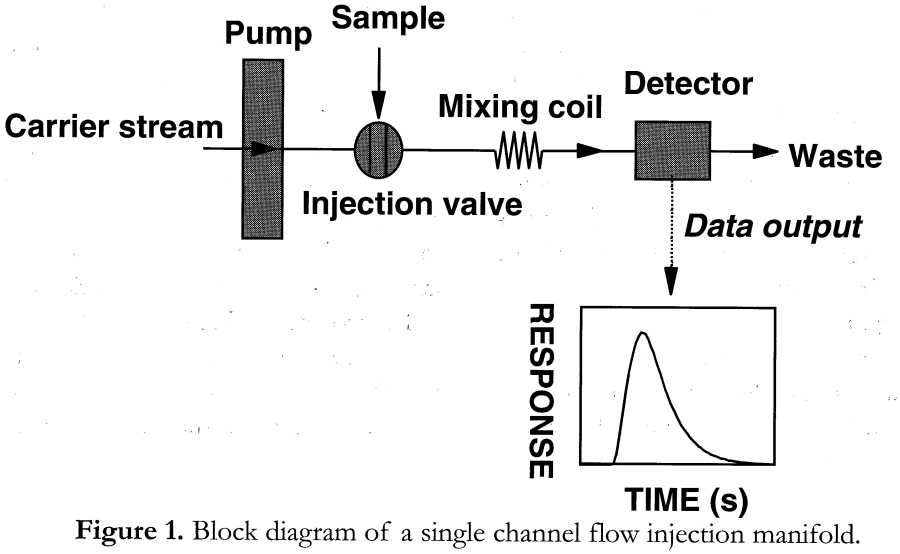

Figure 1. Block diagram of a single channel flow injection manifold.

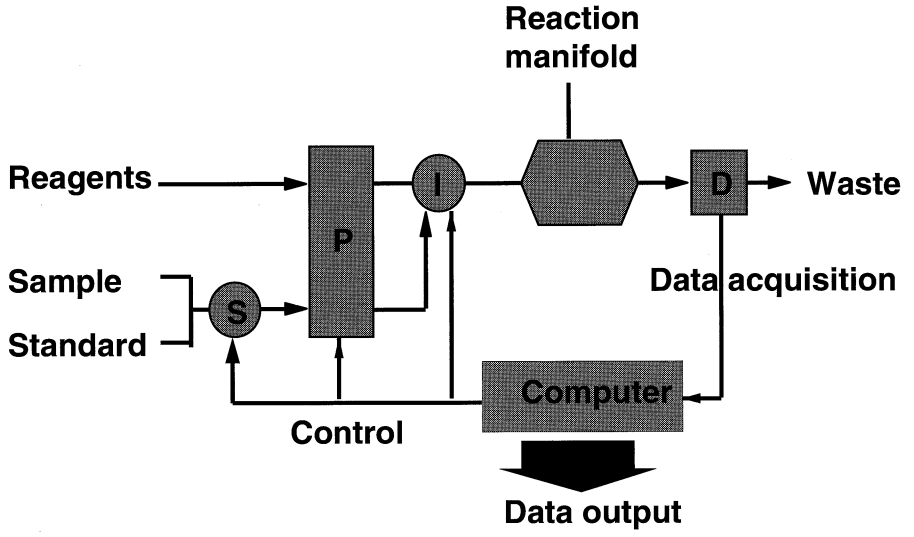

Figure 2. Block diagram of an automated flow injection manifold incorporating a facility for on-board calibration.

3. BIOGEOCHEMISTRY OF NUTRIENTS

3.1 Role of Nutrients

Nutrients are essential elements for biochemical reactions and the growth and maintenance of biomass, with nitrogen and phosphorus being the most important and commonly determined nutrients in aquatic ecosystems.An excess of nitrogen and phosphorus can lead to depletion of dissolved oxygen (DO) in aquatic systems. The cumulative effect of nutrient loading results in eutrophication, an enrichment of water by nutrients, which can lead to an increase in biomass and natural productivity within a given aquatic community structure and deterioration of the quality of water concerned [6-8]. This can cause major ecological changes, e.g. production of algal blooms, reduction in species diversity and major changes in community structure. Thus, assessing nutrient concentrations on a regular basis is of paramount importance for providing an insight into the relative health of aquatic environments.

3.2 Nitrogen

The elemental gas dinitrogen (N_2) is the most abundant but least reactive form of nitrogen in the global environment. However, many biochemical transformations can convert dinitrogen into dissolved inorganic species; e.g. nitrate (NO_3), nitrite (NO_2), ammonium (NH_4^+) and organic nitrogen compounds, in both dissolved and particulate forms. Nitrogen speciation can be operationally defined as total particulate nitrogen (TPN), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) [9]. The atmosphere is the principal nitrogen reservoir, with over 99% of the total in the form of N_2. Nitrogen in terrestrial systems occurs mainly as soil organic matter, with litter and soil inorganic nitrogen accounting for the majority (97%). N_2, in dissolved form, is the most abundant form in the world's oceans. Nitrogen also occurs in various inorganic forms, e.g. nitrate, nitrite, ammonia, hydrazine, nitrous oxide, and nitrogen dioxide and organic forms, e.g. amino acids, amines and amides. However, the organic fraction is not well characterized. Figure 3 shows a schematic representation of the aquatic nitrogen cycle including biochemical transformations. Three of the processes: fixation, nitrification and ammonification convert gaseous nitrogen into bioavailable chemical forms. The fourth, denitrification, converts fixed nitrogen back into gaseous species.

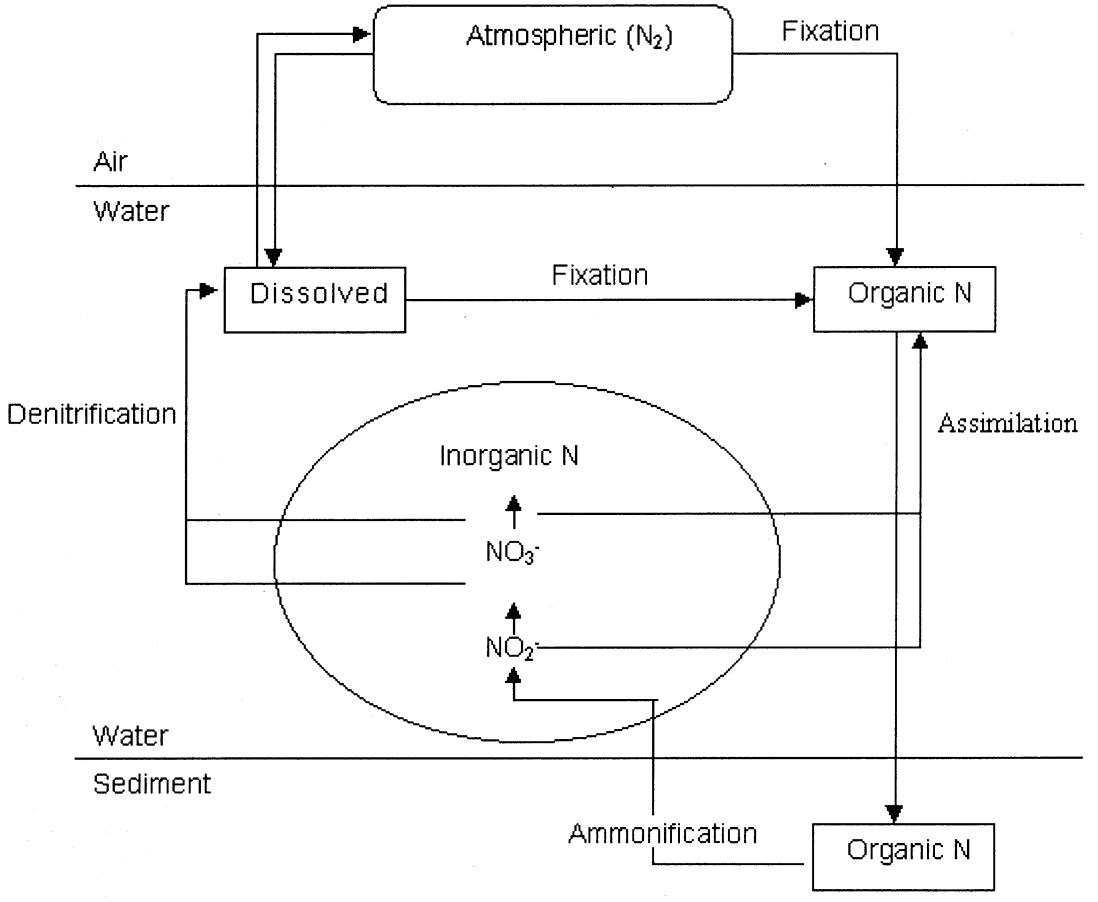

Figure 3. Schematic representation of the aquatic nitrogen cycle

3.3 Phosphorus

Phosphorus occurs in aquatic systems is both particulate and dissolved forms and can be operationally defined as total phosphorus (TP), total reactive phosphorus (TRP), filterable reactive phosphorus (FRP), and total filterable phosphorus (TFP). The distribution and transformation of phosphorus in aquatic systems is shown in Figure 4. Unlike nitrogen, the phosphorus cycle does not have a significant atmospheric component. A chemical distribution of phosphorus between aquatic and particulate components occurs, via e.g. adsorption and precipitation processes. The major reservoir of phosphorus is soils and other bulk sources include marine sediments and crustal rocks.

3.4 Sources of Nutrients in the Aquatic Environment

It is important to assess the input of nutrients into aquatic systems from both diffuse and point sources. Stormwater run-off and catchment discharges are the primary components of diffuse source pollution, with the water quality of the discharge being determined by the dominant land use of the catchment area. Nutrient contamination can originate from fertilizers and pesticides from agricultural and residential lands, and from livestock and human wastes. Point sources are distinct sources of contamination, i.e. those coming from a concentrated point and flowing directly into water bodies at a discrete point. Industrial sources, municipal sewage-treatment facilities and agricultural animal production facilities are the main point sources of nutrients.

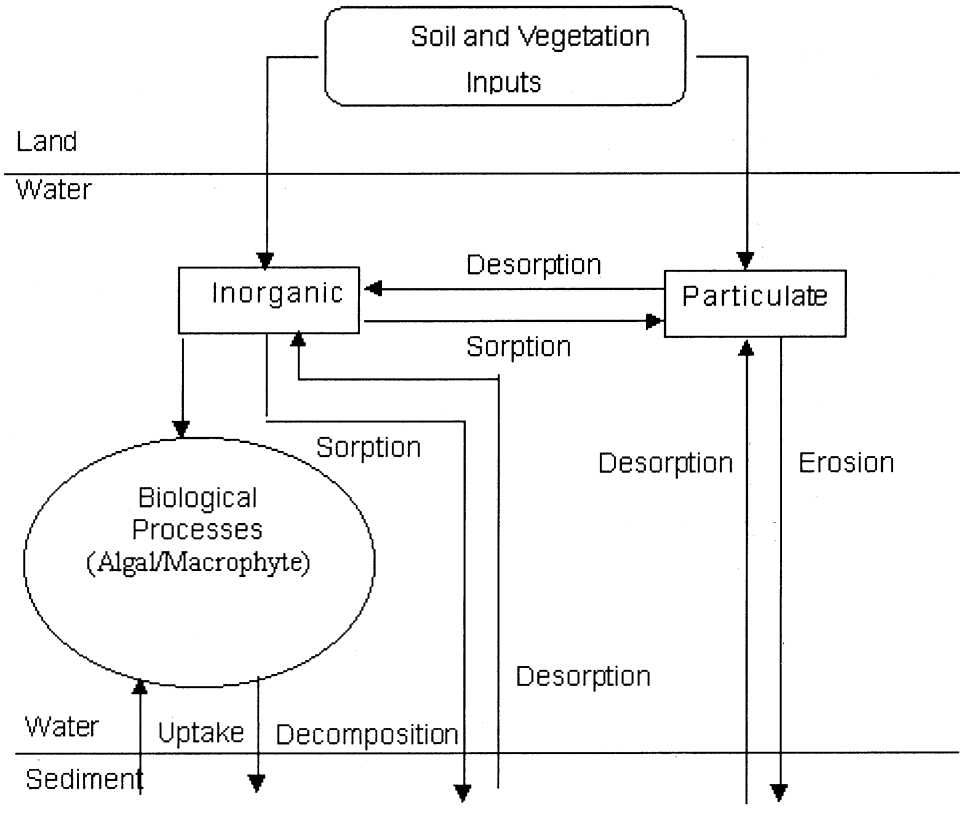

Figure 4. Schematic representation of the aquatic phosphorus cycle

4. Flow Injection Manifolds for the Determination of Nutrients in Natural Waters

4.1 Determination of Nitrate

This manifold (Figure 5) utilises the classical diazotization chemistry for the determination of nitrate following solid phase reduction to nitrite with copperised cadmium. For waters with high suspended solids some form of in-line filtration is recommended, such as a syringe filter or a tangential filter with 0.45 or 0.2 um cut-off. One of the key aspects of the FI approach to *in situ* monitoring is the type of detector. For ease of construction, ruggedness and low cost a solid state detector incorporating a light emitting diode (30 nm bandwidth source) and a photodiode (integrating detector) is the best option. A schematic diagram of the flow cell used is shown in Figure 6.

4.2 Determination of Phosphate

Most methods of phosphorus determination are based on the reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid, which is then reduced to an intensely colored blue compound and determined spectrophotometrically at 840 nm. [10].

Reduction is achieved by the addition of ascorbic acid or tin(II) chloride with the main potential interferences being silicate and arsenate. The phosphorus determined is defined as "molybdate reactive" or soluble reactive phosphorus (SRP). Other phosphorus containing organic compounds and condensed phosphates can be determined using the molybdate reaction following chemical, photochemical, thermal or microwave digestion.

$$PO_4^{3-} + 12\ MoO_4^{2-} + 27\ H^+ \rightarrow H_3PO_4\ (MoO_3)_{12} + 12\ H_2O$$

$$H_3PO_4\ (MoO_3)_{12} \rightarrow \text{Phosphomolybdenum blue}$$

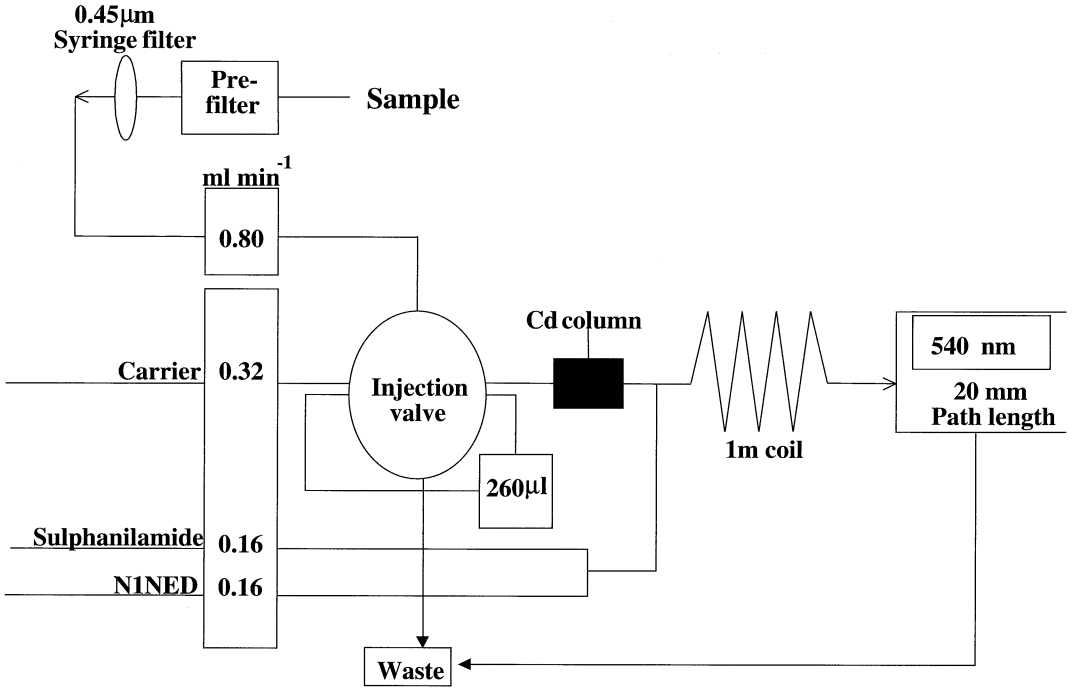

Figure 5. FI manifold for the determination of nitrate in marine waters

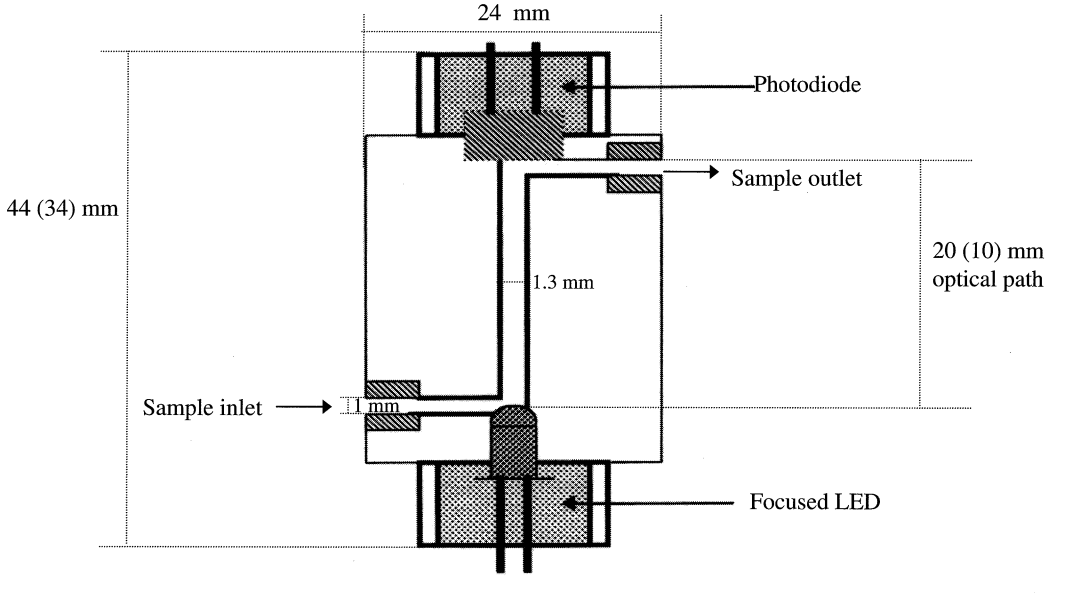

Figure 6. A schematic diagram of a solid state spectrophotometric detector for incorporation in a FI manifold.

5. SUMMARY OF NUTRIENT MONITORING

Nutrients are essential to biochemical structure and function and are found in varying proportions in aquatic ecosystems. The availability of some nutrients, particularly nitrogen and phosphorus, is often limited, and the concentration of these control the rate of primary production. However, an excess of nutrient loading can lead to eutrophication, which may ultimately lead to a deterioration of water quality. An increased public awareness based on environmental, economical and socio-political concerns, has led to the development of water quality monitoring programs.

Ideally the chemical composition of the water being analysed should be measured in situ. FI techniques are ideally suited for this function, particularly when using traditional wet chemistry with solid state spectrophotometric detection. Such instrumentation can be self assembled but if this is not possible, appropriate sampling, collection and storage techniques are required. Currently, there are numerous sampling and storage procedures available but waters vary considerably in composition and what is suitable for preserving nutrient concentrations in one system may not apply to others. It is therefore recommended that laboratories carry out their own experiments and set appropriate procedures.

Monitoring programmes are contingent on good laboratory practices and analytical protocol and the precision and accuracy of measurements must reflect the level of confidence placed on the measurements. There are numerous types of procedures available to measure nutrients in aquatic systems but the measuring systems should be determined by the objectives of the monitoring programme and meet specified objectives. Laboratories should institute strict quality assurance and quality control methods to ensure consistently reliable results. Methods used for this purpose include quality control programmes, intercomparison exercises and the use of certified reference materials.

6. BIOGEOCHEMISTRY OF IRON

Iron is the fourth most abundant element in the Earth's crust, present at a concentration of about 6%. However, like other reactive trace metals, dissolved iron is only present in open-oceanic waters at sub-nanomolar levels. Iron is an essential micro-nutrient, limiting primary production in remote oceanic regions [11-13] such as the Equatorial and North East Pacific and the Southern Ocean, where macro-nutrients are plentiful. Such limitation may have important ramifications for the atmosphere-ocean CO_2 flux and global carbon cycles [14-15]. Seeding experiments during Iron-Ex I and II [13,16] experiments in the Equatorial Pacific, using $FeSO_4$, resulted in enhanced primary productivity, and in the case of Iron-Ex II, also in a noticeable enhanced draw-down of CO_2. Iron exists in two forms in marine systems. Fe(III) is the thermodynamically stable form in oxygenated seawater, existing primarily as insoluble oxy-hydroxides or colloidal matter [17-19]. Fe(II) is a transient species in surface oxic waters, existing via chemical or photochemical Fe(III) reduction [20-22] or via atmospheric deposition [23-25], and is oxidised rapidly by O_2 and H_2O_2 species at seawater pH [26]. Recently, organic complexation has been thought to occur to a significant extent in marine systems [27-29]. Major sources of iron to the world's oceans include aeolian deposition, fluvial transport, hydrothermal venting, continental shelf regeneration and upwelling of Fe-enriched subsurface waters. In order to assess Fe input and removal mechanisms, and to enhance the understanding of redox and chemical speciation of Fe associated with various operationally defined fractions, there is a need for new shipboard analytical methodologies (such as FI) to determine ultratrace levels of iron.

7. FLOW INJECTION MANIFOLD FOR THE DETERMINATION OF IRON IN SEAWATER

The concentration of dissolved iron in seawater is typically less than 1 nM and so spectrophotometric detection is not sufficiently sensitive for this application. Therefore FI

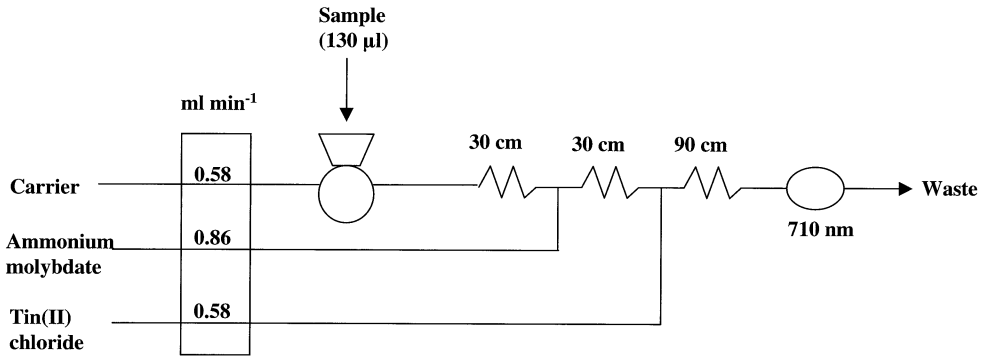

Figure 7. FI manifold for the determination of phosphate in river waters

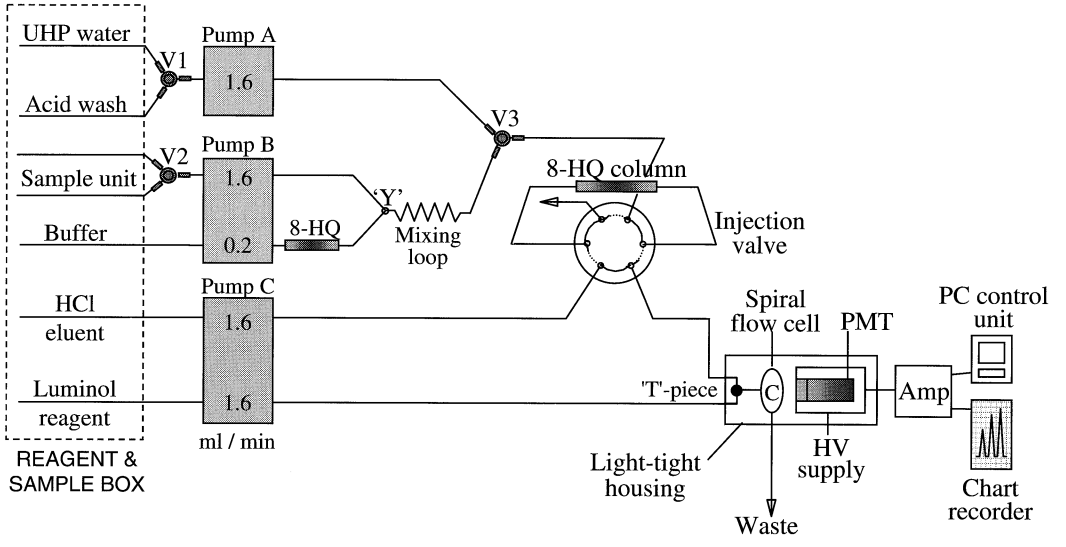

Figure 8. FI-CL manifold for the determination of iron in seawater

is combined with chemiluminescence detection (FI-CL) in order to achieve the desired sensitivity [30].

As with nutrients, it is essential that clean analytical protocols are used if high quality iron data is to be obtained. Contamination from labware is particularly problematic and the following protocol is recommended.

In general laboratory

1. Immerse 30-50 1 L LDPE bottles in 5% detergent bath (e.g. Decon, Merck BDH) for one week.
2. Rinse 3x with distilled water thoroughly until there is no trace of detergent.
3. Rinse 3x with UHP (ultra-high purity; e.g. Milli-Q) water.
4. Immerse in 6 M analytical grade HCl bath (2 weeks, e.g. Aristar, Merck BDH).
5. Rinse 3x with UHP water.
6. Immerse in 3 M analytical grade HNO_3 bath (2 weeks, e.g. Aristar, Merck BDH).
7. Rinse 3x with UHP water.

In clean air (class-100) laboratory

8. Fill with UHP water and acidify to pH 2 with 1 ml ultra-pure Q-HCl (9 M) (or similar for Q-HNO_3) per 1 l UHP sample (ultra-pure Q-acids are sub-boiling quartz distilled reagents).
9. Double bag, seal and store inside large plastic bag within plastic box until use.
10. Sample collection: empty acidified

UHP water, rinse 3x with UHP, rinse 3x with seawater sample, fill and acidify sample (if necessary). Double bag, seal and store for analysis.

8. Summary of Iron Monitoring

The shipboard FI-CL manifold shown above is a low cost, portable, rugged system suitable for on-line determination of both dissolved iron (II+III) (after reduction of Fe(III) with sulfite) and Fe(II) in surface marine waters [31]. Moreover, the generic nature of system components and the use of graphical programming software make it easily adaptable to other CL-detectable analytes (e.g. Co, Cu, Mn, H_2O_2). The performance and reliability of the instrument and the analytical figures of merit are sufficient to allow iron(II) to be determined at picomolar concentrations in real-time over long (>10 h) periods, without user intervention.

In open-ocean environments, iron(II) measurements and observations of iron redox cycling are inherently difficult due to the extremely low dissolved iron(II+III) concentrations, but the FI-CL approach can provide acceptable high resolution data in such settings. This technique is also useful for examining subtle changes in iron redox chemistry during *in situ* iron fertilisation experiments, or in coastal and fresh water environments where dissolved iron(II+III) concentrations are significantly higher. The system could also be used for monitoring biologically-mediated redox cycling and uptake of iron(II) in laboratory culture experiments, deckboard incubations or chemostats.

9. Conclusions

Flow injection is an approach to automating wet chemical methods that is ideally suited to both laboratory and *in situ* determinations of key chemical parameters in natural waters. It is therefore a useful practical tool for high quality data acquisition, with the *in situ* version able to provide high temporal and/or spatial resolution of information. This is an essential prerequisite for the development of reliable systems for water resources management.

References

[1] Andrew K.N., Blundell N.J., Price D. and Worsfold P.J., Flow injection techniques for water monitoring, *Anal. Chem.*, 1994:**66**; 916A-922A.

[2] Ruzicka J., and Hansen E.H., *Flow Injection Analysis*, Wiley, Chichester, 1981; 1-207.

[3] Hanrahan G., Gledhill M., Fletcher P.J. and Worsfold P.J., High temporal resolution field monitoring of phosphate in the River Frome using flow injection with diode array detection, *Anal. Chim. Acta.* , 2001:**440**;55-62.

[4] Worsfold P.J., Achterberg E.P., Bowie A.R., Sandford R.C. and Mantoura R.F.C., Flow injection with chemiluminescence detection for the shipboard monitoring of trace metals, in M.S.Varney (Ed.), *Chemical Sensors in Oceanogra-phy*, Amsterdam : Gordon and Breech, 2000;71-94.

[5] David A.R.J., McCormack T., Morris A.W. and Worsfold P.J., A Submersible Flow Injection-based Sensor for the Determination of Total Oxidised Nitrogen in Coastal Waters, *Anal. Chim. Acta.,* 1998:**361**; 63-72.

[6] Wilson H.M., Gibson M.T. and O'Sullivan P.E., Analysis of Current Policies and Policies and Alternative Strategies for the Reduction of Nutrient Loads on Eutrophication Lakes: the Example of Slapton Ley, Devon, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1993:**3**; 239-251.

[7] Moss B.A., A land awash with nutrients – the Problem of Eutrophication, *Chemical Industry,* 1996:**11**; 407-409.

[8] Young K., Morse G.K., Scrimshaw M.D., Kinniburgh J.H., MacLeod C.L. and Lester J.N., The relation between phosphorus and eutrophication in the Thames catchment, UK, *Science Tot. Env.,* 1999:**228**; 157-183.

[9] Robards K., McKelvie I.D., Benson R.L., Worsfold P.J., Blundell N.J. and Casey H., Determination of carbon, phosphorus, nitrogen and silicon species in waters,

Anal. Chim. Acta, 1994:**287**; 147-190.

[10] McKelvie I.D., Peat D.M.W. and Worsfold P.J., Techniques for the quantification and speciation of phosphorus in natural waters, *Analytical Proc. inc. Analytical Comm.*, 1995:**32**; 437-445.

[11] Martin J.H. and Fitzwater S.E., Iron-deficiency limits phytoplankton growth in the northeast Pacific sub-arctic. *Nature*, 1988:**331**; 341-343.

[12] Martin J.H., Gordon R.M. and Fitzwater S.E., Iron in Antarctic waters. *Nature*, 1990:**345**; 156-158.

[13] Coale K.H., Johnson K.S., Fitzwater S.E., Gordon R.M., Tanner S., Chavez F.P., *et al.*, A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equa-torial Pacific Ocean, *Nature*, 1996:**383**; 495-501.

[14] Martin J.H., Glacial-interglacial CO_2 change: The iron hypothesis, *Paleoceanography*, 1990:**5**; 1-13.

[15] Cooper D.J., Watson A.J. and Nightingale P.D., Large decrease in ocean-surface CO_2 fugacity in response to in-situ iron fertilization, *Nature*, 1996:**383**;511-513.

[16] Martin J.H., Coale K.H., Johnson K.S., Fitzwater S.E., Gordon R.M., Tanner S.J., *et al.*, Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean, *Nature*, 1994:**371**; 123-129.

[17] Stumm W. and Morgan J.J., *Aquatic Chemistry*, New York : Wiley, 1981;5.

[18] Hudson R.J.M. and Morel F.M.M., Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions, *Limnol. Oceanogr.*, 1990:**35**; 1002-1020.

[19] Turner D.R., Whitfield M. and Dickson A.G., The equilibrium speciation of dissolved components in freshwater ans seawater at 25°C and 1atm. pressure, *Geochim. Cosmochim. Acta*, 1981:**45**; 855-882.

[20] O' Sullivan D.W., Hanson A.K., Miller W.L. and Kester D.R., Measurement of Fe(II) in surface water of the equatorial Pacific, *Limnol. Oceanogr.*, 1991:**36**;1727-1741.

[21] Johnson K.S., Coale K.H., Elrod V.A. and Tindale N.W., Iron photochemistry in seawater from the equatorial Pacific, *Mar. Chem.*, 1994:**46**; 319-334.

[22] Gledhill M. and van den Berg C.M.G., Measurement of the redox speciation of iron in seawater by catalytic cathodic stripping voltammetry, *Mar. Chem.*, 1995:**50**;51-61.

[23] Millero F.J., Gonzalez-Davila M. and Santana-Casiano J.M., Reduction of Fe(III) with sulfite in natural waters, *J. Geophys. Res.*, D4, 1995:**100**; 7235-7244.

[24] Zhuang G., Duce R.A. and Kester D.R., The dissolution of atmospheric iron in surface seawater of the open ocean, *J. Geophys. Res. C. Oceans*, 1990: **95**; 16207-16216.

[25] Martin J.H. and Gordon R.M., Northeast Pacific iron distributions in relation to phytoplankton productivity, *Deep-Sea Research Part A*, 1988:**35**;177-196.

[26] King D.W., Lounsbury H.A. and Millero F.J., Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations, *Environ. Sci. Technol.*, 1995:**29**; 818-824.

[27] Gledhill M. and van den Berg C.M.G., Determination of complexation of iron(III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry, *Mar. Chem.*, 1994:**47**; 41-54.

[28] Rue E.L. and Bruland K.W., Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method, *Mar. Chem.*, 1995:**50**; 117-138.

[29] van den Berg C.M.G., Evidence for organic complexation of iron in seawater, *Mar. Chem.*, 1995:**50**; 139-157.

[30] Bowie A.R., Achterberg E.P., Mantoura R.F.C. and Worsfold P.J., Determination of sub-nanomolar levels of iron in seawater using flow injection with chemilu-

minescence detection, *Anal. Chem. Acta*, 1998:**361**; 189-200.

[31] Bowie A.R., Achterberg E.P., Sedwick P.N., Ussher S. and Worsfold P.J., Real-time monitoring of picomolar concentrations of iron(II) in marine waters using automated flow injection-chemiluminescence instrumentation, *Environ. Sci. Technol.*, 2002:**36**;4600-4607.