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NUTRIENT ANALYSIS IN TROPICAL MARINE WATERS

Practical guidance and safety notes for the performance of dissolved micronutrient analysis in sea water with particular reference to tropical waters

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PREFACE

In the context of the regional programme agreed upon at the Third Session of the IOC Regional Committee for the Co-operative Investigation in the North and Central Western Indian Ocean, Vacoas, Mauritius, 14-18 December 1992, and as a follow-up to that meeting, IOC-SAREC organized a Nutrient Intercalibration Exercise, the third in a series of nutrient training activities which began in 1991. The Exercise took place in Zanzibar, Tanzania, between 5 and 16 April 1993.

The goal of the nutrient training series is to build capacity of a core of East African nutrient chemists and to foster interregional co-operation and co-ordination through the standardization of sampling and analytical methods.

This manual, which was prepared by Dr. Ron Johnstone of the Zoology Institute, University of Stockholm, Sweden, and Dr. Martin Preston of the Oceanography Laboratories, University of Liverpool, United Kingdom, is aimed at providing recommendations to East African nutrient chemists with regard to possible methods for nutrient analysis in tropical waters.

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1. Introduction and Background:

It should be stated at the outset that this document is intended to provide some practical guidance to the safe performance of nutrient (nitrate, nitrite, ammonia, and phosphate) analysis with particular reference to the problems associated with the analysis of tropical waters. It is often the case that newcomers to nutrient analysis adopt a set of "recipes", or a guide, and subsequently adhere to this without really considering the possible problems involved or the overall suitability of the given method to their specific environment. For this reason we do not intend this manual to be used as a definitive volume. The recommendations we present are based on the work that we have carried out in a variety of areas around the world and in particular on a series of workshops held in the East African region sponsored by the Intergovernmental Oceanographic Commission of UNESCO (IOC) and the Swedish Agency for Research Cooperation in developing countries (SAREC).

It is hoped that the guidance provided will prove to be of real practical assistance to marine analysts; both in the safe performance of their duties and also in the wider context of improving the quality of data produced. Again, it should be stressed that this document only offers guidance and should not be followed blindly without due regard to local conditions and regulations. In particular, all local/national legislation with regard to the handling of potentially dangerous chemicals must be respected and under no circumstances should any laboratory operation be performed without adequate training of the relevant personnel and the authorisation of the appropriate authorities.

So why should there be an emphasis on tropical waters?

Tropical marine ecosystems have often been described as existing under somewhat of a paradox. They generally exhibit high levels of production (in the same range as some of the most productive terrestrial ecosystems in the world), but this production is achieved in waters which typically have nutrient concentrations which are very low or undetectable. Oceanic coral reefs are particularly notable in this respect as they characteristically exhibit high levels of productivity and species diversity (parallel to rain forests) but they are found in nutrient poor oceanic waters. As a result of this particular characteristic, nutrient studies in tropical marine ecosystems often require the detection of compounds at extremely low levels; often at the lower limits of the methods presently available. Consequently, nutrient analysis in tropical waters requires considerably greater vigilance than might be needed when working with, for example, sewage waters or samples from "richer" temperate coastal marine ecosystems where the majority of nutrient investigations have historically been carried out..

In addition to the lower levels that are to be detected, tropical environments often also present a more demanding environment with regard to the storage of samples and the carrying out of analyses. The often high temperatures and humidity alone can present considerable problems for both the chemist and chemistry; significantly increasing the difficulty in achieving accurate results. Of course, all analyses should ideally be conducted in an environmentally controlled laboratory, however, our own experience in the tropics suggests that this is not always achievable and, given the relative lack of funds for marine research in much of the tropical regions of the world, many researchers presently find themselves having to carry out analyses under difficult and less than ideal circumstances.

Whilst it is beyond the scope of this manual to provide all the answers for overcoming these difficulties, one of the main aims it has is to offer ground level alternatives and hints drawn from our own experiences which can help the newcomer to tropical marine nutrient analysis.

2. Safety and Laboratory Practices

2.1 General safety matters

As first rule, all laboratories should have a properly formulated safety policy which complies with all national health and safety legislation. Clearly, this policy must include a things such spatial plan for the laboratory with items such as emergency exits and fire extinguishers highlighted, as well as integrated presentation of duties and prescribed responses under different situations in line with the specific

laboratory facility. Our experience has been that many laboratories have, at best, a poorly structured laboratory policy and rarely have a policy which is customised to the particular facility. The adoption of a general policy from an external or foreign system may be a good first step but this is of limited use if the facility and infrastructure it pertains to is different to that in question. Further, all laboratory staff should be formally advised of this policy and some managerial mechanism for maintaining these standards should be introduced (if not already in place).

All laboratory exercises involve some hazards and it is vital to ensure that as much is possible is done to understand and minimise them before commencing work. Some of the chemicals described in this document are highly damaging to human health if improperly used. An inventory of these chemicals and a summary of their principal known dangers is presented in Tables 1-3. **This should not be regarded as a definitive statement of all of the risks associated with the chemicals and it is the responsibility of the individual analysts to ensure that they are aware of the most up to date information. This can frequently be obtained from the suppliers of the chemicals or from published databases.**

2.2 General principles of laboratory safety.

1) Never begin any practical work without informing yourself as fully as possible about the hazards associated with it.

2) Make sure that you have everything that you need before beginning work.

3) Wear appropriate protective clothing. This should include, as a minimum standard, a regularly cleaned laboratory overall, safety glasses or other recognised form of eye protection, and closed and non-absorbent footwear. Open sandals are unacceptable. Rubber/latex gloves are also very valuable protective items. These need not be special laboratory gloves, domestic quality rubber gloves are adequate provided they are sufficiently flexible and well fitting to permit safe manipulation of laboratory apparatus.

4) Be aware of the risks to both yourself and the people around you from the various activities in progress. Potentially incompatible activities are very common. For example the use of ammonia solutions in a laboratory attempting trace ammonia analysis will result in disastrous cross-contamination because of the ease with which ammonia can be transmitted though the air between aqueous solutions. Similarly, the use of flammable solvents in an area where there may be naked flames (e.g. Bunsen burners) should be prohibited.

5) You must keep proper records of your work including a full description of your plan of work before you start. In the event of an accident this is frequently of great assistance in identifying the source of the problem. In addition is permits retrospective checking of procedures so that, for example, calculation errors may be identified and corrected.

Full records of procedures (and any deviations from them) are part of what has become known as "Good Laboratory Practice" and are essential if the quality of the data produced by a laboratory are to meet internationally acceptable standards.

6) No unauthorised people should be permitted in laboratory areas.

7) No smoking, food or drink consumption should be permitted in laboratory areas. Laboratory clothing should be removed before leaving the laboratory and hands should be washed. This last point is particularly important if leaving for a meal/drink break when it is very easy for dirty hands to contaminate not only the foodstuffs but any door handles, tables, plates etc. encountered along the way. Also, always remember to take off your gloves and overalls/lab coat for even a momentary departure

from the laboratory. Remember, virtually all chemicals retain their toxicity indefinitely so that an uncleared spillage or a contaminated surface will continue to remain dangerous.

8) Laboratory areas should always be kept well ventilated. In hot climates air conditioning must be regarded as almost essential, not only for working comfort but also to prevent other problems such as the unwanted evaporation of solutions[1]. In hot and humid climates all instrumentation should, as far as possible, be kept in a permanently air-conditioned area to prevent corrosion or other problems. In all cases instruments should be located away from the fumes of potentially damaging chemicals (notable volatile acids).

The provision of a fume cupboard should also be regarded as essential where volatile or otherwise harmful materials are to be handled.

9) Tidiness in laboratories is also essential. All spillage's must be dealt with immediately by the person who caused them. There is no excuse for exposing others to hazards that they may not be aware of and certainly cannot evaluate. There should be no rubbish on floors and proper waste disposal facilities must[2] also be provided.

10) It is not acceptable to allow chemicals to go into general waste containers if there is a risk that untrained and unsuspecting cleaning staff may be exposed to them. This is particularly important when there is the possibility that someone else may try to re-use something which has been discarded; for example plastic chemical containers or plastic syringes.

In the absence of dedicated disposal facilities it is probably least damaging to flush small quantities of soluble materials down sinks with a large excess of water. This should not be done unless it is (i) permitted and (ii) there is no risk of damage to the drainage or waste disposal systems[3].

Volatile solvents must not be discharged to drains because there is a considerable risk of explosion and of damage to waste treatment plants. The second point applies particularly to chlorinated or other halogenated solvents. As a last resort, and only if there are no other good alternatives it is probably best to allow volatile solvents to evaporate in a fume cupboard.

11) Never pipette anything by mouth.

3. General points about nutrient analysis

3.1 Design of sampling programmes

As with other aspects of nutrient analysis, sampling programmes become unmanageable and problematic if there is inadequate planning prior to commencement. Perhaps the single most useful piece of advice to be given is to take the time to effectively plan your sampling; initially, in terms of answering the questions you have set, but also in terms of the logistics of taking the samples and their subsequent handling. Short period time series samples, for example, can prove to be problematic if the time taken to filter and/or handle each replicate sample exceeds the time between subsequent samplings.

Many questions are often asked about where, when and how many samples should be collected. Frequently, these questions miss the key issue which is "what do you want to know?". If the objectives of the field programme are clearly identified at the outset then many of the issues relating to sampling

[1] This is discussed further in section 3.4.

2 It is essential for both safety and environmental reasons that all materials be disposed of correctly. It is **everyones** responsibilty but often not adhered to.

[3] It is always best to consult the person responsible for waste disposal prior to commencing an experiment so that waste disposal methods are safe and, if necessary, planned for.

inevitably derive from them. It obviously makes a difference to sampling strategy as to whether you are trying to identify tidal, seasonal annual or inter-annual variations. Similarly, you would not use the same sampling grid, or sampling frequency, in the study of a coastal lagoon, mangrove region, regional sea or oceanic basin.

It is not possible in this document to give absolute guidelines for all sampling. However, it can be stated that the central criterion for all sampling programmes is that the samples collected should be truly representative of the processes that you wish to study. You would not expect samples collected in the tropical Indian Ocean to tell you anything about process occurring the Antarctic for example. Nor would samples collected once a week tell you anything about tidal or diurnal variability.

The samples must be representative when collected and they must remain representative until analysed. The problems in achieving the latter are addressed in the following section.

3.2 Sample handling

There are considerable problems involved with the treatment and storage of samples for nutrient analysis many of which arise from the very rapid rate at which nutrients are assimilated by both phytoplankton and bacteria. In addition there are difficulties with possible changes occurring because of sample manipulation.

In general it is to be recommended that samples be filtered if there is visible turbidity but not if the water is clear. It is more likely to be necessary to filter waters from close to the land than those from samples obtained from "blue" waters off the continental shelf. If filtration is necessary then this should be performed as soon as possible after collection and the exposure of the sample to the air must be minimised. Dispensing of samples in a way that generates bubbles, for example, should be actively avoided

The easiest and most effective filtration units are those which may be directly attached to a syringe containing the sample. Suitable units are made by a number of manufacturers but the unit made by Millipore may be taken as a reference point. Filter units should be properly cleaned before use and, filters once installed, should be pre-cleaned by passing distilled de-ionised water (ddW)[4] through them. At least two sample volumes of distilled water should be used for cleaning purposes. Also, check that the filter is not ruptured during the installation process. This may easily be done with syringe/filter combinations by gently trying to push air through the wetted filter. If when you release the plunger it springs back then the filter is in good order, if however the plunger does not do this then it is likely that the filter is either not correctly sealed or it is ruptured. A glass fibre filter such as a Whatman GF/F is an appropriate choice (but not if silicate analysis is to be conducted on the sample material).

3.3 Sample storage

The general rule for nutrient analysis is that sample storage should be avoided and that analysis should take place immediately after collection. In many cases, however, this option is not practicable and it is then essential to adopt appropriate storage measures. The best option, especially in warmer tropical environments, is for the samples to be rapidly cooled as soon as possible. In the field, options may include packing the samples in ice in an insulated container (an insulated picnic/food storage box is very convenient for this purpose). Freezing samples in the field may be difficult unless there is access to an electricity supply, however, it is possible to generate solid carbon dioxide (dry ice) from a compressed gas cylinder using a simple attachment. This process is not very efficient, however, and involves access to gas cylinders, but might be worth considering; especially where access to electricity or a field freezer is limited. Further, if methanol or ethanol are available, these can be mixed with dry ice to produce a

4Where ddW is not available then distilled water can be used or in its absence, and if you have excess sample, your sample material can be used.

freezing bath which can be used to snap freeze your samples in the field. The frozen samples can then be held on dry ice or in an insulated container until they can be analysed or transferred to a freezer.

An important point to note here is that **all** samples which are to be frozen must be frozen in an **upright** position. Under freezing there can be some differential freezing leading to a spatial concentration of some compounds within the sample container and, if this happens to be on the lid, the material can be easily lost and/or contaminated. The effect of this is negated if, as standard practise, all samples are treated the same way and frozen in an upright position.

It must be recognised that all storage of samples for nutrient analysis will involve some alteration in the amount of nutrient eventually measured. This is particularly true for ammonia for which any kind of storage should be avoided if at all possible. Whilst some workers have attempted to calibrate for this by storing a sample with a known concentration of the nutrient involved, there can still be many problems and should not necessarily be relied on. It is always best to conduct the analyses as quickly as possible.

3.4 Problems relating particularly to the tropics.

Nutrient concentrations in most tropical waters are extremely low and these are frequently close to the detection limits of the standard methods. It is therefore extremely important that the greatest care is taken to minimise contamination of either the samples or the reagents required.

One point that is well worth making is that the signal to noise ratio for the analysis can be significantly improved by the use of spectrophotometer cells of the greatest practicable length. A 10cm cell will provide a tenfold increase in the measured value of sample absorbance relative to a 1cm cell for a constant value of cell to cell blank and instrumental noise. The same result cannot be obtained by range or scale expansion facilities which amplify signal and noise equally. For tropical waters cell lengths smaller than 4cm should not be used and 10cm cells are probably best if this can be accommodated within the cell compartments.

One aspect of the tropics which often presents a problem for analysts is the warm temperatures and the generally higher humidity. Our experience has been that there are many laboratories in the tropics which still lack adequate air conditioning and so consequently the laboratories are kept cooler by either their location in a shaded section of the building, or by the use of ceiling fans. Provided the temperature obtained is within the limits for the chemistry, these methods are adequate. However, with regard to ceiling fans, the movement of warmer humid air through the laboratory represents a good vector for airborne contamination. Consequently, it is advisable to work with your samples within a small cabinet or under some type of transparent cover so that sample contact with this turbulent air is minimised. Further, given that samples and reagents are undoubtedly less stable in warm climates, every precaution should be undertaken to preserve their integrity. Reagents should be freshly prepared and kept in well stoppered or firmly closed containers. Also, they should be kept cool and away from light; a laboratory refrigerator is an obvious choice for storage purposes. Some specific precautions are given in the individual method sections.

The laboratory must be kept free of potential contaminants. These may arise from obvious sources such as ammonia solutions or nitric acid being used by other workers but may also come from less obvious sources. For example, many domestic cleaning products contain ammonia and, if used by laboratory cleaning staff, these can lead to contamination problems. Similarly, certain marine animals (e.g. prawns) are a rich source of ammonia and should be kept away from the analytical facilities. *This includes the refrigerator or freezer where reagents or samples are stored.* Cigarette smoke (and the fingers of cigarette smokers) are rich in nitrogen oxides and any smoker who is taking samples for nitrate or nitrite analysis must wear gloves and must not smoke during sample collection or around sampling equipment.

Only by rigorous attention to detail and constant checking of methods can contamination problems be rapidly identified and remedied (see quality control)

3.5 Standardisation of methods

A number of important guidelines should be followed in the calibration of all analytical methods. i) it is essential to use a multi-point calibration - single point calibrations are likely to be subject to increased risk of error and should be avoided (see below) ii) the calibration points should encompass all of the sample concentrations. It is not satisfactory to extrapolate below or above the calibration data iii) ensure that all measurements are taken within the linear region of the calibration curve iv) try to avoid taking absorbance readings which lie >0.9 or <0.1 absorbance units because machine accuracy decreases markedly outside this range. If necessary use shorter or longer cell lengths as appropriate to decrease or increase absorbance readings. In tropical waters the usual problem is with readings which are very low - hence the importance of using long cells. However, in virtually any sedimentary system, nutrient concentrations are likely to be much higher and will probably require shorter cells or sample dilution before analysis.

3.6 Blanks

It is essential to make adequate assessments of analytical blanks. In the case of nutrient analysis these will include reagent blanks and cell blanks. Reagent blanks can be measured by treating an aliquot of distilled water as a sample and carrying out the full analysis. Cell blanks are assessed by filling spectrophotometer cells with distilled water and measuring the difference between sample and reference cells. In both cases the blank values must be subtracted from standard and sample readings before any plots/calculations are made.

3.7 Quality control

The requirement for adequate control of the quality of the data produced by a laboratory is paramount both if the data is to be useful to the scientists producing the data and the wider scientific community. It is impossible to compare differences in nutrient concentrations at different times or at different places if the errors associated with the analysis are greater than the differences or, worse, the errors are unknown.

Errors associated with analytical work can conveniently be divided into three categories i) gross, ii) random and iii) systematic and there are a number of well established methods for minimising problems from any of these sources.

3.7.1 *Gross errors*

Gross errors are normally the easiest of the three types of error to identify. They typically arise from bad laboratory practice involving the handling of samples or reagents. The symptoms frequently involve large and unexplained (i.e. random) deviations from normal in calibration lines or between samples which should be the same (e.g. duplicates).

Good laboratory handling procedures and record keeping will help to identify problems. In particular, any peculiarities during the collection of the sample should be noted at the time so that they can be referred to if the sample later produces an abnormal result.

Awareness of what else is happening at the sampling point or in the analytical laboratory is very important. Routine precautions cannot always provide adequate protection against conflicting activities. This is particularly true for species such as nitrate and ammonia which may both be transferred by atmospheric processes.

3.7.2 *Random errors*

Random errors may also arise from the same sources as the gross errors discussed above. However they may also derive from other sources and because they are smaller in magnitude may be harder to identify.

One of the commonest sources of random errors is the improper use of volumetric apparatus. The problems are often associated with pipettes but may also include volumetric flasks, measuring cylinders and burettes. Great care must be taken to use all such apparatus properly. This begins by ensuring that all apparatus is both clean and in good condition. Chipped or otherwise damaged apparatus will not perform to its specifications and great care should be taken to prevent damage and to discard any damaged apparatus.

Laboratory managers and analysts must recognise that the proper use of such apparatus is not intuitive and does involve the application of a number of skills which may need to be taught. A simple test for the use of pipettes which is used in our laboratories is to dispense a number of aliquots of distilled water into a covered beaker on a balance pan. The beaker is weighed after each addition and the weight of the aliquot is calculated. The mean and standard deviation of the results may then be calculated. A skilled analyst should be able to dispense volumes with a standard error ([standard deviation/mean] x 100) of better than 0.1% using a 1ml, Class B bulb pipette. Automatic pipettes also need to be operated properly and equal attention is required to ensure that these are used properly and do not deteriorate with time.

The key test for random errors is to assess the reproducibility of the method. This should be done by measuring the standard deviation of a number (at least 5-10) of replicate samples or standards at environmentally realistic concentrations. This assessment should not be considered to be a 'once is forever' measurement but should be regularly performed so that any changes in operating standards can be rapidly identified. In this way the 'repeatability' of the method may be monitored.

3.7.3 *Systematic errors*

Systematic errors, where there is a constant deviation between the measured and true values, are the most difficult to identify and rectify. These errors may arise from repeated mistakes during the analytical procedure (e.g. a typographical error in a method instruction sheet leading to the incorrect amount of a reagent being weighed out) or from such sources as incorrectly calibrated balances or other analytical devices.

Internal procedures (i.e. within laboratory) can prevent some of these problems. Regular preventative maintenance of equipment is essential and a great deal of this can be accomplished by first reading and then carrying out the manufacturer's instructions in the relevant operating manuals. Balance calibrations should be checked by qualified engineers where possible but if this is difficult the purchase and correct use of a set of calibration weights can at least identify problems when they occur. In view of the fact that balances are probably the most used and abused pieces of laboratory apparatus a regular check (e.g. weekly) should be made.

The most widely adopted techniques for the identification of systematic errors are (i) the use of an intercalibration exercise conducted between a number of laboratories in a country or region and (ii) the analysis of internationally recognised reference standards.

In the case of (i) the normal procedure is for an organising laboratory to be appointed and they issue a series of identical samples to participating laboratories. These are analysed and the results returned to the originating laboratory for compilation and comparison.

Such exercises have to be conducted with considerable skill and diplomacy. Although the only objective is to obtain properly comparable results between laboratories a competitive element between laboratories or countries may sometimes occur with numerous possibilities for difficulties. In the case of trace analysis there are also considerable difficulties in providing samples which can be guaranteed to remain stable and unchanged in the period between preparation and analysis. This is certainly a major problem in the case of dissolved nutrient analysis in sea water. In this and other similar cases there are few alternatives to gathering the relevant scientists in a single location and asking them to perform their standard procedures on a series of common samples obtained locally. This is a relatively expensive option but is a very worthwhile one if it leads to mutual confidence in the results issuing from different laboratories.

An internal intercalibration exercise between a number of scientists within a laboratory can also be a useful way of identifying some systematic errors. This should be conducted under the same conditions as an inter-laboratory exercise.

At present there are not internationally recognised reference samples for nutrient analysis of sea water so intercalibration exercises are really the only option available.

4. Units

A variety of different units are used for the description of nutrient concentrations. These have come about for historical reasons and, in some cases, because it makes elemental budget calculations easier. It is of course very important to be able to make the necessary conversions.

Taking the example of a solution containing 1 µg nitrate l^{-1} (= 1/(14+ (3x16)) = 1.61 x 10^{-2} µmol.l^{-1} of nitrate).

This solution contains 1 x 14/62 = 2.25 x 10^{-1} µmol of nitrogen as nitrate (NO_3-N).l^{-1}.

This is equivalent to 2.25 x 10^{-1}/14 = 1.61 x 10^{-2} µg.atoms of NO_3-N.l^{-1} .

In other words, the unit microgram-atom is the same as the unit micromole but applied to a single element only (in this case nitrogen). The benefits of describing nutrient concentrations in µg.atoms NO_3-N.l^{-1} or µmol NO_3-N.l^{-1} are that what is being stated is the amount of nitrogen present in the sample in a specific chemical form. If it is then required to calculate the total amount of nitrogen present in the system it is only necessary to add up the concentrations of each of the individual chemical species.

5. Standards and Dilutions.

When preparing standard solutions it is normal to begin by weighing out a sufficiently large amount of the standard chemical such that any weighing errors are likely to be small. In practice this normally means amounts of not less than about half a gramme. The problem frequently facing the marine analyst is that such a large quantity of standard produces a primary standard solution which is many times more concentrated than those to be found in the samples. It is therefore necessary to undertake a series of dilutions to prepare a set of working standards which cover the entire range of sample concentrations.

When making dilutions, use large volumes (>1ml) where errors in dispensing are relatively small. Also, only use integer values of volumes. Modern dispensing pipettes may make it relatively easy to dispense fractions of a ml but these introduce increased risks of calculation errors and are best avoided.

A series of worked examples are given below which may be used as guidelines. These are based on the standard materials and weights used in "A Manual of Chemical and Biological Methods for Seawater Analysis" by Parsons, T.R., Maita, Y and Lalli, C.M. (1984) Pergamon Press, Sydney, Australia.

Note: For dilutions and the preparation of reagents it is recommended that where artificial seawater is not applicable, distilled de-ionised water is used and that this be prepared immediately prior to use. This is here after referred to as *ddW*.

Nitrate

Primary standard (A)
1.02 g of analytical grade potassium nitrate in 1000ml of ddW water
This solution is equivalent to 1.02/101.1069 =10.088 millimoles NO_3-N.l^{-1}.
or 10.088 µmoles.ml^{-1}

Secondary standard (B)
Dilute 10ml of A to 1 litre with synthetic sea water.
This solution is equivalent to 100.88µmoles NO_3-N.l^{-1} or 0.10088µmoles.ml^{-1}

Secondary standard (C)
Dilute 10ml of B to 100ml (or 25ml to 250ml if a larger quantity is required) with synthetic sea water.
This solution is equivalent to 10.088µmoles.NO_3-N.l^{-1} or 0.010088µmoles.ml^{-1}.

Working standards

All dilutions should be made with synthetic sea water and an appropriate number (e.g. 5) should be selected from the following list.

Dilution series

50ml B diluted to 100ml = 50.44µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
25ml B diluted to 100ml = 25.22µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
15ml B diluted to 100ml = 15.13µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
10ml B diluted to 100ml = 10.088µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
5ml B diluted to 100ml = 5.044µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
2.5ml B diluted to 100ml = 2.522µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
1.0ml B diluted to 100ml = 1.0088µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})

50ml C diluted to 100ml = 5.044µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
25ml C diluted to 100ml = 2.522µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
15ml C diluted to 100ml = 1.513µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
10ml C diluted to 100ml = 1.0088µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
5ml C diluted to 100ml = 0.5044µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
2.5ml C diluted to 100ml = 0.2522µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})
1.0 ml C diluted to 100ml = 0.10088µmoles.NO_3-N.l^{-1} (or µg.atoms NO_3-N.l^{-1})

Nitrite

Primary standard (A)

0.345 g of analytical grade sodium nitrite in 1000ml of ddW water.
This solution is equivalent to 0.345/68.9953 = 5.00034 millimoles NO_2-N.l^{-1}
or 5.00034 µmoles NO_2-N.ml^{-1}

Secondary standard (B)

Dilute 10ml of A to 1 litre with ddW water.
This solution is equivalent to 50.0034µmoles NO_2-N.l^{-1} or 0.0500034µmoles NO_2-N.ml^{-1}

Secondary standard (C)

Dilute 10ml of B to 100ml with ddW water
This solution is equivalent to 5.00034µmoles NO_2-N.l^{-1} or 0.00500034µmoles NO_2-N.ml^{-1}

Dilution series

50ml C diluted to 100ml = 2.500µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
25ml C diluted to 100ml = 1.250µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
15ml C diluted to 100ml = 0.750µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
10ml C diluted to 100ml = 0.500µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
5ml C diluted to 100ml = 0.250µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})

2.5ml C diluted to 100ml = 0.125µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
1.0 ml C diluted to 100ml = 0.050µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})
1.0ml C diluted to 250ml = 0.020µmoles.NO_2-N.l^{-1} (or µg.atoms NO_2-N.l^{-1})

Ammonia

Primary standard (A)
0.100 g of analytical grade ammonium sulphate in 1000ml of distilled, ammonia-free water.
This solution is equivalent to 0.1/128.10588 x 2 = 1.5612 millimoles NH_4-N.l^{-1}. or 1.5612 µmoles NH_4N.ml^{-1}

Secondary standard (B)
Dilute 10ml of *A* to 1 litre with ddW (ammonium free).
This solution is equivalent to15.14 µmoles NH_4-N.litre or 0.01514 µmoles NH_4-N.ml^{-1}.

Secondary standard (C).
Dilute 25ml of *B* to 100ml with ddW (ammonium free).
This solution is equivalent to 3.785 µmoles NH_4-N.litre or 0.00378 µmoles NH_4-N.ml^{-1}.
Dilution series.
50ml B diluted to 100ml = 7.57 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
25ml B diluted to 100ml = 3.785 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
10ml B diluted to 100ml = 1.514 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
5ml B diluted to 100ml = 0.757 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
2.5ml B diluted to 100ml = 0.379 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
1.0ml B diluted to 100ml = 0.151 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
2.5ml C diluted to 100ml = 0.095 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})
1.0ml C diluted to 100ml = 0.038 µmoles.NH_4-N.l^{-1} (or µg.at. NH_4-N.l^{-1})

6. Methods:

6.1 Determination of Ammonium by Oxidation.

The method presented here is essentially that described by Matsunaga and Nishimura (1974) and is based on the oxidation reaction of ammonium with sodium hypochlorite in an alkaline solution.

The accepted detection range for the analysis is 0.04-10.0 µg-at N.l^{-1} with a mean precision in the range of $0.08/n^{½}$ µg-at N.l^{-1} for *n* determinations.

Sampling and Storage.

Sampling and storage of samples should follow the guidelines given earlier however it is important that analysis be commenced within 1 to 2 hr of sampling. Where samples were not filtered or there is the possibility of water column production within the samples the delay between sampling and analysis should be an absolute minimum or negligible. This is especially true in most tropical water samples where ammonium concentrations are likely to be low.

Where it is not possible to analyse samples within this time, they should be immediately frozen and stored ***upright*** in tightly sealed containers at $\leq -20^{o}C$. As mentioned earlier, there is still the possibility for some changes in concentrations over time and so storage time should also be kept to a minimum.

Reagents.

1. Sulfanilamide solution

5g of sulfanilamide is dissolved in 500ml of 8.5N hydrochloric acid. This solution is stable for some months.

2. Hydrochloric acid solution (8.5N)

500ml of analytical grade concentrated hydrochloric acid (≅ 12N) is diluted with ddW water to 705ml to make 8.5N HCl.

3. Sodium arsenite solution

10g of analytical grade sodium meta-arsenite (Na_2AsO_2) is dissolved in 1000ml of ddW. The solution is stable indefinitely.

4. Potassium bromide solution

175g of analytical grade potassium bromide is dissolved with 250g of sodium hydroxide in 1000ml of de-ionised water. Again, this solution is stable for some months.

5. N-(1-napthyl)-ethylenediamine dihydrochloride solution

0.5g of the napthylethylene is dissolved in 500ml of ddW. This solution is more light sensitive and so should be stored in a dark bottle. It is stable for approximately one month.

6. Hypochlorite solution

In this case it is possible to use commercial sodium hypochlorite such as is commonly sold as bleach (≅1.5N). It should be noted though that in some countries this can contain other substances such as fragrances and these can cause problems. Also, if you use laboratory grade sodium hypochlorite, this should be diluted to ≅1.5N. The strength of the stock solution should be checked periodically to control for decomposition.

The final solution is made by diluting 3.5ml of 1.5N sodium hypochlorite to 100ml with ddW water. This is stable for approximately 2hr.

7. Complexing solution

110g of analytical grade sodium citrate ($C_6H_5O_7Na_3.2H_2O$) and 105g of analytical grade sodium potassium tartrate ($C_4H_4O_6KNa.4H_2O$) is dissolved in 1000ml of de-ionised water. Stable for several months.

Procedure

a) Dispense 50ml of sample into an Erlenmeyer flask and add 2ml of complexing solution. Mix thoroughly by swirling.

b) Add 2ml of potassium bromide solution and mix as above. Place the sample in a water bath and allow it to stand at a temperature between 35^{o} and 45^{o}C.

c) Add 2ml of hypochlorite solution and mix thoroughly. Allow to stand at 35^{o}-45^{o}C for 2 minutes.

d) Add 2ml of sulfanilamide solution and mix thoroughly. The sample must then be allowed to react for between 2 and 8 minutes after which 2ml of the napthylethylene solution is added and immediately mixed thoroughly.

e) The absorbance of the solution is then measured against de-ionised water at 543nm using 1cm or 5cm cuvettes depending on the concentration range. *Note:* The absorbance must be read between 10 minutes and 2 hr after addition of the napthylethylene solution.

Blanks are determined as above using 50ml of ammonium free seawater[5] (seawater blank) and 50ml of de-ionised water (reagent blank).

Calibration standards are made using ammonium free seawater as above.

Points to note:

1. All de-ionised water (ddW) used for preparation of reagents and blanks must be passed through a cation exchange resin immediately prior to use in analysis.

2. As a general rule we have tended to use 5cm cuvettes when the concentration is below 4 µg-at $N.l^{-1}$.

3. A common source of high blank values in this method is the complexing solution. The ammonium in it can be removed, however, by boiling the solution in alkali (pH ≅10-11).

4. There should be no use of other ammonium containing compounds (e.g. Ammonium chloride, ammonium hydroxide) in the laboratory during analysis and preparation of reagents or standards.

5. Under the oxidation stage of this method hydroxilamines can be completely oxidised to nitrite and result in a positive error. Similarly, any nitrite present in the initial sample will give an erroneous result and must consequently be corrected for. This should be particularly noted in samples where nitrite levels are high but ammonium levels are low.

6.2 Determination of Ammonium by the Indophenol method.

Whilst this method also measures the concentration of ammonium in seawater, it is considerably less sensitive than the first method. However, because it requires fewer reagents and is a little more straight forward in its preparation than the previous method, this method is often preferentially used for samples where the ammonium concentrations are higher. The effective detection range of this method is between 0.1 and 10 µg-at/l and, for n determinations, its precision is in the order of $\pm 0.1/n^{1/2}$µg-at/l.

As its name suggests, this method relies on the measurement of an indophenol colour formed by ammonium in the presence of sodium nitroprusside after oxidation with hypochlorite and phenol in an alkaline citrate solution. The method presented here stems essentially from that of Solozarno (1969) and Parsons *et al.*, (1984).

Sampling and Storage.

Again, samples should only be stored if it is absolutely necessary. By contrast to the previous method, however, if freezing of samples is not possible (≤$^{-}$20^{o}C), samples can be stored after the addition of 2ml of the phenol* reagent (Reagent 2 below) to 50ml of sample. Whilst some authors would recommend this for a storage period of approximately 2 weeks, our own experience suggests that 1 week is a safer upper limit and it is important that samples be kept tightly sealed, away from bright light, and at room temperature or lower. Further reading on this storage practise can be obtained from Degobbis (1973).

5. Ammonium free seawater is produced by boiling 1 litre of seawater containing 5ml of 1N NaOH long enough to reduce the volume to approx. 0.7 litres. The volume is then replaced with de-ionised water neuutralised with equivalent HCl and filtered through a glass fiber filter.

Reagents

1. Alkaline citrate solution.

100g of sodium citrate and 5g of sodium hydroxide is dissolved in 500ml of ddW. This is generally stable for ≥4 weeks if stored in a cool (≈8^{o}C), dark place.

2. *Phenol solution.

20g of dry analytical grade phenol is dissolved in 200ml of 95% ethanol.

3. Sodium hypochlorite solution.

As with the previous method, it is possible here to use commercially available hypochlorite provided it has a concentration of approximately 1.5N. Because this solution slowly decomposes it is necessary to check the amount of available hypochlorite in solution.

4. Sodium nitroprusside/catalyst solution.

1.0g of sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) is dissolved in 200ml of ddW. This solution should be stored in a dark bottle and is stable for ≤1 month.

5. Oxidation solution.

This solution is made by mixing 100ml of alkaline solution with 25ml of hypochlorite solution. This solution should be held in a stoppered container when not being used and should be made fresh for every set of analyses and not stored.

Procedure

a) Dispense 50ml of sample into an Erlenmeyer flask and immediately add 2ml of the phenol solution and mix thoroughly by gently swirling.

b) Directly after step a) add 2ml of nitroprusside solution (mix again by swirling) and then 5ml of oxidising solution.

c) After 1hr at room temperature (20^{o} - 27^{o}C) read the absorbance of the solution at 640nm in 10cm cuvettes (or 5cm if these are unavailable).

d) Standard curve for this method is produced using samples of known concentrations over the expected range for the field samples (refer sections 3.5 and 5).

Points to note:

1. The colour formed in this reaction is generally stable for up to 24hr **but** if the temperature is higher than prescribed, this time is considerably reduced and/or the reaction fails to function correctly. We have found this to be particularly so where temperatures exceed 30^{o}C and so it is vital that every effort be made to control laboratory temperature. Of course, one way to overcome this problem in non-air conditioned laboratories is to carry out the analyses at night, when temperatures are lower, or to use a temperature controlled water bath for holding the samples during analysis. Note, however, that any water in the water bath represents a source of contamination and thus it must not come in contact with samples. Consequently it is important that sample containers are thoroughly dried before handling.

2. Whilst it is possible that some samples will require a little more than 5ml of oxidising solution, it is important that sample pH does not exceed 9.8.

3. During the analysis the samples are sensitive to bright light and so it is essential to hold them in a shaded area or dark container.

6.3 Determination of Nitrite.

This method involves the formation of a diazo compound by nitrite and sulfanilamide in acidic solution and the subsequent production of a diazo dye in the presence of N-(1-naphthyl)-ethylenediamine. The effective concentration range for the method is between 0.01 and 2.5µg-at N/l and the estimated precision for n determinations is in the order of $\pm 0.03/n^{1/2}$.

Sampling and storage

(Refer Section 3)

Reagents

1. Sulfanilamide reagent

Add 50ml of concentrated hydrochloric acid to approximately 400ml of ddW and then add 5g of sulfanilamide. Dilute this solution to 500ml and store in a well sealed bottle. The reagent is stable for several months.

2. N-(1-naphthyl)-ethylenediamine dihydrochloride reagent.

0.5g of the naphthyl-ethylenediamine is dissolved in 500ml of ddW. This resulting reagent is light sensitive and should be stored in a dark bottle. Also, the reagent should not be kept for more than about 1 month and should be discarded if a definite brown coloration is observed.

Procedure

a) To a 50ml sample add 1.0ml of sulfanilamide reagent and after mixing by swirling, allow the mixture to stand for between 2 and 10 minutes.

b) Add 1.0ml of the naphthyl-ethylenediamine reagent and, after mixing, allow it to stand for ≥10 minutes but less than 2 hr.

c) Read the absorbance of the resulting solution at 543nm using 10cm cuvettes.

d) A standard curve for this method can be made by analysing ≥4 samples of known concentration over the expected range of field samples (refer sections 3.5 and 5).

6.4 Determination of Nitrate.

This method involves the reduction of nitrate to nitrite by exposure of the sample to copper coated cadmium and the subsequent quantification of nitrite using the sulfanilamide/diazo method as given above. The original method essentially stems from that of Morris and Riley (1963) but with some modifications (e.g. Wood *et al.*, 1967). As presented here, the method adheres closely to that of Parsons *et al.*, (1984) but again with some modifications.

The range of detection for this reaction is between 0.05 and 45µg-at N/l, with an estimated precision of $\pm 0.5/n^{1/2}$ µg-at N/l for n analyses.

Sampling and storage.

As with ammonium, nitrate is rapidly taken up by phytoplankton and so it may be necessary to filter samples in areas where phytoplankton communities are significant or the sample will be stored. Similarly, samples should be stored frozen at $\leq -20^{o}$C. In its standard form, this method requires ≥ 100 ml of sample.

Cadmium reduction column

In order to reduce the nitrate in samples to nitrite the sample is passed through a column (Fig. 1) containing cadmium granules coated. The column described here follows that of Parsons *et al.*,(1984) but provided the given ratios of size, volume and flow are maintained, the column can be modified to facilitate smaller sample sizes. Also, some researchers have successfully substituted cadmium wire for the cadmium granules.

The cadmium granules should be of analytical grade cadmium and in the size range of 0.5 to 2.0mm diameter as determined by sieving. Also, regardless of whether the cadmium is recycled from a previous column or is newly purchased, it is recommended that it be cleaned thoroughly. This is done by first rinsing the granules with 10N HCl and then rinsing them well with ddW. For new cadmium it is strongly recommended that they be first rinsed with diethyl ether to remove any grease or dirt, and then washed as above.

Approximately 100g of cleaned cadmium granules are stirred in a 500ml solution of 2% w/v copper sulphate ($CuSO_4 \cdot 5H_2O$) until the blue colour of the solution has disappeared (note that during this procedure small dark particles of particulate copper will appear in solution). This step is repeated until the copper sulphate solution does not lose colour when mixed with the granules. It is **very important** at and beyond this stage that the granules do not come in contact with air as this will significantly decrease its reduction efficiency.

The granules should then be repeatedly rinsed with ddW until the black colour disappears and the solution is free of particulate copper.

Place a small plug of copper wool or *fine* wire in the bottom of the glass column and gently pour in a slurry of the prepared granules. A good way to do this so that the granules do not come in contact with air is to use a conical flask with a rubber stopper and pipe (Fig. 2). Both the column and the conical flask are kept full of dilute ammonium chloride solution (reagent 2) and the flask is inverted and gently shaken to dispense the granules into the column. Finally, a small plug of glass fibre wool is placed in the top of the filled column.

Ideally, the column should have a flow rate of between 8 to 12 minutes for 100ml and should be repacked if this is not so.

Reagents

1. Sulfanilamide reagent (*see nitrite method*)

2. N-(1-naphthyl)-ethylenediamine dihydrochloride solution (*see nitrite method*)

3. Ammonium chloride stock solution.

125g of analytical grade ammonium chloride (NH_4Cl) is dissolved in 500ml of ddW and stored in an air tight container.

4. Dilute ammonium chloride.

50ml of the stock ammonium chloride solution is diluted to 2000ml with ddW.

Procedure

a) Add 2.0ml of the stock ammonium chloride solution to the sample (volume 100ml). Mix the solution by gently swirling[6] and then dispense 5ml of this onto the top of the column. Allow this to pass through but do not allow the level to drain to the surface of the cadmium.

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6. In order to reduce the chance of airborne contamination it is often useful to cover the top of the sample flask with plastic film between additions and whilst mixing. Note that aluminium foil has been implicated in some problems with this method and so its use is not reccomended.

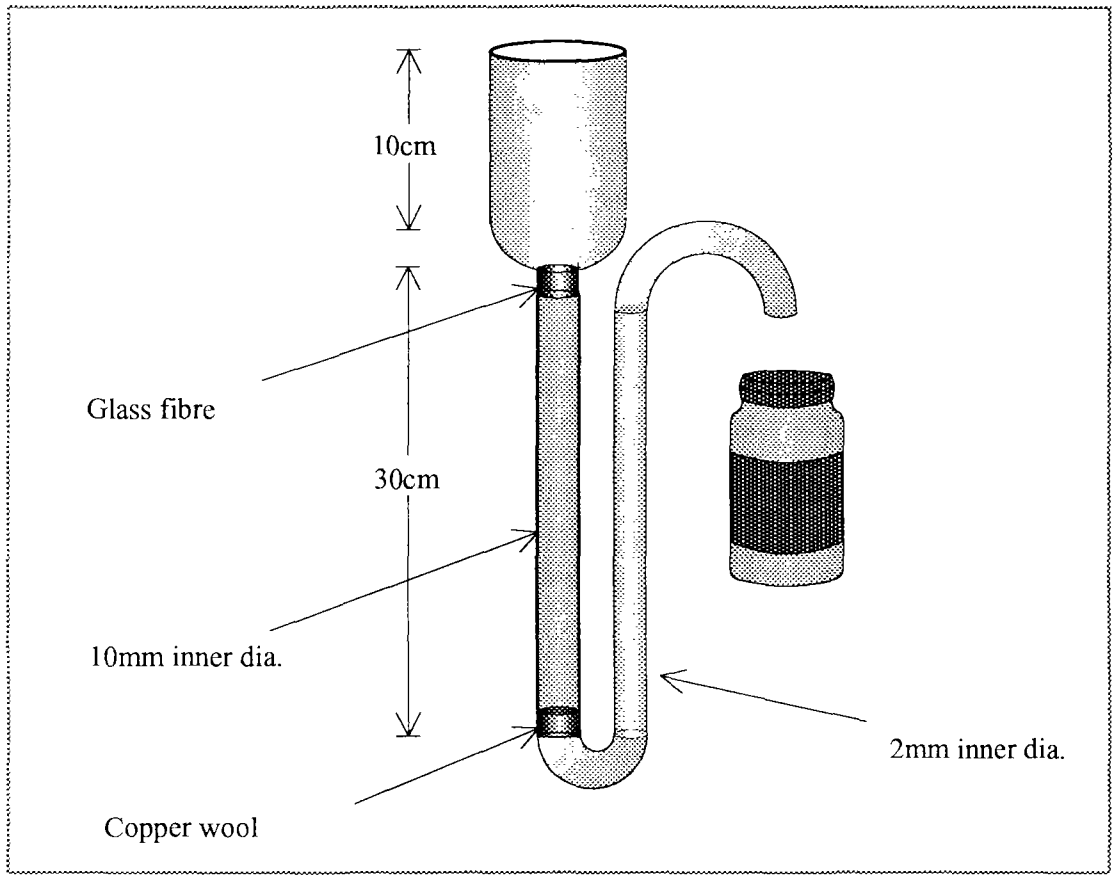

Figure 1. Standard configuration for cadmium column for nitrate analysis.

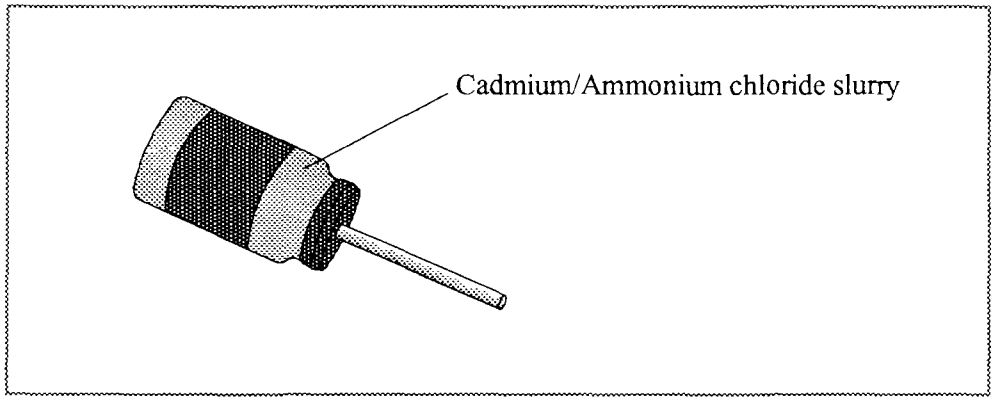

Figure 2. Arrangement for dispensing cadmium granules into the reduction column. Note that the nozzle of the dispenser must be kept under the surface of the ammonium chloride solution in the head of the column.

b) Add the remainder of the sample to the column. The first 40ml passing through the column is collected[7] and discarded. The next 50ml is collected and set aside for further analysis.

c) Allow the column to drain to the minimum level again and add the next 5ml sample (Step a.).

d) To each of the reduced 50ml samples add 1.0ml of sulfanilamide reagent and mix it by swirling. Allow it to stand for between 2 to 8 min. and then add 1.0ml of the naphthylethylenediamine reagent.

e) Allow the sample to stand for between 10 min and 2 hr, and then read its absorbance at 543 nm.

Points to note:

1. Because the cadmium column has a limited life, it is useful to test it's reduction efficiency at the beginning of your analyses and to run regular standards to control for decrease efficiency in the column. The easiest way to check the columns efficiency is to analyse samples of known nitrate, and consequently, nitrite concentrations. By then comparing the amount obtained after reduction with the calculated amount supplied to the column the reduction efficiency can be determined.

2. Given the broad range of concentrations over which this method can be used, it may be necessary to use either 10cm or 5cm cuvettes to bring the absorbance values within the linear range.

6.5 Determination of Reactive Phosphate

This method involves the formation of a complex between soluble reactive phosphate, molybdic acid, ascorbic acid and trivalent antimony. The method presented is adapted from Murphy and Riley (1962), Menzel and Corwin (1965), and Strickland and Parsons (1968).

Sampling and Storage.

See sections 3.2 and 3.3.

Reagents

1. Sulphuric acid solution.

140ml of analytical grade concentrated sulphuric acid (sp.gr.=1.82) is added to 900ml of ddW. This is allowed to cool and stored in a glass bottle.

2. Ascorbic acid solution.

27g of ascorbic acid is dissolved in 500ml of ddW and stored frozen in a plastic bottle[8].

3. Ammonium molybdate reagent.

15g of analytical grade ammonium paramolybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] is dissolved in 500ml of ddW and stored in a glass bottle.

4. Potassium antimonyl-tartrate solution.

0.34g of potassium antimonyl-tartrate is dissolved[9] in 250ml of ddW.

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7. Both the first 40ml and subsequent 50ml is collected in the original sample flask and it is recommended that the initial 40ml be used to rinse the flask prior to discarding it.
8. At room temperature this solution should not be kept for more than 1 week but may be kept frozen for a number of months. To avoid waste and the loss of reagent, it is recommended that the solution be stored in a number of smaller plastic bottles so that one of these can be used for each set of analyses and thus avoid thawing the entire stock.
9. This may require warming of the solution.

5. *Mixed reagent.*

Mix together 100ml ammonium molybdate, 250ml sulphuric acid solution, 100ml ascorbic acid, and 50ml of potassium antimonyl-tartrate. This reagent should be mixed as required and should **not** be stored. The quantity given here is suitable for approximately 50 samples (100ml volume).

Procedure.

a) To a 100ml sample, add 10ml of the mixed reagent whilst mixing by swirling. This is easiest to achieve if a syringe or auto-pipette type dispenser is used for adding the reagent.

b) Allow the sample to stand[10] for between 5 minutes and 2 hr and then read its absorbance at 885nm[11].

Points to note:

1. For tropical samples which may have very low concentrations of phosphate, a modification of the above method has been described by Stephens (1963). This method involves a concentration of the colour formed and provides an almost 5 fold increase in sensitivity.

2. In the event of high turbidity samples should be filtered through a pre-cleaned glass fibre filter prior to analysis (e.g. Whatman GF/F).

10. Colour formation is essentially complete after approximately 5 minutes and this is stable for some time, however, beyond 2 hr we have experienced some changes in colour intensity over time.

11. Prior to analysis, a turbidity check should be made on samples by reading the absorbance at 885nm. If this value exceeds 0.01 (10cm cuvette) a correction should be made for this in the final data.

7. References.

Degobbis (1973) Limnol. Oceanogr.,18:146.

Matsunaga and Nishimura (1974) Anal. Chim. Acta., 73:204.

Menzel and Corwin (1965) Limnol. Oceanogr.,10:280.

Morris and Riley (1963) Anal. Chim. Acta.,29:272.

Murphy and Riley (1962) Anal. Chim. Acta.,27:31.

Parsons *et al.*, (1984) A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press. Potts Point, N.S.W. Australia.

Solozarno (1969) Limnol. Oceanogr.,14:799.

Stephens (1963) Limnol. Oceanogr.,8:361.

Strickland and Parsons (1968) A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada. Ottawa.

Wood *et al.*, (1967) J. Mar. Biol. Assoc. UK., 47:23.

8. Tables

Table 1
UN, Chemical Abstracts Reference Numbers plus Reference Numbers used in this document.

Chemical	UN No	CAS No	Ref. No.
Ammonium chloride		1215-02-9	1
Ammonium molybdate		12054-85-2	2
Ammonium sulphate		7783-20-2	3
Ascorbic acid		50-81-7	4
Cadmium metal		7440-43-9	5
Chloroform	1888	67-66-3	6
Copper sulphate		7758-99-8	7
Ethyl alcohol	1170	64-17-5	8
Hydrochloric acid	1789	7647-01-0	9
Magnesium sulphate		14168-73-1	10
Metol (p-methylaminophenol sulphate)		55-55-0	11
N-(1-naphthyl)-ethylenediamine dihydrochloride		1465-25-4	12
Oxalic acid		6153-56-6	13
Phenol	1671	108-95-2	14
Potassium antimonyl tartrate	1551	28300-74-5	15
Potassium dihydrogen phosphate		7778-77-0	16
Potassium bromide		7758-02-3	17
Potassium iodide		7681-11-0	18
Potassium nitrate	1486	7757-79-1	19
Sodium bicarbonate		144-55-8	20
Sodium chloride		7647-14-5	21
Sodium citrate		6132-04-3	22
Sodium hydroxide	1823	1310-73-2	23
Sodium hypochlorite	1791	7681-52-9	24
Sodium meta-arsenite		7784-46-5	25
Sodium nitrite	1500	7632-00-0	26
Sodium nitroprusside	1588	13755-38-9	27
Sodium potassium tartrate		?	28
Sodium silicofluoride			29
Sodium thiosulphate		7772-98-7	30
Sulphanilamide		63-74-1	31
Sulphuric acid	1830	7664-93-9	32

Table 2
A Summary of the Principal Hazards Associated with Table 1 Chemicals

CHEMICALS HARMFUL AND/OR IRRITATING BY:				OTHER HAZARDS		
Ingestion	Inhalation	Skin contact	Eye contact (dust and/or vapour)	Carcinogen	Mutagen/ teratogen	Fire/ explosion hazard
1[1], 2, 3, 4[1], 6, 7, 8[2], 9, 10[1], 11, 12, 13, 14, 15, 17[1], 18, 19[1], 20[1], 21[1], 22[1], 23, 25*, 26, 27, 28[1], 31, 32	1, 2, 3, 5, 6, 7, 8[2], 9, 11, 12, 13, 14, 15, 16, 22, 23, 24, 25*, 27, 31	7, 9, 11, 12, 13, 14[3], 23, 24, 25*, 27, 31, 32	1, 2, 3, 7, 8, 9[3], 10, 12, 13, 16, 17, 20, 21, 22, 24, 25*, 26, 28, 30, 32	5[4], 6, 14, 15[4], 25*, 31	2, 4, 6, 13, 14, 17, 18, 19, 21, 24, 25*, 31	1[5], 2[5], 3[5], 6[5], 7[5], 8, 9[5], 10[5], 11[5], 12[5], 14[6], 15[5], 16[5], 17[5], 18[5], 22[5], 24[5], 25[5]*, 26[7], 27[5], 30[5], 31[5], 32[5]

1.Large quantities
2.Intoxicating
3.Burns
4.Suspected
5.May evolve toxic fumes in fire
6.Combustible
7.May ignite combustible material
* Sodium meta arsenite is a highly toxic chemical and must be handled with extreme care. It also absorbs water and CO_2 from the air.

Table 3
A Summary of Avoidance Precautions for Table 1 Chemicals

AVOID CONTACT WITH:				
Water	Acids	Bases	Oxidisers	Combustibles
32[1]	5, 20, 23, 24	9, 32	4, 6, 8, 11, 12, 13, 14, 22, 24, 26, 27, 28, 30, 31	19, 26

[1]Concentrated sulphuric acid generates a great deal of heat on dilution with water. **Always add the acid slowly to an excess of water. Allow plenty of time for the solution to cool between acid additions. Never pour water into concentrated acid because explosive evolution of steam can take place which will violently expel the acid into the laboratory.**