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Nutrients: Practical notes on their determination in sea water

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Nutrients: Practical notes on their determination in sea water

1 INTRODUCTION

These notes are aimed primarily at the freshwater chemist beginning to conduct analyses of nutrients in saline waters, but they will also be useful to the complete newcomer to the application of automated colorimetric techniques to natural waters in general. The emphasis is on automated techniques, but much of the material should be of interest to analysts who still use manual methods.

The term 'nutrients' is a little difficult to define precisely, but from the point of view of the marine chemist, phosphate, nitrate, nitrite, ammonia, and silicate are those most commonly encountered. For a more detailed discussion and definitions of terms, see Grasshoff *et al.* (1983).

2 GENERAL CONSIDERATION OF ANALYTICAL TECHNIQUES

Some nutrients have been measured for many decades by manual colorimetric techniques, but the introduction of (segmented) continuous-flow analysis (CFA) brought about an increase in activity in marine chemistry in the 1960s. The advantage of automated over manual techniques is not only their speed of operation but their ability to handle large numbers of samples, treating each one similarly within strictly prescribed and maintained operating conditions. Modern systems are supplied complete with dedicated computer and this greatly simplifies data acquisition, calibration, and the calculation and presentation of results.

For some applications, flow-injection (unsegmented) techniques (FIA) are superseding CFA. FIA is faster than CFA, but as sensitivity is sacrificed to achieve faster sampling rates, FIA is generally not viable where a lack of colorimetric sensitivity is the major limitation, as is the case for nutrients in sea water.

This document dwells on CFA as it is anticipated that this technique will continue to be used widely for many more years, including its latest form, a narrow-bore version with improved dispersion characteristics which generally permit higher sampling rates.

3 CAPABILITIES AND LIMITATIONS

Suppliers of CFA equipment have understated the difficulties of seawater analysis to prospective customers. This is because they have generally lacked hands-on experience with sea water, and the concentration ranges involved, e.g., the determination of phosphate at sub-micromole per litre levels, or, in units more familiar to them and to the freshwater industry, < 0.03 mg P per litre.

Methods may claim, for example, to be suitable for the concentration range 0–10 $\mu\text{mol l}^{-1}$, but a typical supplier's configuration generally does not extract maximum sensitivity from the CFA system, as is necessary for working exclusively in the 0–2 $\mu\text{mol l}^{-1}$ range. Users should be prepared to alter the manufacturer's methods when necessary, but it is important that changes be made in a systematic manner, keeping intact the chemistry of the original manual method on which the CFA version is based.

The range covered by a calibration must be totally appropriate to sample concentrations. In less demanding situations this may not seem important, but at other times it can be critical, as illustrated by the examples below.

- A sample of potable water with a true nitrate content of 8.0 mg l^{-1} (customary freshwater units) is analysed and reported as 9.0 mg l^{-1} . Given that the EU 'MAC' (European Union Maximum Admissible Concentration) for nitrate is 50 mg l^{-1} , the water is evidently acceptable, whatever the source of the 1.0 mg l^{-1} error. In the worst possible case, i.e., where the entire error is caused by a wrongly defined baseline, it follows that a sample containing 1.0 mg l^{-1} would be reported as 2.0 mg l^{-1} , but all is still well if the only concern is whether it is above or below 50 mg l^{-1} .
- Now consider the significance of this 1.0 mg l^{-1} bias for the analysis of a typical North Sea water sample. If its true nitrate concentration is $5.0 \mu\text{mol l}^{-1}$ (customary seawater units), this translates into $\sim 0.3 \text{ mg l}^{-1}$ and with the added bias it would be reported as 1.3 mg l^{-1} . This is a serious error, being too high by a factor of approximately four, and is exactly the kind of situation that can result from an inappropriate calibration and uncritical use of a data system. A calibration curve generated from standards with nitrate concentrations of 10, 20, 30, 40 and 50 mg l^{-1} could have a respectably high correlation coefficient, yet still produce misleading results in this way.

Data systems have impressive work rates, but they are little more than an assemblage of switching devices. They know nothing about chemistry, but they can produce apparently precise and very convincing-looking numbers, and users can easily be misled by them. There is always a temptation to equate apparent precision with implied accuracy, e.g., a nitrate result printed as $3.142 \mu\text{mol l}^{-1}$ implies an uncertainty of ± 0.0005 at the most, whereas ± 0.1 is far more realistic and typical ($\pm 3 \%$).

To guarantee better accuracy than this is possible, but not easy. It can be achieved only if all of the chemical parameters are understood, controlled, and optimized. Then a data system becomes really useful.

4 FLOW CELL CHARACTERISTICS

In CFA, attempts to measure in the $0\text{--}2 \mu\text{mol l}^{-1}$ concentration range generally reveal optical effects which can contribute substantial bias if not taken into account.

First described by Atlas *et al.* (1971), the problem is related to the geometry of individual flow cells. In the interests of good hydraulic performance, typical flow cells have rounded ends and low internal volume; but rounded ends produce effects that masquerade as Absorbance signals. (These effects do not occur in the corresponding manual techniques where the optical surfaces of the cuvettes are aligned at 90° to the incident light beam.) These signals can be demonstrated both in the presence and in the absence of reagents, and they are unrelated to determinand concentrations. They are the result of large changes in the refractive index of the flow cell contents, such as occur when saline samples are separated by a distilled or demineralized water wash. The disruptive effect of these signals ranges from tolerable, in good quality flow cells, to catastrophic in those with an unfavourable internal geometry. In either case, they have to be taken into account when defining the zero-concentration point on the calibration curve (see Section 10.2, below).

5 THE INTER-SAMPLE WASH

Some analysts consider it important to conceal the optical effects described in Section 4 above, by using an inter-sample wash which has a refractive index similar to that of the samples (see Sections 5.1 to 5.3, below). Others, including the author, feel that there is nothing to be gained by concealing these effects, and prefer a pure water inter-sample wash for a variety of reasons; these analysts are content to live with untidy-looking signals at low concentrations, as long as the quality of the analytical chemistry is unimpaired (see Section 10.2, below).

5.1 Artificial Sea Water

One litre of artificial sea water (ASW) (Grasshoff, 1976, p. 152), containing NaCl (32.0 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (14.0 g) and NaHCO_3 (0.15 g), has a nominal salinity of 34.2 parts per thousand, which approximates to 34.2 PSS on the Practical Salinity Scale. (According to F. Culkin (pers. comm., 1992), PSS is preferred to PSU because salinity, as defined by the Practical Salinity Scale, is a ratio and therefore has no units.) Dilution is required where the salinity of samples is markedly lower than 34.2 PSS.

Though this ASW formulation has the required refractive index, contamination problems may limit its usefulness in this context (see Section 7.2, below).

5.2 Natural 'Low-Nutrients' Sea Water

Low-nutrients sea water (LNSW) is, in every possible respect, preferable to ASW. It can be conveniently collected in late spring after the main plankton bloom. If stored in a polyethylene carboy in daylight and at room temperature, internal biological activity will cause the nutrients concentrations in the sea water to continue to deplete naturally and after a few weeks the supernatant can be siphoned off, without filtration, into containers of more convenient size for laboratory use. There is no need for filtration if these operations are carried out carefully. In this way, LNSW can be readily produced with nitrate $< 0.1 \mu\text{mol l}^{-1}$, nitrite $< 0.02 \mu\text{mol l}^{-1}$, phosphate $< 0.02 \mu\text{mol l}^{-1}$, and silicate $\sim 1 \mu\text{mol l}^{-1}$. Residual ammonia concentrations are less predictable because of the potential for airborne contamination (see Section 11.3.4, below).

5.3 Other Preparations

Mostert (1988) described the addition of ethanol to pure water to produce a 9.7 % (by volume) solution which has a refractive index similar to that of sea water. Variations on this theme are mentioned by other workers, but they do not appear to have been widely adopted.

5.4 Pure Water

In practice, 'pure' water is the only liquid that should be relied upon for simultaneous use as a zero-nutrients baseline and zero-nutrients inter-sample wash. Whether originally prepared by demineralization or distillation, its purity is reliably assured *at the point of use* by including a small replaceable ion-exchange resin cartridge within the CFA system, immediately upstream of the auto-sampler's wash point. A suitable device can readily be made from the body of a 20 ml disposable syringe filled with a self-indicating mixed resin such as Duolite MB6113 (Merck) (which changes from dark blue when new, to amber when spent).

5.4.1 Demineralized water

Demineralized water (DMIN) is produced by the demineralization process, which is one of ion exchange and its effectiveness is monitored in terms of electrical conductivity or resistivity. Modern small-scale purification systems, specially designed for laboratory use, generally

employ filtration and reverse osmosis prior to ion exchange, and such systems, according to Mellor (1990), can produce water close to the theoretical resistivity of pure water ($18.15 \text{ Mohm cm}^{-1}$ at 25°C), which is highly suitable for most purposes in nutrients work.

The description 'highly suitable' refers to freshly prepared DMIN. If stored, the effectiveness of the storage regime must be known (see Section 7 on contamination in general, and Section 11.3.4 for notes pertaining specifically to the determination of ammonia).

5.4.2 Distilled water

Users of distilled water must beware of making assumptions about the purity of this water. The distilled product is likely to be purer than the feedstock, but its suitability for nutrients work has to be demonstrated, and not simply assumed.

This warning applies equally to double-distilled water.

6 SAMPLING TECHNIQUES

A full description of sampling techniques is outside the scope of this document, but the arrival on board ship of a rosette sampler containing filled Niskin or similar sampling bottles is taken as the starting point for sample pre-treatment (see Section 8, below). But before commencing any sub-sampling, some appreciation of the role of contamination is necessary.

There is a preferred order for sub-sampling from the rosette bottles, on the basis of perceived contamination risk from the atmosphere, or the susceptibility of the sample to change its composition in some way. The nutrients sub-sample is generally well down the queue, behind sub-samples taken for such determinands as trace organic contaminants, dissolved oxygen, and high-precision salinity.

7 CONTAMINATION

Nutrients concentrations in unpolluted sea water are seldom greater than a few micromoles per litre. Consequently, from the analytical chemist's viewpoint they should be regarded as trace components ($< 1 \text{ mg l}^{-1}$), and the potential for contamination of samples (and reagents) should be appreciated.

7.1 Contamination of Samples

K  rouel and Aminot's (1987) systematic study of contamination appears to be the only one of its kind, and their findings are worth restating here. They ranked nutrients in decreasing order of contamination risk, as follows: urea $>>$ nitrate $>$ ammonia $>$ phosphate $>$ nitrite.

Possible sources of contamination include the ship, sampling equipment, the atmosphere, the analyst, internal (within the sample), or any combination thereof. These sources are described below.

- 1) *The ship*: Sampling must not take place while the ship is discharging sewage or any other wastes, and, where possible, sampling should be carried out on the opposite side of the ship from such discharge points. De-rusting operations generally involve phosphoric acid and should not be undertaken immediately before or during a cruise during which samples for nutrients analyses will be taken.

- 2) *Sampling equipment*: Any surfaces that come into contact with the sample may contaminate it if they are not sufficiently clean. Glass containers present an additional problem. Glass is attacked by sea water and the dissolution rate is significant. For example, a 200 ml borosilicate volumetric flask filled with LNSW will contribute silicate ($\sim 0.5 \mu\text{mol l}^{-1}$) to the contents after 8 hours at room temperature. The dissolution rate for soda glass is two to four times higher (see Aminot *et al.* (1992)).
- 3) *The atmosphere*: Laboratory air can be a source of ammonia contamination, even in the absence of smoking, stored ammonia solutions, and ammonia-producing chemical operations. Exhaust gases from ships' engines are a source of oxides of nitrogen. In ammonia and nitrite determinations, samples awaiting analysis on an autosampler carousel can be protected to some extent from airborne contamination by using a long-reach probe and long (> 10 cm), narrow, partly filled sample tubes. The long, narrow head-space minimizes air circulation at the interface, but, as a general rule, the less time spent on the carousel, the better.
- 4) *The analyst*: Direct finger contact with the sample, or indirect contact via equipment surfaces and/or the atmosphere are sources of contamination (particularly for ammonia and urea).
- 5) *Internal*: Suspended material, organic or inorganic, that is small enough to survive filtration, may contribute physically or chemically (or both) to the Absorbance signal.
 - physical*: Turbidity causes light scatter, which is indistinguishable from absorption in simple colorimetry.
 - chemical*: Reaction conditions, e.g., elevated temperature and/or extremes of pH, may cause otherwise insoluble N, P, Si, etc., to be brought into solution during the actual analysis.

Information of this kind is not generally found in textbooks, but tends to be accumulated slowly and painfully from practical experience.

7.2 Contamination of Reagents

Most aspects of the above discussion of external contamination of samples necessarily pertain also to reagent solutions.

While low-level contamination in constantly flowing reagents may be tolerable, high concentrations will give rise to an excessively noisy baseline signal, the noise being in phase with and characteristic of the action of the pump rollers.

Artificial sea water (ASW), though not strictly speaking a reagent when used as an inter-sample wash, has its own special contamination problems which need to be considered in some detail. Listed below are a typical supplier's specifications for good quality (analytical reagent grade) sodium chloride and magnesium sulphate, the major ingredients of any ASW.

	Maximum concentration limits for specified impurities	
	phosphate (as PO_4)	N compounds (as N)
sodium chloride	0.0005	0.0005
magnesium sulphate	0.001	0.002

Multiple zeros after the decimal point are intended to impress, but the units are percent, and these values represent potential impurity levels that are far from satisfactory. Take, for example, the case where both phosphate and nitrate are present in these two salts at 50 % of their respective maximum concentration limits, and the N is exclusively nitrate. There is a serious problem; the ASW as specified in Section 5.1, above, will contain phosphate and nitrate at 1.6

$\mu\text{mol l}^{-1}$ and $15.7 \mu\text{mol l}^{-1}$, respectively. These concentrations imply that the ASW is completely unsuitable for use as an inter-sample wash.

8 PRE-TREATMENT OF SAMPLES

It is self-evident that visible flora and fauna, microturbids and other suspended material should be removed from the sample, or at least be prevented from taking part in the analytical chemistry. Some samples may be considered free enough from suspended material to permit direct analysis, but for coastal work this may not be the case and the relative merits of filtration and centrifugation need to be considered.

8.1 Filtration

Assuming that the water sub-sample for nutrients analysis requires filtration, a container is needed for the transfer of the sub-sample from the rosette sampler to the filtration apparatus. The use of polyethylene bottles (1-litre) with plastic insert seals and separate screw caps is recommended. Both seal and bottle should be thoroughly rinsed with sample and filled directly from the tap of the rosette bottle, without using drain tubing of any kind.

The filtration process is a potential source of serious contamination but the larger the scale of operation, the lower is the risk (as the ratio of sample volume to surface area of container increases). Where sample size permits, the use of a 1-litre Buchner apparatus is recommended, as well as ample rinsing of the filter with the sample, and of the receiving flask with the filtrate. Glass fibre (GF/C) discs of around 50 mm diameter having a nominal pore size of $\sim 1 \mu\text{m}$ are widely used. The very nature of their composition suggests that glass fibre filters will contribute silicate to any passing filtrate, but in practice, the extent of this contamination is negligible unless a filtration takes an unusually long time to complete.

More thorough filtration can be achieved using polycarbonate membranes if preferred, but, whatever the chosen procedure or scale of operation, the burden is on the analyst to verify its suitability experimentally.

8.2 Centrifugation

To avoid the potential contaminating influence of filtration, K  rouel and Aminot (1987) devised a system whereby the sub-sample from the rosette is collected in a 125 ml polypropylene bottle and, without further transference, this bottle is first centrifuged then presented directly to CFA using a modified commercial sampler. Where facilities are available for centrifugation immediately after sampling, this approach clearly has some possible advantages.

9 STABILITY OF SAMPLES

Nutrients concentrations are liable to change due to the activity of micro-organisms naturally present in sea water. Therefore, as a general rule, samples should not be exposed unnecessarily to any source of light. Analysis within minutes of sampling will always be the preferred and definitive procedure, but where this is not possible, methods of preservation and storage must be considered.

There is ample literature on this subject and the one thing that authors are fully agreed on is that there is no single universally applicable preservation/storage regime that will satisfy all requirements. For example, storage in glass is unacceptable if silicate is to be determined, and

some workers claim that phosphate is removed from the sample by adsorption onto container walls (particularly polyethylene containers).

Some literature claims appear to be openly contradictory, but a large part of the apparent confusion is almost certain to be because authors are never comparing exactly 'like with like'. For example, seawater samples from the same location and depth at different seasons may contain micro-organisms of very different species compositions and concentrations, and it is possible that a given preservative regime could be effective in April, but not in October. Likewise, surface samples obtained simultaneously at nearby locations but from different water masses may have very different contents.

While the debate continues, there are two popular approaches to preservation, both in their own ways intended to arrest the biological processes that cause depletion of nutrients concentrations; these are *refrigeration* and *poisoning*.

9.1 Refrigeration

9.1.1 Freezing

Freezing is the method of choice of many workers, but several important details require attention.

Bottles should be frozen, stored, and thawed in an upright position, and they should not be completely filled. The reason for these recommendations is that during the freezing process the last few millilitres to freeze has a very different composition from the bulk, and if the bottle is completely filled, some of this liquid may be expressed past the seal of the closure during the freezing process. Subsequent thawing produces a sample of unrepresentative composition.

Dissolved silicate is reported to polymerize/crystallize during the freezing process and several authors warn that when samples are thawed prior to analysis, sufficient time must be allowed for depolymerization/redissolution. According to A.J. van Bennekom (pers. comm., 1992), 24 hours is insufficient time for this process, and he recommends that a separate aliquot of sample be reserved for the silicate determination, and stored in darkness at 4 °C.

9.1.2 Non-frozen refrigeration

Non-frozen refrigeration, particularly below 4 °C, is recommended by some workers, but the majority appears to find frozen storage more convincing. For example, Kremling and Wenck (1986) showed that for nitrate and phosphate, storage at 4 °C was totally inadequate for samples of (unfiltered) Atlantic water.

9.2 Poisoning

An alternative approach, particularly where refrigeration is not possible, is the addition of chemicals with the intention of poisoning the species responsible for consuming the nutrients. Of the various chemicals that have been investigated, only three have ever achieved any widespread popularity; they are sulphuric acid, chloroform, and mercuric chloride.

9.2.1 Sulphuric acid and chloroform

Both of these preservatives have had their supporters and critics, and they were recommended individually in specific cases by Standard Methods for the Examination of Water and Wastewater, up to the 12th Edition. However, without explanation, the 13th Edition (1971) and subsequent editions through to the 17th Edition (1989) clearly state "*Preservation with acid or*

chloroform should be avoided". Probable reasons for this change in recommendation, and the topic of chemical preservation in general, are discussed in detail by Kirkwood (1992).

9.2.2 Mercuric chloride

The use of mercuric chloride in this or a similar context was reported before 1920, but Ibáñez (1931) appears to be the first to describe its mode of action in any detail: "The bactericidal action of mercuric chloride prevents the troublesome activity of micro-organisms present in the sea water whilst its very small dissociation constant ($\alpha = 0.013$) is without effect on the pH".

Ibáñez used four drops of a saturated solution per 100 ml of sample, which is approximately 80 μg mercuric chloride per ml of sample, depending on the effect of ambient temperature on concentration. (This is based on translating 'four drops' into 0.12 ml and 'saturated' into 6.9 g/100 ml (the solubility at 20 °C given by the CRC Handbook of Chemistry and Physics).)

Several authors have reported the use of mercuric chloride at a variety of (predominantly lower) concentrations, but over the years it seems to have lost favour, probably due to concurrent interest in the determination of mercury in sea water at nanogram per litre levels, and perceived contamination problems in the use of any form of mercury on research vessels.

In their investigation of the effectiveness of three preservative regimes for nutrients, Kremling and Wenck (1986) were somewhat critical of mercuric chloride. They used 10 μg per ml of sample and concluded that this concentration was "probably . . . inadequate preservation against the ongoing biological activities" at that temperature (4 °C). Their objection to its use at a higher (unspecified) concentration was apparently based on their experience of "erratic nitrate measurements if copperized cadmium reductors are used".

The author (Kirkwood, 1992) has demonstrated that mercuric chloride in samples (20 $\mu\text{g}/\text{ml}$) causes no problems in the methods described in Sections 11.1 to 11.4, below, and that this concentration was an effective preservative, at room temperature, for filtered North Sea (winter) water containing nitrate at $\sim 5.5 \mu\text{mol l}^{-1}$ and phosphate at $\sim 0.5 \mu\text{mol l}^{-1}$. (Six laboratories from the United Kingdom, Norway and France analysed sub-samples of this water over a period of several months and produced remarkably good agreement for both nitrate and phosphate.) As this work and that of Kremling and Wenck are not necessarily contradictory, as they may first appear, the use of mercury in this context should not be totally discouraged. Indeed, in the continued absence of a more acceptable alternative, further investigations with a view to establishing a well-founded recommendable dosage concentration are clearly required.

Note: Mercuric chloride (or any other additive) may cause chemical interference in colour chemistry. The methods described in Sections 11.1 to 11.4, below, have been shown to be unaffected, but this should be verified experimentally by individual analysts, as minor alterations to methods can invalidate claims of this kind (see Section 11.3.4, below).

10 CALIBRATION

Seawater matrix salts affect the kinetics and products of some colour-forming reactions. This is widely known as the *salt effect*, and potential bias due to its influence is countered by ensuring that the composition of calibration solutions is closely matched to that of the samples.

The methods described in Sections 11.1 to 11.4, below, are not seriously affected by salinity differences, but it is important that the analyst be aware of the extent of this source of error in each method.

10.1 Working Calibration Solutions

Working calibration solutions (WCS) (those actually presented to the CFA system) are produced by 'spiking' low-nutrients sea water (LNSW) with standard solutions of nutrient salts.

Diluting, for example, a 1 ml aliquot of a standard solution to 100 ml with LNSW has a negligible effect on the refractive index of LNSW, and the resultant chemical matrix will remain a close approximation to that of a typical seawater sample. (Modern liquid handling devices can dispense a 1 ml aliquot with highly acceptable accuracy and precision, but they should be checked gravimetrically from time to time.)

LNSW, filtered or unfiltered, should be assumed to contain micro-organisms. Consequently, when LNSW has been spiked with a mixture of nutrients, the depletion process is liable to resume. Working calibration solutions are therefore best prepared immediately before their intended use; they may be stored in darkness for short periods but should not be trusted for more than a few hours.

Working calibration solutions consisting simply of nutrient salts in DMIN are appropriate for freshwater samples, but must not be used for the analysis of sea water unless the extent of the salt effect is known and due correction is applied.

10.2 Defining the Zero Concentration in Sea Water

It is self-evident that 'zero-nutrients' sea water would be ideal for defining the zero-concentration signal for each nutrient. In practice, 'near-zero' LNSW is used; ways are then found to determine how close nutrient concentrations are to zero, and the residual concentrations, if significant, are taken into account in the final concentrations assigned to the working calibration solution.

The ultimate zero-nutrients baseline is DMIN in all pumped lines, and the refraction effect described in Section 4, above, can be demonstrated by alternately drawing LNSW and DMIN through the sample line. In practice, the refraction effect is examined under conditions that more closely resemble normal operation, i.e., all reagents flowing, but one vital colour-producing ingredient is omitted.

For example, in the determination of phosphate by the OSU method in Section 11.1.3, omission of hydrazine prevents colour formation, but the presence of relatively high concentrations of the other reagents (sulphuric acid and ammonium molybdate) ensures that normal pH and other conditions pertain.

When omitting an ingredient in this way to produce a 'dummy' reagent, it is important not to alter the concentrations of major components, i.e., those capable of significantly changing the refractive index or the pH of the flow-cell contents. It follows that in the methods described in Sections 11.1 to 11.4, below, ascorbic acid or hydrazine, NEDD, chlorine, and ascorbic acid, respectively, are the 'vital' ingredients.

Using phosphate again as the example, Figures 1 and 2, below, show undamped Absorbance signals for LNSW (35 PSS) in a 50 mm flow cell against a DMIN baseline, using a normal Reagent 1 and a 'dummy' Reagent 2. Sampling times are 1.5 minutes and 6 minutes for Figures 1 and 2, respectively.



Figures 1 to 4. Undamped Absorbance signals; see text below for explanation.

- Appreciable noise levels can be seen at the leading (left) and trailing edges of the signals, but not on the plateau (a). In Figures 1 and 2, (a-b) is ~ 0.011 A, representing a potential error of $\sim 0.2 \mu\text{mol l}^{-1}$. (Each flow cell has its own unique optical characteristics, but these signal shapes and data are typical.)
- The peaks and troughs of the noise are systematic. They are in phase with the action of the pump rollers and are produced only while the refractive index of the flow-cell contents is changing.
- Signal shapes identical to those in Figures 1 and 2 are also obtained when *zero-phosphate LNSW* is sampled with normal Reagents 1 and 2 in place; i.e., position (a) represents zero-phosphate in 35 PSS sea water.
- Figure 3 shows the signal obtained from a 35 PSS LNSW reckoned to contain $0.03 \mu\text{mol l}^{-1}$ phosphate. (The same LNSW was spiked with $0.40 \mu\text{mol l}^{-1}$ phosphate to produce the signal shown in Figure 4, which was then used as a standard-addition calibration, after defining position (a) as zero-phosphate for 35 PSS.)
- In this concentration range, sufficient time must be allowed for the plateau region of the signal to be approached and recognized. (In CFA, 'ideal' signals are not Gaussian peaks, as in chromatography. CFA signals are essentially square-wave, rounded by the effects of dispersion.)
- Errors will result from positions (b) or (c) being wrongly defined as zero-phosphate for samples having salinity 35 PSS; position (a) is correct. Position (b) represents zero-phosphate in DMIN. The peak at (c) is a physical artifact, having no chemical significance. As (a-b) represents the extent of the refraction signal for 35 PSS, the correction required for any given salinity is *pro-rata*.
- The magnitude of the refraction signal (a-b) for a particular flow cell (in Absorbance units) is approximately constant, i.e., independent of wavelength. However, its influence on each determinand should be investigated separately as its value in terms of 'apparent concentration' will differ widely according to colorimetric sensitivity and manifold configuration.

Analysts need to be aware that their data systems require 'educating' in all of these aspects.

10.3 Standard Solutions

Standard solutions are prepared in the laboratory by classical gravimetric and volumetric techniques. The chemicals and water used should be of the purest grades available to the analyst.

Concentrated primary standard solutions (containing hundreds of milligrams of a single salt per litre) can be assumed to have a shelf life of many months if stored in darkness and well protected against evaporation. Silicate is an exception. Solutions containing silicate may be supersaturated and unstable at concentrations exceeding 100 mg per litre.

When using multi-channel CFA for the simultaneous determination of three or four nutrients, it is very convenient to combine the serial dilution of primary standard solutions into a single mixed solution of nutrients of intermediate concentration which can be used as a spiking solution to produce WCS, as described in Section 10.1, above. However, a mixture of this kind should be assumed to have a composition that will support the same kind of biological action as occurs in natural samples; therefore, its working life should be considered very short (hours) unless effective steps are taken to prevent such action.

Kirkwood (1992), with the help of 18 collaborating laboratories, demonstrated excellent stability (> 12 months) for a combined solution of nitrate, phosphate, and silicate stored in polycarbonate containers and protected from biological action by the addition of 20 µg HgCl₂ per ml.

As nitrate is the eventual end product of the oxidation of such nitrogen-containing ions as nitrite and ammonium, solutions containing combinations of these ions need to be treated with some circumspection.

Storage under refrigeration is widely practised, but solutions must be brought to room temperature before use, otherwise systematic volumetric errors will be introduced.

When preparing a new primary standard solution or combined intermediate solution, continuity should always be checked by comparing the old solution with the new solution, before discarding the remains of the old solution.

10.4 Calibration Ranges

The following table shows approximate maximum concentrations of working calibration solutions normally used by the author in the methods described in Sections 11.1 to 11.4, below. They assume the use of a data system capable of handling non-linear calibrations, but in no case is the deviation from linearity greater than ~ 10 % at the maximum concentration. Typical Absorbance values obtained from these concentrations are also shown.

Solution	Maximum concentration (µmol l ⁻¹)	Absorbance (A)	Wavelength (nm)
phosphate	5	0.4	880
nitrate	40	1.2	540
nitrite	5	0.2	540
ammonia	10	0.3	630
silicate	50/140	1.2	810/660

10.5 Certified Reference Materials

Unfortunately, there are no certified reference materials (CRMs) available for nutrients in sea water. Several organizations are presently interested in their development, but a major obstacle to the full certification process is the lack of alternative methods to confirm nutrients concentrations. Some form of ion chromatography may provide the key.

Certified standard solutions specifically for marine work have been commercially available for several years (e.g., Sagami standards, from Wako Chemicals GmbH, Nissanstraße 2, 4040 Neuss 1, Germany). These solutions consist of a sodium chloride matrix of stated nominal salinity and, as their nutrients concentrations are appropriate, they are analysed directly without

dilution. Though it may be said that they are not quite the 'real thing', use of such solutions should be encouraged because the credibility of results for samples is considerably enhanced if the measurement technique produces a simultaneous successful analysis of certified solutions.

11 ANALYTICAL METHODS (CONTINUOUS-FLOW ANALYSIS)

The following Sections 11.1 to 11.4 contain full descriptions of CFA methods suitable for the determination of nutrients at natural concentrations in North Sea waters.

In the author's laboratory, each of these methods is part of a four-channel system supplied by an auto-sampler which is limited to a maximum sample-cup size of ~ 7 ml. As this imposes constraints on sample uptake rates, 0.8 ml/min has been adopted as the maximum rate for any single channel. The chemistry and manifold of each method have been re-scaled accordingly, and, as far as possible, the chemistry remains identical to that of the original method on which it is based.

The author currently uses the SA4000 system manufactured by SKALAR Analytical B.V., Breda, Netherlands, but the methods described in Sections 11.1 to 11.4 should be readily translatable into equally satisfactory methods for systems produced by other manufacturers.

11.1 Determination of Phosphate

11.1.1 Chemistry (phosphate)

Every phosphate method that uses molybdate, sulphuric acid, potassium antimonyl tartrate, and ascorbic acid as reducing agent, whether manual or automated, is descended from Murphy and Riley's (1962) manual method.

A recent re-investigation of Murphy and Riley's method by Pai, Yang and Riley (1990) consisted of a comprehensive study of the effects of variation of acidity and molybdate concentration. The acid/molybdenum ratio was shown to be crucial, influencing not only the composition of the final reduced complex, but also playing an important role in the reaction kinetics.

Their message is clear: the underlying chemistry of the original manual method should not be tampered with. Nevertheless, an ICES Intercomparison Exercise (Kirkwood *et al.*, 1991), which included a survey of phosphate methodology, showed that analysts and equipment suppliers alike make apparently arbitrary changes to their own and to each others' methods, sufficient to cause substantial divergence from the parent methods on which they claim to be based. Conclusions from chemical interference studies on the original method may be invalidated by such changes.

Pai, Yang and Riley's re-investigation confirmed the suitability of Murphy and Riley's (1962) method, and CFA users might be well advised to abandon their individual adaptations unless they adhere closely to the concentrations and conditions originally specified. These consisted of 40 ml of sample plus 8 ml of a 'single mixed reagent', diluted to volume in a 50 ml flask, then maintained at room temperature for a minimum of 10 minutes before Absorbance measurement at 880 nm.

Their mixed reagent was prepared by combining the following ingredients:

sulphuric acid (5 N)	2500	mmol l ⁻¹	125	ml
ammonium molybdate (40 g l ⁻¹)	32.37	mmol l ⁻¹	37.5	ml
ascorbic acid (0.1 M)	100	mmol l ⁻¹	75	ml
potassium antimonyl tartrate (1 mg Sb per ml)	8.21	mmol l ⁻¹	12.5	ml
	consequent volume		250	ml

Final concentrations in the cuvette are as follows (in mmol l⁻¹):

sulphuric acid H ₂ SO ₄	(mol. wt. 98.1)	200
ammonium molybdate (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	(mol. wt. 1235.9)	0.777
ascorbic acid C ₆ H ₈ O ₆	(mol. wt. 176.1)	4.8
potassium antimonyl tartrate KSbC ₄ H ₄ O ₇	(mol. wt. 324.9)	0.0657

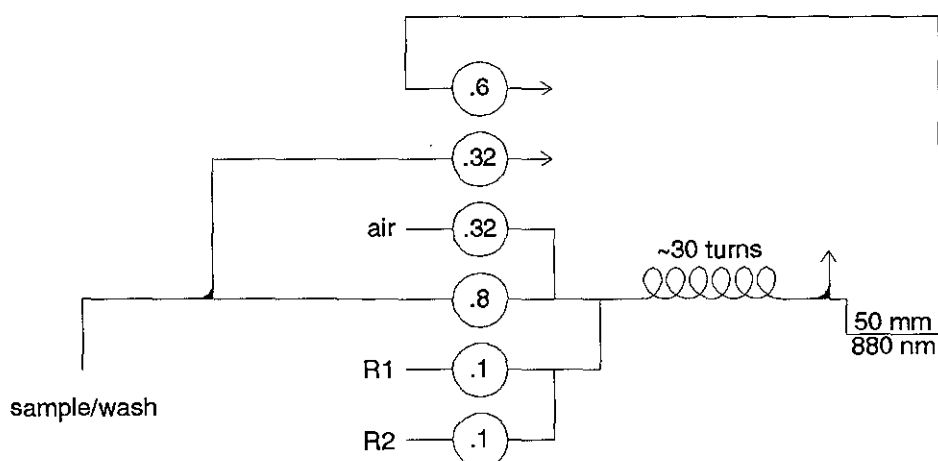
11.1.2 Sensitivity (phosphate)

From Murphy and Riley's (1962) experimental data, a molar absorptivity (ϵ) of $\sim 23,000$ at 882 nm can be derived for the phosphoantimonylmolybdate complex. It follows that a 50 mm path-length cuvette or flow cell containing sample with a phosphate concentration of $1.0 \mu\text{mol l}^{-1}$ (taking account of the small but significant dilution by the added reagents) should produce an Absorbance of 0.091 A. Assuming 0.001 A as an empirical limit of detection for manual colorimetric techniques, it is evident that the measurement of sub-micromolar phosphate concentrations with a precision of $\pm 0.01 \mu\text{mol l}^{-1}$ is about the best that can be expected.

In a CFA manifold, the sample should not be subjected to any more dilution than that of Murphy and Riley's manual method. That is to say, flow cell effluent should contain $\geq 80\%$ sample, otherwise valuable sensitivity is lost.

11.1.3 The CFA manifold (phosphate)

The flow diagram below shows an example of CFA which adheres closely to the parent manual method; it also contains Koroleff's (1967) split-reagent refinement which solves the instability problem associated with Murphy and Riley's single mixed reagent.



Reagent 1	sulphuric acid (5 mol l ⁻¹)	5000	mmol l ⁻¹	200	ml	⊗ 500 ml
	ammonium molybdate (40 g l ⁻¹)	32.37	mmol l ⁻¹	120	ml	
	potassium antimonyl tartrate (5.3 g/100 ml)	163.1	mmol l ⁻¹	2	ml	
Reagent 2	ascorbic acid			4.2	g	⊗ 500 ml
	Aerosol 22® (wetting agent)			0.5	ml	

Assuming that the nominal flow rates for pump tubing are accurate, the final concentrations of these constituents in the flow cell are within $\pm 1\%$ of those of Murphy and Riley's manual method listed in Section 11.1.1, above.

Manufacturers generally include a $37\text{ }^{\circ}\text{C}$ heating cartridge, but this is not entirely necessary. However, if reaction time is to be maintained around or below the 10-minute minimum of the manual method, it is essential that room temperature be above $20\text{ }^{\circ}\text{C}$. Completeness of the reaction should, of course, be checked by addition/subtraction of coils. An excessive number of coils may cause hydraulic problems as well as contribute to adsorption/desorption effects (sometimes referred to as flow-cell coating), that are understood to be a consequence of the colloidal nature of the phosphoantimonylmolybdate complex. When this effect causes peak shapes and separation to deteriorate to an unacceptable level, satisfactory conditions can generally be restored by pumping a solution of sodium hydroxide (1.0 mol l^{-1}) through the sample line for a few minutes.

Atlas *et al.* (1971) described a method known as the 'OSU' (Oregon State University) method, which omits the antimony salt, uses hydrazine as reductant and claims a 15 % increase in sensitivity; but it is not clear whether this increase is in true molar absorptivity terms, or is a consequence of less dilution of the sample. According to L.I. Gordon (pers. comm., 1989), the OSU method is free from the flow-cell coating effects caused by the antimony-containing salt in Murphy and Riley's method. The OSU phosphomolybdate complex is certainly different from Murphy and Riley's complex, as the latter was shown (by them, in 1962) to contain antimony in a 1:1 P:Sb ratio; therefore, it is incorrect to refer to the antimony as a catalyst, as some authors have done.

Using the same manifold as shown in the preceding flow diagram, the author has had highly satisfactory results from the following adaptation of the OSU method. (The reaction is heated at 65 to $70\text{ }^{\circ}\text{C}$ and is thus compatible with the temperature requirements for the ammonia manifold described in Section 11.3, below.)

Reagent 1	sulphuric acid (5 mol l^{-1})	200 ml]	→ 500 ml
	ammonium molybdate (40 g l^{-1})	120 ml		
Reagent 2	hydrazine hydrochloride	0.5 g]	→ 500 ml
	Aerosol 22® (wetting agent)	0.5 ml		

11.2 Determination of Nitrate and Nitrite

Nitrate and nitrite are discussed together as they rely on the same colorimetric procedure.

11.2.1 Chemistry (nitrate and nitrite)

In the absence of a suitable reaction for the direct colorimetric determination of nitrate, the most widely used method relies on reduction to nitrite which then reacts to form a diazo-couple compound. Nitrite originally present in the sample requires separate determination and subtraction, but this is not a serious drawback of the method, as generally nitrate concentrations are very much greater than those of nitrite, and the proportion of the response attributable to nitrite is small relative to the precision attainable for the nitr(ate + ite) determination.

Bendschneider and Robinson (1952) were the first to investigate the use of sulphanilamide as a diazotizing agent and N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) as a coupling agent, in a seawater context. They stated that for maximum colour development, the sample should be mixed with the sulphanilamide and hydrochloric acid before adding the coupling agent, and showed that the final solution should have a $\text{pH} < 2.4$.

Their method uses 50 ml sample to which 1 ml sulphanilamide reagent (containing HCl) is added; then after 2 to 6 minutes, 1 ml of coupling agent is added, and Absorbance measurement is made at 543 nm at least 10 minutes later.

Their two reagents comprised:

- a) sulphanilamide (5 g) in 500 ml of 1.2 N hydrochloric acid;
- b) N-(1-naphthyl)ethylenediamine dihydrochloride (0.5 g) in 500 ml water (NEDD).

Final concentrations in the cuvette are as follows (in mmol l⁻¹):

sulphanilamide C ₆ H ₈ O ₂ N ₂ S	(mol. wt. 172.20)	1.12
hydrochloric acid HCl	(mol. wt. 36.5)	23.1
NEDD C ₁₂ H ₁₄ N ₂ ·2HCl	(mol. wt. 259.2)	0.074

Reduction of nitrate was formerly performed off-line, as in manual methods; but on-line systems based on copperized cadmium in the form of granules, wire or tube, have long been the norm among CFA users.

Several authors stress the need to carry out the reduction at pH ~ 8. Waters with an appreciable salinity naturally have a pH in this region, and have enough buffering capacity to be unaffected by the relatively low concentration (3.0 g l⁻¹) of ammonium chloride included in the method described here.

The role of ammonium chloride is understood to be complexation and removal of ionized cadmium, a by-product of the reduction of nitrate to nitrite, yet some published methods refer to it erroneously as a buffer. A solution containing *only* ammonium chloride does not function as a pH buffer; and if freshwater samples with a naturally low pH are to be analysed, steps must be taken to adjust the ammonium chloride solution to pH 8 to 8.5 by adding, e.g., ammonia, so that it *will then* act as a buffer, otherwise a substantial loss of sensitivity and precision can be expected (Collos *et al.*, 1992).

Various additives to the ammonium chloride, such as EDTA, sodium tetraborate and ammonia, are reputed to prolong reductor life by preventing the formation of cadmium hydroxide or carbonate; but whichever solution is selected, the HCl concentration in the colour-producing reagent must be such that the pH is maintained at < 2.4 in the colour reaction.

Reductor efficiency should be checked by comparing steady-state signals from identical concentrations of nitrate and nitrite (in LNSW) passed separately through the system in nitr(ate + ite) mode. It is important that the efficiency of the nitrate to nitrite reduction be maintained as close to 100 % as possible, and it should preferably not fall below 95 %. When a reductor produces a low yield of nitrite, substantial errors may occur if mixed (nitrate + nitrite) standards have a significantly different nitrate:nitrite ratio from that of typical samples. A recent paper deals with this problem in detail and gives typical examples (Garside, 1993).

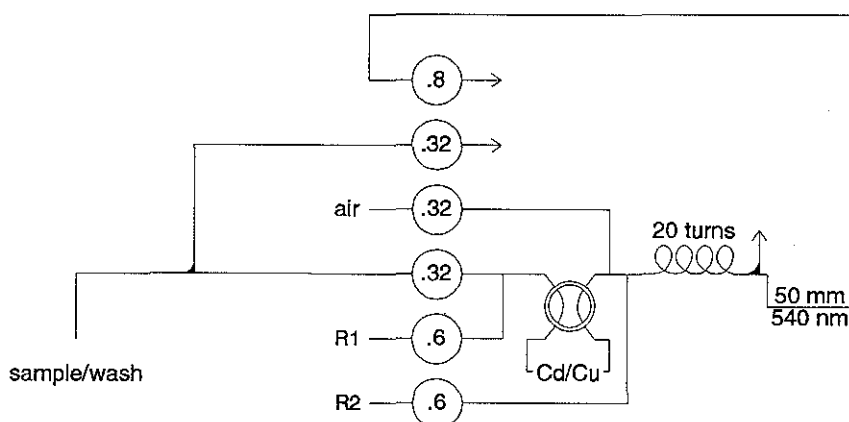
When a reductor is not in use, it should be stored filled with ammonium chloride solution.

11.2.2 Sensitivity (nitrate and nitrite)

From Bendschneider and Robinson's experimental data, a molar absorptivity (ϵ) of ~ 50,000 at 543 nm can be derived. This gives the method a potential sensitivity approximately twice that of Murphy and Riley's phosphate method.

11.2.3 The CFA manifold (nitrate and nitrite)

The flow diagram below shows a dual-purpose manifold which can readily be switched between nitr(ate + ite) and nitrite, and is suitable for typical North Sea (nitrate) concentrations.



Reagent 1	ammonium chloride	3.0 g l ⁻¹	
Reagent 2	sulphanilamide	500 mg	} → 1 litre
	conc. hydrochloric acid (~ 12 mol l ⁻¹)	5 ml	
	NEDD	50 mg	
	10 % BRIJ-35® (wetting agent)	0.5 ml	

Some published methods use air segmentation and mixing coils at the ammonium chloride addition step, but this requires a debubbler before the reductor, then re-segmentation after reduction, all of which adds dead volume. The manifold shown, which includes a single colour reagent, produces good peak shapes, etc., indicating that satisfactory mixing and reaction are achieved despite its simplicity.

A four-way valve (Pharmacia LV-4) protects the reductor from unwanted incursions and is compatible with many CFA components. For example, if a PT II or similar three-way connector is used at the confluence of the sample line and the ammonium chloride supply, it can be mounted directly in a valve port. Using this configuration, the wetting agent need not be added until after the reductor, if at all (see Section 12.3, below). The absence of wetting agent from the reduction stage has a beneficial effect on reductor life and stability.

The working lifetime of a reductor depends heavily on its exposure to nitrate. Gradual dissolution of the metal increases dead volume, which produces poor wash-out characteristics, i.e., deterioration in peak shape and in the separation between consecutive peaks. In the case of SKALAR's glass U-tube packed with Cd/Cu granules, a significant increase in the interstitial volume can easily be seen, and it is a simple matter to isolate the reductor (with the aid of the four-way valve) and add more granules to the inlet end to compensate.

The dual-purpose manifold shown is necessarily a compromise, and if the determination of nitrite is required simultaneously and/or at a higher sensitivity, a separate CFA channel should be dedicated to nitrite. Given that nitrate concentrations are typically an order of magnitude greater than those of nitrite, a greater proportion of sample than that shown (21 %) should be used, and the reagent concentrations altered accordingly, e.g., sample 0.8 ml/min, combined reagent (all ingredients at 3.5 times the stated concentrations) 0.1 ml/min.

11.3 Determination of Ammonia

11.3.1 Chemistry (ammonia)

The most widely used colorimetric method for ammonia is based on the reaction attributed to Berthelot in 1859. Under alkaline conditions (pH 8 to 11.5), ammonia reacts with hypochlorite to form monochloramine, which, in the presence of phenol and excess hypochlorite, forms indophenol blue.

Various authors have adapted this reaction for the direct CFA determination of ammonia in sea water, and the method described here is based on that of Tréguer and LeCorre (1975). Their method produces a response which varies only slightly over the entire salinity range, and in this respect it is understood to be an improvement on other contemporary methods. Following long experience with the method, A. Aminot and R. Kérouel (pers. comm., 1992) have suggested some minor modifications and these have been taken into account in this description.

The main point of difference between Tréguer and LeCorre's method and that described here, is that reagent stability has been improved by altering the combinations used. There has also been a general increase in reagent concentrations accompanied by a lowering of the reaction temperature from 80 °C to 65 °C, in deference to those authors who have expressed fears that extra ammonia may be brought into the determination from hydrolysis, etc., of nitrogen-containing molecules if higher temperatures are used.

Final concentrations in the flow cell are as follows:

		This method (mmol l ⁻¹)	Tréguer and LeCorre (mmol l ⁻¹)
trisodium citrate Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	(mol. wt. 294.1)	61.8	24.2
sodium nitroprusside Na ₂ [Fe(CN) ₅ NO]·2H ₂ O	(mol. wt. 297.9)	0.488	0.274
phenol C ₆ H ₆ O	(mol. wt. 94.1)	17	9.44
sodium hydroxide NaOH	(mol. wt. 40)	25	14
chlorine Cl ₂	(mol. wt. 71)	0.83	0.43

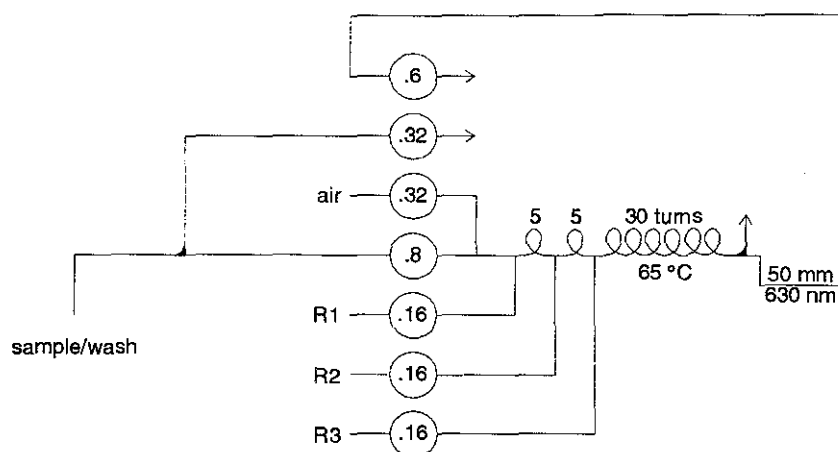
11.3.2 Sensitivity (ammonia)

From Tréguer and LeCorre's experimental data, a molar absorptivity (ε) of ~ 10,000 at 630 nm can be derived for indophenol blue.

Richards and Kletsch (1964) described a method whereby ammonia is oxidized to nitrite, which can then be determined as in Section 11.2, above. While their method is potentially around five times more sensitive than methods based on indophenol blue, the subtraction of nitrite from (ammonia + nitrite) has serious implications for accuracy and precision when concentrations of both are near to detection limits.

11.3.3 The CFA manifold (ammonia)

The flow diagram below shows a CFA manifold for ammonia determination. The entire manifold should be protected from sunlight, otherwise changes in incident light levels may cause baseline instability.



Reagent 1	trisodium citrate	62.5 g	}	→	500 ml
	sodium nitroprusside	0.5 g			
	10 % BRIJ-35® (wetting agent)	0.5 ml			
Reagent 2	phenol	5.0 g		→	500 ml
Reagent 3	sodium hydroxide	3.4 g	}	→	500 ml
	chlorine donor*	1.0 g			

* Hypochlorite has lost favour due to its instability and has generally been replaced by salts (Na or K) of dichloroisocyanuric acid, for example, $C_3Cl_2N_3O_3Na$ (mol. wt. 219.9) (Eastman product no. 10511).

11.3.4 Special contamination problems (ammonia)

The ammonia determination is particularly susceptible to atmospheric contamination. This is generally less of a problem at sea, but it can be particularly serious on land if the laboratory is urban and multi-purpose, as is often the case. In such situations, ammonia may be in use as a reagent or cleaning material elsewhere in the building, yet close enough to affect the analysis. Some ventilation and ducting systems can *move* airborne ammonia very effectively, without actually *removing* it.

Less obvious, but equally important, are the implications for samples in storage prior to analysis. Plastic containers may be permeable to airborne ammonia; therefore, seawater samples awaiting analysis must be stored under very clean conditions, and completely separate from wastewater samples, etc.

Some analysts have the opinion that the results of samples freshly collected and immediately analysed at sea are the only ammonia results that have any chance of being accurate.

11.3.5 Chemical interference from mercuric chloride

During the course of the 1993–1995 QUASIMEME project (Wells *et al.*, 1993), it became clear that several of the fifty laboratories which determined nutrients encountered an interference problem in their ammonia colour-chemistry due to the presence of mercuric chloride in solutions that they were required to analyse in a proficiency testing programme. Mercury was shown to suppress colour formation *in some cases*, and although all of the methods used were based on indophenol blue, their diversity made it impossible to relate the interference to any readily identifiable feature of these methods.

The method described here has been shown to be unaffected by the addition of mercuric chloride to samples, up to at least $20 \mu g ml^{-1}$.

11.4 Determination of Silicate

11.4.1 Chemistry (silicate)

Koroleff thoroughly investigated the determination of silicate in sea water and his 1971 manual method, described in Grasshoff *et al.* (1983), appears to be the first to use ascorbic acid as reductant in this context.

At low pH, dissolved silicate reacts with ammonium molybdate to form silicomolybdic acid, of which there are α and β isomers, and the kinetics of their formation are complex. Once formed, the α isomer is the more stable but its formation is slow. The β isomer is formed rapidly and preferentially, but the presence of seawater salts favours its transformation into the α isomer faster than would be the case in fresh water. Reagent ratios and pH are optimized to favour formation of the β isomer, which is then reduced by ascorbic acid to a stable blue-coloured complex. Oxalic acid prevents the reduction of excess molybdate, which would otherwise interfere in the colorimetry.

Koroleff's manual method used 25 ml sample to which 1.0 ml of an acid/molybdate reagent was added, then after a salinity-dependent time interval, 1.0 ml of oxalic acid solution was added followed immediately by 0.5 ml of ascorbic acid solution, then Absorbance measurement at 810 nm after 30 to 60 minutes. Obviously, a salinity-dependent time interval cannot be accommodated in CFA, but Grasshoff's concentrations (substantially different from Koroleff's) achieve a compromise whereby the response to silicate varies only slightly (3 %) over a wide salinity range; this variation can be neglected in less demanding applications.

Final concentrations in the cuvette/flow cell are as follows:

		Koroleff (mmol l ⁻¹)	Grasshoff (mmol l ⁻¹)
sulphuric acid H ₂ SO ₄	(mol. wt. 98.1)	81.8	25.4
ammonium molybdate (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	(mol. wt. 1235.9)	1.86	1.42
oxalic acid (COOH) ₂ ·2H ₂ O	(mol. wt. 126.1)	28.8	11.9
ascorbic acid C ₆ H ₈ O ₆	(mol. wt. 176.1)	2.89	22.7

11.4.2 Sensitivity (silicate)

Koroleff reported molar absorptivities (ϵ) of 19,000 in sea water and 22,000 in distilled water, and attributed the difference to the effect of salinity on the transformation rate of the β -form into the α -form of silicomolybdic acid.

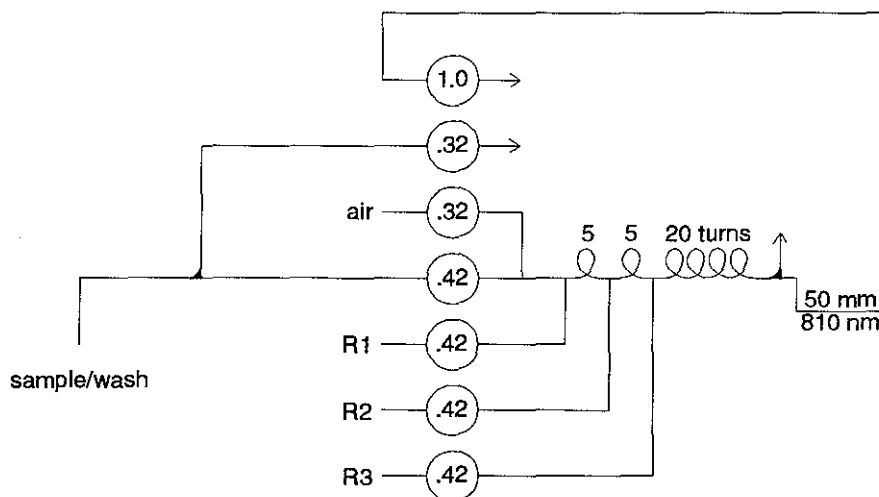
The absorption spectrum of reduced β -silicomolybdate lends itself to the use of alternative wavelengths to extend the range of measurement. Maximum sensitivity is at \sim 810 nm, but an attenuation of \sim 2.5 is available at \sim 660 nm, and the latter wavelength is widely used in oceanic work.

11.4.3 The CFA manifold (silicate)

Grasshoff was evidently aware of a potentially serious temperature dependence, which has also been mentioned by others. He chose to counter this by constructing the entire manifold from polyethylene or Teflon tubing wound around a temperature-controlled cylindrical former. Good results have been obtained in the author's laboratory by winding 0.5 mm i.d. polyethylene tubing externally on a SKALAR 30 mm o.d. heat exchanger, then insulating the entire assembly with 'bubble-wrap' packaging material.

The choice of reaction temperature is less important than its stability, but to ensure that it will not be overtaken by the laboratory temperature, $\sim 30\text{ }^{\circ}\text{C}$ may be more suitable than the $25\text{ }^{\circ}\text{C}$ suggested by Grasshoff.

The flow diagram for this silicate determination is shown below.



Reagent 1	sulphuric acid (5 mol l^{-1})	5000	mmol l^{-1}	9.4	ml	} → 500 ml
	ammonium molybdate (40 g l^{-1})	32.37	mmol l^{-1}	88	ml	
	Aerosol 22® (wetting agent)			0.5	ml	
Reagent 2	oxalic acid			3.0	g	→ 500 ml
Reagent 3	ascorbic acid			8.0	g	→ 500 ml

The chemistry is identical to that of Grasshoff *et al.* (1983, p. 374).

12 MISCELLANEOUS TECHNICAL CONSIDERATIONS

12.1 Multi-channel Systems

Section 11 has already indicated that some compromises may have to be made when assembling a multi-channel system from its component individual methods.

To accommodate the various temperature requirements, some manufacturers supply electrically heated coils for fixed or variable temperature operation. A glance at the temperature requirements of the methods described in Sections 11.1 to 11.4 would suggest that multiple heating units are necessary for the simultaneous operation of these chemistries, but, in practice, a single external circulating water bath is sufficient if jacketed-coil heat exchangers of the type supplied by SKALAR are used.

For all but silicate, the chemistries are such that they operate in a large temperature range above a characteristic minimum. For example, the nitrate/nitrite manifold operates anywhere in the range understood by room temperature, whereas phosphate (Murphy and Riley method) is best described as marginal, needing to be above $20\text{ }^{\circ}\text{C}$ but suffering no adverse effects at $60\text{ }^{\circ}\text{C}$ or even higher. The phosphate (OSU method) and ammonia manifolds produce no worthwhile colour below $40\text{ }^{\circ}\text{C}$, but at around $65\text{ }^{\circ}\text{C}$ both perform well and can be heated by a single circulating water bath supplying the two heat exchangers in parallel.

The silicate manifold has a special requirement for temperature stability (see Section 11.4.3) and, unlike the others, its chemistry produces useful colour only within a restricted temperature

range. If sent *through* a heat exchanger in the normal way, colour development can be shown to collapse above $\sim 40\text{ }^{\circ}\text{C}$. In the configuration described in Section 11.4.3, the heat exchanger around which the silicate manifold is wound can be simultaneously in use for phosphate or ammonia at $65\text{ }^{\circ}\text{C}$ while providing enough waste heat to maintain the silicate chemistry significantly above room temperature, but below $40\text{ }^{\circ}\text{C}$, and at high stability.

12.2 Over-range Concentrations

The foregoing sections are concerned primarily with oceanic or open-sea concentration ranges which generally demand that instrumentation and manifolds be configured in a way that will produce high sensitivity towards the determinands. For estuarine or coastal work, where typical samples may contain very much higher concentrations, there are several points to consider, as noted below:

- A linear relationship between Absorbance and concentration (i.e., compliance with the Beer-Lambert law) must be demonstrated, not assumed.
- Colorimetric non-linearity may be due to instrumental or chemical reasons, or a combination of both. The use of short path length flow-cells and alternative wavelengths provides some instrumental flexibility, but it may be both necessary and preferable to manipulate the chemistry. For example, the lifetime of a cadmium/copper reductor is much reduced by exposure to excessive nitrate concentrations. This exposure can be reduced either by reconfiguration of the manifold to a lower sample uptake rate, or by preliminary dilution of samples, or both.
- Samples may be diluted externally, or within the manifold (by a dilution loop), or in extreme cases, by dialysis. Whichever the method, due attention must be given to the salt effect described in Section 10, above.
- When using a dilution loop, it is not sufficient to calculate the extent of the dilution from the nominal flow rates of pump tubes. High-concentration working calibration solutions, appropriate to the undiluted samples, must be put through the complete system. This applies also to dialysis.
- Where only a small proportion of the samples are over range and the normal high-sensitivity manifold configuration is retained, external dilution of the samples, *using LNSW as diluent*, ensures that the normal calibration procedures remain valid and no errors due to the salt effect are introduced.

12.3 Hydraulics

Some general points to note are the following:

- Unnecessary dead volume should be pursued, and eliminated wherever practical.
- Unsegmented flows, such as between the auto-sampler probe and the point of air segmentation, should be kept as short as possible to minimize mixing across the sample/wash interface.
- Reagent liquid levels, manifold components, and waste outlets should all be in approximately the same horizontal plane, as unnecessary pressure heads generally contribute unwanted signal noise.

- Ionic and non-ionic wetting agents have very different modes of action and they must not be treated as interchangeable. (Analysts discover this accidentally.)
- Recommended concentrations for wetting agents should be treated as maxima. Exceeding them may cause precipitation in high-salinity manifolds and the resultant turbidity will produce false positive Absorbance signals that will bias results. Grasshoff *et al.* (1983, p 369) gives an example of this effect.
- Wetting agents may even be unnecessary. If a system will run smoothly without them, there is no reason to include them.

12.4 Ship-board Operation

The ship's motion may influence the behaviour of CFA systems. Finely balanced manifolds that give trouble-free operation on land may give problems on board ship and must not be assumed to be satisfactory without testing. Debubblers can be weak points in this respect, and manifolds may have to be reconfigured to produce more liquid loss at debubblers. This may degrade wash-out characteristics to a small extent.

Purpose-built research ships have electricity supplies with the required stability for CFA systems, but on other ships this may be a problem.

12.5 Photometer Developments

Recent developments in instrumentation (by SKALAR) employ a dual-wavelength system which is analogous to background correction in atomic absorption spectrophotometry. The refraction component contributes equally to the signal at the analytical wavelength and to that at the (subtracted) non-analytical wavelength. This approach may become increasingly popular as it also implies automatic correction for turbidity in samples. There is, however, some loss of colorimetric sensitivity (typically ~ 25 %) when using the correction mode, so its pros and cons need to be carefully considered on an individual determinand basis.

13 ICES INTERCOMPARISON EXERCISES

Intercomparison (I/C) exercises have played an important role in the development of techniques and in the improvement of analysts' performance in the application of these techniques.

The earliest attempts to study the intercomparability of nutrients determinations were multi-ship events in which freshly obtained samples of sea water were distributed and analysed by participants within hours. The reports by Koroleff (1965) and Grasshoff (1966) describe what were, in effect, intercomparison exercises that can be designated NUTS I/C 1 and NUTS I/C 2, respectively.

Soon afterwards, in 1969/1970 in an ICES/SCOR exercise organized by the ICES Working Group on Chemical Analysis of Seawater, 45 laboratories in twenty countries analysed solutions (not sea water) prepared and distributed by the Sagami Chemical Research Center, Japan. The collection and compilation of results for this exercise, here designated NUTS I/C 3, took some time and the final report by Koroleff *et al.* was published in 1977. (This was the first intercomparison exercise to report on nitrate determinations.)

Some time passed before the conduct of the ICES Fourth Intercomparison Exercise for Nutrients in Sea Water (NUTS I/C 4), when Kirkwood *et al.* (1991), in an attempt to achieve more

realism, made a point of using samples that had originated as natural sea water. A description of these samples follows:

- *Sample 1* was oceanic water from a sampling depth of 1000 m in a water depth of 2700 m off southeastern Greenland. In laboratory experiments, sub-samples from a similar depth and location one year earlier had shown a potentially useful stability after several months of storage in glass bottles, at room temperature and without treatment of any kind. The stability of this water was a surprise to many of the participants, but the measure of agreement achieved for its nitrate and phosphate concentrations seemed to confirm that it was at least stable enough for use in this context.
- *Sample 2* was shelf-sea water that had been filtered, bottled, capped, then sterilized by heat treatment in an autoclave. This appears to be a promising approach to the production of a Standard Reference Material for nutrients (Aminot and K  rouel, 1991).
- *Sample 3* was shelf-sea water that had been allowed to become depleted and therefore should have had nutrients concentrations below the detection limits of participants' techniques. Two bottles (one glass, the other polypropylene) of this sample were supplied, but participants were unaware that these bottles contained the same sample. Their inclusion proved effective in the identification of bias from various sources.

For the 68 participants in NUTS I/C 4 (the great majority of them well accustomed to the determination of nutrients at marine concentrations), it was perhaps something of a surprise to discover that sixteen of them produced unacceptable results for nitrate, and ten produced unacceptable results for phosphate. Surprise or not, it was something well worth finding out, since unacceptable performance can be remedied once recognized. In many cases it would appear that analytical sensitivity and precision were adequate, but the major problem was a lack of attention to important details in the calibration procedures. This is the same conclusion as was reached by Jones and Folkard in their contribution to the NUTS I/C 3 report (Koroleff *et al.*, 1977); twenty years on, not much had changed.

The ICES Fifth Intercomparison Exercise for Nutrients in Sea Water (NUTS I/C 5), with 132 participants, began with a distribution of samples in late 1992. All of the samples were prepared by spiking depleted sea water, then sterilizing in an autoclave (as for Sample 2 of NUTS I/C 4). In NUTS I/C 5 there were no blanks. Concentrations covered a greater range than in NUTS I/C 4 and were at three levels. Nitrite and ammonia were included (but not silicate), and the results showed that the determination of ammonia was a problem for the majority of participants (Aminot and Kirkwood, 1995).

A well-designed intercomparison exercise should not simply come to an end with the listing of results, but should find ways of helping the poorer performers to improve. To aid in this process, it is immensely valuable to the organizers to have detailed information from the participants on how they obtained their results. The ammonia problem made evident by NUTS I/C 5 prompted the distribution of a questionnaire to all participants, and a preliminary report on its outcome was presented (by Kirkwood and Aminot) at the 1995 meeting of the ICES Marine Chemistry Working Group (MCWG) in Reykjavik, Iceland. (An open-literature version is currently in preparation.)

Since techniques, equipment, and personnel all change with time, the skills of individual laboratories will also be subject to change and should be tested by formal intercomparison at regular intervals.

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